Natural Products Peharmaline A and Oxoaplysinopsins: Synthesis, Analogues and their Biological Evaluation

by

Akshay Suhas Kulkarni
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Under the supervision of
Dr. D. Srinivasa Reddy

CSIR-National Chemical Laboratory, Pune

Academy of Scientific and Innovative Research
AcSIR Headquarters, CSIR-HRDC campus
Sector 19, Kamla Nehru Nagar,
Ghaziabad, U.P. – 201 002, India

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Certificate

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Akshay S. Kulkarni
(Research Student)
22.04.2022

Dr. D. Srinivasa Reddy
(Research Supervisor)
22.04.2022

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Signature of the Supervisor
Name : Dr. D. Srinivasa Reddy
Date : 22/04/2022
Place : Pune

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During the entire tenure of my doctoral research, I have been accompanied and supported by many people. Herein I take this opportunity to express my gratitude to all of them.

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Akshay S. Kulkarni

Abbreviations

Ac	acetyl
AChE	acetylcholinesterase
AcOH	acetic acid
ACN	acetonitrile
BBB	blood–brain barrier
BChE	butyrylcholinesterase
Bn	benzyl
brsm	based on recovery of starting material
br.s	broad singlet
^{t}BuOH	*tert*-butanol
Cat.	catalytic
CDI	1,1'-Carbonyldiimidazole
cm^{-1}	1/centimetre
C-C	carbon-hydrogen
C–H	carbon-hydrogen
C–O	carbon-oxygen
°C	degree celcius
DCM	dichloromethane
DIPEA	*N*, *N*-Diisopropylethylamine
DMAP	4-dimethyl aminopyridine
DMF	*N*,*N*-dimethylformamide
DMSO	*N*,*N*-dimethylformamide
Et	ethyl
Et2O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
equiv.	equivalents

Abbreviations

g	gram(s)
h	hour(s)
H_2	hydrogen gas
Hz	hertz
HRMS	High resolution mass spectroscopy
HCl	hydrochloric acid
IR	Infrared
IC_{50}	half maximal inhibitory concentration
i.e.	that is
in vitro	outside a living organism
in vivo	inside a living organism
J	coupling constant
K_2CO_3	potassium carbonate
LDA	Lithium diisopropylamide
LiHMDS	Lithium bis(trimethylsilyl)amide
Me	methyl
mg	milligram(s)
MHz	megahertz
min	minute(s)
mL	millilitre
mmol	millimole(s)
MP	melting point
MW	Microwave Irradiation
m/z	mass to charge ratio
N	normality
NaH	sodium hydride
nM	nanomolar

Abbreviations

NMR	nuclear magnetic resonance
PCC	Pyridinium chlorochromate
PDC	pyridinium dichromate
Ph	phenyl
ppm	parts per million
Pd	palladium
q	quartet
R_f	retention factor
s	singlet
SAR	Structure Activity Relationship
sec	secondary
t	triple
tert	tertiary
TFA	trifluroacetic acid
THF	Tetrahydrofuran
TLC	thin layer chromatography
US-FDA	United States-Food and Drug Administration
vs	versus
WHO	World Health Organization
µM	micromolar

General Remarks

- All reagents, starting materials, and solvents were obtained from commercial suppliers and used as such without further purification. Solvents were dried using standard protocols or through MBRAUN (MB SPS-800) solvent purification system (SPS).
- All reactions were carried out in oven-dried glassware under a positive pressure of argon or nitrogen unless otherwise mentioned with magnetic stirring.
- Air sensitive reagents and solutions were transferred via syringe or cannula and were introduced to the apparatus *via* rubber septa.
- Progress of reactions were monitored by thin layer chromatography (TLC) with 0.25 mm pre-coated silica gel plates (60 F254). Visualization was accomplished with either UV light, Iodine adsorbed on silica gel or by immersion in ethanolic solution of phosphomolybdic acid (PMA), *p*-anisaldehyde, 2,4-DNP, KMnO4, Ninhydrin solution followed by heating with a heat gun for ~15 sec.
- Column chromatography was performed on silica gel (100-200 or 230-400 mesh size).
- Melting points of solids were measured using scientific melting point apparatus (Buchi 565).
- Deuterated solvents for NMR spectroscopic analyses were used as received.
- All ^{1}H NMR, ^{13}C NMR spectra were obtained using a 200 MHz, 400 MHz, 500 MHz spectrometer. Coupling constants were measured in Hertz. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad.
- HRMS (ESI) were recorded on ORBITRAP mass analyzer (Thermo Scientific, QExactive).
- Infrared (IR) spectra were recorded on a FT-IR spectrometer as a thin film.
- Optical rotation values were recorded on P-2000 polarimeter at 589 nm.
- Chemical nomenclature (IUPAC) and structures were generated using Chem Bio Draw Ultra.

Synopsis

AcSIR	**Synopsis of the thesis to be submitted to the Academy of Scientific and Innovative Research for award of the degree of Doctor of philosophy in Chemical Sciences**
Name of the Candidate	**Akshay Suhas Kulkarni**
Enrollment No. and Date	**10CC19J26004 01 January 2019**
Title of the Thesis	**Natural Products Peharmaline A and Oxoaplysinopsins: Synthesis, Analogues and their Biological Evaluation**
Research Supervisor	**Dr. D. Srinivasa Reddy**
Research Co-Guide	

1. Introduction

The field of natural products continues to play an important role in human health, in particular, by providing starting points for the discovery of the drugs. As part of ongoing programs, our lab is engaged in total synthesis and medicinal chemistry of natural products scaffolds. Here, we have chosen two natural products peharmaline and oxoaplysinopsins. (±)-Peharmaline A is a pair of rare β-carboline and vasicinone hybrid alkaloid isolated by Wang *et al*. from seeds of *Peganum harmala* L.[1] and its structure was established through extensive spectroscopic analysis. Interestingly, (±)-peharmaline A showed considerable cytotoxic activities against HL-60, PC-3, and SGC-7901 cancer cell lines with IC_{50} values of 9.2, 21.6, and 25.4 μM, respectively. However it's two of the possible biosynthetic precursor's harmaline and vasicinone were found to be inactive. This implies that hybridity of natural product is playing role for its cytotoxicity.

The second class of natural products oxoaplysinopsins A-G were isolated by Wang *et al*. from *F. reticulata* of the XiSha Islands (Paracel Islands).[2] Although, the structures of these natural products seem to be simple but assigning their stereochemistry is very challenging. Stereochemical assignments were carried out by Wang *et al.* using extensive NMR spectroscopy. Scaffold of parent family aplysinopsin is well studied and their analogues were found to be potent for various biological activities such as neuromodulation, antineoplastic, antiplasmodial antimicrobial *etc*.[3]

2. Statement of Problem

Natural product having potential to act as a druggable candidate are always available in limited amount from natural sources. This scarcity of the material restricts complete bio-assessment of such potential molecules. In-depth study on bio-assessment demands adequate quantity of the material which can be provided by synthetic chemistry. To access the scalable amount and to understand structure activity relationship (SAR) around the natural skeleton, we planned total synthesis of (±)-peharmaline A and oxoaplysinopsins along with their close analogues.

2. Objectives

a. Total synthesis, analogues, cytotoxic evaluation and lead optimization of (±)-peharmaline A
b. Total synthesis of oxoaplysinopsin D, E, F and G
c. Development of a method for one pot oxidation of secondary alcohols to α-hydroxy ketones using pyridinium dichromate (PDC)
d. Synthesis of oxoaplysinopsin B and biological evaluation of the library of oxoaplysinopsins

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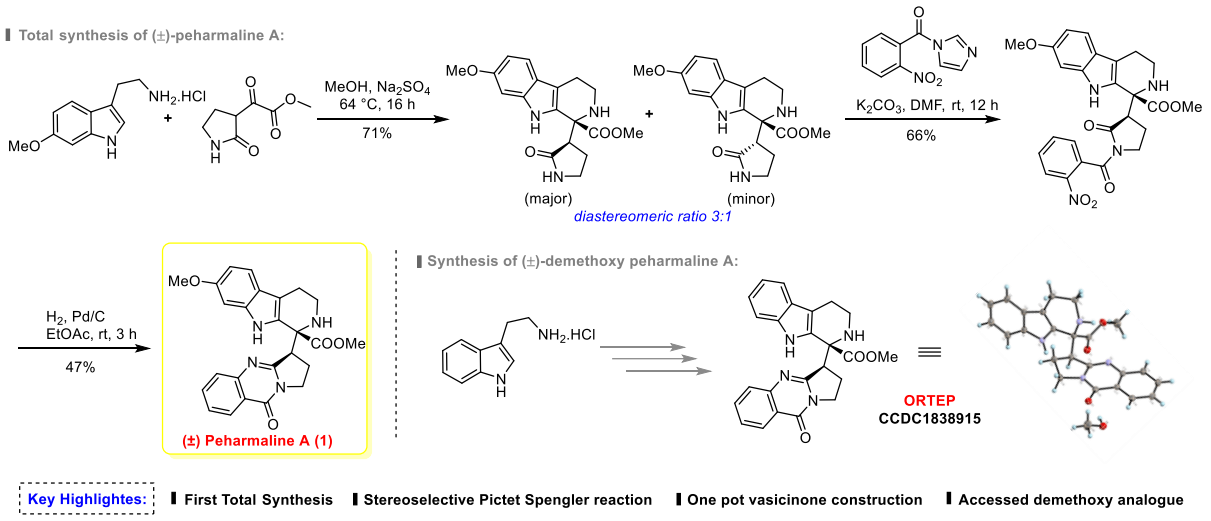
3. Methodology

The thesis is divided into two major chapters. **Chapter 1** is subdivided into two sections. Section 1 deals with the introduction, followed by the total synthesis of (±)-peharmaline A and synthesis of demethoxy peharmaline A. Section 2 describes synthesis of analogues of natural product (±)-peharmaline A, their cytotoxicity evaluation, SAR and lead optimization. **Chapter 2** is further subdivided into three sections, Section 1 introduces about aplysinopsin and oxoaplysinopsin family of natural products followed by total synthesis of oxoaplysinopsin D, E, F and G. Section 2 deals with the PDC-mediated one pot oxidation of secondary alcohols to α-hydroxy ketones. Section 3 describes synthesis of oxoaplysinopsin B and its analogues, followed by biological evaluation of the novel library of oxoaplysinopsins.

Chapter I:
Section 1: First total synthesis of anticancer natural product (±)-peharmaline A

We commenced the synthesis of (±)-peharmaline A from commercially available starting material 6-methoxy tryptamine HCl salt which on Pictet Spengler reaction with ketoester afforded required intermediate as diastereomeric mixture (d.r.=3:1). Further, both diastereomers were subjected separately for acylation reaction and found that both the diastereomers resulted in the formation of same acylation product. This was due to epimerization at the carbon next to pyrrolidinone carbonyl functionality in minor diastereomer. Next, one pot construction of vasicinone was carried out in presence of H_2, Pd/C to give the natural product (±)-peharmaline A. To access demethoxy analogue, tryptamine was subjected for same transformations and afforded demethoxy analogue of natural product and its structure was confirmed by single-crystal X-ray diffraction method.[4]



Section 2: Design, Synthesis and SAR studies of (±)-peharmaline A analogues towards identification of anticancer leads

After successful total synthesis of (±)-peharmaline A, we turned our attention to synthesize various analogues around the scaffold. Accordingly, analogues were synthesized by variation of several structural units such as ring size of vasicinone, substitution on β-carboline unit, changing methyl ester to ethyl ester, demethoxy analogue and several more simplified analogues were synthesized by replacing quinazoline part present in vasicinone.

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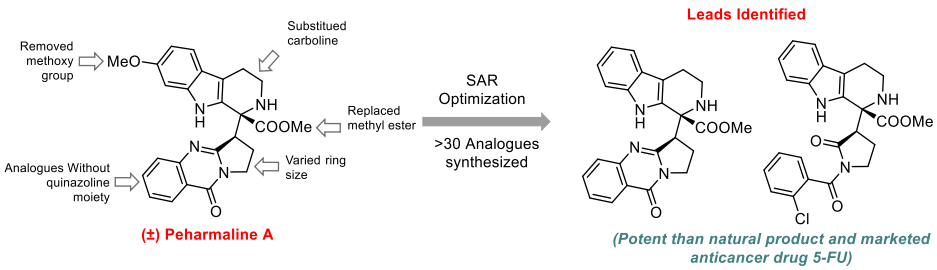
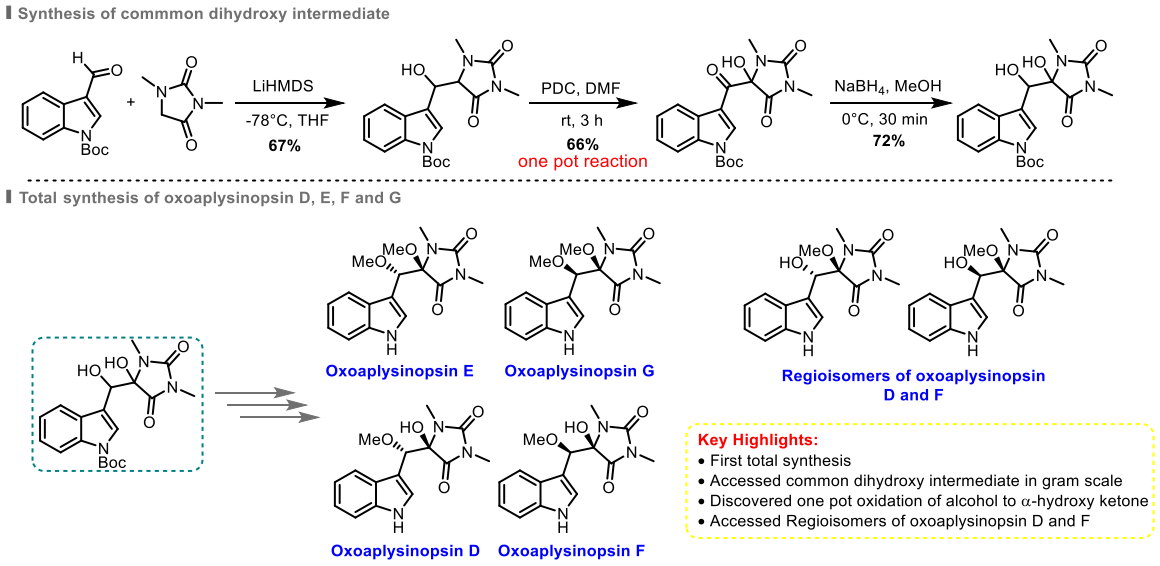


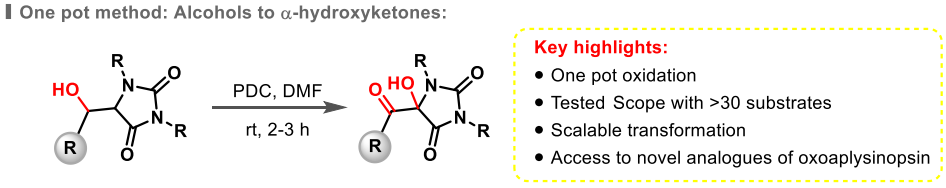
Overall, we have synthesized >30 analogues around natural product scaffold and profiled for their cytotoxic potential against various cancer cell lines. Interestingly, we have identified lead compounds with better cytotoxic activity than natural product and marketed anticancer drug 5-flurouracil.

Chapter II:
Section 1: First total synthesis of oxoaplysinopsin D, E, F and G

Our synthesis of oxoaplysinopsin D-G were planned from common dihydroxy intermediate, which was synthesized from commercially available boc-indole-3-carboxaldehyde, which on reaction with dimethyl hydantoin using LiHMDS as base and THF solvent at -78 °C for 1h yielded aldol adduct on gram scale. Further this aldol adduct was screened for several α-hydroxylation condition with no success. While optimizing the reaction we observed an interesting transformation with use of PDC in DMF solvent gave ketohydroxy compound this method was further discussed in section 2. Ketohydroxy compound was treated with $NaBH_4$ resulted required dihydroxy intermediate on gram scale. Chemoselective methylation of dihydroxy intermediate afforded oxoaplysinopsin D, E, F and G. Moreover, we have accessed regioisomers of oxoaplysinopsin D and F.[5]



Section 2: PDC-mediated one pot oxidation of secondary alcohols to *α*-hydroxy ketones



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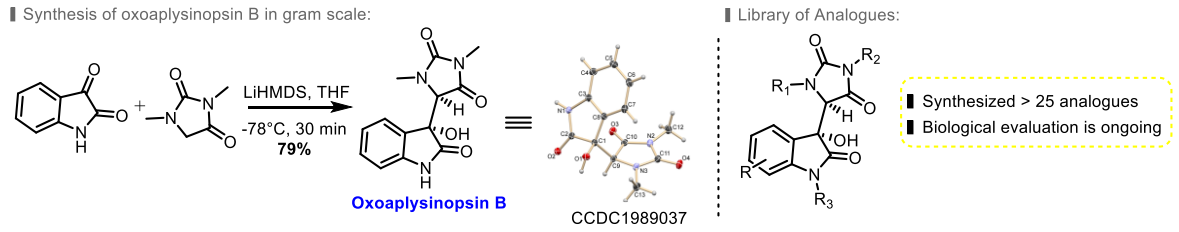
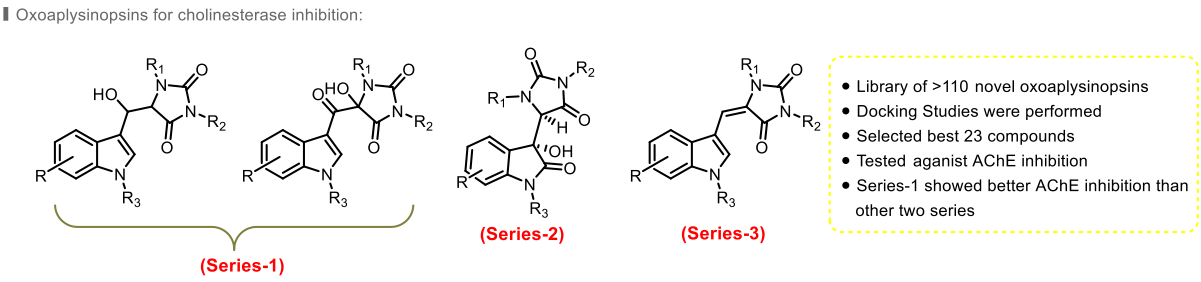
During the synthesis of oxoaplysinopsins, we discovered an interesting transformation for conversion of alcohols to ketohydroxy compounds in one pot using pyridinium dichromate in DMF. The present method is useful for making related natural products. We tested the scope of method by synthesizing more than 30 examples. Overall, using this methodology we have created a library of novel oxoaplysinopsin analogues.[5]

Section 3: Synthesis of oxoaplysinopsin B and biological evaluation of oxoaplysinopsin analogues

Oxoaplysinopsin B has potent activity against tyrosine phosphatase 1B (PTP1B) with an IC_{50} value of 7.67 µM,[2] Oxoaplysinopsin B was prepared in gram scale with one step reaction of dimethyl hydantoin with isatin using LiHMDS as base with 79% yield as single diastereomer. Further, the structure was confirmed with single-crystal X-ray diffraction method. This gram scale access to oxoaplysinopsin B in single step transformation and it's good biologically activity gave us encouragement to synthesize its close analogues. Accordingly, we have synthesized >25 analogues of oxoaplysinopsin and further biological evaluation is ongoing.



Recently, Aplysinopsin was found to inhibit acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and human BACE-1 with IC_{50} values of 33.9, 30.3, and 33.7 µM, respectively and it also showed excellent blood brain barrier permeability (*Pe* =8.92 × 10-6 cm/s).[6] As we were working on oxoaplysinopsin scaffold, we further synthesized 11 new olefinic analogues of oxoaplysinopsin which resulted in a library of more than 110 novel oxoaplysinopsins, we have carried out docking studies and selected 23 best compounds and then tested them for AChE inhibition. We found that series-1 compounds are showing almost similar inhibition as that of aplysinopsin, whereas series-2 and 3 are showing less potency.



4. Summary

a) Accomplished the total synthesis of anticancer natural product (±)-peharmaline A for the first time using short route.

b) Synthesized >30 novel analogues of (±)-peharmaline A and evaluated them against cytotoxic activities.

c) Structure Activity relationship was performed and identified structurally simplified lead compounds having potent activity than natural product (±)-peharmaline A and marketed drug 5-Flurouracil.

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d) Accomplished the total synthesis of oxoaplysinopsin D, E, F and G for the first time from common dihydroxy intermediate.
e) Developed one pot method for conversion of secondary alcohols to α-hydroxy ketones using pyridinium dichromate (PDC).
f) Synthesized oxoaplysinopsin B in one step protocol on gram scale and prepared its 25 analogues.
g) A library of >110 novel oxoaplysinopsin analogues has been generated.

5. Future directions

a) To screen library of novel oxoaplysinopsins in various biological assays.

6. Publications

1. Kulkarni, A. S.; Shingare, R. D.; Dandela, R.; Reddy, D. S. *Eur. J. Org. Chem.* **2018**, 6453–6456.
2. Kulkarni, A. S.; Ramesh, E.; Reddy, D. S. *Eur. J. Org. Chem.* **2021**, 2188-2192.

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2. Wang, Q.; Tang, X. L.; Luo, X. C.; deVoog, N. J.; Li, P. L.; Li, G. Q. *Sci. Rep.* **2019**, *9*, 2248.
3. a) Bialonska, D.; Zjawiony, J. K. *Mar. Drugs* **2009**, *7*, 166; b) Stanovnik, B.; Svete, *J. Mini-Rev. Org. Chem.* **2005**, *2*, 211; c) Beniddir, M. A.; Evanno, L.; Joseph, D.; Skiredj A. *Nat. Prod. Rep.* **2016**, *33*, 820.
4. Kulkarni, A. S.; Shingare, R. D.; Dandela, R.; Reddy, D. S. *Eur. J. Org. Chem.* **2018**, 6453–6456.
5. Kulkarni, A. S.; Ramesh, E.; Reddy, D. S. *Eur. J. Org. Chem.* **2021**, 2188-2192.
6. Nuthakki, V. K.; Yadav Bheemanaboina R. R. Bharate, S. B. *Bioorganic Chemistry*, **2021**, *107*, 104568.

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Section 1: First Total Synthesis of Anticancer Natural Product (±)-Peharmaline A

Section 2: Design, Synthesis and SAR Studies of (±)-Peharmaline A Analogues Towards Identification of Anticancer Leads

Chapter 2

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Chapter-1

Section 1: First Total Synthesis of Anticancer Natural Product (±)-Peharmaline A

Section 1 : First Total Synthesis of Anticancer Natural Product (±)-Peharmaline A

1.1.1 Introduction:

In broad terms, Natural products, are the molecules produced in the nature which includes, molecules derived from living organisms e.g. plants, microbes, animals *etc*. Natural products constitute a large number of diverse structural chemical entities having wide range of biological activities that display various uses, primarily in drug discovery and in agricultural sector.[1] There are more than 23,000 natural products have been isolated from different sources after the discovery of penicillin and their structures were well characterized by various analytical tools and techniques.[2] The creation and evalution process of nature has made natural proudcts well optimized to perform particular biological functions. Moreover, their use in traditional medicine may offer better efficacy and safety. In comparision with any synthetic drug molecule, natural products offer more advantages and challenges because of their wide-range of structural complexity, chemical diversity, higher molecular masses, more numbers of hydrogen bond acceptors and donors, contains large abundance of sp^3 carbon and oxygen atoms, lower clogP (which reflect in higher hydrophilicity), significant molecular rigidity (beneficial in drug discovery which helps to tackle protein–protein interactions), more stereogenic centers and several other molecular assets owing to which they interact with many specific targets present in biological systems.[3] Structural scaffold of natural products serves as starting points to identify new potent, selective and viable drug candidates. Natural products are being chemically synthesized and their structures can also be modified using synthetic and semi-synthetic methods which further help to increase potency and selectivity, improve physiochemical properties, pharmacokinetic properties, increase metabolic stability *etc*.[4] During the past three decades, several natural products or natural product derivatives have been approved as drugs against different diseases. The combined contribution of natural products to total approved drugs remains high, almost at 35-40%. This number increases to around 60%, after considering natural product mimics and other botanical drugs.[5-7] Therefore, numerous drugs that are presently available in market were discovered from Natural products.[8] In fact, the influence of Natural products on drug discovery is so huge that there are many reports and reviews documented in the literature. [9,10]

1.1.1.1 Natural products with carboline unit:

Carbolines are a prominent family of nature-derived heterocyclic compounds, with significant pharmacological potential.[11] Structurally, it contains a tricyclic framework *i.e.* indole ring fused with pyridine. Carboline alkaloids are found extensively in various plants, marine creatures, food products, alcoholic beverages, mammals, insects, microorganisms, human tissues and body fluids *etc*.[12] Carbolines are classified on the basis of position of the nitrogen atom in the pyridine ring as α-, β-, γ- or δ-carboline and degree of saturation present in C ring (totally saturated: 1,2,3,4-tetrahydro, slightly saturated: 3,4-dihydro and unsaturated β -carbolines) as shown in Figure 1.1.1.

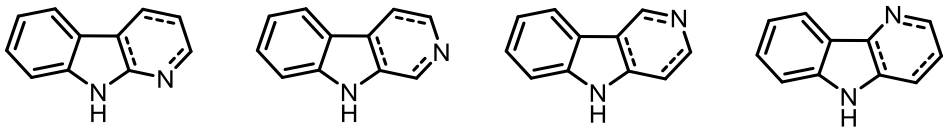


Figure 1.1.1: Classification of carboline unit

Among all the types of carbolines, β-carbolines represent a prominent class of indole alkaloids due to their diverse biological activities.[13,14] The interesting structural diversity and strong biological importance of β-carboline family of alkaloids encourage researchers to plan their synthesis and evaluate their biological potential. Till date, there are several β -carboline containing drugs are marketed (shown in Figure 1.1.2).[11] Vincamine is a carboline containing indole alkaloid isolated from the leaves of *Vinca minor* with a vasodilatory property. It has been an approved drug in Europe for treating vascular and primary degenerative dementia. Moreover, it has been approved by the United States to use as a dietary supplement.[15] Brominated analogue of vincamine called brovincamine is known for its antiglaucoma and vasodilator effect.[16] Moreover, vinpocetine is another synthetic analogue of the vincamine approved in European and several Asian countries for the treatment of cerebrovascular disorders.[17]Abecarnil is another anxiolytic drug from β-carboline family known for its effect on anxiety disorder.[18] Tadalafil is a FDA approved oral medication for the treatment of benign prostatic hyperplasia, pulmonary arterial

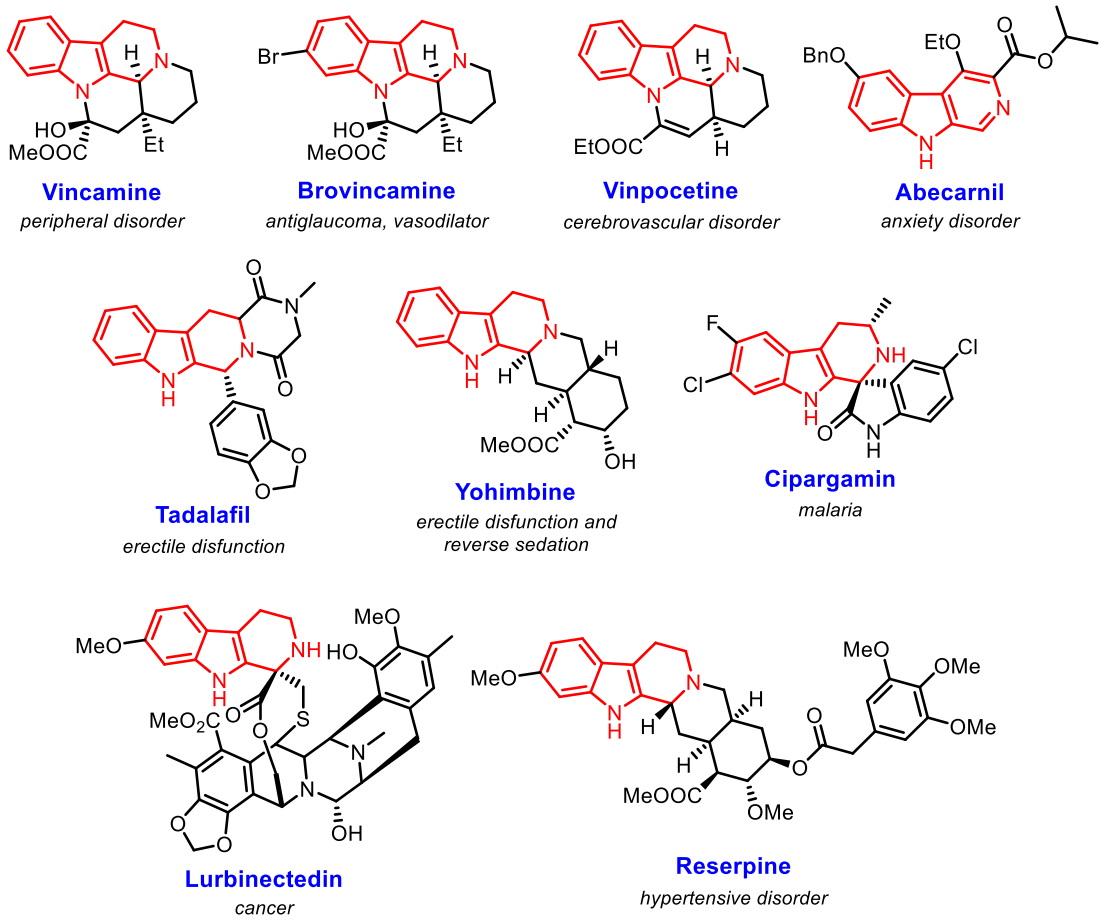


Figure 1.1.2: Marketed drugs having β-carboline unit

hypertension and erectile dysfunction.[19] Yohimbine is a natural indole alkaloid prescribed for erectile dysfunction.[20] Synthetic derivative cipargamin is another molecule belonging to the spiroindolone class and is presently in clinical development for the treatment of malaria.[21] Lurbinectedin is a drug that was approved for use in the United States for the treatment of small cell lung cancer in June 2020.[22] Reserpine is a well-known natural indole alkaloid isolated in 1952 from the dried root of *Rauvolfia serpentine* and used for the treatment of high blood pressure, usually in combination with vasodilator or thiazide diuretic.[23]

Peganum harmala L. is a traditional medicinal plant and rich source of β-carboline alkaloids, which has been used for the treatment of alimentary tract cancers and malaria in Northwest China.[24] β-Carboline alkaloids exhibit a wide range of biological activities such as antitumor, antimalarial, antimicrobial, and anti-inflammatory effects.[25] In this context, (±)-Peharmaline A, a pair of novel β-

carboline–vasicinone hybrid alkaloid enantiomers was isolated by Wang *et al.* in 2017 from the seeds of *Peganum harmala* L.[26] (Figure 1.1.3) Interestingly, compound (±)-peharmaline A displayed significant cytotoxic activity against the HL-60, PC-3, and SGC-7901 cancer cell lines with IC_{50} values of 9.2, 21.6, and 25.4 μM, respectively; whereas, its two biosynthetically related precursors, harmaline and vasicinone were found to be inactive, This clearly implies that the unique dimeric structure is crucial for the cytotoxicity.

Figure 1.1.3: (±)-Peharmaline isolation from *peganum harmala* L. by Wang *et al.*

1.1.2 Present work:

Considering the interesting hybrid alkaloid structure and its anticancer activity, we decided to take up the synthesis and SAR studies around this scaffold to find the anticancer lead(s). Before taking up the synthesis of actual natural product, we optimized the scheme on its demethoxy analogue, and accomplished synthesis of demethoxy-peharmaline A using short sequence. Further same scheme was utilized for synthesis of natural product, and completed first total synthesis of (±)-peharmaline A using Pictet-Spengler reaction followed by one pot vasicinone construction as key steps. Interestingly, we have observed both the diastereomers of Pictet adduct gave natural product (±)-peharmaline A.

1.1.2.1 Total Synthesis of (±)-demethoxy peharmaline A:

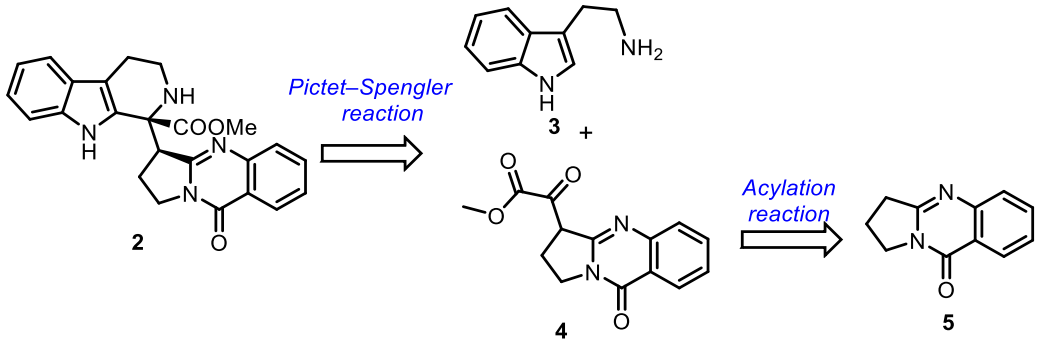
Due to easy commercial availability of tryptamine, we decided to first optimize scheme for synthesis of model compound demethoxy peharmaline A which could further be used for actual natural product synthesis.

1.1.2.1.1 Initial Attempts towards synthesis of (±)-demethoxy peharmaline A:

We have tried few approaches for the total synthesis of demethoxy peharmaline A but with no success, all the failed approaches are explained in the following section.

Approach 1:

Demethoxy peharmaline A **2** was envisioned to be obtained from commercially available tryptamine **3** and acylated deoxyvasicinone **4** using Pictet-Spengler reaction.[27] Acylated deoxyvasicinone **4** was planned from deoxyvasicinone **5** by base mediated acylation reaction using methyl oxalyl chloride. Deoxyvasicinone **5** could be prepared from anthranilic acid by known literature protocol.

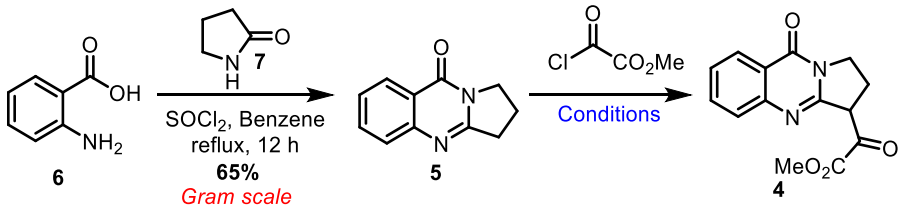


Scheme 1.1.1: Retrosynthetic plan for demethoxy peharmaline A

The synthesis started with an aim to prepare deoxyvasicinone **5** in good quantities following known literature protocol. Accordingly, anthranilic acid **6** was treated with 2-pyrrolidone **7** using $SOCl_2$ in benzene which gave deoxyvasicinone **5**. ^1H NMR of **5** was in complete agreement with known literature data.[28] Further compound **5** was subjected for acylation reaction using various reaction conditions which are mentioned in scheme 1.1.2.

Initially, compound **5** was treated with sodium methoxide in methanol at room temperature and observed no product formation, here we recovered starting material as such, the same observation was noted after changing the base to NaH/THF, NaHMDS/THF, LiHMDS/THF. However, when we changed the base to LDA (2.5 equiv.) in THF at -78 °C it showed formation of compound **4** with very poor yield (5% - 6%). However, addition of HMPA under the same reaction condition resulted in

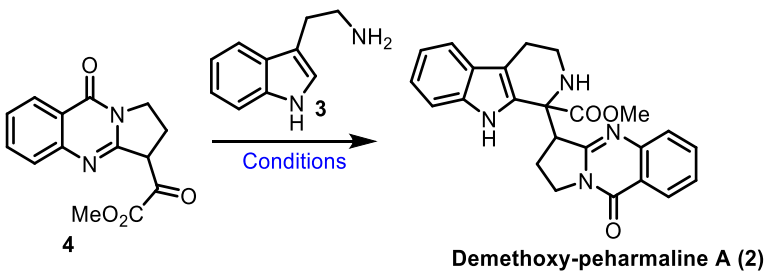
no reaction. Further we did not observe any yield improvement by increasing the equivalent of LDA (4 equiv.). The best condition to obtain this acylated compound was was found to be LDA, THF, -78 °C with 6% yield.



No.	Conditions	Observation
1.	NaOMe, MeOH, rt, 12 h	No reaction
2.	NaH, THF, rt	No reaction
3.	NaH, THF, 60 ºC	No reaction
4.	NaHMDS, THF, -78 °C	No reaction
5.	LiHMDS, THF, -78 °C	No reaction
6.	LDA (2.5 equiv.), THF, -78 ºC, 2 h	5% to 6%
7.	LDA, HMPA, THF, -78 ºC, 2 h	No reaction
8.	LDA (4 equiv.), THF, -78 ºC, 2 h	6%

Scheme 1.1.2: Synthesis of acylated deoxyvasicinone **4**

Having small-scale amount of compound **4** in hand, we decided to utilize it for optimizing Pictet-Spengler reaction. Accordingly, hydrochloride salt of tryptamine **3** was treated with compound **4** in methanol under microwave irradiation at 100 °C for 40 min and afforded trace amount of compound **5** along with starting materials. However, when the same reaction was carried out with conventional heating at 80 °C resulted in no product formation.



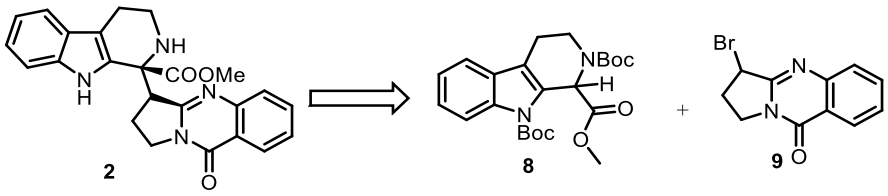
No.	Conditions	Observation
1.	**3**.HCl, MeOH, MW, 100 °C, 40 min	Compound **5** and SM
2.	**3**.HCl, MeOH, 80 °C, 12 h	No reaction
3.	TFA, DCM, rt, 12 h	No reaction
4.	HFIPA, MW, 80 °C	No reaction
5.	AcOH, 60 °C, 12 h	Observed **5**

Scheme 1.1.3: Optimization of Pictet-Spengler reaction

Further, changing the acids to trifluroacetic acid and hexafluoroisopropanol (HFIPA) ended up with no reaction. Compound **5** was obtained after treating tryptamine **3** and compound **4** in presence of acetic acid at 60 °C for 12 h. After screening these few attempts on available small quantity of compound **4** with no success, we decided to modify our approach.

Approach 2:

Demethoxy peharmaline A **2** was further planned from compounds **8** and bromovasicinone **9**. Synthesis of compound **8** and **9** were planned using literature known protocol.

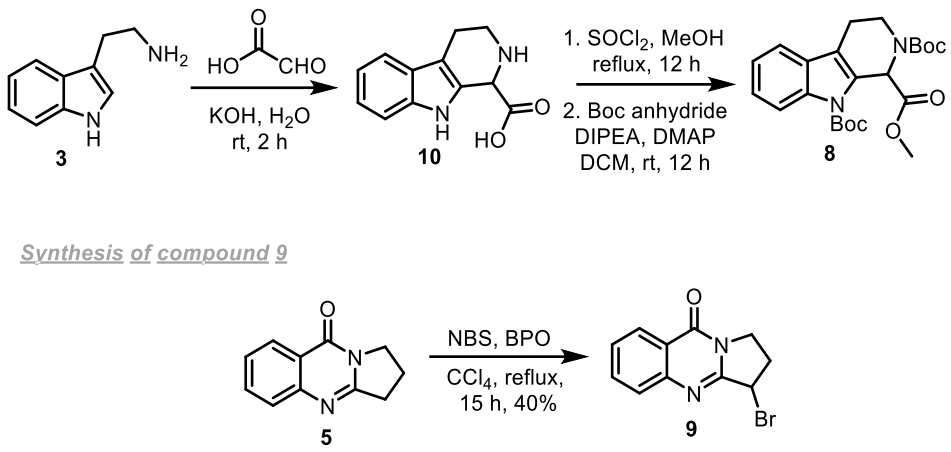


Scheme 1.1.4: Retrosynthetic plan for demethoxy peharmaline A **2**

For the synthesis of compound **8**, commercially available tryptamine **3** was treated with glyoxylic acid using KOH in water gave required acid **10**, which was further subjected for esterification reaction using thionyl chloride in methanol afforded desired compound **10a**. ^{1}H NMR of **10a** is in agreement with reported data in the literature.[29] Compound **10a** was further subjected for diBoc-protection using Boc anhydride, DIPEA and catalytic DMAP resulted in required compound **8**. Formation of compound **8** was confirmed by signal in ^{1}H NMR at δ 1.53 (s., 9 H) belongs to *t*-

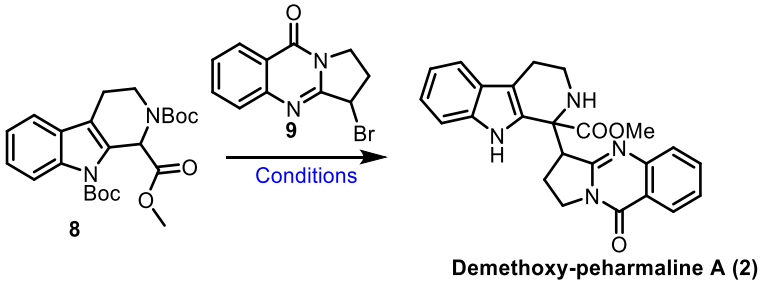
Synthesis of compound 8

Synthesis of compound 9



Scheme 1.1.5: Synthesis of two intermediates **8** and **9**

butyl group from Boc present on secondary amine, whereas peak at δ 1.66 (s, 9 H) showed *t*-butyl group from Boc present on indole nitrogen.Next, compound **5** was subjected for bromination reaction using *N*-bromosuccinimide (NBS) and benzoyl peroxide (BPO) in CCl_4 solvent resulted in compound **9** with 40% yield. Formation of compound **9** was confirmed by comparing ^{1}H NMR data with reported data in literature.[30]



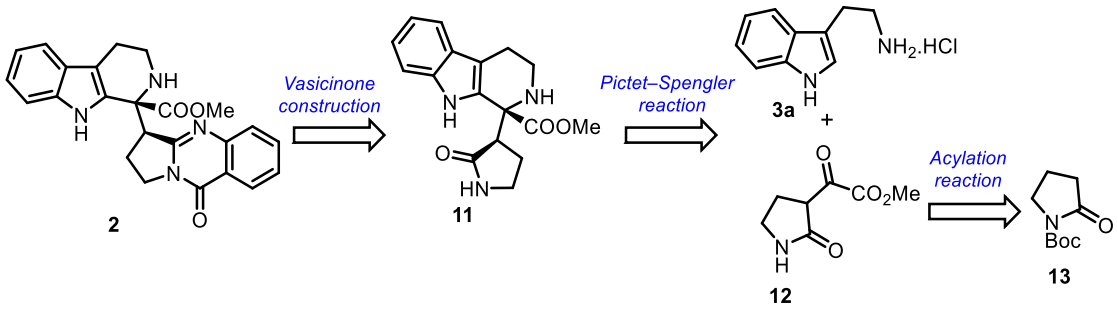
No.	Conditions	Observation
1.	NaHMDS, THF, -78 °C, 2 h	No reaction
2.	LiHMDS, THF, -78 °C, 2 h	No reaction
3.	NaH, THF, rt, 12 h	No reaction
4.	NaH, DMF, rt, 12 h	Decomposed
5.	LDA, THF, -78 °C, 2 h	No reaction

Scheme 1.1.6: Synthesis of demethoxy peharmaline A **2**

After having both compounds in hand, we attempted few conditions to obtain desired compound **2**. Selected conditions are mentioned in scheme 1.1.6. Firstly, compound **8** and compound **9** were treated with commercial NaHMDS in THF at -78 °C resulted no reaction and we recovered starting materials as such. Further changing the base to LiHMDS (freshly prepared) showed no effect on reaction. We also performed the reaction in presence of strong base sodium hydride in THF and observed no reaction. However, same reaction was carried out in DMF and observed decomposition of both the starting materials. Further, freshly prepared LDA was used for same transformation but reaction did not give the desired result.

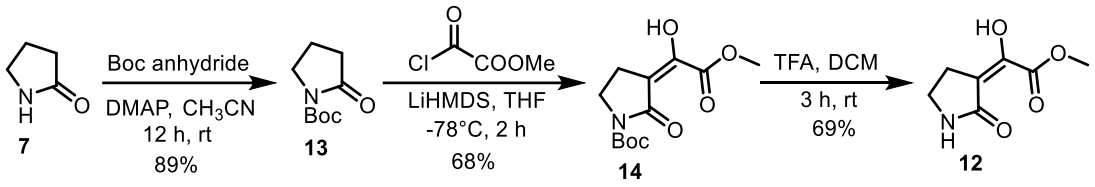
1.1.2.1.2 Revised total synthesis of (±)-demethoxy peharmaline A:

After having a few unsuccessful attempt for the synthesis of demethoxy peharmaline A **2**, we revised our strategy as shown in scheme 1.1.7. Demethoxy peharmaline A **2** was planned from pictet adduct **11** by late stage construction of vasicinone. Pictet adduct **11** could be traced from commercially available tryptamine **3** and ketoester **12** by Pictet-Spengler reaction.



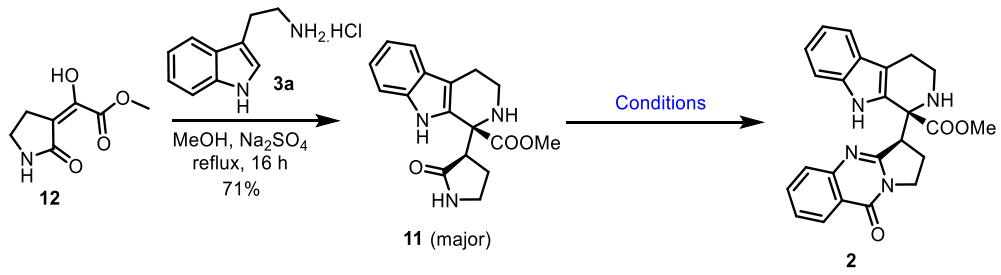
Scheme 1.1.7: Revised restrosynthetic plan

Required ketoester **12** could be accessed from Boc-pyrrolidone **13** by acylation reaction using methyl oxalyl chloride. As planned, first we targeted the synthesis of ketoester **12** on a gram scale, accordingly, commercial available 2-pyrrolidone **7** was subjected for Boc protection using Boc anhydride, DMAP in acetonitrile gave Boc protected compound **13** which was confirmed by comparing ^{1}H NMR with reported data.[31]



Scheme 1.1.8: Synthesis of ketoester **12** on a gram scale

Compound **13** was treated with methyl oxalyl chloride using freshly prepared LiHMDS in THF at -78 °C afforded compound **14** which was subjected for Boc-deprotection using TFA in DCM gave required ketoester **12** in 69% yield (scheme 1.1.8) Formation of compound **12** was confirmed by characteristic peaks at δ 3.88 (s, 3 H) corresponding to methyl ester and further disappearance of *t*-butyl peak (9 H) confirmed Boc deprotection. In addition, HRMS analysis showed peak at 194.0428 corresponding to molecular formula $C_7H_9NO_4Na$ $[M+Na]^+$ confirmed product **12**. After having desired ketoester **12** in hand, it was subjected for Pictet-Spengler reaction with tryptamine hydrochloride salt **3a** using methanol, sodium sulphate on reflux for 16 h gave pictet adduct **11** in 71% yield.

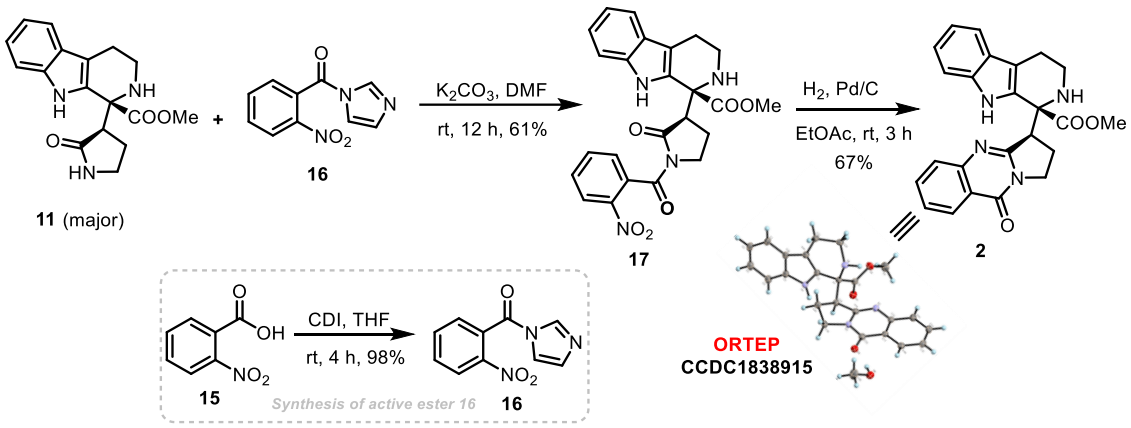


No.	Conditions	Observation
1.	Anthranilic acid, $SOCl_2$, benzene, reflux, 12 h	No reaction
2.	Anthranilic acid, $POCl_3$, toluene, reflux, 8 h	No reaction
3.	Isatoic anhydride, TEA, toluene, MW, 100 ºC, 30 min	No reaction
4.	2-Azidobenzoic acid, $SOCl_2$, reflux , 12 h	No reaction

Scheme 1.1.9: Optimization of vasicinone construction

To our delight, excellent distereoseletivity was observed in this reaction with diastereomeric ratio 9:1 (based on crude NMR). Formation of compound **11** was

confirmed by additional aliphatic peaks in ^{1}H and ^{13}C NMR belongs to pyrrolidone moiety along with peak at 314.1502 in HRMS corresponds to the molecular formula $C_{17}H_{20}N_3O_3$ $[M+H]^+$. The next important task was to construct the tricyclic core of vasicinone, for which we attempted few conditions as mentioned in scheme 1.1.9. First we treated pictet adduct **11** with anthranilic acid **6**, $SOCl_2$, benzene but observed no reaction, additionally use of anthranilic acid, $POCl_3$ in toluene did not work and we recovered starting material **11** as such. Further using literature known protocol for vasicinone construction using isatoic anhydride and 2-azidobenzoic acid did not give any fruitful results.[32] These unproductive results prompted us to perform cyclization reaction in a step-wise fashion. Accordingly, we synthesized stable activated ester **16** using known reaction of 2-nitrobenzoic acid **15** and carbonyldiimidazole (CDI).[33] Activated ester **16** was subjected for acylation reaction with compound **11** using K_2CO_3 as a base in DMF which gave chemoselective acylation product **17** in 67% yield. Formation of compound **17** was confirmed by additional 4 aromatic peaks in ^{1}H NMR and newly formed amide carbonyl group was confirmed by signal at δ 166.2 ppm in ^{13}C NMR, additional confirmation was done by HRMS analysis having peak at 463.1619 corresponds to molecular formula $C_{24}H_{23}N_4O_6$ $[M+H]^+$. In the compound **11** acylation reaction did not work on secondary amine due to present steric effect of methyl ester.



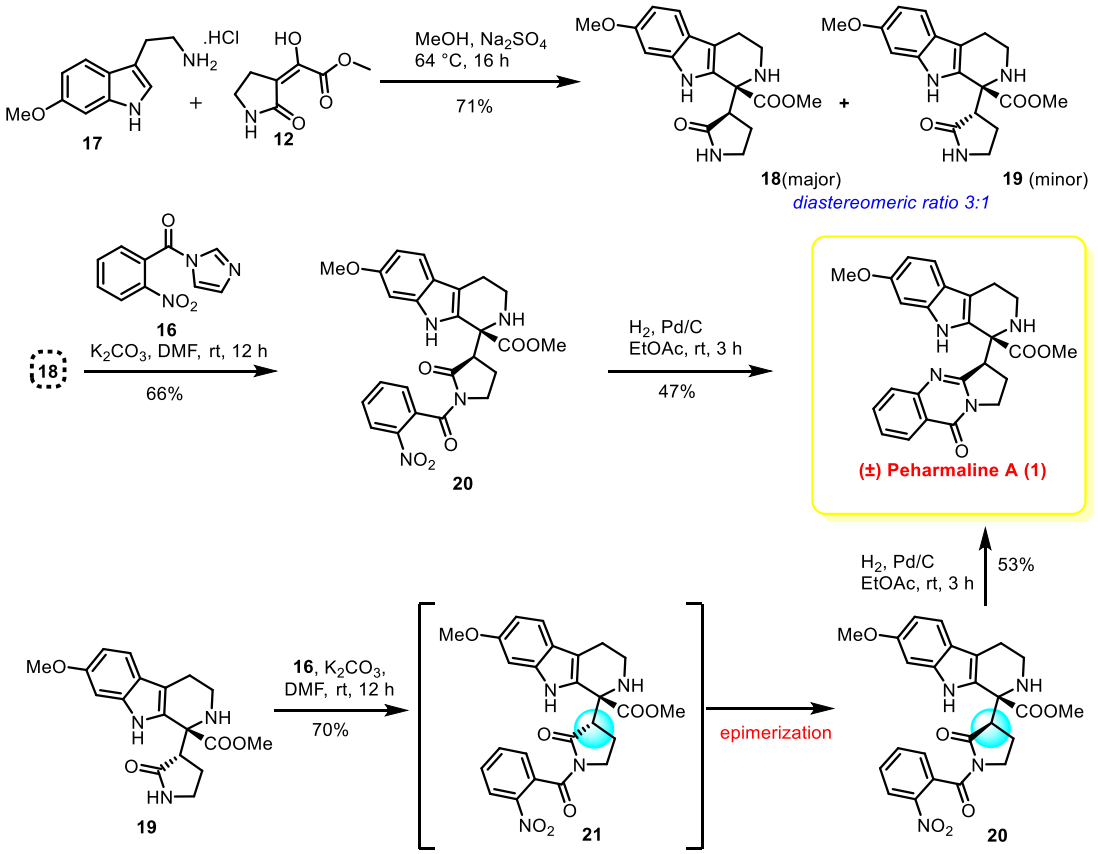
Scheme 1.1.10: Synthesis of (±)-demethoxy peharmaline A

Nitro group present in compound **17** was reduced using 10% palladium on carbon, which resulted spontaneous intramolecular condensation with the amide carbonyl

present in compound **17** to obtain the desired demethoxy peharmaline **2** in 67 % yield. Formation of compound **2** was confirmed by new peak in ^{13}C NMR at δ 158.5 ppm belongs to newly formed junction carbon and also peak at 437.1591 in HRMS analysis corresponds to molecular formula $C_{24}H_{22}N_4O_3Na$ $[M+Na]^+$. Additional confirmation of compound **2** was done by single-crystal X-ray diffraction (scheme 1.1.10).

1.1.2.2 Total Synthesis of (±)-peharmaline A:

After having an optimized route for synthesis of demethoxy peharmaline A **2** in hand, we decided to complete the total synthesis of actual natural product peharmaline A (**1**) which has methoxy group on tryptamine unit at 6-position. For the synthesis, 6-methoxy tryptamine unit was required which was prepared from 6-methoxy indole using known literature protocol.[34]

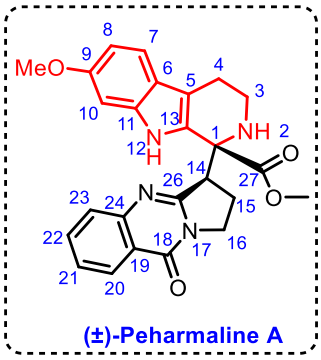


Scheme 1.1.11: Synthesis of (±)-peharmaline A (**1**)

Synthesis of peharmaline A was commenced from 6-methoxytryptamine hydrochloride salt **17** which was subjected for Pictet-Spengler reaction with ketoester

12 using previously optimized condition (Na_2SO_4, MeOH, 64 °C, 16 h) to give diastereomeric mixture of pictet adducts **18** and **19** with diastereomeric ratio 3:1. Here we screened few other conditions as well to obtain better distereoseletivity but with no success. Both the diastereomers were separated using silica gel column chromatography. The major diastereomer **18** was *N*-acylated by treating with active ester **16** using K_2CO_3 as a base in DMF to give compound **20** in 66% yield. Confirmation of compound **20** was done by the appearance of new aromatic peaks corresponding to 4 protons in ^{1}H NMR and ^{13}C NMR having peak at δ 166.2 showed the presence of amide carbonyl, furthermore confirmation was done by HRMS peak at 493.1726 associated with molecular formula $C_{25}H_{25}N_4O_7$ $[M+H]^+$. Next, compound **20** on reductive condensation using H_2, Pd/C gave natural product (±)-peharmaline A **1** in 47% yield. Further minor diastereomer **19** was subjected for acylation using optimized condition (K_2CO_3, DMF, room temp.) gave compound **21** with 70% yield. Interestingly, here we found that all peaks from ^{1}H and ^{13}C NMR of compound **21** was exactly matching with NMR of compound **20** and further structure was also confirmed by HRMS analysis having peak at 493.1726 corresponding to molecular formula $C_{25}H_{25}N_4O_7$ $[M+H]^+$. Further we subjected it for reductive condensation and obtained the final compound with 53% yield. The NMR data of the synthetic compound was exactly matching with NMR of natural product (±)-peharmaline A (**1**) detailed NMR comparison table given in table 1.1.1.[26] In short we have afforded natural product from both major **18** and minor **19** diastereomer. We proposed that due to addition of excess K_2CO_3 base (3 equiv.) epimerization of the α-proton in compound **21** resulted in compound **20**.

Table 1.1.1: Comparison of spectral data of natural and synthetic (±)-peharmaline A



Natural sample in DMSO-d_6			Synthetic sample in DMSO-d_6	
No	**^{1}H (mult, *J* in Hz)**	**^{13}C**	**^{1}H**	**^{13}C**
1	-	64.0	-	64.0
3a	3.10 (ddd, 11.0, 11.0, 4.2, 1 H)	40.4	3.11 (m, 1H)	40.4
3b	3.04 (ddd, 11.0, 11.0, 5.2, 1 H)		3.06 (m, 1H)	-
4a	2.61 (ddd, 11.0, 11.0, 5.2, 1 H)	21.3	2.60 (m, 1H)	21.3
4b	2.55 (m, 1 H)		2.57 (overlapped, 1H)	-
5	-	111.3	-	111.2
6	-	120.4	-	120.3
7	7.30 (d, 8.4, 1 H)	118.6	7.30 (d, 8.5, 1H)	118.6
8	6.64 (dd, 8.4, 2.0, 1 H)	108.5	6.64 (dd, 8.5, 2.4, 1 H)	108.5
9	-	155.8	-	155.7
10	6.91 (d, 2.0, 1 H)	94.7	6.91 (d, 2.4, 1 H)	94.6
11	-	137.3	-	137.2
13	-	128.7	-	128.6
14	4.42 (dd, 9.3, 6.9, 1 H)	51.1	4.42 (dd, 9.2, 7.3, 1 H)	51.1

15a	1.89 (dddd, 18.0, 9.3, 6.9, 6.9, 1 H)	20.7	1.90 (overlapped, 1H)	20.7
15b	1.83 (dddd, 18.0, 9.3, 9.0, 5.2, 1 H)		1.83 (overlapped, 1H)	-
16a	4.00 (ddd, 11.5, 9.3, 5.2, 1 H)	45.2	4.04 (overlapped, 1H)	45.1
16b	3.87 (ddd, 11.5, 9.0, 6.9, 1 H)		3.97 (overlapped, 1H)	-
18	-	160.0	-	160.0
19	-	120.6	-	120.6
20	8.12 (dd, 7.8, 1.0, 1 H)	125.7	8.12 (d, 7.9 1 H)	125.6
21	7.50 (t, 7.8, 1 H)	126.1	7.50 (t, 7.9, 1 H)	126.0
22	7.80 (td, 7.8, 1.0, 1 H)	133.9	7.80 (td, 7.3, 1.2, 1 H)	133.9
23	7.58 (d, 7.8, 1 H)	127.1	7.58 (d, 7.9, 1 H)	127.1
24	-	148.7	-	148.7
26	-	158.6	-	158.5
27	-	173.1	-	173.0
9-OCH_3	3.77 (s, 3 H)	55.1	3.77 (s, 3 H)	55.1
27-OCH_3	3.83 (s, 3 H)	52.3	3.83 (s, 3 H)	52.2
2-NH	1.89 (br. s, 1 H)	-	2.88 (br. s, 1 H)	-
12-NH	10.79 (br. s, 1 H)	-	10.79 (br. s, 1 H)	-

1.1.3 Conclusion:

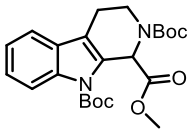
We have synthesized anticancer natural product (±)-peharmaline A using a short route for the first time. Pictet-Spengler reaction and one pot construction of vasicinone are the key steps. Interestingly, we have observed epimerization of a key intermediate and hence both diastereomers obtained from Pictet-Spengler reaction gave desired natural

product. Additionally, we have synthesized one demethoxy analogue of (±)-peharmaline A. The developed route is very useful for the generation of library of molecules around target natural product towards lead optimization as described in next section.

1.1.4 Experimental section

Experimental procedures and characterization data of selected compounds are given below; Data of remaining compounds can be found at (*Eur. J. Org. Chem.* **2018**, 6453; doi.org/10.1002/ejoc.201800949)

2,9-di-tert-butyl 1-methyl 3,4-dihydro-1H-pyrido[3,4-b]indole-1,2,9-tricarboxylate (8)



Yield: 59% over three steps

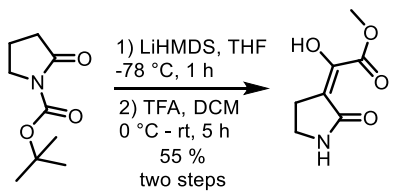
IRυ_{max}(film): 2981, 2351, 1711, 1664, 1151 cm^{-1}

^{1}H NMR (400 MHz,$CDCl_3$) = δ 8.25 - 8.07 (m, 1 H), 7.50 - 7.37 (m, 1 H), 7.35 - 7.29 (m, 1 H), 7.25 (br. s., 1 H), 6.54 – 6.37 (m, 1 H), 6.37 (br. s., 1 H), 3.75 (br. s., 3 H), 3.15 – 3.00 (m, 1 H), 2.85 - 2.71 (m, 2 H), 1.66 (s, 9 H), 1.53 (s., 9 H)

^{13}C NMR (100 MHz,$CDCl_3$) = δ 170.3, 170.0, 154.2, 149.9, 136.3, 129.4, 128.4, 124.7, 122.8, 118.2, 118.1, 116.9, 116.4, 115.6, 84.2, 80.9, 55.7, 54.9, 52.4, 39.7, 38.1, 28.4, 28.1, 21.1, 20.9

HRMS (ESI): *m/z* calculated for $C_{13}H_{30}N_2O_6Na$ $[M+Na]^+$ = 453.1996, Observed = 453.1985

Methyl (E)-2-hydroxy-2-(2-oxopyrrolidin-3-ylidene)acetate (12)



To a stirred solution of *N*-Boc-2-pyrrolidinone **13** (10 g, 0.054 mol) in 100 mL THF, 1M Lithium bis(trimethylsilyl)amide (118 mL, 0.118 mol) was added dropwise at -78

°C for 15 min. Methyl oxalyl chloride (6.6 gm, 0.054 mol) was then added dropwise and the resultant mixture stirred at -78 °C for 1h. The reaction mixture was acidified with 1N HCl and allowed to warm at room temperature. The aqueous layer was extracted with EtOAc (3x200 mL), the combined organic extract washed with brine (300 mL), dried (Na_2SO_4) and concentrated in *vaccuo*. The crude product was purified by column chromatography to afford tert-butyl 3-(2- methoxy-2-oxoacetyl)-2-oxopyrrolidine-1-carboxylate **14** as a white solid (10.6 g, 69% yield) The above product tert-butyl 3-(2-methoxy-2-oxoacetyl)-2-oxopyrrolidine-1-carboxylate **14** (3 g, 0.011 mol)was then diluted with dry dichloromethane (20 mL) and trifluoroacetic acids (4.1 mL, 0.055 mol) was added at 0 °C and allow to stirred at room temperature for 5 h. All the volatile were removed on rotavapour and 5 mL methanol was added and sonicated for 10 min to obtained white solid product. The precipitate obtained was then filtered and dried on *vaccuo* to obtained pure white solid product. (TLC: 40% EtOAc: PE)

Yield: 1.5 gm; 80 %

Melting Point: 165-167 °C

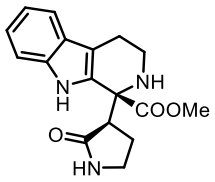
IRυ_{max}(film): 3316, 3023, 1677, 1431, 1030 cm^{-1}

^{1}H NMR (400 MHz,$CDCl_3$) = δ 11.71 (br. s, 1 H), 6.79 (br. s, 1 H), 3.88 (s, 3 H), 3.53 (t, *J* = 6.4 Hz, 2 H), 3.17 - 3.14 (m, 2 H)

^{13}C NMR (100 MHz,$CDCl_3$) = δ 175.8, 163.2, 148.0, 111.7, 52.5, 40.7, 24.7

HRMS (ESI): *m/z* calculated for $C_7H_9NO_4Na$ $[M+Na]^+$ = 194.0424, Observed = 194.0428

(±)-Methyl 1-(2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (11)



A mixture of tryptamine hydrochloride **3a** (70 mg, 0.35 mmol), methyl 2-oxo-2-(2-oxopyrrolidin-3-yl)acetate **12** (61 mg, 0.35 mmol) and Na_2SO_4 (100 mg) in 5 mL methanol was refluxed for 16 h. The reaction mixture was cooled to room temperature, concentrated in *vaccuo* and neutralized with sat.$NaHCO_3$.Then aqueous

layer was extracted with EtOAc (3x 25 mL), the combined organic extract washed with brine (20 mL), dried over sodium sulphate and concentrated in *vaccuo*. The crude product was purified by column chromatography to afford pure product **11** as brown solid. (TLC: 20% EtOAc: DCM)

Yield: 80 mg; 72 %

Melting Point: 124-126 °C

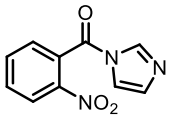
IRυ_{max}(film): 3329, 3022, 1598, 1425, 1032 cm^{-1}

^{1}H NMR (400 MHz,$CDCl_3$) = δ 8.67 (br. s, 1 H), 7.53 (d, *J* = 7.9 Hz, 1 H), 7.32 (d, *J* = 7.9 Hz, 1 H), 7.17 (t, *J* = 7.3 Hz, 1 H), 7.16 - 7.10 (m, 1 H), 6.02 (br. s, 1 H), 3.79 (s, 3 H), 3.49 (q, *J* = 7.3 Hz, 1 H), 3.38 (t, *J* = 9.8 Hz, 1 H), 3.28 - 3.22 (m, 1 H), 3.17 - 3.08 (m, 1 H), 2.99 (dt, *J* = 4.3, 11.0 Hz, 1 H), 2.85 - 2.76 (m, 2 H), 2.39 (br. s, 1 H), 2.32 - 2.25 (m, 1 H), 1.82 - 1.78 (m, 1 H)

^{13}C NMR (100 MHz,$CDCl_3$) = δ 177.7, 175.4, 136.5, 129.6, 126.6, 122.0, 119.2, 118.3, 113.1, 111.4, 63.5, 52.8, 49.8, 40.9, 40.3, 23.7, 22.3

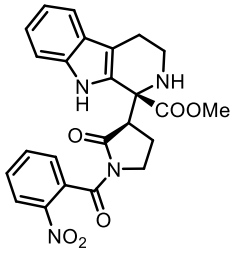
HRMS (ESI): *m/z* calculated for $C_{17}H_{20}N_3O_3$ $[M+H]^+$= 314.1499 Observed = 314.1502

(1H-imidazol-1-yl)(2-nitrophenyl)methanone (16)



To a solution of 2-nitrobenzoic acid **15** (1 g, 5.98 mmol) in dry THF (20 mL), was added *N,N´*- carbonyldiimidazole (1.16 g, 7.18 mmol) and the reaction mixture was stirred at r.t. for 3 h. Reaction mixture was then diluted with water 25 mL and extracted with ethyl acetate (2 x 40 mL). The combined organic layer was washed with sat.$NaHCO_3$, dried over sodium sulphate and concentrated on rotavapour to obtained pure white solid in quantitative yield. Product **16** was obtained was used as such for next step without extensive characterizations.

(±)-Methyl 1-(1-(2-nitrobenzoyl)-2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (17)



To a solution of (±)-methyl 1-(2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate **11** (1 g, 3.19 mmol) in dry DMF (10 mL) was added K_2CO_3 (1.32 g, 9.57 mmol) and (1H-imidazol-1-yl)(2-nitrophenyl)methanone **16** (0.83 g, 3.82 mmol) under positive pressure of argon and stirred for 12 h at room temperature. The reaction mixture was added to cold water and extracted with ethyl acetate (3 x 50 mL). The combined organic layer was washed water (2 x 60 mL), brine (1 x 60 mL) and dried over Na_2SO_4, concentrated under reduced pressure. The crude product was purified by column chromatography to afford pure product **17** as yellow solid. (TLC: 20% EtOAc: DCM)

Yield: 0.898 gm; 61 %

Melting Point: 118-120 °C

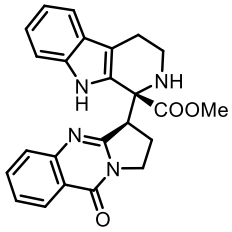
IRυ_{max}(film): 3346, 3022, 2925, 1641, 1426 cm^{-1}

^{1}H NMR (500 MHz, $CDCl_3$) = δ 8.25 - 8.23 (m, 2 H), 7.75 (t, *J* = 7.4 Hz, 1 H), 7.62 - 7.53 (m, 1 H), 7.51 (d, *J* = 7.6 Hz, 1 H), 7.39 (d, *J* = 7.6 Hz, 1 H), 7.34 (d, *J* = 8.0 Hz, 1 H), 7.21 (t, *J* = 7.4 Hz, 1 H), 7.20 - 7.13 (m, 1 H), 4.12 - 4.11 (m, 1 H), 3.77 - 3.74 (m, 2 H), 3.73 (s, 3 H), 3.23 - 3.18 (m, 2 H), 2.86 - 2.76 (m, 1 H), 2.76 - 2.68 (m, 1 H), 2.12 - 2.08 (m, 1 H), 1.7 - 1.75 (m, 1 H)

^{13}C NMR (125 MHz, $CDCl_3$) = δ 173.8, 173.6, 166.2, 145.1, 136.6, 134.5, 132.9, 130.1, 128.8, 128.0, 126.7, 124.2, 122.7, 119.8, 118.6, 113.7, 111.2, 62.6, 53.3, 53.0, 43.8, 41.3, 21.6, 19.2

HRMS (ESI): *m/z* calculated for $C_{24}H_{23}N_4O_6$ $[M+H]^+$= 463.1612 Observed = 463.1619

(±)-Methyl 1-(9-oxo-1,2,3,9-tetrahydropyrrolo[2,1-b]quinazolin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (2)



To a solution of (±) methyl 1-(1-(2-nitrobenzoyl)-2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate **17** (35 mg, 0.08 mmol) in dry ethyl acetate (10 mL) was added 10% Pd/C (7 mg). The reaction mixture was stirred for 3 hours under hydrogen balloon pressure. The mixture was filtered through celite and the solvent was evaporated under reduced pressure. The crude product obtained was purified by silica gel column chromatography to afford pure product **2** as off-white solid; (TLC: 20% EtOAc: DCM)

Yield: 31 mg; 67 %

Melting Point: 137-139 °C

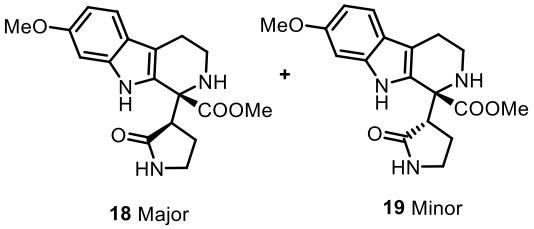
IRυ_{max}(film): 3369, 3013, 1722, 1670, 1618, 1464, 1386 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 11.01 (s, 1 H), 8.13 (d, *J* = 7.9 Hz, 1 H), 7.80 (t, *J* = 7.3 Hz, 1 H), 7.59 (d, *J* = 7.9 Hz, 1 H), 7.50 (t, *J* = 7.3 Hz, 1 H), 7.40 (d, *J* = 7.9 Hz, 1 H), 7.43 (d, *J* = 7.9 Hz, 1 H), 7.10 (t, *J* = 7.6 Hz, 1 H), 7.10 - 6.98 (m, 1 H), 4.48 - 4.44 (m, 1 H), 4.03 - 3.95 (m, 2 H), 3.92 - 3.87 (m, 1 H), 3.84 (s, 3 H), 3.13 - 3.09 (m, 2 H), 2.65 (br. s, 1 H), 1.89 - 1.84 (m, 2 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 173.0, 160.0, 158.5, 148.7, 136.5, 133.9, 130.2, 127.1, 126.1, 125.9, 125.7, 121.5, 120.7, 118.5, 118.0, 111.4, 111.3, 64.1, 52.3, 51.2, 45.2, 40.4, 21.3, 20.8

HRMS (ESI): *m/z* calculated for $C_{24}H_{22}N_4O_3Na$ $[M+Na]^+$ = 437.1584 Observed = 437.1591

(±)-Methyl 7-methoxy-1-(2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (18 & 19)



18 Major **19** Minor

diastereomeric ratio 3:1

A mixture of methyl 2-oxo-2-(2-oxopyrrolidin-3-yl)acetate **12** (113 mg, 0.66 mmol), 6-methoxytryptamine hydrochloride **17** (150 mg, 0.66mmol), and Na_2SO_4 (100 mg) in 5 mL methanolwas stirred at 80 °C for 16 h. The reaction mixture was cooled to rt, concentrated in *vaccuo* and neutralized with sat.$NaHCO_3$.Then aqueous layer was extracted with EtOAc (3 x 30 mL), the combined organic extract washed with brine (20 mL), dried (Na_2SO_4) and concentrated in *vaccuo.* The crude product was purified by column chromatography to afford pure white solid product **18** and **19** in 71% yield with the diastereomeric ratio of 3:1. (TLC: 20% EtOAc: DCM)

Data for major isomer 18

Yield:121 mg

Melting Point: 243-245 °C

IRυ_{max}(film): 3345, 2924, 1724, 1678, 1626, 1450, 1113 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 9.94 (br. s, 1 H), 7.66 (br. s, 1 H), 7.24 (d, *J* = 8.5 Hz, 1 H), 6.93 (br. s, 1 H), 6.60 (d, *J* = 7.9 Hz, 1 H), 3.74 (s, 3 H), 3.63 (s, 3 H), 3.33 (s, 1 H), 3.21 (t, *J* = 8.9 Hz, 1 H), 3.11 (br. s, 2 H), 3.05–3.02 (m, 2 H), 2.90 (br. s., 1 H), 2.10–2.08 (m, 1 H), 1.82 - 1.76 (m, 1 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 175.5, 173.5, 155.4, 136.8, 129.6, 120.7, 118.2, 110.3, 108.1, 95.0, 63.0, 55.2, 52.2, 47.6, 23.4, 21.9

HRMS (ESI): *m/z*calculated for $C_{18}H_{22}N_3O_4$ $[M+H]^+$= 344.1605 Observed = 344.1608

Data for minor 19

Yield: 40 mg

Melting Point: 143-145 °C

IRυ_{max}(film): 3737, 3314, 3222, 2925, 1728, 1677, 1626, 1453, 1359cm^{-1}

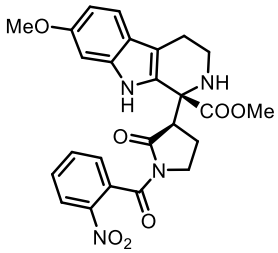
^{1}H NMR (500 MHz, DMSO-*d*$_6$) = δ 10.63 (s, 1 H), 7.78 (br. s, 1 H), 7.25 (d, *J* = 8.8 Hz, 1 H), 6.85 (br. s, 1 H), 6.61 (d, *J* = 8.8 Hz, 1 H), 3.74 (s, 3 H), 3.67 (s, 3 H), 3.52

(t, *J* = 8.8 Hz, 1 H), 3.22 - 3.11 (m, 2 H), 3.08 (d, *J* = 8.0 Hz, 2 H), 2.57 - 2.52 (m, 3 H), 1.83 (t, *J* = 9.9 Hz, 1 H), 1.51 (br. s, 1 H)

^{13}C NMR (125 MHz, DMSO-*d*$_6$) = δ 175.3, 173.7, 155.6, 137.2, 129.1, 120.4, 118.5, 110.4, 108.4, 94.6, 61.8, 55.1, 52.1, 49.0, 40.5, 22.2, 21.3

HRMS (ESI): *m/z* calculated for $C_{18}H_{22}N_3O_4$ $[M+H]^+$= 344.1605 Observed = 344.1604

(±)-Methyl 7-methoxy-1-(1-(2-nitrobenzoyl)-2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (20)



To a solution of (±) methyl 7-methoxy-1-(2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate **18** (80 mg, 0.22 mmol) in dry DMF (4 mL) was added K_2CO_3 (93 mg, 0.67 mmol) and (1H-imidazol-1-yl)(2-nitrophenyl)methanone **16** (58 mg, 0.26 mmol) under a positive pressure of argon and stirred for 12 h at room temperature. The reaction mixture was diluted with cold water (10 mL) and extracted with ethyl acetate (3 x 15 mL). The combined organic layer was washed water (2 x 15 mL), brine (1 x 15 mL) and dried over Na_2SO_4, concentrated under reduced pressure. The crude product was purified by column chromatography to afford **20** pure product as yellow solid. (TLC: 20% EtOAc: DCM)

Yield: 69 mg; 66 %

Melting Point: 188-190 °C

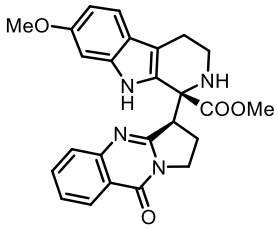
IRυ_{max}(film): 3377, 2923, 1736, 1676, 1630, 1529 cm^{-1}

^{1}H NMR (400 MHz, CDCl$_3$) = δ 8.23 (d, *J* = 7.9 Hz, 1 H), 8.16 (br. s, 1 H), 7.74 (t, *J* = 7.3 Hz, 1 H), 7.73 - 7.61 (m, 1 H), 7.38 (d, *J* = 7.9 Hz, 2 H), 6.85 - 6.84 (m, 1 H), 6.80 - 6.78 (m, 1 H), 4.09 (t, *J* = 9.2 Hz, 1 H), 3.84 (s, 3 H), 3.73 (s, 3 H), 3.20 - 3.16 (m, 2 H), 2.77 - 2.68 (m, 3 H), 2.18 (s, 1 H), 2.11-2.06 (m, 1 H), 1.77 - 1.72 (m, 2 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 173.8, 173.7, 166.2, 156.9, 145.1, 137.4, 134.5, 132.9, 130.1, 128.0, 127.3, 124.2, 121.0, 119.2, 113.7, 109.5, 94.9, 62.6, 55.7, 53.2, 53.0, 43.8, 41.3, 21.6, 19.1

HRMS (ESI): *m/z* calculated for $C_{25}H_{25}N_4O_7$ $[M+H]^+$= 493.1718 Observed = 493.1726

(±)-Peharmaline A (1)



(±) Peharmaline A (1)

To a solution of (±) methyl 7-methoxy-1-(1-(2-nitrobenzoyl)-2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate **20** (50 mg, 0.10 mmol) in dry ethyl acetate (20 mL) was added 10% Pd/C (10 mg). The reaction mixture was stirred for 3 hours under hydrogen balloon at room temperature. The mixture was filtered through celite and the solvent was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography to afford pure product **1** as yellow solid. (TLC: 20% EtOAc: DCM)

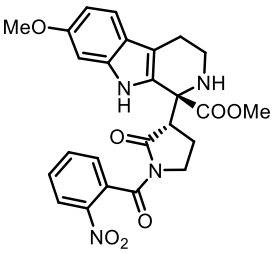
Yield: 21 mg; 47 %

Melting Point: 159-161 °C

IRυ_{max}(film): 3345, 3021, 1660, 1428, 1029 cm^{-1}

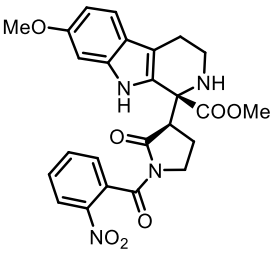
HRMS (ESI): *m/z* calculated for $C_{25}H_{24}N_4O_4Na$ $[M+Na]^+$= 467.1690 Observed = 467.1691

(±)-Methyl 7-methoxy-1-(1-(2-nitrobenzoyl)-2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (20) (Other diastereomer series)



21

Expected



20

Observed

To a solution of (±) methyl 7-methoxy-1-(2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate **19** (200 mg, 0.58 mmol) in dry DMF (10 mL) was added K_2CO_3 (257 mg, 1.86 mmol) and (1H-imidazol-1-yl)(2-nitrophenyl)methanone **16** (151 mg, 0.69 mmol) under a positive pressure of argon and stirred for 12 h at room temperature. The reaction mixture was diluted with cold water (15 mL) and extracted with ethyl acetate (3 x 35 mL). The combined organic layer was washed water (2 x25 mL), brine (1 x 25 mL) and dried over Na_2SO_4, concentrated under reduced pressure. The crude product was purified by column chromatography to afford pure product **20** as yellow solid. (TLC: 20% EtOAc: DCM)

Yield: 202 mg; 70 %

Melting Point: 184-186 °C

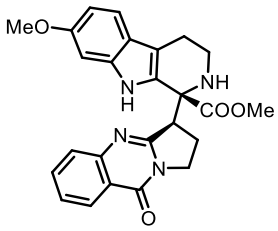
IRυ_{max}(film): 3378, 2922, 1736, 1680, 1626, 1530 cm^{-1}

^{1}H NMR (500 MHz, $CDCl_3$) = δ 8.24 (d,*J* = 7.9 Hz, 1 H), 8.19 (br. s, 1H), 7.74 (t, *J* = 7.6 Hz, 1 H), 7.61 (t, *J* = 7.8 Hz, 1 H), 7.38 (d, *J* = 8.0 Hz, 2 H), 6.84 (s, 1 H), 6.79 (d, *J* = 8.8 Hz, 1 H), 4.09 (t, *J* = 9.2 Hz, 1 H), 3.84 (s, 3 H), 3.72 (s, 3 H), 3.21 - 3.17 (m, 2 H), 2.77 - 2.74 (m, 1 H), 2.69 - 2.66 (m, 1 H), 2.11 - 2.06 (m, 2 H), 1.77 - 1.75 (m, 1 H)

^{13}C NMR (125 MHz, $CDCl_3$) = δ 173.9, 173.7, 166.2, 156.8, 145.0, 137.4, 134.5, 132.9, 130.1, 127.9, 127.3, 124.2, 121.0, 119.2, 113.6, 109.5, 94.9, 62.6, 55.7, 53.2, 53.0, 43.8, 41.3, 21.6, 19.1

HRMS (ESI): *m/z* calculated for $C_{25}H_{25}N_4O_7$ $[M+H]^+$= 493.1718 Observed = 493.1726

(±)-Peharmaline A (1) Obtained from 19 (Other diastereomer series)



(±) Peharmaline A (1)

To a solution of (±) methyl 7-methoxy-1-(1-(2-nitrobenzoyl)-2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate **21** (120 mg, 0.24 mmol) in dry ethyl acetate (20 mL) was added 10% Pd/C (10 mg). The reaction mixture was stirred for 3 hours under hydrogen balloon at room temperature. The mixture was filtered through celite and the solvent was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography to afford pure product as yellow solid. (TLC: 20% EtOAc: DCM)

Yield: 58 mg; 53 %

Note: All the spectral data of the product of this series exactly match with the (±) Peharmaline A (xx)

1.1.5 References

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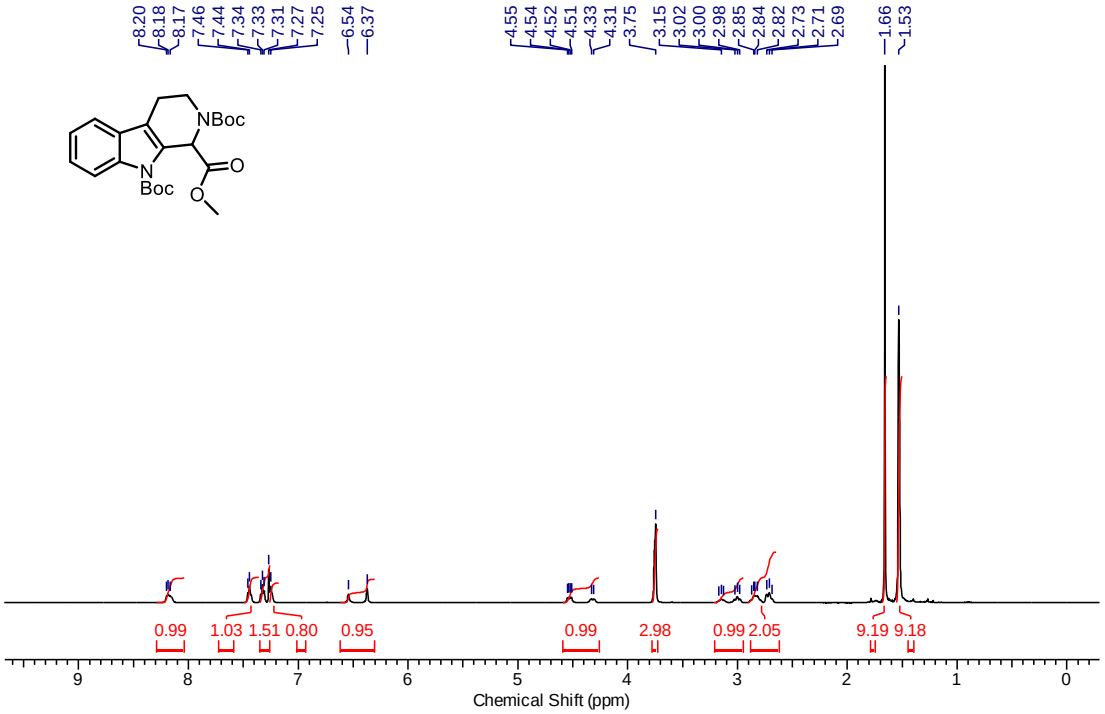
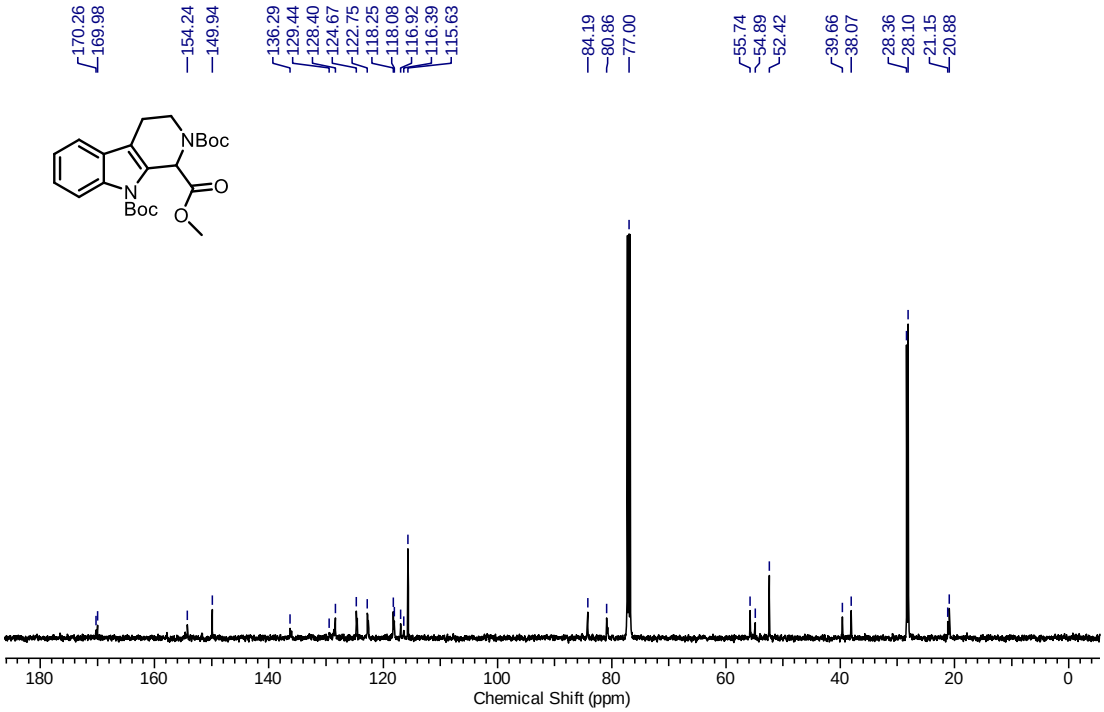
1.1.6 Copies of NMR spectra

^{1}H NMR of Compound 8 in $CDCl_3$ at 400 MHz



^{13}C NMR of Compound 8 in $CDCl_3$ at 100 MHz



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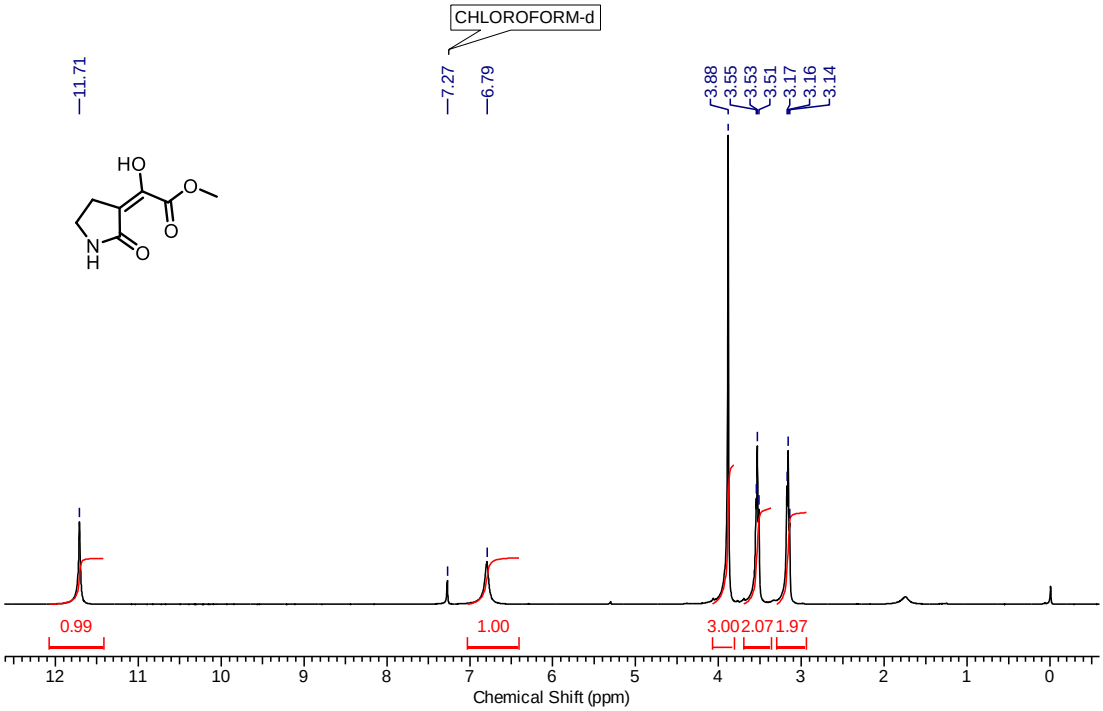

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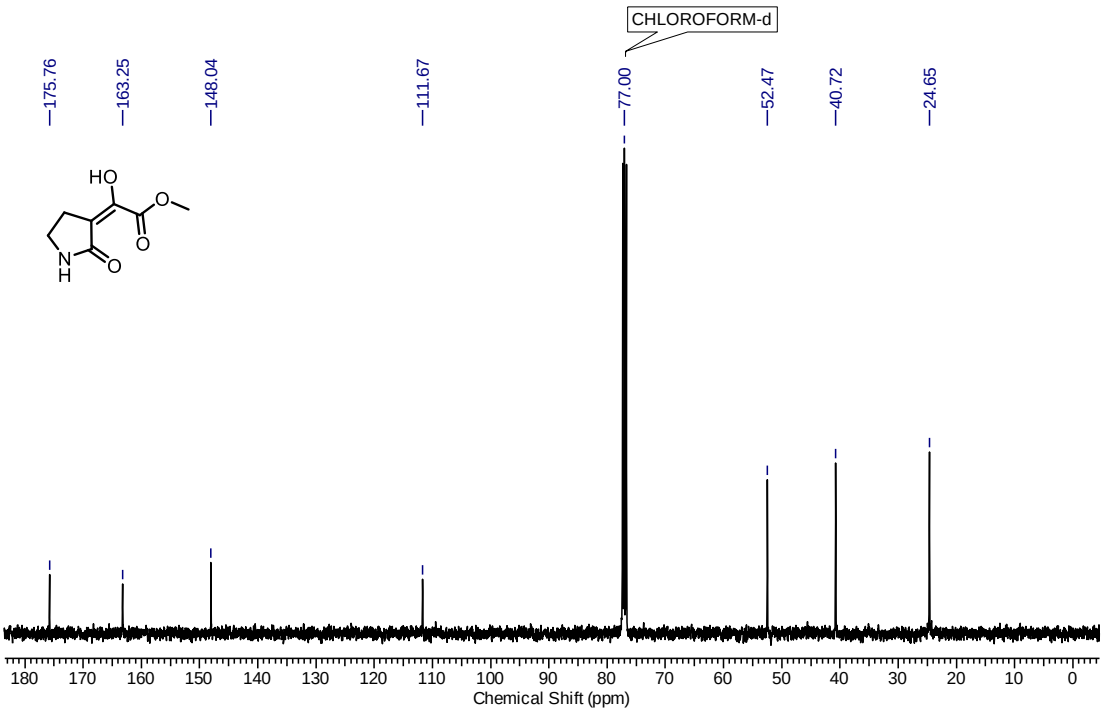
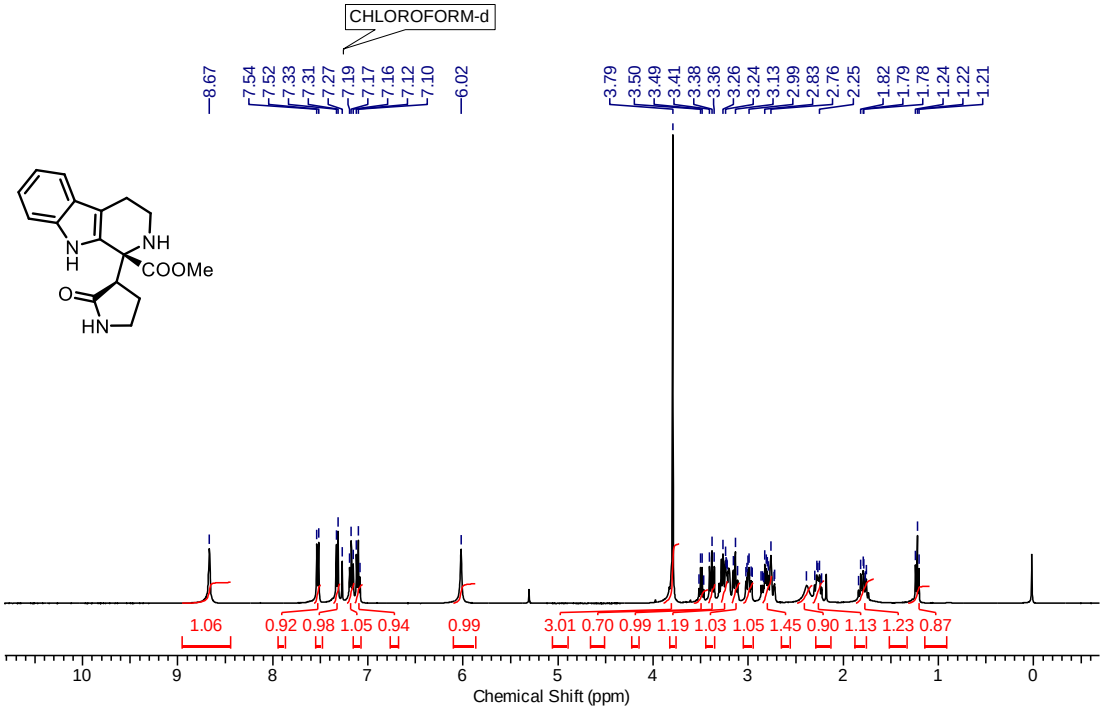
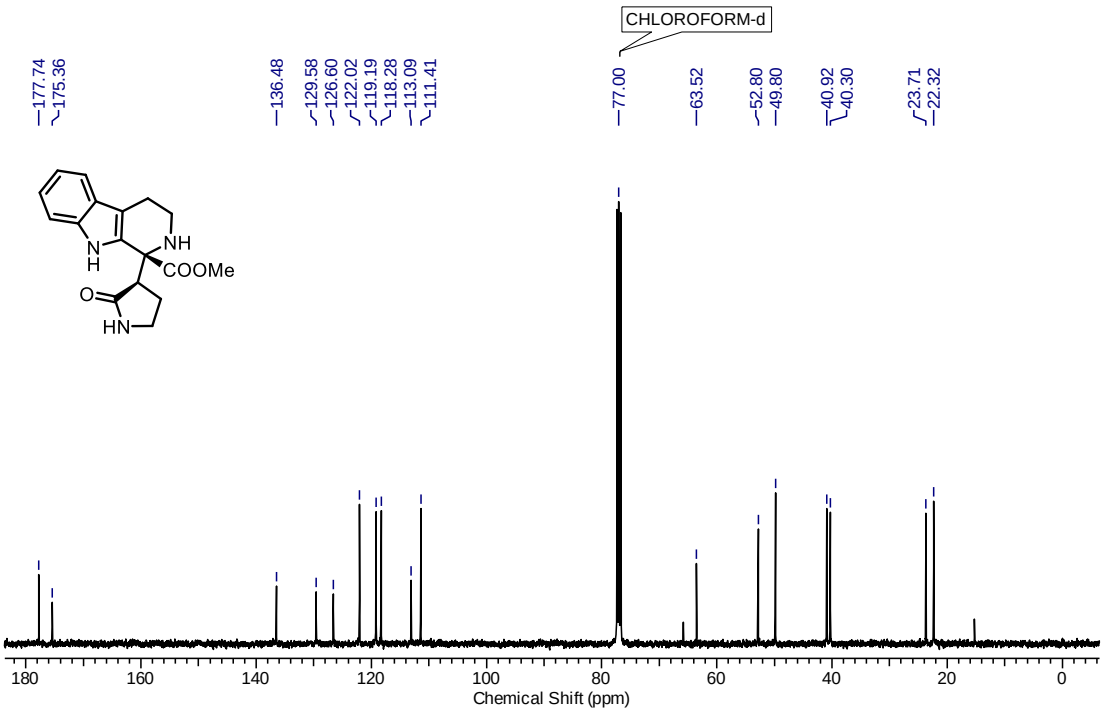


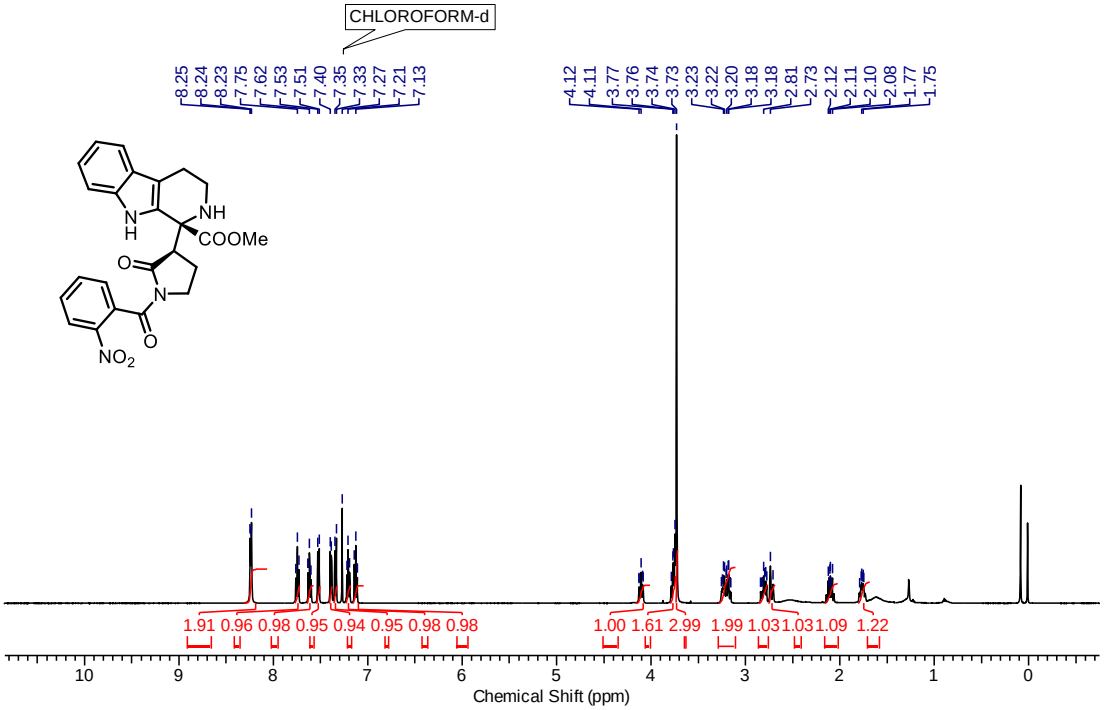
^{1}H NMR of Compound 11 in $CDCl_3$ at 400 MHz



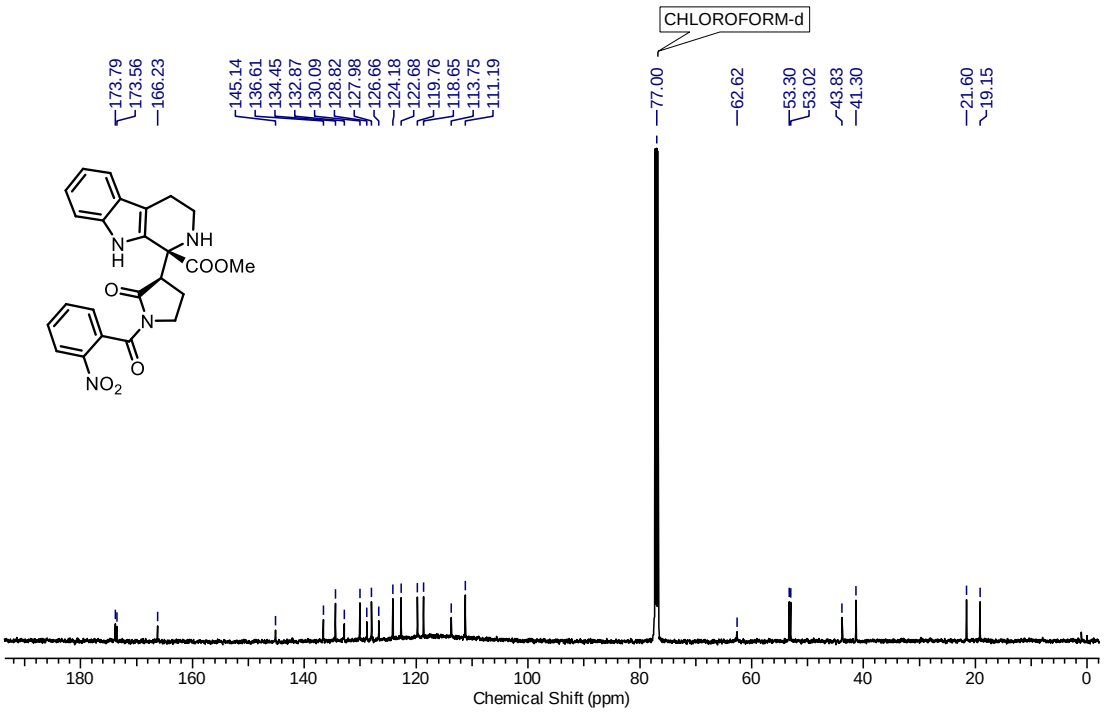
^{13}C NMR of Compound 11 in $CDCl_3$ at 100 MHz



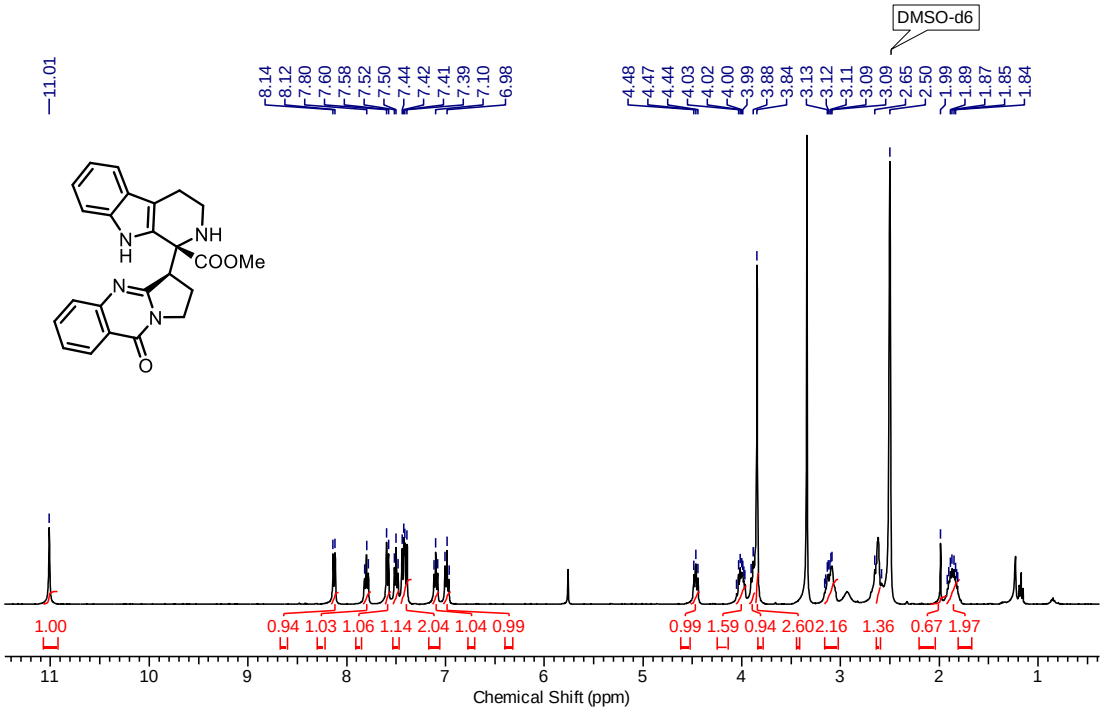
^{1}H NMR of Compound 17 in $CDCl_3$ at 500 MHz



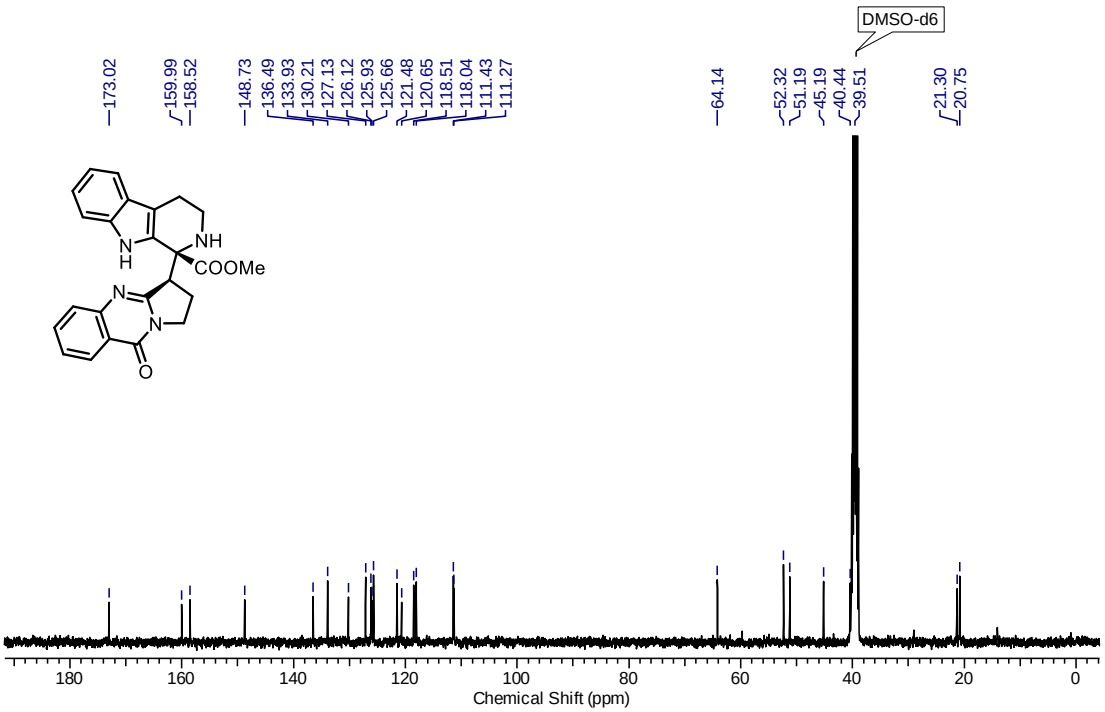
^{13}C NMR of Compound 17 in $CDCl_3$ at 125 MHz



^{1}H NMR of Compound 2 in DMSO-d_6 at 400 MHz



^{13}C NMR of Compound 2 in DMSO-d_6 at 100 MHz



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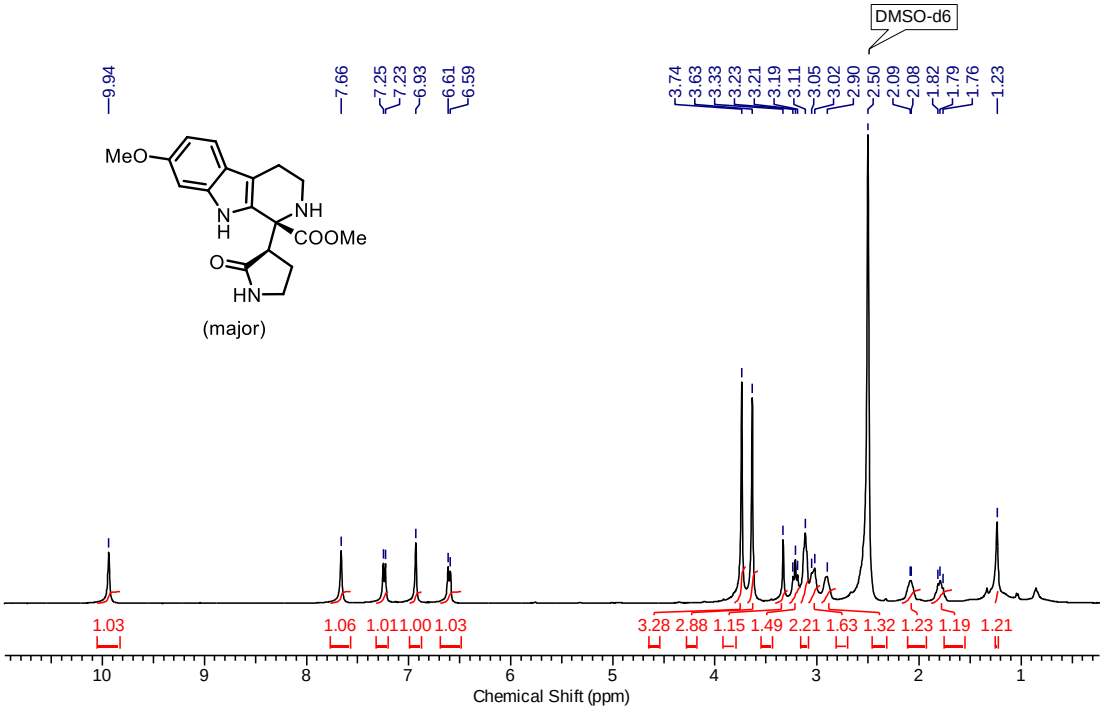
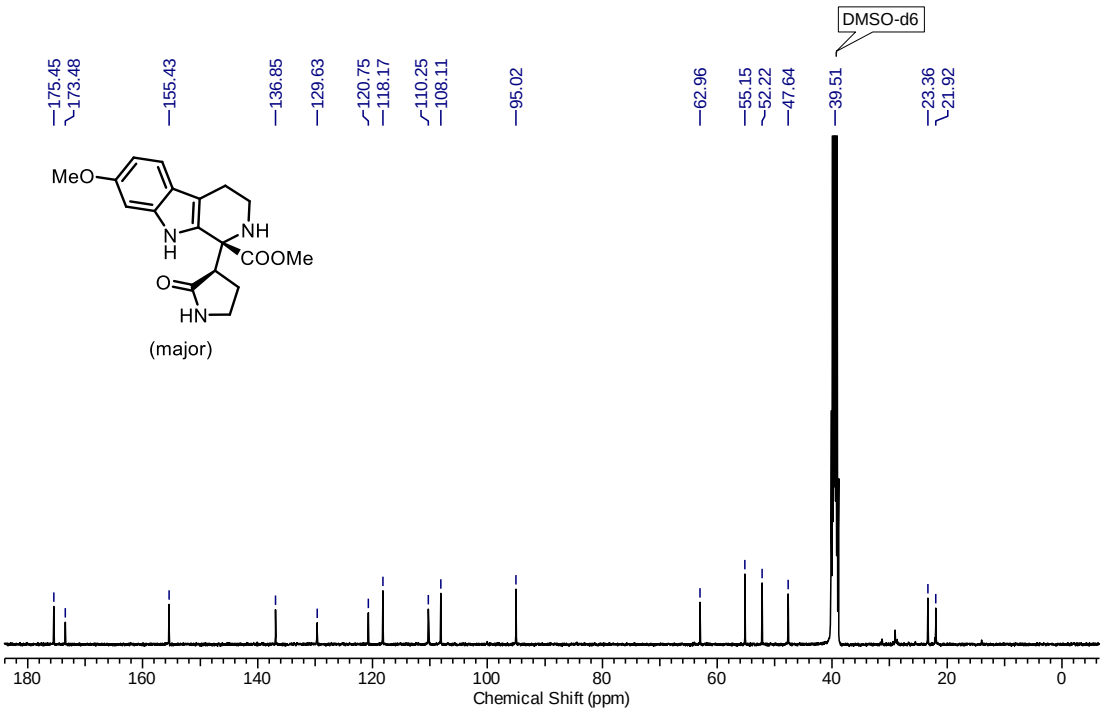
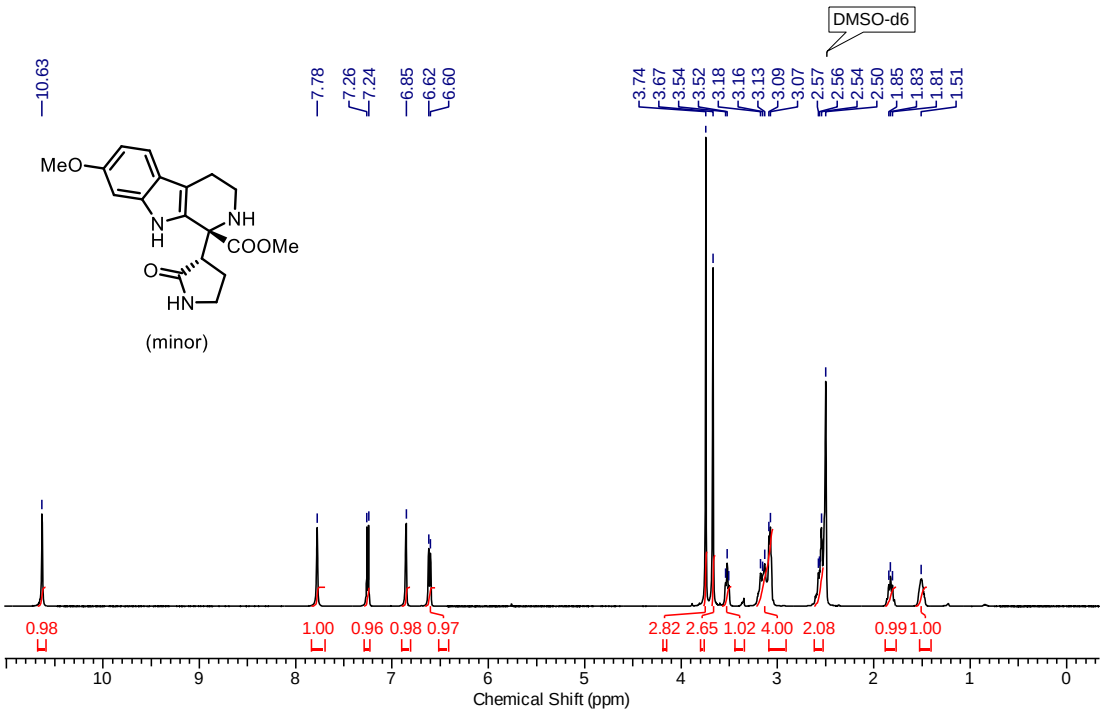


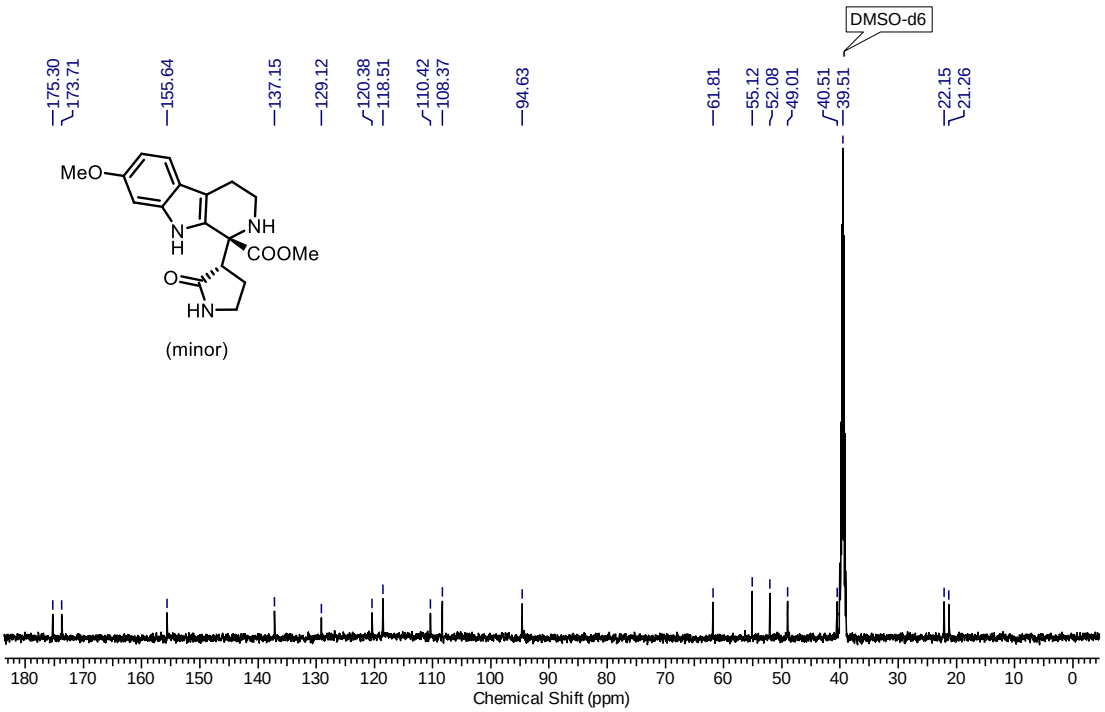
^{13}C NMR of Compound 18 in DMSO-d_6 at 100 MHz



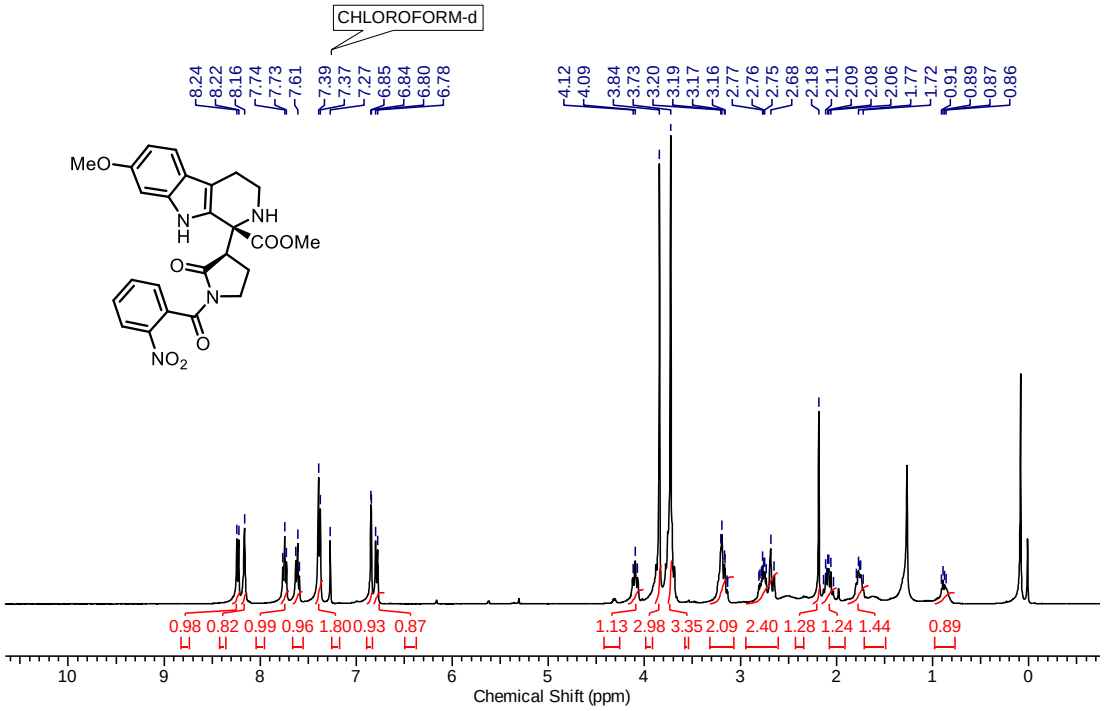
^{1}H NMR of Compound 19 in DMSO-d_6 at 500 MHz



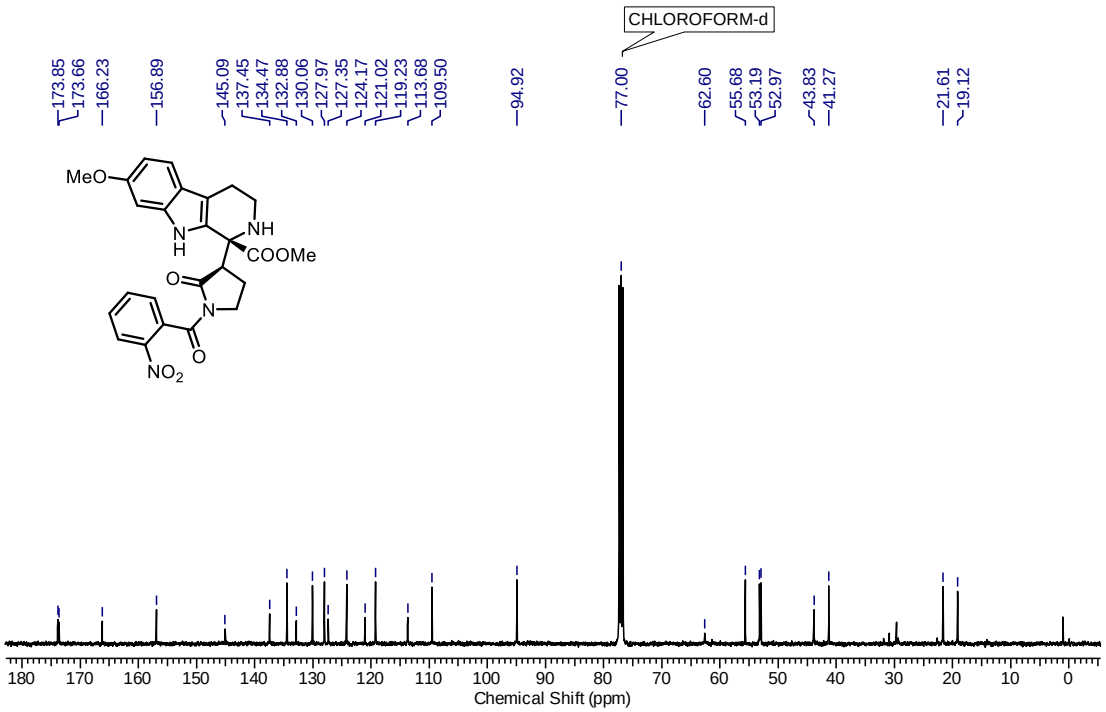
^{13}C NMR of Compound 19 in DMSO-d_6 at 125 MHz



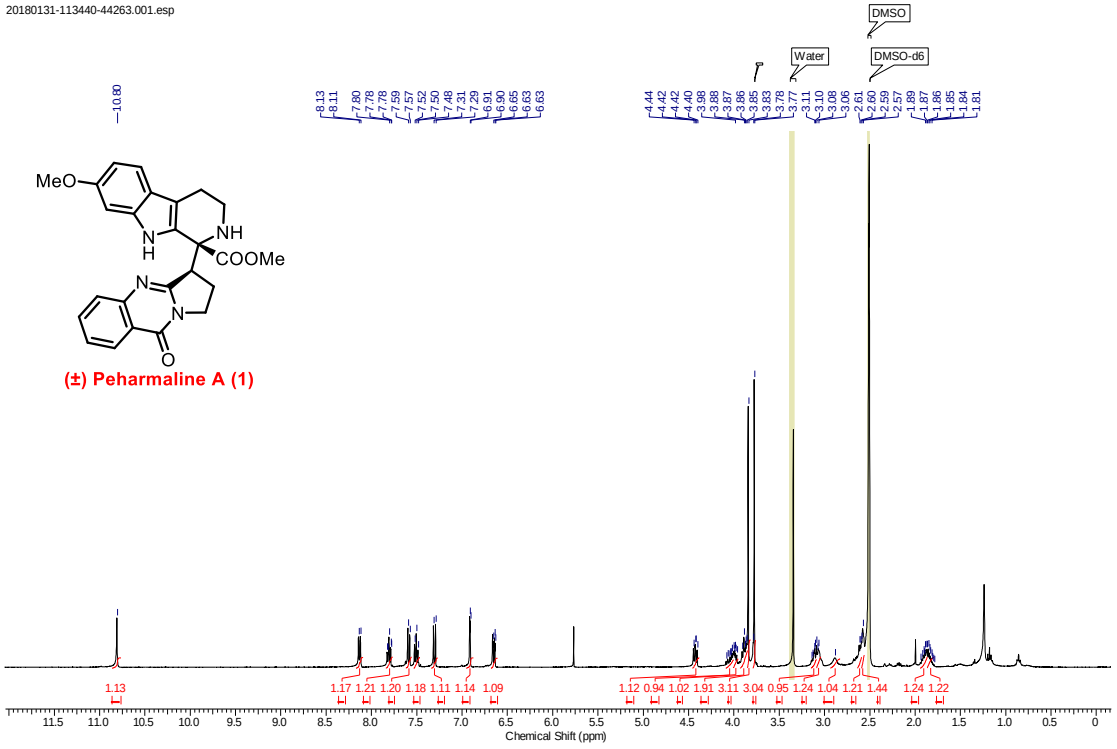
^{1}H NMR of Compound 20 in $CDCl_3$ at 400 MHz



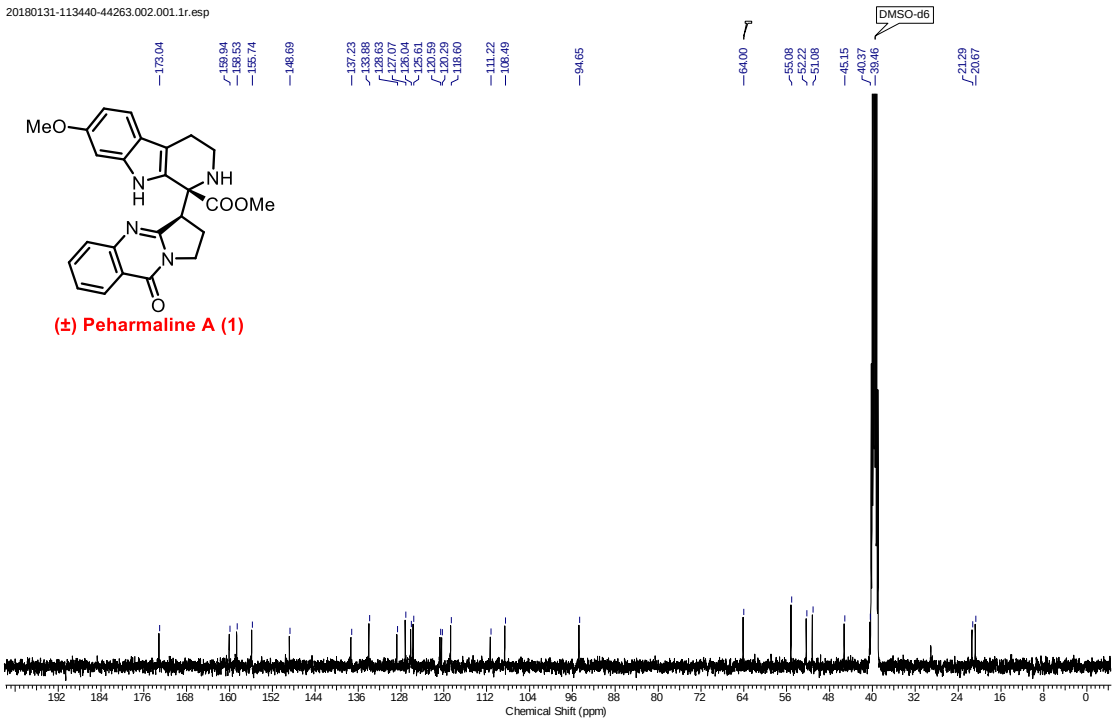
^{13}C NMR of Compound 20 in $CDCl_3$ at 100 MHz



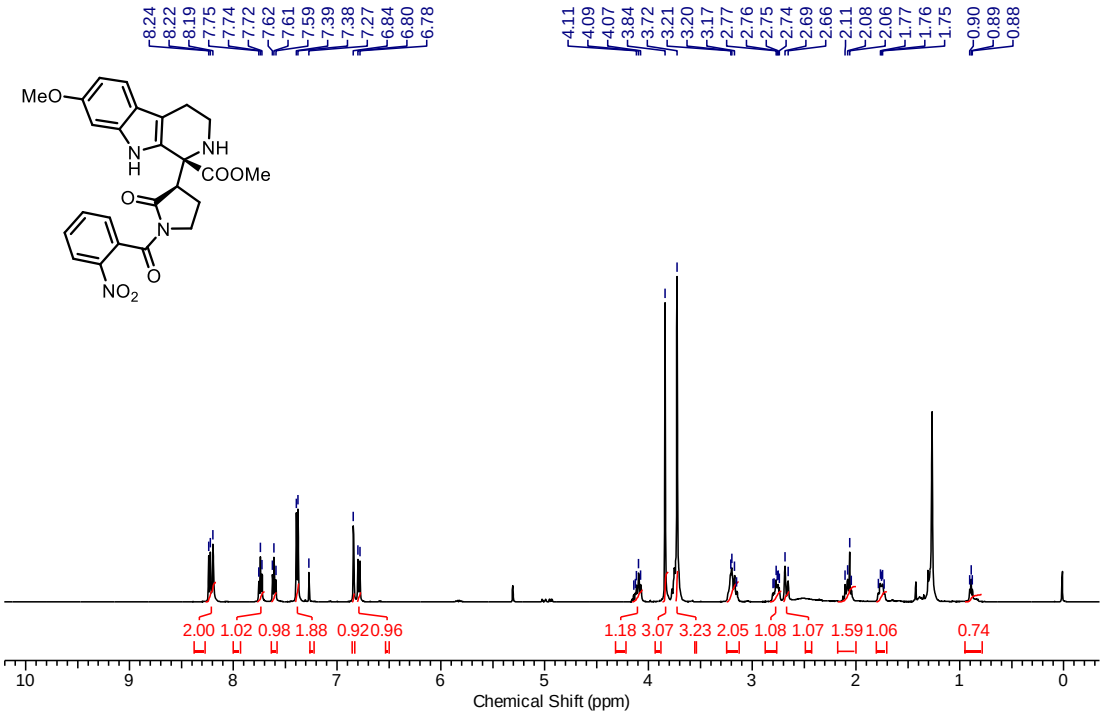
^{1}H NMR of Compound 1 in DMSO-d_6 at 400 MHz



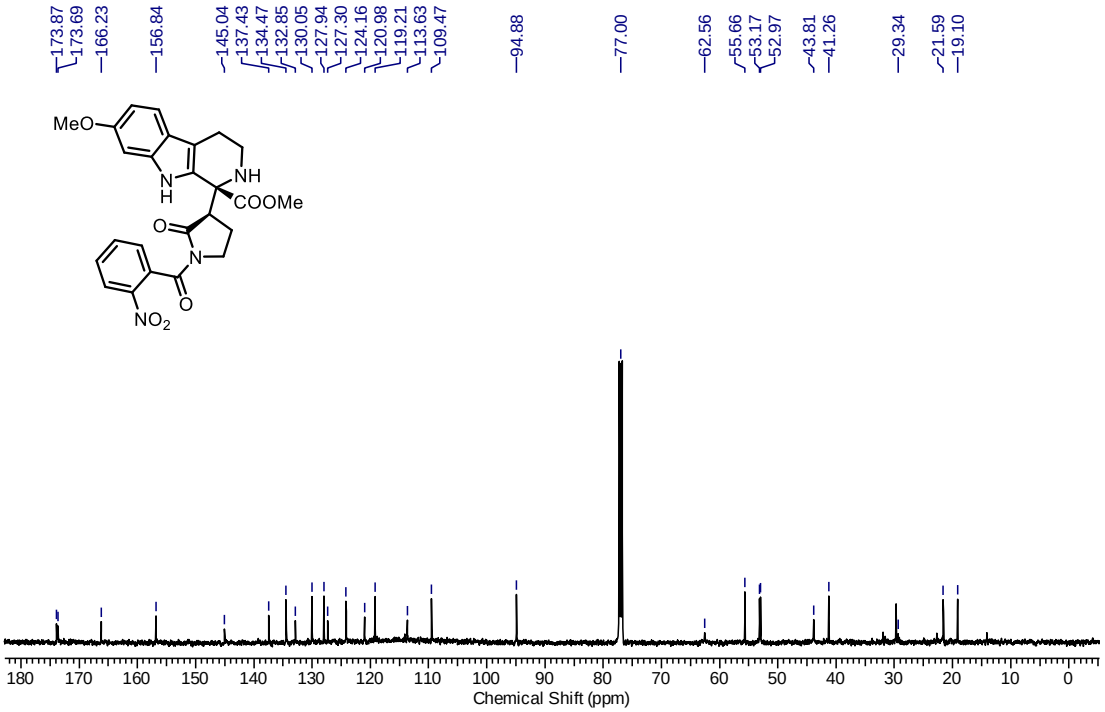
^{13}C NMR of Compound 1 in DMSO-d_6 at 100 MHz



^{1}H NMR of Compound 20 in $CDCl_3$ at 500 MHz (from minor diastereomer)



^{13}C NMR of Compound 20 in $CDCl_3$ at 125 MHz (from minor diastereomer)



Chapter-1

Section 2: Design, Synthesis and SAR Studies of (±)-Peharmaline A Analogues Towards Identification of Anticancer Leads

1.2.1 Introduction:

Cancer is a generic term which underlines diseases characterized by an abnormal proliferation of any kind of cells beyond their usual boundaries which invade adjoining parts and spread to other organs of the body.[1] The initial process of abnormal and uncontrolled growth of cells forms a solid mass termed as tumor and the later process of invasion of such cells in adjoining parts is termed as metastasis. This metastasis is due to malignant tumors and is the leading cause of mortality in cancer patients worldwide.[2] Based on regularity and mortality cancer presents a significant health problem and is a prime cause of deaths worldwide. Recent data of the World Health Organization (WHO) reveals that there are nearly 10 million deaths reported in the year 2020.[3] Breast cancer is observed as a most common occurring cancer, which accounted for about 2.26 million cases, whereas lung cancer accounted about 2.21 million cases and 1.80 million deaths in the same year. Statistical analysis shows, globally, lung cancer hits highest mortality scale amoung all the other types of cancer making it the most threatful type. (Figure 1.2.1)

Cancer cases in 2020	Cancer deaths in 2020
▪ Breast (2.26 million)	▪ Lung (1.80 million)
▪ Lung (2.21 million)	▪ Colon and rectum (935000)
▪ Colon and rectum (1.93 million)	▪ Liver (830000)
▪ Prostate (1.41 million)	▪ Stomach (769000)
▪ Skin (non-melanoma) (1.20 million)	▪ Breast (685000)

Figure 1.2.1: Statistical data given by World Health Organization (WHO)

At present, chemotherapy, surgical procedures, radiotherapy, gene therapy, photo thermal therapy, photodynamic therapy, immunotherapy *etc.* are the most common cancer treatments available, worldwide.[4] Among all of them, chemotherapy is the most prevalent method for treating cancer. Although, continuous research exploration towards the cancer and therapeutics has replaced older classical approaches and newer smarter approaches has recived louder welcoming applause. However,there are many challenges to be addressed by the researchers to come up with a better solution to save innumerous lives affected and suffering from this disease.[5,6] One such challenging

task which led to terminate the early success of research in this sector is 'drug resistance'.[7,8]

Process of understanding the basis of drug resistance in cancer pateints is a complex process as drug resistance in oncology is due to many intrinsic and extrinsic factors and can be acquired by several mechanisms which includes drug inactivation, drug efflux, drug target alteration, DNA damage repair, inherent cell heterogeneity, cell death inhibition, several epigenetic effects *etc.*[9,10] Ultimately, different molecular features of tumour cells makes them sensitive or resistant to different types of treatment. During initial treatment, drug will show better response up to a certain time period since drug may kill some of the sensitive cancer cells and few resistant cells survives invariably. However, after certain period of treatment, resistant cancer cells start multiplying and it helps in the re-growth of tumour. Likewise, drug resistance will develop against cancer cells.[11] (Figure 1.2.2)

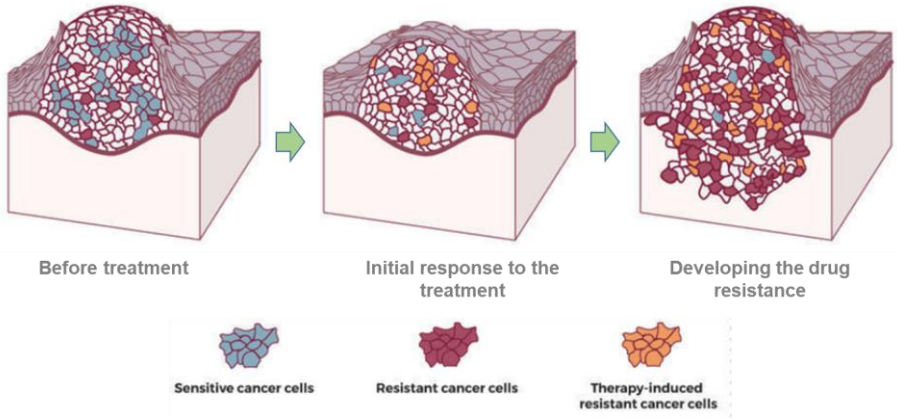


Figure 1.2.2: Drug resistance in cancer (Image source: https://www.cancer.gov/research/annual-plan/scientific-topics/what-is-drug-resistance-infographic)

Hence, to tackle the issue of finding new chemotherapies for the treatment of cancer there is need to discover new molecules with anticancer activity and novel modes of action to replace present available drugs.

1.2.2 Present work:

At present, β-carbolines stands as one of the promising scaffold for the cancer treatment.[12] The successful first total synthesis of β-carboline containing alkaloid (±)-peharmaline A and its anticancer activity encouraged us to design a systematic library of analogues around the natural product scaffold.[13] Our innate aim was to understand in-depth structure activity relationships (SAR), structural simplification and identification of leads with potent anticancer activity. Accordingly, we have created a library of simplified close analogues of (±)-peharmaline A and tested against pannel of five cancer cell lines. Finally, we pulled out three best lead compounds having better activity than parent natural product (±)-peharmaline A and marketed drug 5-flurouracil (5-FU). Details are discussed in following section.

1.2.2.1 Design and synthesis of (±)-peharmaline A analogues:

As discussed in section 1, Wang *et al*. reported cytotoxic activity of peharmaline A against the HL-60, PC-3, and SGC-7901 cancer cell lines with IC_{50} values of 9.2, 21.6, and 25.4 μM, respectively.[14] Besides this they have mentioned that the structural hybridity of natural product is essential for its cytotoxicity as individual components vasicinone and β-carbolines derivatives (Harmaline) did not show significant activity against cancer cell lines. Owing to this interesting activity and structural features we planned analogues having different structural modifications which are depicted below. (Figure 1.2.3)

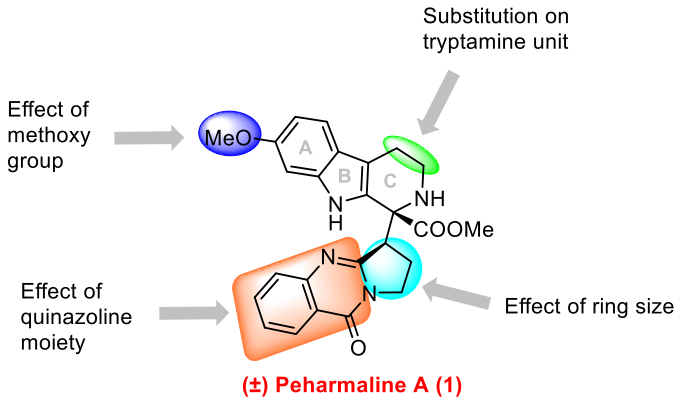


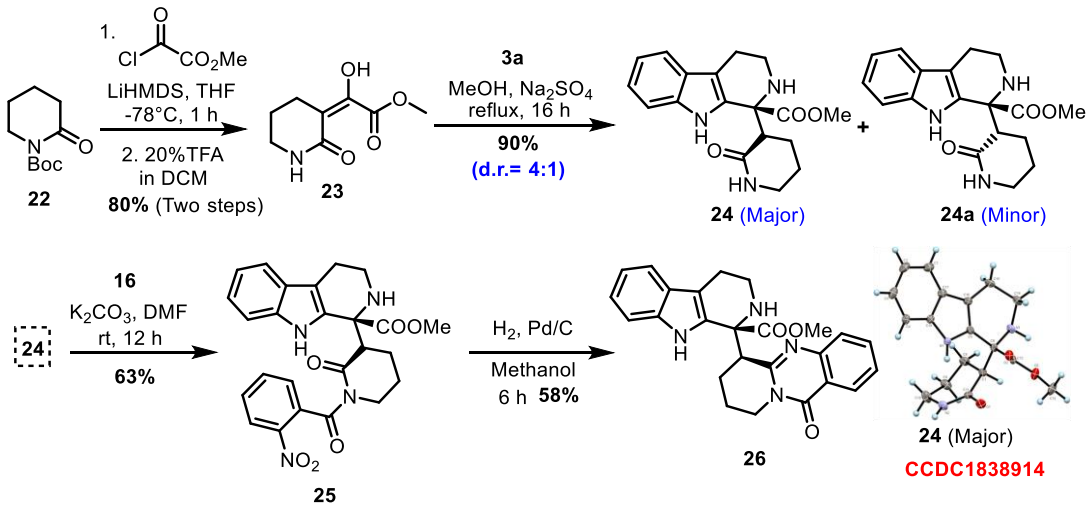
Figure 1.2.3: Planned analogues of (±)-peharmaline A (**1**)

1. Analogues without methoxy group on indole
2. Variation of ring size in vasicinone part
3. Substituted carboline analogues

4. Changing quinazoline moiety with different groups

1.2.2.2 Synthesis of analogues with variation of ring size in vasicinone part:

Synthesis of 6-membered vasicinone analogue of (±)-demethoxy-peharmaline A was commenced from commercially available 2-piperidinone which on Boc protection delivered compound **22**. Compound **22** was further subjected for acylation reaction with methyl oxalyl chloride using LiHMDS followed by Boc deprotection using 20% TFA in DCM delivered compound **23**. Formation of compound **23** was confirmed by peak in ^{1}H NMR at δ 3.86 (s, 3 H) belongs to methyl ester whereas peak at δ 14.77 (br. s, 1 H) showed presence of hydroxy group in enol. Compound **23** was treated with tryptamine hydrochloride salt in methanol and sodium sulphate gave Pictet-Spengler product **24** and **24a** as diastereomeric mixture with ratio 4:1. Next, both diastereomers were separated using column chromatography and major diastereomer **24** was confirmed by ^{1}H NMR spectrum having peak at δ 7.63 (br. s, 1 H), 7.37 (d, *J* = 7.6 Hz, 1 H), 7.41 (d, *J* = 8.0 Hz, 1 H), 7.03 (t, *J* = 7.4 Hz, 1 H), 6.95 (t, *J* = 7.4 Hz, 1 H) showed addition of five aromatic protons from tryptamine, furthermore, peak at 328.1656 in HRMS belongs to molecular formula $C_{18}H_{22}N_3O_3[M+H]^+$ confirmed compound **24**. Relative stereochemistry in compound **24** was also confirmed through X-ray analysis and ORTEP diagram (CCDC1838914) is shown in scheme 1.2.1.

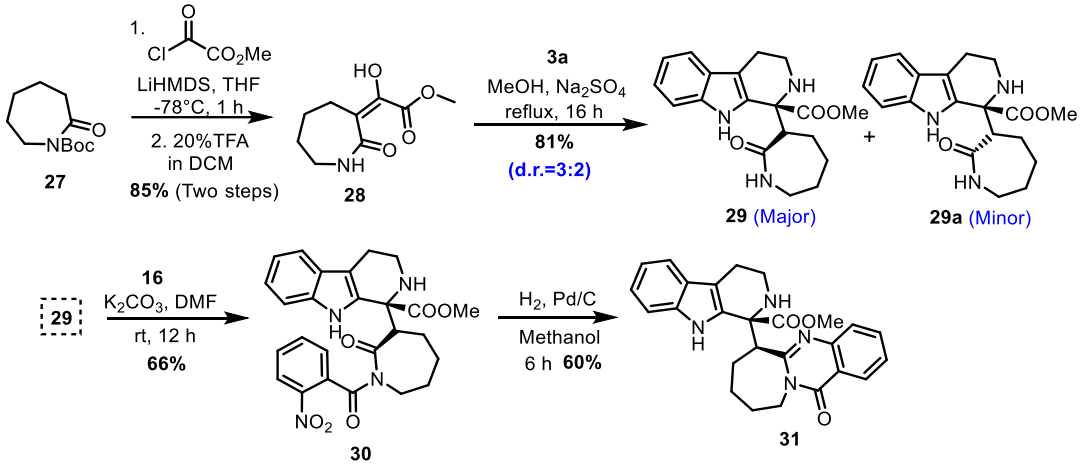


Scheme 1.2.1: Synthesis of 6-membered analogue of (±)-peharmaline A.

Minor diastereomer **24a** was also fully characterized (NMR, IR, HRMS). Further major diastereomer **24** was subjected for acylation reaction with active ester **16**

resulted in compound **25** with 63% yield. Formation of compound **25** was confirmed by addition of four aromatic protons belongs to newly added aromatic part and peak at 477.1766 in HRMS corresponds to molecular formula $C_{25}H_{25}N_4O_6[M+H]^+$. Compound **25** was further treated with H_2, Pd/C gave 6-membered demethoxy analogue **26** of (±)-peharmaline A. Formation of **26** was confirmed by new peak in ^{13}C NMR at δ 154.9 belongs to newly formed tertiary junction carbon, moreover peak in HRMS spectrum at 451.1737 corresponding to molecular formula $C_{25}H_{24}N_4O_3Na$ $[M+Na]^+$.

Synthesis of 7-membered vasicinone analogue was achieved from commercially available ε-caprolactam which on Boc protection gave compound **27,** which was acylated using methyl oxalyl chloride followed by deprotection of Boc resulted in compound **28**. Formation of compound **28** was confirmed by peak in ^1H NMR at δ 3.87 (s, 3 H) belongs to methyl group from acylating part. Compound **28** was subjected for optimized Pictet-Spengler reaction gave Pictet product **29** and **29a**



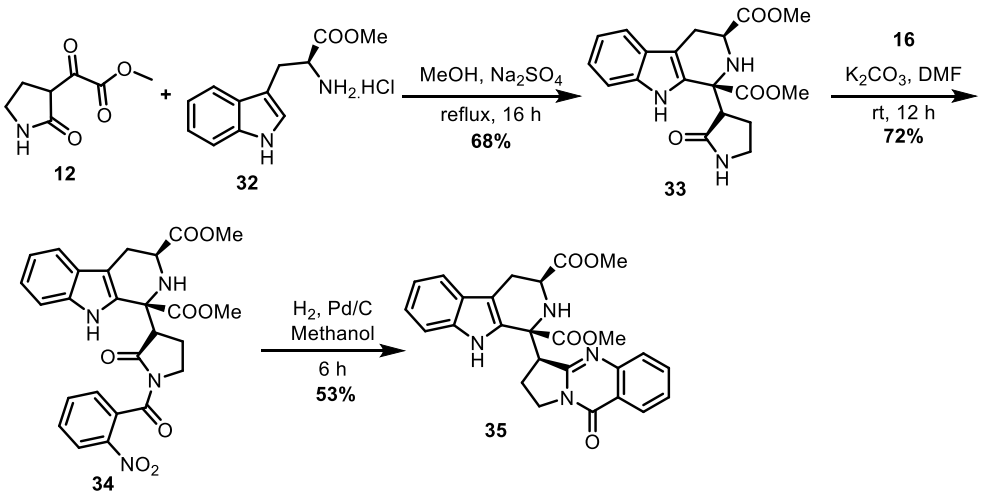
Scheme 1.2.2 Synthesis of 7-membered analogue of (±)-peharmaline A.

with diastereomeric ratio 3:2. Formation of compound **29** and **29a** were confirmed by addition of four aromatic protons in ^1H NMR and with other characterization (IR, HRMS). However, here we forwarded major diastereomer **29** for acylation reaction with active ester **16** which afforded compound **30** in good yield. Further successful one pot vasicinone construction resulted 7-membered vasicinone analogue **31** of (±)-peharmaline A. Structure of **31** was confirmed by peak in ^{13}C NMR at δ 146.5

belongs to newly formed tertiary junction carbon in vasicinone and peak in HRMS at 443.2077 corresponding to molecular formula $C_{26}H_{27}N_4O_3[M+H]^+$. (Scheme 1.2.2)

1.2.2.3 Substituted carboline analogue:

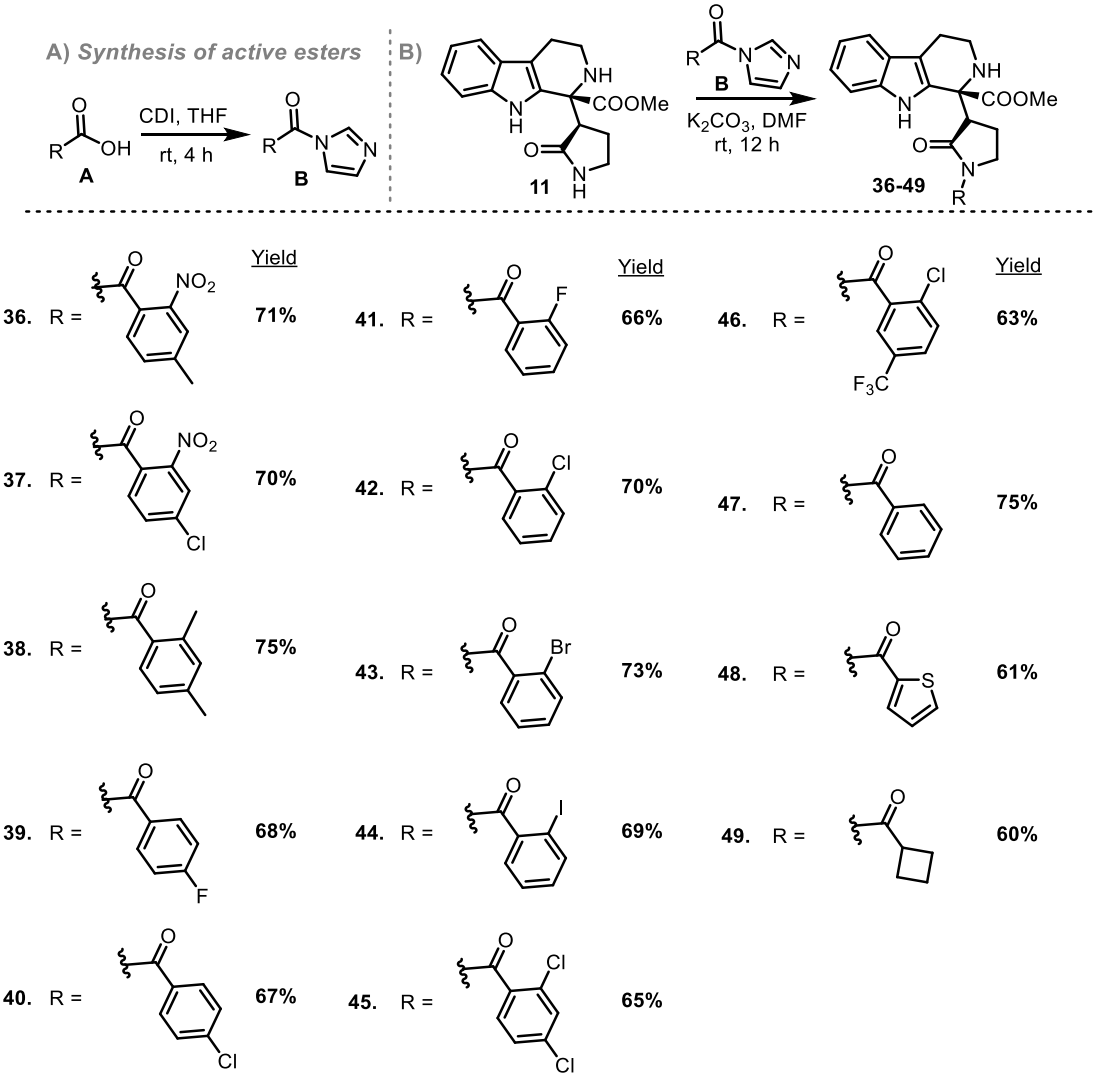
To access a few more analogues tryptophan hydrochloride salt **32** was synthesized from tryptophan using known literature protocol[16], and further it was treated with compound **12** under Pictet-Spengler condition (Na_2SO_4, MeOH, reflux) gave Pictet product **33** (major diastereomer) with 68% yield in this case, we were unable to isolate the minor disatereomer in pure form. Formation of compound **33** was confirmed by new four aromatic protons in ^{1}H NMR at δ 7.53 (d, *J* = 7.6 Hz, 1 H), 7.30 (d, *J* = 8.0 Hz, 1 H), 7.18 (t, *J* = 7.4 Hz, 1 H), 7.13 - 7.10 (m, 1 H) belongs to tryptamine unit and peak in HRMS analysis at 372.1555 corresponding to molecular formula $C_{19}H_{22}N_3O_5[M+H]^+$. Further compound **33** was reacted with active ester **16** under K_2CO_3, DMF at room temperature gave acylated compound **34**. Formation of compound **34** was confirmed by addition of four new aromatic protons in ^{1}H NMR spectrum, and peak in HRMS analysis at 491.1924 belongs to molecular formula $C_{26}H_{27}N_4O_6[M+H]^+$. Compound **34** was further subjected for nitro group reduction which resulted in analogue **35**. (Scheme 1.2.3)



Scheme 1.2.3: Synthesis of substituted carboline analogue of (±)-peharmaline A

1.2.2.4 Analogues with modification of quinazoline core in vasicinone moiety:

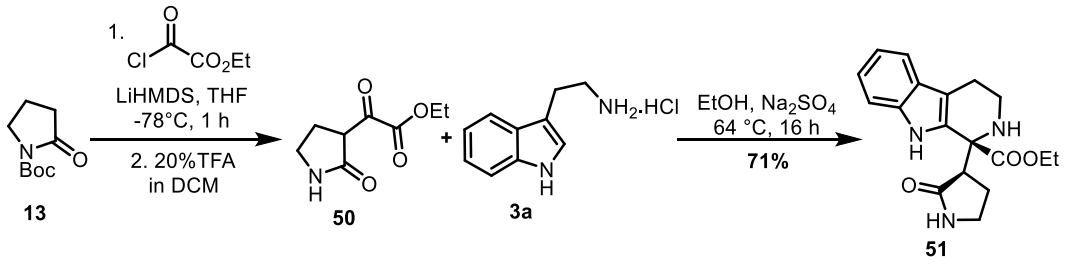
To understand the role of quinazoline moiety by accessing a few simplified analogues of (±)-peharmaline A, we prepared Pictet adduct **11** in gram scale, whereas required active esters (B) were prepared from corroesponding acids (A) using CDI in THF [xx] (Scheme 1.2.4 A), those active esters (B) were used as such without coloumn purification. Pictet product **11** was reacted with different prepared active esters (B) in presence of K_2CO_3 in DMF at room temprature for 12 h resulted in simplified analogues (**36-49**) as shown in scheme 1.2.4. All these new analogues were fully characterized by spectral techniques (^1H,^{13}C NMR, IR, HRMS).



Scheme 1.2.4: Synthesis of simplified analogues of (±)-peharmaline A

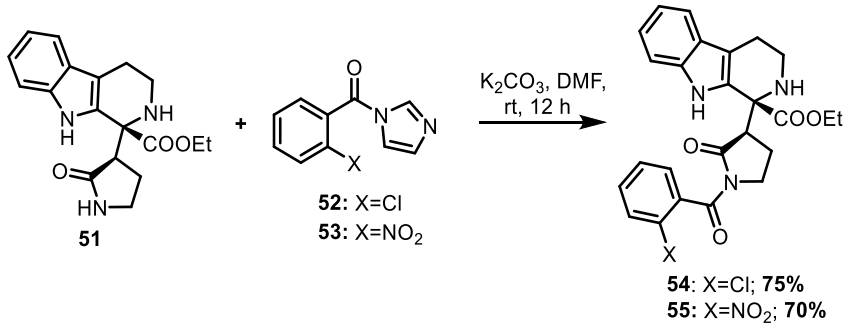
Next, we have planned to change methyl ester to ethyl ester, hence Boc-2-pyrrolidone **13** was subjected for acylation reaction with ethyl oxalyl chloride using LiHMDS

followed by treatment with 20% TFA gave compound **50**. Compound **50** was treated with tryptamine hydrochloride **3a** under Pictet-Spengler condition (Na_2SO_4, EtOH, reflux, 12 h) resulted in Pictet adduct **51** as a single diastereomer**.** Formation of compound **51** was confirmed by addition of four new aromatic protons at δ 7.38 (d, *J* = 8.9 Hz, 2 H), 7.04 – 7.01 (m, 1 H), 6.96 - 6.92 (m, 1 H) corresponding to tryptamine unit, six aromatic carbons in ^{13}C NMR at δ 136.1- 110.1 belongs to aromatic part from indole and characteristic peak at δ 63.07 corresponding to newly form nitrogenated tertiary carbon. Furthermore confirmation was done by HRMS peak at 328.1656 showed molecular formula $C_{18}H_{22}O_3N_3[M+H]^+$. (Scheme 1.2.5)



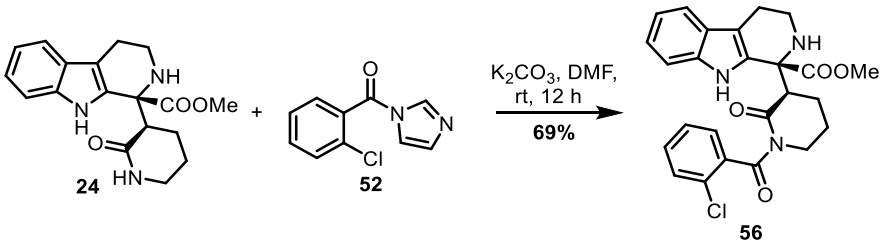
Scheme 1.2.5: Synthesis of Pictet adduct **52** with ethyl ester on gram scale

After having Pictet adduct **51** in hand, we subjected it for acylation reaction with active ester **52** using K_2CO_3 in DMF resulted in the formation of analogue **54** with 75% yield. Formation of compound **54** was confirmed by counting four new protons in aromatic region and peak in HRMS analysis at 466.1523 corresponding to molecular formula $C_{25}H_{25}O_4N_3Cl$ $[M+H]^+$. Further compound **51** was treated with active ester **53** using same optimized reaction gave analogue **55** with 70% yield. Formation of compound **55** was confirmed by appearance of new four aromatic protons in ^1H NMR and peak in HRMS spectrum at 477.1769 belongs to molecular formula $C_{25}H_{25}O_6N_4$ $[M+H]^+$. (Scheme 1.2.6)



Scheme 1.2.6: Synthesis of analogues **55** and **56** with ethyl ester

Next, we planned analogue **56** which is six membered version of analogue **42**, for which we treated Pictet adduct **24** with active ester **52** under optimized condition (K_2CO_3, DMF, rt, 12 h) resulted in analogue **56** with 69% yield. Formation of compound **56** was confirmed by four new aromatic protons in ^{1}H NMR. (Scheme 1.2.7)



Scheme 1.2.7 : Synthesis of analogue **57**

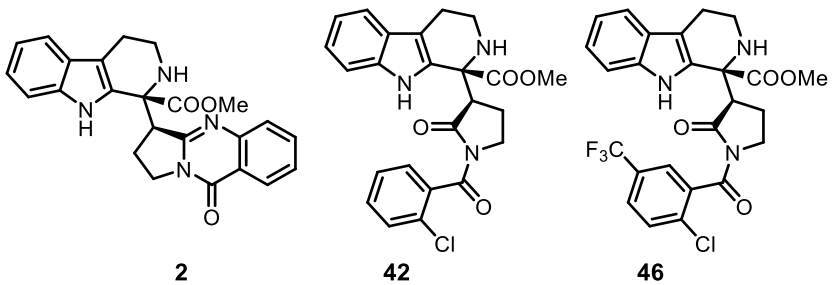
1.2.3 Cytotoxicity of (±)-peharmaline A analogues:

The cytotoxicity of all the analogues with various structural features were evaluated in collaboration with Dr. Anindya Goswami's research group at CSIR-Indian Institute of Integrative Medicine (CSIR-IIIM), Jammu and studied their Structure-Activity Relationships. All the synthesized analogues were tested against five different cancer cell lines HCT-116, MCF-7, A549, HOP-92 and PC-3 using MTT assay and determined their IC_{50} values in µM. Doxorubicin and 5-flurouracil (5-FU) were used as a positive control for MTT assay. The detailed results are shown in table 1.2.1. From the obtained cytotoxicity data we identified three lead compounds (**2**, **42** and **46**) which has better potency against all cancer cell lines than parent natural product **1** and standard drug 5- fluorouracil (5-FU).

Table 1.2.1: Cytotoxicity evaluation of (±)-peharmaline A analogues

No.	HCT-116 (IC_{50}±SD)µM	MCF-7 (IC_{50}±SD)µM	A549 (IC_{50}±SD)µM	HOP-92 (IC_{50}±SD)µM	PC-3 (IC_{50}±SD)µM
1	15.395±0.14	6.22±0.003	5.242±0.08	4.297±0.005	12.21±0.6
2	**1.021±0.002**	**1.406±0.015**	**1.684±0.001**	**0.813±0.001**	**6.049±0.001**
5	>100	>100	>100	>100	>100
11	25.603±0.15	>100	39.75±0.7	66.7±0.798	75.1±0.17
18	>100	81.649±0.4	16.565±0.2	20.231±0.02	>100
19	7.479±0.025	>100	>100	>100	91.651±0.9
20	6.185±0.01	16.129±0.034	12.68±0.01	23.492±0.14	30.978±0.4
24	>100	>100	>100	>100	34.964±0.44

25	42.152±0.463	51.194±0.15	39.087±0.2	>100	>100
26	4.145±0.02	3.283±0.02	6.885±0.03	4.664±0.01	4.948±0.04
29	>100	93.292±0.3	>100	>100	>100
29a	>100	>100	>100	>100	>100
30	15.146±0.01	7.432±0.003	35.777±0.3	15.764±0.02	36.492±0.65
31	5.352±0.003	6.954±0.02	7.911±0.001	2.307±0.025	6.471±0.594
33	27.391±0.3	>100	20.379±0.3	>100	>100
34	>100	>100	19.386±0.9	>100	>100
35	6.260±0.01	6.199±0.035	10.358±0.01	4.184±0.011	13.039±0.04
36	>100	>100	16.257±0.1	38.753±0.45	>100
37	>100	>100	>100	30.987±0.3	>100
38	11.193±0.018	>100	16.337±0.04	10±0.055	59.718±0.08
39	10.12±0.3	5.045±0.003	7.801±0.5	11.386±0.01	17.022±0.01
40	19.677±0.5	>100	20.379±0.O5	13.67±0.029	>100
41	8.649±0.003	6.955±0.02	6.446±0.2	9.473±0.02	5.965±0.03
42	**4.121±0.003**	**1.625±0.001**	**1.121±0.01**	**3.597±0.001**	**2.389±0.001**
43	3.923±0.005	8.122±0.018	3.365±0.002	3.528±0.018	6.225±0.01
44	5.759	8.359	5.023	7.443	7.022±0.03
45	8.789±0.02	8.359±0.14	10.844±0.16	9.376±0.073	11.098±0.04
46	**2.997±0.005**	**4.51±0.02**	**0.845±0.003**	**1.245±0.002**	**13.474±0.05**
47	16.436±0.01	>100	17.349±0.35	31.073±0.1	>100
48	14.031±0.15	15.898±0.01	17.880±0.8	14.843±0.01	94.449±0.51
49	25.603±0.2	>100	23.651±0.5	24.396±0.08	>100
51	>100	>100	>100	>100	>100
54	55.773±0.6	20.379±0.3	72.833±0.5	51.298±0.6	22.787±0.36
55	>100	28.103±0.09	10.694±0.02	83.95±0.56	>100
56	5.342±0.03	47.687±0.15	5.209±0.06	18.147±0.02	43.69±0.26
Dox	0.318	0.11	0.05	0.54	0.599
5-FU	6.15	3.312	2.337	3.45	15.748



2 **42** **46**

Figure 1.2.3: Identified three potent lead compounds

1.2.4 SAR studies of (±)-peharmaline A analogues:

To understand Structure-Activity Relationship in detail, we divided our analogues in to two categories, 1) Close analogues of natural product and 2) Simplified analogues (without quinoxaline part).

Activity of first category of analogues revels that, demethoxy analogue **2** was found to have better activity than parent natural product **1**, Further, increasing the ring size present in compound **2** in vasicinone moiety (compounds **26** and **31**) showed substantial decreases in the activity. Further, substitution on carboline unit in compound **2** (analogue **35**) showed slight decrease in the activity against all the tested cell lines. However, among this close analogue series, compound **2** (5 membered ring size) was found as the best active compound than natural product and drug 5-FU.

Second category consists of simplified analogues *i.e.* analogues without quinoxaline part of natural product. Accordingly, first we tested all Pictet adducts (compound **11, 18**, **19**, **24**, **29**, **29a**, **33** and **52**) against panel of five cancer cell lines, and found only few of them showed moderate activity, however, in this case, we observed increasing the ring size indicated decrease in the activity (**11**, **24** and **29**), changing methyl ester in Pictet adduct **11** to ethyl ester which made compound **9** completely inactive this showed importance of methyl ester for activity. Next, various analogues having *ortho*-nitro acylating part on Pictet adducts (**20**, **25**, **30**, **34**, **36, 37** and **56**) were evaluated and found few of them were moderately active with poor selectivity window. Further changing substitution to *ortho*-chloro **42** showed much increase in activity. However, addition of *para*-trifluoromethyl group **43** showed slight decrease in activity than parent analogue **42**. To understand the role of *ortho*-chloro substitution, we tested compound with different *ortho*-substituted halogens such as with fluoro **41**, bromo **43** and iodo **44**, however, they were found to be potent but less than corresponding chloro compounds **42** and **46**. This clearly indicate that chloro substitution at *ortho* position increases the potency. To further understand SAR in compound **42**, we tested its 6 membered analogue **56** and found that ring size is inversely proportional to potency. Next, changing the methyl ester present in compound **42** to ethyl ester (compound **54)** showed decrease in activity hence, it showed methyl ester is essential for the activity. Among this series of simplified analogues, we have found two

compounds **42** and **46** are the best one and have more potency than parent natural product. Based on SAR, we arrived at conclusions which are as shown in figure 1.2.4.

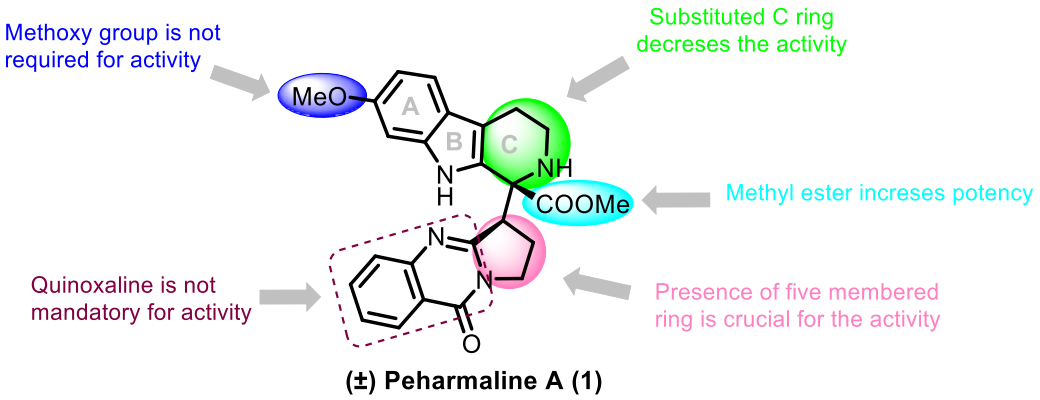


Figure 1.2.4: Structure-Activity Relationship in (±)-peharmaline A (**1**)

With this overall study and SAR, we have found that compound **2** is the best compound against three cancer cell lines, HCT-116, MCF-7 and HOP-92 with an IC_{50} values 1.021, 1.406 and 0.813 µM respectively. Whereas, compound **46** showed highest activity (IC_{50} = 0.845 µM) against A549 cancer cell line.

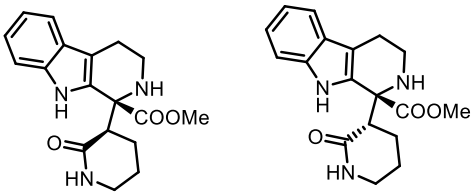
1.2.5 Conclusion:

We have prepared a library of >30 novel analogues around natural product (±)-peharmaline A scaffold using our developed route for total synthesis. All synthesized analogues were tested *in vitro* against a panel of five cancer cell lines, HCT-116, MCF-7, A549, HOP-92 and PC-3 using MTT assay. After having biological activity in hand, and Structure-Activity Relationship (SAR) studies, we have identified three best compounds (**2**, **42**, and **46**) with improved activity than parent natural product (±)-peharmaline A and marketed drug 5-FU. Further optimization and, pharmacokinetic (PK) evaluations and *in vivo* studies of identified compounds are currently in progress.

1.2.6 Experimental section:

Experimental procedures and characterization data of selected compounds are given below; Data of remaining compound can be found at (*Eur. J. Org. Chem.* **2018**, 6453; doi.org/10.1002/ejoc.201800949)

(±)-Methyl 1-(2-oxopiperidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (24 & 24a)



24 Major **24a** Minor

diastereomeric ratio 4:1

A mixture of methyl 2-oxo-2-(2-oxopiperidin-3-yl)acetate **23** (979 mg, 5.28 mmol), tryptamine hydrochloride **3a** (1.2 g, 6.61 mmol) and Na_2SO_4 (500 mg) in 16 mL methanol was stirred at 80°C for 16 h. The reaction mixture was cooled to room temperature, concentrated in *vaccuo* and neutralized with sat. $NaHCO_3$. Then aqueous layer was extracted with EtOAc (3 x 50 mL), the combined organic extract washed with brine (80 mL), dried (Na_2SO_4) and concentrated in *vaccuo*. The crude product was purified by silica gel column chromatography to afford pure desired product **24** and **24a** in 90 % yield with the distereomeric ratio of 4:1.

Major Diastereomer 24

Yield: 1.28 gm

Melting Point: 124-126 °C

IRυ_{max}(film): 3319, 3022, 1649, 1426, 1031 cm^{-1}

^{1}H NMR (500 MHz, DMSO-d_6) = δ 9.59 (s, 1 H), 7.63 (br. s, 1 H), 7.37 (d, *J* = 7.6 Hz, 1 H), 7.41 (d, *J* = 8.0 Hz, 1 H), 7.03 (t, *J* = 7.4 Hz, 1 H), 6.95 (t, *J* = 7.4 Hz, 1 H), 3.59 (s, 3 H), 3.10 - 3.10 (m, 4 H), 2.89 (br. s, 1 H), 2.87 - 2.82 (m, 1 H), 2.65 - 2.53 (m, 2 H), 2.02 (dd, *J* = 4.8, 12.8 Hz, 1 H), 1.74 - 1.53 (m, 2 H), 1.17 - 1.04 (m, 1 H)

^{13}C NMR (125 MHz, DMSO-d_6) = δ 174.2, 172.2, 135.9, 131.7, 126.4, 120.9, 118.3, 117.6, 111.7, 110.5, 63.0, 51.9, 48.8, 40.6, 22.2, 22.0, 21.7

HRMS (ESI): *m/z* calculated for $C_{18}H_{22}N_3O_3[M+H]^+$= 328.1656 Observed = 328.1656

Minor Diastereomer 24a

Yield: 305 mg

Melting Point:105-108 °C

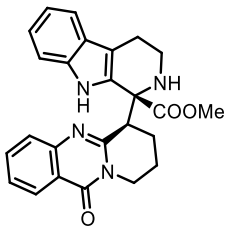
IRυ_{max}(film): 3342, 2937, 1720, 1650, 1444, 1311, 1111 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 9.57 (s, 1 H), 7.61 (br. s, 1 H), 7.37 (d, *J* = 7.9 Hz, 1 H), 7.40 (d, *J* = 7.9 Hz, 1 H), 7.03 (t, *J* = 7.3 Hz, 1 H), 6.98 - 6.95 (m, 1 H), 3.59 (s, 3 H), 3.14 –3.01 (m, 4 H), 2.95 - 2.77 (m, 2 H), 2.67 - 2.53 (m, 2 H), 2.03 (dd, *J* = 4.9, 12.8 Hz, 1 H), 1.69 - 1.66 (m, 2 H), 1.13 - 1.10 (m, 1 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 174.1, 172.1, 135.9, 131.6, 126.4, 120.8, 118.2, 117.6, 111.7, 110.5, 63.0, 51.8, 48.7, 40.6, 22.1, 22.0, 21.6

HRMS (ESI): *m/z* calculated for $C_{18}H_{22}N_3O_3[M+H]^+$= 328.1656 Observed = 328.1654

(±)-Methyl 1-(11-oxo-6,8,9,11-tetrahydro-7H-pyrido[2,1-b]quinazolin-6-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (26)



The compound **26** was synthesized by reductive condensation of **25,** following the same synthetic procedure as mentioned for compound **2;** section 1.

Yield: 31 mg; 58 %

Melting Point: 155-158 °C

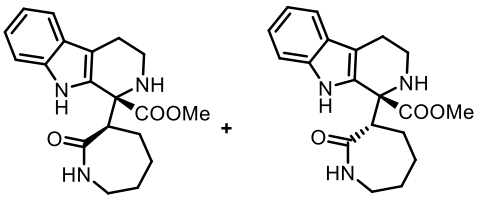
IRυ_{max}(film): 3250, 2913, 1731, 1682, 1605, 1534, 1030 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 9.43 (br. s, 1 H), 8.27 (d, *J* = 7.3 Hz, 1 H), 7.73 (t, *J* = 7.0 Hz, 1 H), 7.62 - 7.57 (m, 1 H), 7.53 (dd, *J* = 8.2, 16.8 Hz, 1 H), 7.46 - 7.42 (m, 2 H), 7.32 (t, *J* = 7.6 Hz, 1 H), 7.22 (t, *J* = 7.6 Hz, 1 H), 7.16 - 7.14 (m, 1 H), 4.17 (t, *J* = 8.9 Hz, 1 H), 4.10 (t, *J* = 6.1 Hz, 1 H), 4.01-3.91 (d, 3 H), 3.39 - 3.20 (m, 1 H), 3.05 - 2.95 (m, 2 H), 2.83 - 2.64 (m, 1 H), 2.08 - 1.91 (m, 2 H), 1.89 - 1.75 (m, 1 H), 1.61 (br. s, 1 H), 1.51 - 1.40 (m, 1 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 164.7, 162.2, 154.9, 150.1, 147.4, 136.8, 134.2, 126.6, 126.4, 126.2, 126.1, 125.4, 124.8, 120.4, 120.1, 118.2, 112.3, 53.1, 49.5, 42.3, 32.0, 22.1, 19.3, 18.7

HRMS (ESI): *m/z* calculated for $C_{25}H_{24}N_4O_3Na$ $[M+Na]^+$ = 451.1741 Observed = 451.1737

(±)-Methyl 1-(2-oxoazepan-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (29 & 29a)



A mixture of methyl 2-oxo-2-(2-oxoazepan-3-yl)acetate **28** (1g, 5.02 mmol) , tryptamine hydrochloride **3a** (1.28g, 6.52 mmol) and Na_2SO_4 (500 mg) in methanol (15 mL)was stirred at 80°C for 16 h. The reaction mixture was cooled to rt, concentrated in *vaccuo* and neutralized with sat. $NaHCO_3$.Then aqueous layer was extracted with EtOAc (3 x 80 mL), the combined organic extract washed with brine (100 mL), dried (Na_2SO_4) and concentrated in *vaccuo*. The crude product was purified by silica gel column chromatography to afford pure product **29** and **29a** in 81% yield with the diastereomeric ratio of 3:2.

Major diastereomer 29:

Yield: 851 mg

Melting Point: 183-185 °C

IRυ_{max}(film): 3370, 2928, 2849, 1718, 1650, 1475 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 9.66 (br. s, 1 H), 7.53 (d, *J* = 7.9 Hz, 1 H), 7.35 (d, *J* = 7.9 Hz, 1 H), 7.17 (t, *J* = 7.3 Hz, 1 H), 7.15 - 7.09 (m, 1 H), 6.26 (br. s, 1 H), 3.69 (s, 3 H), 3.40 - 3.37 (m, 1 H), 3.22 (dd, *J* = 4.3, 11.0 Hz, 2 H), 3.19 - 3.04 (m, 2 H), 2.92 - 2.72 (m, 2 H), 2.07 (br. s, 1 H), 1.94 (t, *J* = 15.0 Hz, 2 H), 1.83 (d, *J* = 13.4 Hz, 1 H), 1.53 - 1.40 (m, 1 H), 1.40 (m, 1 H), 0.99 - 0.96 (m, 1 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 180.1, 176.3, 135.3, 130.1, 126.4, 121.5, 118.7, 118.1, 111.6, 111.2, 63.6, 54.1, 52.4, 42.8, 40.9, 30.1, 29.1, 24.9, 22.1

HRMS (ESI): *m/z* calculated for $C_{19}H_{24}N_3O_3[M+H]^+$= 342.1812 Observed = 342.1814

Minor diastereomer 29a:

Yield: 541 mg

Melting Point: 264-266 °C

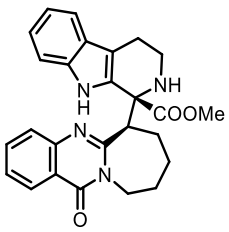
IRυ_{max}(film): 3372, 3022, 2626, 1654, 1431, 1215 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.50 (br. s, 1 H), 7.54 (d, *J* = 7.3 Hz, 1 H), 7.37 (d, *J* = 7.9 Hz, 1 H), 7.20 (t, *J* = 7.3 Hz, 1 H), 7.18 - 7.13 (m, 1 H), 6.09 (br. s, 1 H), 3.72 (s, 3 H), 3.58 - 3.55 (m, 1 H), 3.45 (dt, *J* = 4.0, 11.1 Hz, 1 H), 3.32 - 3.26 (m, 2 H), 3.17 (dd, *J* = 6.7, 13.4 Hz, 1 H), 2.89 - 2.69 (m, 2 H), 1.85 (d, *J* = 12.8 Hz, 1 H), 1.76 (d, *J* = 10.4 Hz, 1 H), 1.59 - 1.50 (m, 2 H), 1.43 - 1.27 (m, 3 H)

^{13}C NMR (100 MHz, $CDCl_3$) δ 178.8, 177.0, 136.4, 130.1, 127.0, 122.1, 119.4, 118.4, 114.1, 111.2, 64.3, 53.2, 52.5, 42.7, 40.8, 29.9, 29.2, 25.2, 21.6

HRMS (ESI): *m/z* calculated for $C_{19}H_{24}N_3O_3[M+H]^+$= 342.1812 Observed = 342.1812

(±)-Methyl 1-(12-oxo-6,7,8,9,10,12-hexahydroazepino[2,1-b]quinazolin-6-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (31)



The compound **31** was synthesized by reductive condensation of **30,** following the same synthetic procedure as mentioned for compound **2;** section 1.

Yield: 80 mg; 60 %

Melting Point: 170-172 °C

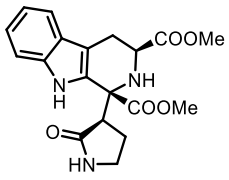
IRυ_{max}(film): 3362, 2927, 2311, 1676, 1596, 1431 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.27 (d, *J* = 7.6 Hz, 1 H), 8.22 (s, 1 H), 7.72 - 7.60 (m, 1 H), 7.58 (t, *J* = 8.6 Hz, 2 H), 7.46 (t, *J* = 7.6 Hz, 1 H), 7.39 (d, *J* = 8.0 Hz, 1 H), 7.22 (t, *J* = 7.4 Hz, 1 H), 7.22 - 7.15 (m, 1 H), 5.36 (dd, *J* = 5.5, 14.3 Hz, 1 H), 4.04 (d, *J* = 9.5 Hz, 1 H), 3.67 (s, 3 H), 3.54 - 3.51 (m, 2 H), 3.33 (dd, *J* = 4.0, 11.3 Hz, 1 H), 2.85 - 2.80 (m, 2 H), 2.09 - 1.96 (m, 1 H), 1.76 - 1.76 (m, 2 H), 1.63 - 1.58 (m, 2 H), 1.47 - 1.43 (m, 2 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 161.9, 159.5, 146.5, 136.5, 134.1, 130.2, 127.2, 127.1, 126.6, 122.4, 120.4, 119.7, 118.5, 114.9, 111.2, 65.2, 53.4, 52.3, 42.6, 40.9, 29.1, 27.6, 27.1, 21.7

HRMS (ESI): *m/z* calculated for $C_{26}H_{27}N_4O_3[M+H]^+$= 443.2078 Observed = 443.2077

(±)-Dimethyl (3S)-1-(2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1,3-dicarboxylate (33)



33 Major

A mixture of methyl 2-oxo-2-(2-oxopyrrolidin-3-yl) acetate **12** (201 mg, 1.18 mmol), hydrochloride salt of L-tryptophan methylester **32** (300 mg, 1.18 mmol) and Na_2SO_4 (100 mg) in methanol (10 mL)was stirred at 80 °C for 16 h. The reaction mixture was cooled to rt, concentrated in *vaccuo* and neutralized with sat. $NaHCO_3$.Then aqueous layer was extracted with EtOAc (3 x 50 mL), the combined organic extract washed with brine (50 mL), dried (Na_2SO_4) and concentrated in *vaccuo*. The crude product was purified by silica gel column chromatography to afford pure product **33** as major product in 68% yield.

Major diastereomer 33:

Yield: 302 mg; 68 %

Melting Point: 155-158°C

IRυ_{max}(film): 3381, 2944, 1733, 1688, 1441, 1117 cm^{-1}

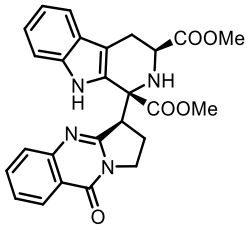
^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.61 (s, 1 H), 7.53 (d, *J* = 7.6 Hz, 1 H), 7.30 (d, *J* = 8.0 Hz, 1 H), 7.18 (t, *J* = 7.4 Hz, 1 H), 7.13 - 7.10 (m, 1 H), 6.22 (br. s, 1 H), 3.83 (s, 3 H), 3.75 (s, 3 H), 3.51 (t, *J* = 9.9 Hz, 1 H), 3.28 - 3.20 (m, 1 H), 3.17 (m, 1 H), 3.11 (t, *J* = 9.3 Hz, 1 H), 2.92 (s, 1 H), 2.83 (dd, *J* = 11.3, 14.7 Hz, 1 H), 2.38 - 2.33 (m, 1 H), 1.69 (t, *J* = 11.6 Hz, 1 H), 1.29 - 1.27 (m, 1 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 177.7, 174.7, 172.6, 136.7, 129.5, 126.2, 122.3, 119.4, 118.2, 112.1, 111.6, 63.5, 53.3, 52.9, 52.3, 49.8, 40.3, 25.5, 23.8

HRMS (ESI): *m/z* calculated for $C_{19}H_{22}N_3O_5[M+H]^+$= 372.1554 Observed = 372.1555

Minor diastereomer 33a: Due to close spot we are unable to afford minor product in pure form

(±)-Dimethyl (3S)-1-(9-oxo-1,2,3,9-tetrahydropyrrolo[2,1-b]quinazolin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1,3-dicarboxylate (35)



The compound **35** was synthesized by reductive condensation of **34**, following the same synthetic procedure as mentioned for compound **2;** section 1.

Yield: 96 mg; 53 %

Melting Point: 107-109 °C

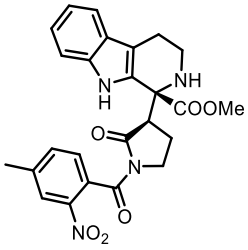
IRυ_{max}(film): 3347, 3022, 1610, 1428, 1215 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.62 (br. s., 1 H), 8.30 (d, *J* = 7.9 Hz, 1 H), 7.74 (t, *J* = 8.5 Hz, 1 H), 7.62 (d, *J* = 8.5 Hz, 1 H), 7.56 (d, *J* = 7.9 Hz, 1 H), 7.47 (t, *J* = 7.3 Hz, 1 H), 7.41 (d, *J* = 7.9 Hz, 1 H), 7.24 (d, *J* = 7.3 Hz, 1 H), 7.17 (t, *J* = 7.3 Hz, 1 H), 4.39 (t, *J* = 9.2 Hz, 1 H), 4.28 - 4.22 (m, 1 H), 4.09 (dd, *J* = 3.7, 11.0 Hz, 1 H), 3.95 (s, 3 H), 3.91 - 3.84 (m, 1 H), 3.76 (s, 3 H), 3.21 (dd, *J* = 3.7, 15.3 Hz, 1 H), 3.12 (br. s., 1 H), 2.84 (dd, *J* = 11.3, 15.0 Hz, 1 H), 2.25 (qd, *J* = 9.2, 12.7 Hz, 1 H), 1.96 - 1.84 (m, 1 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 173.9, 172.6, 160.8, 157.2, 148.8, 136.9, 134.0, 129.4, 127.3, 126.5, 126.4, 122.9, 120.8, 120.0, 118.6, 111.9, 111.4, 63.5, 53.7, 53.0, 52.7, 52.2, 44.8, 24.8, 21.2

HRMS (ESI): *m/z* calculated for $C_{26}H_{25}N_4O_5Na[M+Na]^+$= 495.1639 Observed = 495.1635

(±)-Methyl 1-(1-(4-methyl-2-nitrobenzoyl)-2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (36)



The compound **36** was synthesized by *N*-acylation of **11**, following the same synthetic procedure as mentioned for compound **17;** section 1.

Yield: 71%

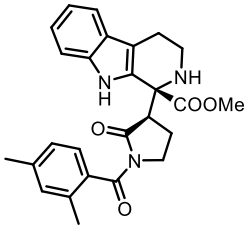
IRυ_{max}(film): 3397, 2914, 1730, 1688, 1336 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.25 (br. s., 1 H), 7.94 (s, 1 H), 7.44 (d, *J* = 7.8 Hz, 2 H), 7.25 (s, 1 H), 7.22 - 7.17 (m, 1 H), 7.16 - 7.08 (m, 1 H), 7.07 - 7.04 (m, 1 H), 4.04 - 3.97 (m, 1 H), 3.75 - 3.66 (m, 2 H), 3.65 (s, 3 H), 3.24 - 3.07 (m, 2 H), 2.75 - 2.61 (m, 2 H), 2.42 (s, 3 H), 2.01 (d, *J* = 9.9 Hz, 1 H), 1.77 - 1.67 (m, 2 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 173.7, 166.4, 145.2, 141.0, 136.6, 135.0, 130.0, 127.9, 126.6, 124.5, 122.7, 119.7, 118.6, 113.6, 111.2, 62.6, 53.2, 53.1, 43.9, 41.3, 21.4, 21.2, 19.1

HRMS (ESI): *m/z* calculated for $C_{25}H_{25}O_6N_4$ $[M+H]^+$ = 477.1769, Observed = 477.1765.

(±)-Methyl 1-(1-(2,4-dimethylbenzoyl)-2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (38)



The compound **38** was synthesized by *N*-acylation of **11**, following the same synthetic procedure as mentioned for compound **17;** section 1.

Yield: 75%

IRυ_{max}(film): 3376, 2935, 1752, 1688, 1319 cm^{-1}

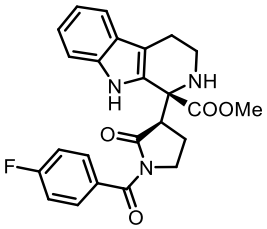
^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.39 (br. s., 1 H), 7.53 (d, *J* = 7.8 Hz, 1 H), 7.35 (d, *J* = 8.0 Hz, 1 H), 7.24 - 7.17 (m, 2 H), 7.17 - 7.10 (m, 1 H), 7.08 - 7.06 (m, 2 H), 4.00 (t, *J* = 9.8 Hz, 1 H), 3.82 (dd, *J* = 9.1, 10.8 Hz, 1 H), 3.76 (s, 3 H), 3.74 - 3.63 (m, 1

H), 3.35 - 3.22 (m, 2 H), 2.93 - 2.81 (m, 1 H), 2.81 - 2.72 (m, 1 H), 2.36 (s, 3 H), 2.32 (s, 3 H), 2.16 (t, *J* = 10.6 Hz, 1 H), 1.78 (br. s., 1 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 173.0, 170.4, 140.4, 136.5, 135.5, 132.2, 131.4, 127.6, 126.6, 126.1, 122.7, 119.8, 118.6, 113.1, 111.2, 62.3, 53.7, 53.2, 43.9, 41.4, 21.4, 21.3, 19.5, 19.4

HRMS (ESI): *m/z* calculated for $C_{26}H_{28}O_4N_3$ $[M+H]^+$ = 446.2074, Observed = 446.2067.

(±)-Methyl 1-(1-(4-fluorobenzoyl)-2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (39)



The compound **39** was synthesized by *N*-acylation of **11**, following the same synthetic procedure as mentioned for compound **17;** section 1.

Yield: 68%

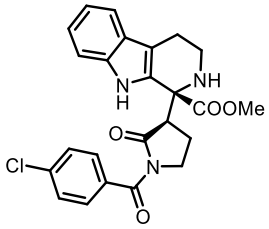
IRυ_{max}(film): 3390, 2912, 1727, 1669, 1303 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.32 (br. s., 1 H), 7.70 - 7.65 (m, 2 H), 7.54 (d, *J* = 7.88 Hz, 1 H), 7.37 - 7.35 (m, 1 H), 7.24 - 7.22 (m, 1 H), 7.17 - 7.13 (m, 1 H), 7.13 - 7.08 (m, 2 H), 3.96 - 3.91 (m, 1 H), 3.83 - 3.80 (m, 1 H), 3.79 (s, 3 H), 3.78 - 3.69 (m, 1 H), 3.37 - 3.30 (m, 2 H), 2.88 - 2.75 (m, 2 H), 2.19 - 2.08 (m, 1 H), 1.79 - 1.72 (m, 1 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 173.8, 173.6, 169.1, 166.3 (d, J_{C-F} = 253.29 Hz), 136.6, 131.9 (d, J_{C-F} = 9.15 Hz), 130.0 (d, J_{C-F} = 3.05 Hz), 129.0, 126.7, 122.7, 119.8, 118.6, 115.2 (d, J_{C-F} = 22.12 Hz), 115.0, 113.4, 111.2, 62.4, 53.9, 53.1, 44.7, 41.4, 21.6, 19.5

HRMS (ESI): *m/z* calculated for $C_{24}H_{23}O_4N_3F$ $[M+H]^+$ = 436.1667, Observed = 436.1664.

(±)-Methyl 1-(1-(4-chlorobenzoyl)-2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (40)



The compound **40** was synthesized by *N*-acylation of **11**, following the same synthetic procedure as mentioned for compound **17;** section 1.

Yield: 67%

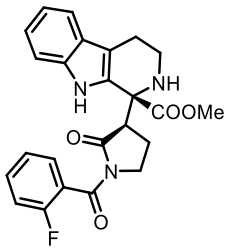
IRυ_{max}(film): 3392, 2918, 1730, 1672, 1300 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.35 (s, 1 H), 7.60 - 7.56 (m, 2 H), 7.54 (d, *J* = 7.9 Hz, 1 H), 7.44 - 7.41 (m, 1 H), 7.41 - 7.39 (m, 1 H), 7.36 (d, *J* = 8.0 Hz, 1 H), 7.22 (ddd, *J* = 1.2, 7.1, 8.1 Hz, 1 H), 7.17 - 7.10 (m, 1 H), 3.94 (ddd, *J* = 1.9, 9.1, 11.1 Hz, 1 H), 3.84 - 3.80 (m, 1 H), 3.79 (s, 3 H), 3.74 - 3.68 (m, 1 H), 3.33 - 3.23 (m, 2 H), 2.89 - 2.78 (m, 2 H), 2.14 - 2.04 (m, 2 H), 1.82 - 1.78 (m, 1 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 173.7, 173.6, 169.2, 138.4, 136.6, 132.3, 130.6, 130.3, 128.8, 128.4, 128.2, 126.6, 122.7, 119.8, 118.7, 113.4, 111.2, 62.4, 53.8, 53.2, 44.6, 41.5, 21.6, 19.5

HRMS (ESI): *m/z* calculated for $C_{24}H_{23}O_4N_3Cl$ $[M+H]^+$ = 452.1372, Observed = 452.1367.

(±)-Methyl 1-(1-(2-fluorobenzoyl)-2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (41)



The compound **41** was synthesized by *N*-acylation of **11**, following the same synthetic procedure as mentioned for compound **17;** section 1.

Yield: 66%

IRυ_{max}(film): 3387, 2913, 1736, 1672, 1320 cm^{-1}

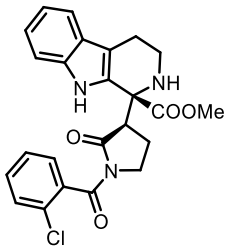
^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.32 - 8.27 (m, 1 H), 7.56 - 7.45 (m, 3 H), 7.35 (d, *J* = 8.1 Hz, 1 H), 7.25 - 7.19 (m, 2 H), 7.17 - 7.14 (m, 2 H), 4.02 (ddd, *J* = 2.0, 9.4, 11.4

Hz, 1 H), 3.82 (t, *J* = 9.8 Hz, 1 H), 3.78 (s, 3 H), 3.70 (ddd, *J* = 7.7, 10.0, 11.3 Hz, 1 H), 3.31 - 3.22 (m, 2 H), 2.89 - 2.79 (m, 1 H), 2.74 (td, *J* = 2.8, 14.9 Hz, 1 H), 2.17 - 2.05 (m, 1 H), 1.79 - 1.74 (m, 1 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 173.8, 173.1, 165.3, 160.8 (d, J_{C-F} = 251.01 Hz), 136.6, 132.9 (d, J_{C-F} = 8.39 Hz), 129.8 (d, J_{C-F} = 3.05 Hz), 129.0, 126.7, 124.2, 124.2, 123.8 (d, J_{C-F} = 15.26 Hz), 122.6, 119.7, 118.6, 115.7 (d, J_{C-F} = 21.36 Hz), 113.6, 111.2, 62.5, 53.6, 53.0, 44.0, 41.3, 21.6, 19.0

HRMS (ESI): *m/z* calculated for $C_{24}H_{23}O_4N_3F$ $[M+H]^+$ = 436.1667, Observed = 436.1669

(±)-Methyl 1-(1-(2-chlorobenzoyl)-2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (42)



The compound **42** was synthesized by *N*-acylation of **11**, following the same synthetic procedure as mentioned for compound **17;** section 1.

Yield: 70%

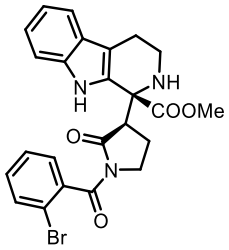
IRυ_{max}(film): 3389, 2905, 2356, 1736, 1676, 1323 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.21 (br. s., 1 H), 7.45 (d, *J* = 7.8 Hz, 1 H), 7.34 - 7.30 (m, 2 H), 7.29 - 7.22 (m, 3 H), 7.14 (dt, *J* = 1.1, 7.6 Hz, 1 H), 7.08 - 7.06 (m, 1 H), 3.98 (ddd, *J* = 1.9, 9.6, 11.4 Hz, 1 H), 3.75 (dd, *J* = 9.1, 10.6 Hz, 1 H), 3.68 (s, 3 H), 3.62 (ddd, *J* = 7.8, 10.1, 11.3 Hz, 1 H), 3.18 - 3.12 (m, 2 H), 2.80 - 2.67 (m, 2 H), 2.08 - 1.99 (m, 2 H), 1.67 - 1.64 (m, 1 H), 0.82 - 0.80 (m, 1 H)

^{13}C NMR (100 MHz, $CDCl_3$) = 173.7, 173.0, 167.0, 136.6, 135.6, 130.9, 130.4, 129.4, 128.8, 128.1, 126.9, 126.7, 122.7, 119.7, 118.6, 113.5, 111.2, 62.4, 53.4, 53.1, 43.5, 41.3, 21.5, 19.1

HRMS (ESI): *m/z* calculated for $C_{24}H_{23}O_4N_3Cl$ $[M+H]^+$ = 452.1372, Observed = 452.1374

(±)-Methyl 1-(1-(2-bromobenzoyl)-2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (43)



The compound **43** was synthesized by *N*-acylation of **11**, following the same synthetic procedure as mentioned for compound **17;** section 1.

Yield: 73%

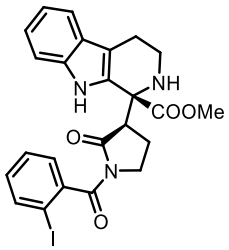
IRυ_{max}(film): 3395, 2347, 1738, 1675, 1321 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.34 (br. s., 1 H), 7.60 - 7.56 (m, 1 H), 7.53 (d, *J* = 7.8 Hz, 1 H), 7.42 - 7.26 (m, 4 H), 7.21 (dt, *J* = 1.1, 7.6 Hz, 1 H), 7.18 - 7.13 (m, 1 H), 4.06 (ddd, *J* = 2.0, 9.6, 11.4 Hz, 1 H), 3.85 (dd, *J* = 9.1, 10.6 Hz, 1 H), 3.76 (s, 3 H), 3.69 (ddd, *J* = 7.8, 10.0, 11.4 Hz, 1 H), 3.33 - 3.19 (m, 2 H), 2.84 (d, *J* = 9.0 Hz, 1 H), 2.79 - 2.72 (m, 1 H), 2.20 - 2.06 (m, 1 H), 1.84 - 1.73 (m, 1 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 172.9, 167.7, 137.7, 136.6, 132.5, 131.0, 127.9, 127.4, 126.6, 122.7, 119.8, 118.8, 118.6, 113.4, 111.2, 62.4, 53.3, 53.1, 43.5, 41.3, 21.4, 19.2

HRMS (ESI): *m/z* calculated for $C_{24}H_{23}O_4N_3Br$ $[M+H]^+$ = 496.0866, Observed = 496.0879

(±)-Methyl 1-(1-(2-iodobenzoyl)-2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (44)



The compound **44** was synthesized by *N*-acylation of **11**, following the same synthetic procedure as mentioned for compound **17;** section 1.

Yield: 69%

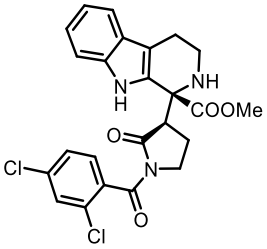
IRυ_{max}(film): 3397, 2338, 1737, 1677, 1317 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.31 (br. s., 1 H), 7.80 - 7.72 (m, 1 H), 7.46 (d, *J* = 7.9 Hz, 1 H), 7.38 - 7.33 (m, 1 H), 7.29 (s, 1 H), 7.19 - 7.12 (m, 2 H), 7.12 - 7.06 (m, 2 H), 3.99 - 3.93 (m, 1 H), 3.79 (t, *J* = 9.8 Hz, 1 H), 3.70 (s, 3 H), 3.63 (dt, *J* = 7.9, 10.5 Hz, 1 H), 3.27 - 3.14 (m, 2 H), 2.89 - 2.73 (m, 1 H), 2.73 - 2.61 (m, 1 H), 2.20 - 2.04 (m, 1 H), 1.99 (s, 1 H), 1.74 (br. s., 1 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 172.9, 169.0, 141.8, 138.8, 136.6, 130.8, 128.1, 127.4, 126.6, 122.8, 119.8, 118.7, 111.2, 91.5, 62.5, 53.3, 53.2, 43.5, 41.3, 21.3, 19.3

HRMS (ESI): *m/z* calculated for $C_{24}H_{23}O_4N_3I$ $[M+H]^+$ = 544.0728, Observed = 544.0730

(±)-Methyl 1-(1-(2,4-dichlorobenzoyl)-2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (45)



The compound **45** was synthesized by *N*-acylation of **11**, following the same synthetic procedure as mentioned for compound **17;** section 1.

Yield: 65%

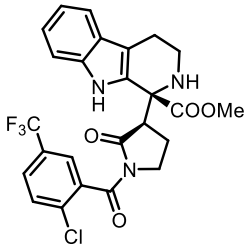
IRυ_{max}(film): 3388, 2924, 2355, 1735, 1679, 1317 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.25 (s, 1 H), 7.53 (d, *J* = 7.8 Hz, 1 H), 7.42 (d, *J* = 1.9 Hz, 1 H), 7.36 - 7.30 (m, 2 H), 7.25 (d, *J* = 8.1 Hz, 1 H), 7.23 - 7.18 (m, 1 H), 7.16 - 7.11 (m, 1 H), 4.04 (ddd, *J* = 2.0, 9.5, 11.5 Hz, 1 H), 3.82 (dd, *J* = 9.0, 10.6 Hz, 1 H), 3.78 (s, 3 H), 3.68 (ddd, *J* = 7.8, 9.9, 11.4 Hz, 1 H), 3.24 (dd, *J* = 3.1, 7.8 Hz, 2 H), 2.82 - 2.75 (m, 2 H), 2.20 - 2.04 (m, 1 H), 1.73 (dddd, *J* = 2.0, 7.7, 9.1, 12.7 Hz, 1 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 173.8, 173.1, 166.1, 136.6, 136.4, 134.1, 131.4, 129.4, 129.0, 128.8, 127.3, 126.7, 122.7, 119.8, 118.7, 113.6, 111.2, 62.4, 53.5, 53.1, 43.6, 41.3, 21.6, 19.1

HRMS (ESI): *m/z* calculated for $C_{24}H_{22}O_4N_3Cl_2$ $[M+H]^+$ = 486.0982, Observed = 486.0992

(±)-Methyl 1-(1-(2-chloro-5-(trifluoromethyl)benzoyl)-2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (46)



The compound **46** was synthesized by *N*-acylation of **11**, following the same synthetic procedure as mentioned for compound **17;** section 1.

Yield: 63%

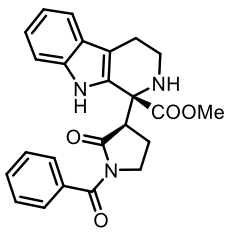
IRυ_{max}(film): 3402, 2896, 1738, 1680, 1301 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.23 (s, 1 H), 7.67 - 7.61 (m, 1 H), 7.58 (d, *J* = 1.5 Hz, 1 H), 7.53 (dd, *J* = 3.4, 8.0 Hz, 2 H), 7.35 (d, *J* = 7.6 Hz, 1 H), 7.25 - 7.18 (m, 1 H), 7.18 - 7.10 (m, 1 H), 4.06 (ddd, *J* = 2.3, 9.7, 11.6 Hz, 1 H), 3.82 (dd, *J* = 9.2, 10.7 Hz, 1 H), 3.77 (s, 3 H), 3.70 (ddd, *J* = 7.6, 9.7, 11.6 Hz, 1 H), 3.28 - 3.21 (m, 2 H), 2.86 - 2.70 (m, 2 H), 2.19 - 2.13 (m, 1 H), 1.79 - 1.74 (m, 1 H), 0.91 - 0.88 (m, 1 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 173.8, 173.2, 165.5, 136.6, 136.3, 134.4, 130.0, 129.6, 129.3, 128.7, 127.6, 126.7, 125.3 (q, $J_{C\text{-}F3}$ = 3.83 Hz), 122.7 (q, $J_{C\text{-}F3}$ = 2.88 Hz), 119.8, 118.7, 113.7, 111.2, 62.4, 53.4, 53.1, 43.5, 41.3, 21.6, 19.2

HRMS (ESI): *m/z* calculated for $C_{25}H_{22}O_4N_3ClF_3$ $[M+H]^+$ = 520.1245, Observed = 520.1257.

(±)-Methyl 1-(1-benzoyl-2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (47)



The compound **47** was synthesized by *N*-acylation of **11**, following the same synthetic procedure as mentioned for compound **17;** section 1.

Yield: 75%

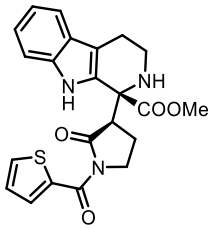
IRυ_{max}(film): 3396, 2916, 1731, 1670, 1301 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.36 (s, 1 H), 7.65 - 7.63 (m, 2 H), 7.60 - 7.50 (m, 2 H), 7.50 - 7.40 (m, 2 H), 7.36 (d, *J* = 8.1 Hz, 1 H), 7.22 (dt, *J* = 1.1, 7.6 Hz, 1 H), 7.19 - 7.07 (m, 1 H), 3.95 (ddd, *J* = 1.8, 9.1, 11.1 Hz, 1 H), 3.84 - 3.80 (m, 1 H), 3.78 (s, 3 H), 3.75 - 3.72 (m, 1 H), 3.35 - 3.28 (m, 2 H), 2.90 - 2.71 (m, 2 H), 2.21 - 2.04 (m, 2 H), 1.83 - 1.74 (m, 1 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 173.9, 173.5, 170.4, 136.5, 134.0, 132.1, 129.1, 129.0, 127.9, 126.6, 122.6, 119.7, 118.6, 113.3, 111.2, 62.3, 53.8, 53.1, 44.6, 41.4, 21.6, 19.5

HRMS (ESI): *m/z* calculated for $C_{24}H_{24}O_4N_3$ $[M+H]^+$ = 418.1761, Observed = 418.1753.

(±)-Methyl 1-(2-oxo-1-(thiophene-2-carbonyl)pyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (48)



The compound **48** was synthesized by *N*-acylation of **11**, following the same synthetic procedure as mentioned for compound **17;** section 1.

Yield: 61%

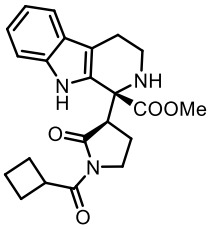
IRυ_{max}(film): 3371, 2902, 2357, 1732, 1647, 1291 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.37 (br. s., 1 H), 7.91 (d, *J* = 2.9 Hz, 1 H), 7.64 (d, *J* = 4.1 Hz, 1 H), 7.54 (d, *J* = 7.8 Hz, 1 H), 7.37 (d, *J* = 8.0 Hz, 1 H), 7.22 (t, *J* = 7.6 Hz, 1 H), 7.17 - 7.06 (m, 2 H), 3.96 - 3.88 (m, 1 H), 3.84 (s, 3 H), 3.80 - 3.70 (m, 1 H), 3.49 (d, *J* = 7.0 Hz, 1 H), 3.35 - 3.26 (m, 2 H), 2.90 - 2.72 (m, 2 H), 2.20 - 2.06 (m, 1 H), 1.81 - 1.73 (m, 2 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 173.8, 173.3, 162.9, 136.6, 136.4, 135.0, 133.5, 128.9, 127.4, 126.7, 122.7, 119.8, 118.6, 113.4, 111.2, 62.5, 54.0, 53.1, 45.3, 41.4, 21.6, 19.5

HRMS (ESI): *m/z* calculated for $C_{22}H_{22}O_4N_3S$ $[M+H]^+$ = 424.1326, Observed = 424.1317.

(±)-Methyl 1-(1-(cyclobutanecarbonyl)-2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (49)



The compound **49** was synthesized by *N*-acylation of **11**, following the same synthetic procedure as mentioned for compound **17;** section 1.

Yield: 60%

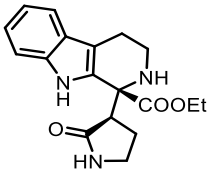
IRυ_{max}(film): 3388, 2934, 2356, 1717, 1239 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.37 (br. s., 1 H), 7.52 (d, *J* = 7.8 Hz, 1 H), 7.36 (d, *J* = 8.1 Hz, 1 H), 7.24 - 7.18 (m, 1 H), 7.16 - 7.10 (m, 1 H), 4.04 (quin, *J* = 8.4 Hz, 1 H), 3.87 (s, 3 H), 3.86 (d, *J* = 2.0 Hz, 1 H), 3.80 (dd, *J* = 9.2, 10.9 Hz, 1 H), 3.53 - 3.42 (m, 1 H), 3.27 (dd, *J* = 2.9, 7.8 Hz, 2 H), 2.88 - 2.69 (m, 2 H), 2.37 - 2.18 (m, 4 H), 2.09 - 1.94 (m, 2 H), 1.93 - 1.79 (m, 1 H), 1.71 - 1.66 (m, 1 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 175.5, 173.6, 136.6, 128.7, 126.6, 122.6, 119.7, 118.6, 113.3, 111.2, 62.4, 53.9, 53.1, 43.5, 41.4, 39.7, 24.6, 24.3, 21.4, 19.1, 17.9

HRMS (ESI): *m/z* calculated for $C_{22}H_{26}O_4N_3$ $[M+H]^+$ = 396.1918, Observed = 396.1916.

(±)-Ethyl 1-(2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (51)



The compound **51** was synthesized by, following the same synthetic procedure as mentioned for compound **11;** section 1.

Yield: 71%

IRυ_{max}(film): 3384, 2905, 2366, 1690, 1224 cm^{-1}

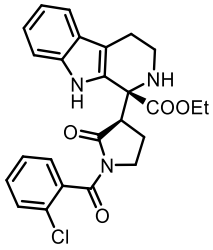
^{1}H NMR (400 MHz, DMSO-d_6) = δ 10.23 (s, 1 H), 7.64 (s, 1 H), 7.38 (d, *J* = 8.9 Hz, 2 H), 7.04 – 7.01 (m, 1 H), 6.96 - 6.92 (m, 1 H), 4.15 - 4.10 (m, 2 H), 3.27 (t, *J* = 9.1

Hz, 1 H), 3.18 - 3.10 (m, 2 H), 3.10 - 2.91 (m, 3 H), 2.64 - 2.52 (m, 2 H), 2.18 - 2.03 (m, 1 H), 1.92 - 1.78 (m, 1 H), 1.19 (t, *J* = 7.1 Hz, 3 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 175.3, 172.6, 136.1, 131.3, 126.3, 120.8, 118.1, 117.6, 111.5, 110.1, 63.1, 61.0, 47.4, 23.2, 21.9, 14.1

HRMS (ESI): *m/z* calculated for $C_{18}H_{22}O_3N_3$ $[M+H]^+$ = 328.1656, Observed = 328.1656.

(±)-Ethyl 1-(1-(2-chlorobenzoyl)-2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (54)



The compound **54** was synthesized by *N*-acylation of **51**, following the same synthetic procedure as mentioned for compound **17;** section 1.

Yield: 75%

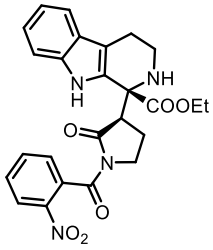
IRυ_{max}(film): 3386, 2908, 2358, 1737, 1677, 1321 cm^{-1}

^{1}H NMR (400 MHz, CDCl$_3$) = δ 8.36 (br. s., 1 H), 7.53 (d, *J* = 7.8 Hz, 1 H), 7.41 - 7.37 (m, 2 H), 7.37 - 7.31 (m, 3 H), 7.21 (dt, *J* = 1.1, 7.6 Hz, 1 H), 7.16 - 7.13 (m, 1 H), 4.31 - 4.14 (m, 2 H), 4.11 - 3.99 (m, 1 H), 3.84 (t, *J* = 9.9 Hz, 1 H), 3.69 (ddd, *J* = 7.8, 10.0, 11.3 Hz, 1 H), 3.34 - 3.20 (m, 2 H), 2.93 - 2.80 (m, 1 H), 2.80 - 2.67 (m, 1 H), 2.27 - 2.10 (m, 1 H), 1.85 - 1.67 (m, 2 H), 1.25 (t, *J* = 7.2 Hz, 3 H)

^{13}C NMR (100 MHz, CDCl$_3$) = δ 172.9, 167.0, 136.6, 135.6, 130.9, 130.4, 129.4, 128.1, 126.8, 126.6, 122.7, 119.8, 118.6, 111.2, 62.5, 62.3, 53.3, 43.5, 41.4, 19.2, 14.0

HRMS (ESI): *m/z* calculated for $C_{25}H_{25}O_4N_3Cl$ $[M+H]^+$ = 466.1528, Observed = 466.1523.

(±)-Ethyl 1-(1-(2-nitrobenzoyl)-2-oxopyrrolidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (55)



The compound **55** was synthesized by *N*-acylation of **51**, following the same synthetic procedure as mentioned for compound **17;** section 1.

Yield: 70%

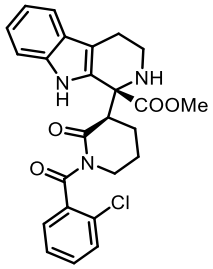
IRυ_{max}(film): 3395, 2920, 1732, 1685, 1330 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 8.34 (s, 1 H), 8.23 (dd, *J* = 1.0, 8.3 Hz, 1 H), 7.74 (dt, *J* = 1.1, 7.5 Hz, 1 H), 7.64 - 7.57 (m, 1 H), 7.53 (d, *J* = 7.8 Hz, 1 H), 7.39 (dd, *J* = 1.3, 7.5 Hz, 1 H), 7.34 (d, *J* = 8.1 Hz, 1 H), 7.21 (dt, *J* = 1.2, 7.6 Hz, 1 H), 7.16 - 7.13 (m, 1 H), 4.21 - 4.14 (m, 2 H), 4.14 - 4.06 (m, 1 H), 3.81 - 3.69 (m, 2 H), 3.31 - 3.19 (m, 2 H), 2.87 - 2.74 (m, 2 H), 2.12 - 2.06 (m, 1 H), 1.82 - 1.69 (m, 1 H), 1.23 (t, *J* = 7.1 Hz, 3 H), 0.91 - 0.88 (m, 1 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 173.8, 173.0, 166.3, 145.1, 136.6, 134.5, 132.9, 130.1, 128.9, 128.0, 126.6, 124.1, 122.6, 119.7, 118.6, 113.6, 111.2, 62.2, 53.2, 43.8, 41.3, 29.7, 21.6, 19.1, 14.0

HRMS (ESI): *m/z* calculated for $C_{25}H_{25}O_6N_4$ $[M+H]^+$ = 477.1769, Observed = 477.1769

(±)-Methyl 1-(1-(2-chlorobenzoyl)-2-oxopiperidin-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (56)



The compound **57** was synthesized by *N*-acylation of **24**, following the same synthetic procedure as mentioned for compound **17;** section 1.

Yield: 69%

IRυ_{max}(film): 3392, 2893, 2366, 1685, 1304 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.17 (br. s., 1 H), 7.52 (d, *J* = 7.8 Hz, 1 H), 7.40 - 7.31 (m, 5 H), 7.21 (dt, *J* = 1.1, 7.6 Hz, 1 H), 7.15 - 7.10 (m, 1 H), 4.06 - 3.92 (m, 1 H), 3.86 - 3.69 (m, 2 H), 3.63 (s, 3 H), 3.32 - 3.15 (m, 2 H), 2.83 - 2.65 (m, 2 H), 2.02 - 1.78 (m, 4 H), 1.42 - 1.36 (m, 1 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 174.6, 173.6, 169.8, 137.1, 136.4, 130.5, 129.4, 129.2, 128.5, 127.0, 126.8, 122.5, 119.7, 118.6, 114.0, 111.1, 62.9, 53.3, 52.7, 44.7, 41.2, 21.9, 21.5

1.2.7 References:

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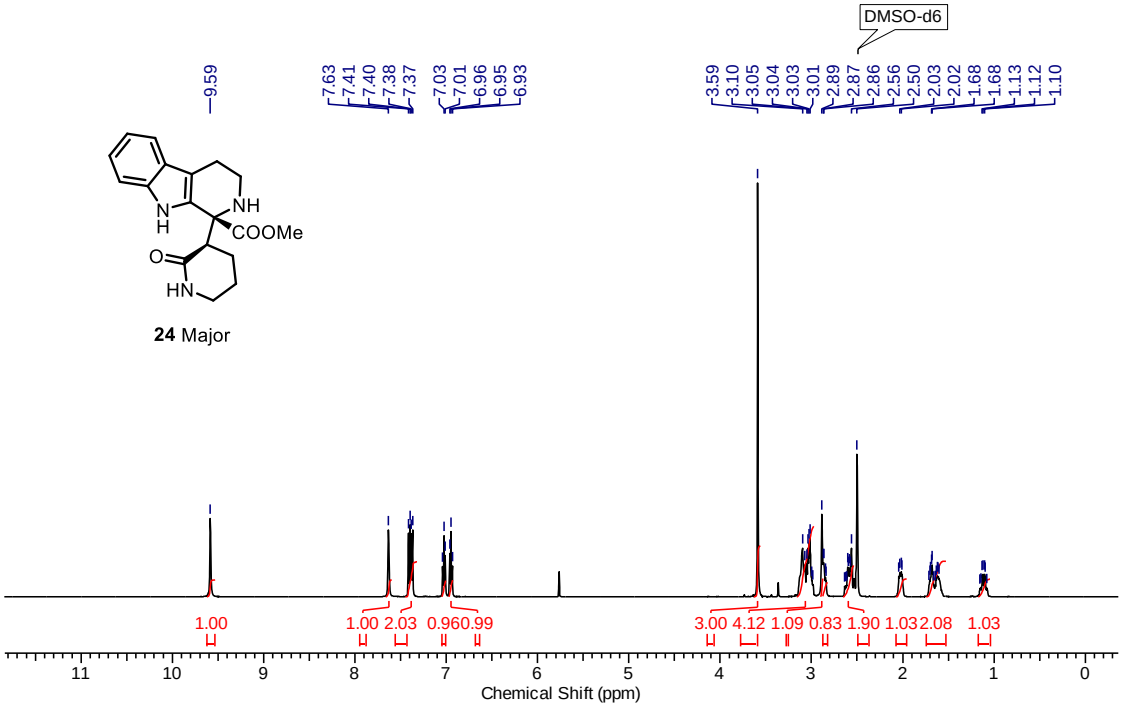
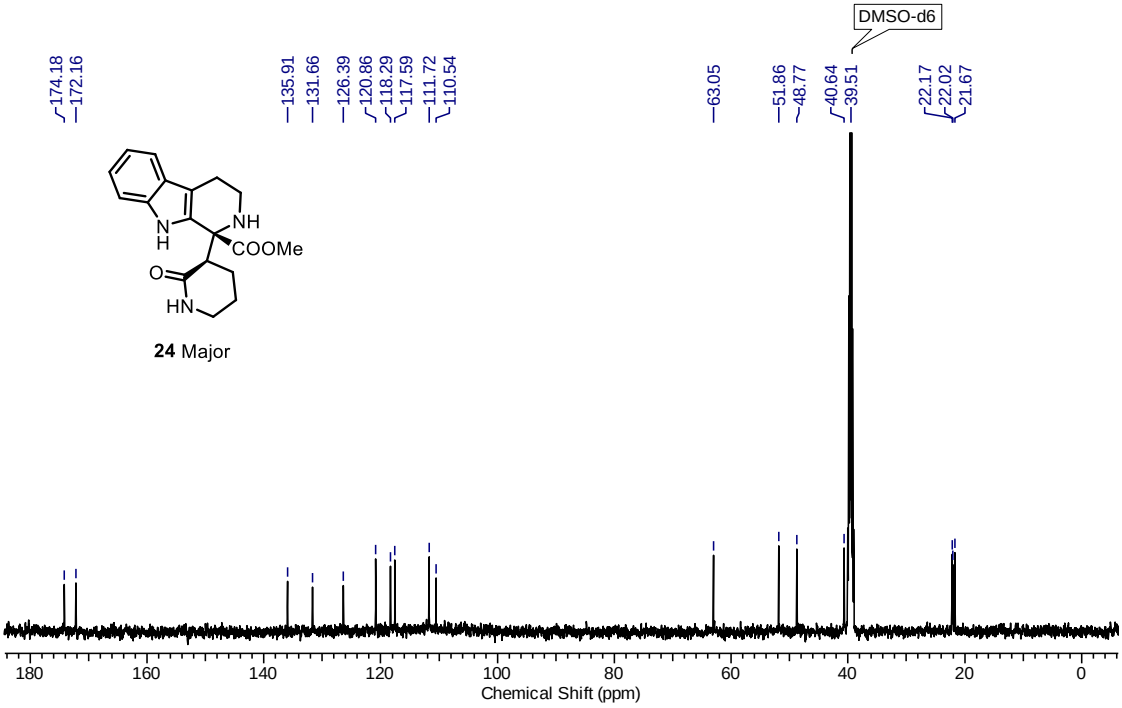
1.2.8 Copies of NMR spectra

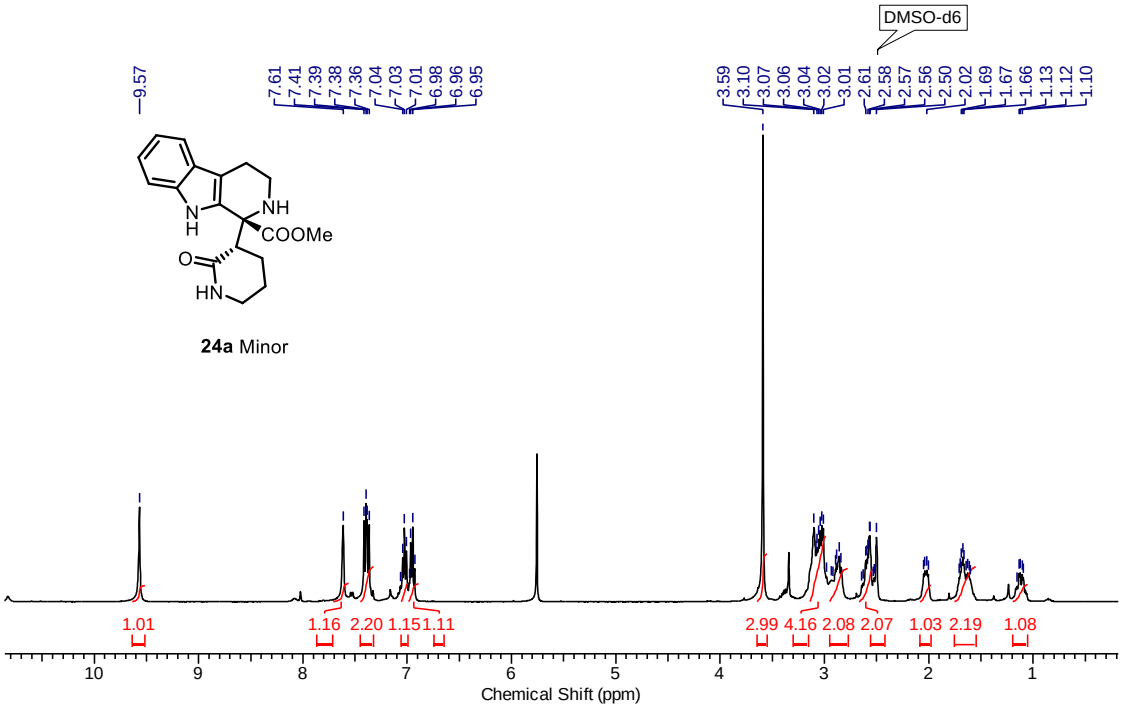
^{1}H NMR of Compound 24 in DMSO-d_6 at 500 MHz



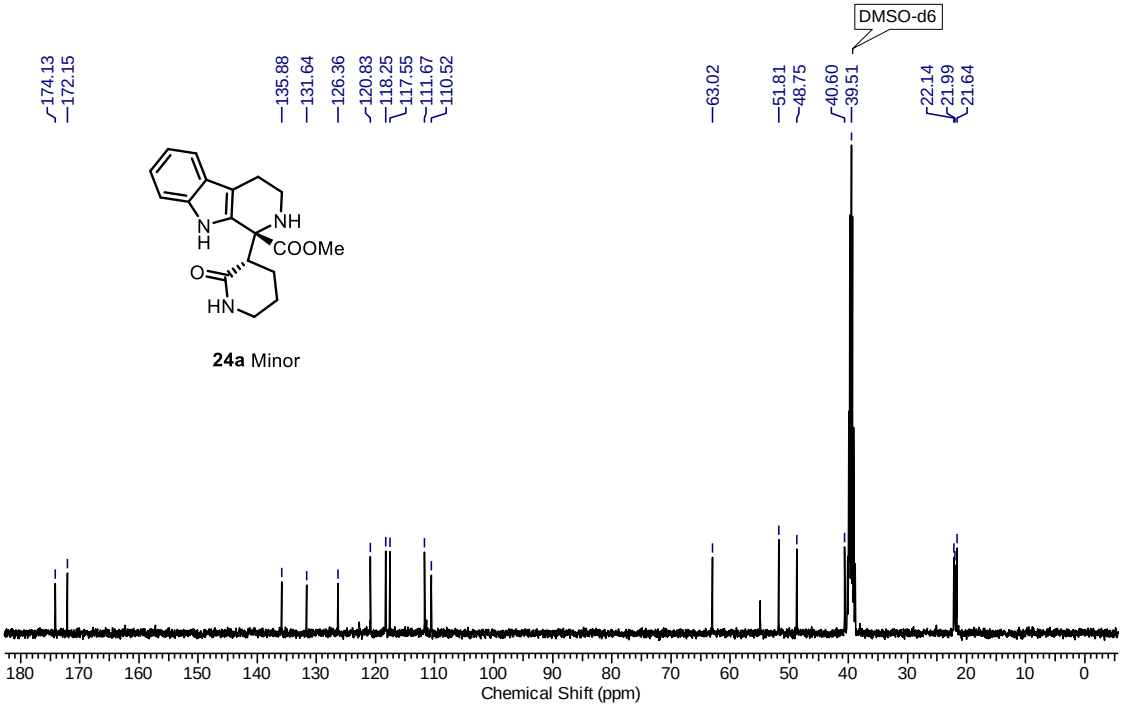
^{13}C NMR of Compound 24 in DMSO-d_6 at 125 MHz



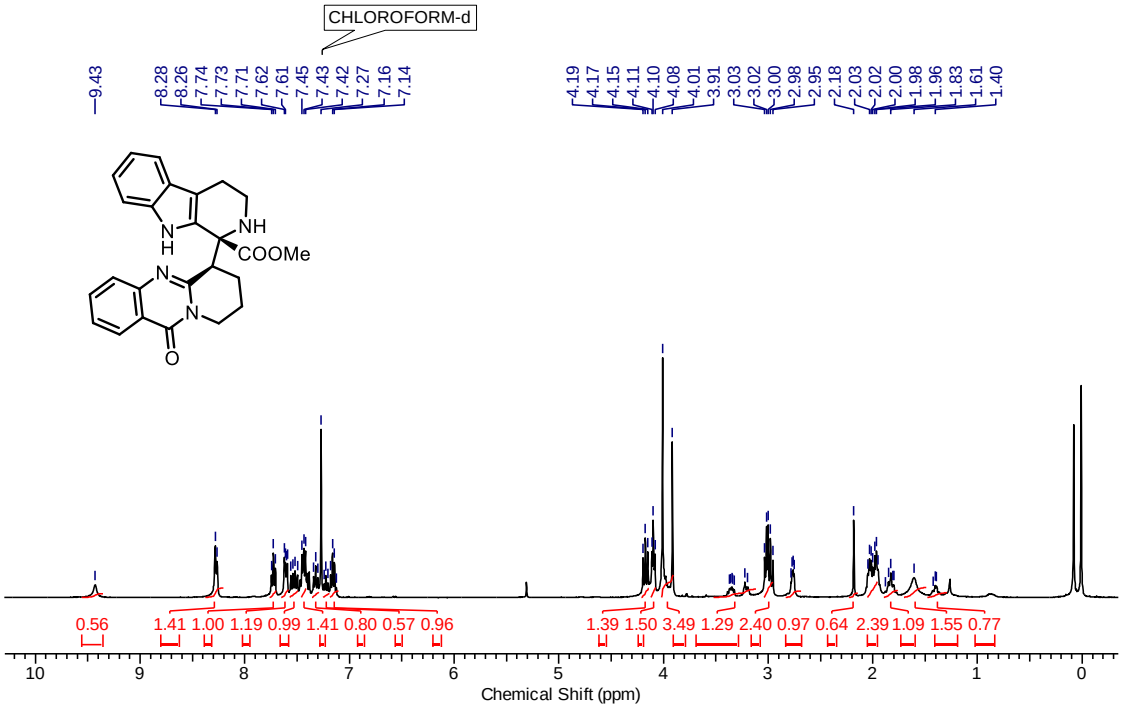
^{1}H NMR of Compound 24a in DMSO-d_6 at 500 MHz



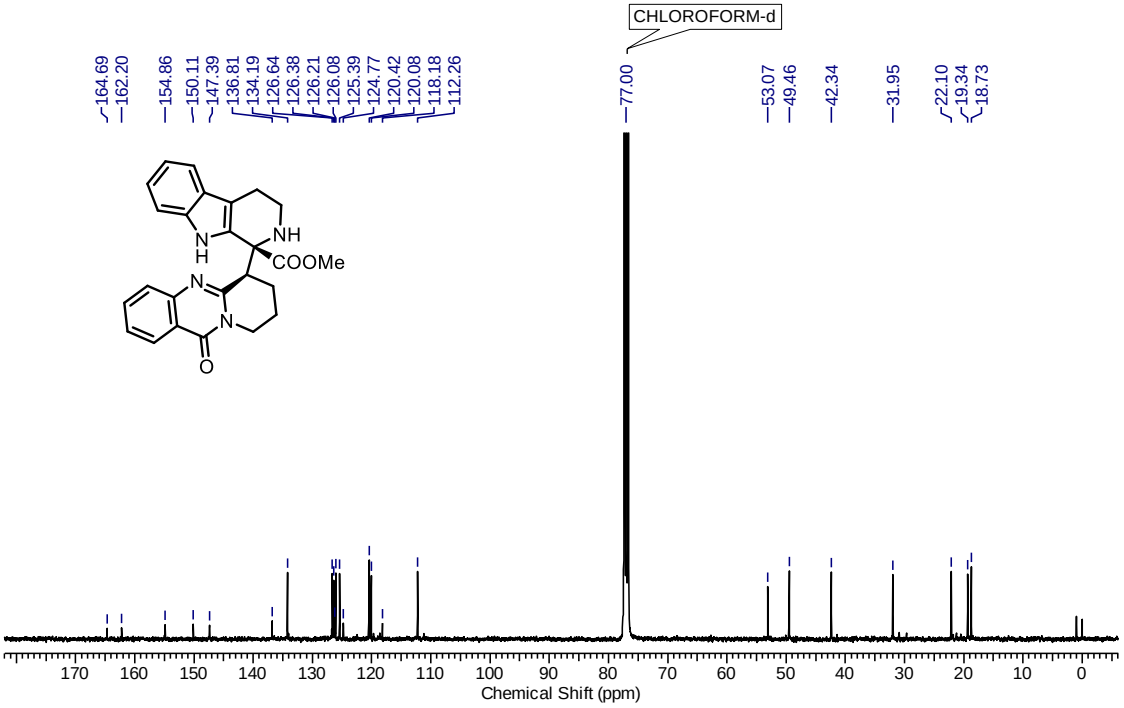
^{13}C NMR of Compound 24a in DMSO-d_6 at 125 MHz



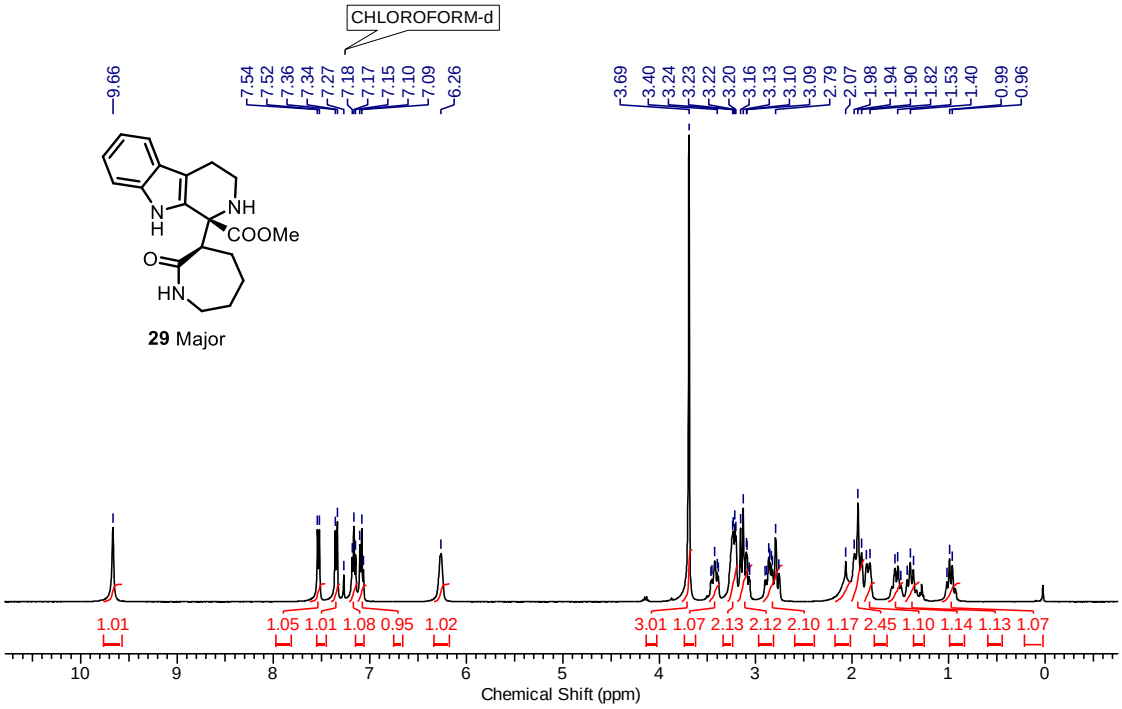
^{1}H NMR of Compound 26 in $CDCl_3$ at 400 MHz



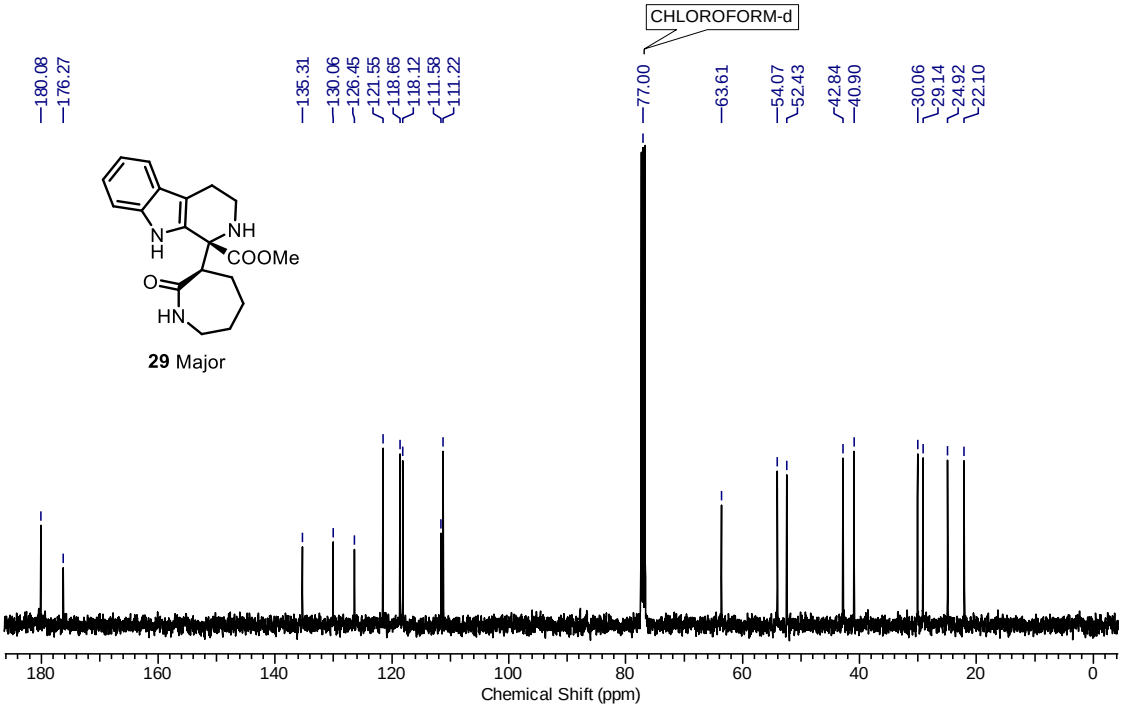
^{13}C NMR of Compound 26 in $CDCl_3$ at 100 MHz



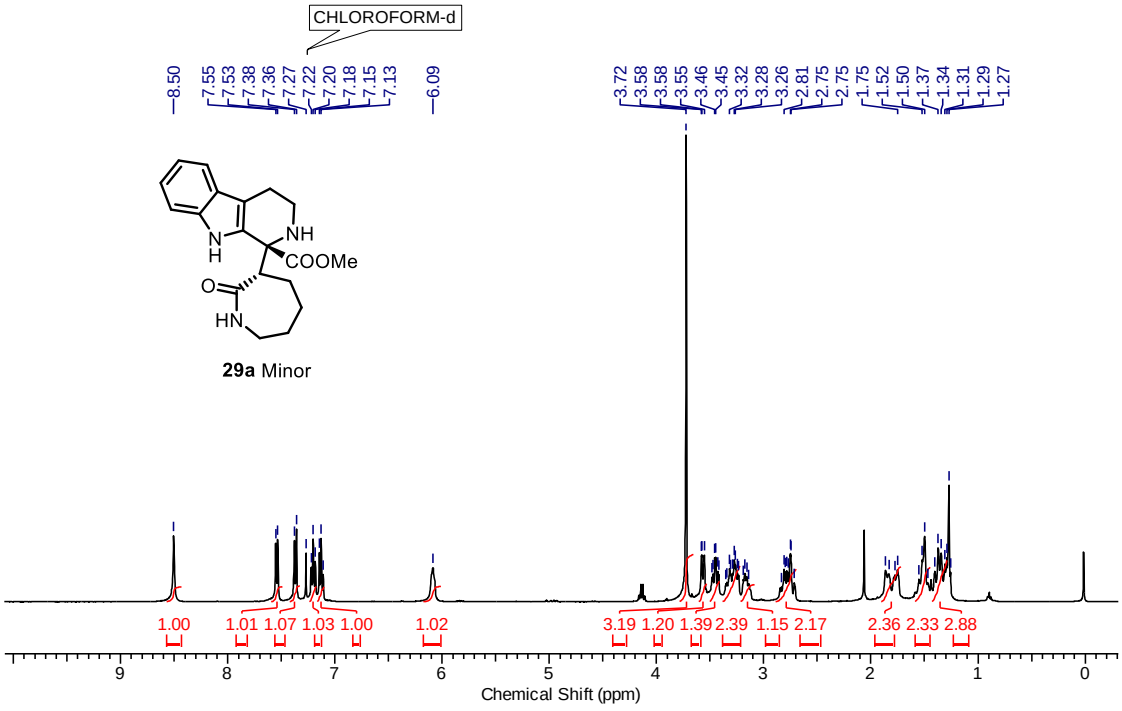
^{1}H NMR of Compound 29 in $CDCl_3$ at 400 MHz



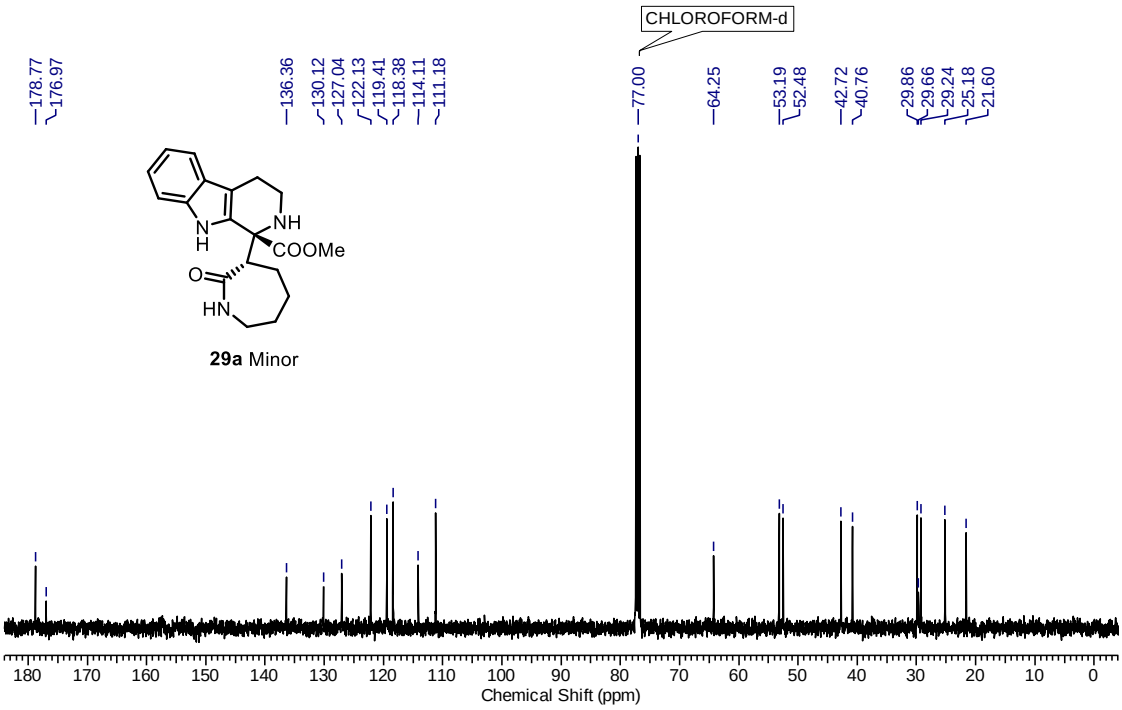
^{13}C NMR of Compound 29 in $CDCl_3$ at 100 MHz



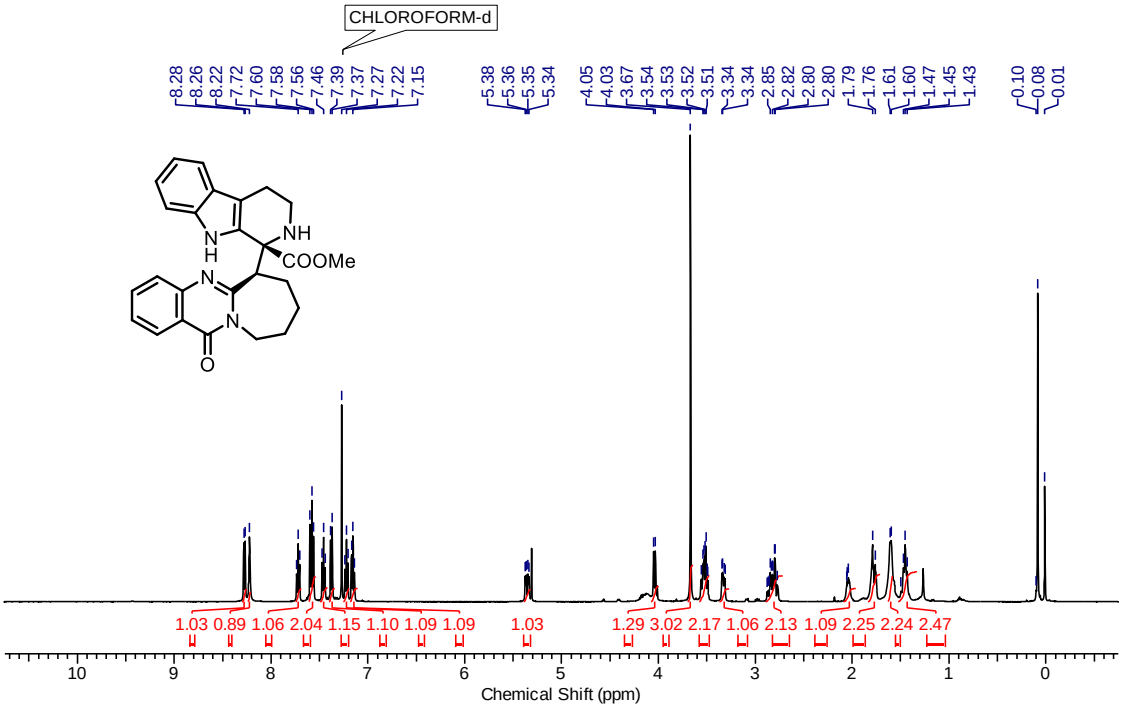
^{1}H NMR of Compound 29a in $CDCl_3$ at 400 MHz



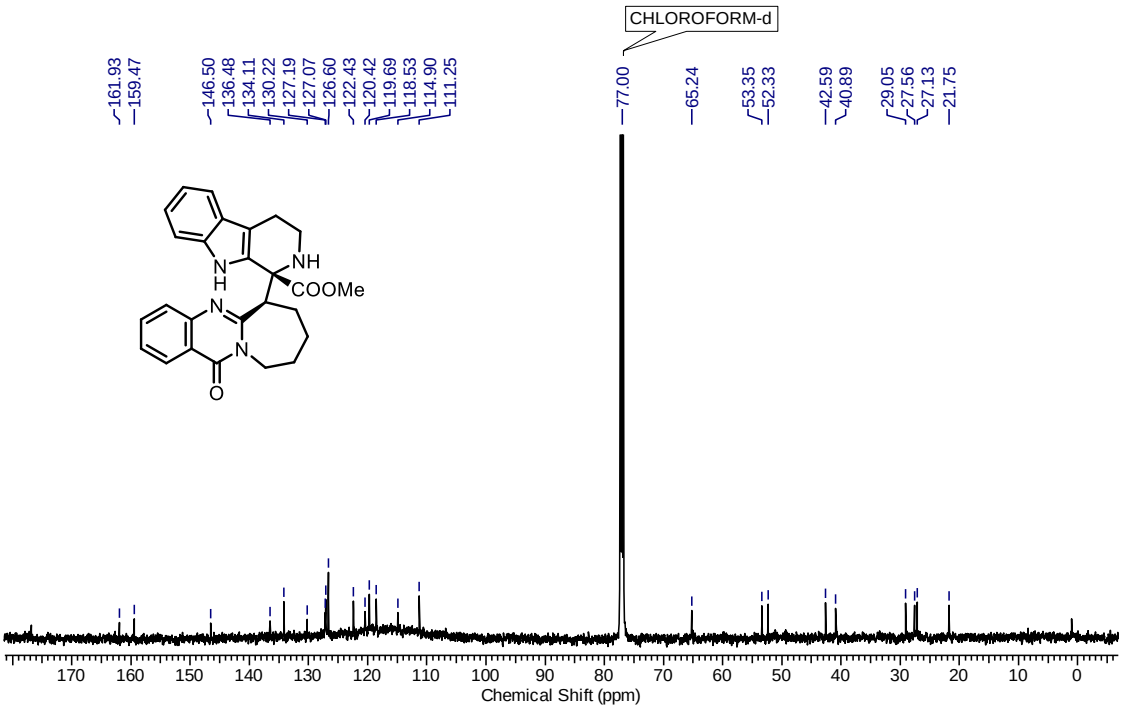
^{13}C NMR of Compound 29a in $CDCl_3$ at 100 MHz



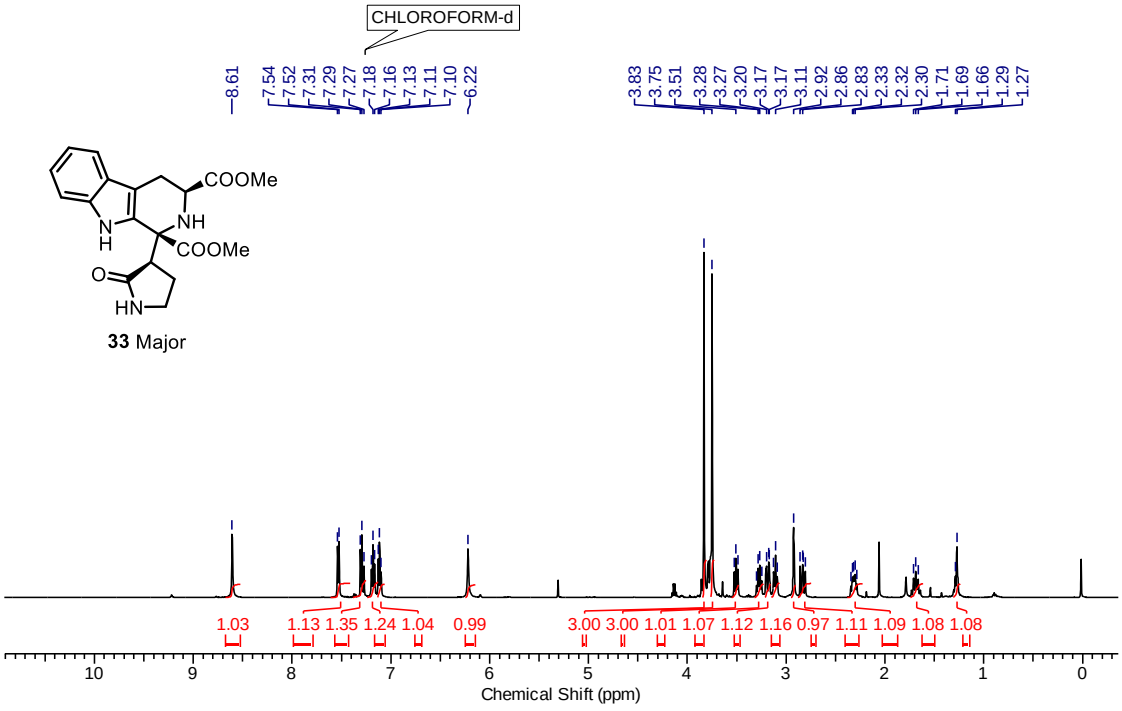
^{1}H NMR of Compound 31 in $CDCl_3$ at 400 MHz



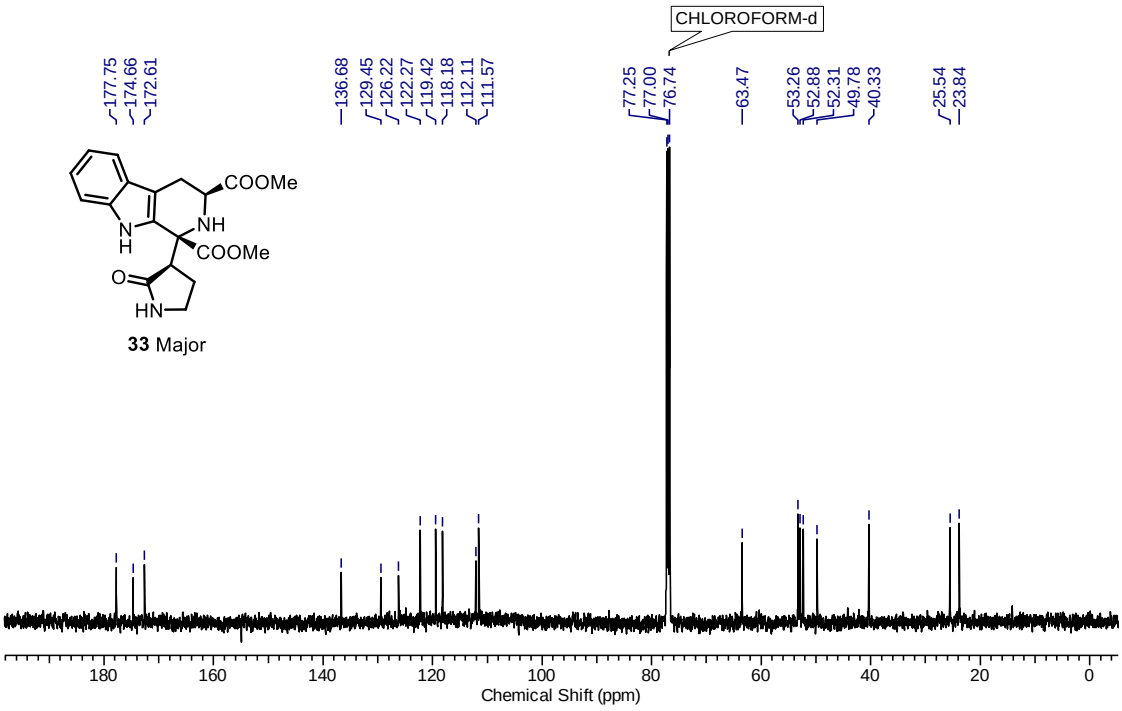
^{13}C NMR of Compound 31 in $CDCl_3$ at 100 MHz



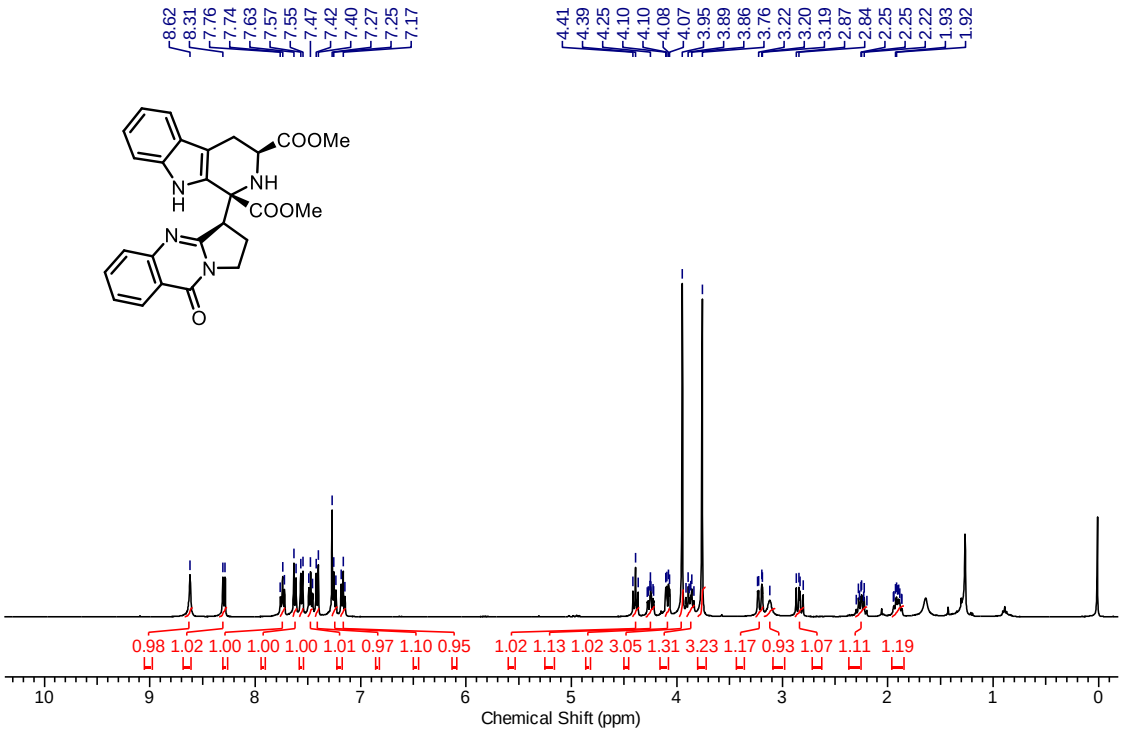
^{1}H NMR of Compound 33 in $CDCl_3$ at 400 MHz



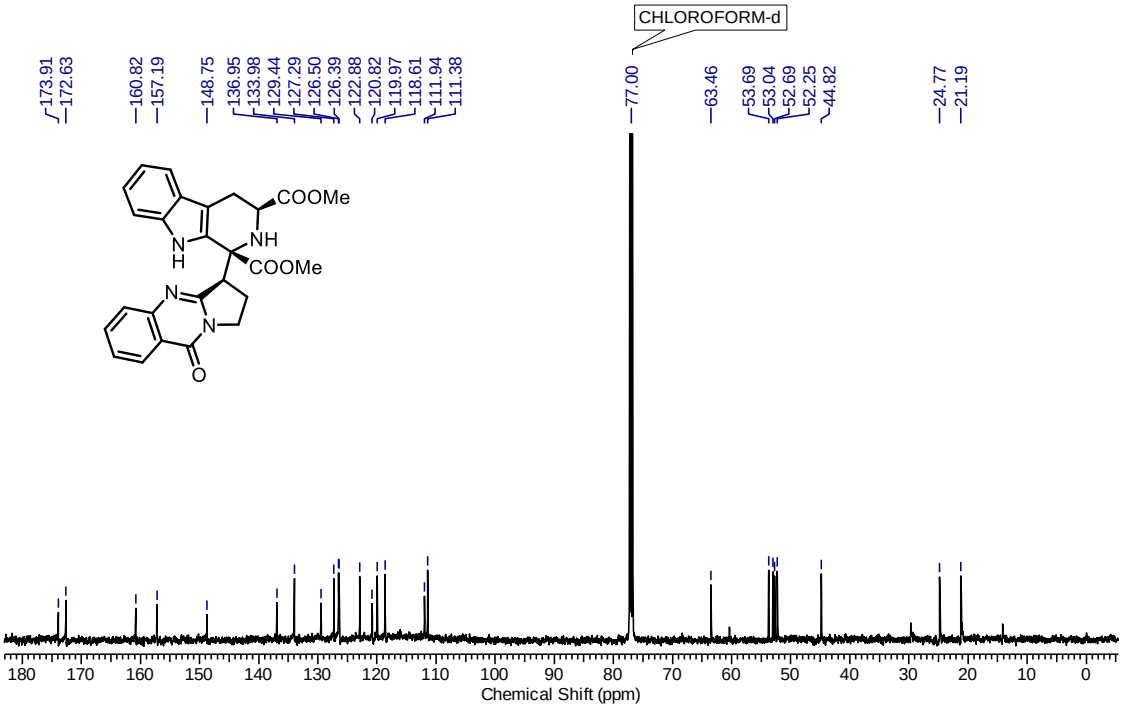
^{13}C NMR of Compound 33 in $CDCl_3$ at 100 MHz



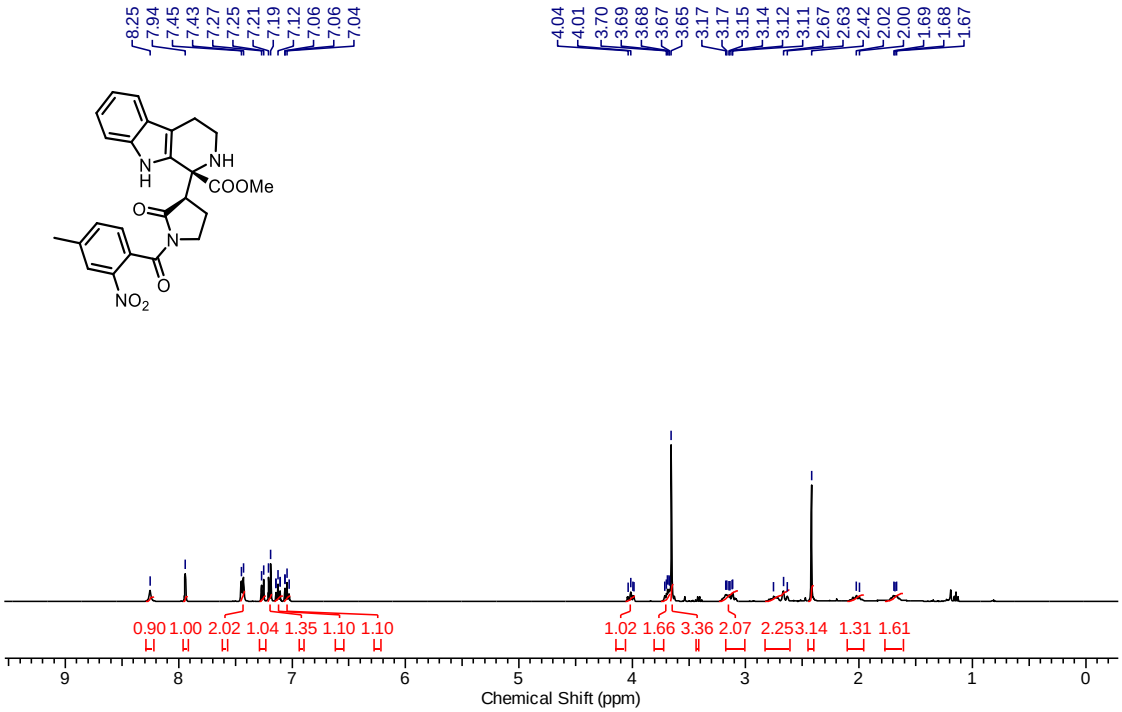
^{1}H NMR of Compound 35 in $CDCl_3$ at 400 MHz



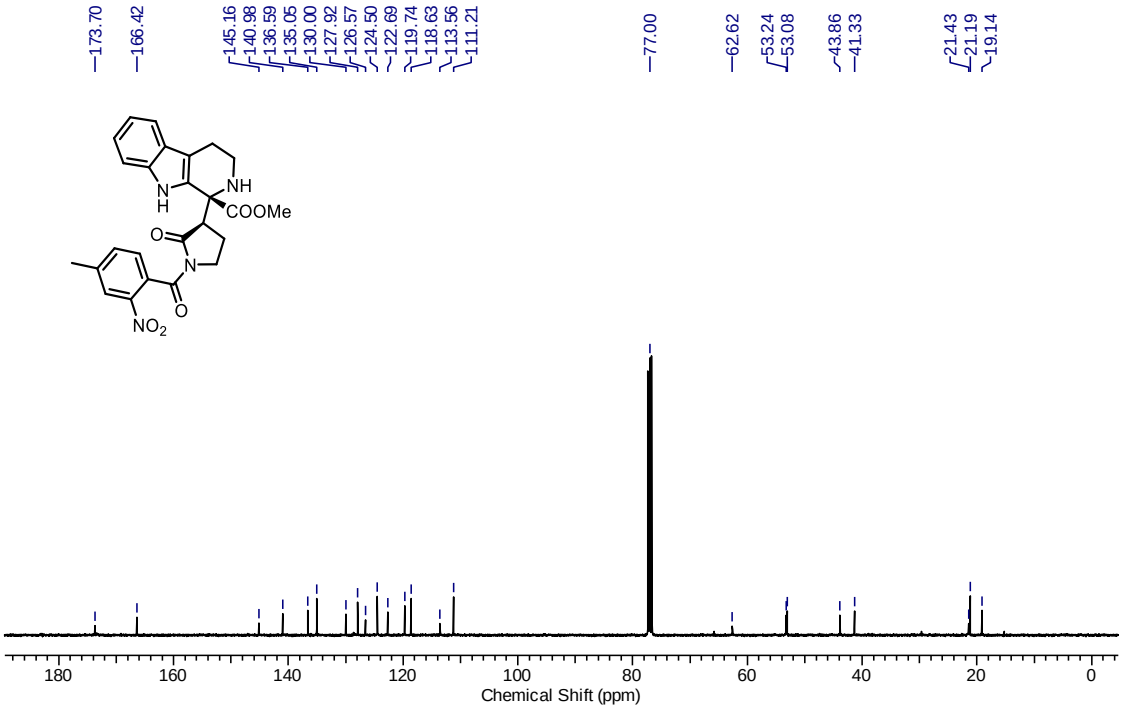
^{13}C NMR of Compound 35 in $CDCl_3$ at 100 MHz



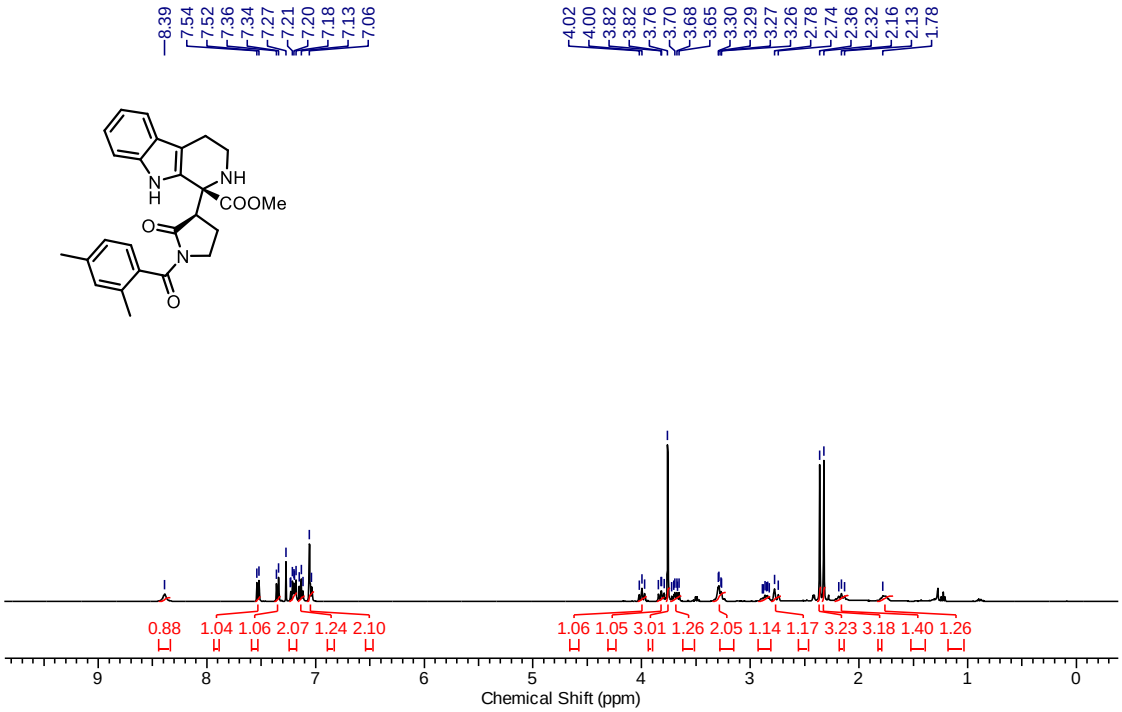
^{1}H NMR of Compound 36 in $CDCl_3$ at 400 MHz



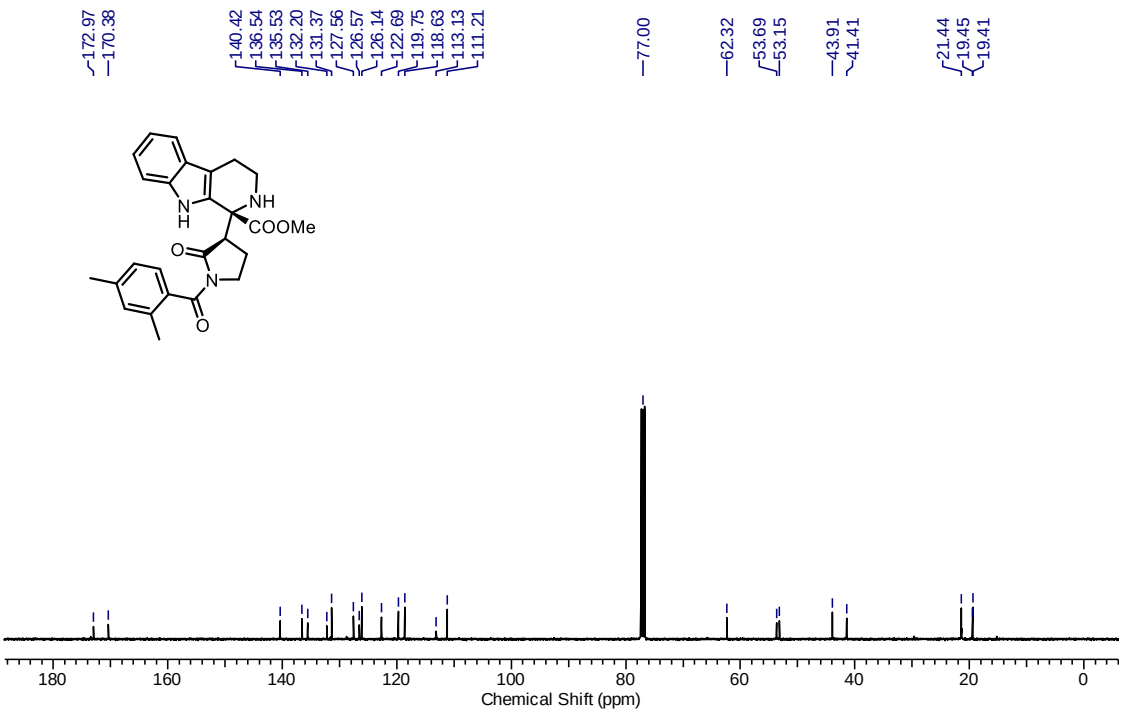
^{13}C NMR of Compound 36 in $CDCl_3$ at 100 MHz



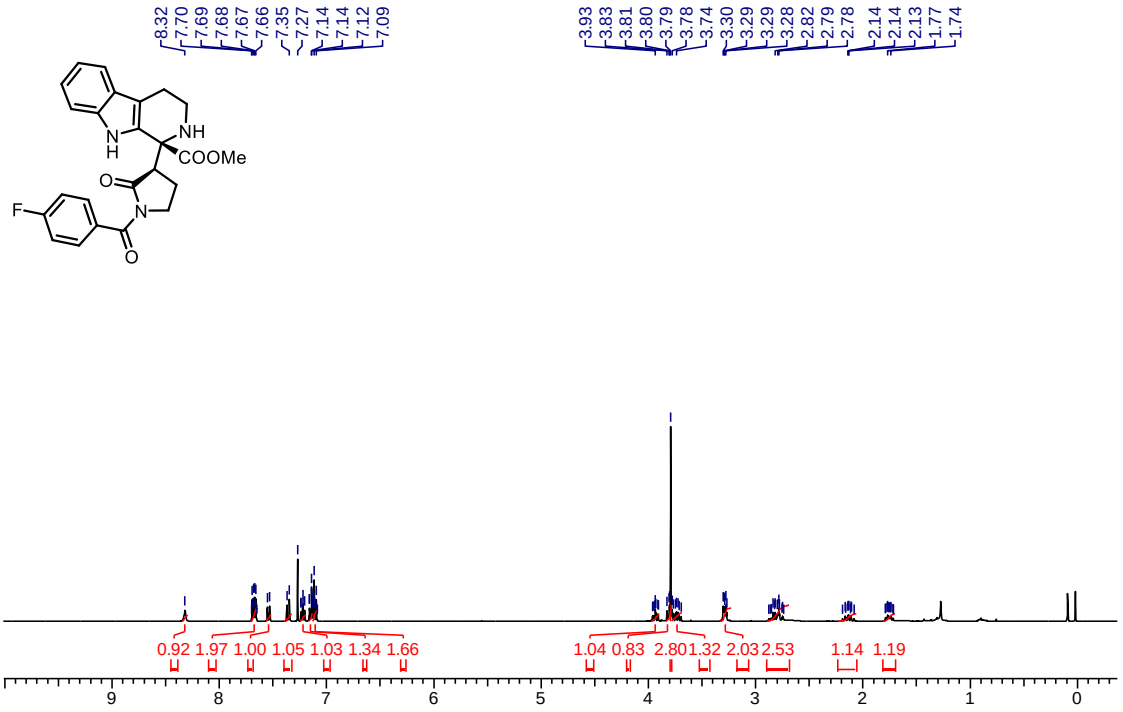
^{1}H NMR of Compound 38 in $CDCl_3$ at 400 MHz



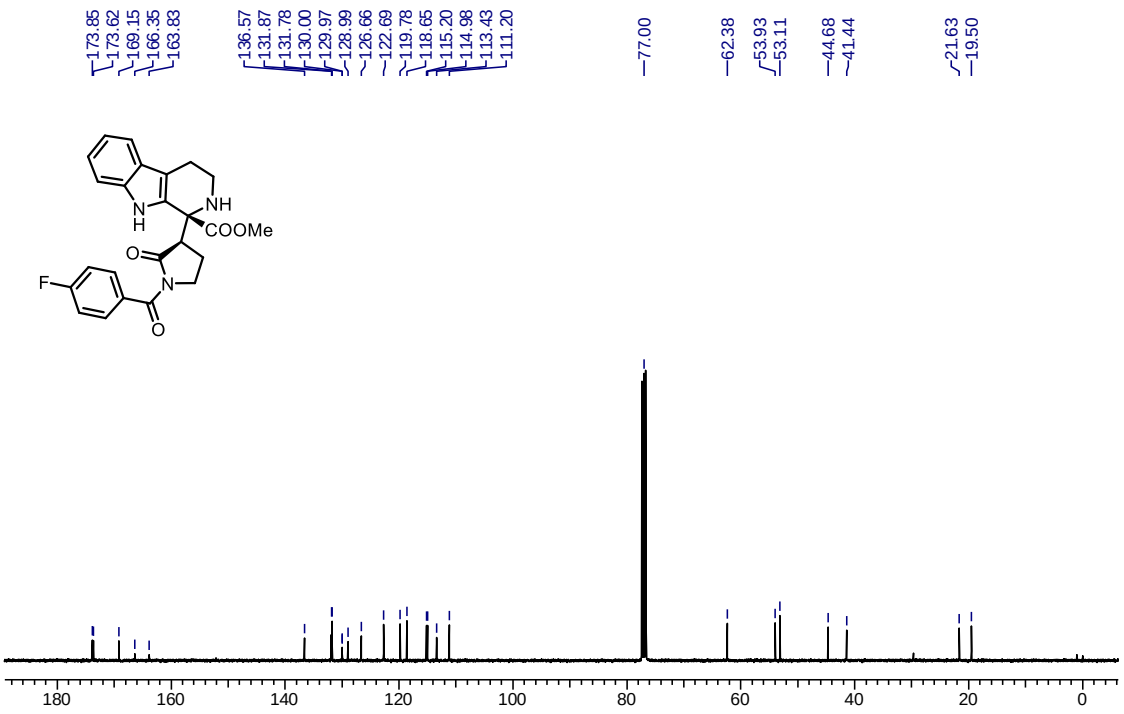
^{13}C NMR of Compound 38 in $CDCl_3$ at 100 MHz



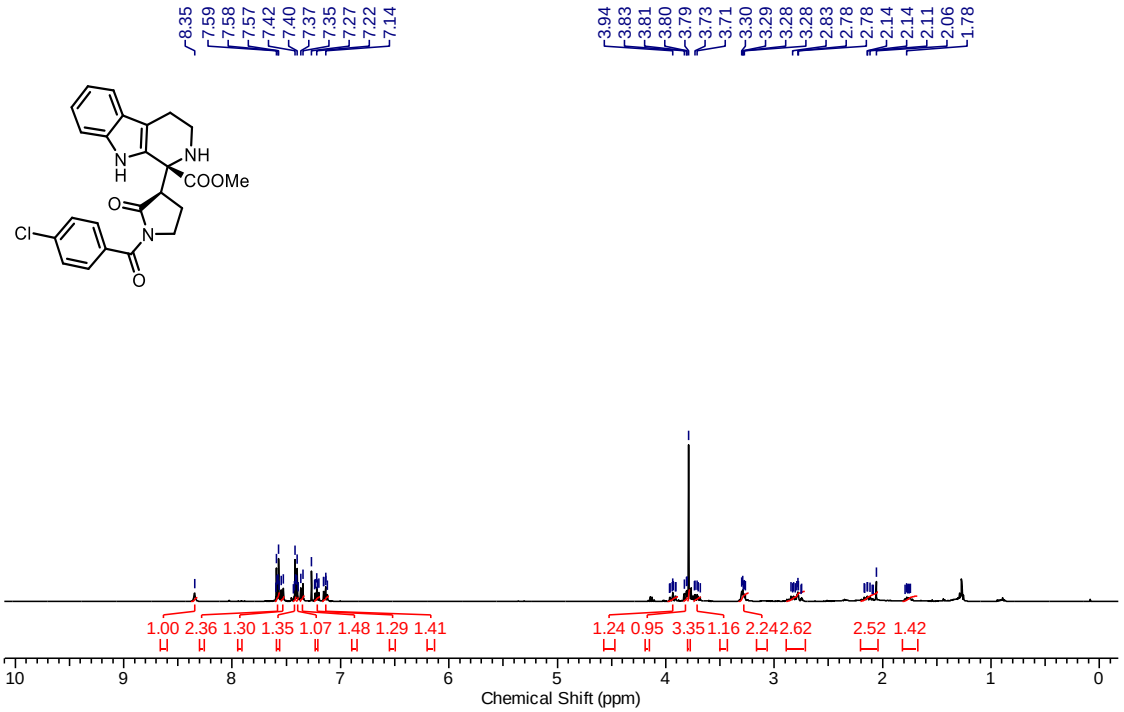
^{1}H NMR of Compound 39 in $CDCl_3$ at 400 MHz



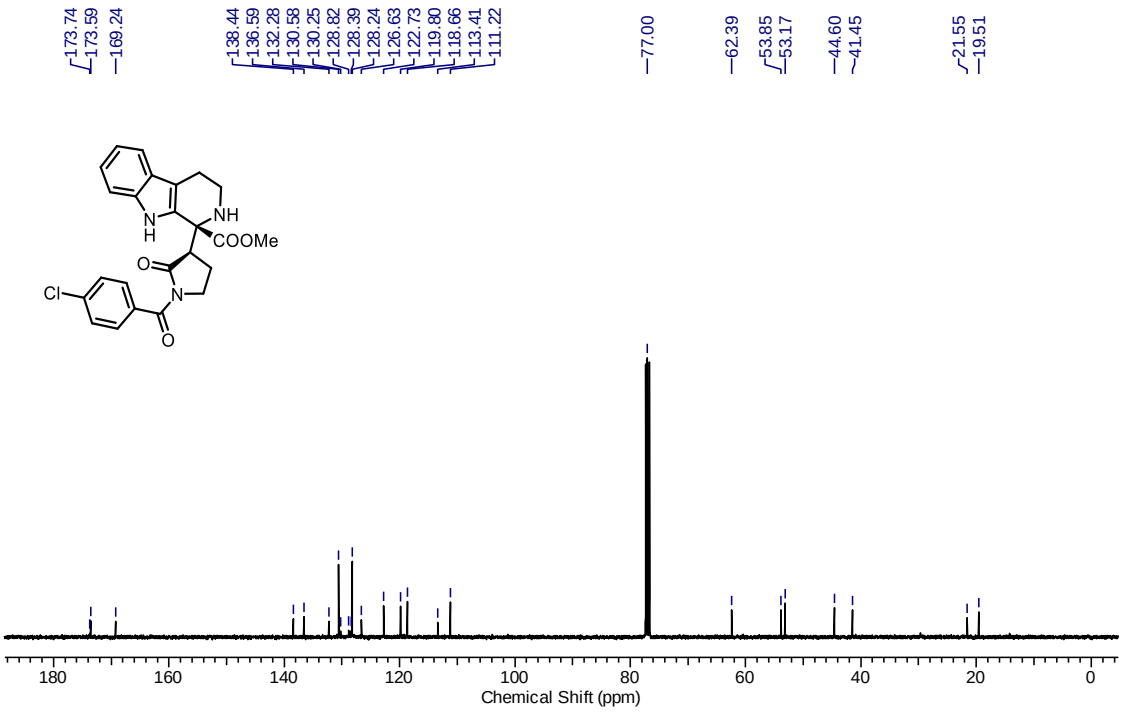
^{13}C NMR of Compound 39 in $CDCl_3$ at 100 MHz



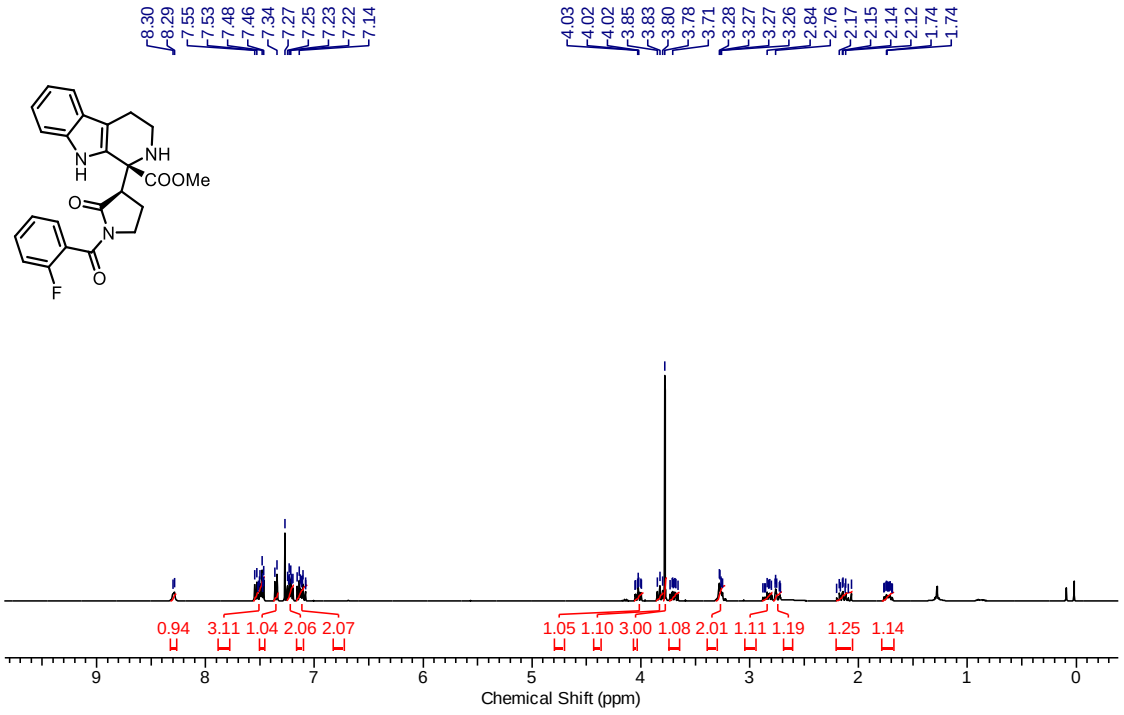
^{1}H NMR of Compound 40 in $CDCl_3$ at 400 MHz



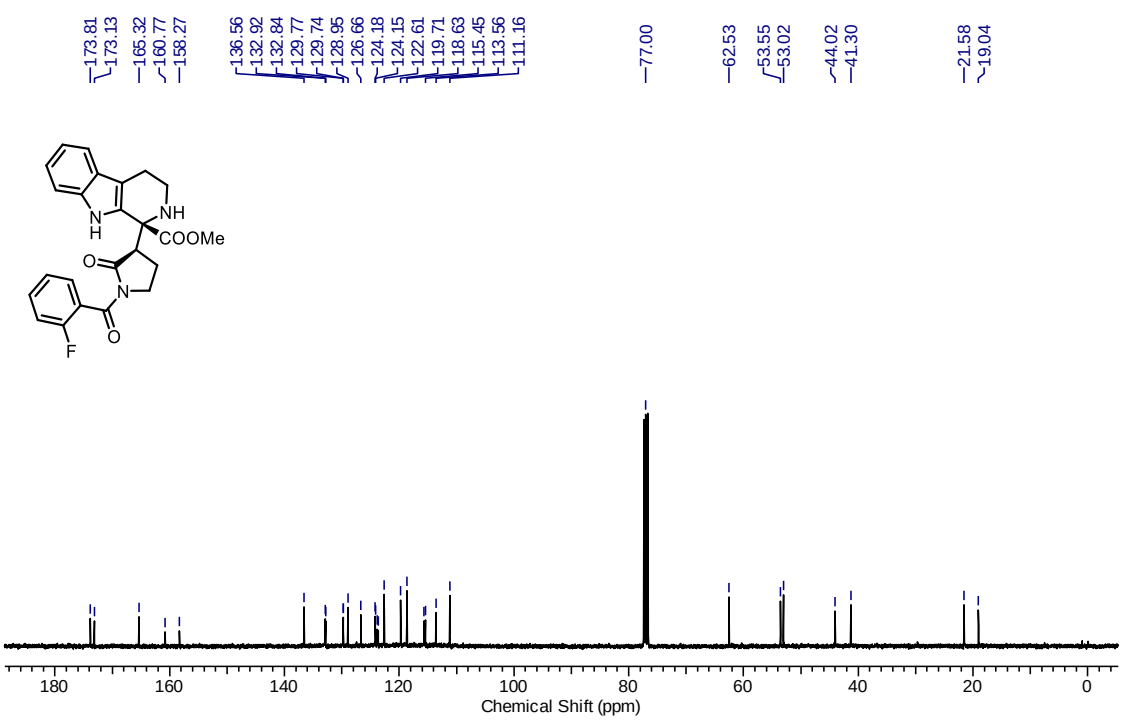
^{13}C NMR of Compound 40 in $CDCl_3$ at 100 MHz



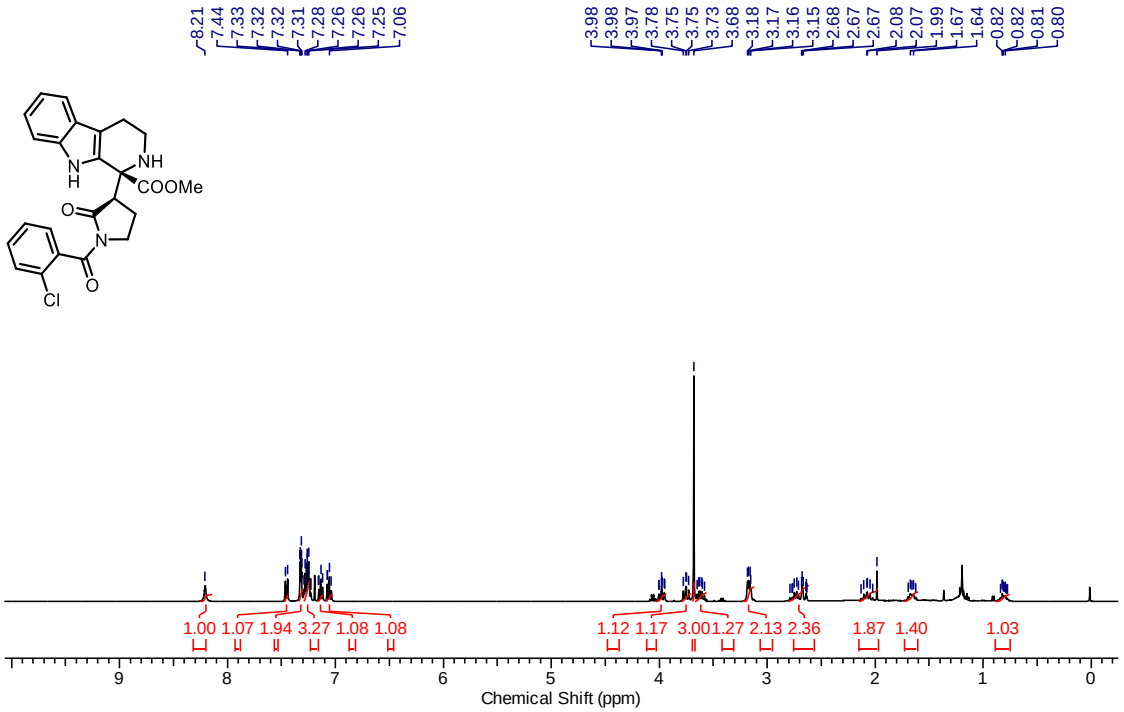
^{1}H NMR of Compound 41 in $CDCl_3$ at 400 MHz



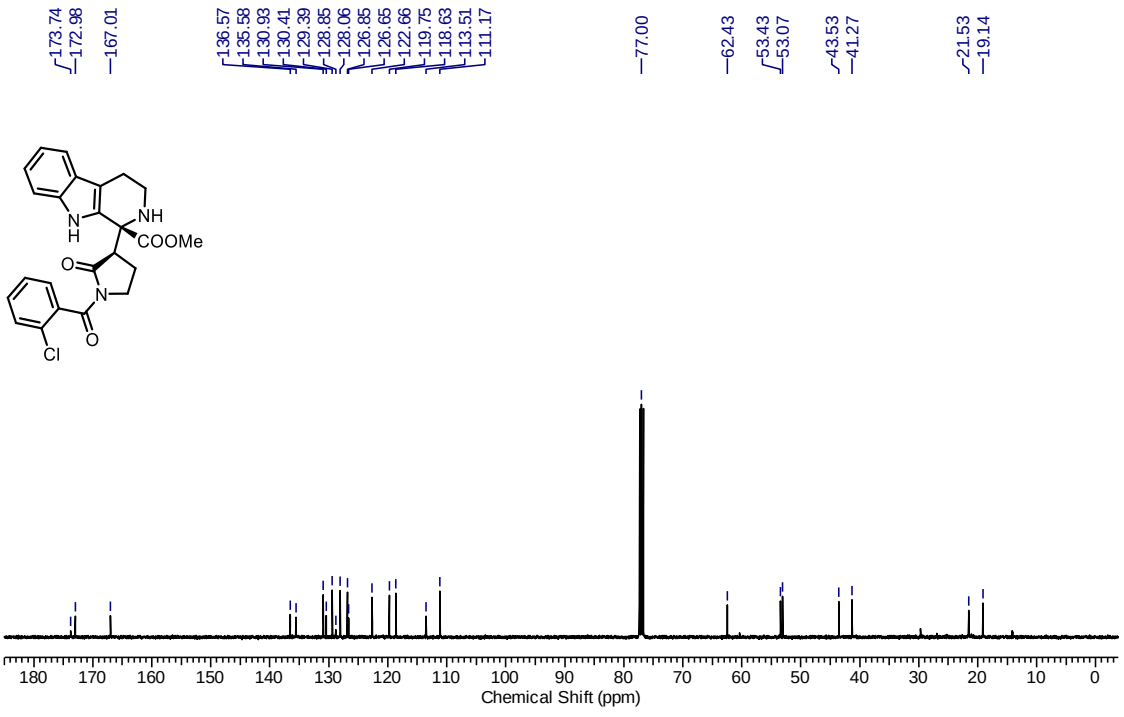
^{13}C NMR of Compound 41 in $CDCl_3$ at 100 MHz



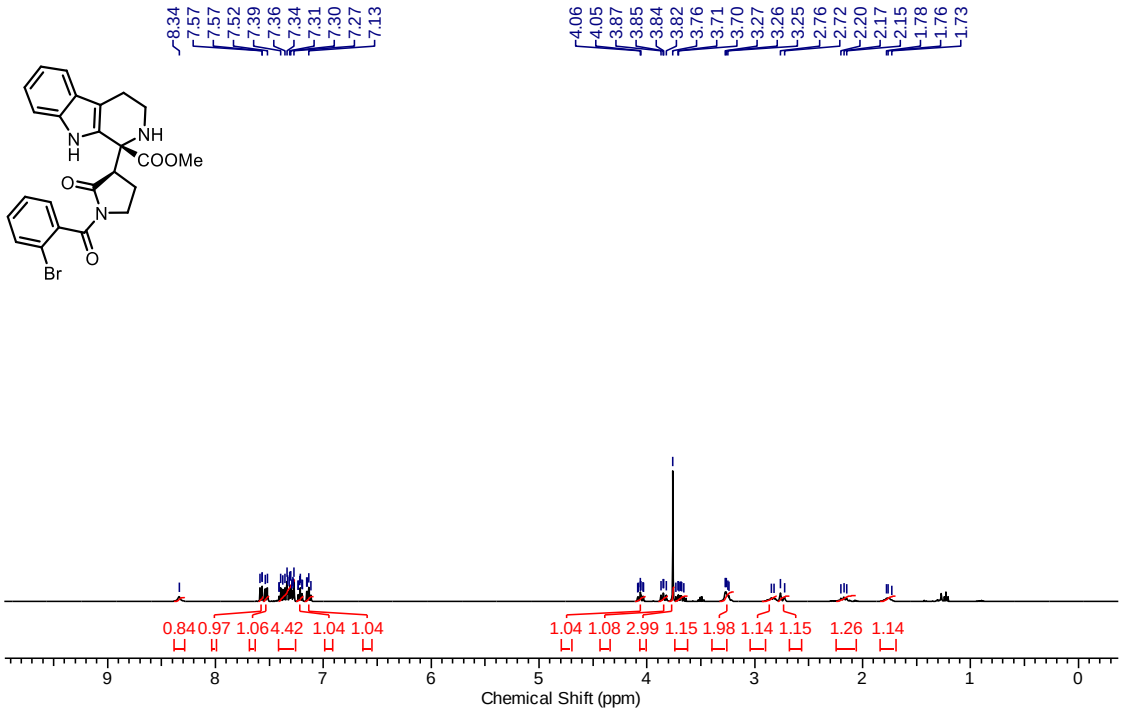
^{1}H NMR of Compound 42 in $CDCl_3$ at 400 MHz



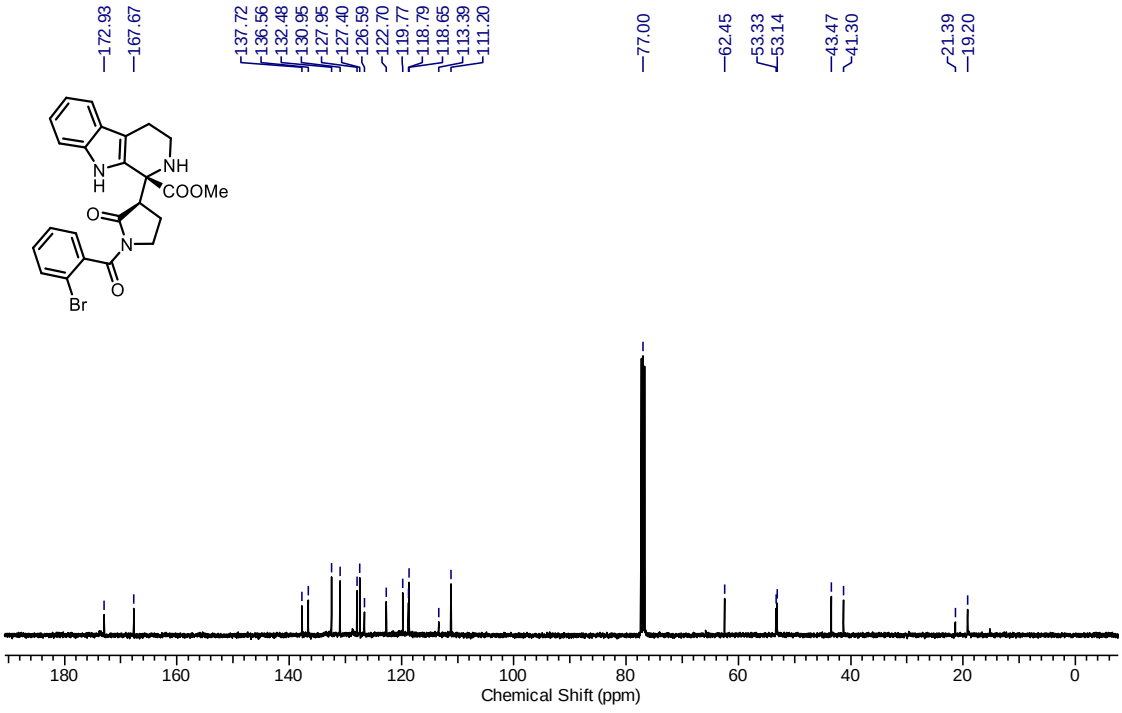
^{13}C NMR of Compound 42 in $CDCl_3$ at 100 MHz



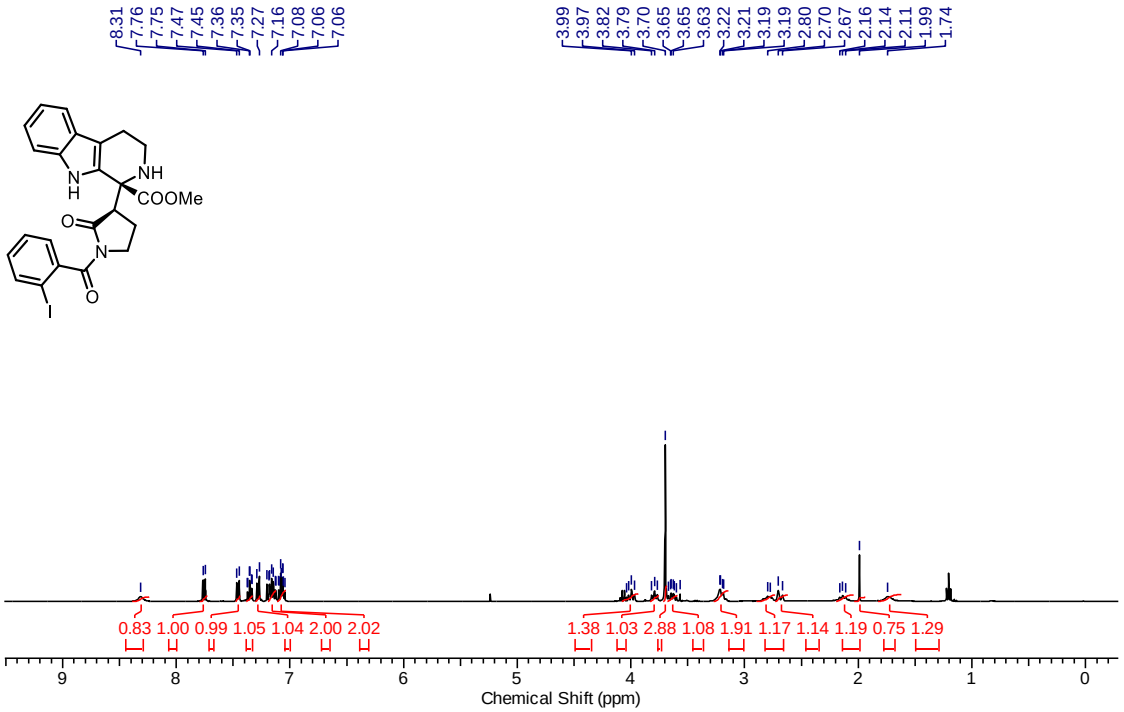
^{1}H NMR of Compound 43 in $CDCl_3$ at 400 MHz



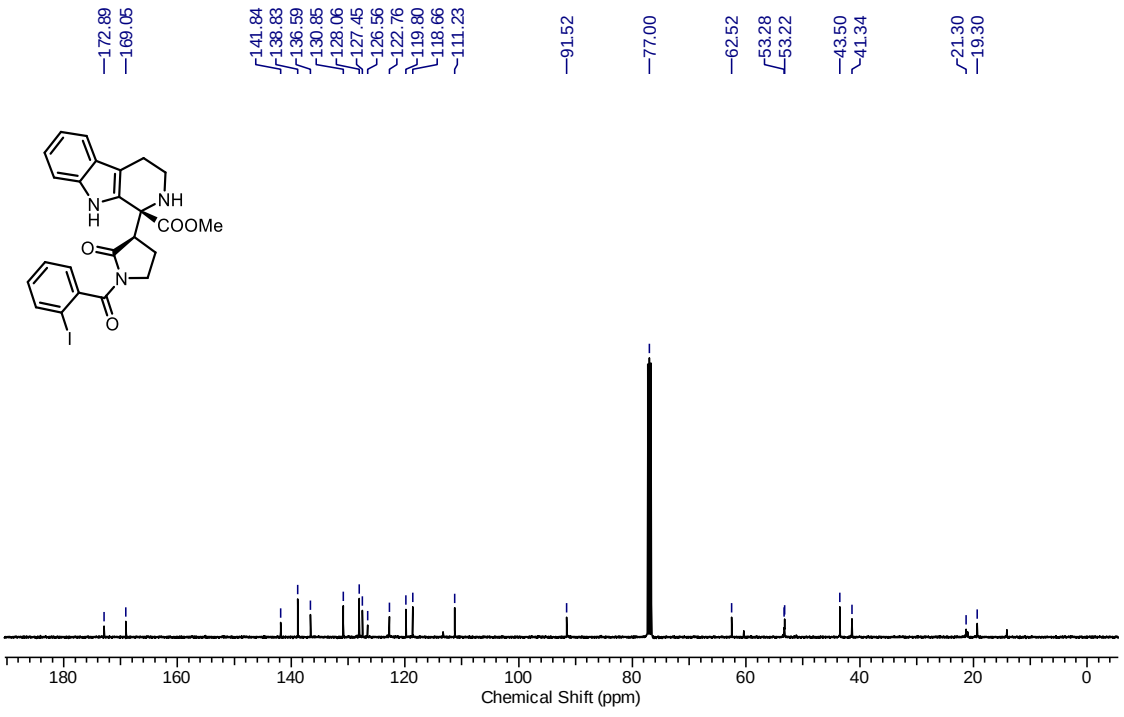
^{13}C NMR of Compound 43 in $CDCl_3$ at 100 MHz



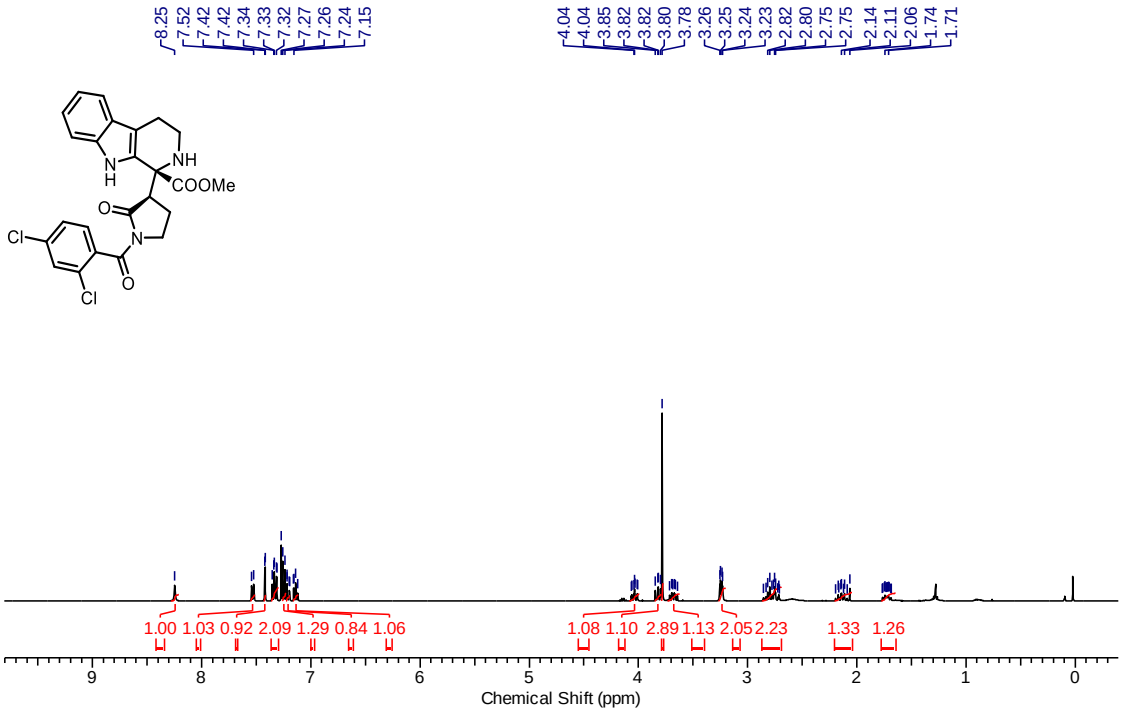
^{1}H NMR of Compound 44 in $CDCl_3$ at 400 MHz



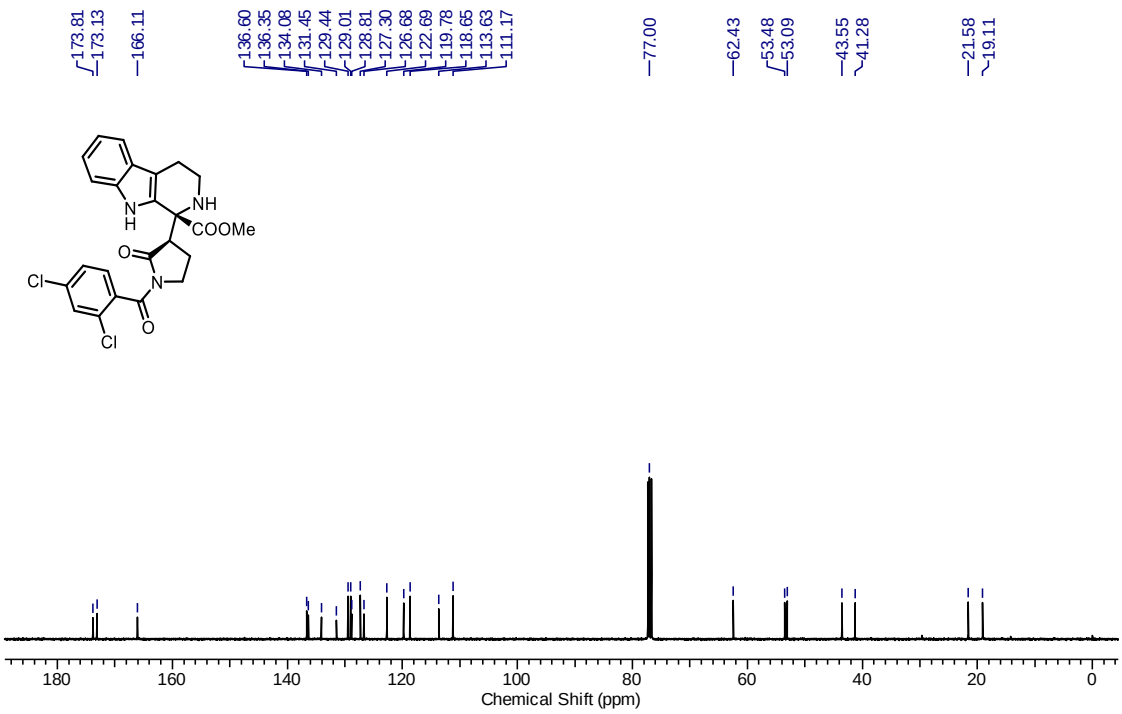
^{13}C NMR of Compound 44 in $CDCl_3$ at 100 MHz



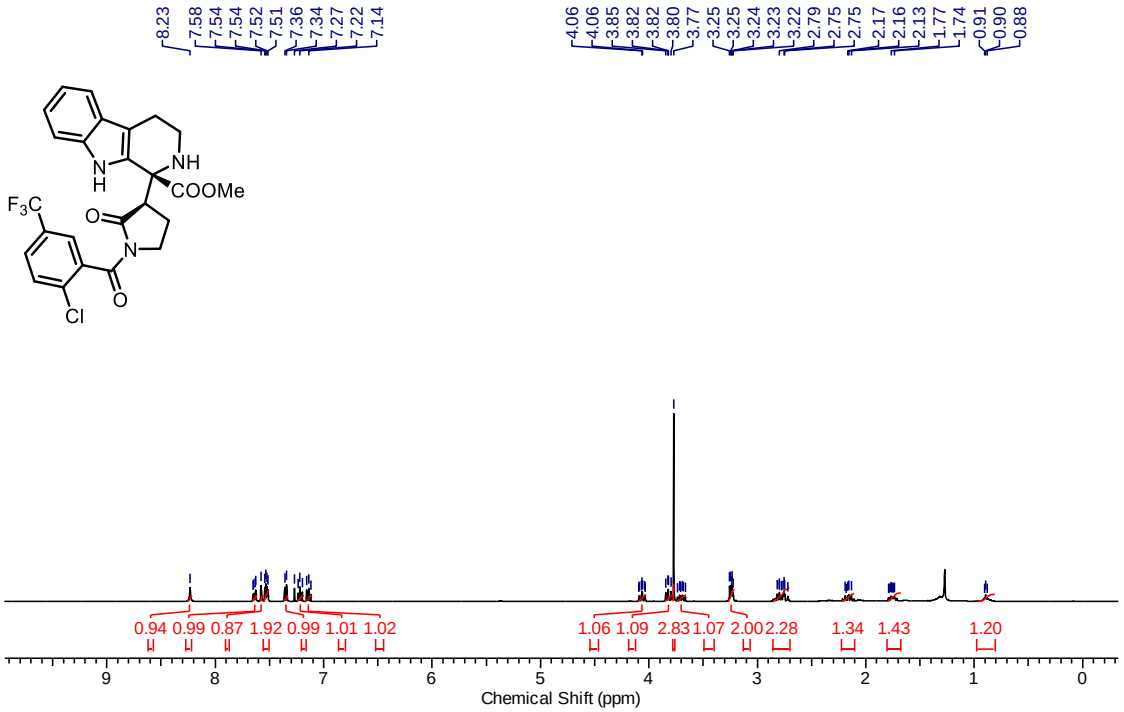
^{1}H NMR of Compound 45 in $CDCl_3$ at 400 MHz



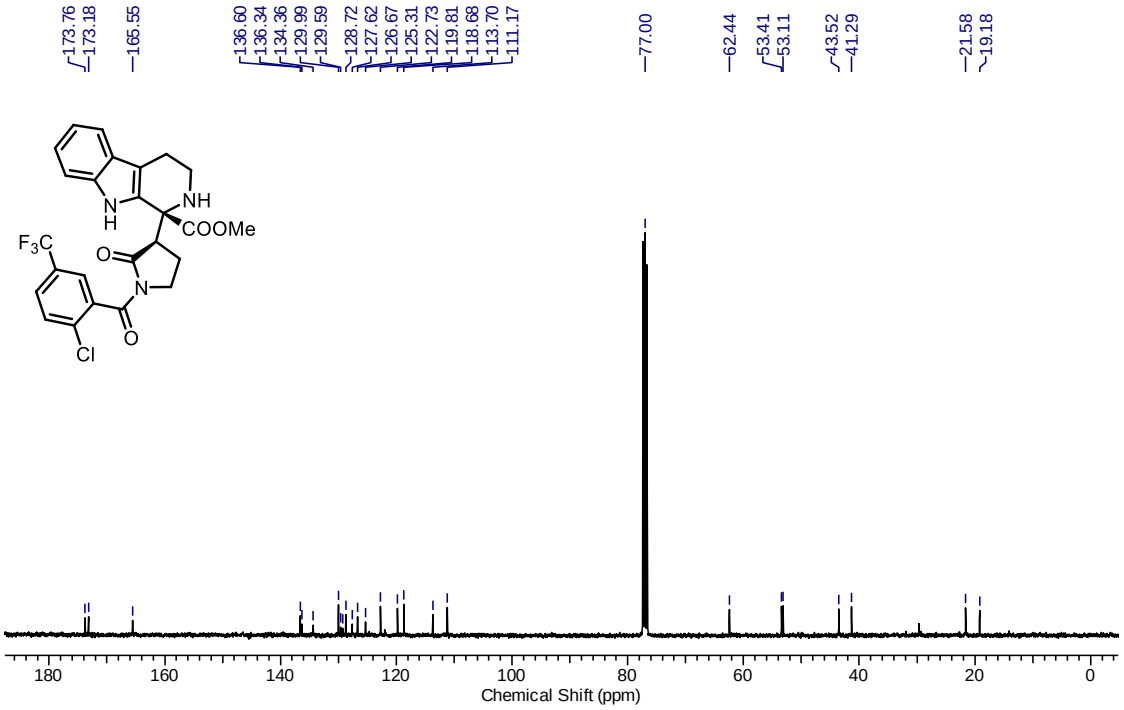
^{13}C NMR of Compound 45 in $CDCl_3$ at 100 MHz



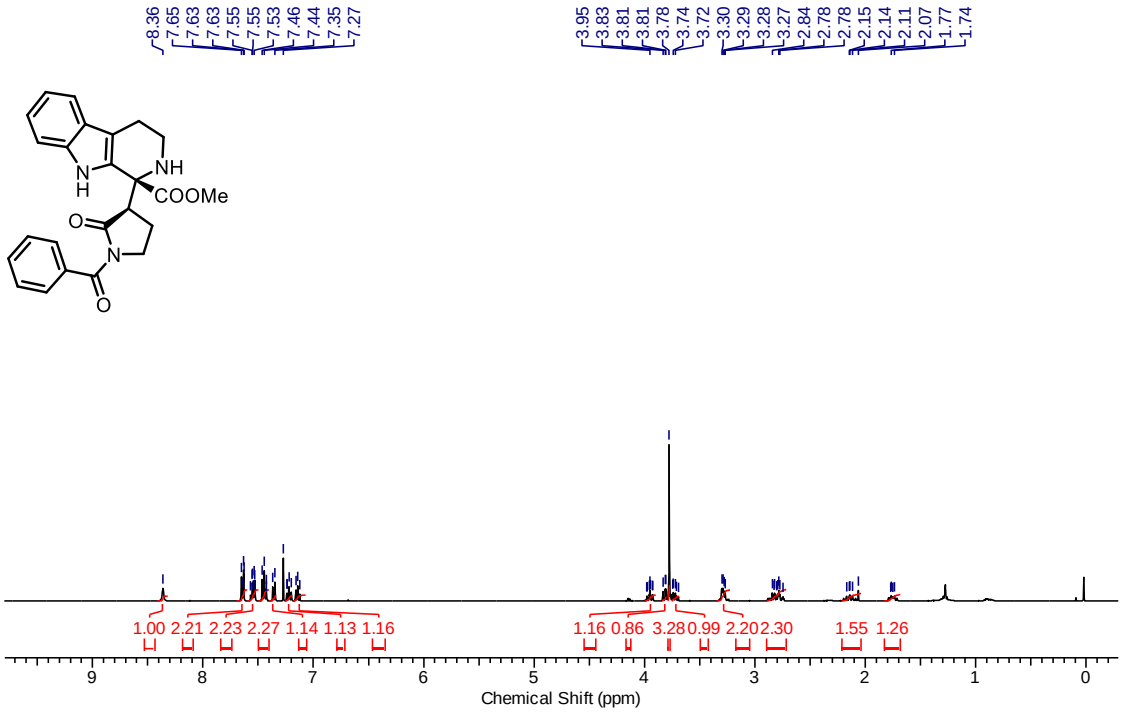
^{1}H NMR of Compound 46 in $CDCl_3$ at 400 MHz



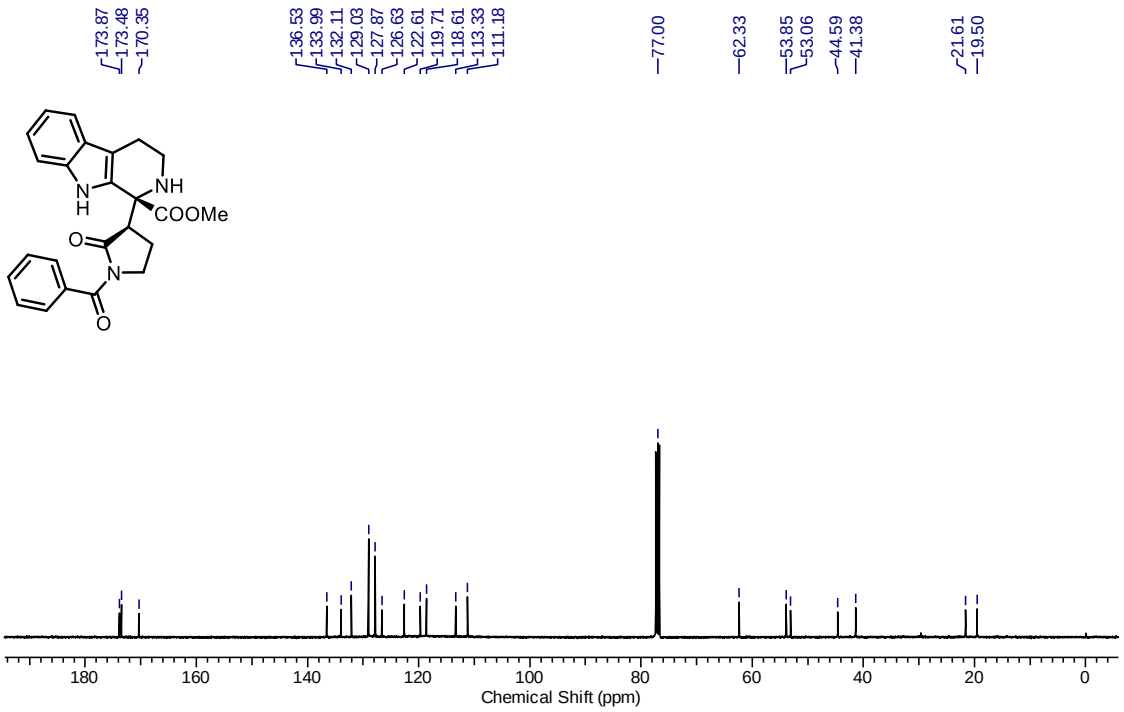
^{13}C NMR of Compound 46 in $CDCl_3$ at 100 MH



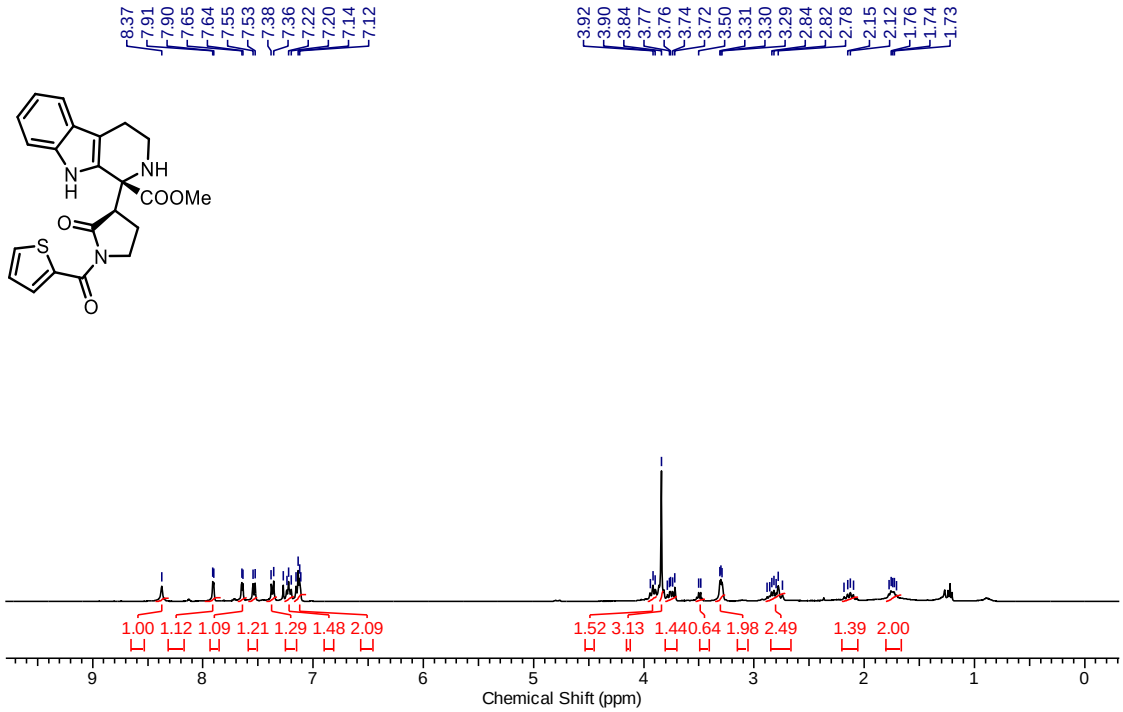
^{1}H NMR of Compound 47 in $CDCl_3$ at 400 MHz



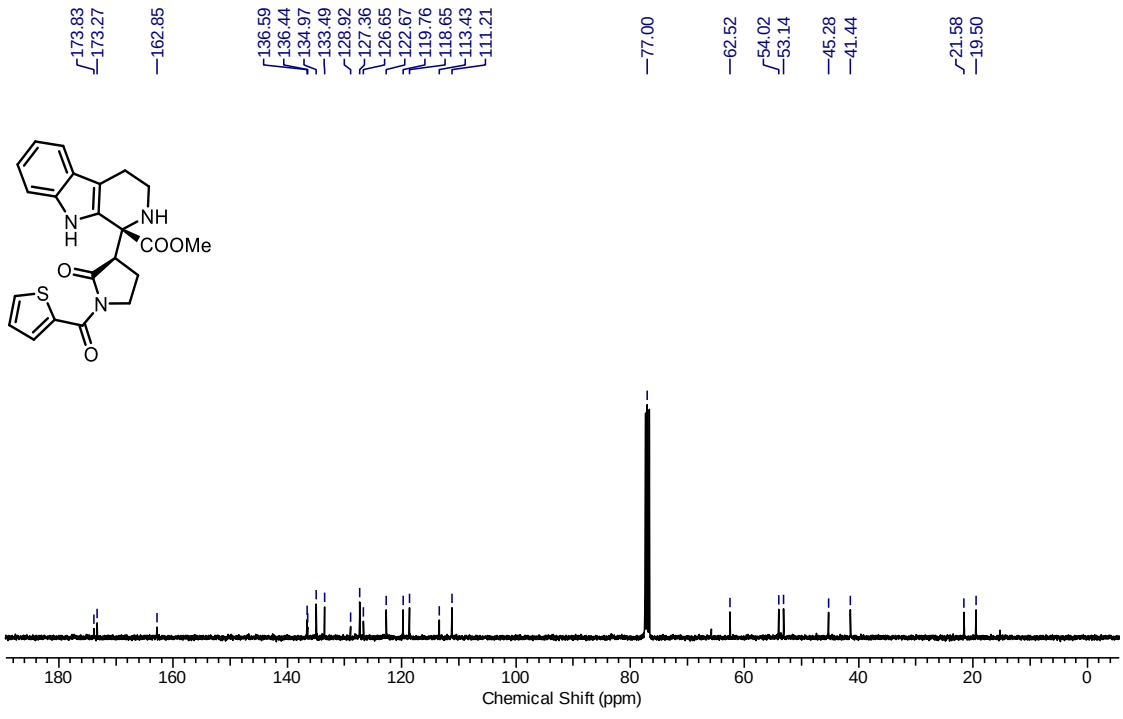
^{13}C NMR of Compound 47 in $CDCl_3$ at 100 MHz



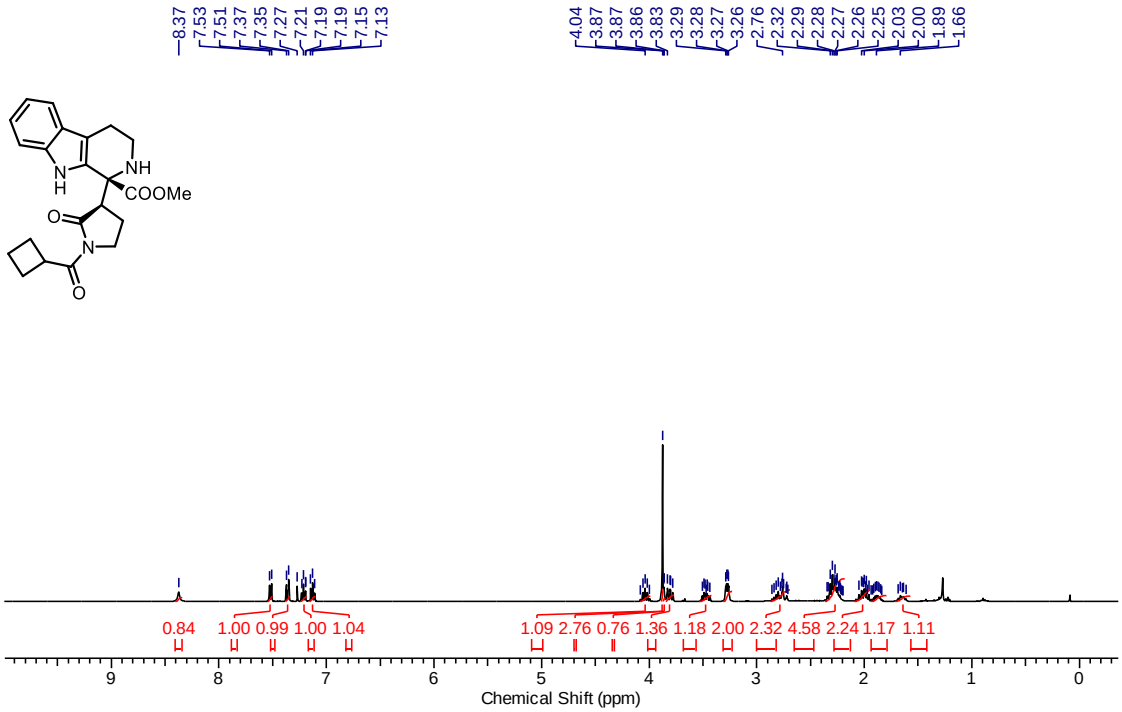
^{1}H NMR of Compound 48 in $CDCl_3$ at 400 MHz



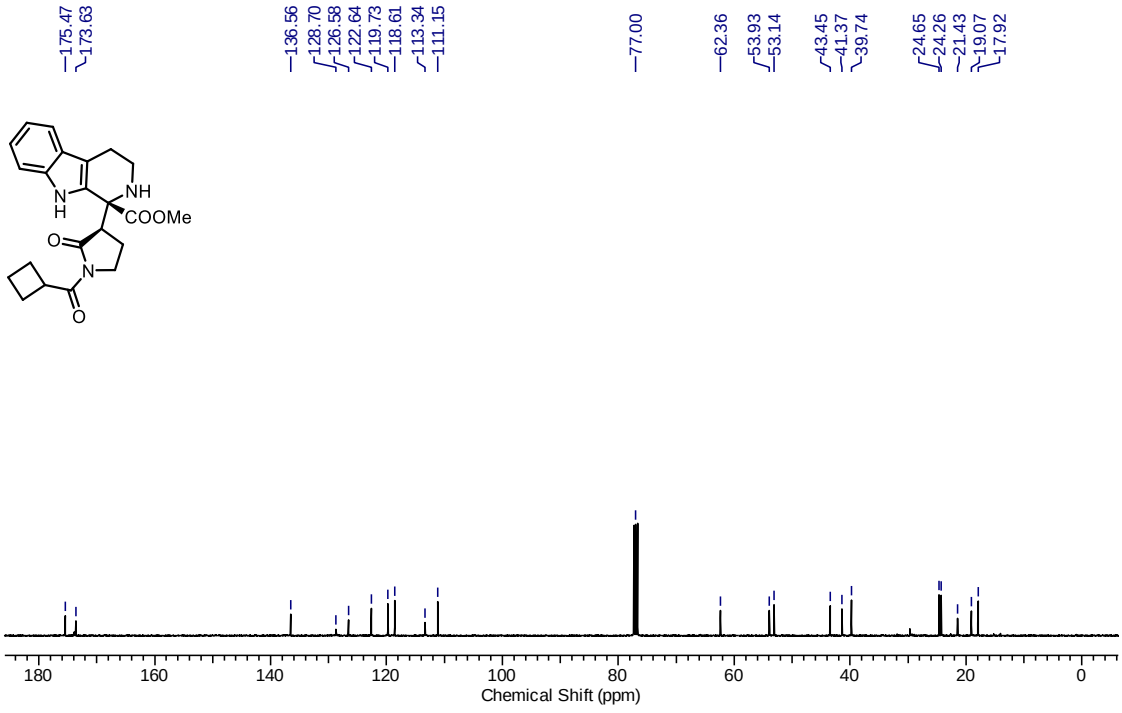
^{13}C NMR of Compound 48 in $CDCl_3$ at 100 MHz



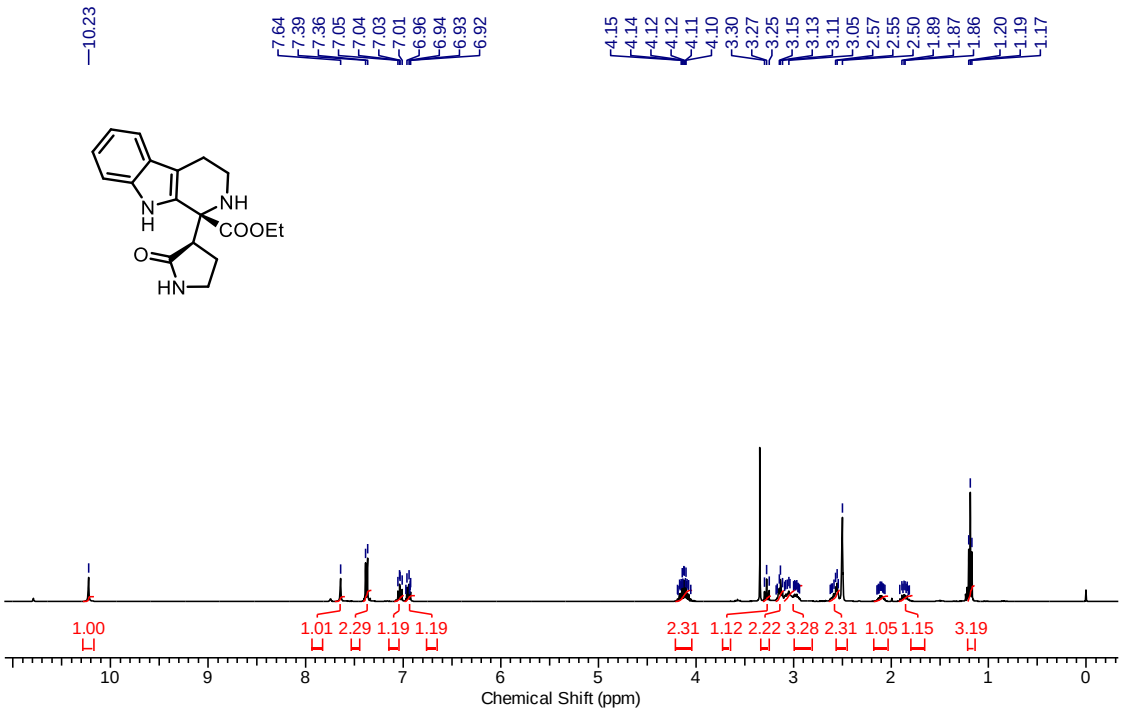
^{1}H NMR of Compound 49 in $CDCl_3$ at 400 MHz



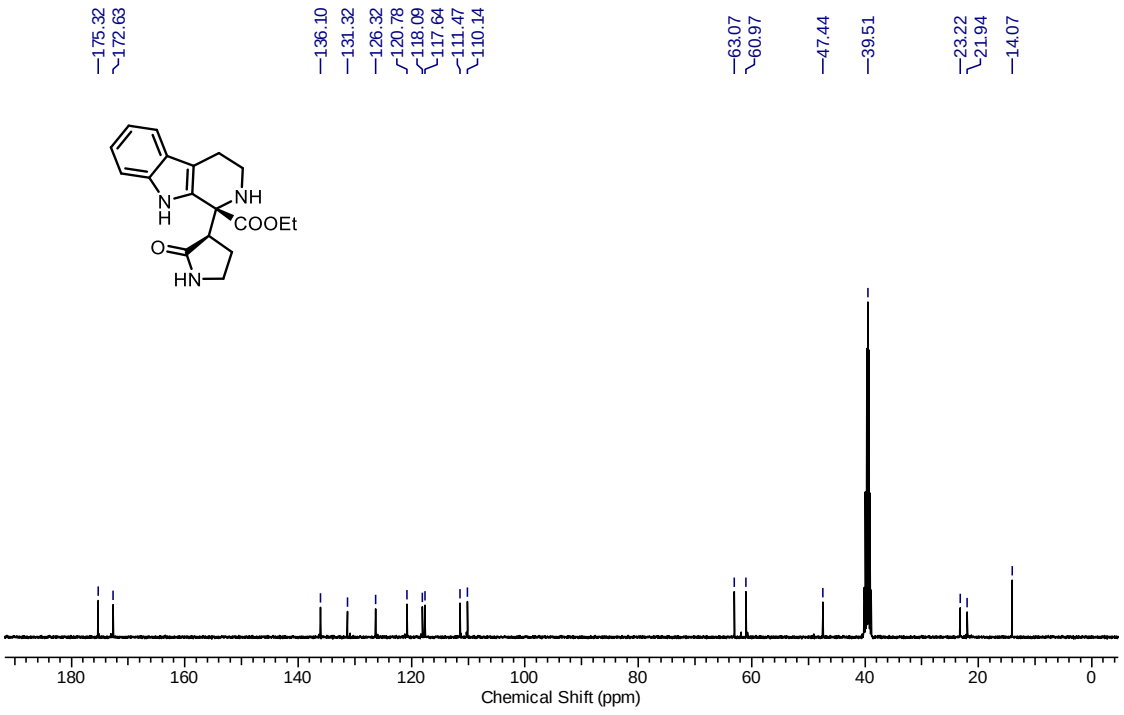
^{13}C NMR of Compound 49 in $CDCl_3$ at 100 MHz



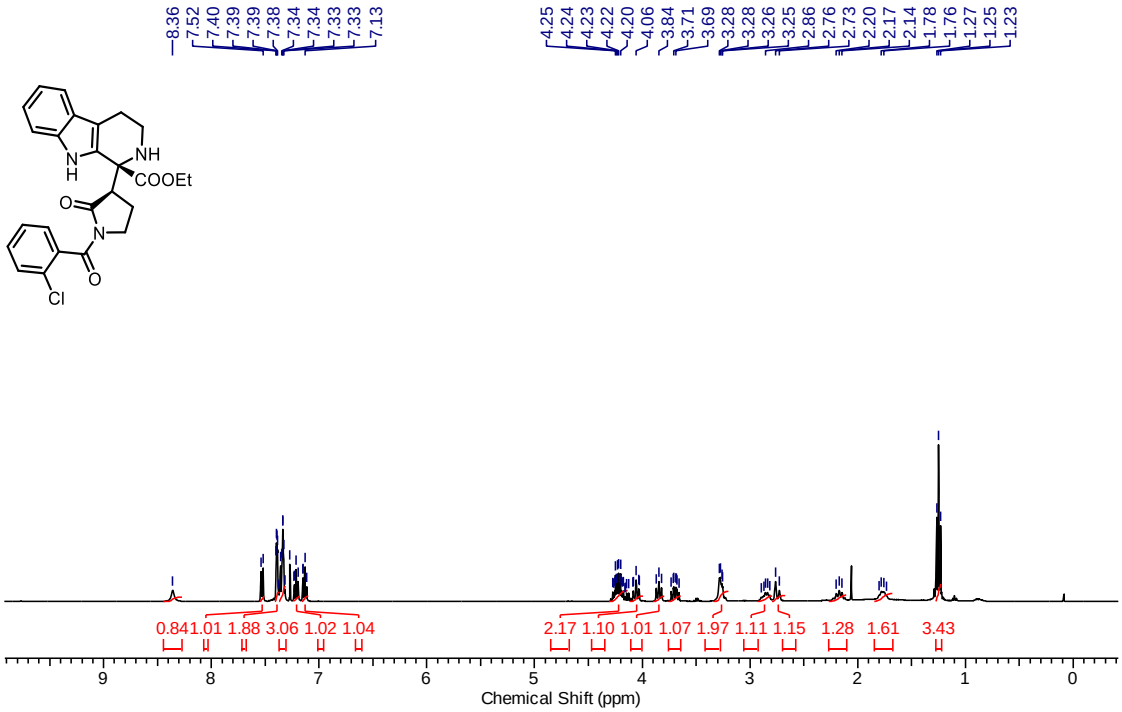
^{1}H NMR of Compound 51 in DMSO-d_6 at 400 MHz



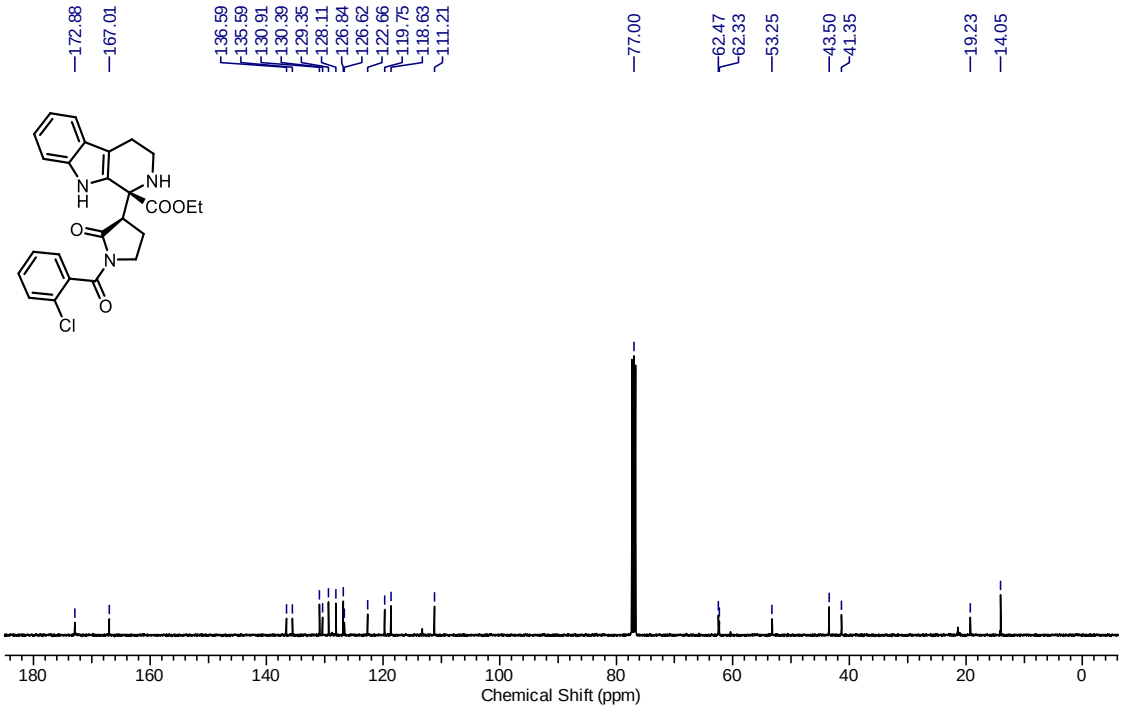
^{13}C NMR of Compound 51 in DMSO-d_6 at 100 MHz



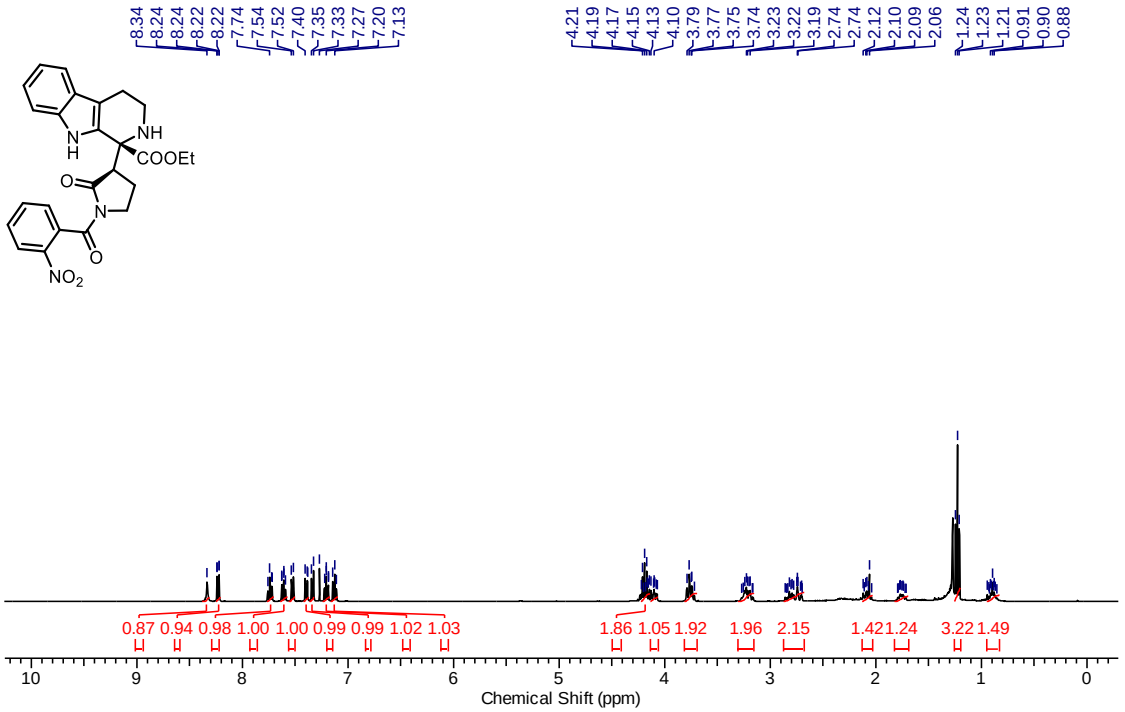
^{1}H NMR of Compound 54 in $CDCl_3$ at 400 MHz



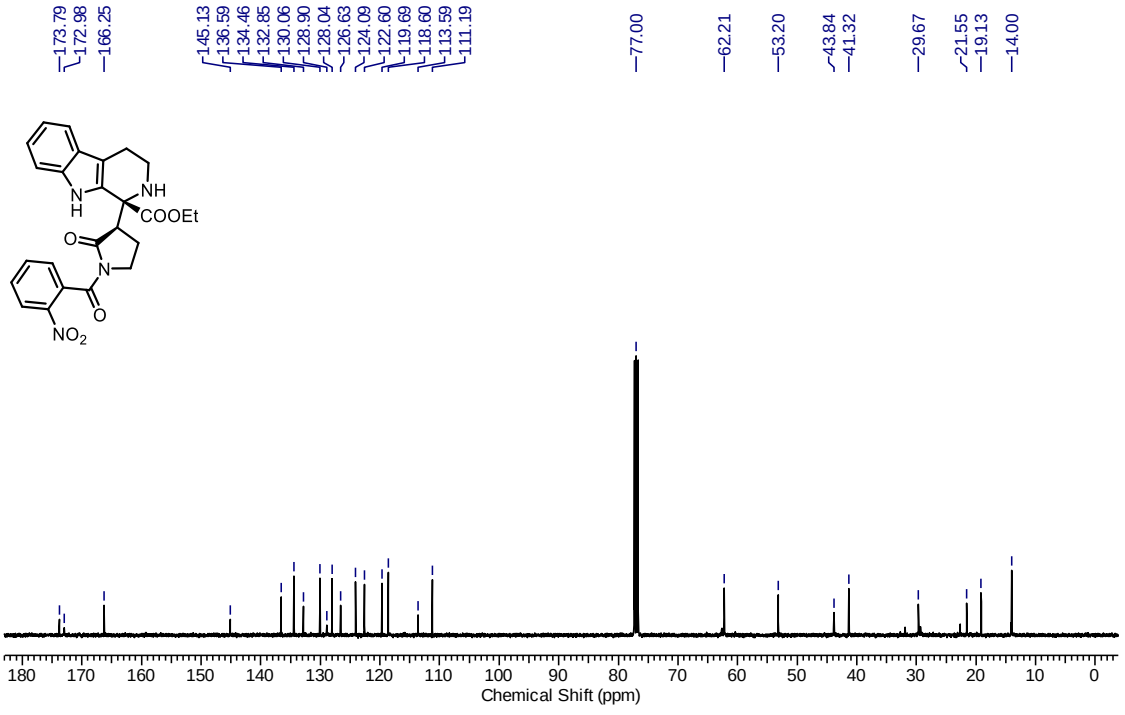
^{13}C NMR of Compound 54 in $CDCl_3$ at 100 MHz



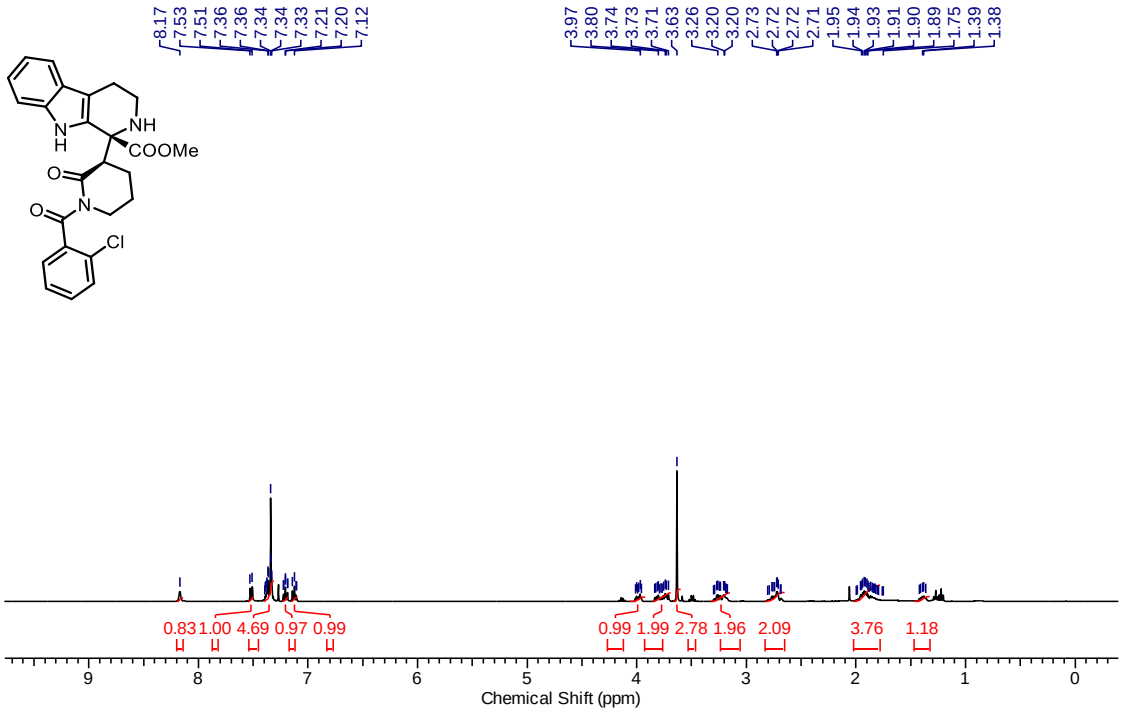
^{1}H NMR of Compound 55 in $CDCl_3$ at 400 MHz



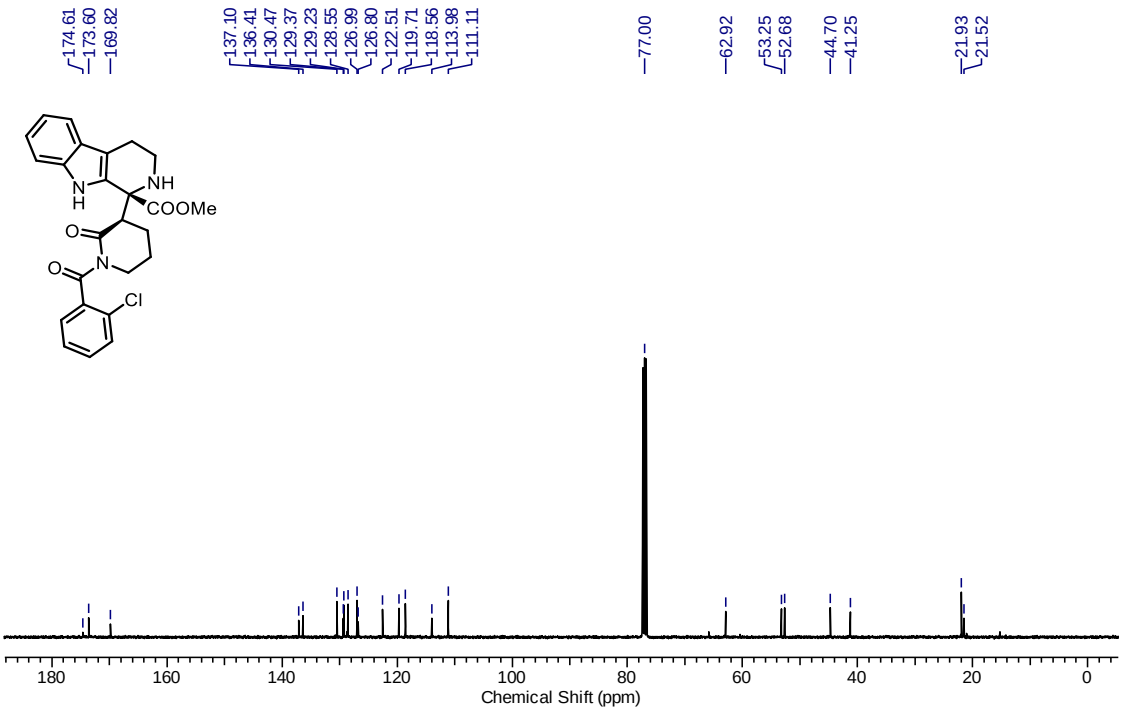
^{13}C NMR of Compound 55 in $CDCl_3$ at 100 MHz



^{1}H NMR of Compound 56 in $CDCl_3$ at 400 MHz



^{13}C NMR of Compound 56 in $CDCl_3$ at 100 MHz



Chapter-2

Section 1: Total Synthesis of Oxoaplysinopsin D, E, F and G

2.1.1 Introduction

In earlier times, medicines derived from the natural products were mostly from terrestrial origins due to ease of access. However, in recent times, identification of marine natural products (MNPs) became much easier owing to several technological advancements such as use of underwater drones, SCUBA *etc*.[1-5] According to a report by Kong *et al.*, about 71% present molecular scaffolds of MNPs were obtained entirely in marine creature.[6] Although, only a very little percentage of the ocean has been explored so far. Approximately, more than 28000 of MNPs with a broad spectrum of biological activities have been identified till date.[7,8] Amongst these wide range of bio-activities, cytotoxic and anticancer activities plays a crucial role.[9] In spite of such impressive therapeutic properties, development of drugs based on MNPs happens to be a challenging task due to the scarcity of these NPs from natural sources. In this context, total synthesis or semi-synthesis possess a promising solution to this. However, in few cases, biotechnological techniques are being used where, cultivation of invertebrates or large-scale fermentation of the grower microorganism are done. But in most of the cases this technique turns out to be difficult.[10] Food and Drug Administration (FDA) of the United States has approved few marine natural products to be used as drugs (selected examples are shown in Figure 2.1.1) and there are many other promising MNPs undergoing clinical trials.[11]

Anticancer drug cytarabine and antiviral agent vidarabine are the two nucleosides which were obtained from two natural arabino-nucleosides and approved by US FDA in 1969 and 1976 respectively. Currently, cytarabine is used for cancer therapy whereas vidarabine was discontinued in the US and in Europe . However structural template of these MNPs can be considered for other marketed antiviral drugs.[12] Trabectedin isolated from marine source *Tunicate Ecteinascidia Turbinata* was approved in 2007 by Europe for the treatment of advanced soft tissue sarcoma and later in 2009 for the treatment of recurrent platinum-sensitive ovarian cancer by the European Medicines Agency (EMEA). Later, it was approved by FDA for anticancer treatment in 2015.[13] Eribulin mesylate, an anticancer agent which was approved by US FDA in 2010 is a synthetic truncated derivative of the polyketide MNP halichondrin B.[10,14] In 2011, brentuximab vedotin was approved by FDA and in 2015 by Europe as anticancer drug.

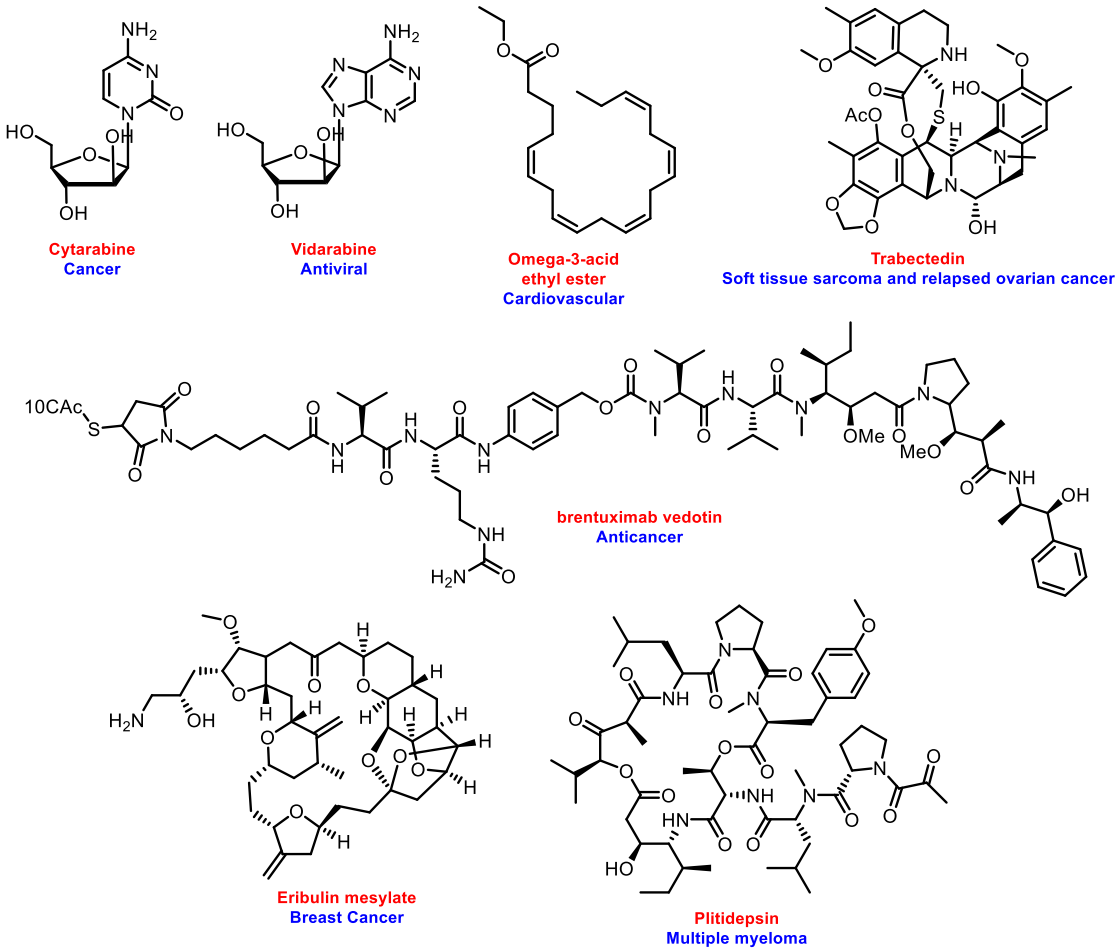


Figure 2.1.1: Selected marketed drugs from marine source

It has been used as antibody-drug conjugate (ADC) for the treatment of several lymphoma's.[15] Plitidepsin is another natural product approved by Australia as a remedy for relapsed and refractory multiple myeloma in patients.[16]

Marine indole alkaloids are a special class of MNPs consisting of diverse biological activities which make them attractive starting points for the drug discovery and development.[2] It also received significant interest due to their structural similarities with endogenous neurotransmitters and is to be useful in treating various central nervous system (CNS) disorders. Accordingly, many indole alkaloids are in the market for the treatment of CNS disorders.[17] e.g triptans, useful for the treatment of migraines are as shown in Figure 2.1.2.

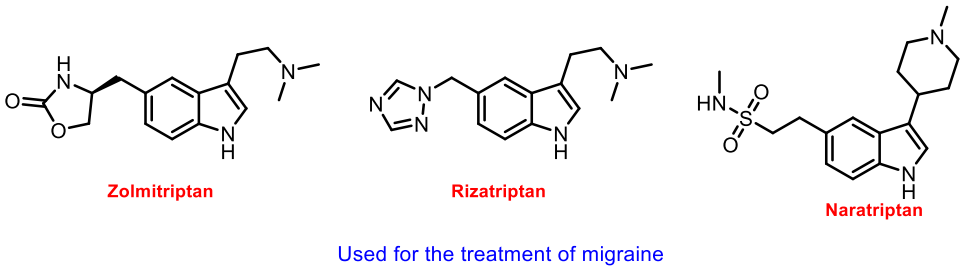


Figure 2.1.2: Marketed triptans for the treatment of migraines

2.1.1.1 Aplysinopsins:

Aplysinopsins is a distinct group of indole alkaloids that has fascinated many researchers in the field. Aplysinopsin **1** was first isolated by Kazlauskas *et al*. from Indo-Pacific sponges named as *aplysinopsin genera* which was also known with previous name *genera Thorecta*.[18] Various aplysinopsin analogues were also isolated from mollusks, corals and sea anemones.[19-21] Aplysinopsin consists of two heterocyclic units, indole and imidazolidinone as shown in Figure 2.1.3.

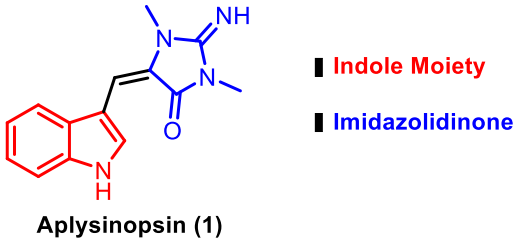


Figure 2.1.3: Structure of natural product aplysinopsin

There are small structural variations in natural analogues of aplysinopsin specifically differ in:

a) Bromination pattern present on indole ring
b) Number and position of methyl groups on the NH functionality
c) Stereochemistry of olefin
d) Absence of olefin moiety
e) Dimers of aplysinopsins

a) Bromination pattern present on indole ring:

Natural products containing organobromine compounds are mostly found in the marine organisms. Kalzlauskas *et al*. reported first monobrominated aplysinopsin, but unable to elucidate its structure due to unavailability of sufficient amount.[18] Further, several

brominated aplysinopsins were reported from different marine sources such as corals, sponges, mollusk and anemone. Among all aplysinopsin isolated till date, almost half of them are mostly halogenated at the 6-position of indole component **2** with bromine, with an exceptional report of only one compound with di-bromo functionality at 5 and 6 positions (**3**).[22] (Figure 2.1.4)

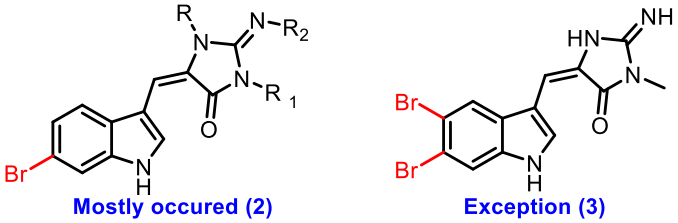
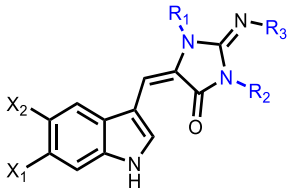


Figure 2.1.4: Brominated aplysinopsins

b) Number and position of methyl groups on NH:

Different aplysinopsins were isolated which differ in their methylation pattern *i.e.* number and position of *N*-methyl functional group. (Figure 2.1.5)[3]



No.	R_1	R_2	R_3	X_1	X_2
1	CH_3	CH_3	H	H	H
3	H	CH_3	H	Br	Br
4	H	CH_3	H	H	H
5	H	CH_3	H	Br	H
6	CH_3	CH_3	H	Br	H
7	CH_3	H	H	Br	H
8	CH_3	CH_3	CH_3	H	H
9	H	CH_3	CH_3	H	H
10	H	CH_3	CH_3	Br	H
11	CH_3	H	CH_3	H	H
12	CH_3	CH_3	CH_2CH_3	H	H

Figure 2.1.5: Naturally isolated substituted aplysinopsins

c) Stereochemistry of olefins:

Aplysinopsin possesses an olefin at C8-C1', configuration of which was assigned to be *E*. However in later year, Guella *et al*. published a simple NMR technique that could differentiate between *E* and *Z* geometrical isomers of aplysinopsin.[23] According to this study, alkyl group present on amidine nitrogen contribute to play a crucial role in favouring the *E* configuration of the natural product. On the other hand the absence of the concerned alkyl functionality favours *Z*-configuration. (Figure 2.1.6)

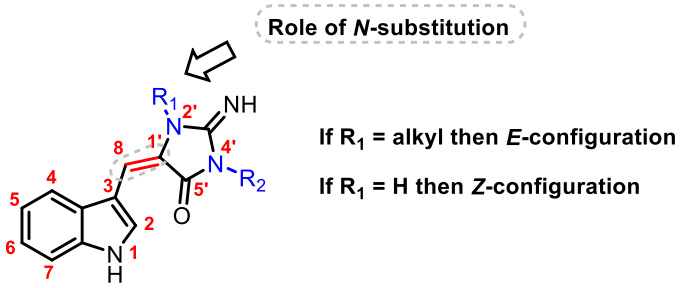


Figure 2.1.6: Guella *et al.* hypothesis on stereochemistry of olefin

d) Without olefin moiety:

Compounds **13-17** were isolated from Indo-Pacific sponges which are structurally slightly different than the parent aplysinopsin. These compounds lacks the presence of the olefin functionality.[24] (Figure 2.1.7)

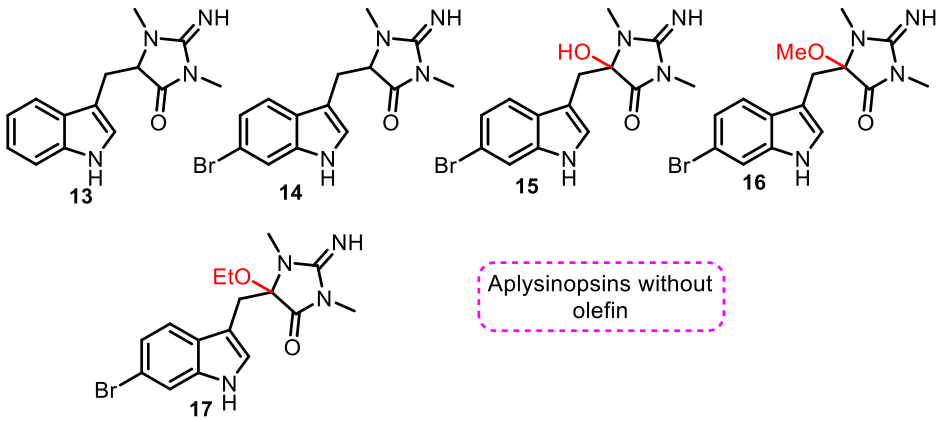


Figure 2.1.7: Olefin functionalized aplysinopsins

e) Dimers of aplysinopsins:

Dimers of aplysinopsin were isolated for the first time in 2000 from the coral source *Tubastraea faulkneri*.[25] Later in 2003, first spectroscopic studies of these dimers were

reported and named as tubastrindoles A–C **18-20**.[26] Moreover, isolated two more cycloaplysinopsins A **21** and B **22** from dendrophylliid coral.[27] Further, Meyer *et al.* described the isolation of cycloaplysinopsins C **23** in 2009.[28] (Figure 2.1.8)

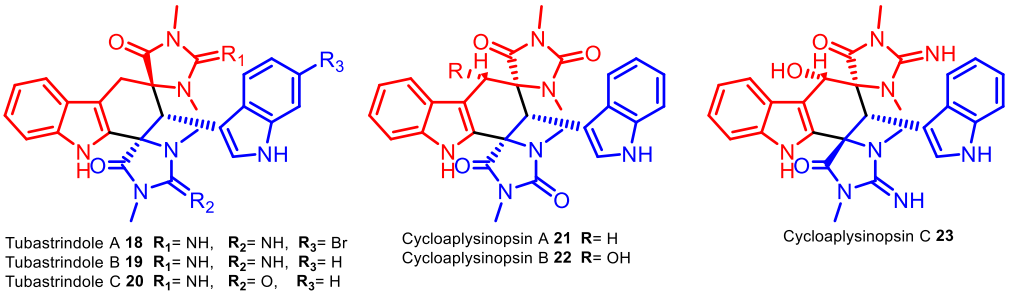


Figure 2.1.8: Aplysinopsin dimers

2.1.1.2 Biological activities of aplysinopsins:

Aplysinopsin and their derivatives have been well known for their various biological activities. For the first time antitumor activity of aplysinopsin **1** in mice has been reported by Hollenbeak *et al.* in 1977.[29] Later, in 1994, Kondo *et al.* reported cytotoxicity against murine lymphoma L-1210 with IC_{50} = 11.5 μg/mL of a new analogue of aplysinopsin called Isoplysin A (**11**).[30] Moreover, they have also showed aplysinopsin **1** and methylaplysinopsin **8** possess cytotoxicity against the LH-1210 cell line with IC_{50} values of 2.3 and 3.5 μg/mL, respectively. Further, Hu *et al.* isolated a series of aplysinopsins from the sponge *Smenospongia aurea* and evaluated their antimalarial activity.[31] Isolated compounds were tested against *Plasmodium falciparium* and found to be moderately active antimalarial compounds. Three compounds, 6-Bromoaplysinopsin **6**, Isoplysin A **11** and 6-bromo-2′-de-*N*-methylaplysinopsin **3** showed IC_{50} values 0.34, 0.97 and 1.1 μg/mL respectively. But 6-Bromoaplysinopsin **6** was found to be inactive *in vivo* assay.

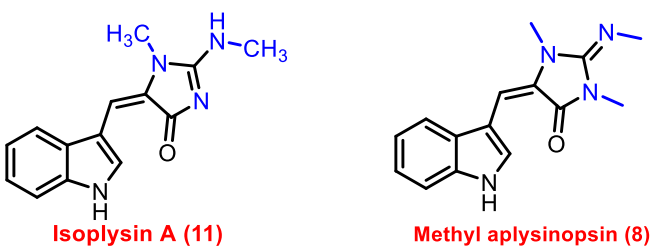


Figure 2.1.9: Biologically active derivatives of aplysinopsin

Later, several studies have shown that aplysinopsin and their derivatives have a wide range of biological activities, including anti-cancer, anti-proliferative, anti-malarial, anti-microbial, CNS disorders *etc*.[3]

2.1.2 Isolation details of oxoaplysinopsins A-G

Recently, seven new oxygenated aplysinopsins were isolated by Qiang Li group from the Xisha Islands sponge *Fascaplysinopsis reticulate* and named as oxoaplysinopsin A-G **24-30** (Figure 2.1.10).[32] This oxoaplysinopsin's family showed remarkable stereochemical diversity which could have possibly originated from biosynthetic olefinic precursors **31** and **32**. Structures of these natural product seems to be simple, but stereochemical determination of each natural product is a challenging task using NMR spectroscopy, Wang *et al*. have assigned stereochemistry to all individual oxoaplysinopsins.

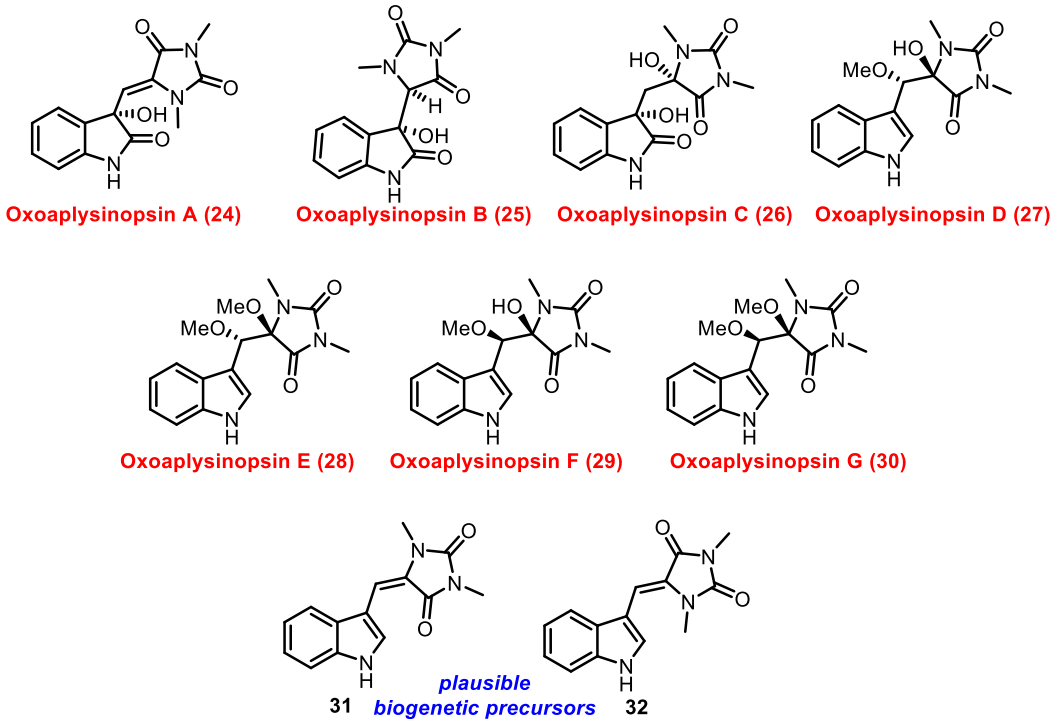


Figure 2.1.10: Oxoaplysinopsins A-G isolated by Wang *et al.*

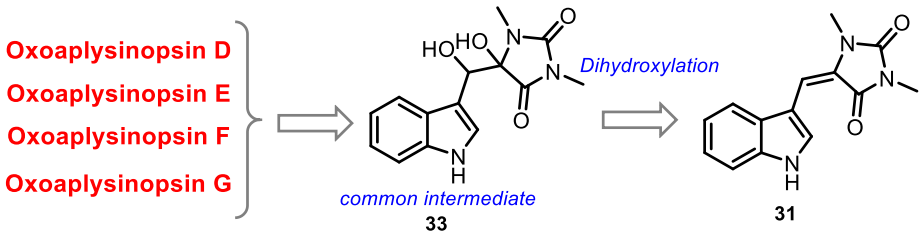
2.1.3 Total Synthesis of oxoaplysinopsins D, E, F and G:

Inspired by the structural features and biological activity, we became interested in the total synthesis of oxoaplysinopsins D, E, F and G. We have accomplished these

synthesis from common dihydroxy intermediate by employing one pot ketohydroxylation using pyridinium dichromate and aldol reaction as the key steps. This work has been discussed in detail in the following sections.

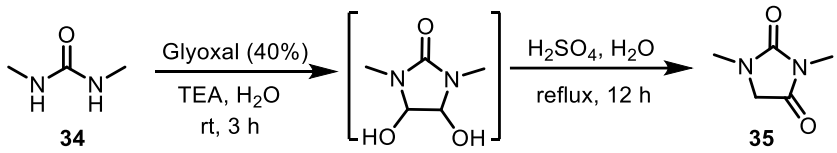
2.1.3.1 Initial attempt towards oxoaplysinopsins:

Oxoaplysinopsins D, E, F and G share a common structural core hence their synthesis were planned from common dihydroxy intermediate **33**, which was traced by dihydroxylation reaction on well-known oxoaplysinopsin **31**. Oxoaplysinopsin **31** could be prepared using known literature protocol from commercially available indole-3-caboxaldehyde and *N*,*N*-dimethyl hydantoin. (Scheme 2.1.1)



Scheme 2.1.1: Retrosynthesis of oxoaplysinopsins D, E, F and G

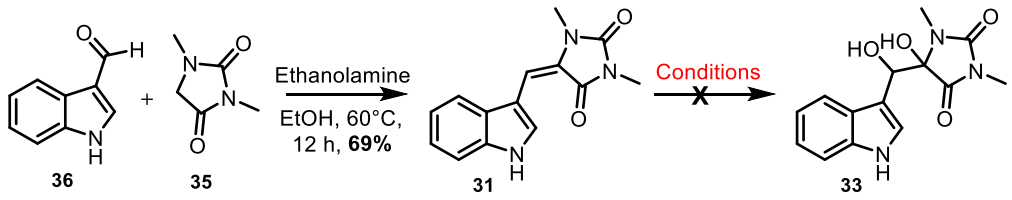
We commenced our strategy with an aim to synthesize *N*,*N*-dimethyl hydantoin **35** in good quantities following the known literature procedures.[33] Accordingly, commercially available *N*,*N*-dimethyl urea **34** was treated with triethylamine and glyoxal (40% in water) in water at room temperature to get desired dihydroxy intermediate which on further treatment with conc. H_2SO_4 in water resulted *N*,*N*-dimethyl hydantoin **35** in good yield.



Scheme 2.1.2: Synthesis of *N*,*N*-dimethyl hydantoin on gram scale

After having good quantity of *N*,*N*-dimethyl hydantoin **35** in hand, we subjected it for condensation reaction with commercially available indole-3-caboxaldehyde **36** using ethanolamine in ethanol as solvent so as to obtain required oxoaplysinopsin **31** in good

yield. NMR data of the synthesized compound **31** was in complete agreement with the data reported in the literature.[34] Oxoaplysinopsin **31** was further subjected to various dihydroxylation conditions as mentioned in following Scheme 2.1.3.



Sr. No.	Conditions	Observations
1	OsO_4, NMO, Acetone : H_2O, rt, 4 h	Aldehyde **36** was observed
2	OsO_4, NMO, ^tBuOH : H_2O	Aldehyde **36** was observed
3	OsO_4, NMO, THF : H_2O	Aldehyde **36** was observed
4	OsO_4, Pyridine, rt, 5 h	No reaction
5	*m*CPBA, DCM, rt, 2 h	Aldehyde **36** was observed

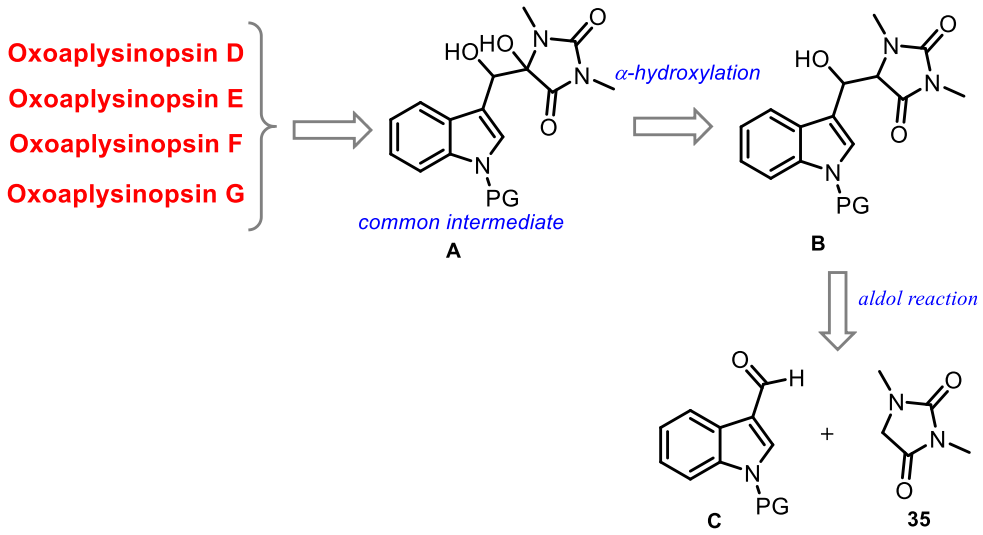
Scheme 2.1.3: Attempted dihydroxylations on oxoaplysinopsin

Osmium tetroxide in various solvents resulted in the cleavage of olefin which subsequently led to the formation of aldehyde **36**. However, use of osmium tetroxide in pyridine showed no progress in the reaction. Further, we tried epoxidation of the olefin using *m*CPBA and alkaline hydrogen peroxide, but the reaction failed to yield the desired product and the starting material was recovered as such with indole aldehyde **36**. Having no desired outcome from the attempts towards dihydroxylation, we planned to revise our approach towards retrosynthesis.

2.1.3.2 Revised synthesis:

In light of above results, we have modified our retrosynthetic plan as shown in Scheme 2.1.4. Common dihydroxy intermediate **A** was planned to be synthesized from aldol adduct **B** via α-hydroxylation reaction. Aldol adduct **B** could be traced by base mediated aldol reaction of Boc-indole-3-caboxaldehyde **C** and *N*,*N*-dimethyl hydantoin

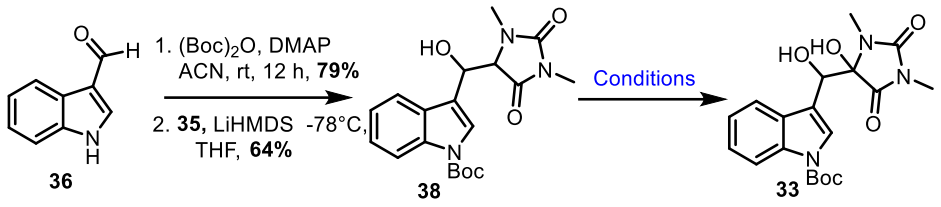
35. However, the main challenge in the designed route was to carry out the α-hydroxylation reaction on aldol adduct **B**.



Scheme 2.1.4: Modified retrosynthetic approach

As per the plan, our synthesis commenced with Boc-protection of commercially available indole-3-carboxaldehyde **36** using Boc anhydride and catalytic amount of DMAP in acetonitrile which yielded Boc-indole-3-caboxaldehyde **37**. The formation of Boc protected product was confirmed using ^{1}H NMR spectroscopy.[35] Next, compound **37** was subjected to aldol reaction with *N*,*N*-dimethyl hydantoin **35** using LiHMDS base at -78 °C in THF and furnished gram scale amount of the required aldol adduct **38** as a diastereomeric mixture (dr = 4 : 1). The presence of diastereomeric mixture was confirmed with NMR spectroscopy, where characteristic peaks at 5.57- 5.55 (m, 1 H) corresponding to proton attached to newly formed hydroxy group and 4.33 - 4.28 (m, 1 H) corresponding to proton at nitrogenated tertiary carbon was observed. The diastereomeric mixture was taken forward as such for further transformation. Next task was to install hydroxy group at nitrogenated tertiary center, for which diastereomeric mixture of aldol adduct **38** was subjected to several α-hydroxylation conditions[36] as mentioned in Scheme 2.1.5. In an initial attempt, reaction was carried out in the presence of iodine in DMSO at 60 °C, which gave a retro-aldol reaction leading to the exclusive formation of Boc-indole-3-caboxaldehyde **37** and *N*,*N*-dimethyl hydantoin **35**. In order to overcome this issue, same reaction was carried out at room temperature for 12 h which resulted in zero reaction progress and the starting material was recovered

as such. Identical retro aldol products were also observed, when reaction was performed in the presence of NaOAc under oxygen in THF at room temperature. Next we tried

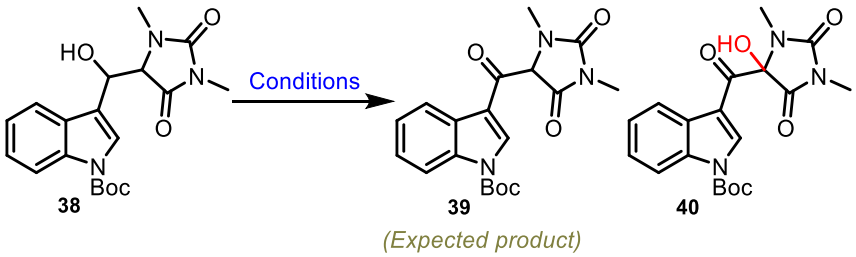


Sr. No.	Conditions	Observation
1	Iodine, DMSO, 60 °C 12 h	Retro-aldol product
2	Iodine, DMSO, rt, 12 h	No reaction
3	Iodine, NaOAc, O_2, THF, rt	Retro-aldol product
4	Iodine, TBHP, DMSO, 60 °C 12 h	Retro-aldol product
5	NBS, DMSO, rt, 3 h	No reaction
6	Oxone, ACN: H_2O, rt	Retro-aldol product

Scheme 2.1.5: Attempted conditions for α-hydroxylation of aldol adduct (**38**)

reaction using iodine, TBHP in DMSO[36a] at 60 °C, but ended up with retro-aldol products as well. Furthermore, we also treated the same aldol adduct with *N*-Bromosuccinimide in DMSO at room temperature[36b] but observed no desired reaction. While use of oxone in combination of acetonitrile and water[36c] at room temperature also resulted in the formation of retro-aldol products. From all of the above failed attempts it was concluded that synthesized aldol adduct **38** is very sensitive under heating and acidic media which probably resulted in the formation of retro-aldol products. In order to avoid the formation of retro-aldol products, we changed our strategy and planned oxidation of aldol adduct **38** to obtain corresponding ketone **39**, followed by α-hydroxylation. Accordingly, aldol adduct **38** was subjected to several oxidation conditions as mentioned in Scheme 2.1.6. For oxidation of aldol adduct **38** to ketone **39**, compound **38** was treated with DMP, $NaHCO_3$ in DCM at room temperature for 6

h, but it resulted in retro-aldol adducts. Same results were observed, even when the reaction was performed at 0 °C. In order to avoid the retro-aldol reaction, we used mild

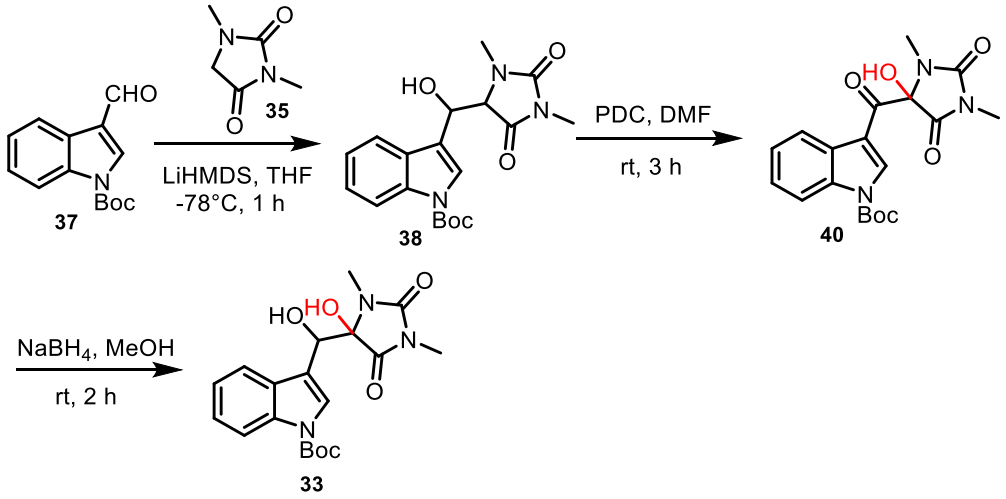


Sr. No.	Conditions	Observations
1.	DMP, $NaHCO_3$, rt, 6 h	Retro-aldol product
2.	DMP, $NaHCO_3$, 0 °C, 1 h	Retro-aldol product
3.	MnO_2, DCM, rt, 4 h	**39** (20%) + retro-aldol
4.	IBX, DMSO, rt, 3 h	**39** (31%) + retro-aldol
5.	PCC (3 equiv.), DCM, rt, 3 h	**39** (37%) + **40** (30%)

Scheme 2.1.6: Oxidation of aldol adduct **38**

oxidizing reagent MnO_2 in DCM which resulted in the formation of required ketone **39**, but in poor yield (20%) along with the retro-aldol products. Formation of ketone **39** was confirmed by disappearance of hydroxy attached proton at δ 5.57- 5.55 region in ^1H NMR analysis. Use of IBX in DMSO showed slight improvement in yield of ketone **39** (31%) along with retro-aldol adducts. Use of PCC (3 equiv.) for oxidation led to the formation of the desired ketone **39** in 37% yield along with the formation of another by-product (TLC analysis) which turned out to be hydroxyketone **40** as confirmed by NMR spectroscopy through the appearance of characteristic peak at δ 5.81 (s, 1 H) in ^1H NMR corresponding to proton from newly formed hydroxy group at nitrogenated tertiary carbon, however when NMR was recorded in MeOH-d_4, we observed disappearance of observed peak δ 5.81 (s, 1 H). Moreover, DEPT NMR analysis also showed carbon peak at δ 86.79 was not bearing any proton. HRMS spectra showed peak at 410.1320 corresponding to molecular formula $C_{19}H_{21}N_3O_6Na$ $[M+Na]^+$ with

calculated value 410.1323 further confirmed the product formation. From the optimization studies, it was found that PDC (3 equiv.) in DMF at room temperature for

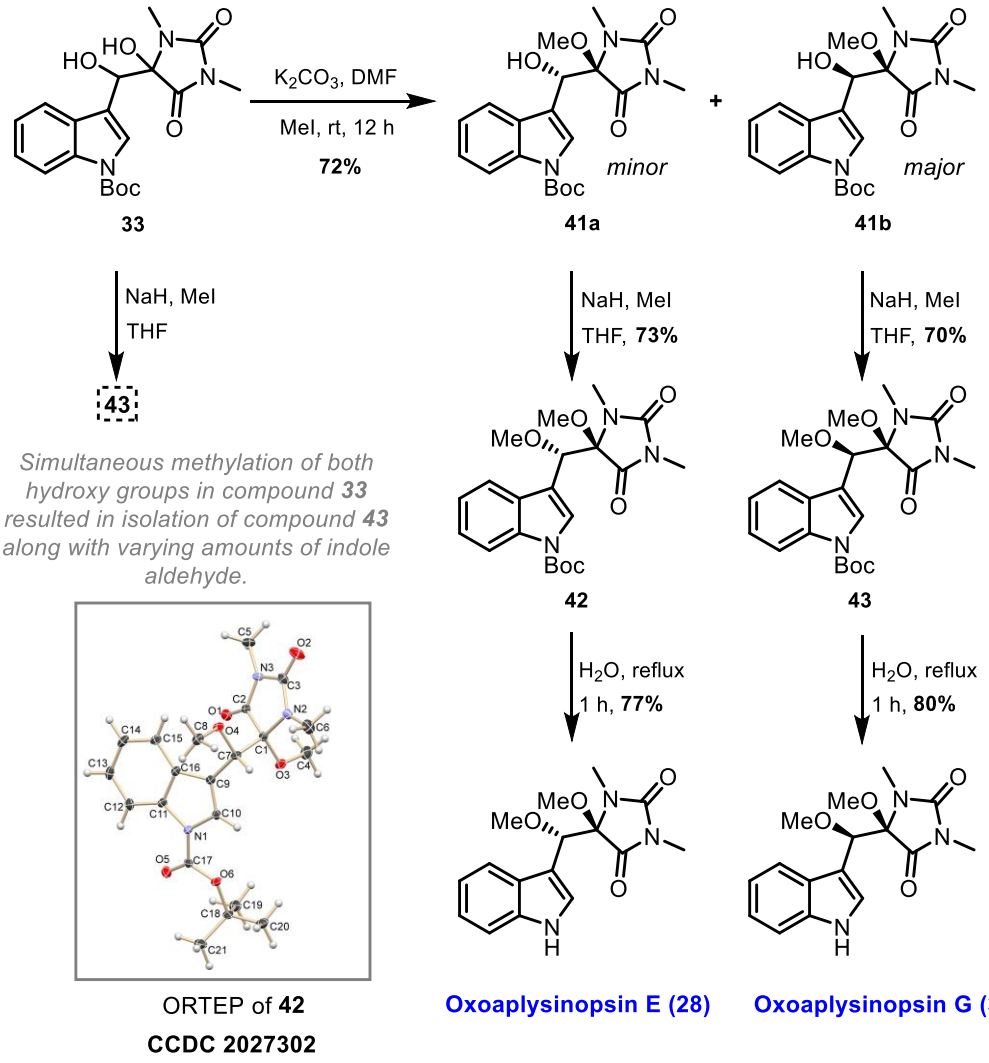


Scheme 2.1.7: Synthesis of common dihydroxy intermediate on gram scale

3 h was the best conditions for the desired transformation to obtain hydroxy ketone **40** exclusively. (Detailed optimization table is given in chapter 2 - section 2). Next, obtained hydroxy ketone **40** was treated with sodium borohydride in methanol at room temperature to furnish required dihydroxy intermediate **33** as diastereomeric mixture. Formation of dihydroxy compound **33** was indicated by presence of ^{1}H NMR at δ 5.42 – 5.25 region corresponding to proton attached to hydroxyl group. Further, this common dihydroxy intermediate **33** was synthesized in gram scale as shown in Scheme 2.1.7.

2.1.3.2.1 Syntheis of oxoaplysinopsin E and G:

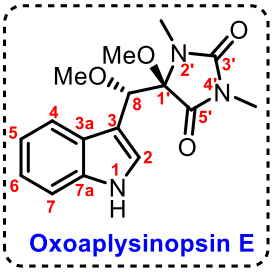
After having dihydroxy compound **33** in hand, it was further treated with sodium hydride in THF at room temperature to obtain dimethylated compound as diastereomeric mixture. However, we isolated only one diastereomer **43** along with indole aldehyde **37** as result of retro-aldol reaction, instead of getting diastereomeric mixture of two compounds **42** and **43**.



Scheme 2.1.8: Synthesis of oxoaplysinopsin E and G

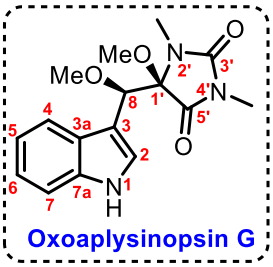
Based on the above observation, we have used mild base K_2CO_3 which resulted in the formation of monomethylated compound as diastereomeric mixture which could be easily separated using silica coloumn chromatography to obtain both diastereomers **41a** and **41b**. (Stereochemistry was given to both diastereomers on the basis of Scheme 2.1.9 and Scheme 2.1.12). Compound **41a** was confirmed by signal in ^{1}H NMR at δ 3.15 (s, 3H) and signal in ^{13}C NMR at δ 52.48 corresponds to methyl group, whereas newly introduced methyl group in **41b** was confirmed by signal in ^{1}H NMR at 3.13 (s, 3H) and carbon signals ^{13}C NMR at δ 52.41. Next minor diastereomer **41a** was treated with NaH in THF at room temperature which resulted in the formation of dimethylated compound **42** in 73% yield, structure and stereochemistry of this dimethylated compound **42** were confirmed using X-ray crystallography.

Table 2.1.1: Comparison of spectral data of natural and synthetic oxoaplysinopsin E (**28**) in DMSO-d_6



Oxoaplysinopsin E	^{1}H NMR δ ppm		^{13}C NMR δ ppm	
	Natural	Synthetic	Natural	Synthetic
1	11.18, br s, 1H	11.18, br s, 1H	-	-
2	7.17, d, 1 H (2.2)	7.17, d, 1 H (2.3)	124.7, CH	124.8, CH
3	-	-	107.9, C	107.9, C
3a	-	-	127.0, C	127.0, C
4	7.53, d, 1 H (8.0)	7.53, d, 1 H (7.8)	118.9, CH	118.9, CH
5	6.97, dd, 1 H (7.8, 7.2)	6.97, dd, 1 H (7.8, 7.3)	118.8, CH	118.8, CH
6	7.06, dd, 1 H (7.2, 7.9)	7.06, dd, 1 H (7.2, 7.9)	120.9, CH	121.0, CH
7	7.35, d, 1 H (8.1)	7.35, d, 1 H (8.2)	111.4, CH	111.5, CH
7a	-	-	135.7, C	135.7, C
8	4.94, s, 1 H	4.94, s, 1 H	77.9, CH	77.9, CH
1’	-	-	92.4, C	92.5, C
3’	-	-	155.6, C	155.7, C
5’	-	-	169.2, C	169.2, C
2’-NCH_3	3.02, s, 3 H	3.02, s, 3 H	25.6, CH_3	25.6, CH_3
4’-NCH_3	2.60, s, 3 H	2.60, s, 3 H	23.9, CH_3	23.9, CH_3
8-OCH_3	3.19, s, 3 H	3.19, s, 3 H	57.1, CH_3	57.1, CH_3
1’-OH	-	-	-	-
1’-OCH_3	3.03, s, 3 H	3.03, s, 3 H	51.3, CH_3	51.4, CH_3

Table 2.1.2: Comparison of spectral data of natural and synthetic oxoaplysinopsin G (**30**) in DMSO-d_6



Oxoaplysinopsin G	^{1}H NMR δ ppm		^{13}C NMR δ ppm	
	Natural	Synthetic	Natural	Synthetic
1	11.20, br. S	11.19, br. s	-	-
2	7.29, s	7.29, d	125.8, C	125.8, C
3	-	-	108.4, C	108.4, C
3a	-	-	126.8, C	126.8, C
4	7.58, d, 1H (8.0)	7.58, d, 1H (7.8)	120.4, CH	120.4, CH
5	7.00, dd, 1H (7.3, 7.7)	6.99, dt, 1H (7.4)	119.5, CH	119.5, CH
6	7.08, dd, 1H (7.2, 7.9)	7.08, m, 1H	121.5, CH	121.6, CH
7	7.37, d, 1H (8.1)	7.37, d, 1H (7.8)	112.1, CH	112.1, CH
7a	-	-	136.8, C	136.8, C
8	4.85, s, 1H	4.84, s, 1H	79.4, CH	79.4, CH
1’	-	-	92.3, C	92.3, C
3’	-	-	156.6, C	156.6, C
5’	-	-	171.4, C	171.4, C
2’-NCH_3	2.33, s, 3H	2.33, s, 3H	26.1, CH_3	26.1, CH_3
4’-NCH_3	2.90, s, 3H	2.90, s, 3H	24.7, CH	24.7, CH
8-OCH_3	3.19, s, 3H	3.19, s, 3H	57.6, CH_3	57.6, CH_3
1’-OCH_3	3.01, s, 3H	3.02, m, 3H	51.8, CH_3	51.8, CH_3

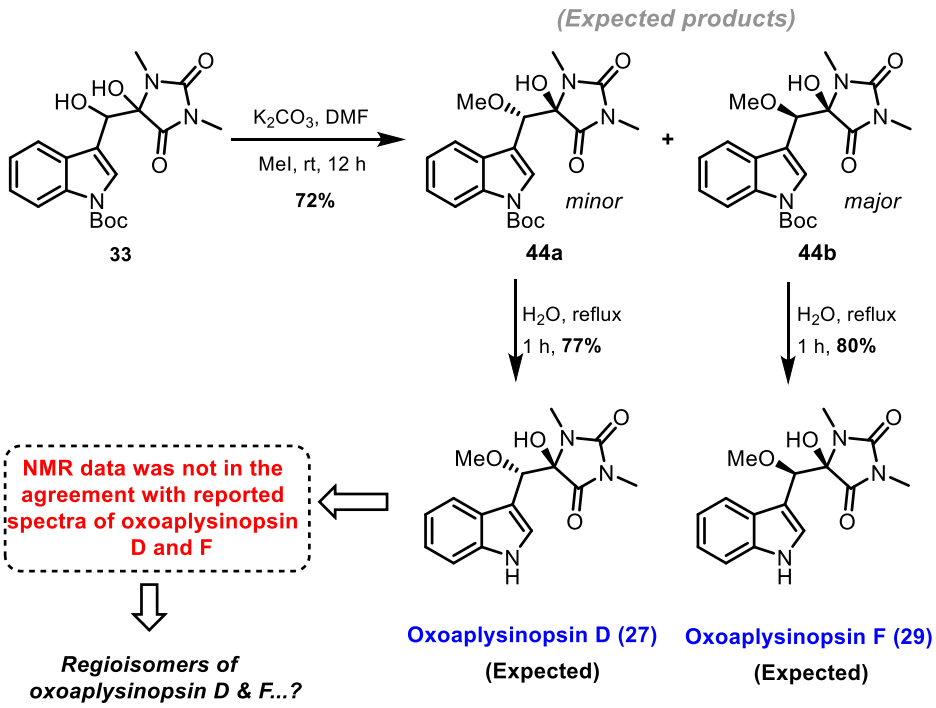
Further, Boc-deprotection of **42** was carried out in refluxed water[37] resulted in the formation of oxoaplysinopsin E **28**. All the spectroscopic data of oxoaplysinopsin E was in good agreement with the reported data by Wang’s group.[32] Next, major diastereomer **41b** was subjected to methylation using NaH in THF solvent at room temperature which gave dimethylated compound **43** in 70% yield. Formation of

compound **43** was confirmed by ^{1}H NMR peaks at δ 3.35 (s, 3H) belongs to newly added methyl group and with HRMS analysis which showed peak at 440.1792 corresponds to molecular formula $C_{21}H_{27}N_3O_6Na$ $[M+Na]^+$. Boc-deprotection of compound **43** was carried out in refluxed water[37] resulting in oxoaplysinopsin G **30** in 80% yield (Scheme 2.1.8). All the spectroscopic data of oxoaplysinopsin G was in agreement with the reported data by Wang's group.[32] Complete ^{1}H and ^{13}C comparison tables of synthesized oxoaplysinopsin E and G with their natural samples are given in tables 2.1.1 and 2.1.2.

2.1.3.2.2 Syntheis of oxoaplysinopsin D and F:

During the synthesis of oxoaplysinopsin E and G, common dihydroxy intermediate **33** was treated with methyl iodide in presence of K_2CO_3 in DMF to obtain two diastereomers **41a** and **41b** (Scheme 2.1.8). However, the regioselectivity of this reaction was not clear *i.e.* whether the methylation was occurred at secondary hydroxy group or tertiary hydroxy group. Based on the literature, we hypothesized that the secondary alcohol is more reactive than that of tertiary alcohol. Accordingly, further transformation was planned.

Greater reactivity of secondary hydroxyl group over tertiary:

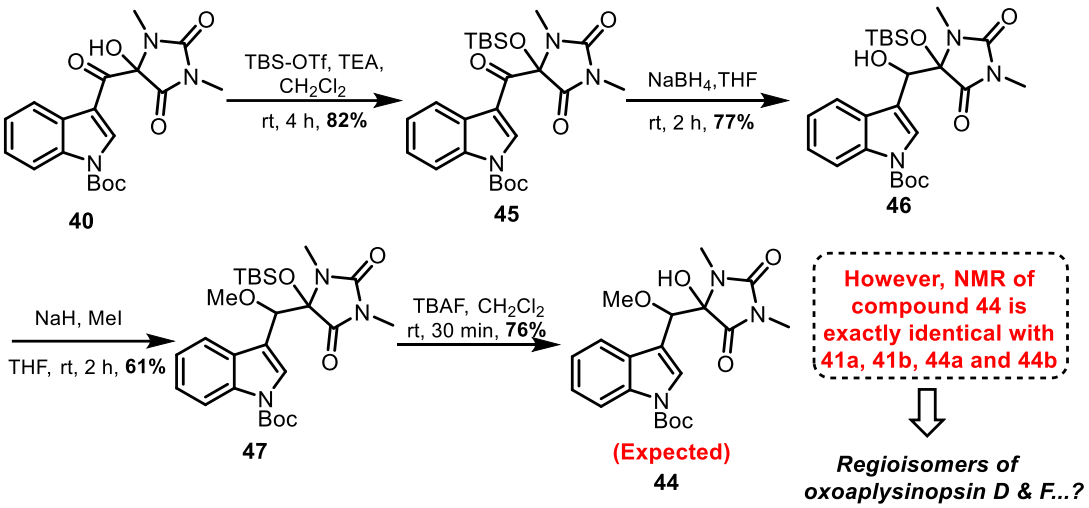


Scheme 2.1.9: Attempted synthesis of oxoaplysinopsin D and F

On the basis of this hypothesis, we expected that methylation of dihydroxy intermediate **33** using K_2CO_3 and methyl iodide would result in methylation of secondary hydroxy group which was expected to furnish compound **44a** and **44b**. Those compounds were subjected for Boc deprotection in water[37] under reflux for 1 h resulted deprotected compounds **27** and **29.** Both compounds **27** and **29** were confirmed by disappearance of Boc-related signals in ^1H and ^{13}C NMR, furthermore confirmation of **27** and **29** has been done by HRMS analysis having peak at 326.1112 and 326.1113 respectively, those peaks corresponds to molecular formula $C_{15}H_{17}N_3O_4Na$ $[M+Na]^+$. (Scheme 2.1.9). To our surprise, NMR data of synthesized compounds were not in agreement with reported data by isolation group,[32] although HRMS data, proton counts as well as carbon count are identical. This can result in two possibilities, 1) Structural revision of oxoaplysinopsin D and F or 2) Nitrogenated tertiary alcohol was more reactive than secondary alcohol which resulted in formation of regioisomers of oxoaplysinopsin D & F in Scheme 2.1.9. To solve this issue, we decided to protect nitrogenated tertiary alcohol.

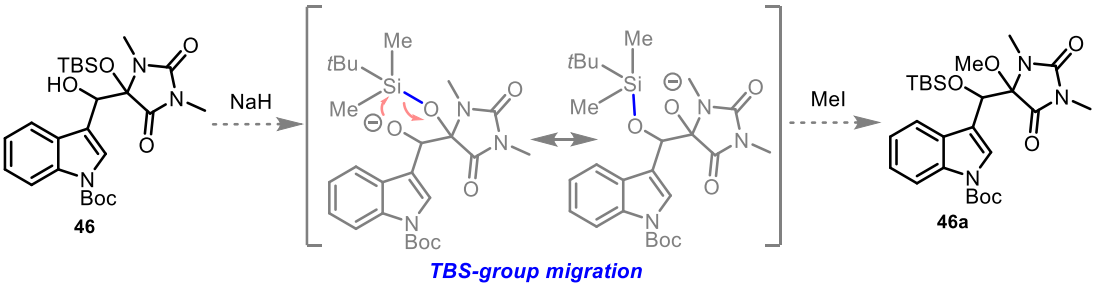
Protection of nitrogenated tertiary alcohol (TBS-protecting group):

Compound **40** was subjected for TBS protection using TBS-triflate and triethylamine in DCM resulted compound **45** which was confirmed by signals in ^1H NMR spectrum at δ 0.15 (s, 3H), δ 0.32 (s, 3H) δ 0.98 (s, 9H) corresponds to TBS group. Next compound **45** on further treatment with sodium borohydride in methanol gave compound **46** as diastereomeric mixture which was confirmed by characteristic diastereomeric proton signal in ^1H NMR spectrum at δ 5.33, 1H and diastereomeric signals in ^{13}C NMR at δ 70.76, δ 71.17 belongs to newly generated chiral centre. Compound **46** was further subjected for methylation using methyl iodide and sodium hydride in THF afforded compound **47**. The formation of **47** was confirmed by signal in ^{13}C NMR at δ 51.89 belongs to CH_3 group and peak in HRMS analysis at 540.2499 corresponds to molecular formula $C_{26}H_{39}N_3O_6SiNa$ $[M+Na]^+$. Next TBS-deprotection of compound **47** was carried out using TBAF in DCM to afford compound **44** as diastereomeric mixture. But to our surprise NMR data is exactly matching with compound **44a** and **44b** from Scheme 2.1.10 and **41a** and **41b** from Scheme 2.1.9.



Scheme 2.1.10: TBS-protection strategy for synthesis of oxoaplysinopsin D & F

However, there was possibility of unexpected product formation due to TBS migration as shown in Scheme 2.1.11, Compound **46** when treated with sodium hydride can result in migration of TBS group from tertiary alcohol to secondary alcohol to give unexpected compound **46a.**



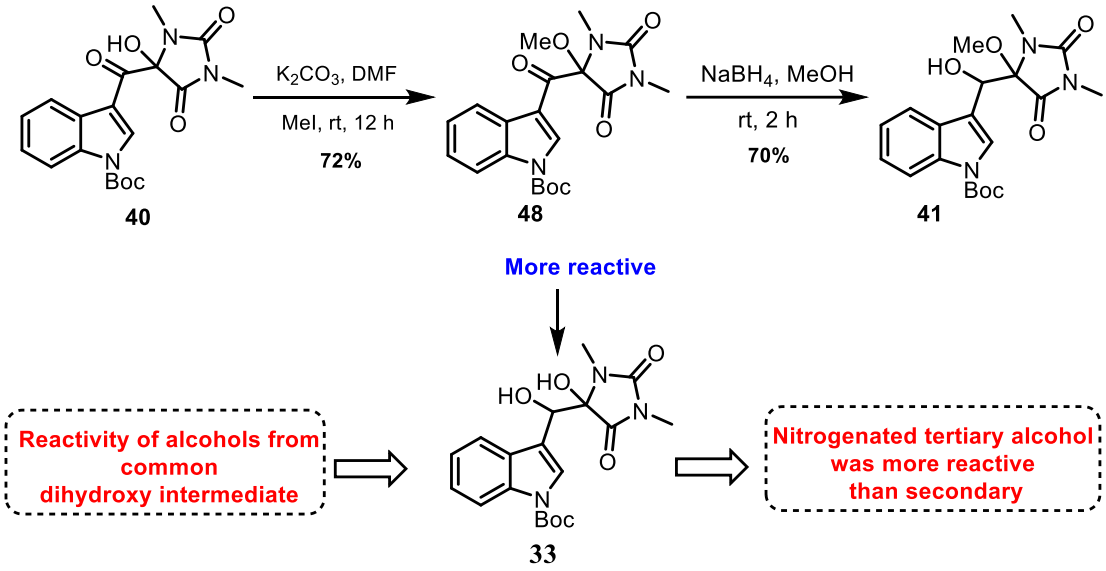
Scheme 2.1.11: Plaussible TBS-migration in compound **46**

To understand exact reactivity of dihydroxy compound **33** and TBS migration possibility, we decided to make exact regioisomers of natural product oxoaplysinopsin D and F.

Confirmation of regioselectivity during methylation:

To confirm the regioselectivity between secondary and tertiary alcohols, we have treated compound **40** with methyl iodide in presence of K_2CO_3 gave methylated compound **48**. Formation of **48** was confirmed by appearance of signal in ^1H NMR at δ 3.44 (s, 3H) and ^{13}C NMR at δ 52.23 corresponding to newly added CH_3 group.

Further, compound **48** on reduction using sodium borohydride in methanol afford compound **41** as diastereomeric mixture. Interestingly, NMR data of afforded compounds were in exact agreement with compound **41a** and **41b** from Scheme 2.1.8, **44a** and **44b** in Scheme 2.1.9 and **44** from Scheme 2.1.10



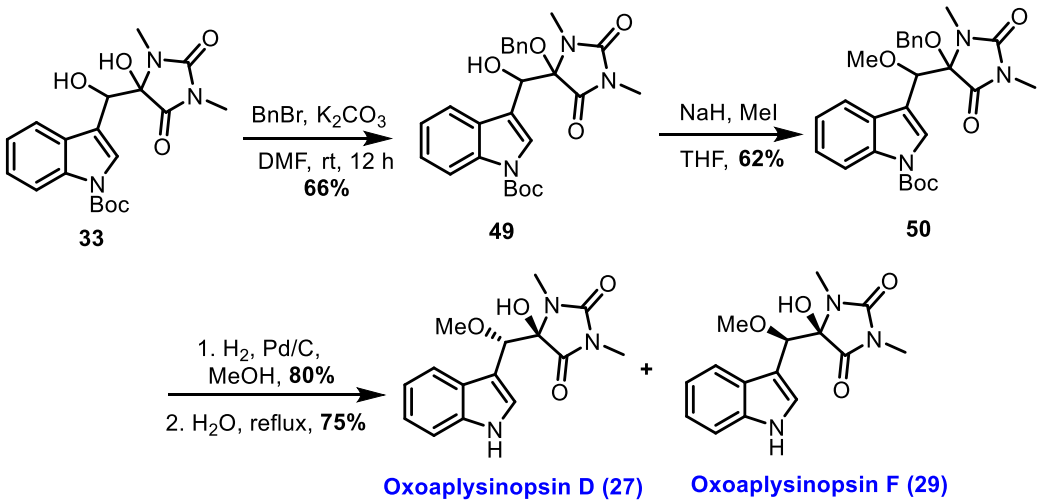
Scheme 2.1.12: Confirmation of reactivity of dihydroxy compound

Thus, we have confirmed that in K_2CO_3 mediated alkylation reaction, tertiary alcohol in the dihydroxy intermediate was more reactive than secondary alcohol. With this experiment it was also clear that in case of Scheme 2.1.9 we have got regioisomers of oxoaplysinopsin D and F and we also confirmed that we have observed TBS migration from nitrogenated tertiary alcohol to secondary alcohol. (Scheme 2.1.10)

Protection of nitrogenated tertiary alcohol (Bn-Protecting group):

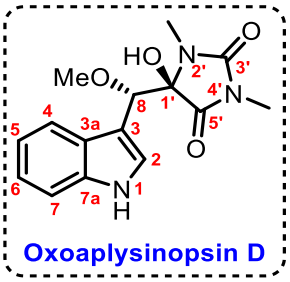
To avoid unwanted TBS-migration we decided to use benzyl protecting group. For which common dihydroxy intermediate **33** was treated with benzyl bromide, K_2CO_3 in DMF to give protected compound **49** which was confirmed by ^{1}H NMR having peak at 4.19 - 4.42 (m, 2 H) belongs to benzylic CH_2 and additional five protons in aromatic region showed the presense of benzyl group, ^{13}C NMR showed diastereomeric peaks at δ 67.23 and δ 67.49 corresponds to CH_2. Compound **49** was treated with methyl iodide and sodium hydride in THF to furnish methylated compound **50**. Compound **50** was confirmed by ^{13}C NMR diastereomeric peak at δ 78.27 and 78.88 corresponds CH_3

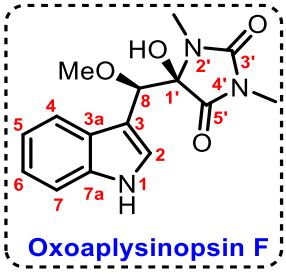
group, HRMS analysis showed peak at 516.2103 for the molecular formula $C_{27}H_{31}N_3O_6Na$ $[M+Na]^+$. Further deprotection of benzyl group from compound **50** was carried out using 10% Pd/C in methanol gave deprotected compound **51** in good yield which was confirmed by disappearance of peak corresponds to benzylic CH_2 at δ 4.40 - 4.16 (m, 2 H) and disappearance of aromatic protons and HRMS analysis which showed peak at 426.1636 belongs to molecular formula $C_{20}H_{25}N_3O_6Na$ $[M+Na]^+$. Further Boc-deprotection of **51** was carried out in refluxed water to afford oxoaplysinopsin D and F as diastereomeric mixture (Scheme 2.1.13). All the spectroscopic data of oxoaplysinopsin D and F was in agreement with reported data by Wang's group.[32] Complete comparison is shown in table 2.1.3.



Scheme 2.1.13: Synthesis of oxoaplysinopsins D and F

Table 2.1.3: Comparison of ^{13}C spectral data of natural and synthetic oxoaplysinopsin D (**27**) and oxoaplysinopsin F (**29**)





Oxoaplysinopsin D & F	Oxoaplysinopsin D		Oxoaplysinopsin F	
	^{13}C NMR δ ppm		^{13}C NMR δ ppm	
	Natural	Synthetic	Natural	Synthetic
1	-	-	-	-
2	124.6, CH	124.6, CH	125.3, CH	125.3, CH
3	108.5, C	108.5, C	108.3, C	108.3, C
3a	127.0, C	127.0, C	126.5, C	126.5, C
4	119.0, CH	119.0, CH	120.2, CH	120.3, CH
5	118.7, CH	118.7, CH	118.9, CH	118.9, CH
6	120.9, CH	120.9, CH	121.0, CH	121.0, CH
7	111.4, CH	111.4, CH	111.5, CH	111.5, CH
7a	135.7, C	135.7, C	136.3, C	136.3, C
8	78.7, CH	78.7, CH	79.2, CH	79.2, CH
1’	87.4, C	87.4, C	86.8, C	86.8, C
3’	155.6, C	155.6, C	156.6, C	156.6, C
5’	171.8, C	171.8, C	173.7, C	173.7, C
2’-NCH_3	25.5, CH_3	25.5, CH_3	25.6, CH_3	25.6, CH_3
4’-NCH_3	23.9, CH_3	23.9, CH_3	24.2, CH_3	24.2, CH_3
8-OCH_3	57.3, CH_3	57.3, CH_3	57.0, CH_3	57.1, CH_3

2.1.4 Conclusion:

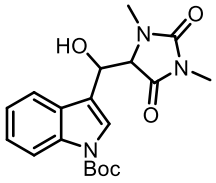
Thus, we have accomplished the first total synthesis of four natural products, oxoaplysinopsin D, E, F and G using a short and simple route. All four oxoaplysinopsins were synthesized from novel common dihydroxy intermediate which was accessed in a gram scale. Aldol reaction and one pot PDC mediated oxidation of alcohol to hydroxy ketone were the key steps of the synthesis. We have also confirmed reactivity of secondary alcohol *vs* alcohol on tertiary nitrogenated carbon present in

dihydroxy intermediate and showed that alcohol on tertiary nitrogenated carbon is more reactive than secondary alcohol. Moreover, while synthesizing oxoaplysinopsins D and F, we have observed interesting TBS-migration which afforded regioisomers of natural product oxoaplysinopsins D and F. All the spectral data of synthesized natural products are in complete agreement with that of natural products isolated marine organism.

2.1.5 Experimental section

Experimental procedures and characterization data of selected compounds are given below; Data of remaining compounds can be found at (*Eur. J. Org. Chem.* 2021, 2188; doi.org/10.1002/ejoc.202100184)

***tert*-butyl 3-((1,3-dimethyl-2,5-dioxoimidazolidin-4-yl)(hydroxy)methyl)-1H-indole-1- carboxylate (38):**



To a stirred solution of *N*,*N*-dimethyl hydantoin (1.0 equiv.) in 20 mL THF, 1M lithium bis(trimethylsilyl)amide (2.0 equiv.) was added dropwise at -78 °C for 15 min. indole-3-carboxaldehyde (1.2 equiv.) in THF (20 ml) was then added dropwise and the resultant mixture was stirred at -78 °C for 1 h. The reaction mixture was quenched with saturated NH_4Cl and allowed to warm to room temperature. The aqueous layer was extracted with EtOAc (3x30 mL), the combined organic extract washed with brine, dried (Na_2SO_4) and concentrated in *vaccuo*. The crude product was purified by column chromatography to afford white solid **38** as diastereomeric mixtures.

Yield= 67% (mixture of diastereomers, dr ratio = 4 : 1)

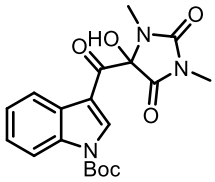
IRυ_{max}(film): 3437, 2355, 2324, 1707 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.19 (d, *J* = 8.2 Hz, 1 H), 8.17 - 7.56 (m, 2 H), 7.29 - 7.25 (m, 2 H), 5.57- 5.55 (m, 1 H), 4.33 - 4.28 (m, 1 H), 3.32 (br. s., 1H), 3.02 (s, 2 H), 2.89 (s, 1 H), 2.84 (s, 1 H), 2.65 (s, 2 H), 1.78 (s, 1 H), 1.67 (s, 8 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 171.9, 171.6, 157.8, 157.3, 149.4, 135.7, 127.5, 125.0, 124.9, 124.6, 123.4, 123.0, 122.9, 119.7, 119.4, 119.0, 118.0, 115.7, 115.4, 84.3, 67.0, 65.6, 65.6, 30.2, 30.0, 28.1, 25.0, 24.9

HRMS (ESI): *m/z* calculated for $C_{19}H_{23}N_3O_5Na$ $[M+Na]^+$ = 396.1530, Observed = 396.1530.

***tert*-butyl 3-(4-hydroxy-1,3-dimethyl-2,5-dioxoimidazolidine-4-carbonyl)-1H-indole-1-carboxylate (40):**



To a stirred solution of **38** (1 equiv.) in DMF (5 mL), pyridinium dichromate (3 equiv.) was added at 0 °C and resultant mixture stirred for 3 h at room temperature. After completion of reaction EtOAc was added and decanted the solvent thrice. Combined organic extract and washed with water (30 mL), dried on Na_2SO_4 and concentrated in *vaccuo*. The crude product was purified by column chromatography to afford desired products **40** as white solid.

Yield= 66%

Melting point: 159 -162 °C

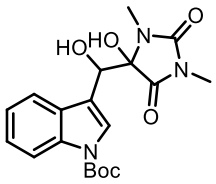
IRυ_{max}(film): 3364, 2982, 1720, 1537 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.38 (d, *J* = 6.7 Hz, 1 H), 8.16 (d, *J* = 7.9 Hz, 1 H), 8.06 (s, 1 H), 7.47 - 7.27 (m, 2 H), 5.81 (s, 1 H), 3.20 (s, 3 H), 2.85 (s, 3 H), 1.70 (s, 9 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 185.9, 169.6, 156.1, 148.0, 135.2, 132.7, 127.5, 126.5, 125.3, 122.6, 115.2, 113.5, 86.7, 86.5, 28.0, 25.3, 24.9

HRMS (ESI): *m/z* calculated for $C_{19}H_{21}N_3O_6Na$ $[M+Na]^+$ = 410.1323, Observed = 410.1320.

***tert*-butyl-3-(hydroxy(4-hydroxy-1,3-dimethyl-2,5-dioxoimidazolidin-4-yl)methyl)-1H-indole-1-carboxylate (33):**



To a stirred solution of *tert*-butyl 3-(4-hydroxy-1,3-dimethyl-2,5-dioxoimidazolidine4-carbonyl)-1H-indole-1-carboxylate **40** (1 g, 2.583 mmol) in THF (30 mL), added $NaBH_4$ (195 mg, 5.163 mmol) at 0 °C and reaction mixture was stirred for 3 h at room temperature, the reaction mixture was quenched with saturated NH_4Cl. The aqueous layer was extracted with EtOAc (3x20 mL), the combined organic extract washed with brine (20 mL), dried (Na_2SO_4) and concentrated in *vaccuo*. The crude product was purified by column chromatography to afford compound **33** as white solid.

Yield= 720 mg, 72% (mixture of diastereomers, dr ratio= 3 : 2)

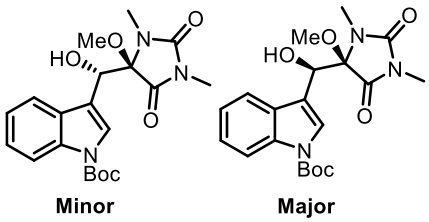
IRυ_{max}(film): 3398, 2361, 2334, 1706 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.12 - 8.09 (m, 1 H), 7.68 - 7.54 (m, 1 H), 7.52 - 7.50 (m, 1 H), 7.31 - 7.19 (s, 2 H), 5.42 – 5.25 (s, 1 H), 2.97 (s, 2 H), 2.90 (s, 1 H), 2.79 (s, 1 H), 2.76 (s, 2 H), 1.66 (m, 9 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 173.6, 172.3, 156.0, 155.9, 149.4, 149.3, 135.3, 135.2, 135.1, 128.6, 128.2, 125.0, 124.8, 124.6, 123.9, 122.9, 122.8, 122.5, 122.4, 119.8, 119.3, 116.7, 116.4, 115.3, 115.3, 86.7, 86.5, 84.4, 84.4, 77.2, 70.3, 69.3, 28.1, 25.8, 25.2, 24.6, 24.5

HRMS (ESI): *m/z* calculated for $C_{19}H_{23}N_3O_6Na$ $[M+Na]^+$ = 412.1479, Observed = 412.1479.

(±)-*tert*-butyl 3-(hydroxy(4-methoxy-1,3-dimethyl-2,5-dioxoimidazolidin-4-yl)methyl)-1H-indole-1-carboxylate (41a & 41b):



Minor **Major**

To a solution of *ter*t-butyl 3-(hydroxy(4-hydroxy-1,3-dimethyl-2,5-dioxoimidazolidin4-yl)methyl)-1H-indole-1-carboxylate **33** (300 mg, 0.7704 mmol) in

dry DMF (5 mL) was added K_2CO_3 (212 mg, 1.5408 mmol) and excess methyl iodide (0.4 mL) under positive pressure of argon and stirred for 12 h at room temperature. The reaction mixture was added to cold water and extracted with ethyl acetate (3 x30 mL). The combined organic layer was washed with water (2 x 20 mL), brine (20 mL) and dried over Na_2SO_4, concentrated under reduced pressure. The crude product was purified by column chromatography to afford pure product as yellow solid **41a** and **41b** as diastereomeric mixture (2:3).

Yield: 223 mg; 72%

Data for minor isomer (41a):

Yield= 56 mg; White solid

Melting point: 159 -161 °C

IRυ_{max}(film): 3452, 3021, 2254, 1720 cm^{-1}

^{1}H NMR (400 MHz, CD_3OD) = δ 8.10 (d, *J* = 8.2 Hz, 1 H), 7.63 - 7.60 (m, 2 H), 7.27 (dt, *J* = 0.9, 7.8 Hz, 1 H), 7.21 - 7.17 (m, 1 H), 5.31 (d, *J* = 0.9 Hz, 1 H), 3.15 (s, 3 H), 3.10 (s, 3 H), 2.82 (s, 3 H), 1.68 (s, 9 H)

^{13}C NMR (100 MHz, CD_3OD) = δ 171.5, 158.1, 151.1, 136.7, 130.9, 126.0, 125.4, 123.6, 121.5, 119.5, 116.0, 94.9, 85.2, 69.6, 52.5, 28.5, 25.7, 24.6

HRMS (ESI): *m/z* calculated for $C_{20}H_{25}N_3O_6Na$ $[M+Na]^+$ = 426.1636, Observed = 426.1634.

Data for major isomer (41b):

Yield= 167 mg; White solid

Melting point: 168 - 170 °C

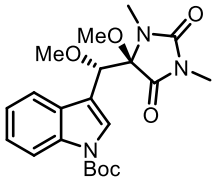
IRυ_{max}(film): 3453, 3020, 2252, 1721 cm^{-1}

^{1}H NMR (400 MHz, CD_3OD) = δ 8.11 (d, *J* = 8.2 Hz, 1 H), 7.83 (d, *J* = 7.8 Hz, 1 H), 7.64 (d, *J* = 0.9 Hz, 1 H), 7.29 - 7.19 (m, 2 H), 5.30 (d, *J* = 0.9 Hz, 1 H), 3.13 (s, 3 H), 3.02 (s, 3 H), 2.54 (s, 3 H), 1.67 (s, 9 H)

^{13}C NMR (100 MHz, CD_3OD) = δ 173.2, 158.7, 151.0, 137.1, 130.8, 125.9, 125.6, 123.8, 122.8, 121.1, 116.1, 95.1, 85.3, 70.3, 52.4, 28.5, 26.9, 24.8

HRMS (ESI): *m/z* calculated for $C_{20}H_{25}N_3O_6Na$ $[M+Na]^+$ = 426.1636, Observed = 426.1633.

(±)-*tert*-butyl 3-(methoxy(4-methoxy-1,3-dimethyl-2,5-dioxoimidazolidin-4-yl)methyl)-1H-indole-1-carboxylate (42):



To a solution of (±)-*tert*-butyl 3-(hydroxy(4-methoxy-1,3-dimethyl-2,5-dioxoimidazolidin-4-yl)methyl)-1H-indole-1-carboxylate **41a** (50 mg, 0.1240 mmol) in dry THF (8 mL) was added NaH (6 mg, 0.1488 mmol) and excess methyl iodide (0.4 mL) at 0 °C under positive pressure of argon and stirred for 2 h at room temperature. The reaction was quenched by adding saturated NH_4Cl solution and extracted with ethyl acetate (3x20 mL). The combined organic layer was washed water (2x20 mL), brine (20 mL) and dried over Na_2SO_4, concentrated under reduced pressure. The crude product was purified by column chromatography to afford pure product **42** as white solid.

Yield= 41 mg, 73%

Melting point: 68 - 70 °C

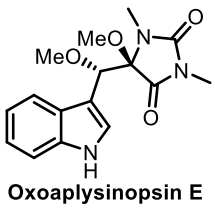
IRυ_{max}(film): 3746, 2361, 1725, 1456 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.11 (d, *J* = 8.1 Hz, 1 H), 7.59 - 7.56 (m, 2 H), 7.31 - 7.23 (m, 2 H), 4.91 (s, 1 H), 3.36 (s, 3 H), 3.17 (s, 3 H), 3.11 (s, 3 H), 2.82 (s, 3 H), 1.68 (s, 9 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 169.2, 156.3, 149.5, 135.2, 129.4, 125.6, 124.4, 122.6, 119.9, 115.1, 114.2, 92.5, 84.1, 78.4, 58.2, 52.0, 28.2, 25.6, 24.3

HRMS (ESI): *m/z* calculated for $C_{21}H_{27}N_3O_6Na$ $[M+Na]^+$ = 440.1792, Observed = 440.1789.

(±)-5-((1H-indol-3-yl)(methoxy)methyl)-5-methoxy-1,3-dimethylimidazolidine2,4-dione (28):



Oxoaplysinopsin E

In a 10 mL round bottle flask filled with 5 mL of water, (±)-*tert*-butyl 3-(methoxy(4-methoxy-1,3-dimethyl-2,5-dioxoimidazolidin-4-yl)methyl)-1H-indole-1-carboxylate **42** (25 mg) was added. Then reaction was refluxed at 110 °C for 1 h, the reaction mixture was cooled down after 1 h and was extracted with ethyl acetate (40 mL×3). The extract was washed with brine, dried over anhydrous Na_2SO_4, and then concentrated in *vacuum*. The residue was purified by column chromatography on silica gel with ethyl acetate/petroleum ether to afford the product **28** as yellow solid.

Yield= 14 mg, 77%

Melting point: 143 - 145 °C

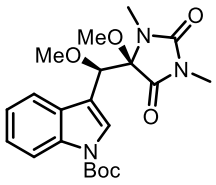
IRυ_{max}(film): 3348, 2932, 1715, 1465 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 11.17 (br. s., 1 H), 7.53 (d, *J* = 7.8 Hz, 1 H), 7.35 (d, *J* = 8.2 Hz, 1 H), 7.17 (d, *J* = 2.3 Hz, 1 H), 7.06 (dd, *J* = 7.2, 7.9 Hz, 1 H), 6.97 (dd, *J* = 7.8, 7.3 Hz, 1 H), 4.94 (s, 1 H), 3.19 (s, 3 H), 3.03 (s, 3 H), 3.02 (s, 3 H), 2.60 (s, 3 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 169.2, 155.7, 135.7, 127.0, 124.8, 121.0, 118.9, 118.8, 111.5, 107.9, 92.5, 77.9, 57.1, 51.4, 25.6, 23.9

HRMS (ESI): *m/z* calculated for $C_{16}H_{19}N_3O_4Na$ $[M+Na]^+$ = 340.1268 Observed = 340.1268.

(±)-*tert*-butyl 3-(methoxy(4-methoxy-1,3-dimethyl-2,5-dioxoimidazolidin-4-yl)methyl)-1H-indole-1-carboxylate (43):



To a solution of (±)-*tert*-butyl 3-(hydroxy(4-methoxy-1,3-dimethyl-2,5-dioxoimidazolidin-4-yl)methyl)-1H-indole-1-carboxylate **41b** (30 mg, 0.0744 mmol) in dry THF (8 mL) was added NaH (4 mg, 0.0893 mmol) and excess methyl iodide (0.4 mL) at 0 °C under positive pressure of argon and stirred for 2 h at room temperature. The reaction was quenched by adding saturated NH_4Cl solution and extracted with ethyl acetate (3 x20 mL). The combined organic layer was washed with water (2 x20 mL), brine (20 mL) and dried over Na_2SO_4, concentrated under reduced pressure. The crude

product was purified by column chromatography to afford pure product **43** as white solid.

Yield= 22 mg, 71%

Melting point: 111 -113 °C

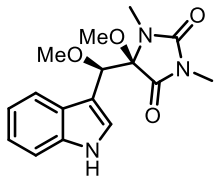
IRυ_{max}(film): 2927, 2360, 2318, 1725 cm^{-1}

^{1}H NMR (500 MHz, $CDCl_3$) = δ 8.18 - 8.16 (m, 1 H), 7.83 (d, *J* = 7.6 Hz, 1 H), 7.61 (br. s., 1 H), 7.37 - 7.34 (m, 1 H), 7.30 - 7.26 (m, 1 H), 4.93 (s, 1 H), 3.35 (s, 3 H), 3.14 (d, *J* = 9.5 Hz, 6 H), 2.52 (s, 3 H), 1.72 (s, 9 H)

^{13}C NMR (125 MHz, $CDCl_3$) = δ 171.4, 157.0, 149.5, 135.5, 129.3, 125.6, 124.5, 122.9, 121.4, 115.1, 92.3, 84.1, 78.7, 58.2, 51.6, 28.2, 26.3, 24.6

HRMS (ESI): *m/z* calculated for $C_{21}H_{27}N_3O_6Na$ $[M+Na]^+$ = 440.1792, Observed = 440.1792.

(±)-5-((1H-indol-3-yl)(methoxy)methyl)-5-methoxy-1,3-dimethylimidazolidine2,4-dione (30):



Oxoaplysinopsin G

In a 10 mL round bottle flask filled with 5 mL of water, (±)-*tert*-butyl 3-(methoxy(4-methoxy-1,3-dimethyl-2,5-dioxoimidazolidin-4-yl)methyl)-1H-indole-1-carboxylate **43** (20 mg) was added. Then reaction was refluxed at 110 °C for 1 h, the reaction mixture was cooled down after 1 h and was extracted with ethyl acetate (15 mL×3). The extract was washed with brine, dried over anhydrous Na_2SO_4, and then concentrated in *vacuum*. The residue was purified by column chromatography on silica gel with ethyl acetate/petroleum ether to afford the product **30** as yellow solid.

Yield= 12 mg, 80%;

Melting point: 148 -150 °C

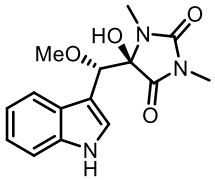
IRυ_{max}(film): 3346, 2354, 1716, 1215 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6) = δ 11.19 (br. s., 1 H), 7.58 (d, *J* = 7.9 Hz, 1 H), 7.37 (d, *J* = 8.0 Hz, 1 H), 7.29 (d, *J* = 2.4 Hz, 1 H), 7.13 - 7.04 (m, 1 H), 7.04 - 6.92 (m, 1 H), 4.89 - 4.80 (m, 1 H), 3.19 (s, 3 H), 3.01 (s, 3 H), 2.90 (s, 3 H), 2.33 (s, 3 H)

^{13}C NMR (100 MHz, DMSO-d_6) = δ 171.40, 156.6, 136.8, 126.8, 125.8, 121.6, 120.4, 119.5, 112.1, 108.4, 92.3, 79.4, 57.7, 51.8, 26.2, 24.7

HRMS (ESI): *m/z* calculated for $C_{16}H_{19}N_3O_4Na$ $[M+Na]^+$ = 340.1268, Observed = 340.1267.

(±)-5-(hydroxy(1H-indol-3-yl)methyl)-5-methoxy-1,3-dimethylimidazolidine-2,4-dione (Expected 27): (Obtained through Scheme 2.1.9)



Expected

In a 10 mL round bottle flask filled with 5 mL of water, (±)-*tert*-butyl 3-(hydroxy(4-methoxy-1,3-dimethyl-2,5-dioxoimidazolidin-4- yl)methyl)-1H-indole-1-carboxylate **41a (expected 44a)** (25 mg) was added. Then the reaction mixture was refluxed at 110 °C for 1 h, then it was cooled down after 1 h and was extracted with ethyl acetate (30 mL×3). The extract was washed with brine, dried over anhydrous Na_2SO_4, and then concentrated in *vacuum*. The residue was purified by column chromatography on silica gel with ethyl acetate/petroleum ether to afford the product **27 (expected)** as sticky solid.

Yield= 14 mg, 78%

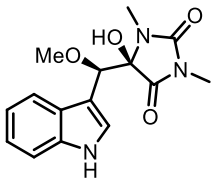
IRυ_{max}(film): 3452, 3021, 2245, 1705 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6) = δ 11.01 (br. s., 1 H), 7.51 (d, *J* = 7.6 Hz, 1 H), 7.32 (d, *J* = 8.4 Hz, 1 H), 7.18 (d, *J* = 2.3 Hz, 1 H), 7.07 - 7.00 (m, 1 H), 6.98 - 6.89 (m, 1 H), 5.86 (d, *J* = 4.6 Hz, 1 H), 5.22 (d, *J* = 4.6 Hz, 1 H), 3.03 (d, *J* = 6.1 Hz, 6 H), 2.62 (s, 3 H)

^{13}C NMR (100 MHz, DMSO-d_6) = δ 169.7, 155.7, 135.6, 126.4, 124.0, 120.7, 119.2, 118.4, 112.1, 111.3, 93.1, 68.2, 51.4, 25.4, 23.9

HRMS (ESI): *m/z* calculated for $C_{15}H_{17}N_3O_4Na$ [M+Na]+ = 326.1111, Observed = 326.1112.

(±)-5-(hydroxy(1H-indol-3-yl)methyl)-5-methoxy-1,3-dimethylimidazolidine-2,4-dione (Expected 29): (Obtained through Scheme 2.1.9)



Expected

In a 10 mL round bottle flask filled with 5 mL of water, (±)-*tert*-butyl 3-(hydroxy(4-methoxy-1,3-dimethyl-2,5-dioxoimidazolidin-4- yl)methyl)-1H-indole-1-carboxylate **41b (expected 44b)** (25 mg) was added. Then reaction was refluxed at 110 °C for 1 h, The reaction mixture was cooled down after 1.5 h and was extracted with ethyl acetate (30 mL×3). The extract was washed with brine, dried over anhydrous Na_2SO_4, and then concentrated in *vacuum*. The residue was purified by column chromatography on silica gel with ethylacetate/petroleum ether to afford the product **29 (expected)** as sticky solid.

Yield= 13 mg, 73%

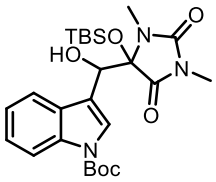
IRυ_{max}(film): 3455, 3025, 2262, 1715 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6) = δ 11.14 - 10.84 (m, 1 H), 7.66 (d, *J* = 7.8 Hz, 1 H), 7.34 (d, *J* = 7.8 Hz, 1 H), 7.28 (d, *J* = 1.8 Hz, 1 H), 7.09 - 7.01 (m, 1 H), 7.00 - 6.89 (m, 1 H), 5.89 (d, *J* = 5.5 Hz, 1 H), 5.21 - 5.12 (m, 1 H), 3.01 (s, 3 H), 2.91 (s, 3 H), 2.37 (s, 3 H)

^{13}C NMR (100 MHz, DMSO-d_6) = δ 171.4, 156.3, 136.2, 126.4, 124.3, 120.8, 120.5, 118.6, 113.2, 111.3, 93.2, 68.7, 51.4, 25.9, 24.1

HRMS (ESI): *m/z* calculated for $C_{15}H_{17}N_3O_4Na$ $[M+Na]^+$ = 326.1111, Observed = 326.1113.

***tert*-butyl 3-((4-((tert-butyldimethylsilyl)oxy)-1,3-dimethyl-2,5-dioxoimidazolidin4-yl)(hydroxy)methyl)-1H-indole-1-carboxylate (46):**



To a stirred solution of *tert*-butyl 3-(4-((tert-butyldimethylsilyl)oxy)-1,3-dimethyl-2,5-dioxoimidazolidine-4-carbonyl)-1H-indole-1-carboxylate **45** (300 mg, 0.598 mmol) in THF (20 mL), added $NaBH_4$ (195 mg, 5.163 mmol) at 0 °C and reaction mixture was stirred for 3 h at room temperature, the reaction mixture was quenched with saturated NH_4Cl. The aqueous layer was extracted with EtOAc (3x20 mL), the combined organic extract washed with brine (20 mL), dried (Na_2SO_4) and concentrated in *vaccuo*. The crude product was purified by column chromatography to afford **46** as white solid.

Yield= 231 mg, 77% (mixture of diastereomers, dr ratio= 3 : 2)

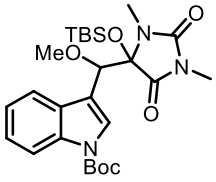
IRυ_{max}(film): 3374, 2934, 2362, 1711 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.12 (dd, *J* = 8.3, 14.8 Hz, 1 H), 8.09 – 7.61 (m, 2 H), 7.33 - 7.23 (m, 2 H), 5.33 (s, 1 H), 3.94 (br. s., 1 H), 3.07 (s, 2 H), 2.96 (s, 1 H), 2.78 (s, 2 H), 2.60 (s, 1 H), 1.68 (d, *J* = 1.8 Hz, 9 H), 0.93 (s, 5 H), 0.87 (s, 4 H), 0.13 (s, 2 H), 0.03 (s, 1 H), -0.13 (s, 2 H), -0.22 (s, 1 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 173.3, 171.3, 156.9, 155.9, 149.3, 135.5, 135.1, 128.8, 128.1, 125.4, 124.8, 124.7, 122.8, 121.5, 119.5, 117.6, 116.8, 115.2, 115.1, 87.7, 87.2, 84.2, 84.2, 77.2, 71.2, 70.8, 28.2, 26.4, 25.6, 25.6, 25.4, 24.6, 24.5, 18.0, 17.9, -4.5, -4.6, -5.5, -5.8

HRMS (ESI): *m/z* calculated for $C_{25}H_{37}N_3O_6SiNa$ $[M+Na]^+$ = 526.2344, Observed = 526.2340.

***tert*-butyl 3-((4-((tert-butyldimethylsilyl)oxy)-1,3-dimethyl-2,5-dioxoimidazolidin4-yl)(methoxy)methyl)-1H-indole-1-carboxylate (47):**



To a solution of *tert*-butyl 3-((4-((tert-butyldimethylsilyl)oxy)-1,3-dimethyl-2,5-dioxoimidazolidin-4-yl)(hydroxy)methyl)-1H-indole-1-carboxylate **46** (150 mg,

0.2982 mmol) in dry THF (10 mL) was added NaH (15 mg, 0.5964 mmol) and excess methyl iodide (0.5 mL) at 0 °C under positive pressure of argon and stirred for 2 h at room temperature. The reaction was quenched by adding saturated NH_4Cl solution and extracted with ethyl acetate (3 x20 mL). The combined organic layer was washed with water (2 x20 mL), brine (20 mL) and dried over Na_2SO_4, concentrated under reduced pressure. The crude product was purified by column chromatography to afford pure product **47** as white solid.

Yield= 94 mg, 61% (mixture of diastereomers)

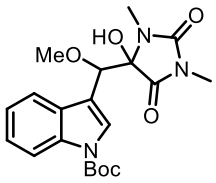
IRυ_{max}(film): 2933, 2357, 1724, 1456 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.12 (dd, *J* = 8.4, 16.8 Hz, 1 H), 7.82 - 7.55 (m, 2 H), 7.32 - 7.22 (m, 2 H), 5.35 - 5.29 (m, 1 H), 3.10 (dd, 6 H), 2.79 (s, 1 H), 2.54 (s, 2 H), 1.69 (br. s., 9 H), 0.87 (s, 9 H), 0.10 (s, 1 H), 0.02 (s, 2 H), -0.11 (s, 1 H), -0.20 (s, 2 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 171.4, 169.6, 157.1, 156.2, 149.5, 135.5, 135.1, 129.2, 128.8, 125.5, 125.5, 124.4, 124.2, 122.5, 122.4, 121.9, 120.0, 118.9, 117.8, 115.0, 114.9, 93.7, 93.2, 83.9, 83.8, 70.6, 70.3, 51.9, 51.9, 28.2, 26.7, 25.6, 24.6, 24.2, 18.0, 17.8, 0.0, -4.5, -4.9, -5.2, -5.8

HRMS (ESI): *m/z* calculated for $C_{26}H_{39}N_3O_6SiNa$ $[M+Na]^+$ = 540.2500, Observed = 540.2499.

(±)-*tert*-butyl 3-(hydroxy(4-methoxy-1,3-dimethyl-2,5-dioxoimidazolidin-4 yl)methyl)-1H-indole-1-carboxylate (44) (Obtained through Scheme 2.1.10):



Expected

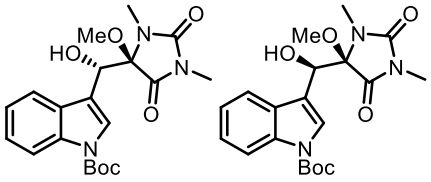
To a stirred solution of *tert*-butyl 3-((4-((tert-butyldimethylsilyl)oxy)-1,3-dimethyl2,5-dioxoimidazolidin-4-yl)(methoxy)methyl)-1H-indole-1-carboxylate **47** (200 mg, 0.3866 mmol) in THF (10 mL), added tetrabutyl ammonium fluoride 1M in THF solution (0.7 mL, 0.7732 mmol) at 0 °C and allow to stirred at room temperature for 1 h, the reaction mixture was quenched with water. The aqueous layer was extracted with EtOAc (3x20 mL), the combined organic extract washed with brine (20 mL), dried on

Na_2SO_4 and concentrated in *vaccuo* to get diastereomeric mixture of **44 (expected)** with 2:1 diastereomeric ratio (130 mg, 84%) as white solid.

^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.10 (t, *J* = 7.6 Hz, 1 H), 7.82 - 7.55 (m, 2 H), 7.34 - 7.14 (m, 2 H), 5.31 (dd, *J* = 0.9, 3.7 Hz, 1 H), 3.14 (d, *J* = 5.0 Hz, 3 H), 3.10 (s, 2 H), 3.02 (s, 1 H), 2.81 (s, 2 H), 2.55 (s, 1 H), 1.68 (s, 9 H)

^{13}C NMR (100 MHz, $CDCl_3$) = 173.2, 171.5, 158.7, 158.1, 151.0, 137.1, 136.7, 130.9, 130.8, 126.0, 125.9, 125.6, 125.4, 123.8, 123.6, 122.8, 121.5, 121.1, 119.5, 116.0, 95.1, 94.8, 85.3, 85.2, 70.3, 69.6, 52.5, 52.4, 28.5, 26.9, 25.7, 24.8, 24.6

***tert*-butyl 3-(hydroxy(4-methoxy-1,3-dimethyl-2,5-dioxoimidazolidin-4-yl)methyl)-1H-indole-1-carboxylate (41a and 41b)** (Obtained through Scheme 2.1.12):



Major **Minor**

To a stirred solution of *tert*-butyl 3-(4-methoxy-1,3-dimethyl-2,5-dioxoimidazolidine-4-carbonyl)-1Hindole-1-carboxylate **48** (300 mg, 0.598 mmol) in THF (20 mL), added $NaBH_4$ (195 mg, 5.163 mmol) at 0 °C and reaction mixture was stirred for 3 h at room temperature, the reaction mixture was quenched with saturated NH_4Cl. The aqueous layer was extracted with EtOAc (3x20 mL), the combined organic extract washed with brine (20 mL), dried (Na_2SO_4) and concentrated in *vaccuo*. The crude product was purified by column chromatography to afford **41a** and **41b** as white solid.

Data for major (41a):

Yield= 142 mg

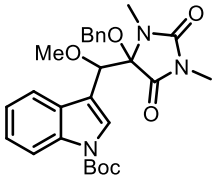
^{1}H NMR (200MHz, CD_3OD) = δ 8.09 (d, *J* = 7.5 Hz, 1 H), 7.67 - 7.51 (m, 2 H), 7.34 - 7.05 (m, 2 H), 5.31 (d, *J* = 0.9 Hz, 1 H), 3.12 (d, *J* = 8.0 Hz, 6 H), 2.82 (s, 3 H), 1.68 (s, 9 H)

Data for minor (41b):

Yield= 70 mg

^{1}H NMR (200MHz, CD_3OD) = δ 8.11 (d, *J* = 7.6 Hz, 1 H), 7.92 - 7.76 (m, 1 H), 7.64 (d, *J* = 1.0 Hz, 1 H), 7.25 (ddd, *J* = 1.4, 7.9, 9.7 Hz, 2 H), 5.30 (d, *J* = 1.1 Hz, 1 H), 3.13 (s, 3 H), 3.02 (s, 3 H), 2.55 (s, 3 H), 1.68 (s, 9 H)

***tert*-butyl 3-((4-(benzyloxy)-1,3-dimethyl-2,5-dioxoimidazolidin-4-yl)(methoxy)methyl)-1H-indole-1-carboxylate (50):**



To a solution of *tert*-butyl 3-((4-(benzyloxy)-1,3-dimethyl-2,5-dioxoimidazolidin-4-yl)(hydroxy)methyl)-1H-indole-1-carboxylate **49** (400 mg, 0.834 mmol) in dry THF (10 mL) was added NaH (30 mg, 1.251 mmol) and excess methyl iodide (0.5 mL) at 0 °C under positive pressure of argon and stirred for 2 h at room temperature. The reaction was quenched by adding saturated NH_4Cl solution and extracted with ethyl acetate (3 x20 mL). The combined organic layer was washed water (2 x20 mL), brine (20 mL) and dried over Na_2SO_4, concentrated under reduced pressure. The crude product was purified by column chromatography to afford pure product **50** as white solid.

Yield= 308 mg, 75% (mixture of diastereomers)

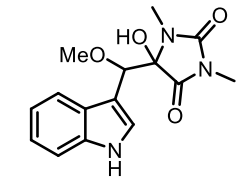
IRυ_{max}(film): 1719, 1458, 1344, 1215 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.13 (t, *J* = 7.1 Hz, 1 H), 7.73 - 7.62 (m, 1 H), 7.58 - 7.56 (m, 1 H), 7.29 - 7.26 (m, 4 H), 7.26 - 7.20 (m, 3 H), 4.99 (d, *J* = 1.1 Hz, 1 H), 4.40 - 4.16 (m, 2 H), 3.36 (d, *J* = 8.6 Hz, 3 H), 3.13 (s, 1 H), 3.09 - 3.04 (m, 2 H), 2.87 (s, 2 H), 2.48 (s, 1 H), 1.66 (d, *J* = 7.8 Hz, 9 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 171.3, 169.0, 168.4, 156.8, 156.3, 155.9, 149.5, 149.4, 136.2, 135.9, 135.8, 135.5, 135.3, 129.5, 129.1, 128.8, 128.3, 128.1, 128.0, 128.0, 127.8, 127.7, 127.6, 127.6, 125.5, 125.5, 124.5, 124.3, 122.7, 122.5, 121.7, 120.3, 120.0, 115.1, 115.0, 115.0, 114.9, 114.4, 113.5, 92.1, 91.8, 84.0, 83.3, 78.9, 78.3, 66.8, 66.5, 58.5, 58.3, 28.2, 28.2, 27.6, 26.6, 25.5, 24.7

HRMS (ESI): *m/z* calculated for $C_{27}H_{31}N_3O_6Na$ $[M+Na]^+$ = 516.2105, Observed = 516.2103.

5-((1H-indol-3-yl)(methoxy)methyl)-5-hydroxy-1,3-dimethylimidazolidine-2,4-dione (27 and 29) :



Oxoaplysinopsin D and F

In a 10 mL round bottle flask filled with 5 mL of water, *tert*-butyl 3-((4-hydroxy-1,3-dimethyl-2,5-dioxoimidazolidin-4-yl)(methoxy)methyl)-1H-indole-1-carboxylate **51** (50 mg) was added. Then reaction was refluxed at 110 °C for 1 h, the reaction mixture was cooled down after 1 h and was extracted with ethyl acetate (30 mL×3). The extract was washed with brine, dried over anhydrous Na_2SO_4, and then concentrated in *vacuum*. The residue was purified by column chromatography on silica gel with ethyl acetate/petroleum ether to afford the product **27** and **29** as diastereomeric mixture.

Yield= 30 mg, 80% (mixture of diastereomers)

IRυ_{max}(film): 3356, 2357, 1709, 1469 cm^{-1}

^{1}H NMR (200 MHz, DMSO-d_6) = δ 11.27 - 11.02 (m, 1 H), 7.76 - 7.47 (m, 1 H), 7.33 (dd, *J* = 7.4, 12.9 Hz, 1 H), 7.20 - 6.80 (m, 3 H), 3.87 (s, 1 H), 3.78 (s, 1 H), 3.65 (s, 1 H), 3.18 (d, *J* = 4.6 Hz, 2 H), 2.99 (s, 1 H), 2.86 (s, 2 H), 2.53 (br. s., 1 H), 2.21 (s, 2 H)

^{13}C NMR (100 MHz, DMSO-d_6) = δ 173.7, 171.8, 156.6, 155.6, 136.3, 135.8, 127.0, 126.5, 125.3, 124.6, 121.0, 121.0, 120.3, 119.0, 118.9, 118.8, 111.5, 111.4, 108.5, 108.3, 87.5, 86.8, 79.2, 78.8, 57.3, 57.1, 25.6, 25.5, 24.2, 23.9

HRMS (ESI): *m/z* calculated for $C_{15}H_{17}N_3O_4Na$ $[M+Na]^+$ = 326.1111, Observed = 326.1113.

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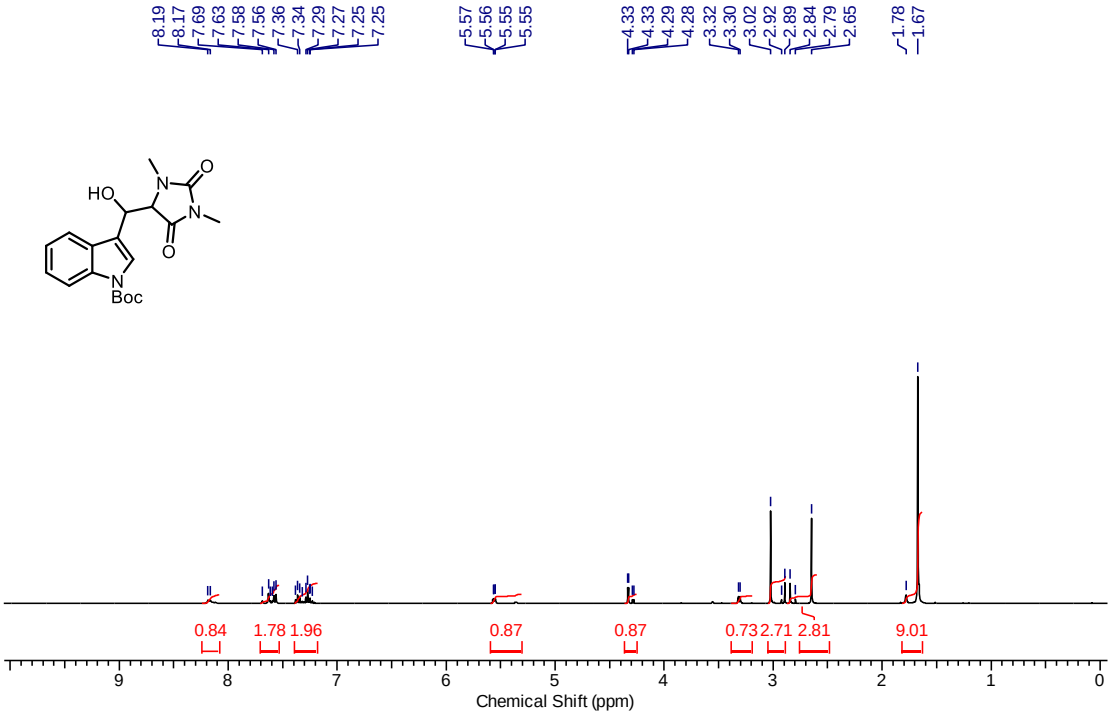
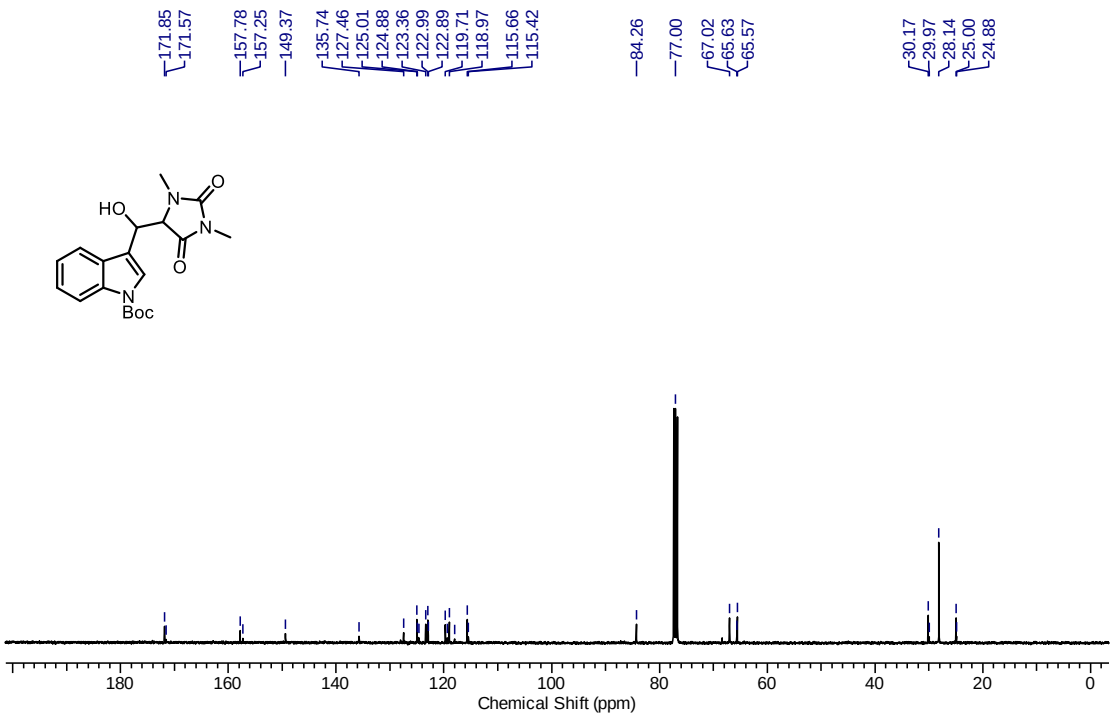
36. a) Siddaraju, Y.; Prabhu, K. R. *Org. Biomol. Chem.*, **2015**, *13*, 6749 b) Liang, Y. F.; Wu, K.; Song, S.; Li, X.; Huang, X.; Jiao, N. *Org. Lett.* **2015**, *17*, 876. c) Yu, J.; Cui, J.; Zang, C. *Eur. J. Org. Chem.* **2010**, 7020. d) Miao, C. B.; Wang, Y. H.; Xing, M. L.; Lu, X. W.; Sun, X. Q.; Yang, H. T. *J. Org. Chem.* **2013**, *78*, 11584.
37. Wang, J.; Liang, Y. L.; Qu, J. *Chem.Commun.* **2009**, 5144.

2.1.7 Copies of NMR spectra

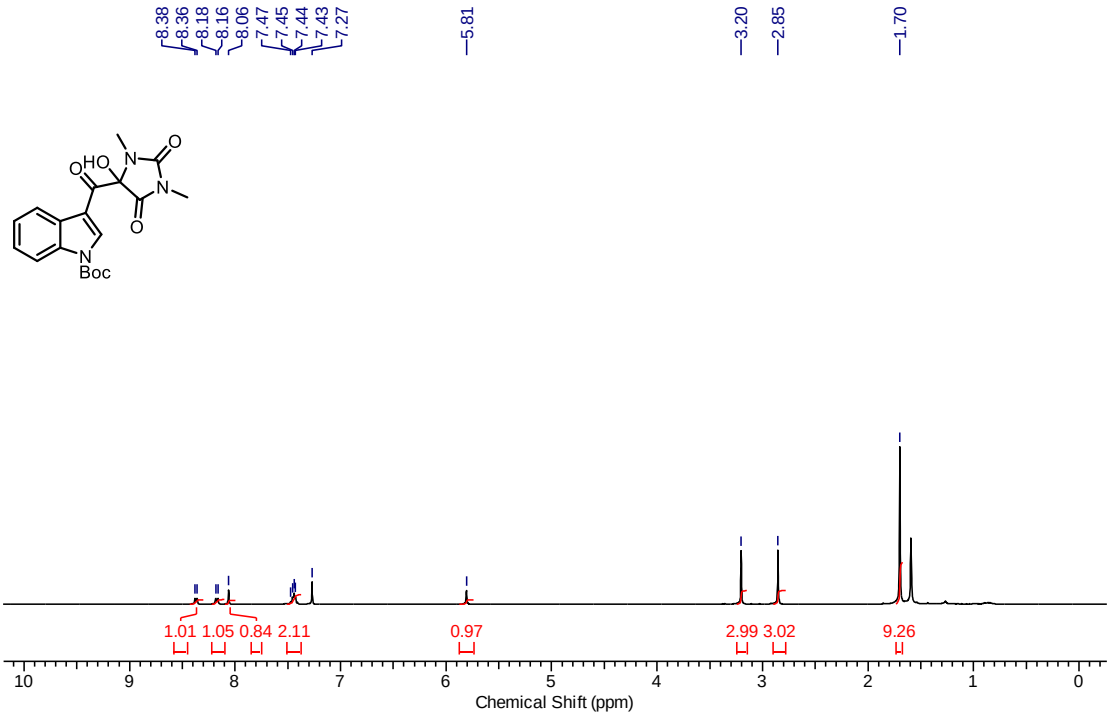
^{1}H NMR of Compound 38 in $CDCl_3$ at 400 MHz



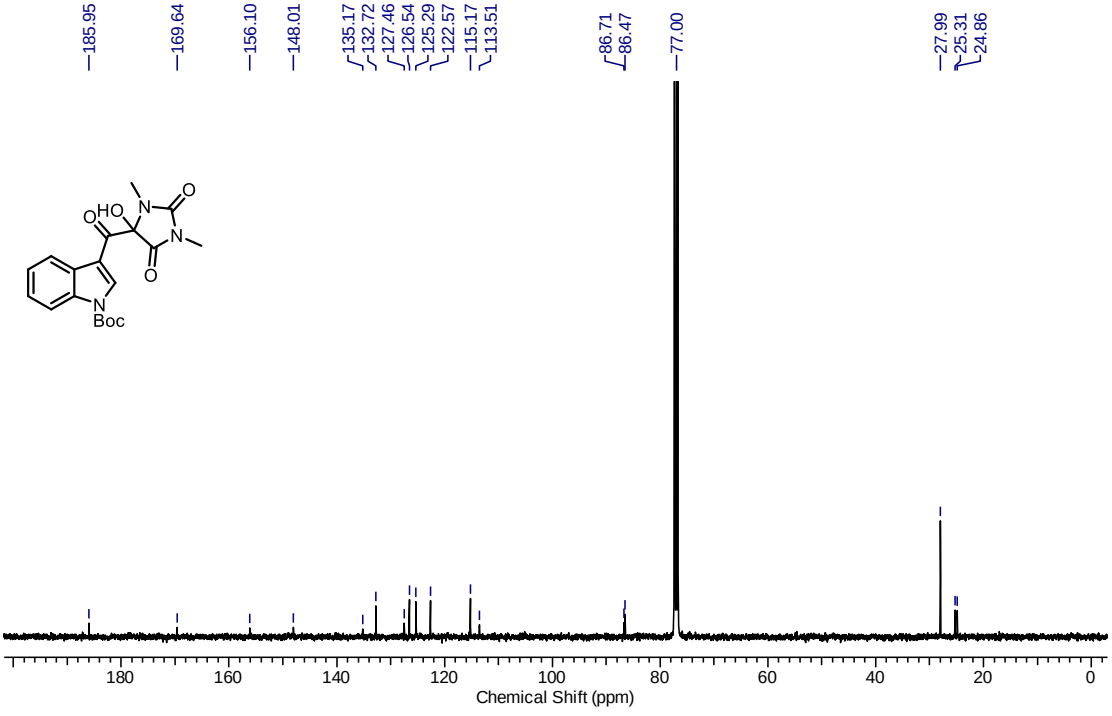
^{13}C NMR of Compound 38 in $CDCl_3$ at 100 MHz



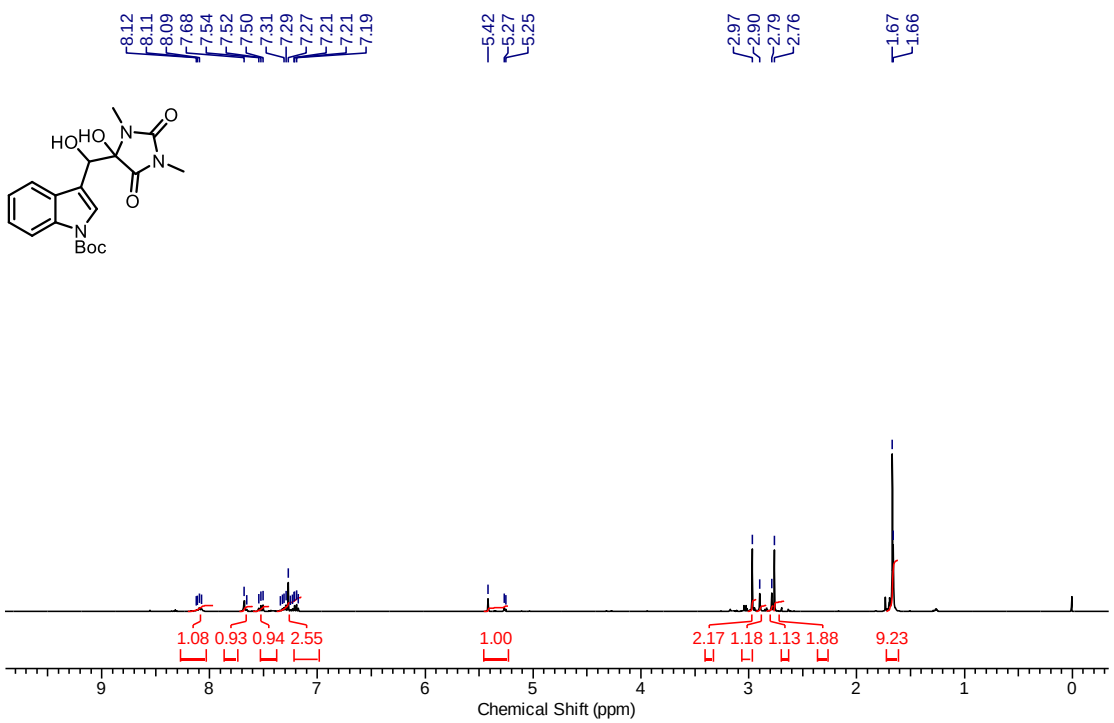
^{1}H NMR of Compound 40 in $CDCl_3$ at 400 MHz



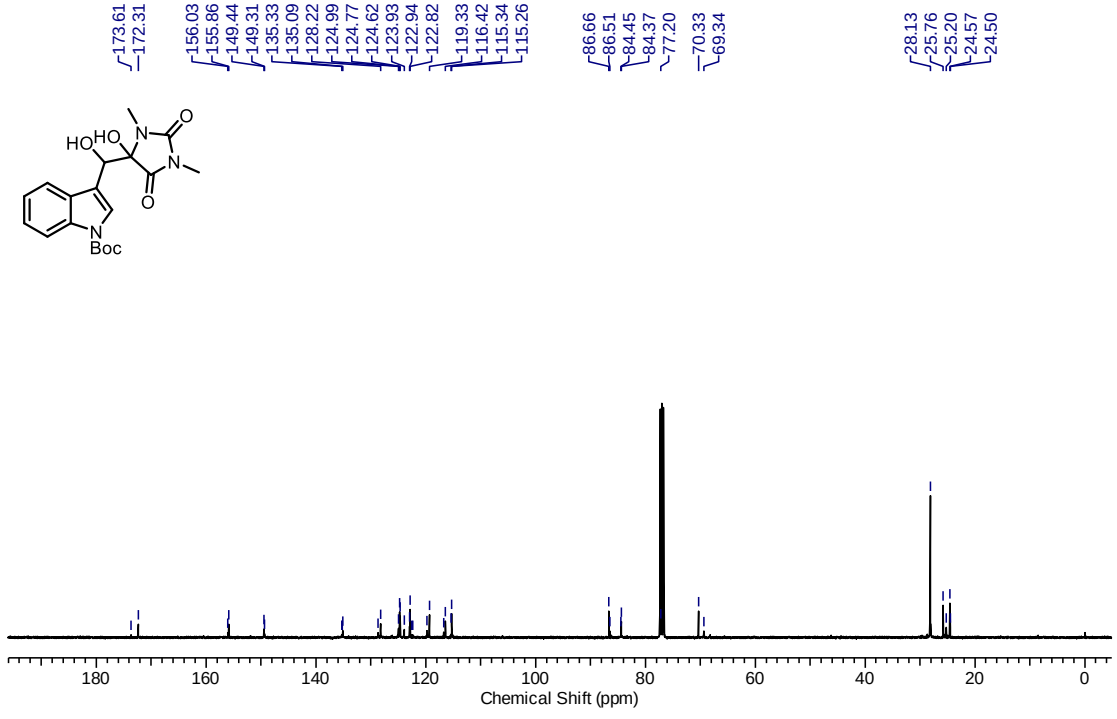
^{13}C NMR of Compound 40 in $CDCl_3$ at 100 MHz



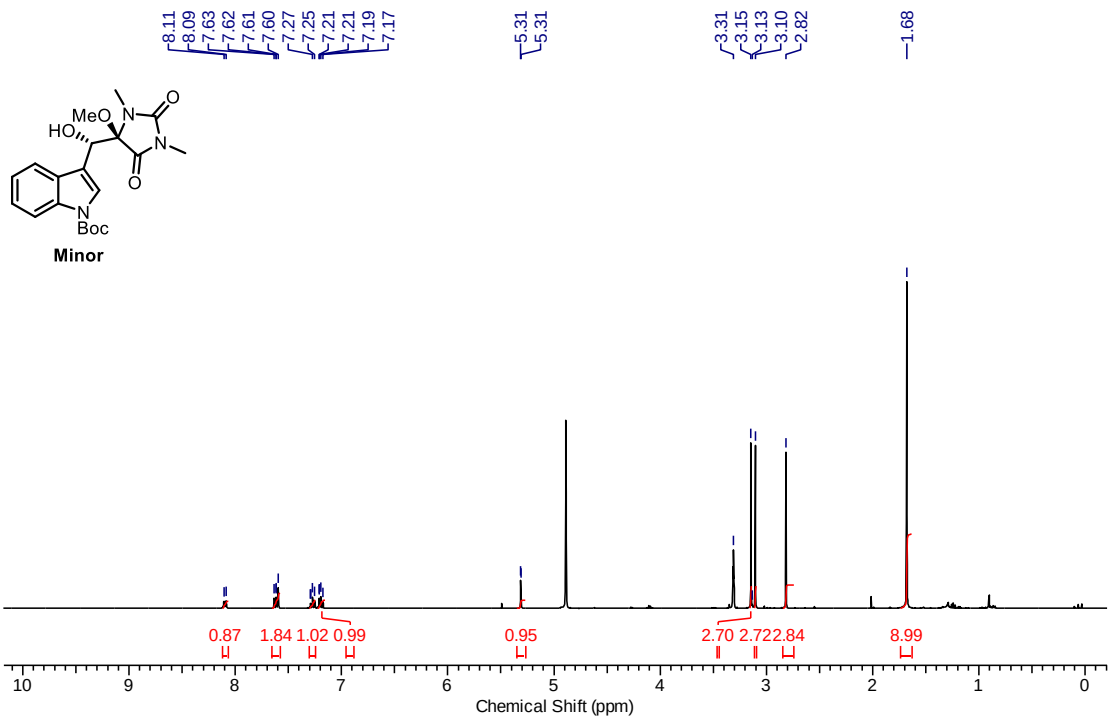
^{1}H NMR of Compound 33 in $CDCl_3$ at 400 MHz



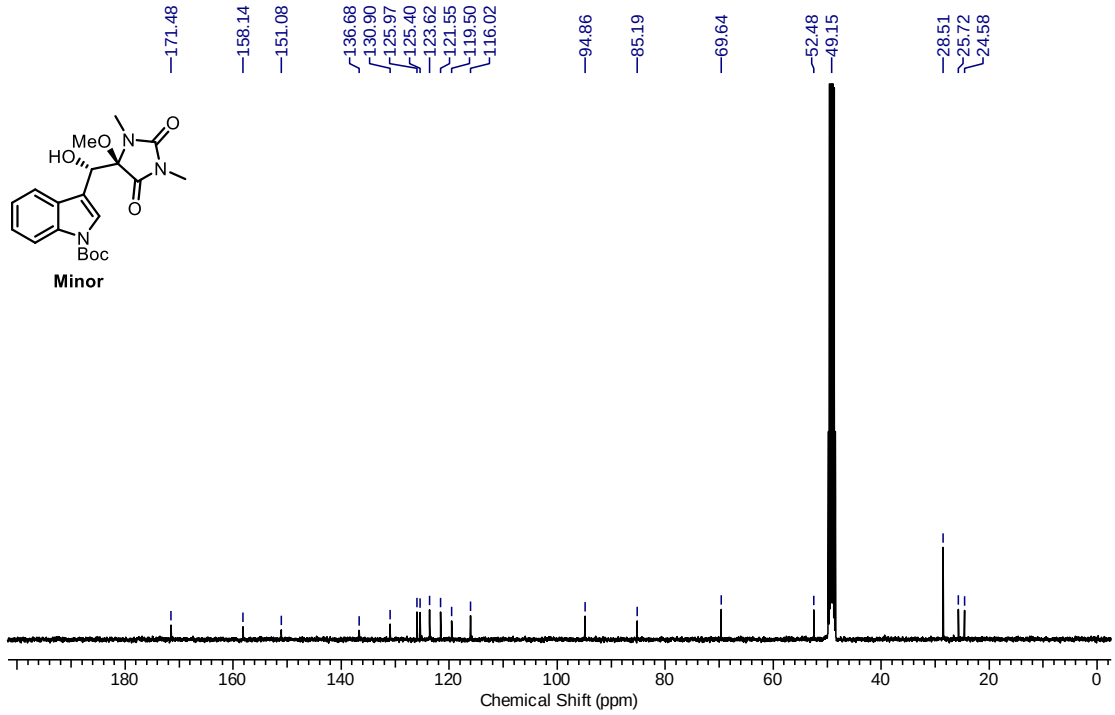
^{13}C NMR of Compound 33 in $CDCl_3$ at 100 MHz



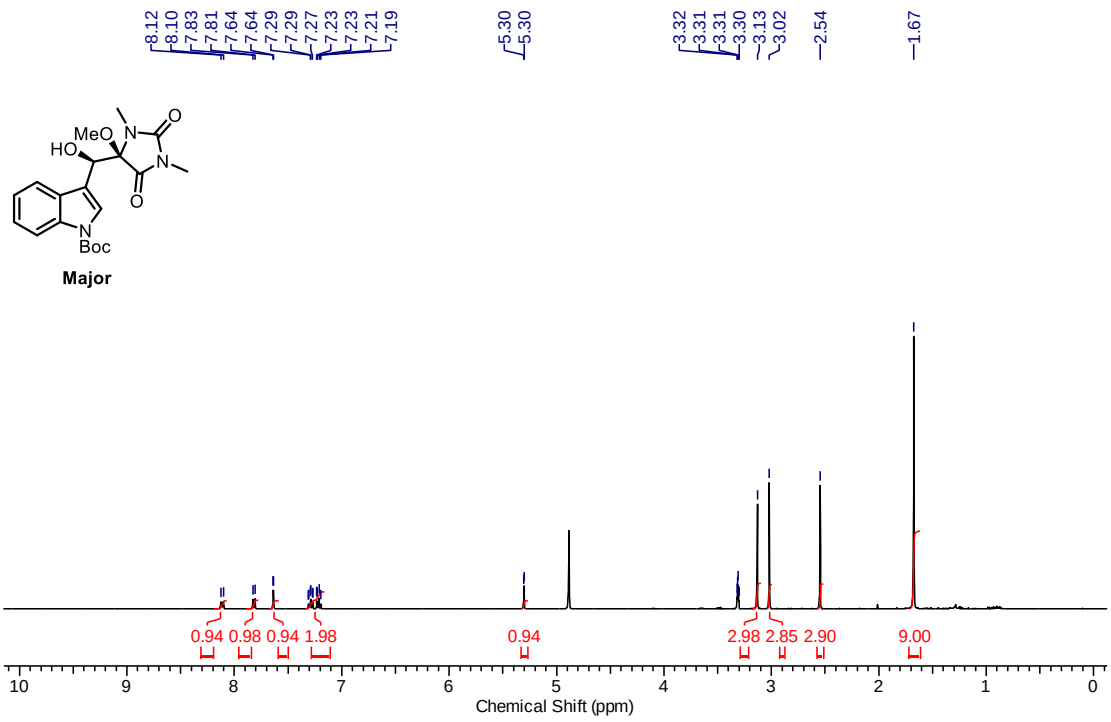
^{1}H NMR of Compound 41a in CD_3OD at 400 MHz



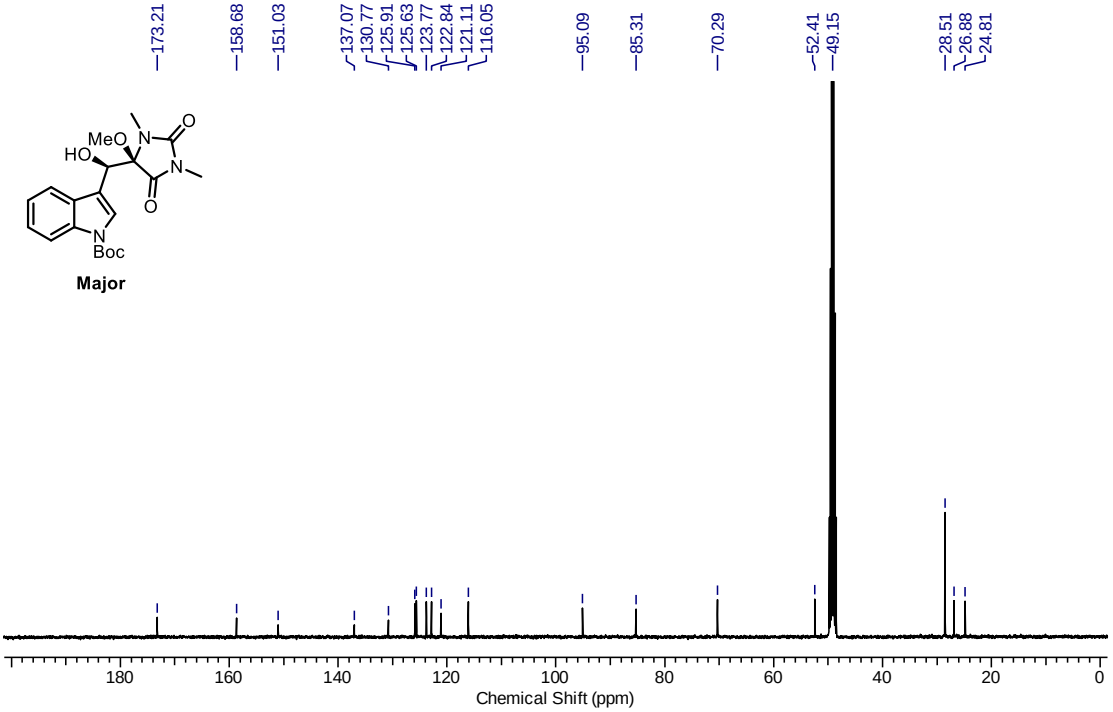
^{13}C NMR of Compound 41a in CD_3OD at 100 MHz



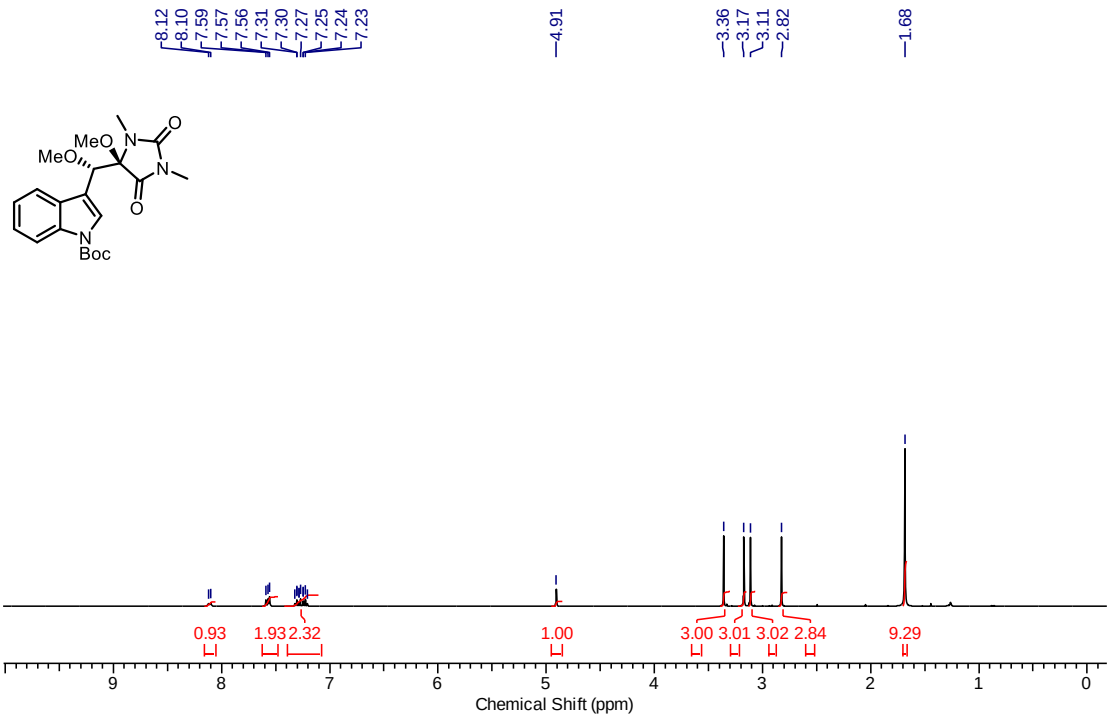
^{1}H NMR of Compound 41b in CD_3OD at 400 MHz



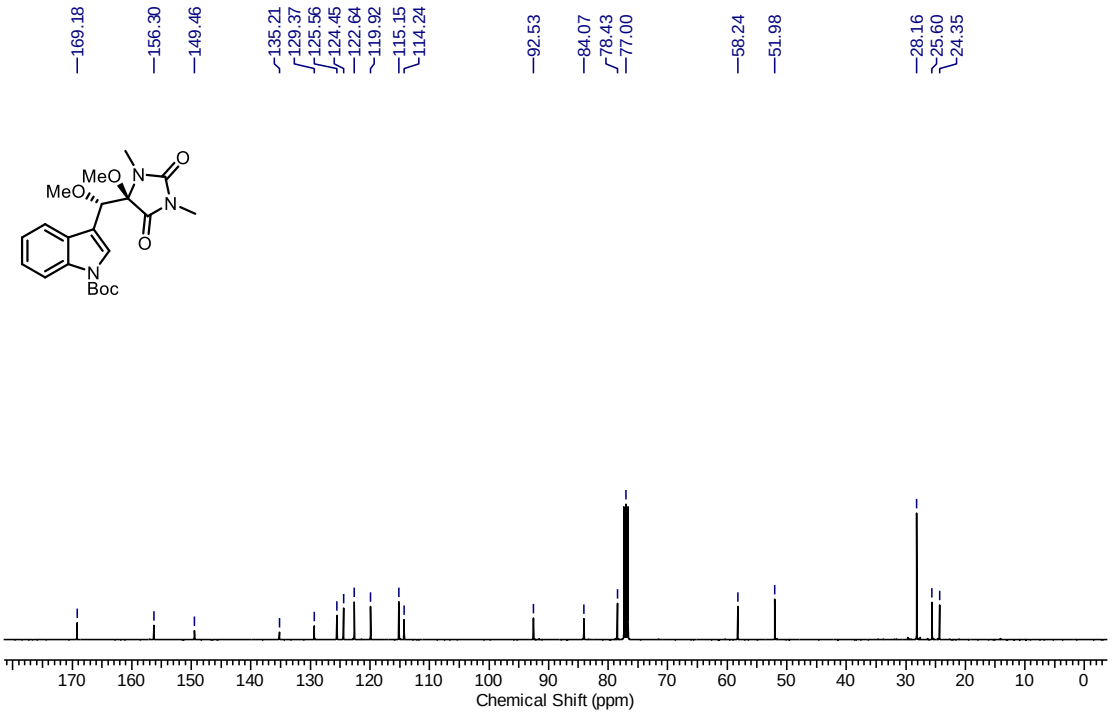
^{13}C NMR of Compound 41b in CD_3OD at 100 MHz



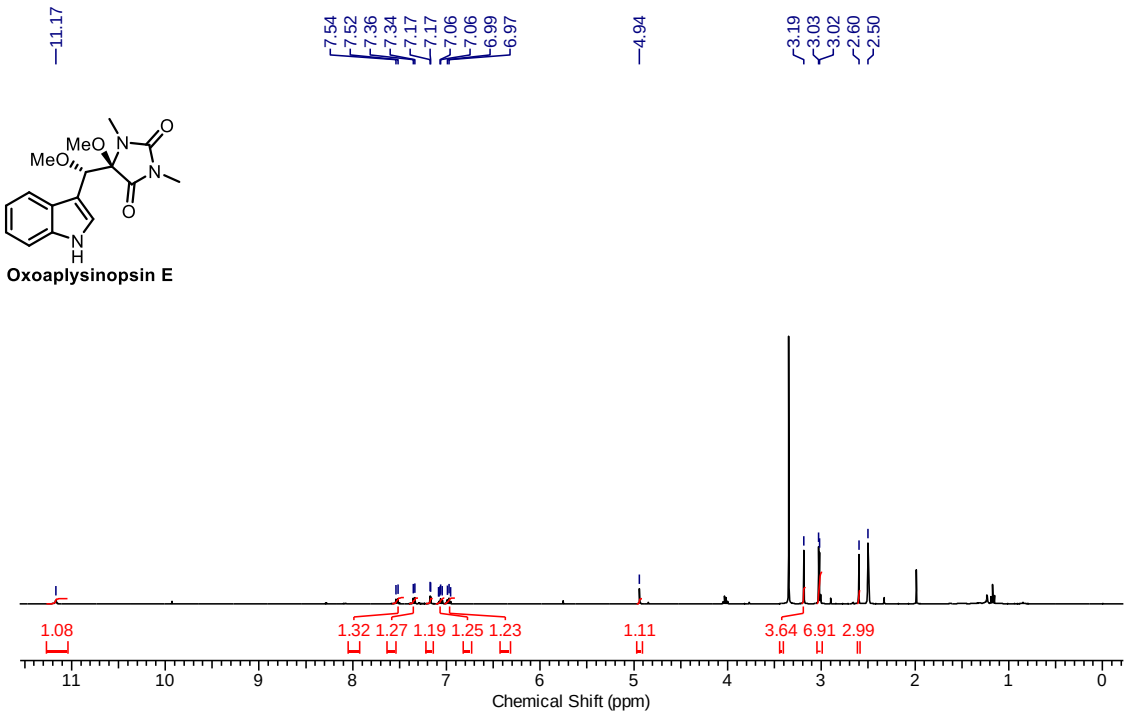
^{1}H NMR of Compound 42 in $CDCl_3$ at 400 MHz



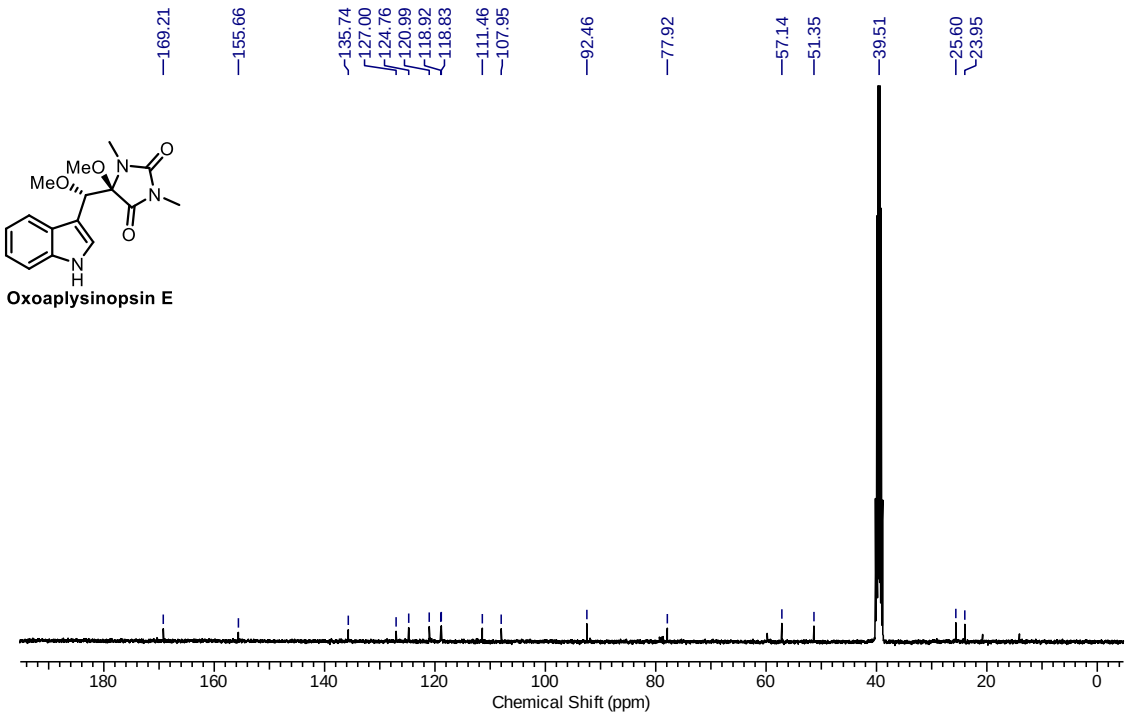
^{13}C NMR of Compound 42 in $CDCl_3$ at 100 MHz



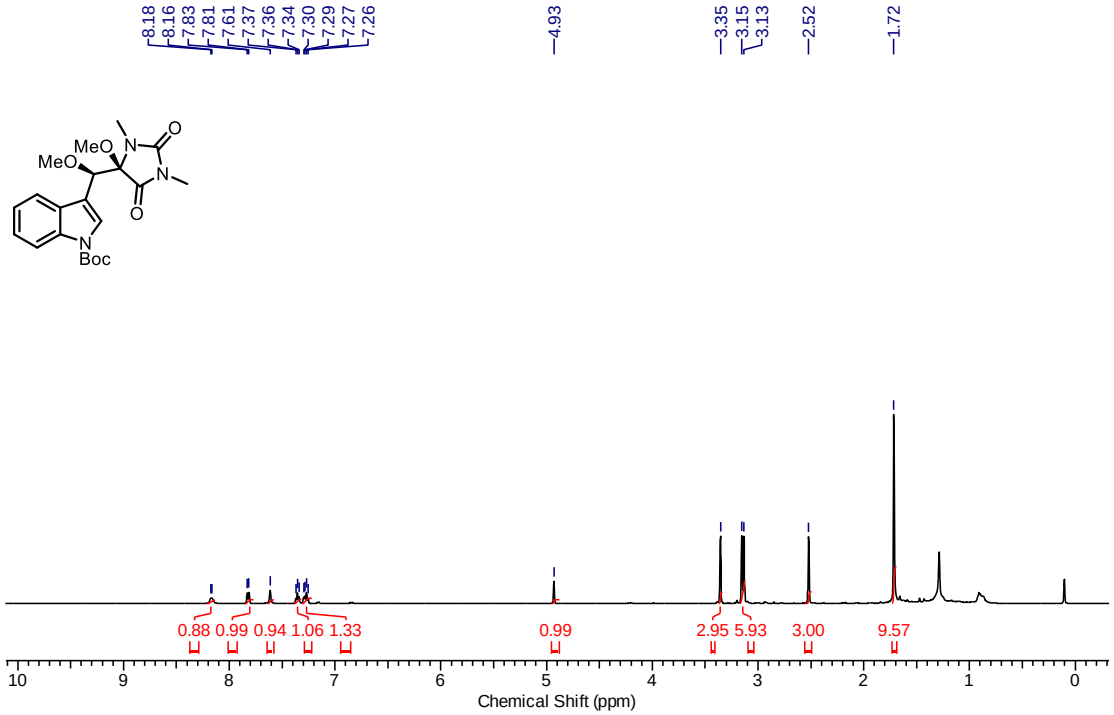
^{1}H NMR of Compound 28 in DMSO-d_6 at 400 MHz



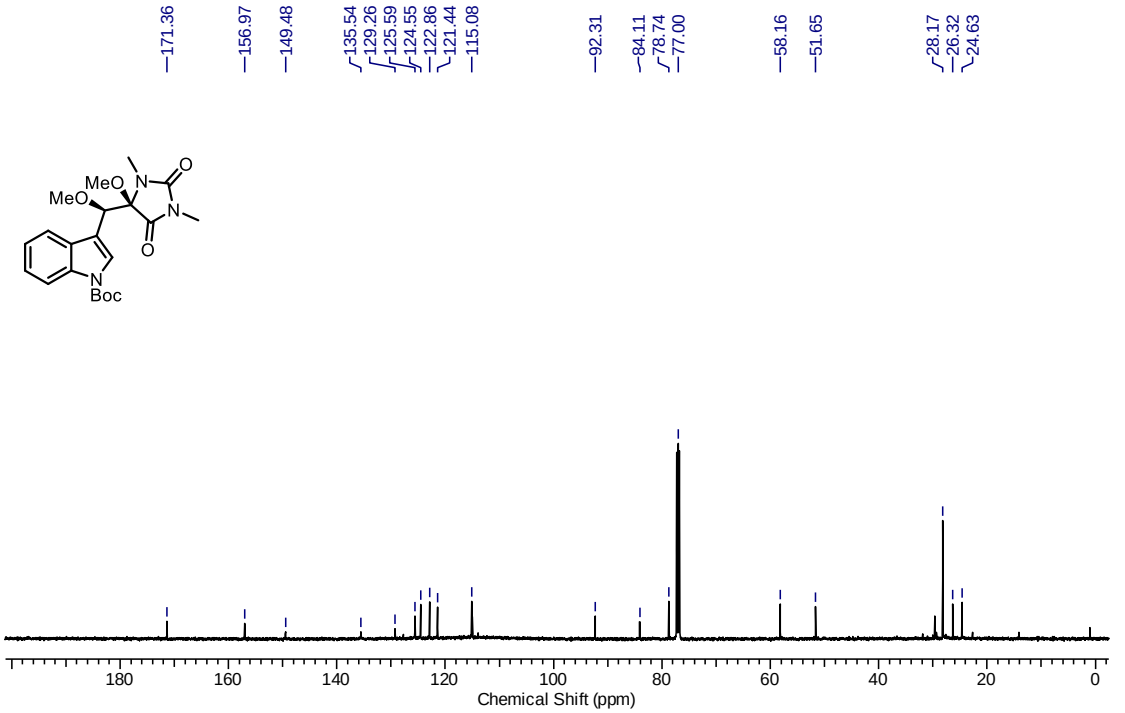
^{13}C NMR of Compound 28 in DMSO-d_6 at 100 MHz



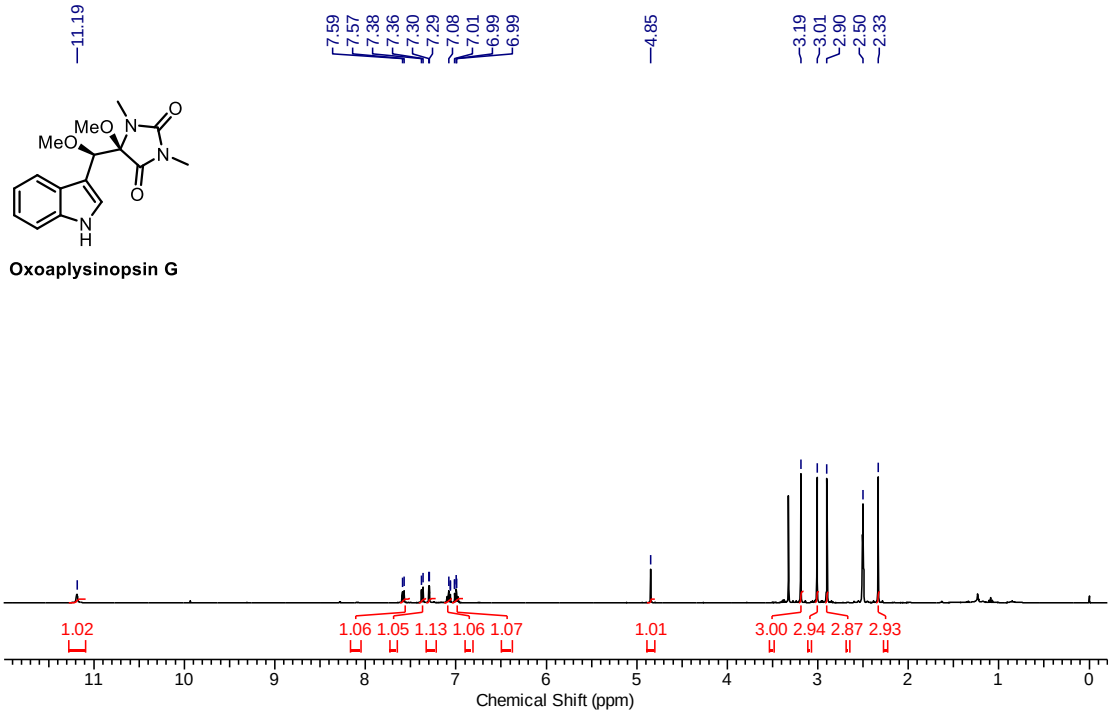
^{1}H NMR of Compound 43 in $CDCl_3$ at 500 MHz



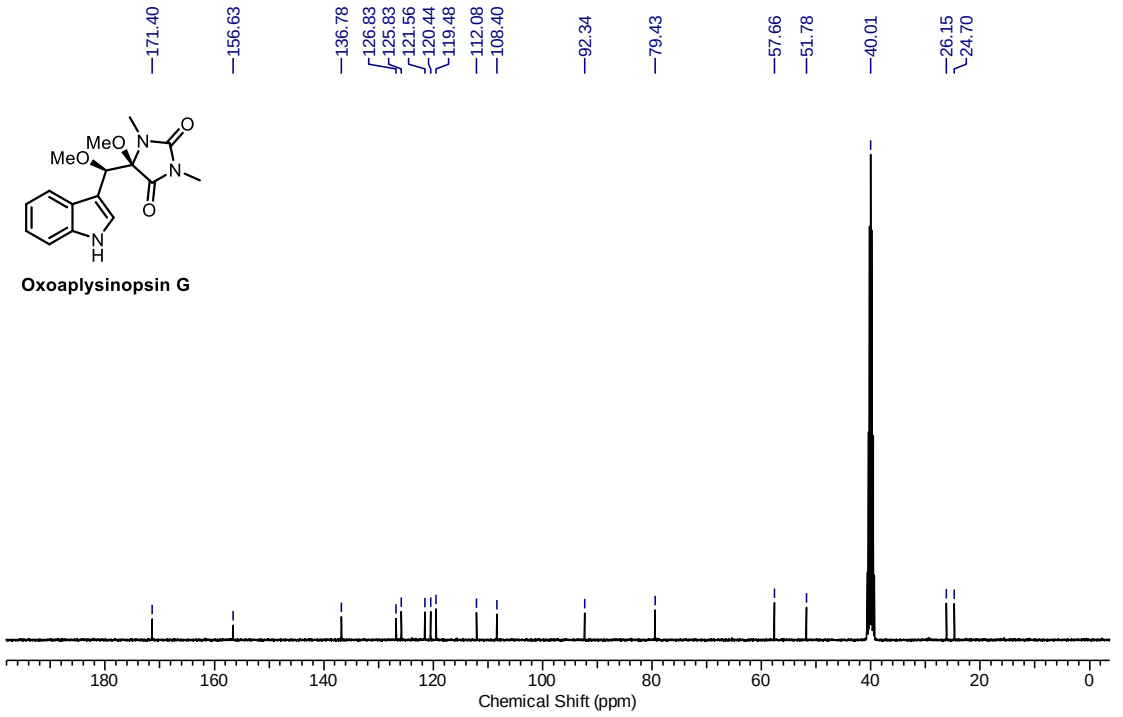
^{13}C NMR of Compound 43 in $CDCl_3$ at 125 MHz



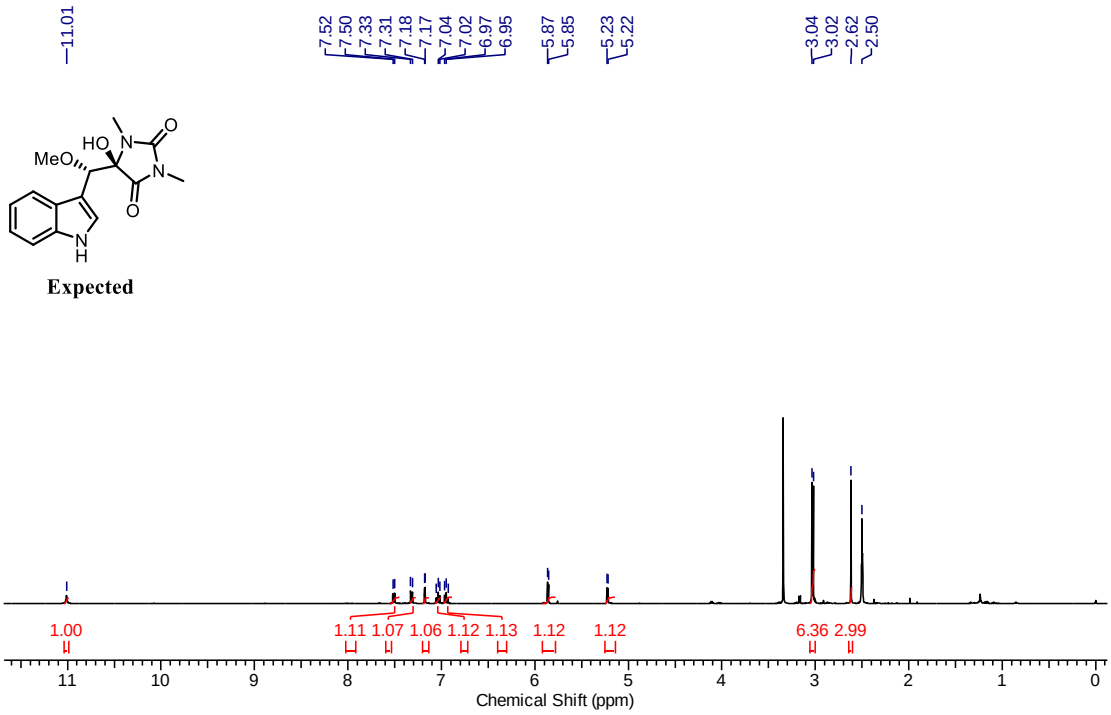
^{1}H NMR of Compound 30 in DMSO-d_6 at 400 MHz



^{13}C NMR of Compound 30 in DMSO-d_6 at 100 MHz

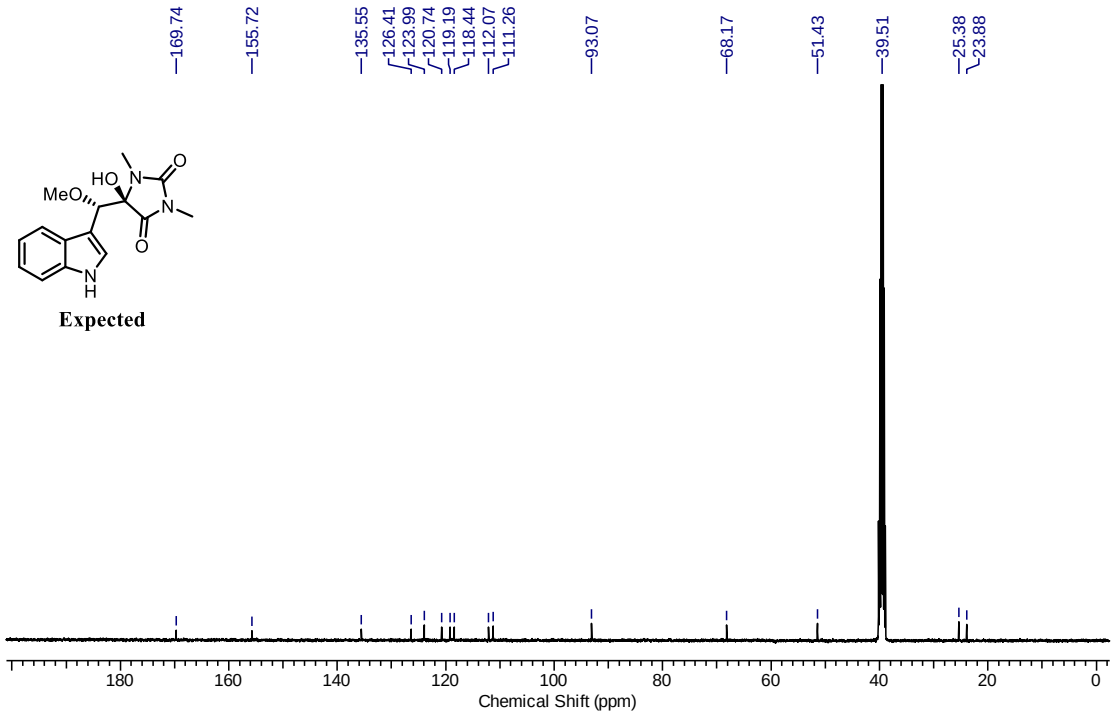


^{1}H NMR of Compound 27 (Expected) in DMSO-d_6 at 400 MHz



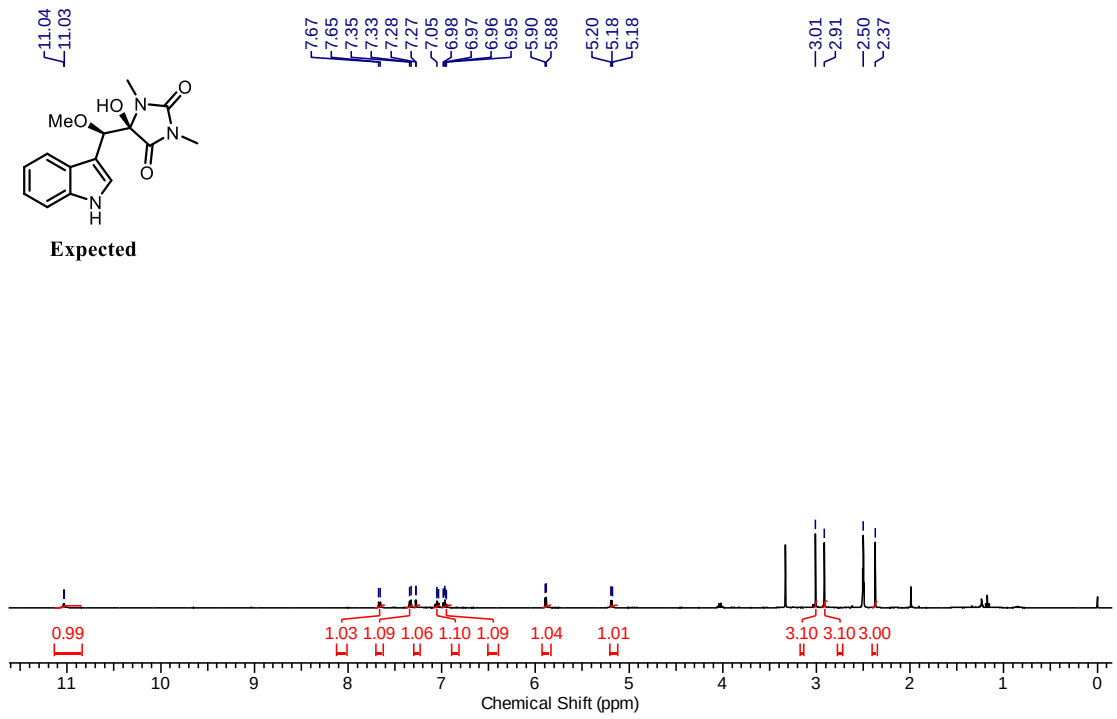
Expected

^{13}C NMR of Compound 27(Expected) in DMSO-d_6 at 100 MHz

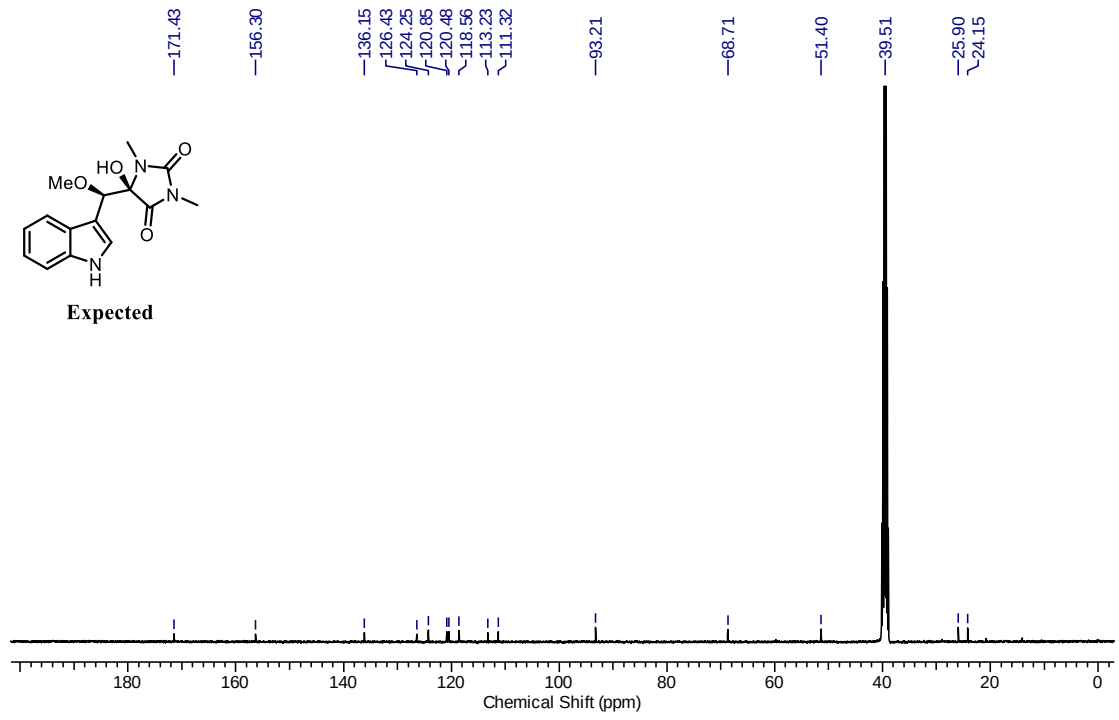


Expected

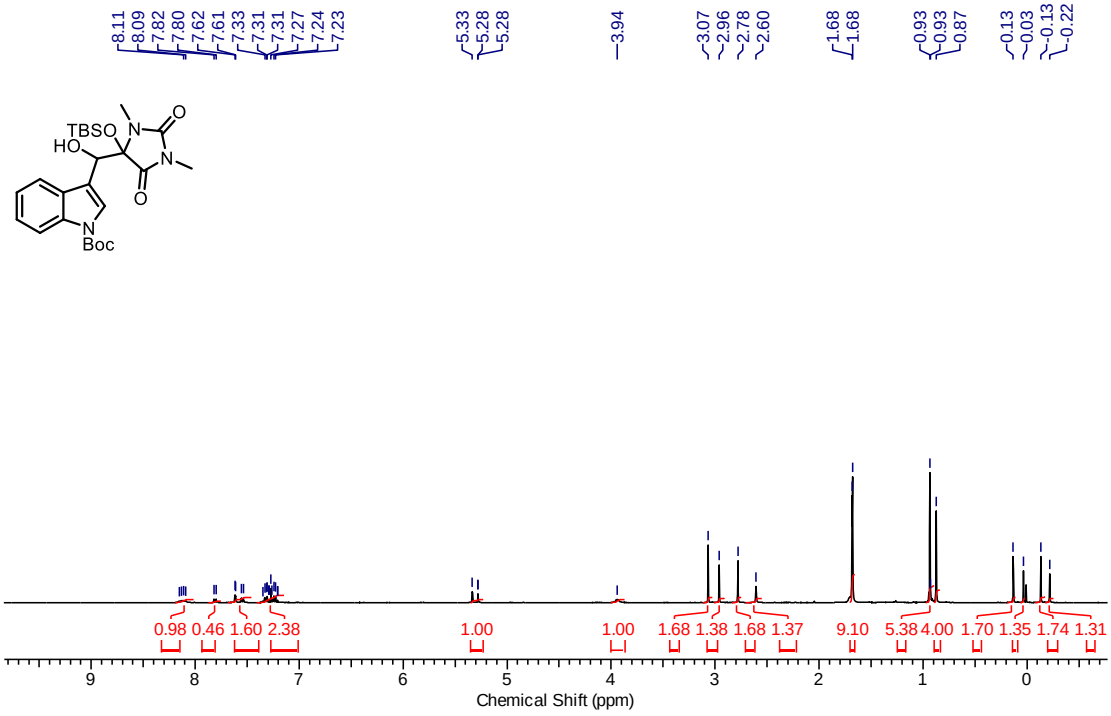
^{1}H NMR of Compound 29 (Expected) in DMSO-d_6 at 400 MHz



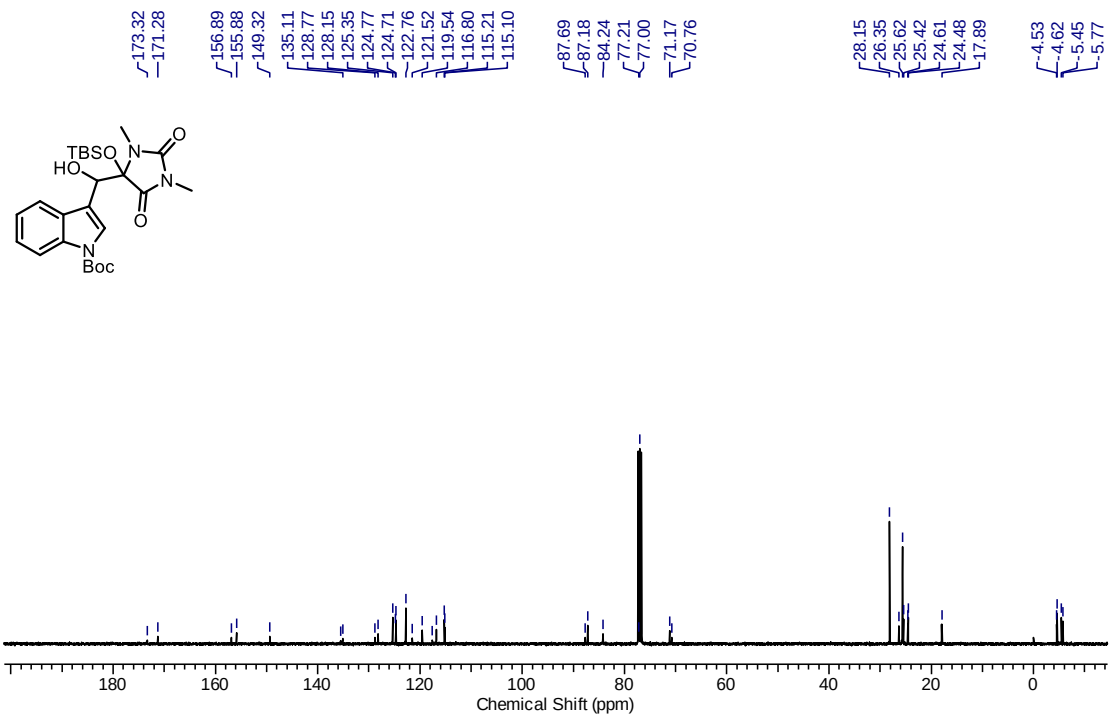
^{13}C NMR of Compound 29 (Expected) in DMSO-d_6 at 100 MHz



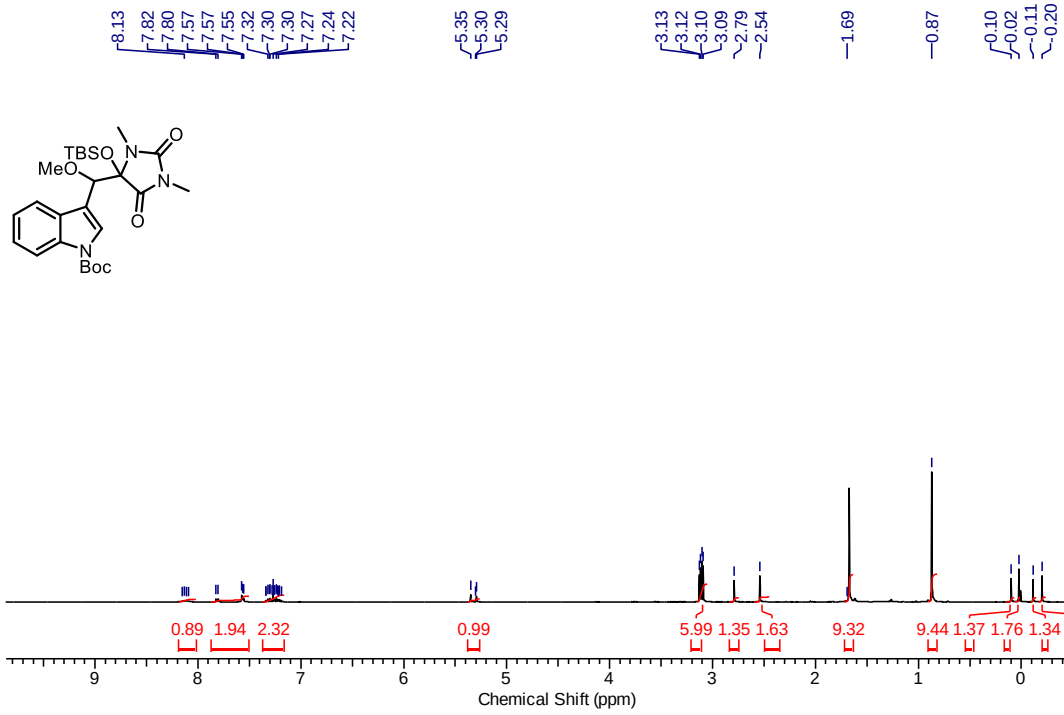
^{1}H NMR of Compound 46 in $CDCl_3$ at 400 MHz



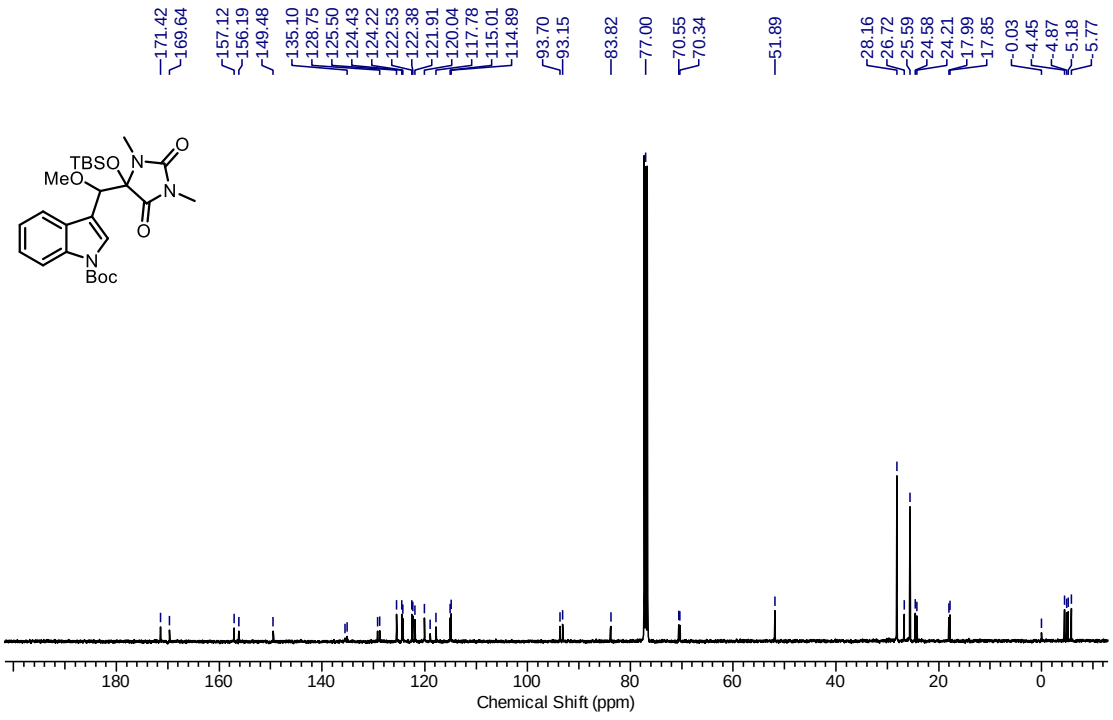
^{13}C NMR of Compound 46 in $CDCl_3$ at 100 MHz



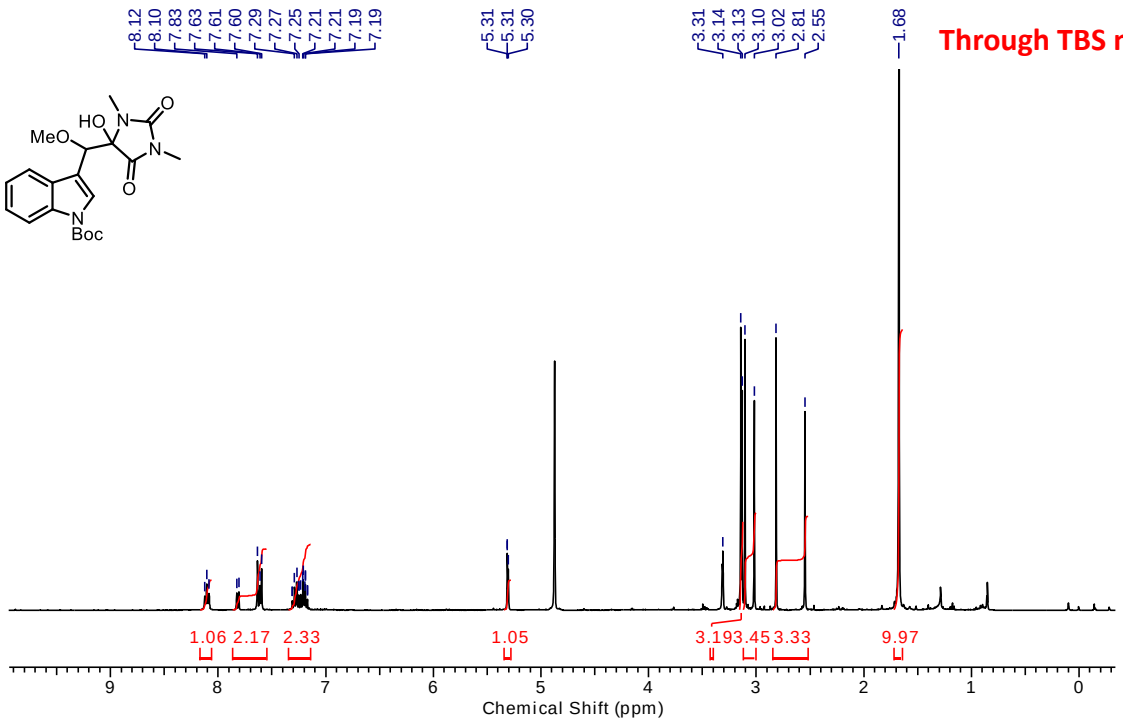
^{1}H NMR of Compound 47 in $CDCl_3$ at 400 MHz



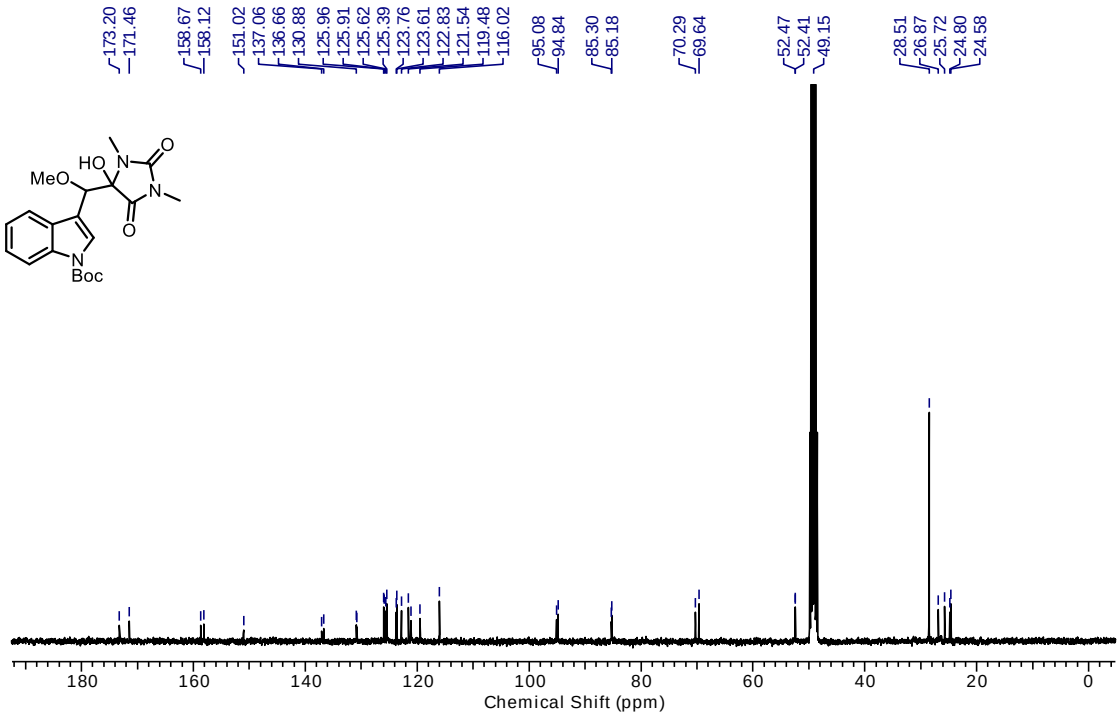
^{13}C NMR of Compound 47 in $CDCl_3$ at 100 MHz



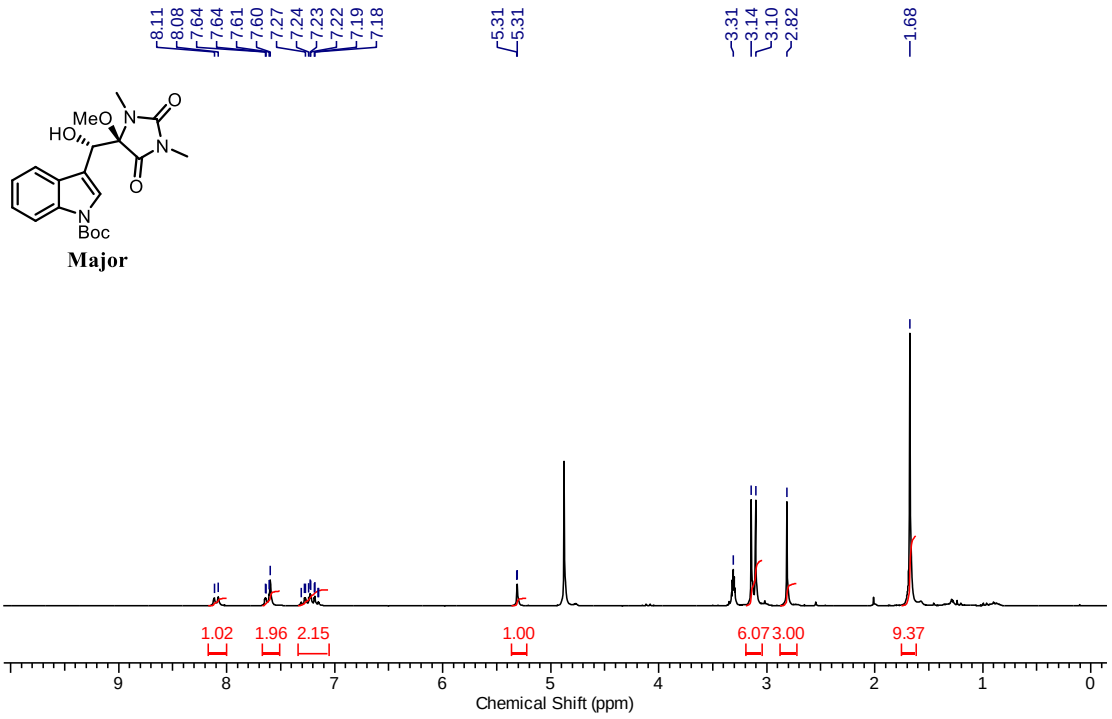
^{1}H NMR of Compound 44 (Expected) in $CDCl_3$ at 400 MHz



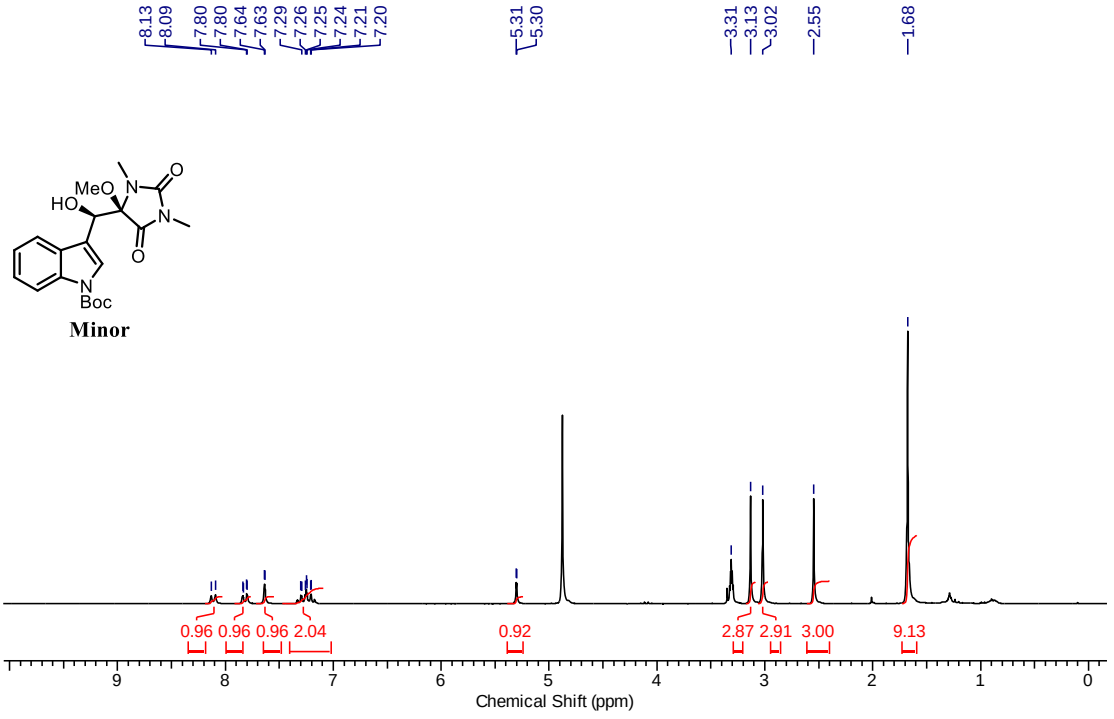
^{13}C NMR of Compound 44 (Expected) in $CDCl_3$ at 100 MHz



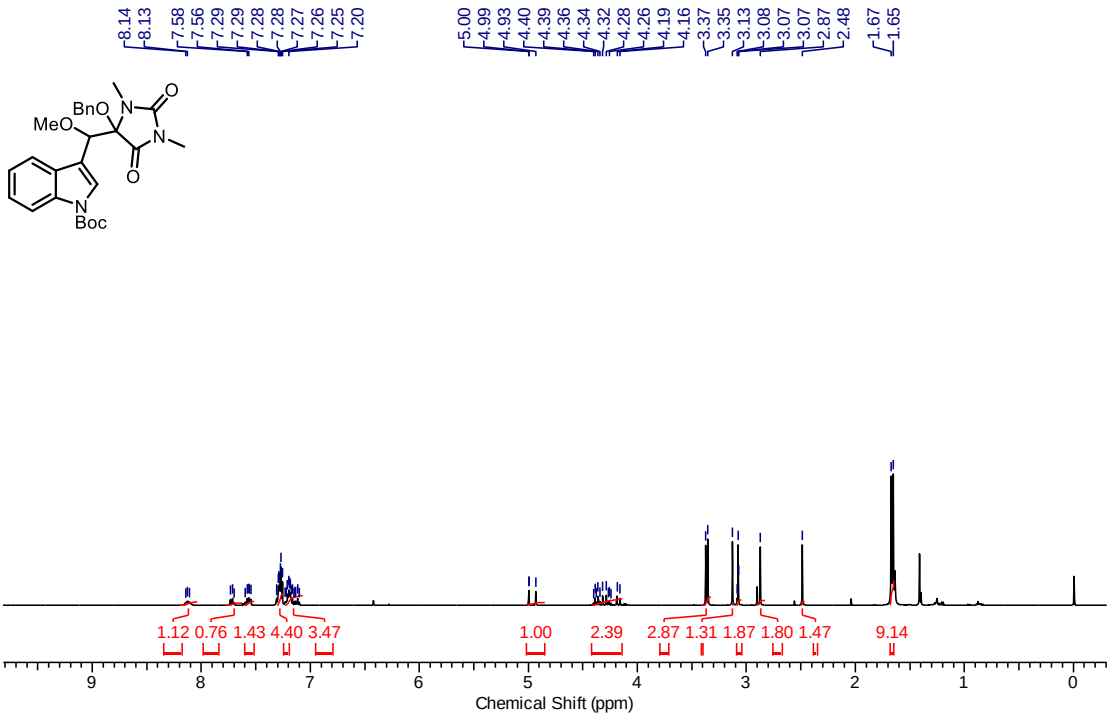
^{1}H NMR of Compound 41a in CD_3OD at 200 MHz



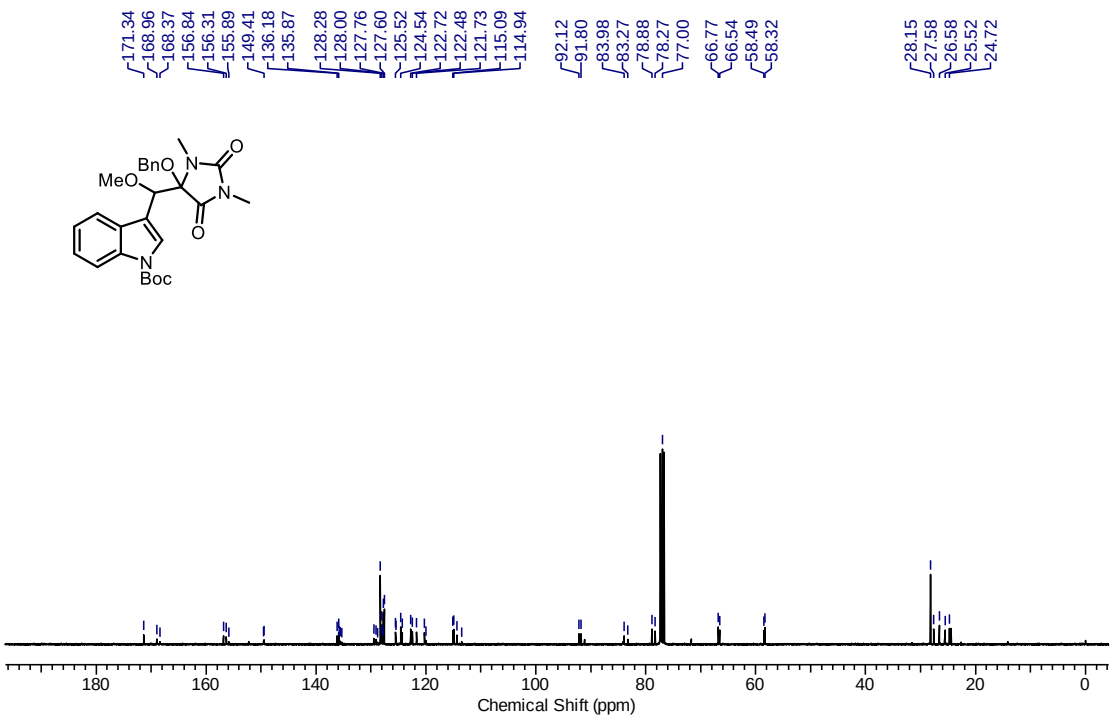
^{1}H NMR of Compound 41b in CD_3OD at 200 MHz



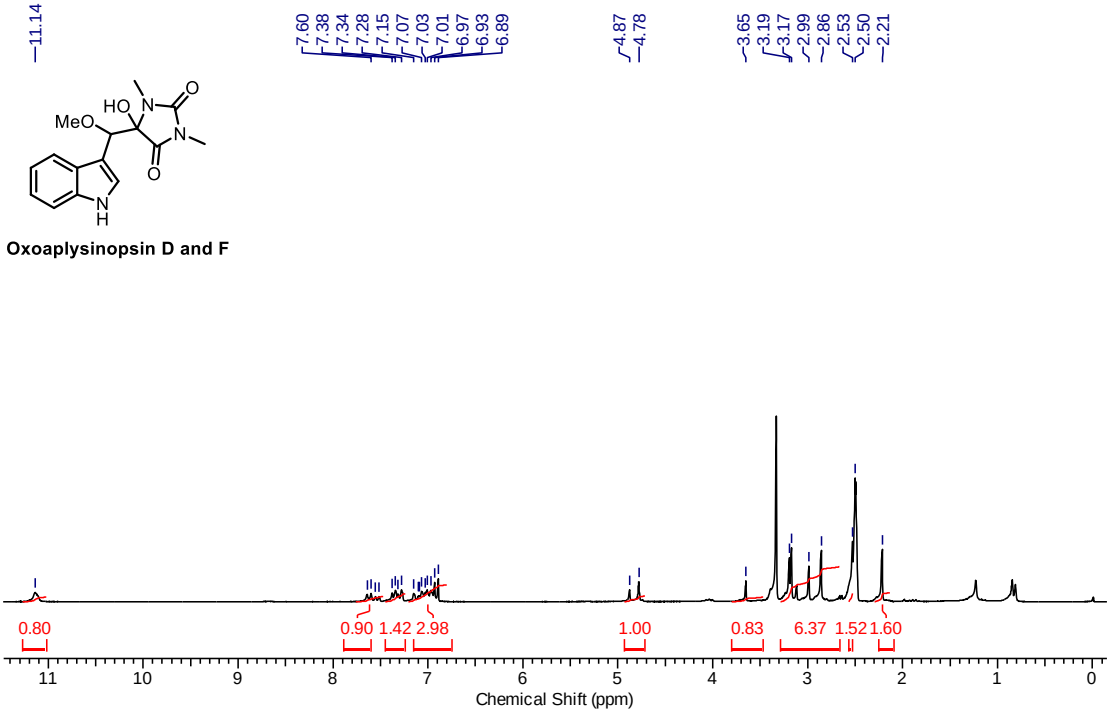
^{1}H NMR of Compound 50 in $CDCl_3$ at 400 MHz



^{13}C NMR of Compound 50 in $CDCl_3$ at 100 MHz

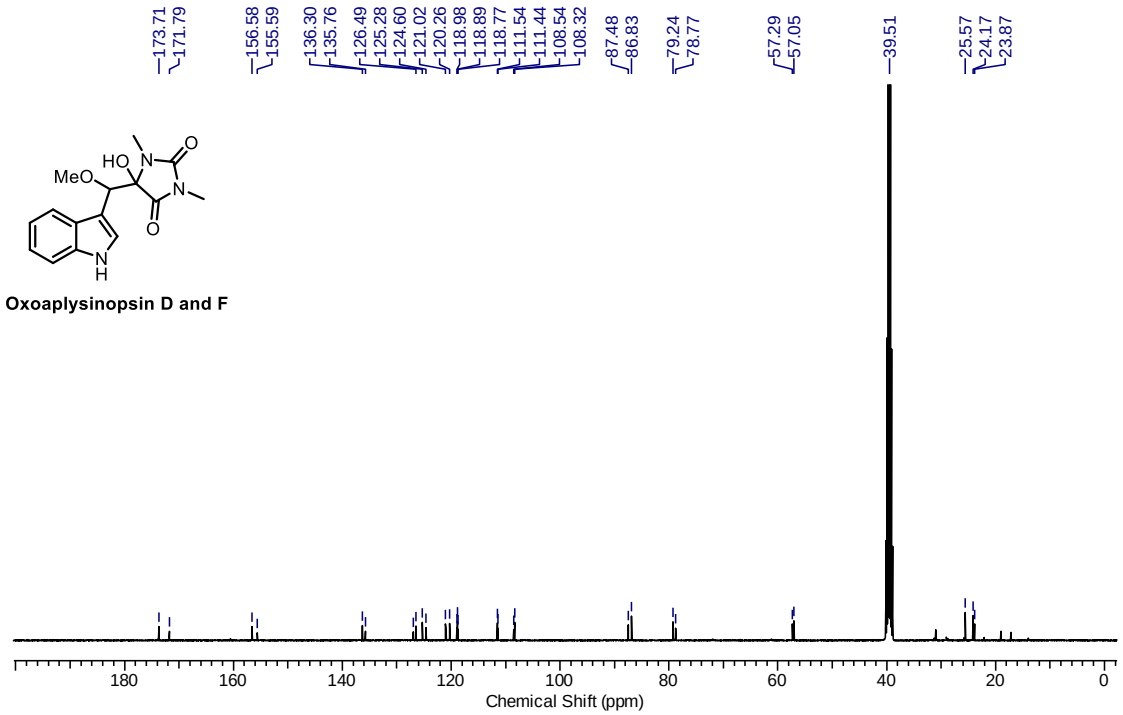


^{1}H NMR of Compound 27 & 29 in DMSO-d_6 at 200 MHz



Oxoaplysinopsin D and F

^{13}C NMR of Compound 27 & 29 in DMSO-d_6 at 100 MHz



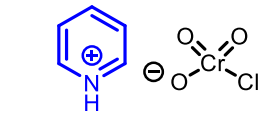
Oxoaplysinopsin D and F

Chapter-2

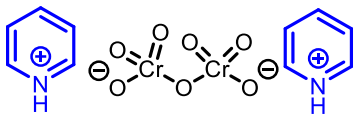
Section 2: PDC Mediated One-Pot Oxidation of Secondary Alcohols to α-Hydroxy Ketones

2.2.1 Introduction

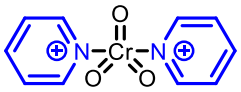
In recent times, oxidation reactions, particularly, identifying selective and mild oxidation reactions have gained significant attention. In this context, chromium (VI) reagents are widely used as oxidizing agents in synthetic organic chemistry and deserves a special mention. For the first time Sarett and co-workers have reported chromium-pyridine salt as a mild oxidizing reagent to oxidize steroidal alcohols with an advantage of its solubility in organic solvent.[1] Among this class of oxidizing agents, pyridinium chlorochromate (PCC), pyridinium dichromate (PDC) and chromic oxide-pyridine (Collins reagent) are the most popular ones (Figure 2.2.1). However, Collins reagent has certain limitations in comparison with PDC and PCC which includes difficulty in preparation, compromised stability of the reagent and efficiency. On the other hand, PCC and PDC are stable reagents, easy to handle and have better selectivity than Collin's reagent.[2]



Pyridinium chlorochromate (PCC)



Pyridinium dichromate (PDC)



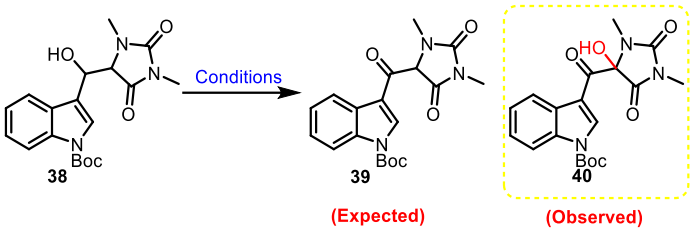
Chromic oxide-pyridine (Collins reagent)

Figure 2.2.1: Popular chromium reagents for oxidation

The selective use of PCC and PDC depends on the stability of the alcohol substrate. PDC is closer to neutral pH and thus well tolerated by acid sensitive substrates leading to a broader substrate scope. PDC was introduced in 1962 by chemist Sir John Warcup Cornforth hence it is also called 'Cornforth reagent'.[3] However, it was further developed in 1979 by E. J. Corey and G. Schmidt and reported that alcohols can oxidized to carboxylic acid directly instead of stopping at aldehyde stage using PDC in DMF solvent.[4] Besides this, Prof. Chandrasekaran also reported a well known method for allylic and benzylic oxidations using ^{t}BuOOH-PDC in 1987.[5] Till date, PDC is well known for many applications such as oxidation of alcohols,

rearrangement of allylic hydroxyl groups,[6] construction of heterocycles,[7] oxidation of carbon-boron bonds,[8] synthesis of metal free dienones,[9,10] preparation of enones[11] *etc*.

As discussed in section 1, we desired to oxidize aldol adduct **38** to corresponding ketone **39**, for which different oxidizing reagents were tried. (Section 1; Table 2.1.6)



Scheme 2.2.1: Oxidation of aldol adduct showed unexpected product formation

While optimizing the reaction, use of PCC in dichloromethane led to the formation of ketone **39** in 37% yield along with the formation of a side-product (observed in TLC analysis) which was isolated and characterized. From ^{1}H NMR analysis it was found that proton attached to secondary hydroxy was absent, indicating oxidation of the aldol adduct **38** to ketone. Moreover, we observed a broad peak at δ (5.81, 1H) corresponding to –OH (Figure 2.2.2). Meanwhile, mass of this compound appeared to be equal to hydroxyketone **40**.

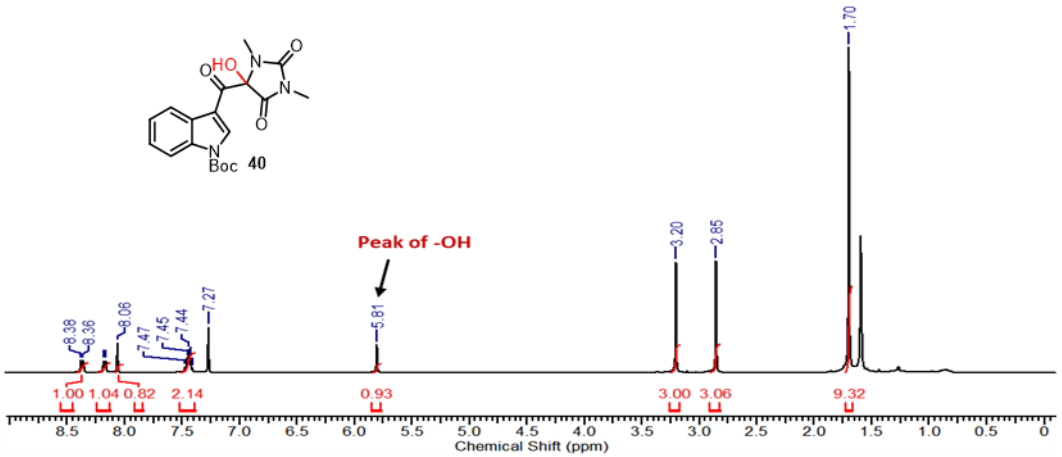


Figure 2.2.2: ^{1}H NMR of hydroxyketone **40** in $CDCl_3$

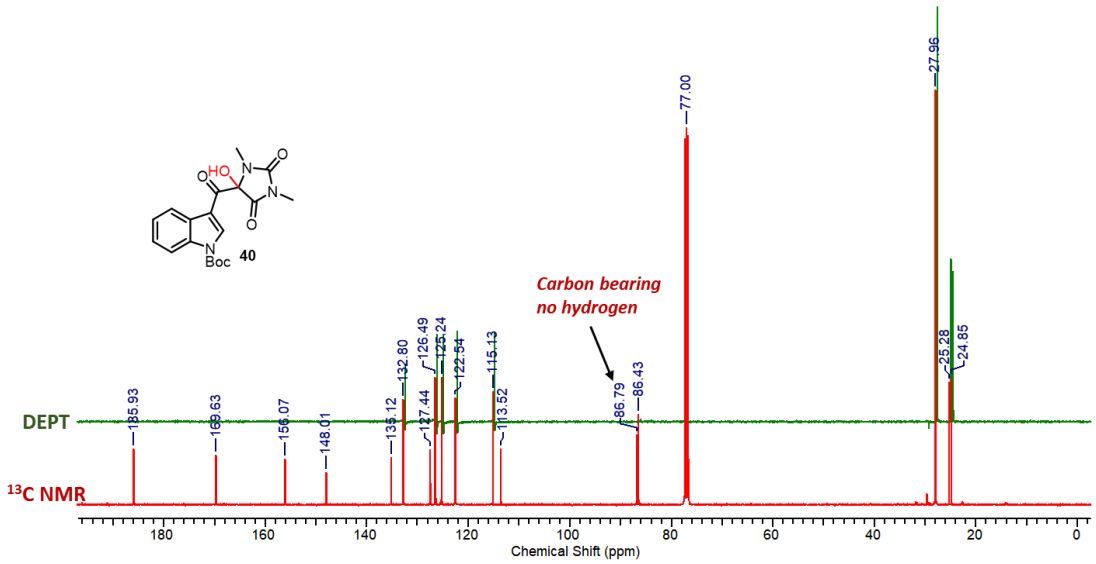


Figure 2.2.3: ^{13}C NMR of hydroxyketone **40** in $CDCl_3$

For an unambiguious assignment, ^{1}H NMR of same compound was recorded in CD_3OD leading to the disappearance of the concerned ^{1}H NMR peak at δ (5.81, 1H) which further confirmed its correspondence to the hydroxy group present at nitrogenated tertiary carbon. The structure was further confirmed through peak at δ 86.71 belongs to tertiary nitrogenated carbon in ^{13}C NMR. This interesting one-pot oxidation protocol is a much useful procedure to synthesize medicinally and synthetically important α-hydroxy ketones. Moreover, with the help of this methodology we can access library of novel oxoaplysinopsin analogues as well.

Literature survey reveals that, the synthesis of α-hydroxy ketones were documented from ketones, allene and enolates.[12] Whereas Sekar group has reported a protocol for synthesis of α-hydroxy ketones from corresponding benzylic secondary alcohols using catalytic iodine in DMSO (Figure 2.2.4). [13] However, it's worth mentioning that this reaction condition did not work on our substrate. It was further found that, a similar sort of transformation under PCC condition was observed by Mehta *et al*. during the synthesis of Secoprezizaane[14], and Paterson *et al*. during the synthesis of Jiadifenolide[15]. Keeping this in mind, we decided to optimize this interesting transformation and understand the substrate scope.

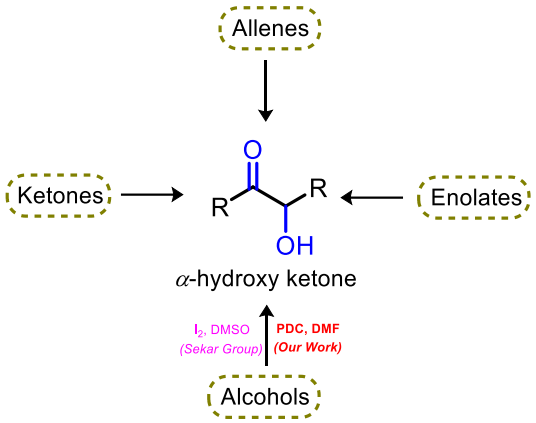


Figure 2.2.4: Selected known protocol for making α-hydroxy ketones

2.2.2 Optimization of reaction condition:

Encouraged by above results, we initiated optimization studies with the aim to find the best condition that can exclusively give compound **40** in good yield from aldol adduct **38**. Accordingly, aldol adduct **38** was initially subjected for oxidation using 6 equiv. of pyridinium chlorochromate (PCC) in dichloromethane at room temperature for 12 h and a slight increase in the yield of α-hydroxy ketone compound **40** along with formation of ketone **39** (entry-2) was observed. Furthermore, changing solvent from dichloromethane to DMF did not improved the yield of reaction as such (entry-3).

To understand the role of water and oxygen in oxidation, we have carried out the concerned reaction in presence of water and under oxygen atmosphere but did not observed any yield improvement (entry-4 and 5). Besides, the use of pyridinium dichromate (PDC) in dichloromethane did not improve the yield as well (entry-6). To our delight, only α-hydroxy ketone compound **40** was observed after using PDC (3 equiv.) in DMF with 66% yield (entry-7). Moreover through this condition, we found that reaction was proceeding very fast from aldol adduct to α-hydroxy ketone compound **40** without the formation of ketone **39**. To understand role of DMF in the reaction, we have replaced DMF with DMSO and observed slight decrease in the yield of α-hydroxy ketone compound **40** along with formation of ketone **39** in 15% yield (entry-8).

Table 2.2.1: Optimization of reaction condition

Entry	Condition	Observation
1.	PCC (3 eq.), DCM, rt, 3 h	**39** (37%) + **40** (30%)
2.	PCC (6 eq.), DCM, rt, 12 h	**39** (30%) + **40** (40%)
3.	PCC, DMF, rt, 4 h	**39** (31%) + **40** (38%)
4.	PCC, DMF, H_2O, rt, 4 h	**39** (35%) + **40** (36%)
5.	PCC , DMF, O_2, rt, 4 h	**39** (33%) + **40** (35%)
6.	PDC (3 eq.), DCM, rt, 3 h	**39** (31%) + **40** (39%)
7.	PDC (3 eq.), DMF, rt, 3 h	Only **40** (66%)
8.	PDC (3 eq.), DMSO, rt, 3 h	**39** (15%) + **40** (58%)
9.	CrO_3 (VI), DCM, rt, 12 h	No reaction
10.	CrO_3 (VI), DMF, rt, 12 h	No reaction

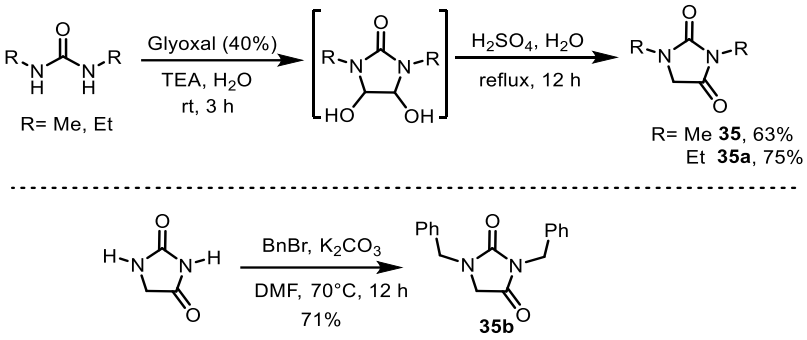
Next, we performed reaction using chromium trioxide in both DCM and DMF solvent but did not observed any progress in the reaction. Starting materials were recovered as such (entry 9 and 10). Finally, PDC in DMF (entry- 7) was found to be the best suitable condition for this transformation. (Table 2.2.1)

2.2.3 Substrate scope:

After having the best condition to get exclusively α-hydroxy ketone **40** from secondary alcohol **38**, we focused on understanding the substrate scope for this transformation. For this purpose, we devided our substrates in to two categories:

1) Substrates around oxoaplysinopsin scaffold (indole and hydantoins)
2) Substrates apart from oxoaplysinopsin scaffold

Initially, we targeted substrates from category 1. Accordingly, we have synthesized different substituted hydantoins using known protocols in the literature.[16] *N*,*N*-dimethyl hydantoin **35** and *N*,*N*-diethyl hydantoin **35a** were synthesized on gram scale from corresponding dimethyl urea and diethyl urea respectively. Moreover, *N*,*N*-dibenzyl hydantoin **35b** was synthesized from commercial hydantoin using benzyl bromide and K_2CO_3 in DMF at 70 °C for 12 h. Here we afforded *N*,*N*-dibenzyl hydantoin **35b** with 71% yield.[16] (Scheme 2.2.2)



Scheme 2.2.2: Synthesis of substituted hydantoins

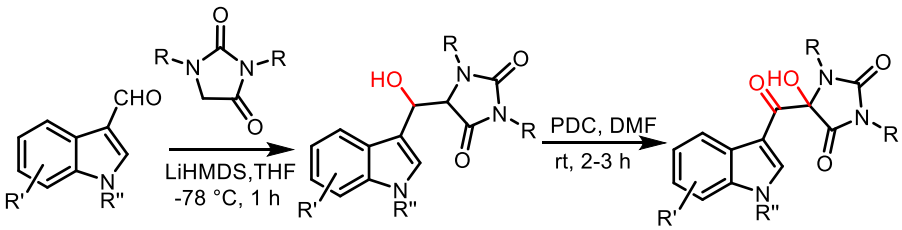
Next, we synthesized several substituted indole-3-carboxaldehydes starting from substituted indole by Vilsmeier Haack formylation[17] using phosphorous oxychloride and DMF followed by Boc protection using Boc anhydride and catalytic DMAP in acetonitrile. Moreover, different *N*-protected indole-3-carboxaldehydes were prepared using known literature protocol from indole-3-carboxaldehydes.[17]

For the synthesis of aldol adducts as substrates, we have subjected different substituted aldehydes with *N*,*N*-dimethyl, *N*,*N*-diethyl and *N*,*N*-dibenzyl hydantoins under base mediated aldol reaction using LiHMDS in THF. Synthesized substrates **38a-38t** were characterized by ^1H, ^{13}C NMR, IR and HRMS analysis.

Initially, substrates **38, 38a** and **38b** with different substituents on hydantoin moiety were subjected to optimized oxidation condition and found that all of them produced corresponding products **40**, **40a** and **40b** in good yields, respectively. Further changing the substituent on indole and hydantoin moieties (**38c -38o)** noconsiderable effect on the yield of reaction was observed. Additionally, substrates with different protection on indole NH (**38p-38s**) were successfully converted to corresponding

oxidation products **40p**-**40s**. Further, to understand the role of *N*-indole protection, we have synthesized substrate **38t** with absence of protecting group, from compound **38** by Boc-deprotection in refluxed water, and successfully converted this to ketohydroxy product **40t**.

Table 2.2.2: Scope of method

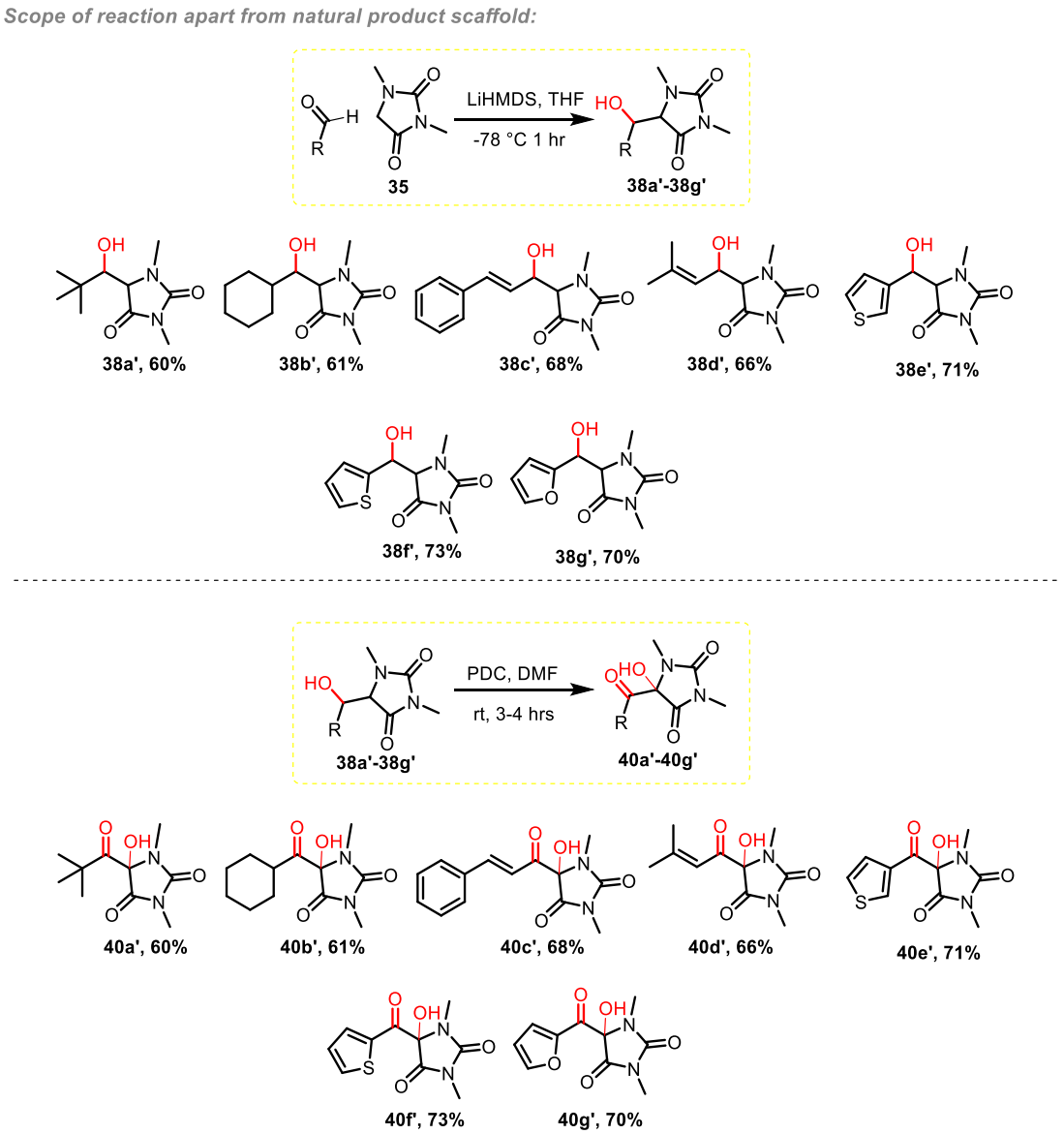


aldol products	Yield, dr	α-hydroxy ketones	Yield
38 : R = Me, R' = H, R'' = Boc	**67%**, 4:1	**40** : R = Me, R' = H, R'' = Boc	**66%**
38a : R = Et, R' = H, R'' = Boc	**61%**, 2:1	**40a** : R = Et, R' = H, R'' = Boc	**67%**
38b : R = Bn, R' = H, R'' = Boc	**63%**, 2:1	**40b** : R = Bn, R' = H, R'' = Boc	**80%**
38c : R = Me, R' = 5-*o*-tolyl, R'' = Boc	**64%**, 1:1	**40c** : R = Me, R' = 5-*o*-tolyl, R'' = Boc	**71%**
38d : R = Me, R' = 5-bromo, R'' = Boc	**73%**, 7:2	**40d** : R = Me, R' = 5-bromo, R'' = Boc	**60%**
38e : R = Et, R' = 5-bromo, R'' = Boc	**66%**, 3:2	**40e** : R = Et, R' = 5-bromo, R'' = Boc	**62%**
38f : R = Bn, R' = 5-bromo, R'' = Boc	**79%**, 1:1	**40f** : R = Bn, R' = 5-bromo, R'' = Boc	**69%**
38g : R = Me, R' = 5-fluoro, R'' = Boc	**66%**, 3:2	**40g** : R = Me, R' = 5-fluoro, R'' = Boc	**64%**
38h : R = Me, R' = 6-bromo, R'' = Boc	**73%**, 3:2	**40h** : R = Me, R' = 6-bromo, R'' = Boc	**78%**
38i : R = Me, R' = 5-phenyl, R'' = Boc	**63%**, 4:1	**40i** : R = Me, R' = 5-phenyl, R'' = Boc	**76%**
38j : R = Me, R' = 5-methoxy, R'' = Boc	**73%**, 3:2	**40j** : R = Me, R' = 5-methoxy, R'' = Boc	**82%**
38k : R = Et, R' = 5-methoxy, R'' = Boc	**71%**, 3:2	**40k** : R = Et, R' = 5-methoxy, R'' = Boc	**64%**
38l : R = Bn, R' = 5-methoxy, R'' = Boc	**78%**, 7:3	**40l** : R = Bn, R' = 5-methoxy, R'' = Boc	**61%**
38m : R = Me, R' = 6-methoxy, R'' = Boc	**40%**, 7:3	**40m** : R = Me, R' = 6-methoxy, R'' = Boc	**40%**
38n : R = Et, R' = 6-methoxy, R'' = Boc	**60%**, 3:2	**40n** : R = Et, R' = 6-methoxy, R'' = Boc	**60%**
38o : R = Bn, R' = 6-methoxy, R'' = Boc	**72%**, 1:1	**40o** : R = Bn, R' = 6-methoxy, R'' = Boc	**58%**
38p : R = Me, R' = H, R'' = Me	**69%**, 1:0	**40p** : R = Me, R' = H, R'' = Me	**68%**
38q : R = Me, R' = H, R'' = Bn	**71%**, 3:2	**40q** : R = Me, R' = H, R'' = Bn	**70%**
38r : R = Me, R' = H, R'' = n-hexyl	**72%**, 1:1	**40r** : R = Me, R' = H, R'' = n-hexyl	**84%**
38s : R = Me, R' = H, R'' = tosyl	**56%**, 2:1	**40s** : R = Me, R' = H, R'' = tosyl	**77%**
38t* : R = Me, R' = H, R'' = H	**70%**, 7:3	**40t** : R = Me, R' = H, R'' = H	**64%**

38t* was synthesized from **38 by boc-deprotection*

Next, as per our plan, we focused on substrates apart from the natural product scaffold, Accordingly, we subjected different commercially available non aromatic aldehydes including pivaldehyde, cyclohexanecarboxaldehyde and 3-methyl-2-butenal for aldol reaction with *N,N*-dimethyl hydantoin **35** which afforded corresponding pivalyl **38a'**, cyclohexyl **38b'** and 3-methyl-2-butenyl **38d'** aldol adducts respectively.

Table 2.2.3: Scope of method apart from natural product scaffold

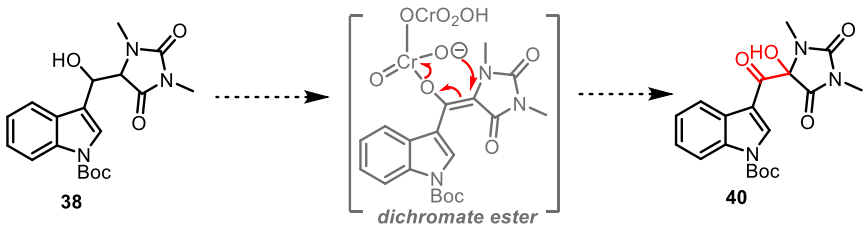


Moreover, aromatic aldehydes including thiophene-3-carboxaldehyde, thiophene-2-carboxaldehyde, furan-2-carboxaldehyde and cinnamaldehyde were subjected to aldol

reaction with *N,N*-dimethyl hydantoin to furnish required aldol adducts **38e'**, **38f'**, **38g'** and **38c'** respectively. All synthesized aldol adducts **38a'-38g'** were successfully converted to their corresponding α-hydroxy ketones **40a'-40g'** and they were characterized by NMR analysis, IR and HRMS.

All substrates and products from table 2.2.2 and 2.2.3 were considered as analogues of oxoaplysinopsin and their structures were subjected for docking studies and identified docking score against acetylcholinesterase (AchE), butyrylcholinesterase (BChE) and BACE-1 for Alzheimer's disease (discussed in section 3).

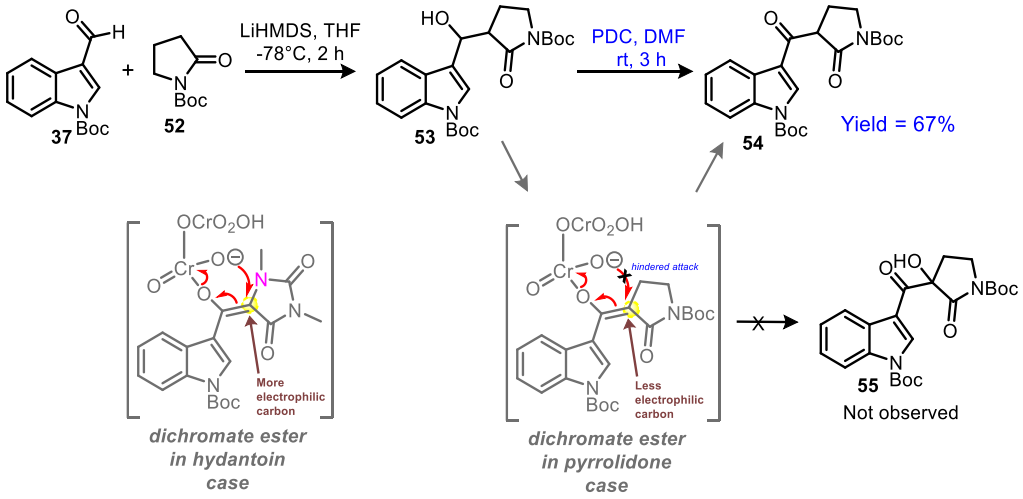
Plausible mechanism for transformation of **38** to **40** based on the result in this work and known literature reports is shown in Scheme 2.2.3. Firstly, compound **38** undergo oxidation using PDC to form ketone **39** which exist in keto-enol tautomer, which further react with PDC to form dichromate ester. Further attack of oxygen on electrophilic carbon present in between carbonyl and nitrogen atom, gives α-hydroxy ketone **40**.



Scheme 2.2.3: Plausible reaction mechanism

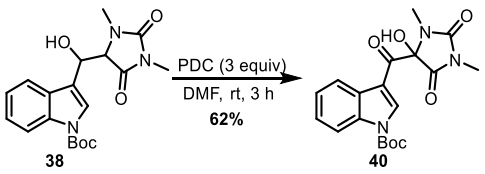
To further understand the role of hydantoin in the developed method, we planned substrate **53** without hydantoin moiety. Synthesis of planned substrate **53** started from Boc-indole-3-carboxaldehyde **37**, which on aldol reaction with Boc-2-pyrrolidone **52** using LiHMDS at -78 °C for 2 h furnished compound **53**. Compound **53** was then subjected to the optimized reaction condition (PDC, DMF, rt, 3 h) to exclusively afford ketone **54** without formation of α-hydroxy ketone **55**. Formation of compound **54** was confirmed by peak in ^{1}H NMR at δ 4.85 (t, *J* = 8.5 Hz, 1 H) corresponding to proton between two carbonyl units and new pyrrolidone proton peaks, whereas signal in ^{13}C NMR at δ 53.1 belongs to tertiary carbon between two carbonyl, presence of CH was confirmed by DEPT and peak at δ 191.4 showed ketone carbonyl . Further

confirmation was also done by peak in HRMS at 451.1830 corresponding to molecular formula $C_{23}H_{28}O_6N_2Na$ $[M+Na]^+$. This interesting finding showed the tertiary nitrogenated carbon present in the substrate should be more electrophilic. The reaction stops at ketone stage in the case of pyrrolidone due to less electrophilic tertiary carbon atom present in dichromate ester, which resulted in the exclusive formation of ketone as the oxidation product. (Scheme 2.2.4)



Scheme 2.2.4: Scope of reaction without hydantoin

We have also performed a gram-scale experiment on aldol adduct **38** and prepared α-hydroxy ketone **40** with 62% isolated yield. This gram scale experiment showed the consistency of our one-pot oxidation protocol. Moreover, α-hydroxy ketone **40** was utilized in section 1 for the synthesis of oxoaplysinopsin D, E, F and G.



Scheme 2.2.5: Gram-scale synthesis of compound **40**

2.2.4 Conclusion

We have developed a one pot oxidation method that converts secondary alcohols to corresponding α-hydroxy ketones using pyridinium dichromate (PDC) in DMF at room temperature. We have tested the scope of reaction with more than 30 examples,

which generated a library of >100 novel analogues of oxoaplysinopsin. Moreover, utility and consistency of one pot oxidation were showed by performing a gram-scale reaction.

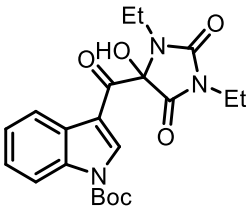
2.2.5 Experimental section:

Experimental procedures and characterization data of selected compounds are given below; Data of remaining compounds can be found at (*Eur. J. Org. Chem.* 2021, 2188; doi.org/10.1002/ejoc.202100184)[19]

General procedure for the synthesis of aldol products (38a-38s and **38a'-38g'):**
To a stirred solution of substituted hydantoin (1.0 equiv.) in 20 mL THF, 1M lithium bis(trimethylsilyl)amide (2.0 equiv.) was added dropwise at -78 °C for 15 min. aldehyde (1.2 equiv.) in THF (20 ml) was then added dropwise and the resultant mixture was stirred at -78 °C for 1 h. The reaction mixture was quenched with saturated NH_4Cl and allowed to warm to room temperature. The aqueous layer was extracted with EtOAc (3x30 mL), the combined organic extract washed with brine, dried (Na_2SO_4) and concentrated in *vaccuo*. The crude product was purified by column chromatography to afford aldol product as diastereomeric mixtures. Spectral data of all aldol products **38a-38s** and **38a'-38g'** is given in our published article.[19]

General procedure for one-pot hydroxylation (40a-40t and **40a'-40g'):**
To a stirred solution of **38a-38t** and **38a'-38g'** (1 equiv.) in DMF (5 mL), pyridinium dichromate (3 equiv.) was added at 0 °C and resultant mixture stirred for 3 h at room temperature. After completion of reaction EtOAc was added and decanted the solvent thrice. Combined organic extract and washed with water (30 mL), dried on Na_2SO_4 and concentrated in *vaccuo*. The crude product was purified by column chromatography to afford desired products **40a-40t** and **40a'-40g'**.

***tert*-butyl 3-(1,3-diethyl-4-hydroxy-2,5-dioxoimidazolidine-4-carbonyl)-1H-indole-1-carboxylate (40a):**



The compound **40a** was synthesized by following general procedure for one-pot hydroxylation and obtained as sticky solid.

Yield= 67%

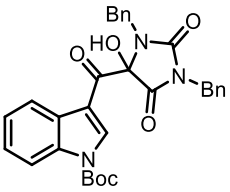
IRυ_{max}(film): 3382, 2982, 2342, 1720 cm^{-1}

^{1}H NMR (500 MHz, CD_3OD) = δ 9.02 (s, 1 H), 8.28 (d, *J* = 7.6 Hz, 1 H), 8.17 (d, *J* = 8.0 Hz, 1 H), 7.41 - 7.32 (m, 2 H), 3.58 (quin, *J* = 7.2 Hz, 3 H), 3.22 (dd, *J* = 7.2, 14.5 Hz, 1 H), 1.72 (s, 9 H), 1.21 (q, *J* = 7.2 Hz, 6 H)

^{13}C NMR (125 MHz, CD_3OD) = δ 190.1, 171.8, 157.4, 150.3, 138.7, 136.4, 129.6, 126.9, 125.8, 123.4, 117.0, 116.2, 92.7, 87.2, 36.8, 35.1, 28.4, 15.1, 13.7.

HRMS (ESI): *m/z* calculated for $C_{21}H_{25}N_3O_6Na$ $[M+Na]^+$ = 438.1636, Observed = 438.1630.

***tert*-butyl 3-(1,3-dibenzyl-4-hydroxy-2,5-dioxoimidazolidine-4-carbonyl)-1H-indole-1-carboxylate (40b):**



The compound **40b** was synthesized by following general procedure for one-pot hydroxylation and obtained as white solid.

Yield= 80%

Melting point: 55 - 58 °C

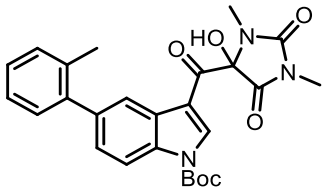
IRυ_{max}(film): 3387, 2979, 2363, 1724 cm^{-1}

^{1}H NMR (400 MHz, CD_3OD) = δ 8.77 (s, 1 H), 8.10 (t, *J* = 8.4 Hz, 2 H), 7.36 - 7.21 (m, 9 H), 7.20 – 7.07 (m, 3 H), 4.72 (s, 2 H), 4.58 (d, *J* = 16.0 Hz, 1 H), 4.46 (d, *J* = 15.3 Hz, 1 H), 1.68 (s, 9 H)

^{13}C NMR (100 MHz, CD_3OD) = δ 189.8, 171.8, 157.7, 150.1, 138.2, 138.1, 137.3, 136.1, 129.8, 129.7, 129.4, 129.1, 129.0, 128.5, 126.8, 125.7, 123.4, 116.9, 116.0, 92.4, 87.0, 45.3, 43.6, 28.3.

HRMS (ESI): *m/z* calculated for $C_{31}H_{29}N_3O_6Na$ $[M+Na]^+$ = 562.1949, Observed = 562.1964.

***tert*-butyl 3-(4-hydroxy-1,3-dimethyl-2,5-dioxoimidazolidine-4-carbonyl)-5-(o-tolyl)-1Hindole-1-carboxylate (40c):**



The compound **40c** was synthesized by following general procedure for one-pot hydroxylation and obtained as white solid.

Yield= 71%

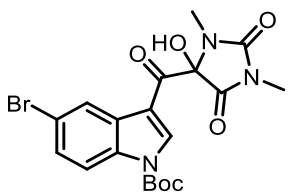
IRυ_{max}(film): 3342, 2979, 2363, 1723 cm^{-1}

^{1}H NMR (500 MHz, DMSO-d_6) = δ 9.06 (s, 1 H), 8.27 (s, 1 H), 8.17 - 8.15 (m, 2 H), 7.44 - 7.42 (m, 1 H), 7.30 - 7.23 (m, 4 H), 2.92 (s, 3 H), 2.80 (s, 3 H), 2.21 (s, 3 H), 1.70 (s, 9 H)

^{13}C NMR (125 MHz, DMSO-d_6) = δ 188.1, 169.7, 155.2, 148.3, 141.2, 138.2, 137.9, 134.7, 133.1, 130.3, 129.6, 127.6, 127.3, 126.9, 125.9, 121.8, 114.9, 114.6, 90.8, 86.1, 27.5, 24.9, 24.6, 20.1.

HRMS (ESI): *m/z* calculated for $C_{26}H_{27}N_3O_6Na$ $[M+Na]^+$ = 500.1792, Observed = 500.1789.

***tert*-butyl 5-bromo-3-(4-hydroxy-1,3-dimethyl-2,5-dioxoimidazolidine-4-carbonyl)-1Hindole-1-carboxylate (40d):**



The compound **40d** was synthesized by following general procedure for one-pot hydroxylation and obtained as white solid.

Yield= 60%

Melting point: 170 -172 °C

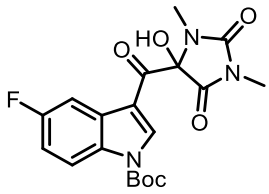
IRυ_{max}(film): 3342, 2979, 2363, 1723 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6) = δ 9.00 (s, 1 H), 8.34 - 8.30 (m, 2 H), 8.03 (d, *J* = 8.4 Hz, 1 H), 7.61 - 7.59 (m, 1 H), 2.92 (s, 3 H), 2.80 (s, 3 H), 1.67 (s, 9 H)

^{13}C NMR (100 MHz, DMSO-d_6) = δ 188.0, 169.6, 155.2, 148.0, 138.8, 133.1, 129.3, 128.4, 123.9, 117.4, 116.9, 114.1, 90.8, 86.5, 27.5, 24.9, 24.7.

HRMS (ESI): *m/z* calculated for $C_{19}H_{20}BrN_3O_6Na$ $[M+Na]^+$ = 488.0428, Observed = 488.0428.

***tert*-butyl 5-fluoro-3-(4-hydroxy-1,3-dimethyl-2,5-dioxoimidazolidine-4-carbonyl)-1Hindole-1-carboxylate (40g):**



The compound **40g** was synthesized by following general procedure for one-pot hydroxylation and obtained as white solid.

Yield= 64%

Melting point: 153 -156 °C

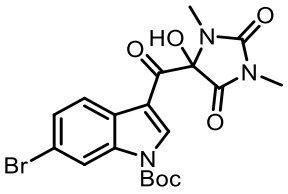
IRυ_{max}(film): 3400, 2980, 2363, 1722 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6) = δ 9.04 (s, 1 H), 8.29 (s, 1 H), 8.08 (dd, *J* = 4.6, 9.2 Hz, 1 H), 7.87 (dd, *J* = 3.1, 9.2 Hz, 1 H), 7.26 (dt, *J* = 3.1, 9.2 Hz, 1 H), 2.93 (s, 3 H), 2.81 (s, 3 H), 1.67 (s, 9 H)

^{13}C NMR (100 MHz, DMSO-d_6) = δ 188.0, 169.7, 159.0 (d, J_{C-F} = 238.66 Hz), 155.2, 148.1, 139.1, 130.7, 128.7 (d, J_{C-F} = 0.23 Hz), 116.5 (d, J_{C-F} = 9.16 Hz), 114.6 (d, J_{C-F} = 3.83 Hz), 113.7 (d, J_{C-F} = 25.88 Hz), 107.3 (d, J_{C-F} = 25.88 Hz), 90.9, 86.3, 27.5, 24.9, 24.7.

HRMS (ESI): *m/z* calculated for $C_{19}H_{20}FN_3O_6Na$ $[M+Na]^+$ = 428.1228, Observed = 428.1220.

***tert*-butyl 6-bromo-3-(4-hydroxy-1,3-dimethyl-2,5-dioxoimidazolidine-4-carbonyl)-1Hindole-1-carboxylate (40h):**



The compound **40h** was synthesized by following general procedure for one-pot hydroxylation and obtained as white solid.

Yield= 78%

Melting point: 108 -111 °C

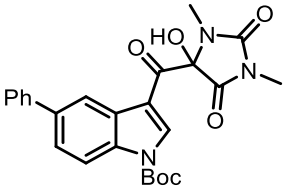
IRυ_{max}(film): 3363, 2981, 2363, 1720 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6) = δ 8.98 (s, 1 H), 8.30 - 8.26 (m, 2 H), 8.15 - 8.08 (m, 1 H), 7.53 (dd, *J* = 1.5, 8.4 Hz, 1 H), 2.92 - 2.91 (m, 3 H), 2.80 - 2.77 (m, 3 H), 1.67 (s, 9 H)

^{13}C NMR (100 MHz, DMSO-d_6) = δ 188.0, 169.6, 155.2, 148.0, 138.2, 134.9, 127.6, 126.7, 123.4, 118.4, 117.8, 114.7, 90.9, 90.8, 86.5, 27.4, 24.9, 24.7.

HRMS (ESI): *m/z* calculated for $C_{19}H_{20}BrN_3O_6Na$ $[M+Na]^+$ = 488.0428, Observed = 488.0420.

***tert*-butyl 3-(4-hydroxy-1,3-dimethyl-2,5-dioxoimidazolidine-4-carbonyl)-5-phenyl-1Hindole-1-carboxylate (40i):**



The compound **40i** was synthesized by following general procedure for one-pot hydroxylation and obtained as white solid.

Yield= 76%

Melting point: 140 - 142 °C;

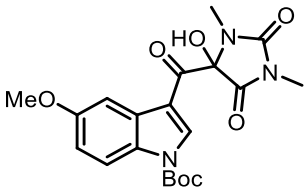
IRυ_{max}(film): 3362, 2980, 2363, 1722 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 9.05 (s, 1 H), 8.49 (d, *J* = 1.5 Hz, 1 H), 8.29 (s, 1 H), 8.17 (d, *J* = 8.6 Hz, 1 H), 7.77 - 7.68 (m, 3 H), 7.50 - 7.47 (m, 1 H), 7.38 (d, *J* = 7.3 Hz, 1 H), 2.93 (m, 3 H), 2.83 (s, 3 H), 1.70 (s, 9 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 188.2, 169.8, 155.2, 148.3, 140.2, 138.4, 137.0, 133.6, 129.0, 128.3, 127.4, 126.9, 124.8, 119.6, 115.4, 115.0, 90.9, 86.1, 27.5, 27.5, 24.9, 24.6.

HRMS (ESI): *m/z* calculated for $C_{25}H_{25}N_3O_6Na$ $[M+Na]^+$ = 486.1636, Observed = 486.1656.

***tert*-butyl 3-(4-hydroxy-1,3-dimethyl-2,5-dioxoimidazolidine-4-carbonyl)-5-methoxy1H-indole-1-carboxylate (40j):**



The compound **40j** was synthesized by following general procedure for one-pot hydroxylation and obtained as white solid.

Yield= 82%

Melting point: 83 - 85 °C;

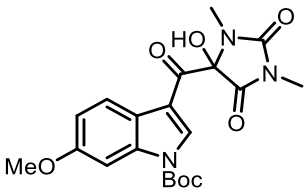
IRυ_{max}(film): 2925, 2362, 1725, 1459 cm^{-1}

^{1}H NMR (400 MHz, CD_3OD) = δ 8.99 (s, 1 H), 8.00 (d, *J* = 8.5 Hz, 1 H), 7.78 (d, *J* = 2.4 Hz, 1 H), 6.96 (dd, *J* = 2.4, 9.2 Hz, 1 H), 3.84 (s, 3 H), 3.03 (s, 3 H), 2.91 (s, 3 H), 1.72 (s, 9 H)

^{13}C NMR (100 MHz, CD_3OD) = δ 189.2, 171.9, 159.0, 157.7, 150.3, 139.6, 130.8, 130.5, 116.9, 116.5, 115.7, 105.5, 87.0, 56.2, 28.4, 25.6, 25.3.

HRMS (ESI): *m/z* calculated for $C_{20}H_{23}N_3O_7Na$ $[M+Na]^+$ = 440.1428, Observed = 440.1429

***tert*-butyl 3-(4-hydroxy-1,3-dimethyl-2,5-dioxoimidazolidine-4-carbonyl)-6-methoxy1H-indole-1-carboxylate (40m):**



The compound **40m** was synthesized by following general procedure for one-pot hydroxylation and obtained as white solid.

Yield= 40%

Melting point: 69 - 71 °C

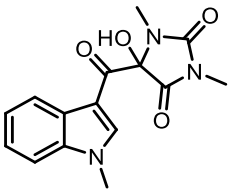
IRυ_{max}(film): 3367, 2978, 2363, 1723 cm^{-1}

^{1}H NMR (400 MHz, CD_3OD) = δ 8.90 (s, 1 H), 8.10 (d, *J* = 9.2 Hz, 1 H), 7.71 (d, *J* = 2.3 Hz, 1 H), 6.93 (dd, *J* = 2.3, 8.4 Hz, 1 H), 3.85 (s, 3 H), 3.02 (s, 3 H), 2.90 (s, 3 H), 1.72 (s, 9 H)

^{13}C NMR (100 MHz, CD_3OD) = δ 189.1, 171.9, 160.2, 157.7, 150.4, 138.1, 137.4, 123.9, 122.9, 116.9, 114.6, 100.2, 92.6, 87.0, 56.1, 28.4, 25.6, 25.3

HRMS (ESI): *m/z* calculated for $C_{20}H_{23}N_3O_7Na$ $[M+Na]^+$ = 440.1428, Observed = 440.1423.

5-hydroxy-1,3-dimethyl-5-(1-methyl-1H-indole-3-carbonyl)imidazolidine-2,4-dione (40p):



The compound **40p** was synthesized by following general procedure for one-pot hydroxylation and obtained as white solid.

Yield= 68%

Melting point: 194-196° C

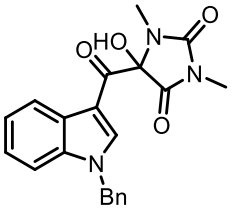
IRυ_{max}(film): 3847, 2364, 1724, 1602 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 8.44 - 8.42 (m, 1 H), 7.48 (s, 1 H), 7.41 - 7.39 (m, 3 H), 6.12 (s, 1 H), 3.86 (s, 3 H), 3.18 (s, 3 H), 2.85 (s, 3 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 183.3, 170.2, 156.6, 137.1, 135.5, 127.2, 124.7, 124.1, 122.9, 110.1, 110.0, 86.5, 34.1, 25.3, 24.8.

HRMS (ESI): *m/z* calculated for $C_{15}H_{15}N_3O_4Na$ $[M+Na]^+$ = 324.0955, Observed = 324.0947

5-(1-benzyl-1H-indole-3-carbonyl)-5-hydroxy-1,3-dimethylimidazolidine-2,4-dione (40q):



The compound **40q** was synthesized by following general procedure for one-pot hydroxylation and obtained as white solid.

Yield= 70%

Melting point: 202 -205 °C

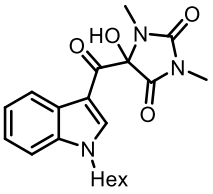
IRυ_{max}(film): 3743, 2976, 2363, 1721 cm^{-1}

^{1}H NMR (500 MHz, DMSO-d_6) = δ 8.90 (s, 1 H), 8.23 (dd, *J* = 2.7, 6.5 Hz, 1 H), 7.97 (s, 1 H), 7.57 (dd, *J* = 2.7, 6.1 Hz, 1 H), 7.35 - 7.25 (m, 7 H), 5.64 (s, 2 H), 2.92 (s, 3 H), 2.80 (s, 3 H)

^{13}C NMR (125 MHz, DMSO-d_6) = δ 186.3, 170.4, 155.5, 141.2, 136.7, 135.8, 128.8, 127.8, 127.3, 123.5, 123.0, 121.6, 111.7, 111.4, 90.8, 49.8, 24.9, 24.5.

HRMS (ESI): *m/z* calculated for $C_{21}H_{19}N_3O_4Na$ $[M+Na]^+$ = 400.1268, Observed = 400.1262.

5-(1-hexyl-1H-indole-3-carbonyl)-5-hydroxy-1,3-dimethylimidazolidine-2,4-dione (40r):



The compound **40r** was synthesized by following general procedure for one-pot hydroxylation and obtained as white solid.

Yield= 84%

Melting point: 168 -170 °C

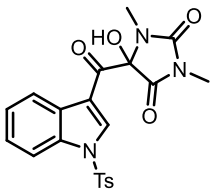
IRυ_{max}(film): 3325, 2932, 2362, 1721 cm^{-1}

^{1}H NMR (400 MHz, CD_3OD) = δ 8.66 (s, 1 H), 8.28 (m, 1 H), 7.49 (d, *J* = 7.6 Hz, 1 H), 7.32 - 7.26 (m, 2 H), 4.26 (t, *J* = 7.2 Hz, 2 H), 3.02 (s, 3 H), 2.90 (s, 3 H), 1.87 (t, *J* = 6.9 Hz, 2 H), 1.35 - 1.31 (m, 6 H), 0.89 - 0.86 (m, 3 H)

^{13}C NMR (100 MHz, CD_3OD) = δ 187.4, 172.7, 158.0, 142.2, 137.8, 129.2, 124.9, 124.3, 123.4, 113.2, 111.7, 92.6, 32.6, 30.9, 27.5, 25.6, 25.3, 23.7, 14.4

HRMS (ESI): *m/z* calculated for $C_{20}H_{25}N_3O_4Na$ $[M+Na]^+$ = 394.1737, Observed = 394.1726

5-hydroxy-1,3-dimethyl-5-(1-tosyl-1H-indole-3-carbonyl)imidazolidine-2,4-dione (40s):



The compound **40s** was synthesized by following general procedure for one-pot hydroxylation and obtained as white solid.

Yield= 77%

Melting point: 110 -113 °C

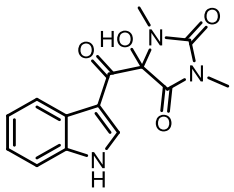
IRυ_{max}(film): 3743, 2960, 2363, 1723 cm^{-1}

^{1}H NMR (400 MHz, CD_3OD) = δ 9.12 (s, 1 H), 8.23 - 8.21 (m, 1 H), 7.95 - 7.88 (m, 3 H), 7.36 - 7.28 (m, 4 H), 3.07 (s, 1 H), 3.01 (m, 2 H), 2.92 (s, 2 H), 2.84 (s, 1 H), 2.29 (s, 3 H)

^{13}C NMR (100 MHz, CD_3OD) = δ 188.9, 171.7, 157.6, 148.0, 139.9, 135.6, 135.4, 131.5, 131.5, 129.7, 128.6, 128.5, 127.0, 126.3, 123.7, 117.6, 114.5, 92.9, 25.7, 25.4, 21.7.

HRMS (ESI): *m/z* calculated for $C_{21}H_{19}N_3O_6SNa$ $[M+Na]^+$ = 464.0887, Observed = 464.0879.

5-hydroxy-5-(1H-indole-3-carbonyl)-1,3-dimethylimidazolidine-2,4-dione (40t)



The compound **40t** was synthesized by following general procedure for one-pot hydroxylation and obtained as white solid.

Yield= 64%

Melting point: 203 -205° C

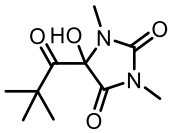
IRυ_{max}(film): 3345, 2995, 2318, 1704 cm $^{-1}$

^{1}H NMR (400 MHz, CD_3OD) = δ 8.65 (s, 1 H), 8.28 - 8.26 (m, 1 H), 7.48 - 7.46 (m, 1 H), 7.26- 7.23 (m, 2 H), 3.03 (s, 3 H), 2.90 (s, 3 H)

^{13}C NMR (100 MHz, CD_3OD) = δ 187.9, 172.7, 158.0, 139.4, 137.7, 128.3, 124.9, 124.0, 123.1, 114.2, 113.1, 92.4, 25.6, 25.3

HRMS (ESI): *m/z* calculated for $C_{14}H_{13}N_3O_4Na$ $[M+Na]^+$ = 310.0803, Observed = 310.0826.

5-hydroxy-1,3-dimethyl-5-pivaloylimidazolidine-2,4-dione (40a'):



The compound **40a'** was synthesized by following general procedure for one-pot hydroxylation and obtained as sticky solid.

Yield= 60%

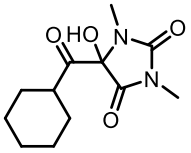
IRυ_{max}(film): 3375, 2974, 1713, 1465 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 5.55 (s, 1 H), 3.09 (s, 3 H), 2.76 (s, 3 H), 1.21 (s, 9 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 207.5, 169.3, 156.6, 86.7, 43.8, 27.0, 25.3, 25.1.

HRMS (ESI): *m/z* calculated for $C_{10}H_{16}N_2O_4Na$ $[M+Na]^+$ = 251.2010, Observed = 277.2011.

5-(cyclohexanecarbonyl)-5-hydroxy-1,3-dimethylimidazolidine-2,4-dione (40b'):



The compound **40b'** was synthesized by following general procedure for one-pot hydroxylation and obtained as white solid.

Yield= 61%

Melting point: 115 -117° C

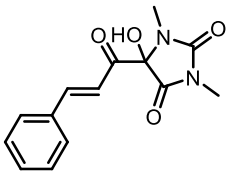
IRυ_{max}(film): 3395, 2933, 1781, 1462 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) = δ 5.23 (br. s., 1 H), 3.12 (d, *J* = 0.8 Hz, 3 H), 2.77 (s, 3 H), 2.54 - 2.48 (m, 1 H), 1.78 - 1.64 (m, 5 H), 1.23 - 1.21 (m, 5 H)

^{13}C NMR (100 MHz, $CDCl_3$) = δ 205.3, 168.8, 156.6, 87.6, 44.8, 29.4, 28.5, 25.3, 25.2, 25.1, 24.9.

HRMS (ESI): *m/z* calculated for $C_{12}H_{18}N_2O_4Na$ $[M+Na]^+$ = 277.1159, Observed = 277.1158.

5-cinnamoyl-5-hydroxy-1,3-dimethylimidazolidine-2,4-dione (40c'):



The compound **40c'** was synthesized by following general procedure for one-pot hydroxylation and obtained as white solid.

Yield= 68%

Melting point: 142 -144° C

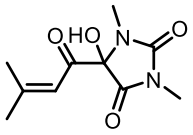
IRυ_{max}(film): 3738, 2927, 1716, 1601 cm^{-1}

^{1}H NMR (400 MHz, CD_3OD) = δ 7.83 (d, *J* = 16.0 Hz, 1 H), 7.71 - 7.69 (m, 2 H), 7.45 - 7.30 (m, 4 H), 3.02 (s, 3 H), 2.84 (s, 3 H)

^{13}C NMR (100 MHz, CD_3OD) = δ 193.0, 172.0, 157.8, 148.2, 135.7, 132.7, 130.3, 130.3, 120.5, 90.7, 25.4, 25.3

HRMS (ESI): *m/z* calculated for $C_{14}H_{14}N_2O_4Na$ $[M+Na]^+$ = 297.0846, Observed = 297.0844

5-hydroxy-1,3-dimethyl-5-(3-methylbut-2-enoyl)imidazolidine-2,4-dione (40d'):



The compound **40d'** was synthesized by following general procedure for one-pot hydroxylation and obtained as sticky solid.

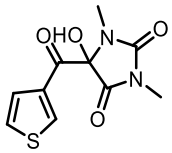
Yield= 66%

IRυ_{max}(film): 3606, 2954, 1719, 1462 cm^{-1}

^{1}H NMR (400 MHz, CD_3OD) = δ 6.50 (td, *J* = 1.2, 2.5 Hz, 1 H), 3.00 (s, 3 H), 2.78 (s, 3 H), 2.21 (d, *J* = 1.1 Hz, 3 H), 2.02 (d, *J* = 1.3 Hz, 3 H)

^{13}C NMR (100 MHz, CD_3OD) = δ 192.3, 172.1, 165.8, 157.9, 118.9, 90.4, 28.5, 25.3, 25.2, 21.9.

5-hydroxy-1,3-dimethyl-5-(thiophene-3-carbonyl)imidazolidine-2,4-dione (40e'):



The compound **40e'** was synthesized by following general procedure for one-pot hydroxylation and obtained as white solid.

Yield= 71%

Melting point: 110 -112° C

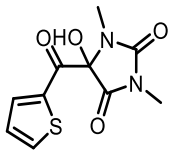
IRυ_{max}(film): 3314, 2325, 1717, 1465 cm^{-1}

^{1}H NMR (400 MHz, CD_3OD) = δ 8.79 (dd, *J* = 1.1, 2.9 Hz, 1 H), 7.66 (dd, *J* = 1.1, 5.1 Hz, 1 H), 7.46 (dd, *J* = 2.9, 5.1 Hz, 1 H), 3.01 (s, 3 H), 2.86 (s, 3 H)

^{13}C NMR (100 MHz, CD_3OD) = δ 187.7, 171.6, 157.6, 139.5, 138.7, 129.6, 127.0, 92.1, 25.5, 25.3

HRMS (ESI): *m/z* calculated for $C_{10}H_{10}N_2O_4SNa$ $[M+Na]^+$ = 277.0259, Observed = 277.0222.

5-hydroxy-1,3-dimethyl-5-(thiophene-2-carbonyl)imidazolidine-2,4-dione (40f'):



The compound **40f'** was synthesized by following general procedure for one-pot hydroxylation and obtained as white solid.

Yield= 73%

Melting point: 125 -127° C

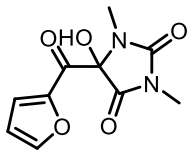
IRυ_{max}(film): 3095, 2351, 1718, 1467 cm^{-1}

^{1}H NMR (400 MHz, CD_3OD) = δ 8.29 (dd, *J* = 1.1, 4.0 Hz, 1 H), 7.97 (dd, *J* = 1.1, 5.0 Hz, 1 H), 7.24 (dd, *J* = 3.9, 4.9 Hz, 1 H), 3.02 (s, 3 H), 2.86 (s, 3 H)

^{13}C NMR (100 MHz, CD_3OD) = δ 186.5, 171.4, 157.6, 140.2, 138.8, 138.4, 129.6, 91.7, 25.5, 25.3

HRMS (ESI): *m/z* calculated for $C_{10}H_{11}N_2O_4S$ $[M+H]^+$ = 255.0439, Observed = 255.0451.

5-(furan-2-carbonyl)-5-hydroxy-1,3-dimethylimidazolidine-2,4-dione (40g'):



The compound **40g'** was synthesized by following general procedure for one-pot hydroxylation and obtained as white solid.

Yield= 70%

Melting point: 132 -134° C

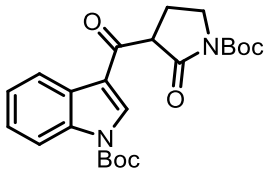
IRυ_{max}(film): 3097, 2350, 1719, 1466 cm^{-1}

^{1}H NMR (400 MHz, CD_3OD) = δ 7.87 (dd, *J* = 0.8, 1.8 Hz, 1 H), 7.77 (dd, *J* = 0.8, 3.8 Hz, 1 H), 6.71 (dd, *J* = 1.7, 3.7 Hz, 1 H), 3.05 (s, 3 H), 2.81 (s, 3 H)

^{13}C NMR (100 MHz, CD_3OD) = δ 181.0, 171.4, 157.8, 151.0, 150.6, 125.4, 114.2, 90.2, 25.4, 25.3

HRMS (ESI): *m/z* calculated for $C_{10}H_{11}N_2O_5$ $[M+H]^+$ = 239.0667, Observed = 239.0653.

***tert*-butyl 3-(1-(tert-butoxycarbonyl)-2-oxopyrrolidine-3-carbonyl)-1H-indole-1-carboxylate (54)**



The compound **54** was synthesized by following general procedure for for one-pot hydroxylation and obtained as white solid.

Yield= 62%

IRυ_{max}(film): 2981, 1736, 1660, 1368, 1146 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6) = δ 8.72 (s, 1 H), 8.25 (d, *J* = 7.6 Hz, 1 H), 8.12 (d, *J* = 8.3 Hz, 1 H), 7.49 - 7.33 (m, 2 H), 4.85 (t, *J* = 8.5 Hz, 1 H), 3.87 - 3.74 (m, 1 H), 3.74 - 3.63 (m, 1 H), 2.46 - 2.33 (m, 1 H), 2.27 - 2.13 (m, 1 H), 1.68 (s, 9 H), 1.45 (s, 9 H)

^{13}C NMR (100 MHz, DMSO-d_6) = δ 191.4, 170.2, 149.5, 148.5, 135.9, 135.0, 126.8, 125.7, 124.5, 121.8, 119.2, 115.0, 85.8, 82.1, 53.1(CH), 44.8, 27.6, 20.6.

HRMS (ESI): *m/z* calculated for $C_{23}H_{28}O_6N_2Na$ $[M+Na]^+$= 451.1840, Observed = 451.1830.

2.2.6 References

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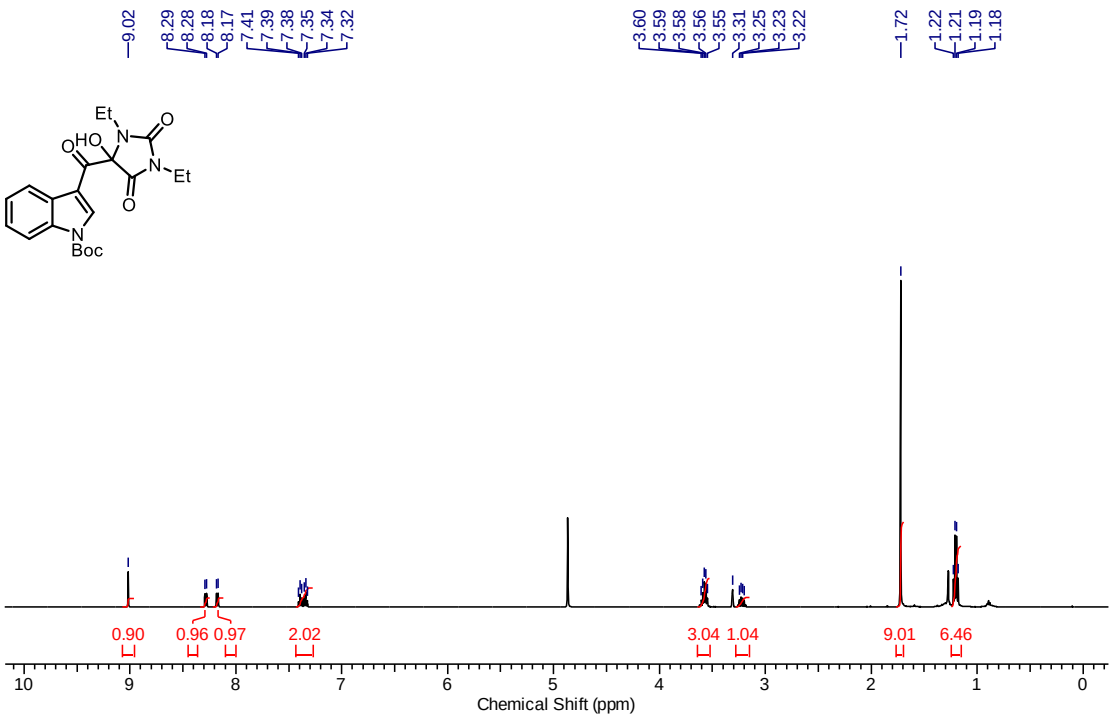
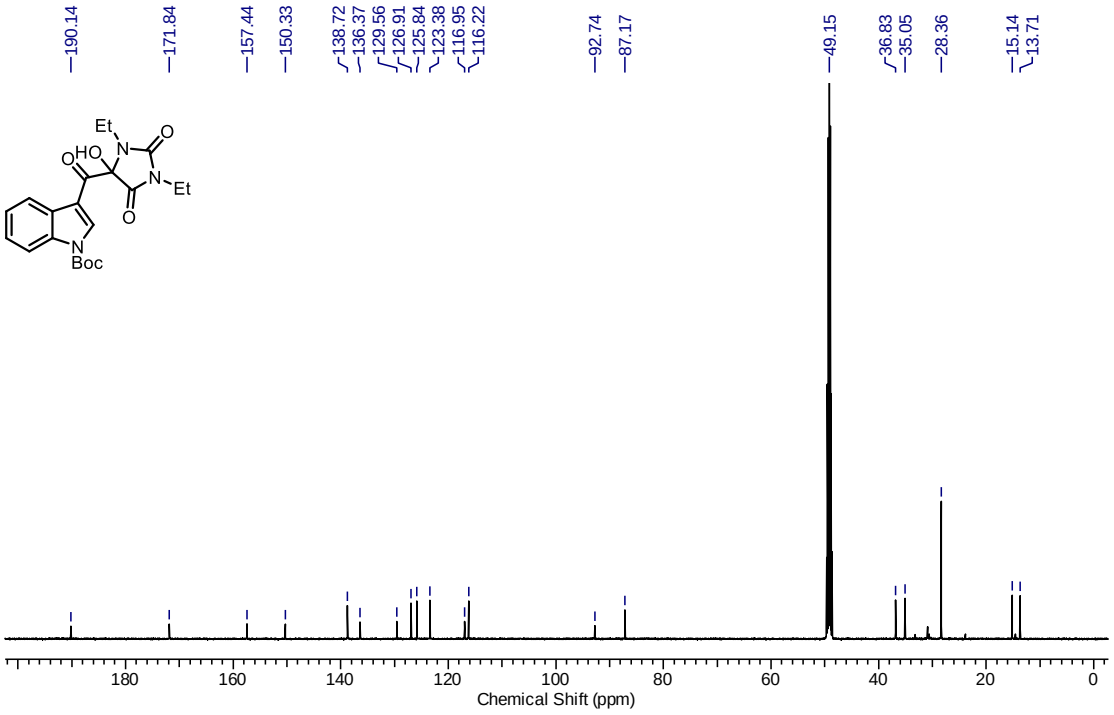
2.2.7 NMR Spectra:

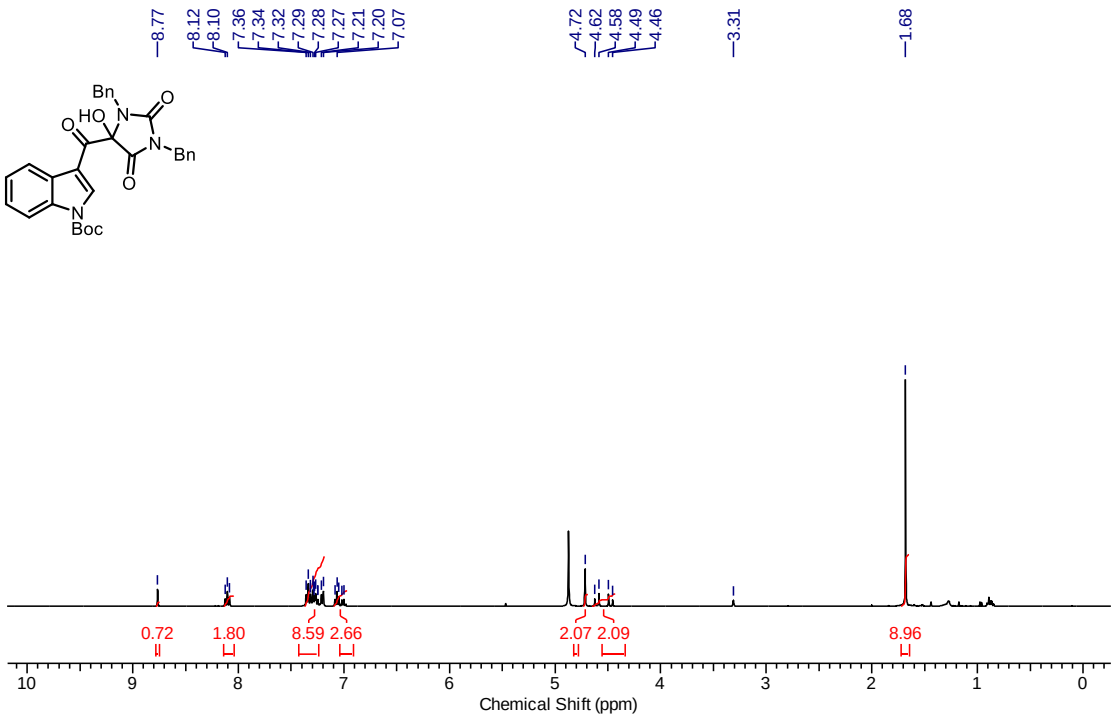
^{1}H NMR of Compound 40a in CD_3OD at 500 MHz



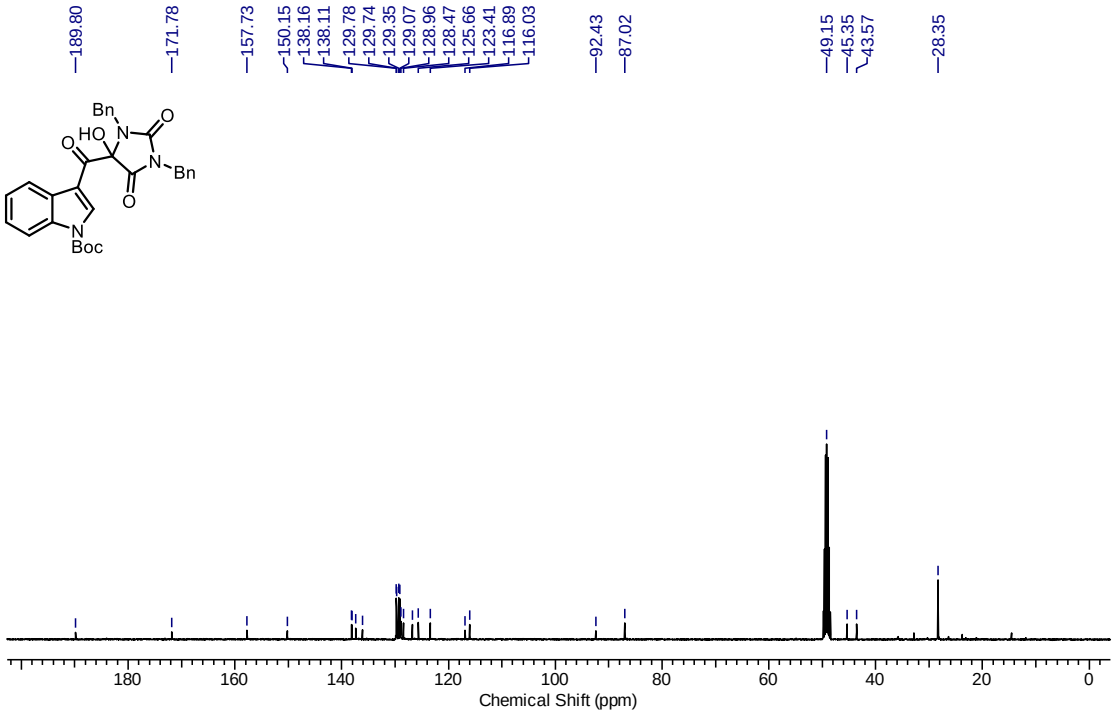
^{13}C NMR of Compound 40a in CD_3OD at 125 MHz



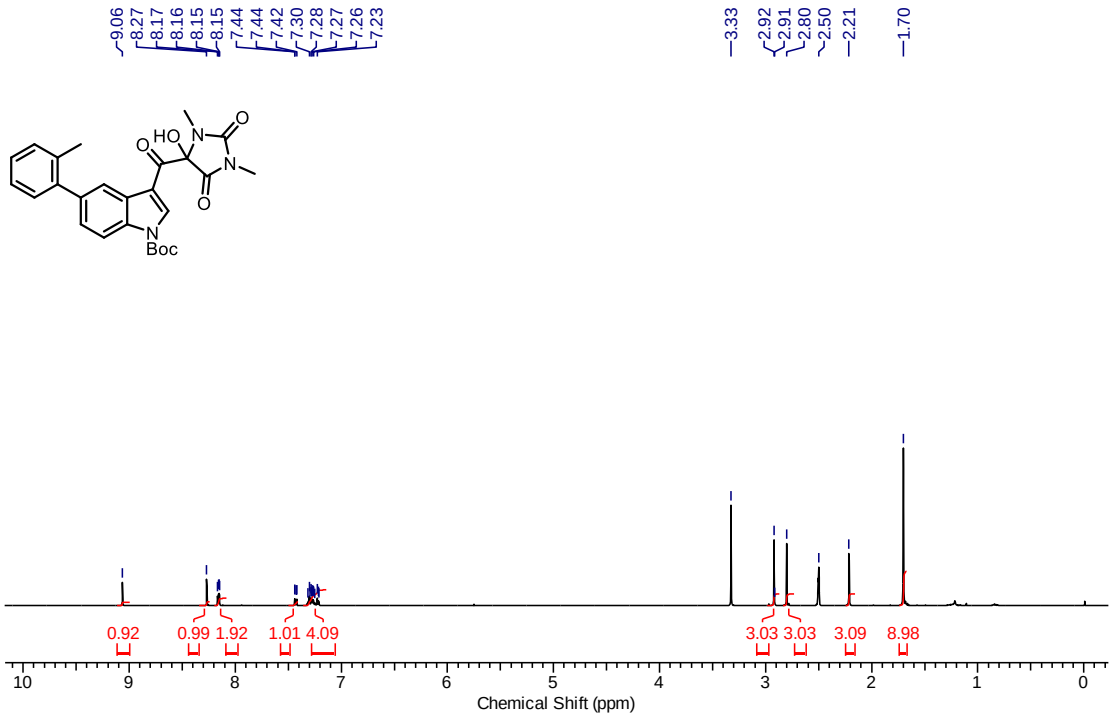
^{1}H NMR of Compound 40b in CD_3OD at 400 MHz



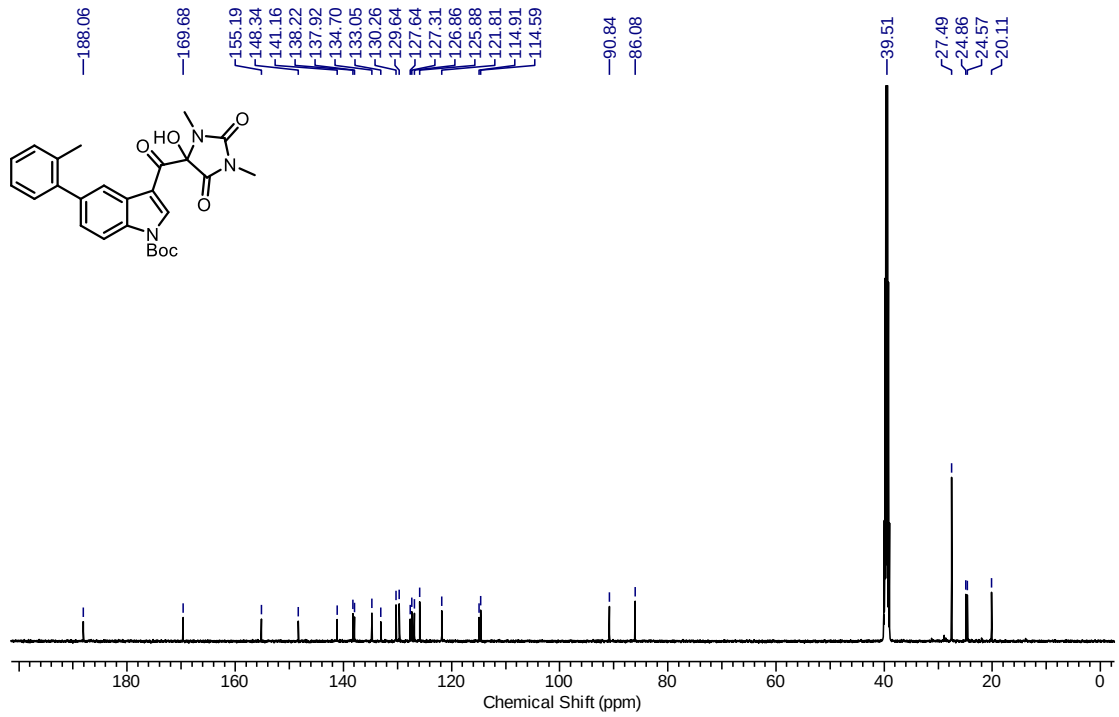
^{13}C NMR of Compound 40b in CD_3OD at 100 MHz



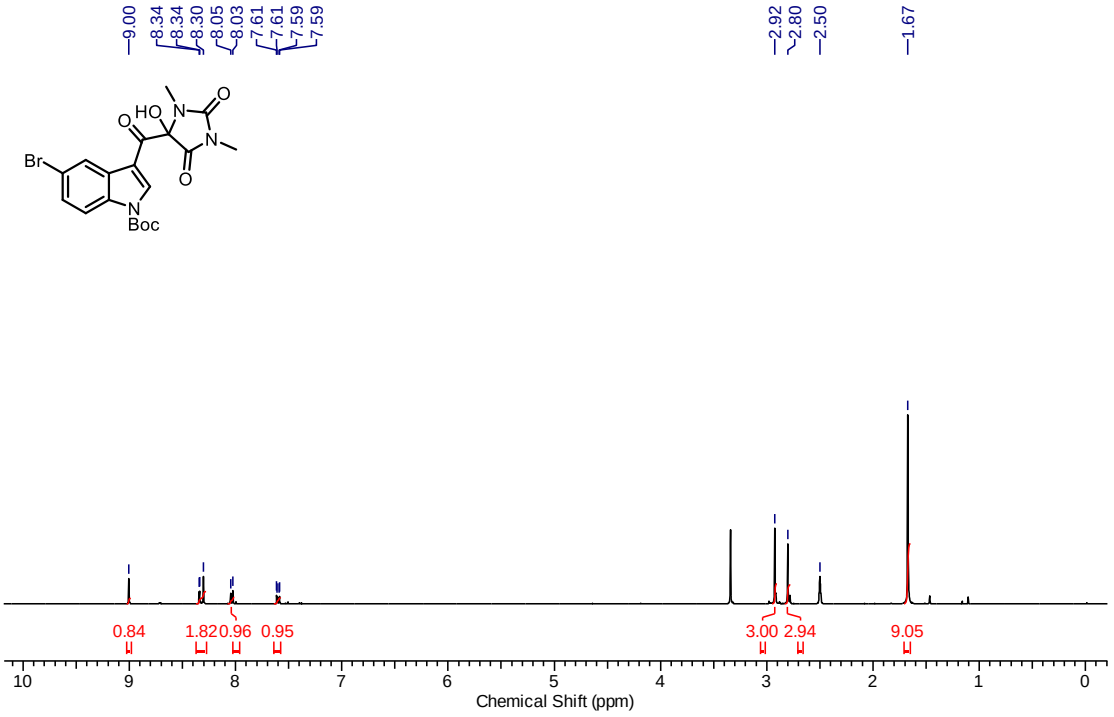
^{1}H NMR of Compound 40c in DMSO-d_6 at 500 MHz



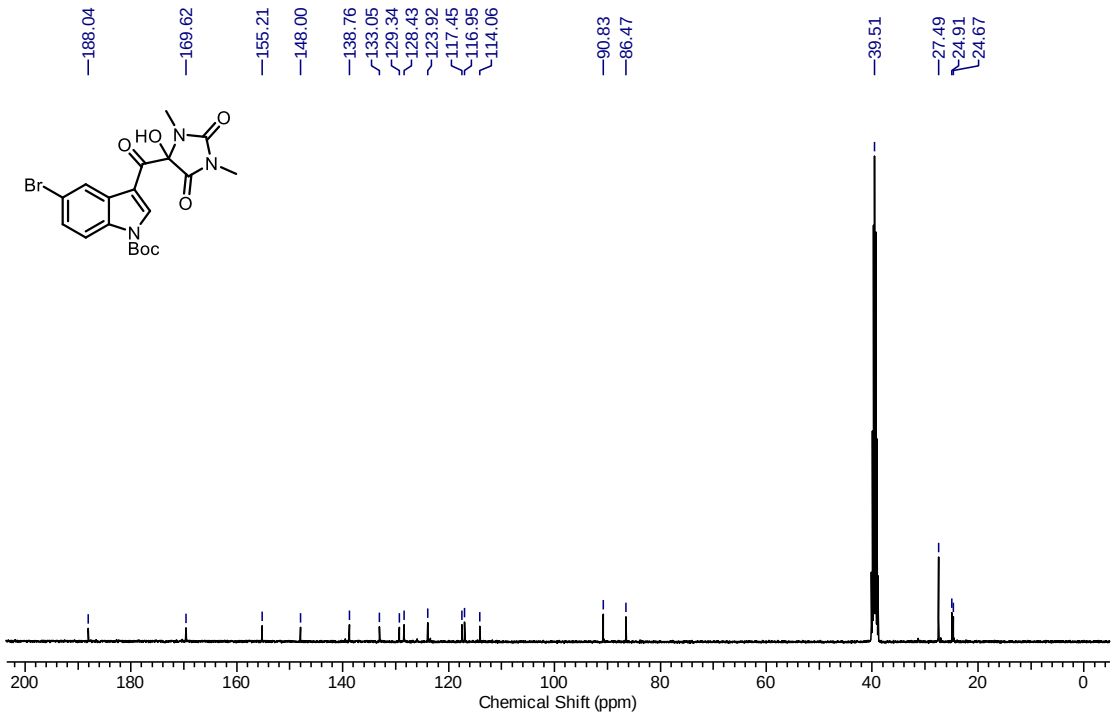
^{13}C NMR of Compound 40c in DMSO-d_6 at 100 MHz



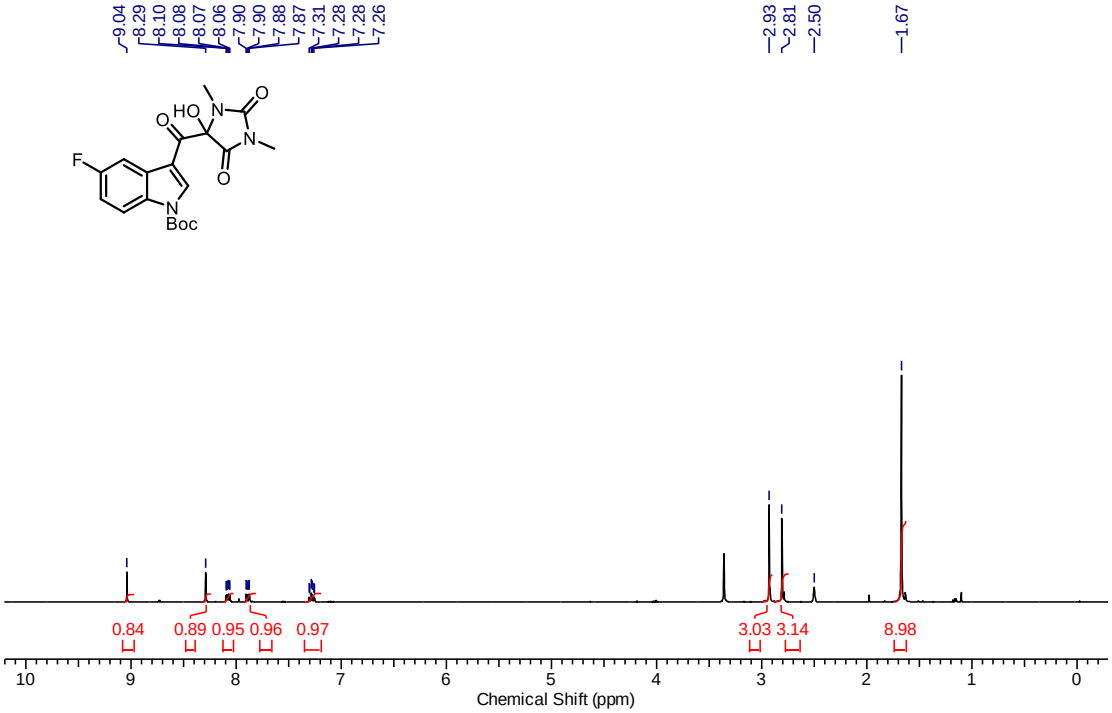
^{1}H NMR of Compound 40d in DMSO-d_6 at 400 MHz



^{13}C NMR of Compound 40d in DMSO-d_6 at 100 MHz



^{1}H NMR of Compound 40g in DMSO-d_6 at 400 MHz



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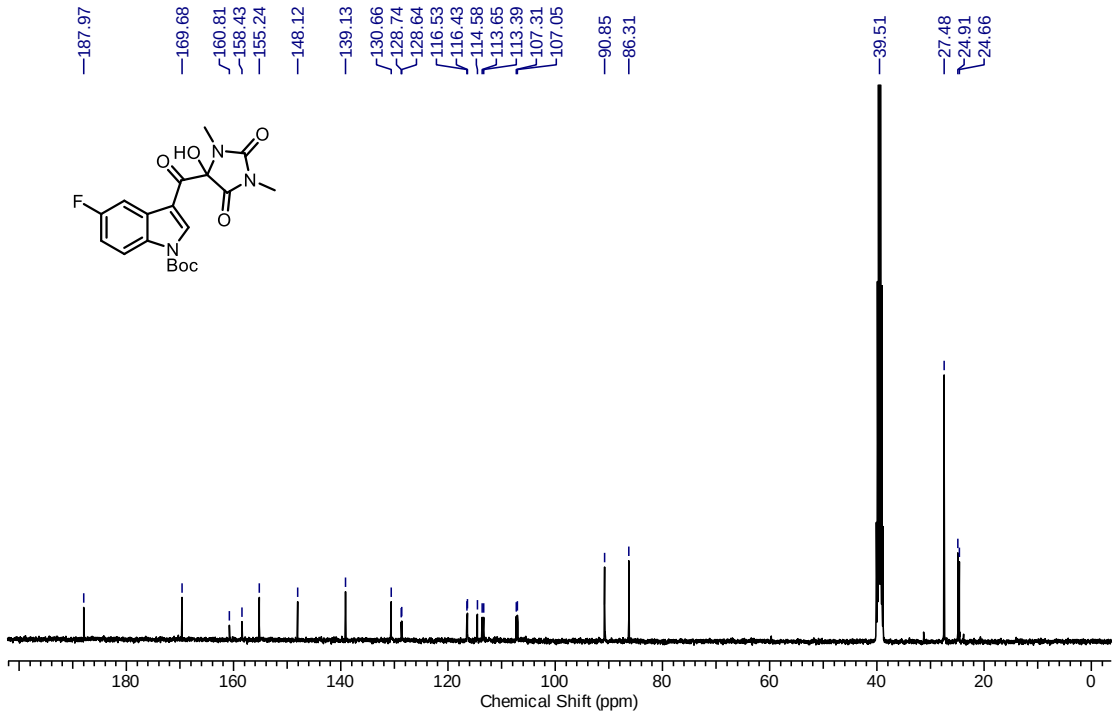
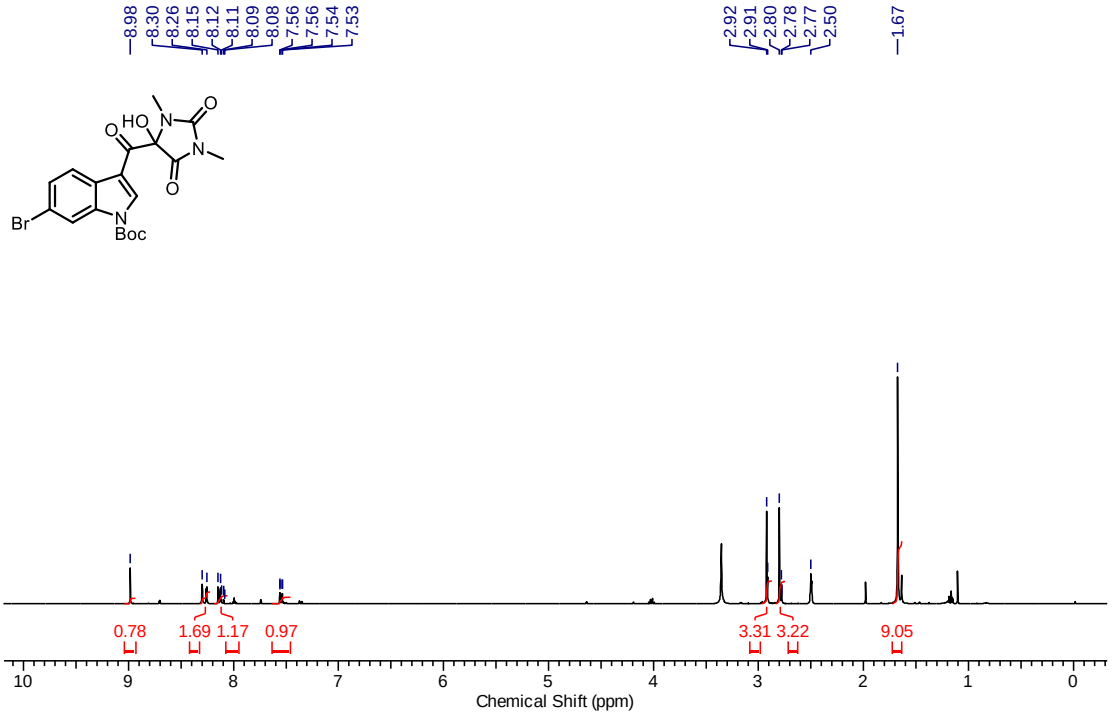
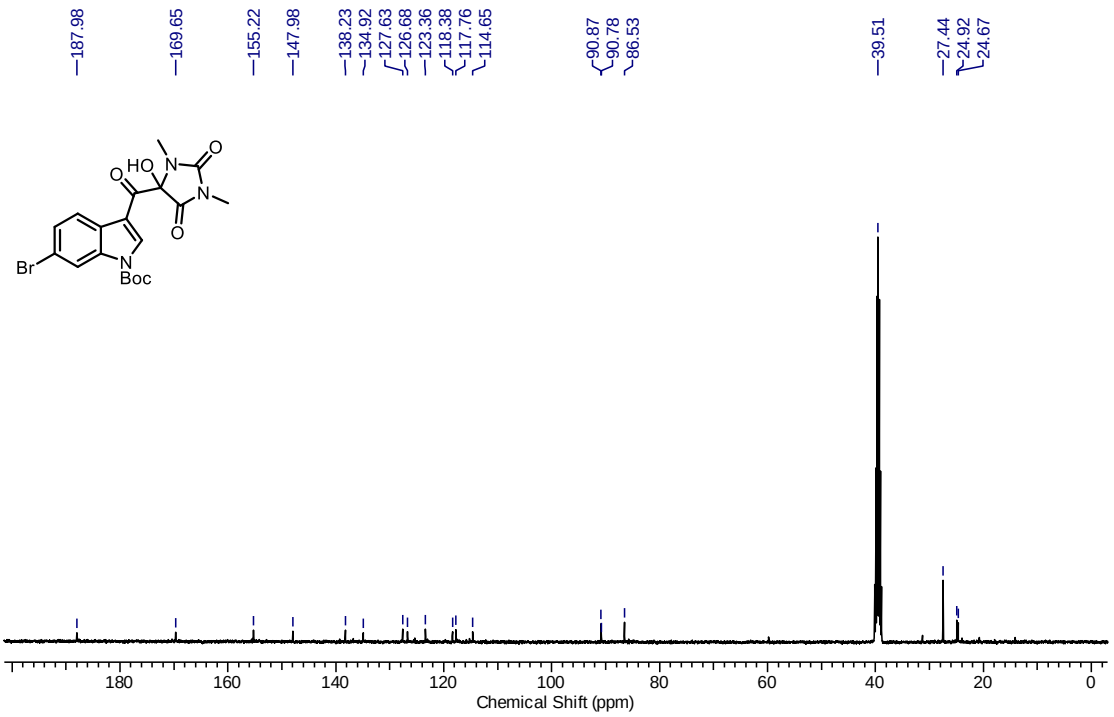


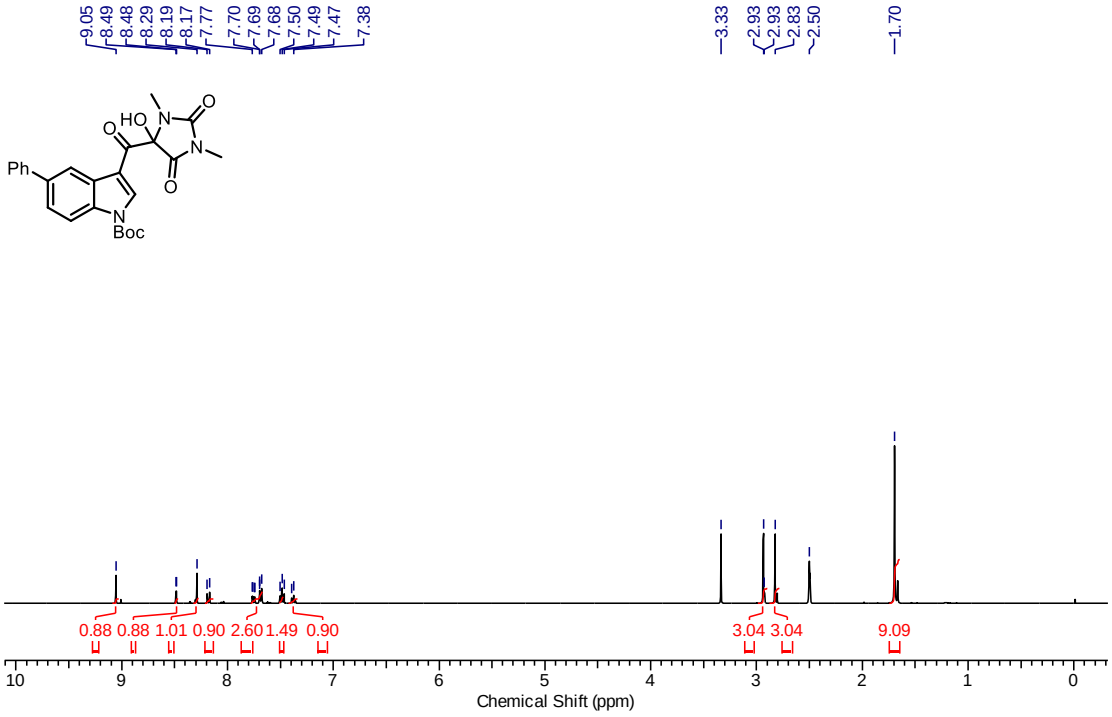
^{1}H NMR of Compound 40h in DMSO-d_6 at 400 MHz



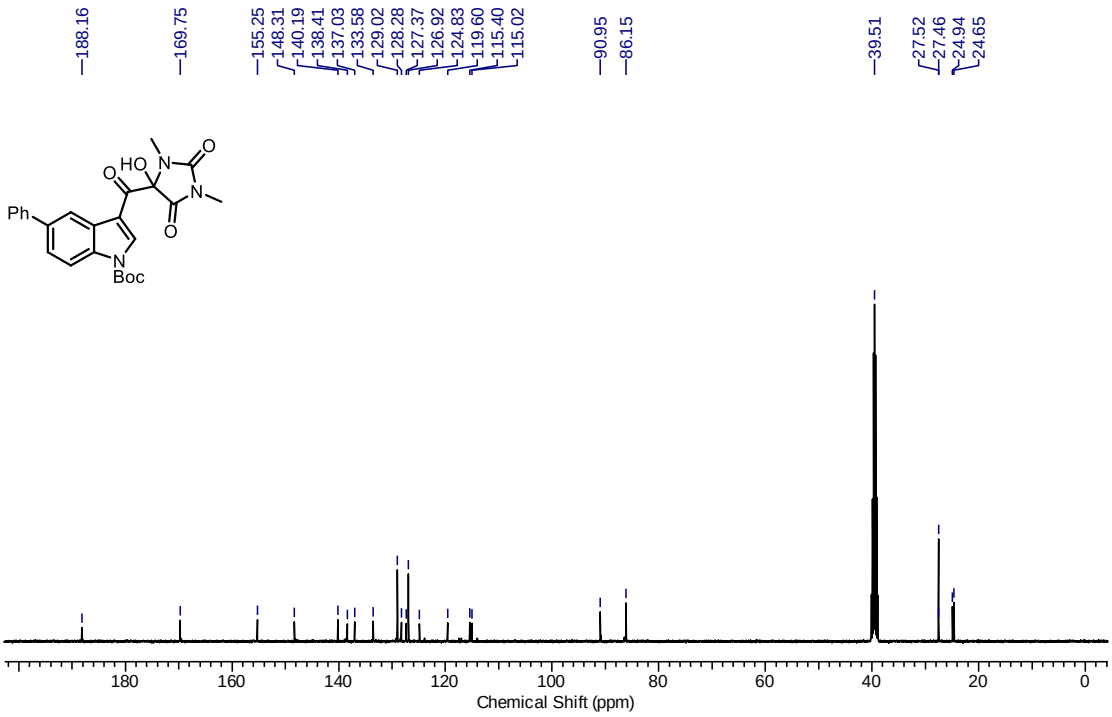
^{13}C NMR of Compound 40h in DMSO-d_6 at 100 MHz



^{1}H NMR of Compound 40i in DMSO-d_6 at 400 MHz



^{13}C NMR of Compound 40i in DMSO-d_6 at 100 MHz



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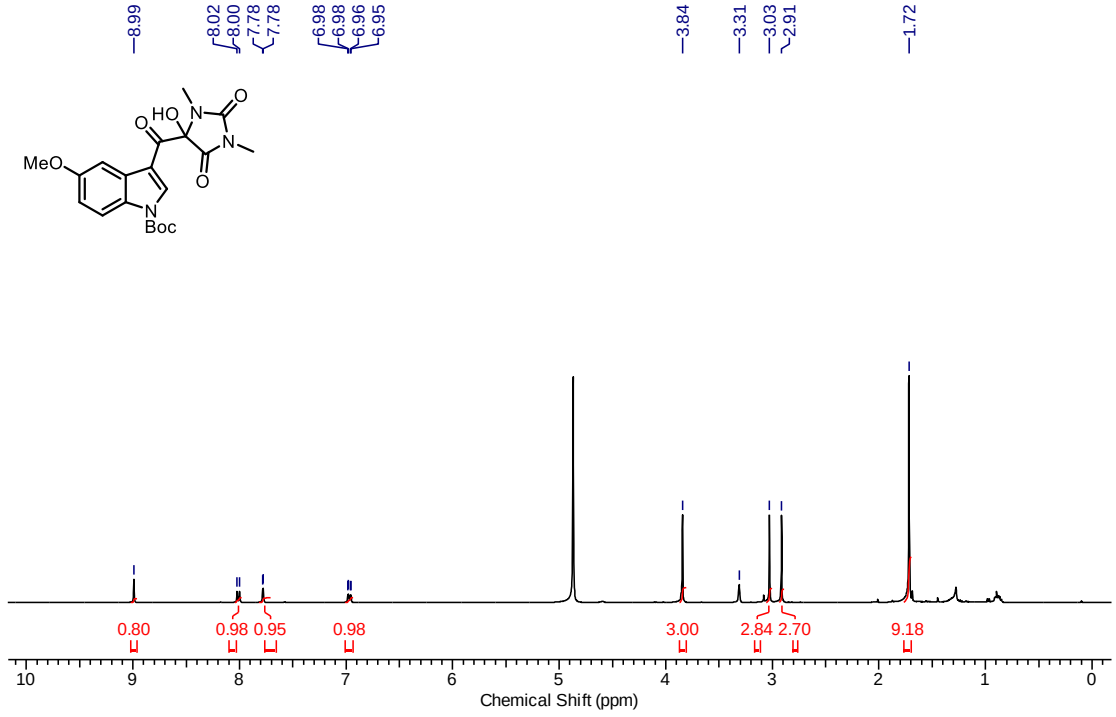
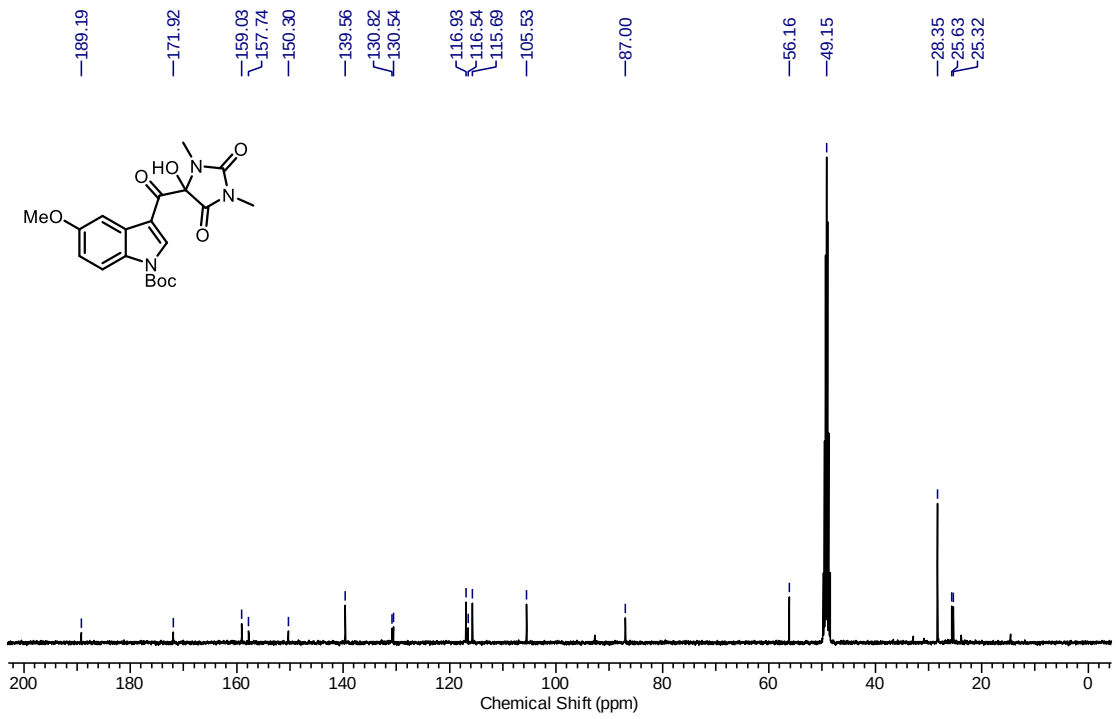
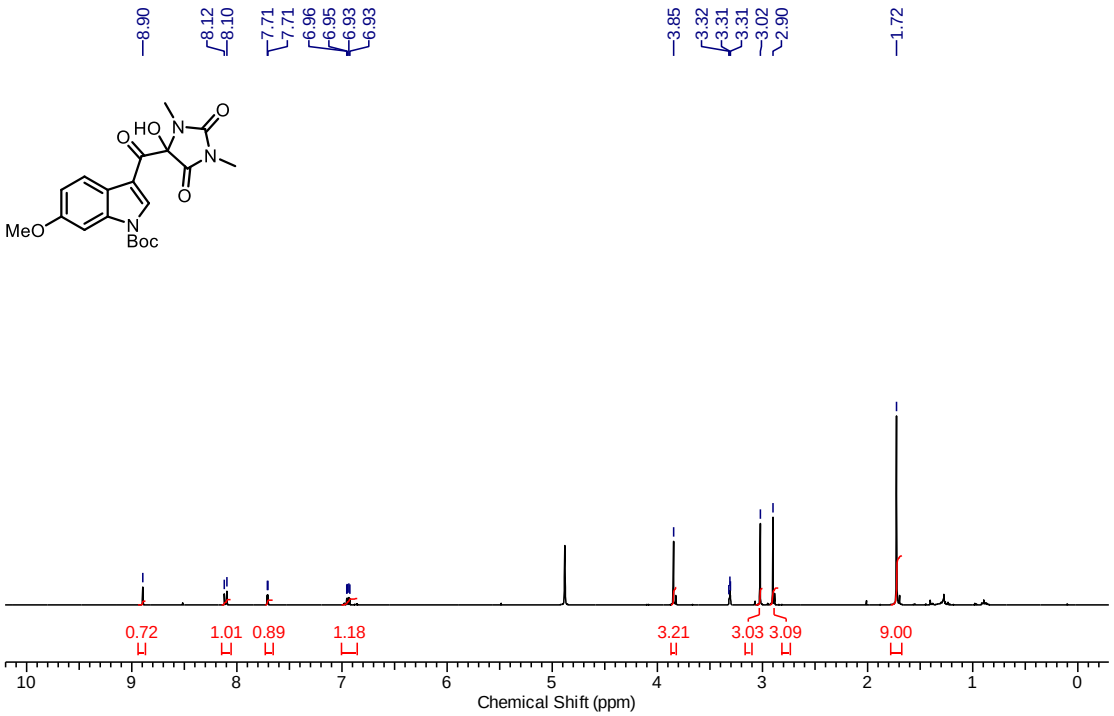


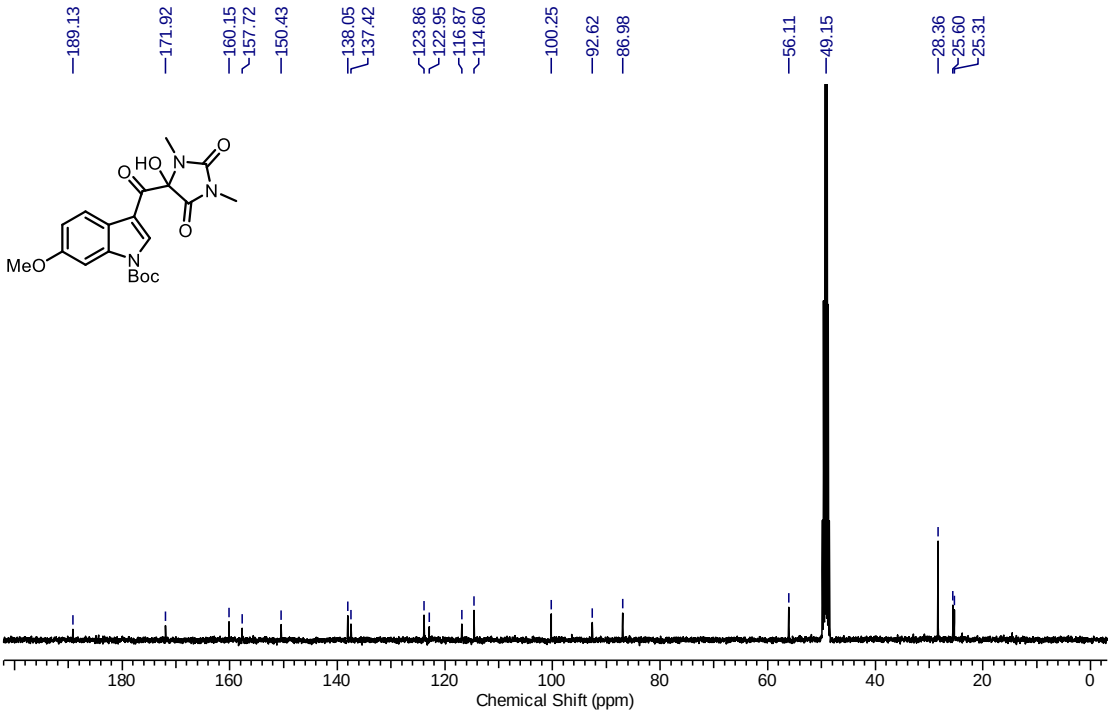
^{13}C NMR of Compound 40j in CD_3OD at 100 MHz



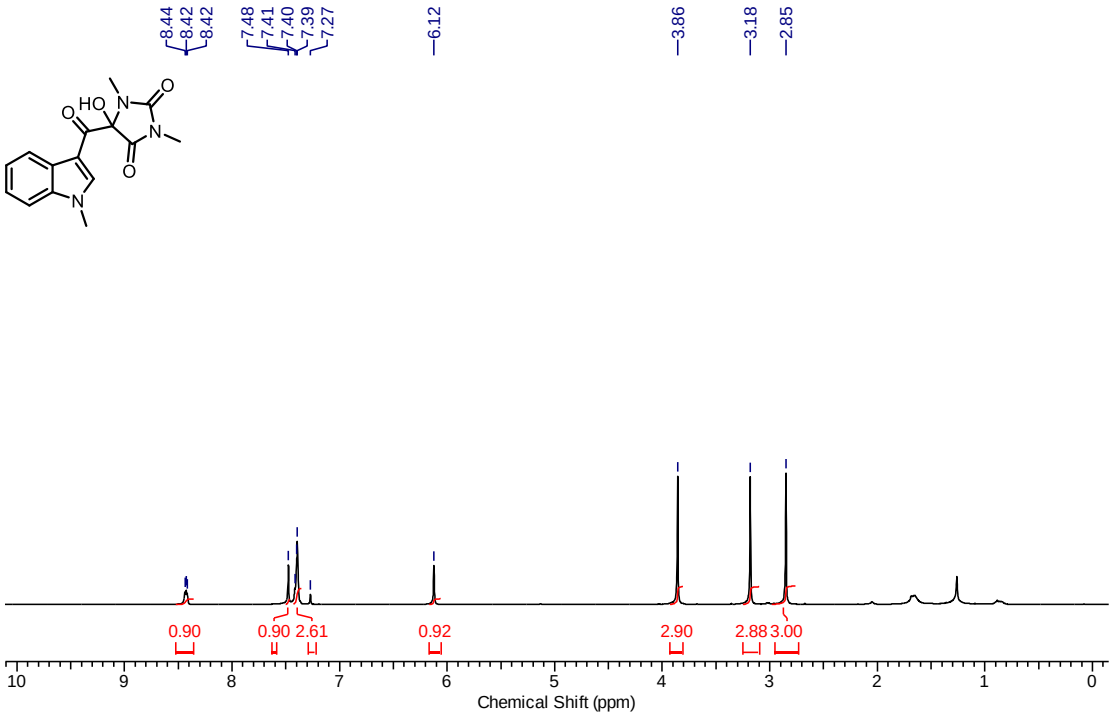
^{1}H NMR of Compound 40m in CD_3OD at 400 MHz



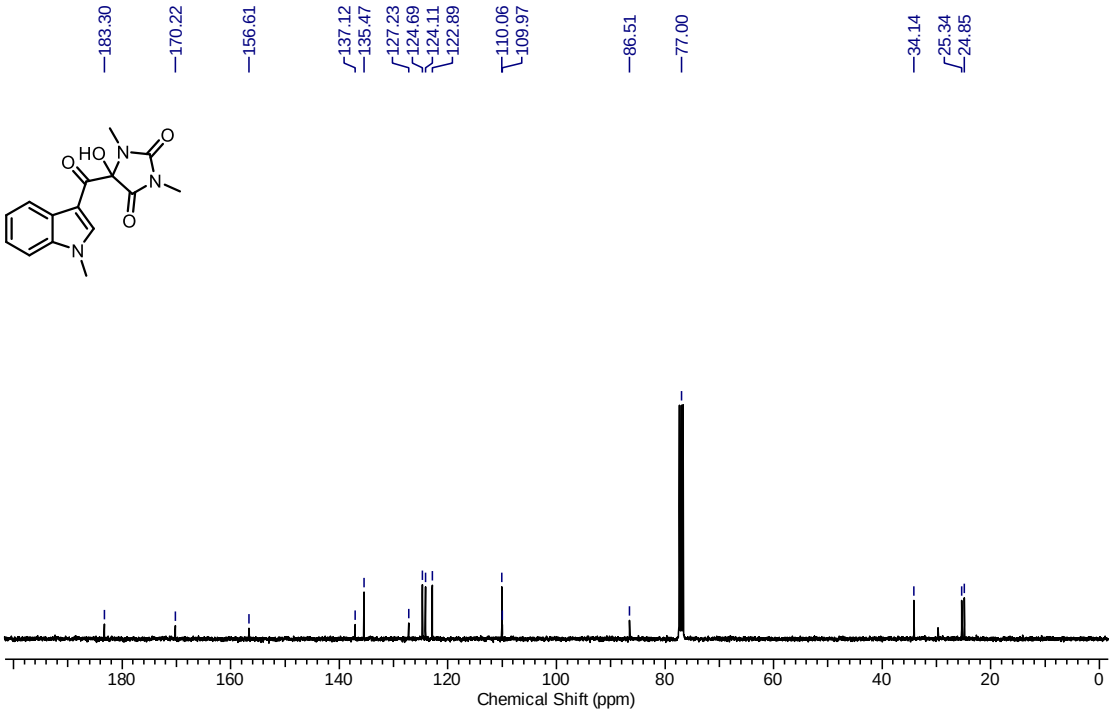
^{13}C NMR of Compound 40m in CD_3OD at 100 MHz



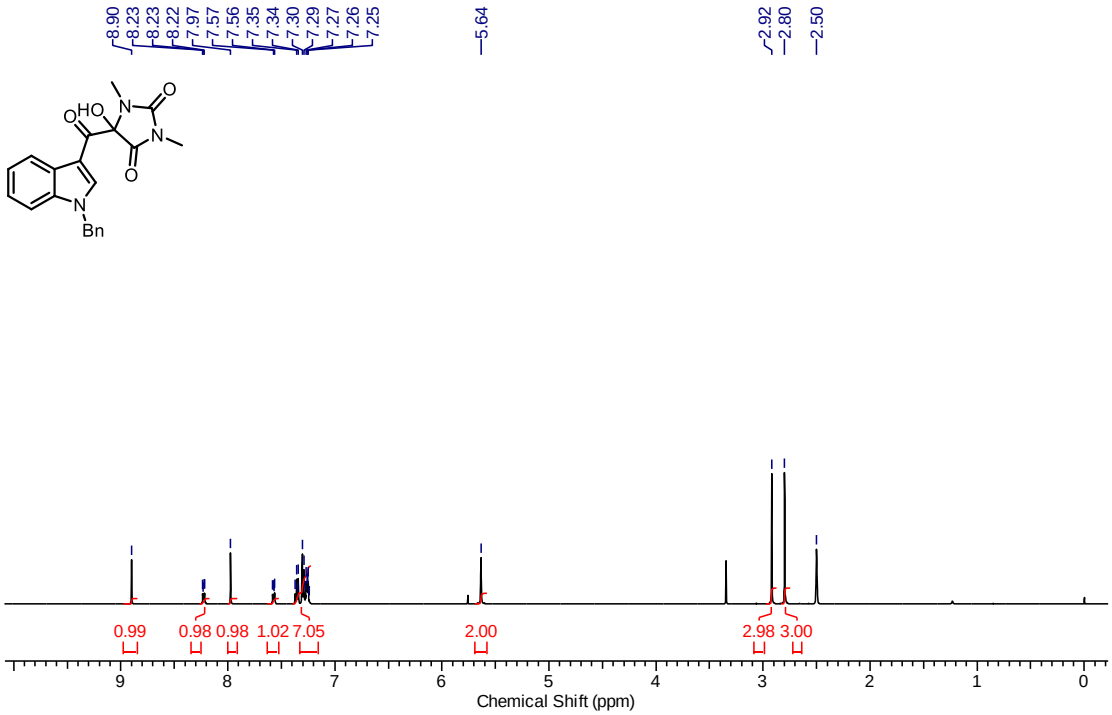
^{1}H NMR of Compound 40p in $CDCl_3$ at 400 MHz



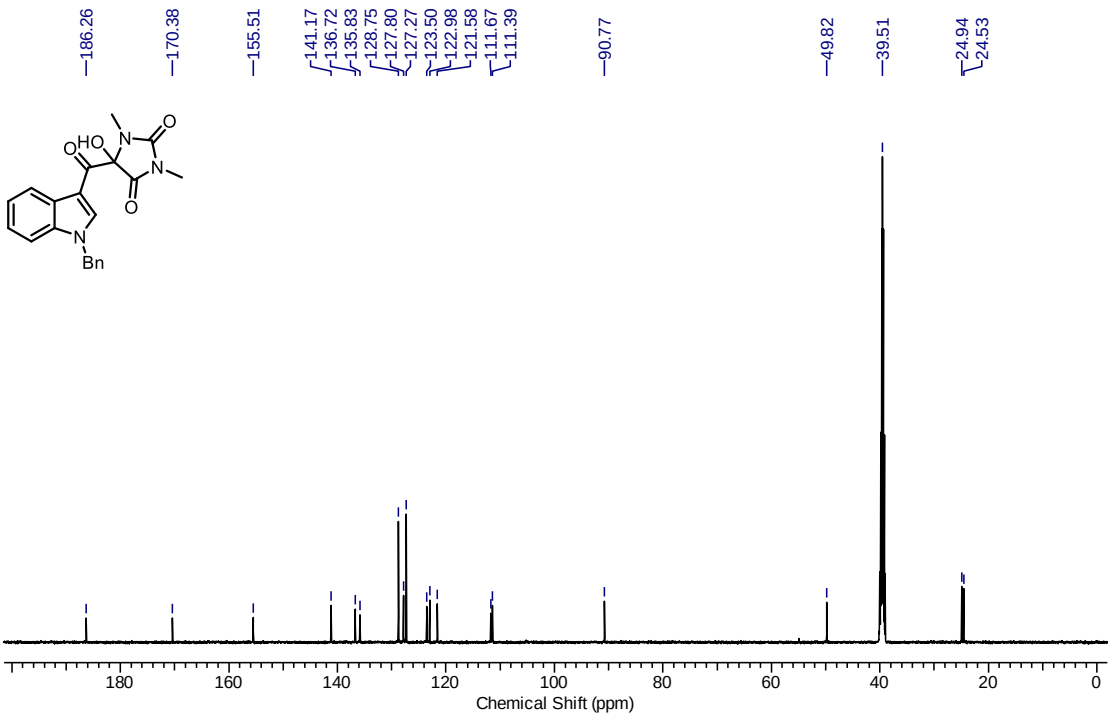
^{13}C NMR of Compound 40p in $CDCl_3$ at 100 MHz



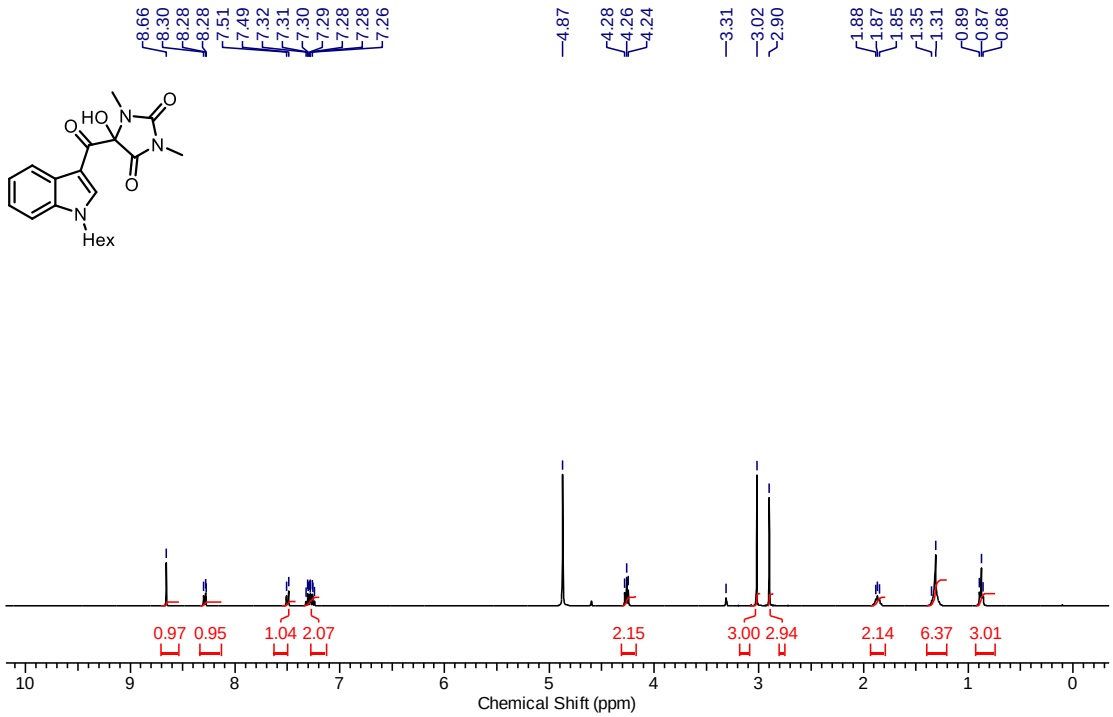
^{1}H NMR of Compound 40q in DMSO-d_6 at 500 MHz



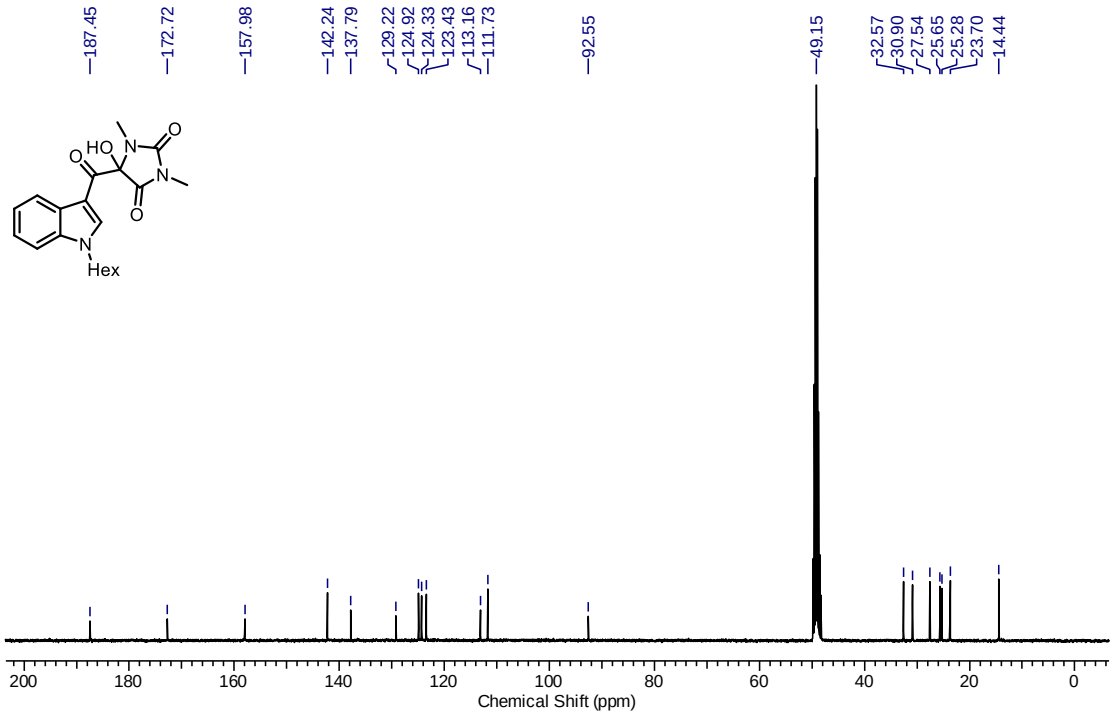
^{13}C NMR of Compound 40q in CD_3OD at 125 MHz



^{1}H NMR of Compound 40r in CD_3OD at 400 MHz



^{13}C NMR of Compound 40r in CD_3OD at 100 MHz



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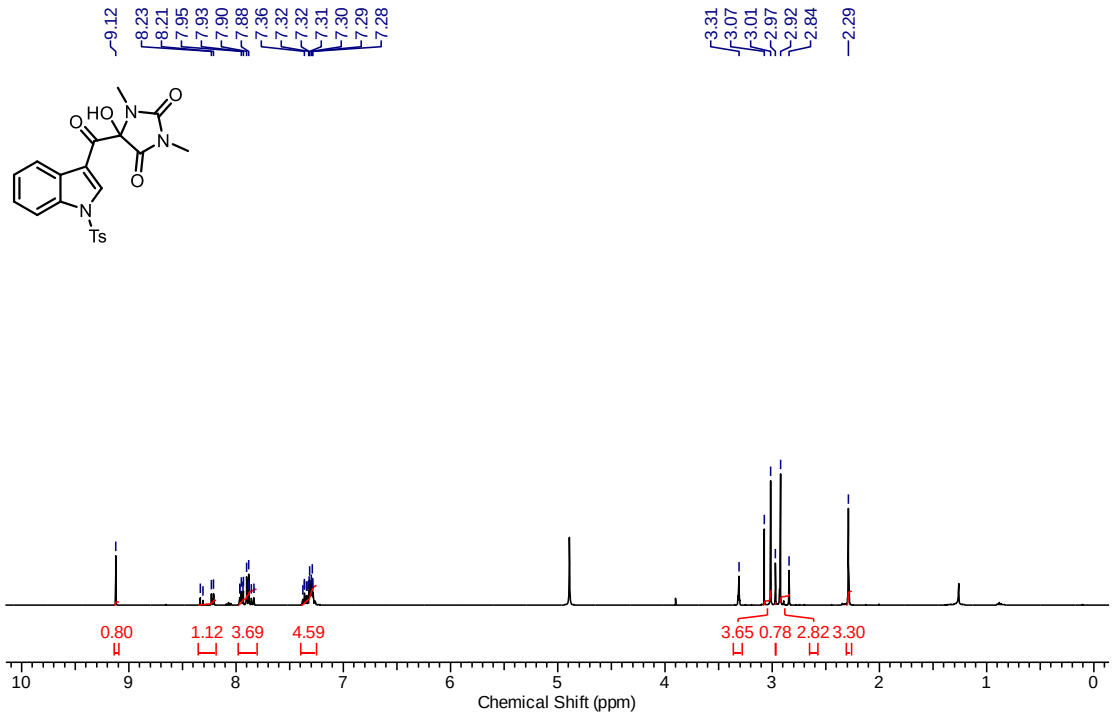
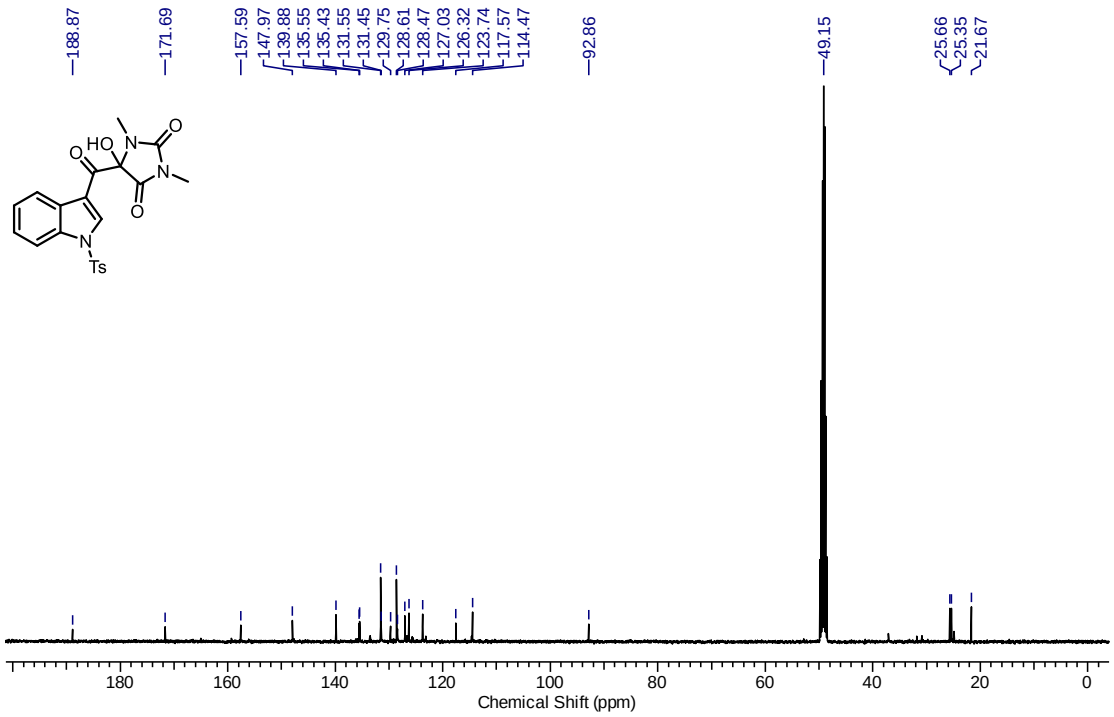
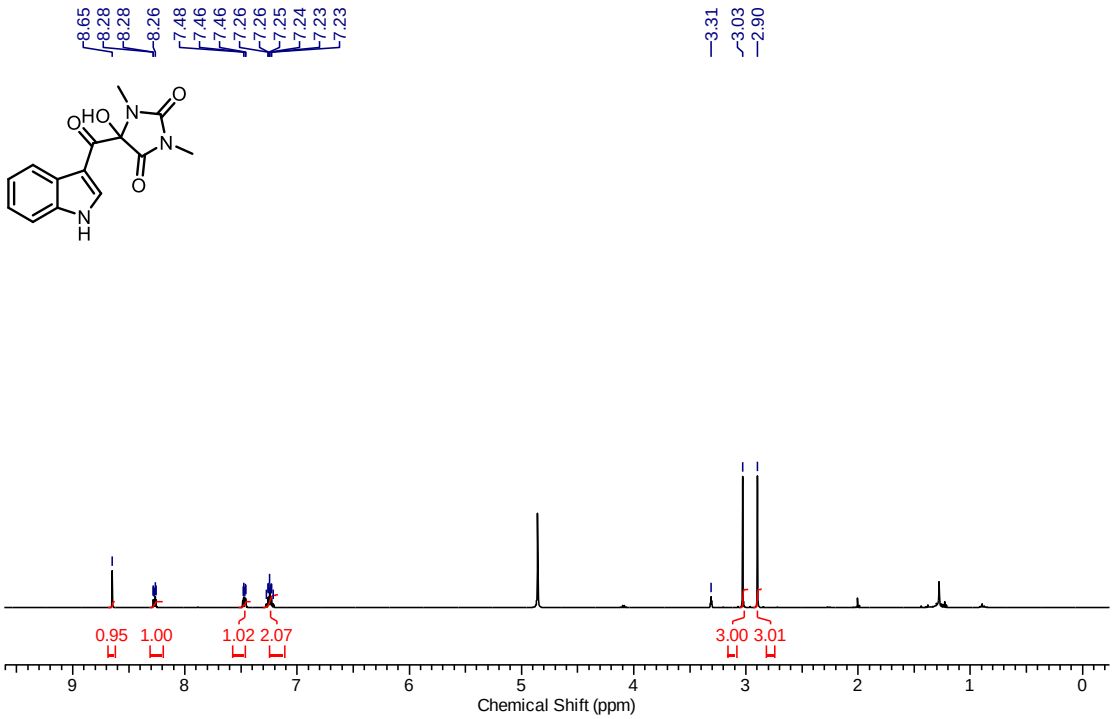


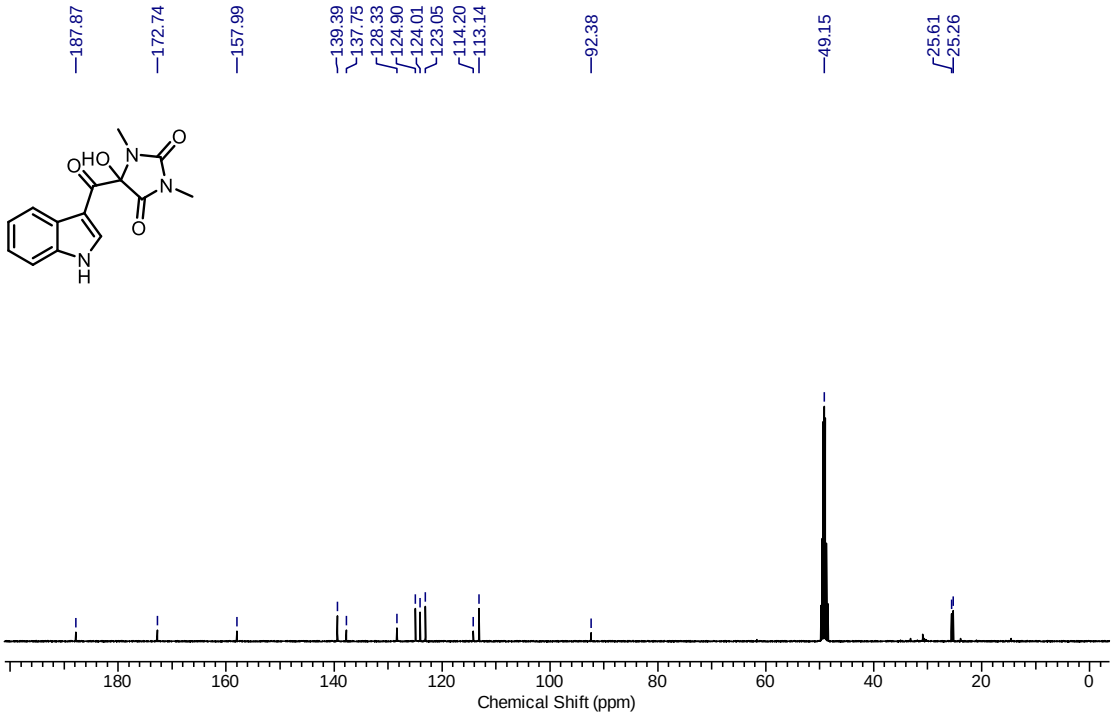
^{13}C NMR of Compound 40s in CD_3OD at 100 MHz



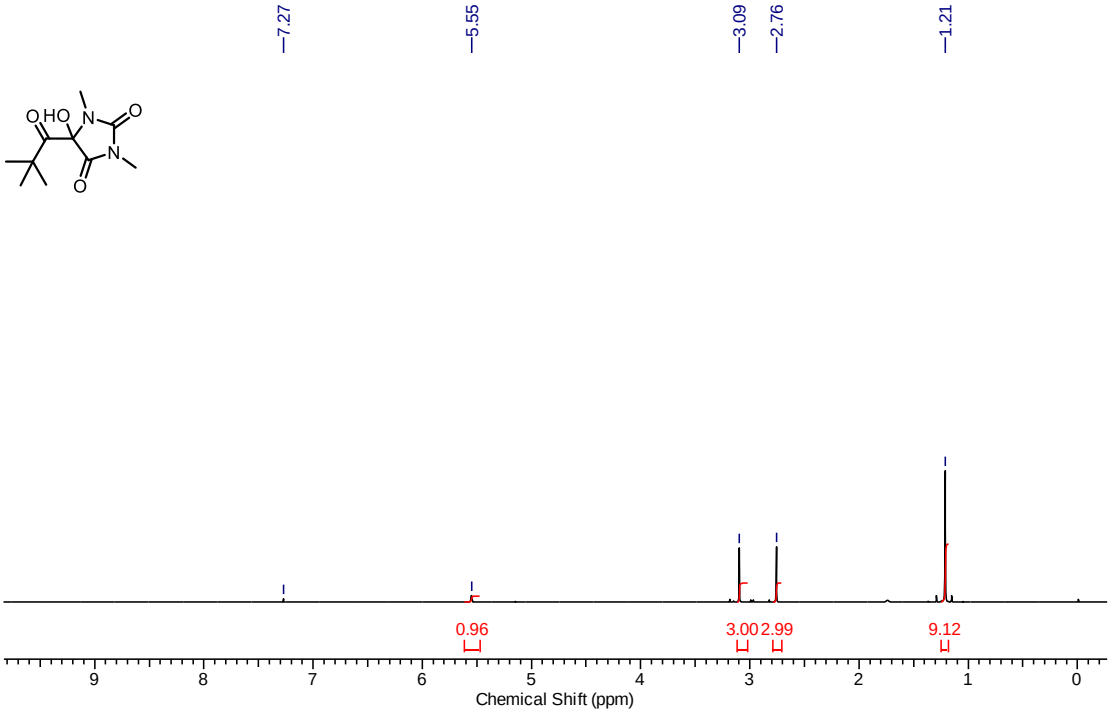
^{1}H NMR of Compound 40t in CD_3OD at 400 MHz



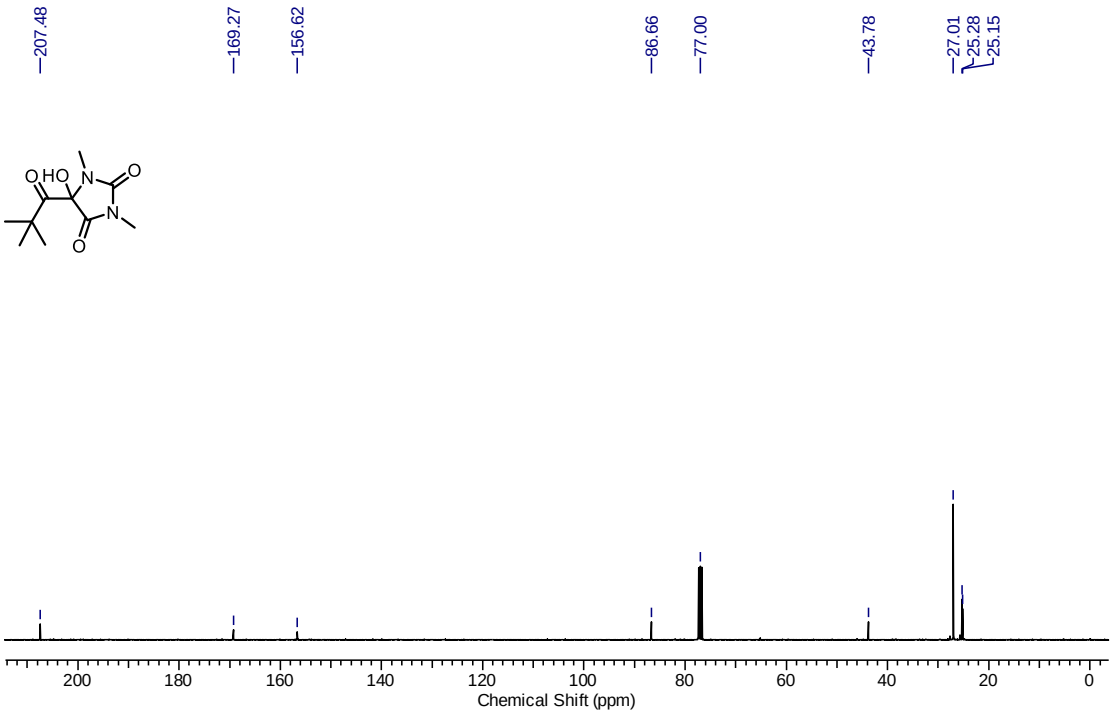
^{13}C NMR of Compound 40t in CD_3OD at 100 MHz



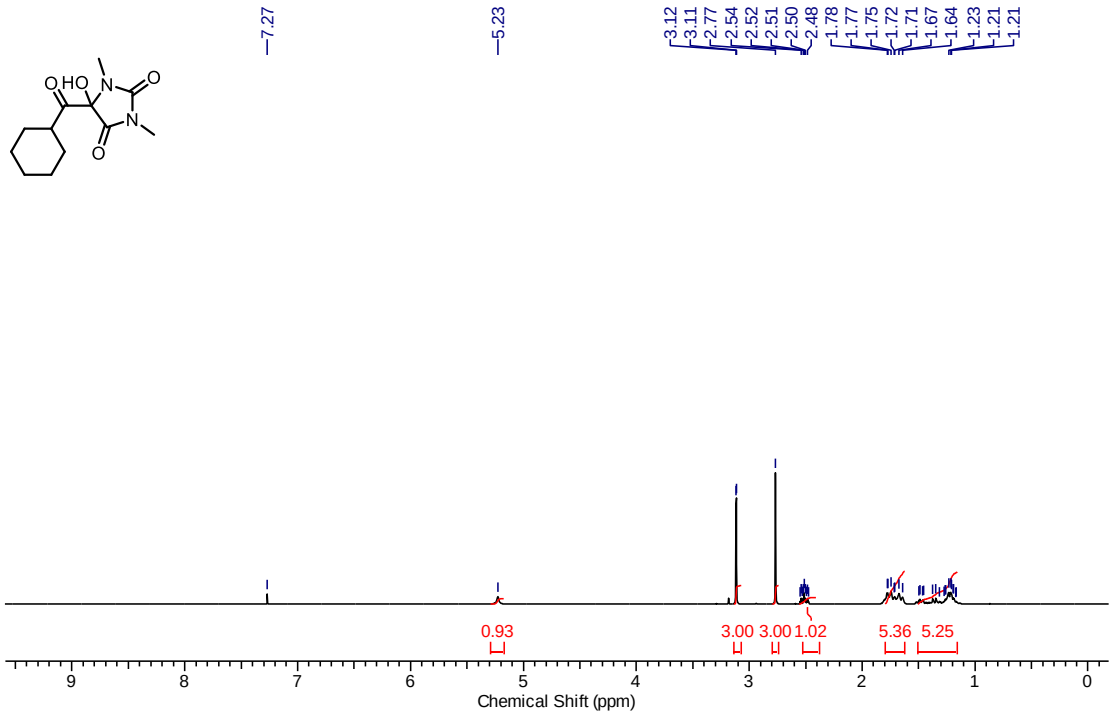
^{1}H NMR of Compound 40a' in $CDCl_3$ at 400 MHz



^{13}C NMR of Compound 40a' in $CDCl_3$ at 100 MHz



^{1}H NMR of Compound 40b' in $CDCl_3$ at 400 MHz



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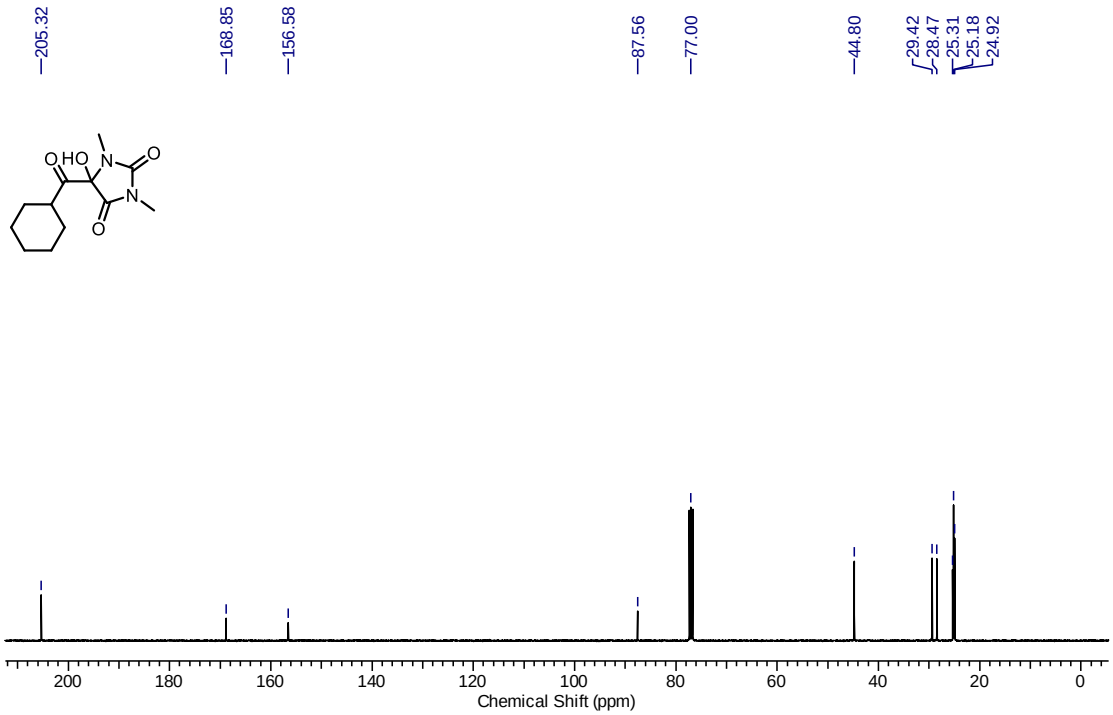
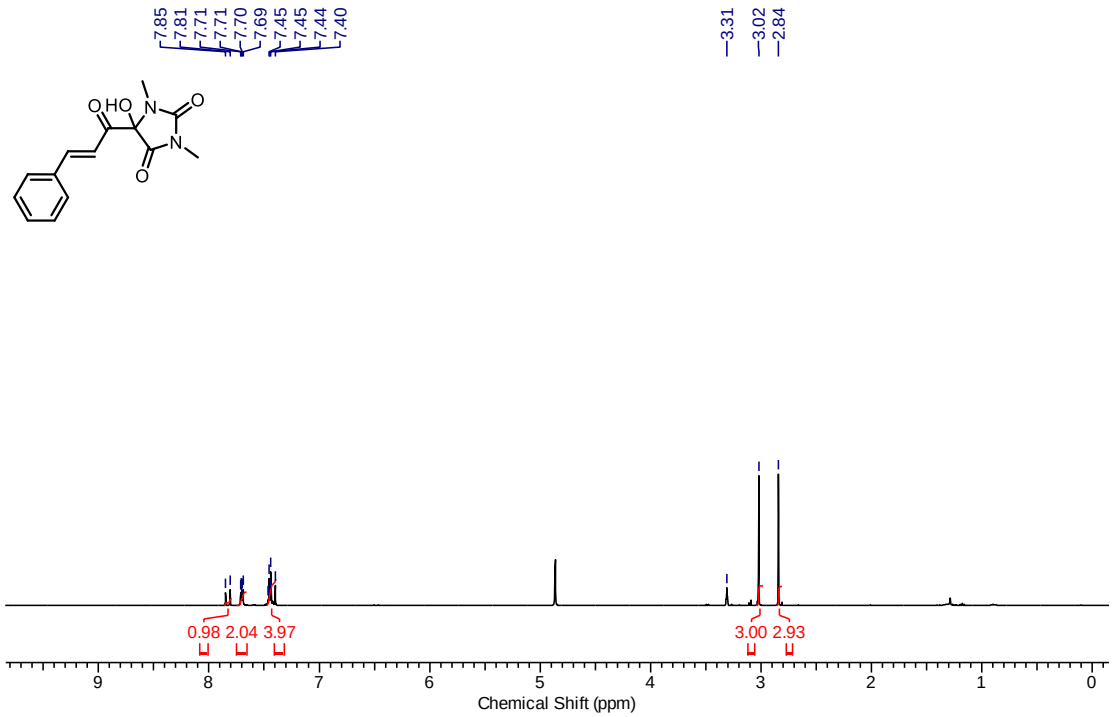
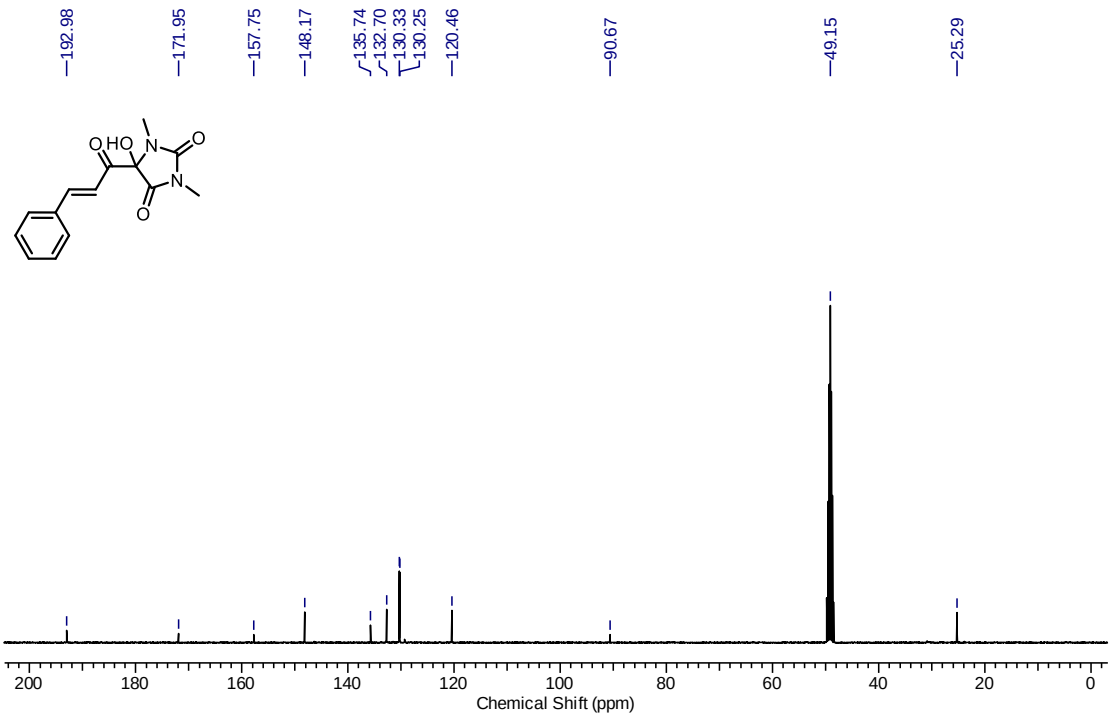


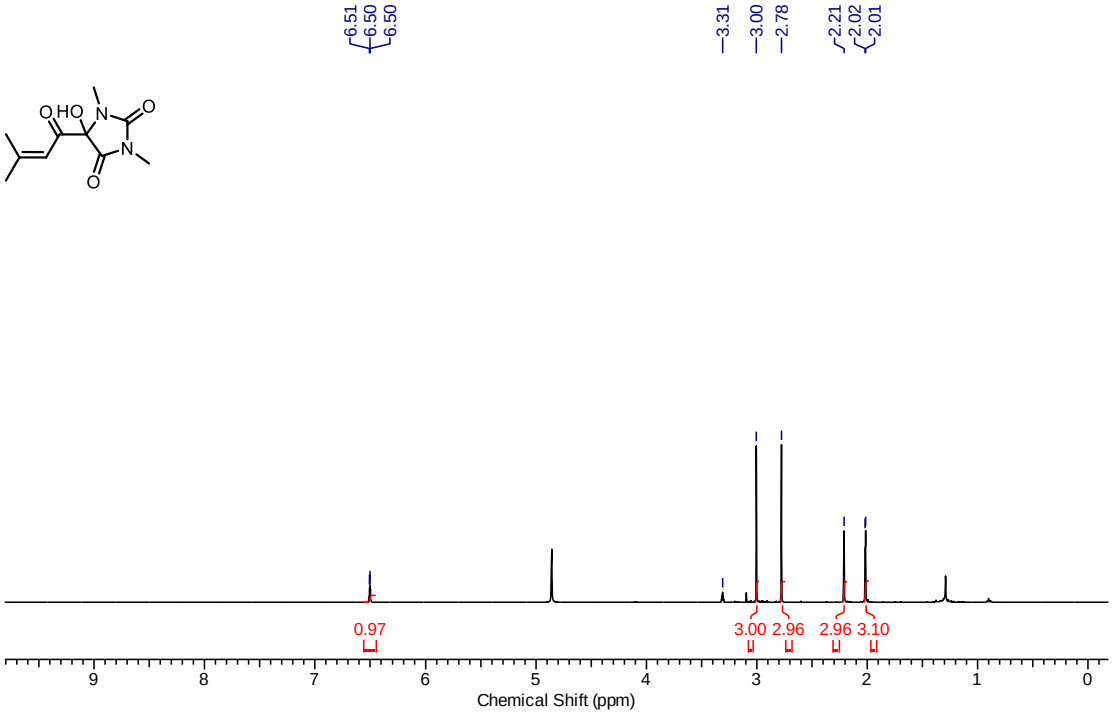
^{1}H NMR of Compound 40c' in CD_3OD at 400 MHz



^{13}C NMR of Compound 40c' in CD_3OD at 100 MHz



^{1}H NMR of Compound 40d' in CD_3OD at 400 MHz



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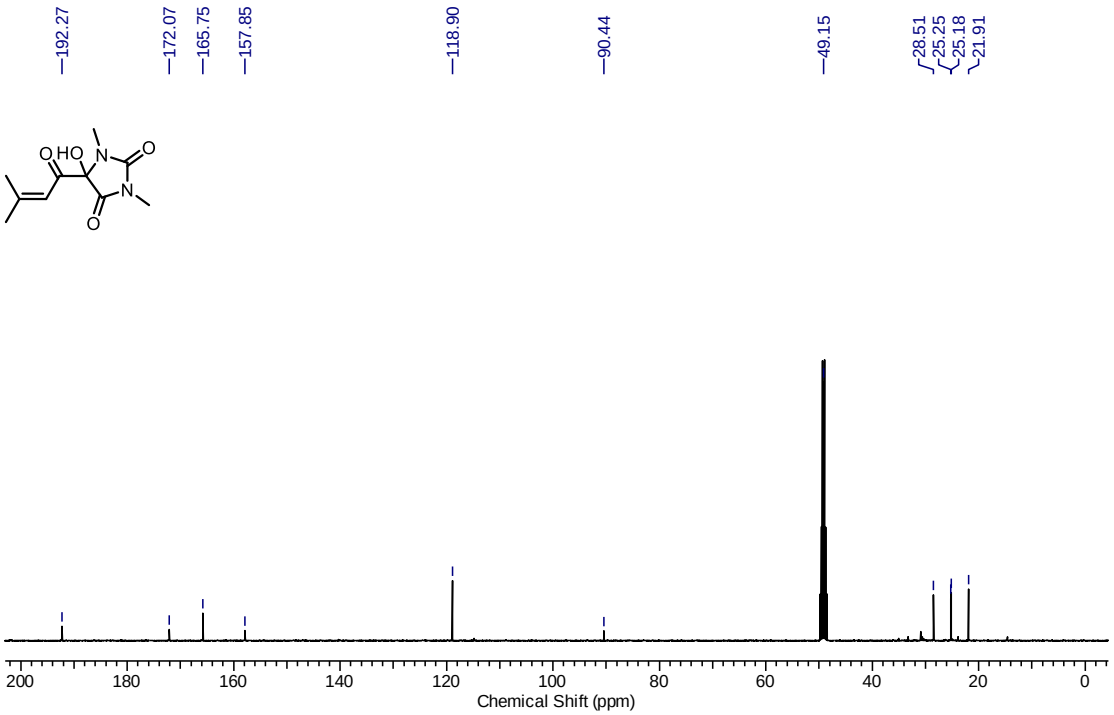
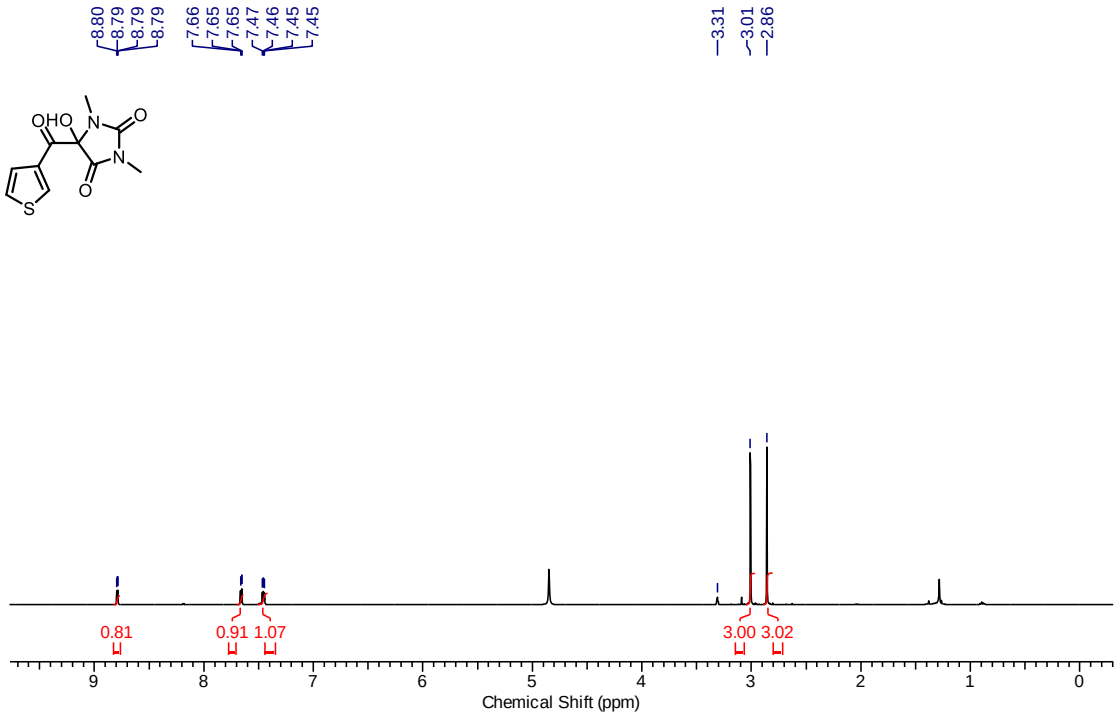


^{1}H NMR of Compound 40e' in CD_3OD at 400 MHz



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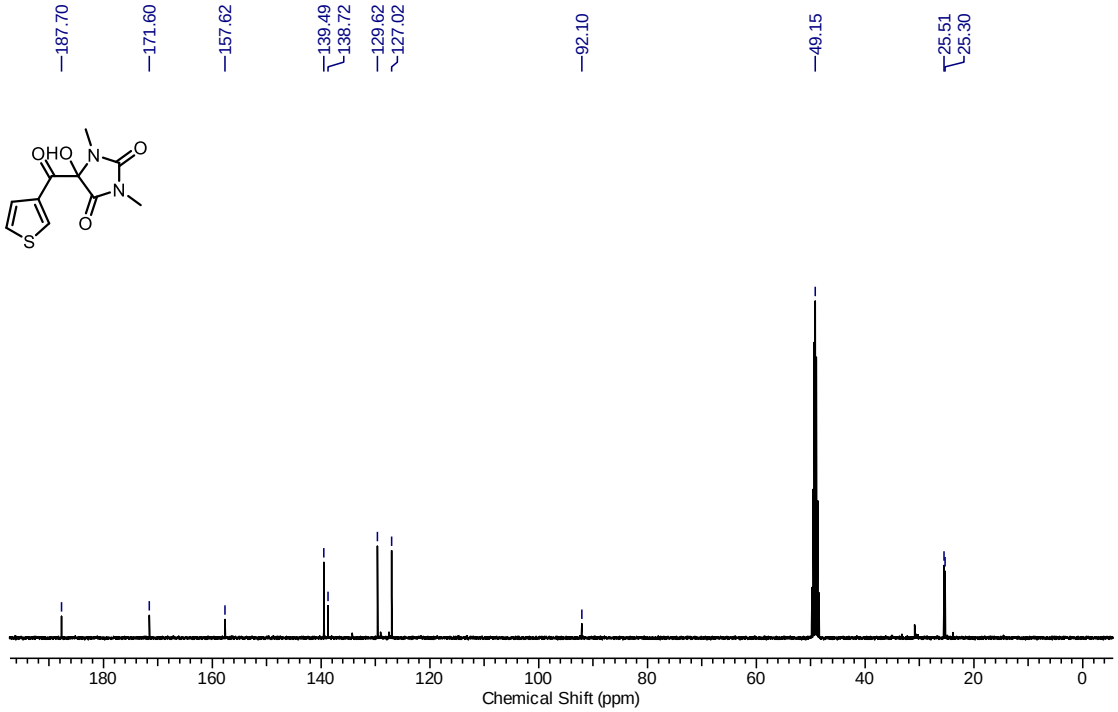
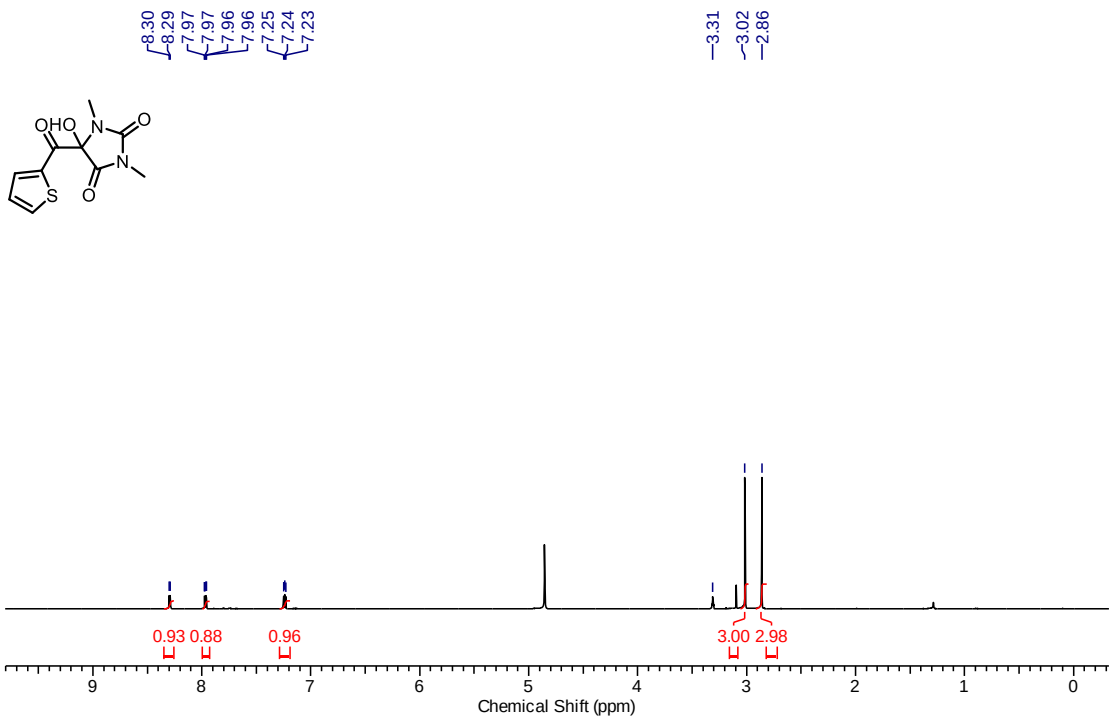
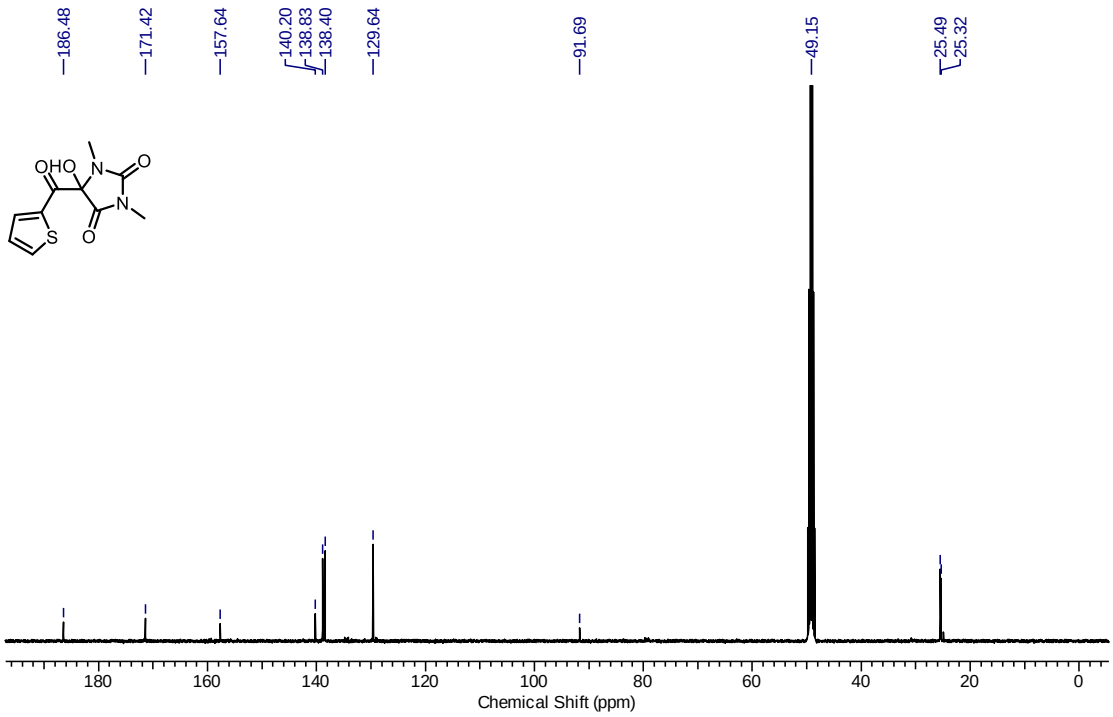


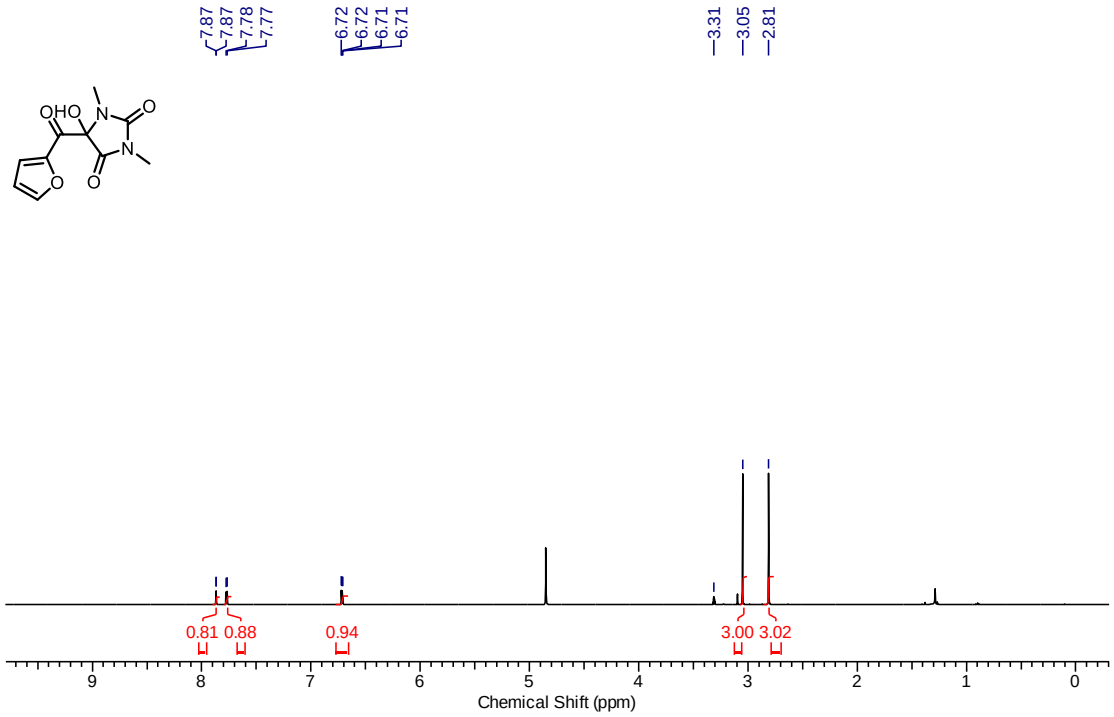
^{1}H NMR of Compound 40f' in CD_3OD at 400 MHz



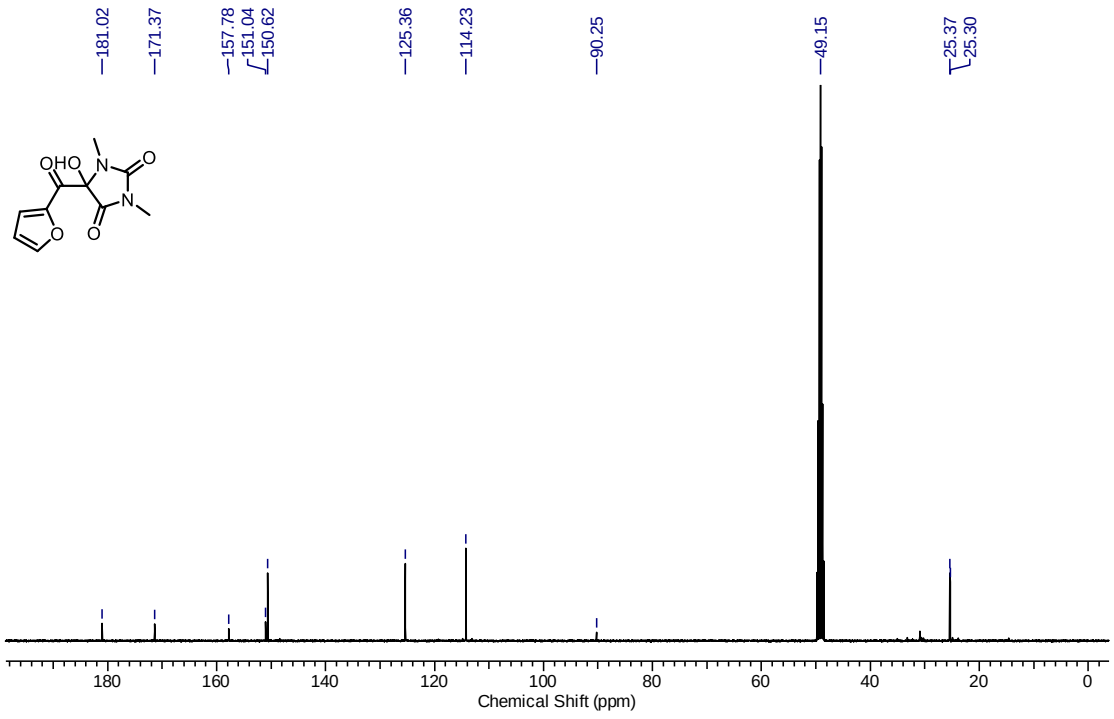
^{13}C NMR of Compound 40f' in CD_3OD at 100 MHz



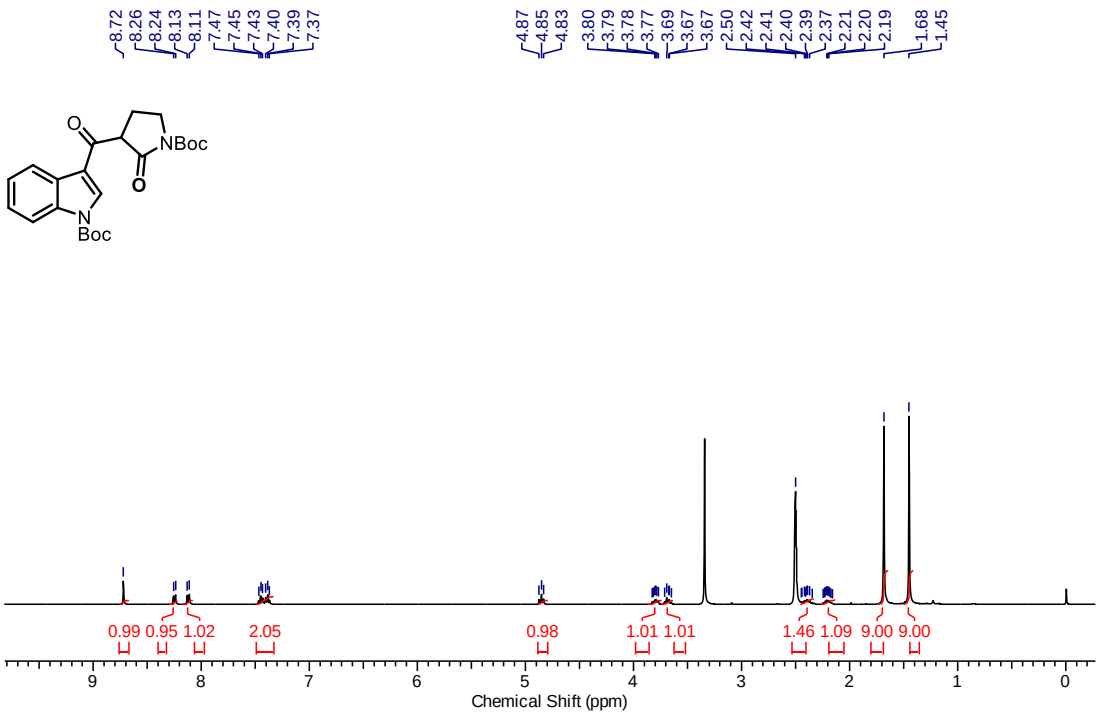
^{1}H NMR of Compound 40g' in CD_3OD at 400 MHz



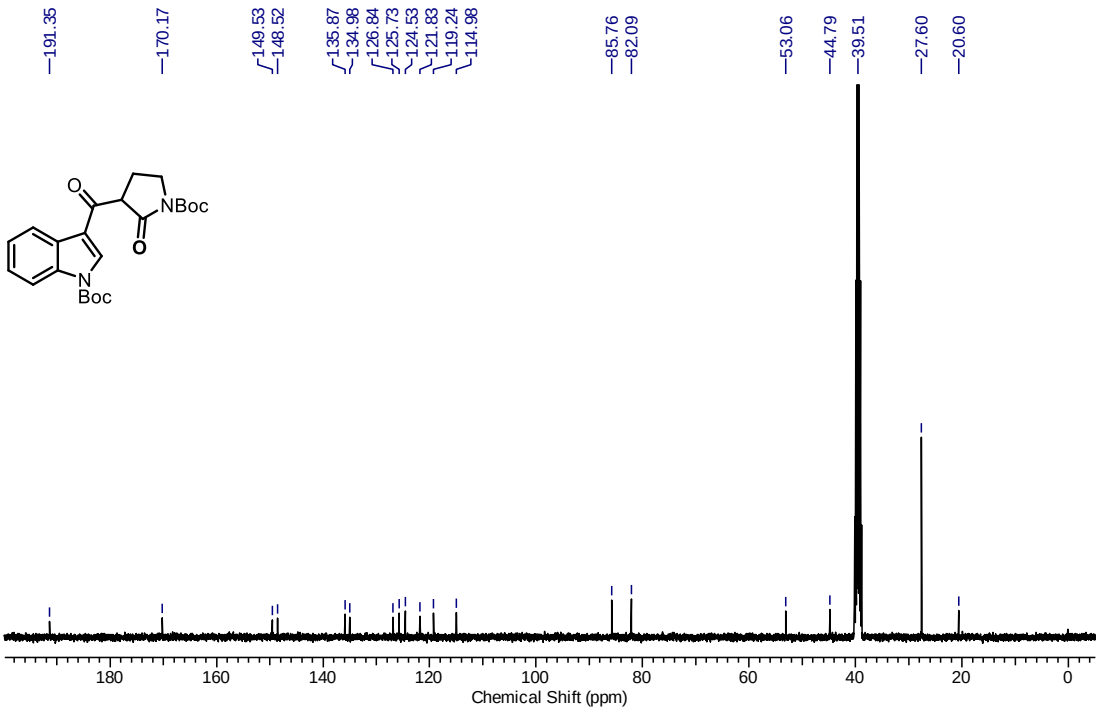
^{13}C NMR of Compound 40g' in CD_3OD at 100 MHz



^{1}H NMR of Compound 54 in DMSO-d_6 at 400 MHz



^{13}C NMR of Compound 54 in DMSO-d_6 at 100 MHz



Chapter-2

Section 3: Synthesis of Oxoaplysinopsin B and Biological Evaluation of Oxoaplysinopsin Analogues

2.3.1 Introduction

Heterocyclic compounds are one of the most important class of organic compounds having vital biological and pharmacological properties.[1] Oxindole scaffolds are commonly observed in natural products, several of them showed various biological activities including anticancer, antimicrobial, antiviral, antitubercular, antileishmanial, antirheumatoid arthritis *etc*.[2] hence, it is known to be a privileged structural scaffold in drug discovery. Currently, many of oxindole based compounds are in clinical development for the treatment of numerous diseases. Among these compounds, 3-hydroxy-2-oxindole containing natural products received significant attention in the recent times due to their promising biological activities.[3] TMC-95A is a well-known natural product which inhibits selective proteasome in non-covalent and reversible fashion with remarkable bioactivity profiles.[4] SM-130686 is presently used as the potent orally active GHSR (growth hormone secretagogue receptor) agonist.[5] YK-4-279 is another molecule which effectively inhibit Ewing's sarcoma growth by restricting the interaction between the RNA helicase A (RHA) and oncogenic protein EWS-FLI1. However, (S)-YK-4-279 is more potent than (R)-YK-4-279 and racemic compound is effective in inhibiting the RHA/EWS-FLI1 interactions.[6] Convolutamydine-A is a natural product with potent activity for the differentiation of HL-60 human promyelocytic leukemia cells. (Figure 2.3.1) [7]

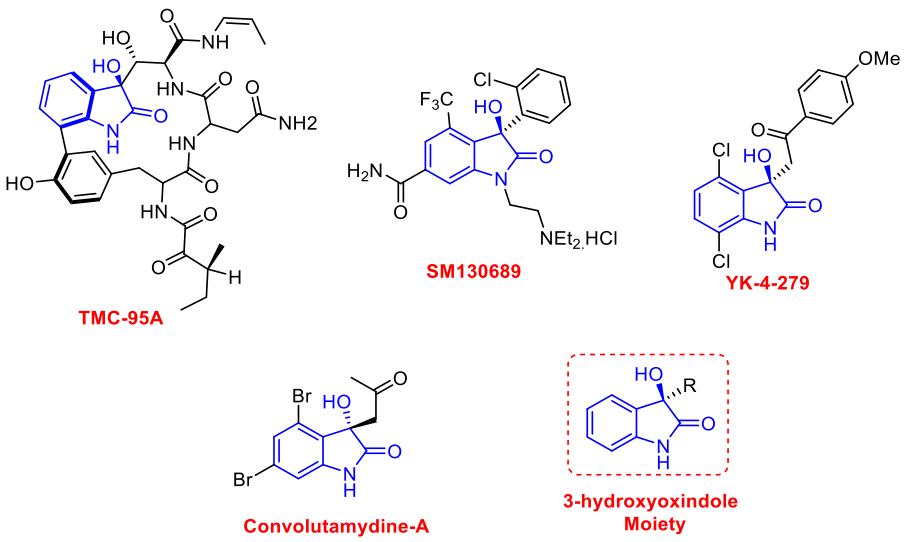


Figure 2.3.1: Natural products with 3-substituted-3-hydroxy-2-oxindole core

Oxoaplysinopsin B **25** was isolated by Wang *et al.* from *F. reticulata* of the XiSha Islands.[8] It showed moderate activity against tyrosine phosphatase 1B (PTP1B) with an IC_{50} value of 20.8 µM. However, dextrorotary (+)-enantiomer of oxoaplysinopsin B showed a slightly better activity than levorotary (-)-enantiomer. While we are working on this project, Nagarajan's group reported the first synthesis of oxoaplysinopsin B. Details are discussed below.

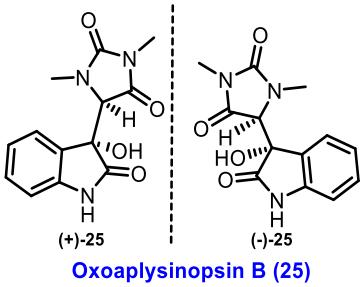
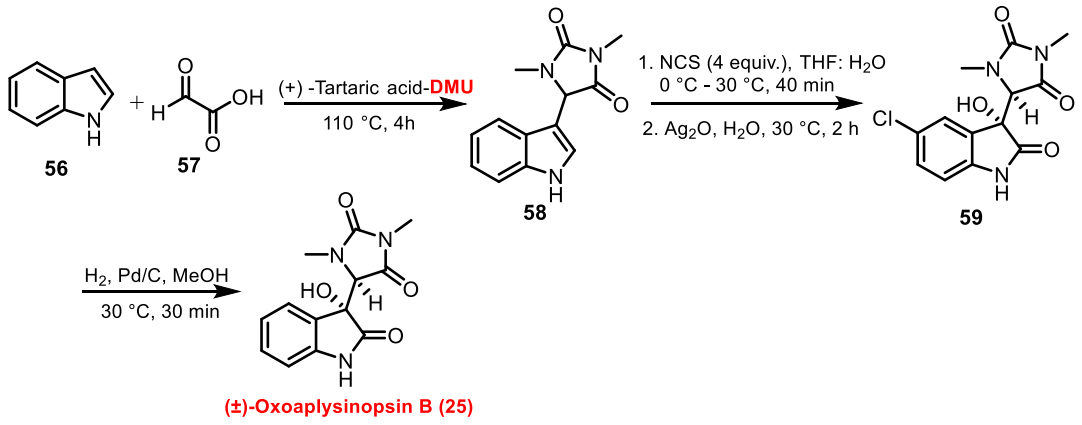


Figure 2.3.2: Structure of oxoaplysinopsin B

2.3.1.1 Synthesis of oxoaplysinopsin B by Nagarajan group:

In 2020, Nagarajan and co-workers reported first total synthesis of oxoaplysinopsin B as application of a methodology developed in their lab. Synthesis of oxoaplysinopsin B **25** started from commercially available indole **56** which on one pot reaction with glyoxylic acid **57** using dimethyl urea and tartaric acid gave product **58**. Further oxidation of indole moiety present in compound **58** was done by 4 equiv. of *N*-Chlorosuccinimide (NCS) followed by reaction with silver oxide to afford required 5-chloro analogue of oxoaplysinopsin **59**.

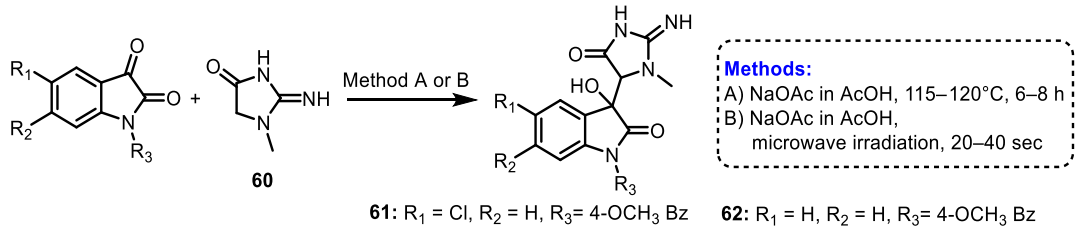


Scheme 2.3.1: First synthesis of oxoaplysinopsin B by Nagarajan group

Dechloro-hydrogenation of compound **59** was carried out using H_2, Pd/C in methanol to get desired oxoaplysinopsin B **25**. In summary, Nagarajan group synthesized oxoaplysinopsin B **25** in four steps with overall yield of 48% for the first time as a part of of showcasing the utility of their methodology.[9]

2.3.1.2 Isatin and Creatinine hybrids as anticancer agents:

In 2010, Crooks *et al.*[10] synthesized 3-hydroxy-3-(2-imino-3-methyl-5-oxoimidazolidin-4-yl)indolin-2-one derivatives by condensation reaction of substituted isatins with creatinine **60** by employing both conventional heating (method A) and microwave irradiation (method B) using sodium acetate in acetic acid as shown in scheme 2.3.2.

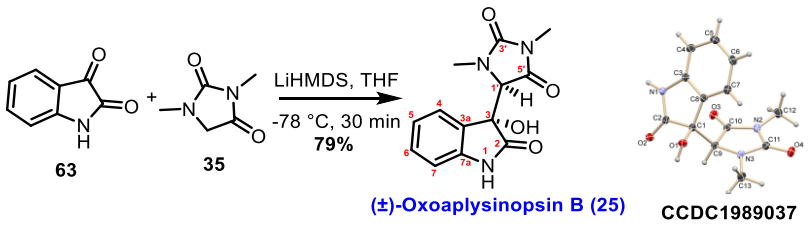


Scheme 2.3.2 Synthesis of 3-hydroxy-3-(2-imino-3-methyl-5-oxoimidazolidin-4-yl)indolin-2-one analogues by Crooks group

However, they have found that microwave irradiation method was much faster (20-40 secs) than conventional heating method (6 h). Further, these synthesized derivatives were subjected for in *vitro* cytotoxicity evaluation against a panel of 57 tumor cell lines and two compounds **61** and **62** were found as lead compounds. Compound **61** showed GI_{50} of 190 nM and 750 nM against non-small cell lung cancer A549/ATTC cell line and LOX IMVI melanoma cell line respectively. Moreover, Both **61** and **62** exhibited GI_{50} values ranging from 2 to 5 µM against several leukaemia cell lines including HL-60(TB), CCRF-CEM, MOLT-4, K-562, and RPMI-8226.

2.3.2 Our synthesis of oxoaplysinopsin B:

We have planned oxoaplysinopsin B using simple aldol reaction of isatin and *N*,*N*-dimethyl hydantoin. Accordingly, *N*,*N*-dimethyl hydantoin was subjected for aldol reaction with isatin using LiHMDS in THF at -78 °C afforded oxoaplysinopsin B. (Scheme 2.3.3)



Scheme 2.3.3 Synthesis of oxoaplysinopsin B on multi-gram scale by our group

Spectroscopic data of synthesized oxoaplysinopsin B was in complete agreement with the isolation data given by Wang *et al.* (Table 2.3.1)[8] Moreover we have confirmed structure with X-ray crystallographic analysis. Further using same reaction protocol we have synthesized oxoaplysinopsin B in multi-gram scale.

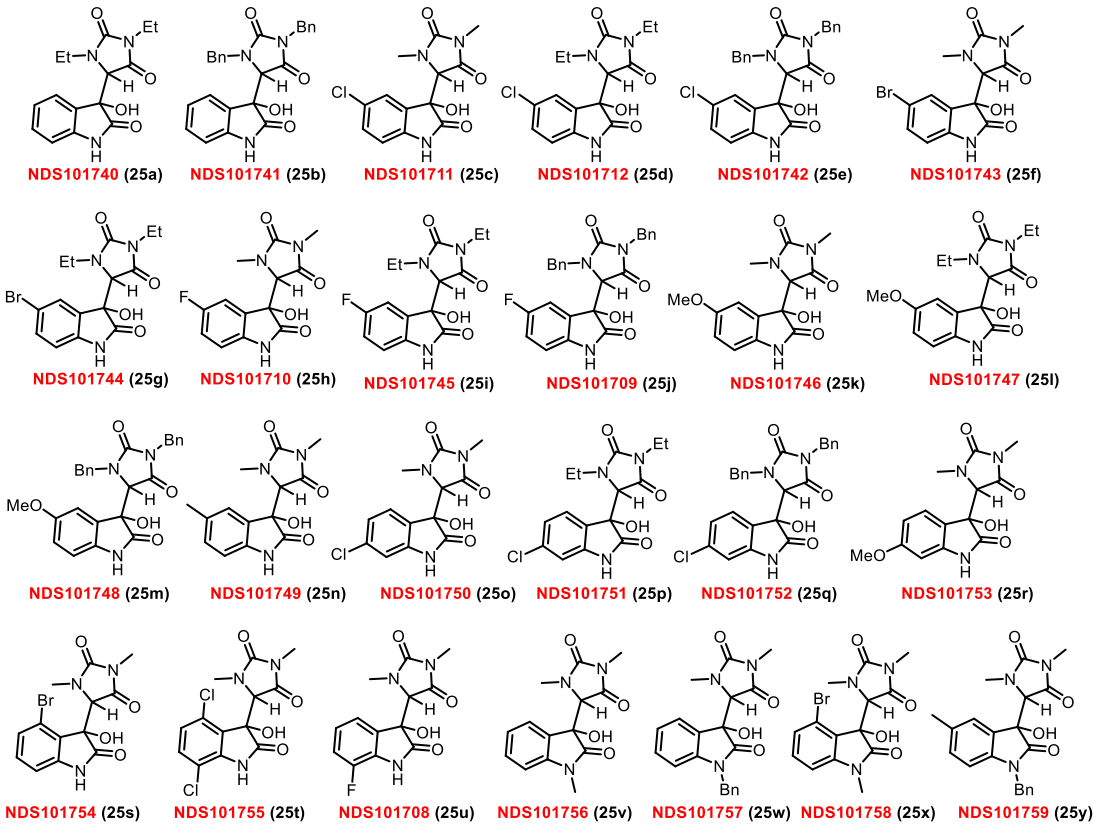
Table 2.3.1: Comparison of spectral data of natural and synthetic oxoaplysinopsin B in DMSO-d_6

Oxoaplysinopsin B	**^{1}H NMR δ ppm**		**^{13}C NMR δ ppm**	
	Natural	Synthetic	Natural	Synthetic
1	10.43, br. s	10.42, br. S	-	-
2	-	-	175.3, C	175.3, C
3	-	-	76.2, C	76.3, C
3a	-	-	126.9, C	126.9, C
4	7.08, d, 1H (7.5)	7.08, d, 1H (7.2)	124.1, CH	124.1, CH
5	6.90, dd, 1H (7.5, 7.5)	6.90, t, 1H (7.4)	121.5, CH	121.5, CH
6	7.22, dd, 1H (7.7, 7.7)	7.22, m, 1H	130.1, CH	130.1, CH
7	6.78, d, 1 H (7.7)	6.78, d, 1 H (7.6)	109.9, CH	109.9, CH
7a	-	-	142.6, C	142.6, C
1’	4.40, s, 1 H	4.40, s, 1 H	66.1, CH	66.1, CH
3’	-	-	157.2, C	157.2, C
5’	-	-	168.6, C	168.6, C

2'-NCH_3	3.14, s, 3 H	3.14, s, 3 H	31.3, CH_3	31.3, CH_3
4'-NCH_3	2.50, s, 3 H	2.50, s, 3 H	24.2, CH_3	24.2, CH_3
3-OH	6.62, s	6.61, s	-	-

2.3.2.1 Synthesis of oxoaplysinopsin B analogues:

Considering the interesting biological activities of isatin-creatinine hybrids showed by Crooks group and moderate PTP1B activity of the natural product oxoaplysinopsin B,[10] We planned synthesis of analogues around natural product skeleton and evaluate their biological activities, in particular, anticancer potential. The targeted analogues were envisioned using similar one step protocol used for the synthesis of oxoaplysinopsin B.



Scheme 2.3.4 Synthesis of oxoaplysinopsin B analogues

Accordingly, various commercial substituted isatins were treated with different substituted hydantoins (**35**, **35a**, **35b**) under the aldol reaction using LiHMDS as base

to afford corresponding oxoaplysinopsin B analogues (**25a-25y**) as shown in scheme 2.3.4. All analogues were characterized by NMR, IR and HRMS.

2.3.2.2 Cytotoxicity evaluation of oxoaplysinopsin B analogues:

After having all the analogues in hand, we evaluated the cytotoxicity of the compounds in collaboration with Dr. Anindya Goswami, CSIR-Indian Institute of Integrative Medicine (CSIR-IIIM) Jammu. The cytotoxic activities of the newly accessed oxoaplysinopsin B analogues were tested by MTT assay using Doxorubicin

Table 2.3.2: In *vitro* cytotoxic activities of oxoaplysinopsin analogues

Compound Code	IC50(µM)		Compound Code	IC50(µM)	
	MCF-7	A549		MCF-7	A549
NDS101740	>100 µM	>100 µM	NDS101754	>100 µM	>100 µM
NDS101741	**56 µM**	>100 µM	NDS101755	**96.76 µM**	>100 µM
NDS101742	**26.65 µM**	>100 µM	NDS101756	>100 µM	>100 µM
NDS101743	>100 µM	>100 µM	NDS101757	>100 µM	>100 µM
NDS101744	>100 µM	>100 µM	NDS101758	>100 µM	>100 µM
NDS101745	>100 µM	>100 µM	NDS101759	>100 µM	>100 µM
NDS101746	>100 µM	**50.88 µM**	NDS101707	>100 µM	>100 µM
NDS101747	>100 µM	**28.93 µM**	NDS101708	>100 µM	>100 µM
NDS101748	>100 µM	**31.54 µM**	NDS101709	**51.19 µM**	**41.79 µM**
NDS101749	>100 µM	**83.84 µM**	NDS101710	>100 µM	>100 µM
NDS101750	>100 µM	>100 µM	NDS101711	>100 µM	>100 µM
NDS101751	>100 µM	>100 µM	NDS101712	>100 µM	>100 µM
NDS101752	**3.8 µM**	**7.16 µM**	DOXORUBICIN	0.11 µM	0.05 µM
NDS101753	>100 µM	>100 µM	5-FU	3.31 µM	2.33 µM

and 5-fluorouracil 5-FU as a positive control. Two cancer cell lines were used: MCF-7 (breast cancer cell line) and A549 (human lung cancer cell line). All the 26 synthesized derivatives were subjected for cytotoxicity evaluation and *in vitro* activities are compiled in table 2.3.2, among them only few compounds NDS101741, NDS101742, NDS101752, NDS101755 and NDS101709 showed moderate activity against MCF-7 cancer cell line. However, NDS101746, NDS101747, NDS101748,

NDS101749, NDS101752 and NDS101709 showed moderate activity against A549. NDS101752 was the best identified compound among this series which displayed better activity against both MCF-7 and A549 with IC_{50} value 3.8 µM and 7.16 µM respectively. These results indicate that dibenzyl substitution on hydantoin increases activity and the compounds with substitution on isatin NH (NDS101756, NDS101757, NDS101758 and NDS101759) were found to be inactive, indicating that free NH of isatin may be required for the activity.

2.3.3 Cholinesterase inhibition potential of aplysinopsins:

Dr. Sandip Bharate group have reported that aplysinopsin inhibits acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and human BACE-1 with IC_{50} values of 33.9, 30.31 and 33.69 µM respectively.[11] Moreover, they have found that aplysinopsin showed excellent blood–brain barrier (BBB) permeability (*Pe* = 8.92 x 10^{-6} cm/sec). Further, they have synthesized related analogues and found two lead compounds **64** and **65** which were more potent than parent aplysinopsin **1.** (Figure 2.3.5)

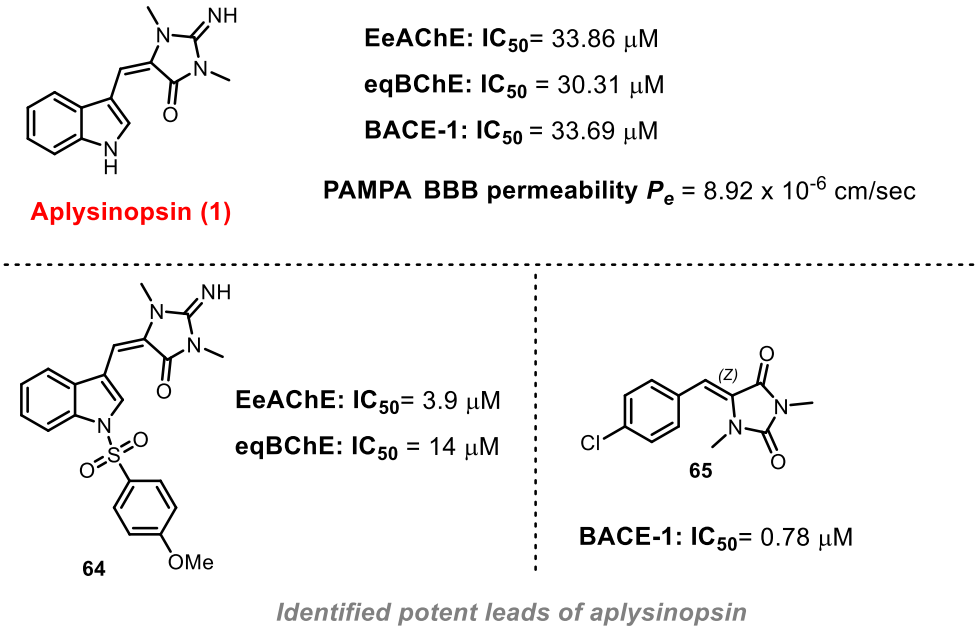
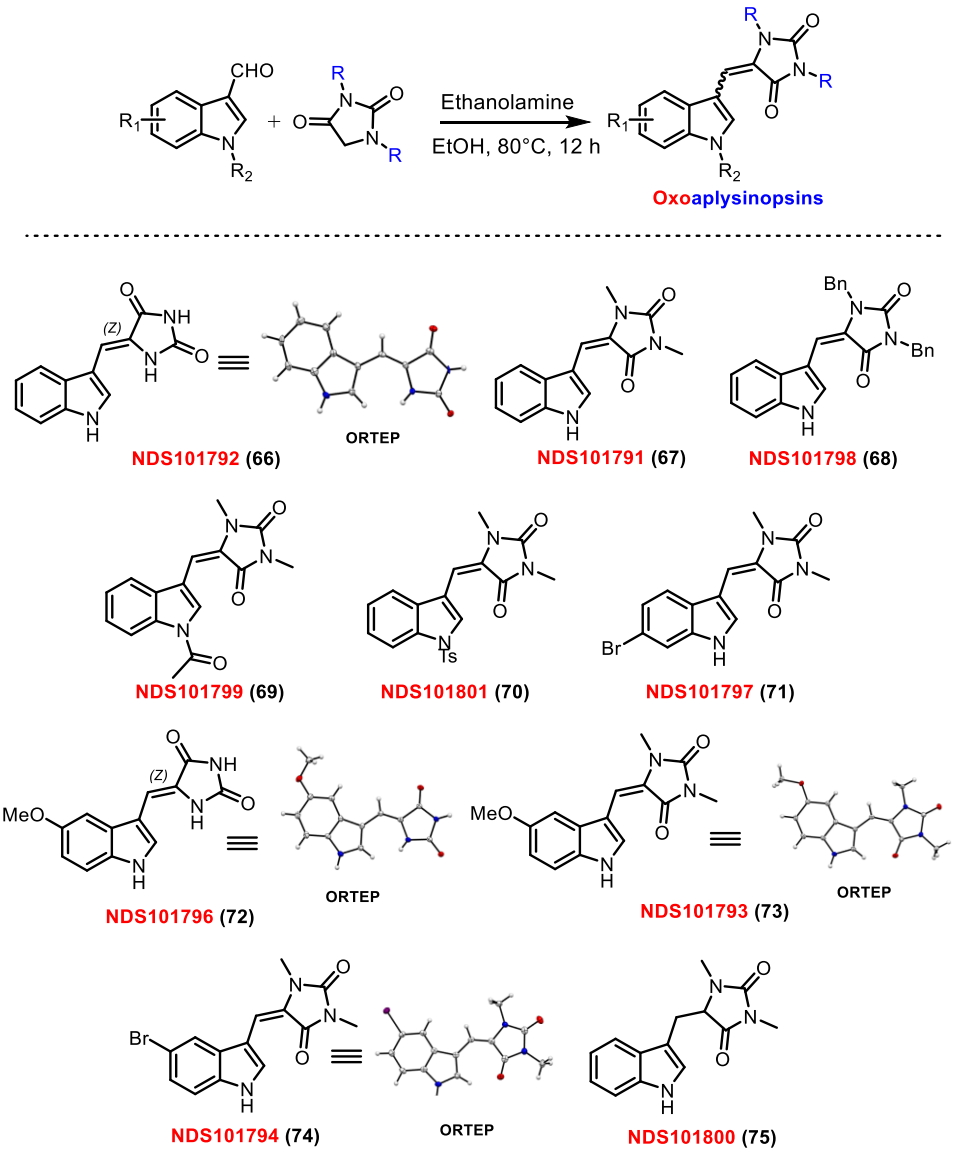


Figure 2.3.5: Aplysinopsin and lead identification for Alzheimer's disease

2.3.3.1 Synthesis of olefinic analogues of oxoaplysinopsins:

Since, we were also working on oxoaplysinopsin family of natural products which is oxygenated version of aplysinopsin. Considering the work done by Bharate's group, we decided to make olefinic analogues of oxoaplysinopsin and test their effect on cholinesterases. Accordingly, commercially available indoles were subjected for condensation reaction with substituted hydantoins using ethanolamine in ethanol[12] at 80 °C resulting in olefinic oxoaplysinopsins **66**-**75**.



Scheme 2.3.6 Synthesis of olefinic oxoaplysinopsin

Next important task was to understand stereochemistry of olefin for which we applied Guella *et al.* simplest hypothesis to understand stereochemistry of olefin present in

aplysinopsin analogues.[13] It states that, amide *N*-substitution plays an important role in deciding olefin stereochemistry, *i.e.* presence of alkyl group on amidine nitrogen attributes to *E*- configuration while absence of the substituent denotes olefin as *Z*-olefin. (Figure 2.2.3) To study the validity of this hypothesis in case of oxoaplysinopsin we crystalized four compounds in ethyl acetate and performed X-ray crystallography. X-ray crystal structure and the corresponding ORTEP presentation clearly showed, hypothesis given by Guella *et al.* fits perfectly for oxoaplysinopsins to identify olefin stereochemistry as shown in scheme 2.3.6.

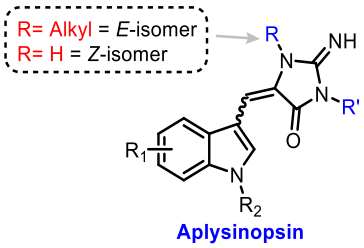
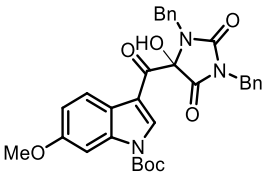
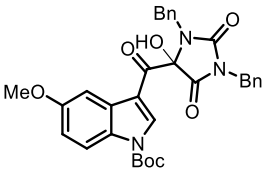
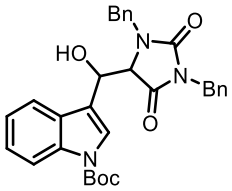
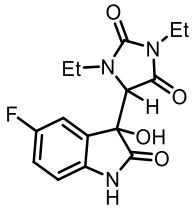
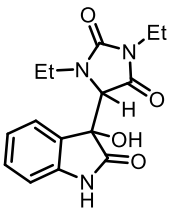
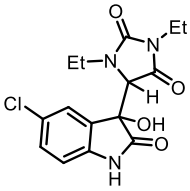
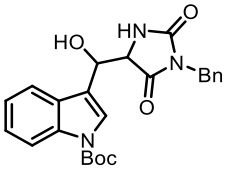


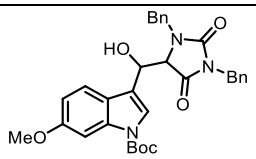
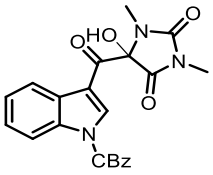
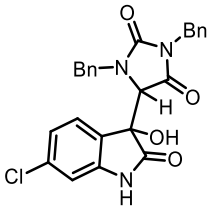
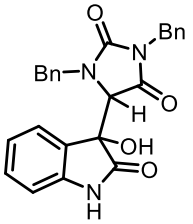
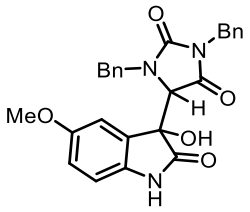
Figure 2.3.3: Guella *et al.* hypothesis on olefin steriochemistry

2.3.3.2 Cholinesterase inhibition of oxoaplysinopsins:

The addition of these newly synthesized olefinic analogues around oxoaplysinopsin scaffold raised the number of synthesized compounds to 115. Then we collaborated with Bharate's group to screen all these novel derivatives against cholinesterase. However, It was difficult to screen all of them in biological assay therefore structures of all oxoaplysinopsins were subjected for molecular docking studies against three targets of Alzheimer's disease *i.e.* acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and beta-secretase (BACE-1) and identified 12 best compounds which showed better docking score. (Table 2.3.3)

Table 2.3.3 Docking score of all the analogues

Sr. No.	NDS Code	Structures	Docking Score		
			AChE	BChE	BACE-1
1	NDS101785		-13.1	-7.11	-7.9
2	NDS101786		-9.54	-6.86	-8.4
3	NDS101787		-9.43	-6.8	-8.04
4	NDS101745		-9.33	-10.49	-6.95
5	NDS101740		-8.49	-10.31	-5.66
6	NDS101712		-7.98	-10.24	-5.64
7	NDS101788		-11.78	-9.09	-7.57

8	**NDS101789**		-12.48	-6.06	-5.41
9	**NDS101790**		-12.05	-3.18	-5.9
10	**NDS101752**		-11.96	-5.64	-5.47
11	**NDS101741**		11.77	-5.4	-5.26
12	**NDS101748**		-11.75	-5.27	-5.18

After having the 12 best identified compounds along with olefinic oxoaplysinopins (**66-76**) were subjected for biological evaluation against acetylcholinesterase (AChE) inhibition. We determined % inhibition of all selected compounds and compared it with known aplysinopsin. For comparison studies we divided our compounds in three different series.

1) Analogues with functionalized olefin moiety: In this series of compounds, two of them NDS101785 and NDS101786 showed small increase in % inhibition as compared with aplysinopsin, However, NDS101787 and NDS101789 are moderately active against AChE. These results indicate that α-hydroxy derivatives are the better compounds than corresponding aldol adducts. However, dibenzyl substitution on hydantoin increases the activity.

Sr. No.	NDS Code	% inhibition
1	NDS101785	30.1
2	NDS101786	28.9
3	NDS101787	23.5
4	NDS101789	22.3
5	NDS101788	11.4
6	NDS101790	10.9

2) Analogues of oxoaplysinopsin B: In this series, we did not observed any best compound as in comparison with parent aplysinopsin, however one of the analogue NDS101752 showed moderate activity i.e. 15.4% inhibition against AChE than other analogues.

Sr. No.	NDS Code	% inhibition
1	NDS101752	15.4
2	NDS101748	7.2
3	NDS101740	2.9
4	NDS101741	2.5
5	NDS101745	0.9
6	NDS101712	0.4

3) Olefinic analogues of oxoaplysinopsin: This series of compounds were tested without checking their docking scores, however, we found only one compound NDS101799 showed better % inhibition than parent aplysinopsin. Interestingly, if we compare activity of oxoaplysinopsin (NDS101791) with aplysinopsin, it clearly indicate aplysinopsin enhances activity.

No.	NDS Code	% inhibition	No.	NDS Code	% inhibition
1	NDS101799	27.4	**7**	NDS101792	10.4
2	NDS101794	12.9	**8**	NDS101796	8.2

3	NDS101801	11.6	**9**	NDS101800	7.1
4	NDS101793	11.6	**10**	NDS101797	1.8
5	NDS101791	10.8		**Aplysinopsin**	**25.1**
6	NDS101798	10.5			

Finally, after evaluation of all analogues of oxoaplysinopsin against AChE inhibition, we found that compounds from series-1 are showing better than series-2 and series-3.

2.3.4 Conclusion:

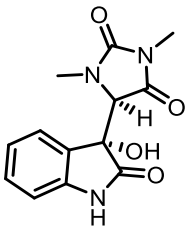
We have synthesized oxoaplysinopsin B in a gram scale using one step aldol reaction protocol, Further synthesized 25 close analogues around oxoaplysinopsin B scaffold and evaluated their cytotoxicity against MCF-7 and A549 cell lines. However only a few compounds showed moderate activity and NDS101752 found to have activity against MCF-7 and A549 cell lines. Following this, we also synthesized 11 new olefinic analogues of oxoaplysinopsin to understand their potential towards Cholinesterase inhibition. All the analogues were classified into three series based on their structure and tested against AChE and series-1 showed better % inhibition and was comparable to aplysinopsin.

2.3.5 Experimental section:

General procedure for the synthesis of aldol products (25, 24a-24y) :

To a stirred solution of substituted hydantoins (1 equiv.) in 20 mL THF, 1M lithium bis(trimethylsilyl)amide (2 equiv.) was added dropwise at -78 °C for 15 min. Isatin derivatives (1 equiv.) in THF (15 mL) was then added dropwise and the resultant mixture stirred at -78 °C for 1 h. The reaction mixture was quenched with saturated NH_4Cl and allowed to warm at room temperature. The aqueous layer was extracted with EtOAc (3x30 mL), the combined organic extract washed with brine, dried (Na_2SO_4) and concentrated in *vaccuo*. The crude product was purified by column chromatography to afford aldol products **25, 24a-24y**.

5-(3-hydroxy-2-oxoindolin-3-yl)-1,3-dimethylimidazolidine-2,4-dione (25):



The compound **25** was synthesized by following general procedure for aldol reaction and obtained as white solid.

Yield= 76%;

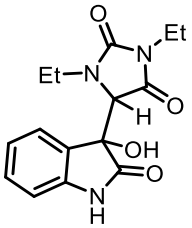
IRυ_{max}(film): 3263, 1706, 1474, 754 cm^{-1}

^{1}H NMR (500 MHz, DMSO-*d*$_6$) = δ 10.42 (br. s, 1 H), 7.26 - 7.17 (m, 1 H), 7.08 (d, *J* = 7.2 Hz, 1 H), 6.90 (t, *J* = 7.4 Hz, 1 H), 6.78 (d, *J* = 7.6 Hz, 1 H), 6.61 (s, 1 H), 4.40 (s, 1 H), 3.14 (s, 3 H), 3.50 (s, 3 H)

^{13}C NMR (125 MHz, DMSO-*d*$_6$) = δ 175.3, 168.6, 157.2, 142.6, 130.1, 126.9, 124.1, 121.5, 109.9, 76.3, 66.1, 31.3, 24.2.

HRMS (ESI): *m/z* calculated for $C_{13}H_{12}N_3O_4$ $[M-H]^+$= 274.0822, Observed = 274.0833.

1,3-diethyl-5-(3-hydroxy-2-oxoindolin-3-yl)imidazolidine-2,4-dione (25a):



The compound **25a** was synthesized by following general procedure for aldol reaction and obtained as yellow solid

Yield= 63% (mixture of diastereomers, dr ratio = 3 : 2)

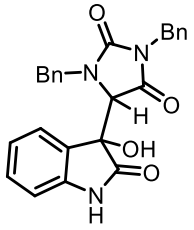
IRυ_{max}(film): 3275, 1699, 1464, 752 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 10.48 - 10.42 (m, 1 H), 7.23 - 7.17 (m, 2 H), 6.95 - 6.80 (m, 1 H), 6.78 (dd, *J* = 4.1, 7.7 Hz, 1 H), 6.57 - 6.51 (m, 1 H), 4.45 (d, *J* = 14.1 Hz, 1 H), 3.54 - 3.52 (m, 1 H), 3.53 (dd, *J* = 7.1, 14.1 Hz, 1 H), 3.30 - 3.24 (m, 1 H), 3.07 (td, *J* = 7.1, 14.7 Hz, 1 H), 1.25 (t, *J* = 7.0 Hz, 1 H), 1.07 (t, *J* = 7.0 Hz, 2 H), 0.99 (t, *J* = 7.1 Hz, 2 H), 0.44 (t, *J* = 7.1 Hz, 1 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 176.1, 175.3, 169.1, 168.2, 156.3, 156.2, 142.7, 142.4, 130.0, 129.9, 128.0, 126.7, 124.4, 124.3, 121.7, 121.4, 109.8, 109.7, 76.6, 75.5, 63.7, 61.9, 38.6, 37.7, 32.9, 32.4, 13.2, 13.0, 12.8, 12.2.

HRMS (ESI): *m/z* calculated for $C_{15}H_{16}N_3O_4$ $[M-H]^+$= 302.1135, Observed = 302.1145.

1,3-dibenzyl-5-(3-hydroxy-2-oxoindolin-3-yl)imidazolidine-2,4-dione (25b):



The compound **25b** was synthesized by following general procedure for aldol reaction and obtained as white solid.

Yield= 42% (mixture of diastereomers, dr ratio = 1 : 2)

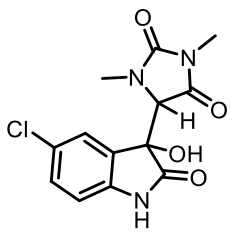
IRυ_{max}(film): 3323, 1708, 1451, 751 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 10.47 (d, *J* = 12.4 Hz, 1 H), 7.40 - 7.26 (m, 6 H), 7.26 - 7.02 (m, 5 H), 6.91 (d, *J* = 7.5 Hz, 1 H), 6.86 - 6.69 (m, 2 H), 6.45 (d, *J* = 7.3 Hz, 1 H), 5.13 - 4.85 (m, 1 H), 4.52 (d, *J* = 3.6 Hz, 1 H), 4.47 - 4.20 (m, 3 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 176.0, 175.1, 168.6, 168.0, 156.8, 156.8, 142.7, 142.3, 136.8, 136.7, 136.0, 135.6, 130.3, 129.8, 128.5, 128.5, 128.4, 128.3, 128.3, 127.7, 127.6, 127.5, 127.5, 127.4, 126.8, 126.6, 125.9, 124.8, 124.3, 121.8, 110.0, 109.9, 76.6, 75.6, 63.6, 62.0, 46.7, 46.2, 41.7, 41.2.

HRMS (ESI): *m/z* calculated for $C_{25}H_{20}N_3O_4$ $[M-H]^+$= 426.1448, Observed = 426.1463.

5-(5-chloro-3-hydroxy-2-oxoindolin-3-yl)-1,3-dimethylimidazolidine-2,4-dione (25c):



The compound **25c** was synthesized by following general procedure for aldol reaction and obtained as white solid .

Yield= 75% (mixture of diastereomers, dr ratio = 1 : 1)

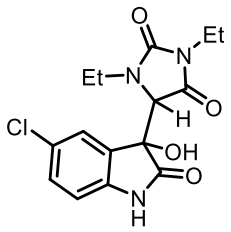
IRυ_{max}(film): 3280, 1703, 1470, 752 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6) = δ 10.58 (d, *J* = 14.0 Hz, 1 H), 7.31 - 7.05 (m, 2 H), 6.82 - 6.73 (m, 2 H), 4.49 – 4.42 (m, 1 H), 3.13 (s, 2 H), 2.96 (s, 1 H), 2.74 (s, 1 H), 2.55 (s, 2 H)

^{13}C NMR (100 MHz, DMSO-d_6) = δ 175.4, 175.0, 169.0, 168.5, 157.3, 156.7, 141.5, 141.1, 130.6, 129.9, 129.6, 129.0, 125.8, 125.4, 124.4, 124.0, 111.4, 111.2, 76.3, 75.5, 66.1, 65.1, 31.5, 30.9, 24.4, 24.3.

HRMS (ESI): *m/z* calculated for $C_{13}H_{11}ClN_3O_4$ $[M-H]^+$= 308.0433, Observed = 308.0445.

5-(5-chloro-3-hydroxy-2-oxoindolin-3-yl)-1,3-diethylimidazolidine-2,4-dione (25d):



The compound **25d** was synthesized by following general procedure for aldol reaction and obtained as yellow solid.

Yield= 61% (mixture of diastereomers, dr ratio = 2 : 1)

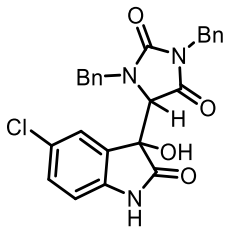
IRυ_{max}(film): 3282, 1699, 1460, 1195 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6) = δ 10.61 - 10.60 (m, 1 H), 7.31 - 7.26 (m, 2 H), 6.87 - 6.79 (m, 1 H), 6.72 (s, 1 H), 4.58 - 4.46 (m, 1 H), 3.59 - 3.55 (m, 1 H), 3.36 - 3.34 (m, 1 H), 3.33 - 3.28 (m, 1 H), 3.26 - 3.11 (m, 1 H), 1.24 (t, *J* = 7.1 Hz, 1 H), 1.10 (t, *J* = 7.1 Hz, 2 H), 0.98 (t, *J* = 7.2 Hz, 2 H), 0.47 (t, *J* = 7.1 Hz, 1 H)

^{13}C NMR (100 MHz, DMSO-d_6) = δ 175.5, 175.0, 168.8, 168.1, 156.3, 156.0, 141.6, 141.2, 130.4, 129.8, 129.6, 128.8, 125.7, 125.4, 124.6, 124.1, 111.4, 111.2, 76.6, 75.6, 63.5, 61.9, 38.8, 37.9, 32.9, 32.5, 13.1, 13.0, 12.8, 12.1.

HRMS (ESI): *m/z* calculated for $C_{15}H_{15}ClN_3O_4$ $[M-H]^+$= 336.0746, Observed = 336.0758.

1,3-dibenzyl-5-(5-chloro-3-hydroxy-2-oxoindolin-3-yl)imidazolidine-2,4-dione (25e):



The compound **25e** was synthesized by following general procedure for aldol reaction and obtained as white solid.

Yield= 60% (mixture of diastereomers, dr ratio = 1 : 1)

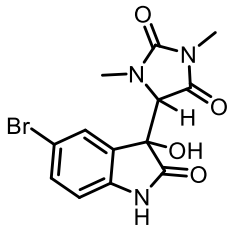
IRυ_{max}(film): 3314, 1706, 1447, 751 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 10.58 (d, *J* = 12.1 Hz, 1 H), 7.45 - 7.21 (m, 6 H), 7.21 - 7.06 (m, 3 H), 7.01 (d, *J* = 2.3 Hz, 1 H), 6.93 (s, 1 H), 6.87 (s, 1 H), 6.77 (dd, *J* = 3.8, 8.3 Hz, 1 H), 6.54 (d, *J* = 7.1 Hz, 1 H), 5.02 (d, *J* = 15.1 Hz, 1 H), 4.94 - 4.82 (m, 1 H), 4.67 (d, *J* = 15.9 Hz, 1 H), 4.61 - 4.32 (m, 2 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 175.2, 174.9, 168.2, 167.8, 157.0, 156.6, 141.5, 141.0, 137.0, 136.9, 136.1, 135.6, 130.5, 130.2, 129.6, 128.5, 128.5, 128.4, 128.3, 127.6, 127.5, 127.3, 127.2, 127.0, 126.9, 125.9, 125.9, 125.8, 124.7, 124.7, 111.4, 111.2, 76.6, 75.8, 63.8, 62.7, 47.2, 46.5, 41.6, 41.2.

HRMS (ESI): *m/z* calculated for $C_{25}H_{19}ClN_3O_4$ $[M-H]^+$= 460.1059, Observed = 460.1077.

5-(5-bromo-3-hydroxy-2-oxoindolin-3-yl)-1,3-dimethylimidazolidine-2,4-dione (25f):



The compound **25f** was synthesized by following general procedure for aldol reaction and obtained as white solid.

Yield= 63% (mixture of diastereomers, dr ratio = 1 : 1)

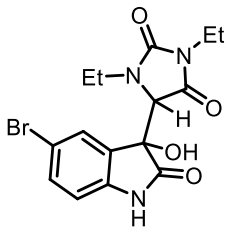
IRυ_{max}(film): 3268, 1703, 1471, 754 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 10.58 (d, *J* = 13.8 Hz, 1 H), 7.43 - 7.35 (m, 2 H), 6.79 - 6.72 (m, 2 H), 4.49 - 4.41 (m, 1 H), 3.13 (s, 1 H), 2.96 (s, 2 H), 2.74 (s, 2 H), 2.56 (m, 1 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 175.3, 174.9, 169.0, 168.5, 157.3, 156.7, 142.0, 141.5, 132.8, 132.4, 130.9, 129.4, 127.1, 126.8, 113.4, 112.9, 112.0, 111.7, 76.3, 75.4, 66.1, 65.1, 31.5, 30.9, 24.4, 24.3.

HRMS (ESI): *m/z* calculated for $C_{13}H_{13}BrN_3O_4$ $[M+H]^+$= 354.0084, Observed = 354.0079.

5-(5-bromo-3-hydroxy-2-oxoindolin-3-yl)-1,3-diethylimidazolidine-2,4-dione (25g):



The compound **25g** was synthesized by following general procedure for aldol reaction and obtained as brown solid.

Yield= 61% (mixture of diastereomers, dr ratio = 3 : 2)

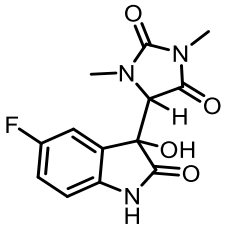
IRυ_{max}(film): 3270, 1699, 1461, 753 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 10.62 (s, 1 H), 7.44 - 7.19 (m, 2 H), 6.87 - 6.72 (m, 2 H), 4.57 – 4.45 (m, 1 H), 3.58 - 3.32 (m, 2 H), 3.31 - 3.26 (m, 2 H), 1.24 (t, *J* = 7.1 Hz, 1 H), 1.17 - 1.06 (m, 2 H), 0.99 (t, *J* = 7.2 Hz, 2 H), 0.47 (t, *J* = 7.1 Hz, 1 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 175.4, 174.9, 168.8, 168.1, 156.3, 156.1, 142.0, 141.6, 132.6, 132.5, 130.7, 129.2, 127.3, 126.9, 113.4, 113.0, 111.9, 111.7, 76.6, 75.5, 63.6, 61.9, 38.9, 37.9, 32.9, 32.5, 13.1, 13.0, 12.8, 12.1

HRMS (ESI): *m/z* calculated for $C_{15}H_{17}BrN_3O_4$ $[M+H]^+$= 382.0397, Observed = 382.0393.

5-(5-fluoro-3-hydroxy-2-oxoindolin-3-yl)-1,3-dimethylimidazolidine-2,4-dione (25h):



The compound **25h** was synthesized by following general procedure for aldol reaction and obtained as white solid.

Yield= 63% (mixture of diastereomers, dr ratio = 1 : 1)

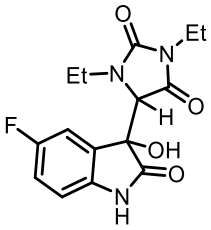
IRυ_{max}(film): 3266, 1708, 1481, 680 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 10.47 (d, *J* = 7.9 Hz, 1 H), 7.08 – 7.06 (m, 2 H), 6.79 - 6.71 (m, 2 H), 4.44 (d, *J* = 19.9 Hz, 1 H), 3.13 (s, 2 H), 2.95 (s, 1 H), 2.74 (s, 1 H), 2.55 (s, 2 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 175.7, 175.3, 169.0, 168.5, 159.2, 158.8, 157.2, 156.8, 156.7, 156.4, 138.8, 138.8, 138.4, 130.2, 130.1, 128.6, 128.5, 116.5, 116.3, 116.1, 115.9, 112.0, 111.9, 111.8, 111.6, 110.8, 110.8, 110.6, 110.6, 76.5, 75.7, 75.6, 66.1, 65.1, 31.4, 30.8, 24.4, 24.3

HRMS (ESI): *m/z* calculated for $C_{13}H_{11}FN_3O_4$ $[M-H]^+$= 292.0728, Observed = 292.0738.

1,3-diethyl-5-(5-fluoro-3-hydroxy-2-oxoindolin-3-yl)imidazolidine-2,4-dione (25i):



The compound **25i** was synthesized by following general procedure for aldol reaction and obtained as white solid.

Yield= 81% (mixture of diastereomers, dr ratio = 3 : 2)

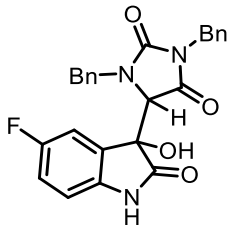
IRυ_{max}(film): 3280, 1697, 1468, 752 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 10.48 (d, *J* = 3.1 Hz, 1 H), 7.10 - 7.06 (m, 2 H), 6.83 - 6.69 (m, 2 H), 4.56 (m, 1 H), 3.73 - 3.57 (m, 1 H), 3.36 - 3.29 (m, 1 H), 3.27 - 3.12 (m, 1 H), 3.11 - 3.09 (m, 1 H), 1.24 (t, *J* = 7.1 Hz, 1 H), 1.11 (t, *J* = 7.1 Hz, 2 H), 0.97 (t, *J* = 7.1 Hz, 2 H), 0.49 (t, *J* = 7.1 Hz, 1 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 175.7, 175.3, 168.7, 168.0, 156.3, 156.0, 138.5, 138.4, 130.1, 130.0, 116.1, 116.1, 115.9, 112.3, 112.1, 110.5, 110.5, 79.2, 76.8, 75.8, 75.8, 63.6, 62.0, 38.8, 37.9, 32.9, 32.5, 13.1, 12.9, 12.8, 12.2.

HRMS (ESI): *m/z* calculated for $C_{15}H_{15}FN_3O_4$ $[M-H]^+$= 320.1041, Observed = 320.1050.

1,3-dibenzyl-5-(5-fluoro-3-hydroxy-2-oxoindolin-3-yl)imidazolidine-2,4-dione (25j):



The compound **25j** was synthesized by following general procedure for aldol reaction and obtained as yellow solid.

Yield= 61% (mixture of diastereomers, dr ratio = 1 : 1)

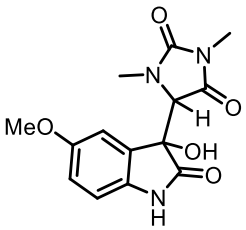
IRυ_{max}(film): 3319, 1709, 1452, 752 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 10.49 (d, *J* = 7.5 Hz, 1 H), 7.40 - 7.21 (m, 7 H), 7.21 - 7.02 (m, 3 H), 6.99 - 6.90 (m, 1 H), 6.90 - 6.64 (m, 2 H), 6.54 (d, *J* = 7.3 Hz, 1 H), 5.07 - 4.75 (m, 1 H), 4.63 - 4.30 (m, 4 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 175.6, 175.1, 168.3, 167.8, 159.1, 158.9, 156.9, 156.8, 156.6, 156.6, 138.8, 138.3, 136.9, 136.8, 136.0, 135.7, 130.0, 129.9, 128.5, 128.4, 128.3, 128.1, 128.0, 127.6, 127.5, 127.4, 127.2, 127.0, 126.2, 116.8, 116.6, 116.2, 115.9, 112.5, 112.4, 112.2, 112.2, 110.9, 110.8, 110.7, 110.6, 76.8, 76.0, 63.7, 62.5, 47.0, 46.4, 41.6, 41.3.

HRMS (ESI): *m/z* calculated for $C_{25}H_{19}FN_3O_4$ $[M-H]^+$= 444.1354, Observed = 444.1373.

5-(3-hydroxy-5-methoxy-2-oxoindolin-3-yl)-1,3-dimethylimidazolidine-2,4-dione (25k):



The compound **25k** was synthesized by following general procedure for aldol reaction and obtained as brown solid.

Yield= 53%

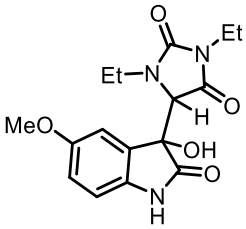
IRυ_{max}(film): 3266, 1709, 1483, 1206 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 10.26 (s, 1 H), 6.82 - 6.76 (m, 1 H), 6.72 - 6.65 (m, 2 H), 6.62 (s, 1 H), 4.39 - 4.38 (m, 1 H), 3.65 (s, 3 H), 3.13 (s, 3 H), 2.53 (s, 3 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 175.1, 168.6, 157.2, 154.5, 135.7, 128.1, 114.5, 111.2, 110.3, 76.6, 66.0, 55.5, 31.3, 24.2.

HRMS (ESI): *m/z* calculated for $C_{14}H_{16}N_3O_5$ $[M+H]^+$= 306.1084, Observed = 306.1078.

1,3-diethyl-5-(3-hydroxy-5-methoxy-2-oxoindolin-3-yl)imidazolidine-2,4-dione (25l):



The compound **25l** was synthesized by following general procedure for aldol reaction and obtained as orange solid.

Yield= 64% (mixture of diastereomers, dr ratio = 1 : 1)

IRυ_{max}(film): 3306, 1704, 1473, 1208 cm^{-1}

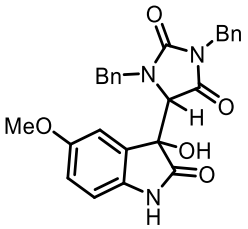
^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 10.29 (d, *J* = 17.8 Hz, 1 H), 6.83 - 6.76 (m, 2 H), 6.76 - 6.61 (m, 2 H), 4.42 (s, 1 H), 3.71 (d, *J* = 6.4 Hz, 1 H), 3.69 (s, 1 H), 3.64 (s,

2 H), 3.58 - 3.47 (m, 1 H), 3.31 - 3.22 (m, 1 H), 3.15 - 2.91 (m, 1 H), 1.25 (t, *J* = 7.1 Hz, 2 H), 1.08 (t, *J* = 7.1 Hz, 1 H), 0.98 (t, *J* = 7.1 Hz, 2 H), 0.45 (t, *J* = 7.1 Hz, 1 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 175.9, 175.2, 169.0, 168.1, 156.4, 156.2, 154.8, 154.6, 135.8, 135.5, 129.3, 127.8, 114.6, 114.2, 111.5, 111.1, 110.3, 110.1, 76.9, 75.9, 63.7, 61.9, 55.5, 55.4, 38.7, 37.7, 32.9, 32.4, 13.2, 13.0, 12.8, 12.2

HRMS (ESI): *m/z* calculated for $C_{16}H_{20}N_3O_5$ $[M+H]^+$= 334.1397, Observed = 334.1393.

1,3-dibenzyl-5-(3-hydroxy-5-methoxy-2-oxoindolin-3-yl)imidazolidine-2,4-dione (25m):



The compound **25m** was synthesized by following general procedure for aldol reaction and obtained as brown solid.

Yield= 37% (mixture of diastereomers, dr ratio = 3 : 2)

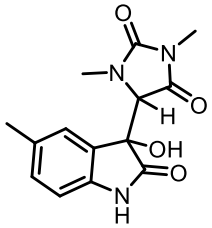
IRυ$_{max}$(film): 3312, 1707, 1448, 753 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 10.30 (d, *J* = 13.8 Hz, 1 H), 7.49 - 7.20 (m, 7 H), 7.20 - 6.97 (m, 2 H), 6.89 - 6.60 (m, 4 H), 6.50 (d, *J* = 7.4 Hz, 1 H), 4.95 - 4.70 (m, 1 H), 4.65 - 4.36 (m, 3 H), 4.36 - 4.06 (m, 1 H), 3.61 (s, 2 H), 3.49 (s, 1 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 175.7, 175.1, 168.5, 168.0, 157.0, 156.8, 154.9, 154.7, 137.0, 136.9, 136.1, 135.8, 135.7, 135.4, 129.3, 128.5, 128.5, 128.4, 128.2, 127.6, 127.4, 127.3, 127.0, 126.9, 126.0, 115.1, 114.5, 111.3, 111.2, 110.4, 110.3, 76.8, 76.0, 64.0, 62.5, 55.3, 55.3, 47.2, 46.3, 41.6, 41.2;

HRMS (ESI): *m/z* calculated for $C_{26}H_{24}N_3O_5$ $[M+H]^+$= 458.1710, Observed = 458.1704.

5-(3-hydroxy-5-methyl-2-oxoindolin-3-yl)-1,3-dimethylimidazolidine-2,4-dione (25n):



The compound **25n** was synthesized by following general procedure for aldol reaction and obtained as white solid.

Yield= 64% (mixture of diastereomers, dr ratio = 3 : 2)

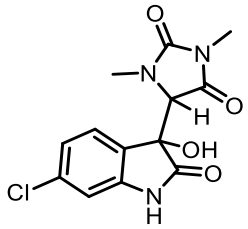
IRυ_{max}(film): 3301, 1706, 1483, 755 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 10.36 - 10.32 (m, 1 H), 7.04 - 6.90 (m, 2 H), 6.69 - 6.48 (m, 2 H), 4.38 - 4.35 (m, 1 H), 3.13 (s, 2 H), 2.87 (s, 1 H), 2.75 (s, 1 H), 2.52 (s, 2 H), 2.23 (s, 1 H), 2.19 (s, 2 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 176.1, 175.3, 169.5, 168.7, 157.2, 156.9, 140.1, 139.9, 130.6, 130.2, 130.2, 130.0, 128.1, 127.1, 124.7, 124.7, 109.6, 109.5, 76.4, 75.4, 66.1, 65.0, 31.3, 30.6, 24.3, 24.2, 21.1, 20.6.;

HRMS (ESI): *m/z* calculated for $C_{14}H_{16}N_3O_4$ $[M+H]^+$= 290.1135, Observed = 290.1129.

5-(6-chloro-3-hydroxy-2-oxoindolin-3-yl)-1,3-dimethylimidazolidine-2,4-dione (25o):



The compound **25o** was synthesized by following general procedure for aldol reaction and obtained as yellow solid.

Yield= 66% (mixture of diastereomers, dr ratio = 1 : 1)

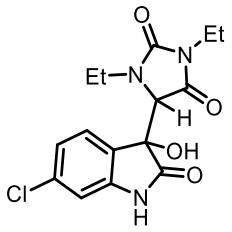
IRυ_{max}(film): 3617, 1707, 1510, 654 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 10.61 (s, 1 H), 7.18 – 6.98 (m, 2 H), 6.81 - 6.66 (m, 2 H), 4.42 (d, *J* = 2.4 Hz, 1 H), 3.14 (s, 1 H), 2.91 (s, 2H), 2.74 (s, 2 H), 2.54 (s, 1 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 175.9, 175.3, 169.1, 168.6, 157.2, 156.8, 144.2, 143.8, 134.4, 134.0, 127.3, 125.9, 125.7, 125.6, 121.6, 121.3, 110.0, 109.9, 75.8, 75.1, 66.1, 65.0, 31.4, 30.8, 24.4, 24.3.

HRMS (ESI): *m/z* calculated for $C_{13}H_{11}ClN_3O_4$ $[M-H]^+$= 308.0433, Observed = 308.0448.

5-(6-chloro-3-hydroxy-2-oxoindolin-3-yl)-1,3-diethylimidazolidine-2,4-dione (25p):



The compound **25p** was synthesized by following general procedure for aldol reaction and obtained as white solid.

Yield= 61% (mixture of diastereomers, dr ratio = 3 : 2)

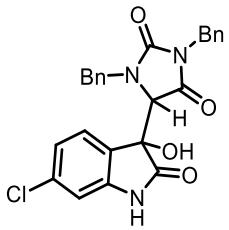
IRυ$_{max}$(film): 3268, 1696, 1455, 754 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 10.61 (s, 1 H), 7.23 (d, *J* = 8.0 Hz, 1 H), 7.07 - 6.94 (m, 1 H), 6.85 - 6.76 (m, 1 H), 6.65 (s, 1 H), 4.53 - 4.45 (m, 1 H), 3.71 - 3.55 (m, 1 H), 3.35 - 3.32 (m, 1 H), 3.30 - 3.25 (m, 1 H), 3.12 - 3.08 (m, 1 H), 1.24 (t, *J* = 7.1 Hz, 1 H), 1.10 (t, *J* = 7.1 Hz, 2 H), 0.97 (t, *J* = 7.1 Hz, 2 H), 0.51 (t, *J* = 7.1 Hz, 1 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 175.7, 175.3, 168.7, 168.1, 156.3, 156.0, 144.2, 143.8, 134.4, 134.0, 127.3, 125.9, 125.7, 125.7, 121.4, 121.2, 109.9, 109.8, 76.2, 75.3, 63.6, 62.0, 38.8, 37.9, 32.9, 32.5, 13.2, 12.9, 12.8, 12.2

HRMS (ESI): *m/z* calculated for $C_{15}H_{17}ClN_3O_4$ $[M+H]^+$= 338.0902, Observed = 338.0898.

1,3-dibenzyl-5-(6-chloro-3-hydroxy-2-oxoindolin-3-yl)imidazolidine-2,4-dione (25q):



The compound **25q** was synthesized by following general procedure for aldol reaction and obtained as white solid.

Yield= 57% (mixture of diastereomers, dr ratio = 1 : 1)

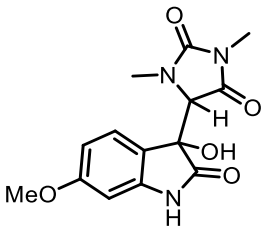
IRυ_{max}(film): 3300, 1703, 1446, 752 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6) = δ 10.62 (d, *J* = 2.8 Hz, 1 H), 7.39 - 7.15 (m, 8 H), 7.14 - 7.09 (m, 2 H), 6.94 - 6.85 (m, 1 H), 6.85 - 6.65 (m, 2 H), 6.54 (d, *J* = 7.3 Hz, 1 H), 5.10 - 4.82 (m, 1 H), 4.52 - 4.31 (m, 4 H)

^{13}C NMR (100 MHz, DMSO-d_6) = δ 175.7, 175.0, 168.3, 167.9, 156.7, 156.7, 144.2, 143.7, 136.7, 136.7, 136.0, 135.6, 134.7, 134.0, 128.5, 128.4, 128.3, 128.1, 127.6, 127.5, 127.5, 127.4, 127.3, 127.0, 126.9, 126.2, 126.2, 125.8, 125.4, 121.6, 121.4, 110.1, 109.9, 79.2, 76.2, 75.3, 63.5, 62.2, 46.8, 46.4, 41.7, 41.3

HRMS (ESI): *m/z* calculated for $C_{25}H_{21}ClN_3O_4$ $[M+H]^+$= 462.1215, Observed = 462.1212.

5-(3-hydroxy-6-methoxy-2-oxoindolin-3-yl)-1,3-dimethylimidazolidine-2,4-dione (25r):



The compound **25r** was synthesized by following general procedure for aldol reaction and obtained as white solid

Yield= 69% (mixture of diastereomers, dr ratio = 9 : 1)

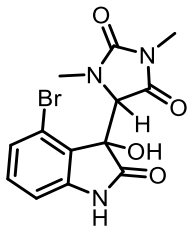
IRυ_{max}(film): 3460, 2853, 1713, 1468 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 10.38 (s, 1 H), 6.98 (d, *J* = 8.3 Hz, 1 H), 6.48 - 6.36 (m, 2 H), 6.32 (d, *J* = 2.3 Hz, 1 H), 4.37 - 4.32 (m, 1 H), 3.73 - 3.71 (m, 3 H), 3.13 (s, 3 H), 2.54 (s, 3 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 176.7, 175.8, 169.6, 168.7, 160.9, 160.8, 157.2, 156.9, 144.0, 143.8, 125.1, 119.7, 118.8, 106.6, 106.3, 96.6, 76.0, 75.1, 66.1, 65.0, 55.2, 48.5, 31.3, 30.5, 24.4, 24.2

HRMS (ESI): *m/z* calculated for $C_{14}H_{15}N_3O_5Na$ $[M+Na]^+$= 328.0904, Observed = 328.0901.

5-(4-bromo-3-hydroxy-2-oxoindolin-3-yl)-1,3-dimethylimidazolidine-2,4-dione (25s):



The compound **25s** was synthesized by following general procedure for aldol reaction and obtained as white solid

Yield= 66% (mixture of diastereomers, dr ratio = 3 : 2)

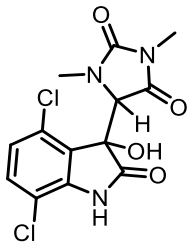
IRυ$_{max}$(film): 3313, 1705, 1615, 755 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 10.72 -10.50 (m, 1 H), 7.18 - 7.13 (m, 2 H), 6.82 - 6.80 (m, 1 H), 6.80 - 6.48 (m, 1 H), 4.90 - 4.57 (m, 1 H), 3.12 (s, 1 H), 2.84 - 2.83 (m, 1 H), 2.72 - 2.70 (m, 4 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 174.9, 174.3, 169.4, 168.3, 157.2, 156.4, 145.0, 144.1, 132.0, 131.4, 127.1, 126.5, 126.1, 125.6, 118.9, 109.5, 109.0, 78.3, 77.0, 64.5, 63.0, 31.2, 31.0, 24.5, 24.4

HRMS (ESI): *m/z* calculated for $C_{13}H_{13}BrN_3O_4$ $[M+H]^+$= 354.0084, Observed = 354.0082.

5-(4,7-dichloro-3-hydroxy-2-oxoindolin-3-yl)-1,3-dimethylimidazolidine-2,4-dione (25t):



The compound **25t** was synthesized by following general procedure for aldol reaction and obtained as white solid

Yield= 87% (mixture of diastereomers, dr ratio = 1 : 1)

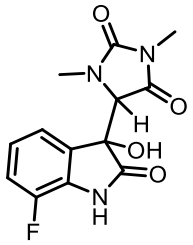
IRυ_{max}(film): 3300, 1715, 1610, 750 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6) = δ 11.20 - 11.01 (m, 1H), 7.36 (dd, *J* = 2.4, 8.6 Hz, 1 H), 7.14 - 6.94 (m, 1 H), 6.83 (s, 1 H), 4.87 - 4.52 (m, 1 H), 3.11 (s, 1 H), 2.91 (s, 2 H), 2.72 (s, 1 H), 2.66 (s, 2 H)

^{13}C NMR (400 MHz, DMSO-d_6) = δ 174.9, 174.1, 169.0, 168.2, 156.9, 156.3, 142.4, 141.4, 131.5, 131.0, 129.3, 129.1, 127.0, 126.2, 124.0, 123.8, 113.2, 112.8, 78.8, 77.1, 65.7, 63.0, 31.1, 24.5, 24.4

HRMS (ESI): *m/z* calculated for $C_{13}H_{11}Cl_2N_3O_4Na$ $[M+Na]^+$= 366.0019, Observed = 366.0017.

5-(7-fluoro-3-hydroxy-2-oxoindolin-3-yl)-1,3-dimethylimidazolidine-2,4-dione (25u):



The compound **25u** was synthesized by following general procedure for aldol reaction and obtained as yellow solid.

Yield= 63% (mixture of diastereomers, dr ratio = 4: 1);

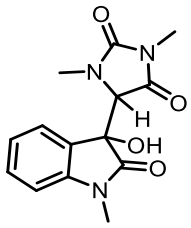
IRυ_{max}(film): 3317, 1705, 1476, 750 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 10.99 (s, 1 H), 7.23 - 7.10 (m, 1 H), 7.08 - 6.91 (m, 2 H), 6.80 (s, 1 H), 4.43 (s, 1 H), 3.15 (s, 2 H), 2.93 (s, 1 H), 2.74 (s, 1 H), 2.52 (s, 2 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 175.7, 175.1, 169.1, 168.6, 157.2, 156.8, 147.5, 145.1, 129.9, 129.9, 129.7, 129.6, 122.6, 122.6, 120.2, 120.2, 117.3, 117.1, 76.3, 76.3, 75.6, 75.5, 66.1, 65.1, 31.4, 30.8, 24.4, 24.3.;

HRMS (ESI): *m/z* calculated for $C_{13}H_{12}FN_3O_4Na$ $[M+Na]^+$= 316.0704, Observed = 316.0699.

5-(3-hydroxy-1-methyl-2-oxoindolin-3-yl)-1,3-dimethylimidazolidine-2,4-dione (25v):



The compound **25v** was synthesized by following general procedure for aldol reaction and obtained as white solid.

Yield= 92%;

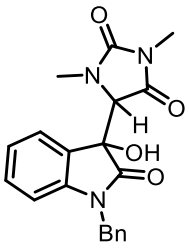
IRυ$_{max}$(film): 3305, 1701, 1485, 757 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 7.33 (dt, *J* = 1.3, 7.7 Hz, 1 H), 7.19 - 7.09 (m, 1 H), 7.06 - 6.93 (m, 2 H), 6.72 (s, 1 H), 4.45 (s, 1 H), 3.17 (s, 3 H), 3.10 (s, 3 H), 2.47 (s, 3 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 173.7, 168.6, 157.1, 143.9, 130.2, 126.2, 123.7, 122.2, 108.7, 76.1, 66.4, 31.3, 25.9, 24.2

HRMS (ESI): *m/z* calculated for $C_{14}H_{15}O_4N_3Na$ $[M+Na]^+$= 312.0955, Observed = 312.0947.

5-(1-benzyl-3-hydroxy-2-oxoindolin-3-yl)-1,3-dimethylimidazolidine-2,4-dione (25w):



The compound **25w** was synthesized by following general procedure for aldol reaction and obtained as white solid

Yield= 79%;

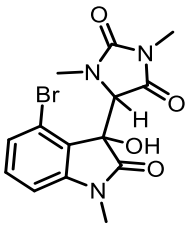
IRυ_{max}(film): 3306, 1704, 1425, 750 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 7.44 (d, *J* = 7.4 Hz, 2 H), 7.39 - 7.11 (m, 5 H), 7.01 - 6.91 (m, 1 H), 6.85 (s, 1 H), 6.77 (d, J = 7.8 Hz, 1 H), 4.93 - 4.81 (m, 2 H), 4.55 (s, 1 H), 3.17 (s, 3 H), 2.53 (s, 3 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 174.1, 168.7, 157.2, 143.1, 136.0, 130.1, 128.5, 127.4, 127.3, 126.4, 124.0, 122.4, 109.5, 76.0, 66.2, 42.9, 31.4, 24.3

HRMS (ESI): *m/z* calculated for $C_{20}H_{19}O_4N_3Na$ $[M+Na]^+$= 388.1268, Observed = 388.1260.

5-(4-bromo-3-hydroxy-1-methyl-2-oxoindolin-3-yl)-1,3-dimethylimidazolidine-2,4-dione (25x):



The compound **25x** was synthesized by following general procedure for aldol reaction and obtained as white solid

Yield= 51%

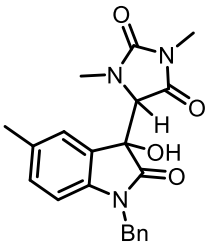
IRυ_{max}(film): 3301, 1708, 1420, 754 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 7.27 (t, *J* = 7.9 Hz, 1 H), 7.19 (d, *J* = 7.5 Hz, 1 H), 7.10 - 6.98 (m, 1 H), 6.63 (s, 1 H), 4.57 (s, 1 H), 3.10 (s, 3 H), 2.83 (s, 3 H), 2.64 (s, 3 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 173.4, 169.2, 157.1, 146.2, 132.0, 126.9, 125.7, 118.6, 108.3, 78.2, 65.5, 31.2, 26.2, 24.4

HRMS (ESI): *m/z* calculated for $C_{14}H_{14}O_4N_3BrNa$ $[M+Na]^+$= 390.0060, Observed = 390.0054.

5-(1-benzyl-3-hydroxy-5-methyl-2-oxoindolin-3-yl)-1,3-dimethylimidazolidine-2,4-dione (25y):



The compound **25y** was synthesized by following general procedure for aldol reaction and obtained as white solid

Yield= 79%;

IRυ_{max}(film): 3307, 1704, 1426, 752 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 7.42 - 7.38 (m, 2 H), 7.38 - 7.20 (m, 3 H), 7.14 - 6.92 (m, 2 H), 6.79 (s, 1 H), 6.64 (d, *J* = 7.9 Hz, 1 H), 4.90 - 4.79 (m, 2 H), 4.53 - 4.49 (m, 1 H), 3.15 (s, 3 H), 2.55 (s, 3 H), 2.19 (s, 3 H)

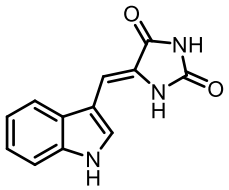
^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 174.0, 168.8, 157.3, 140.7, 136.1, 131.3, 130.2, 128.4, 127.3, 127.3, 126.6, 124.6, 109.2, 76.1, 66.1, 42.9, 31.5, 24.3, 20.6.;

HRMS (ESI): *m/z* calculated for $C_{21}H_{21}O_4N_3Na$ $[M+Na]^+$= 402.1424, Observed = 402.1411.

Procedure for the synthesis of olefinic oxoaplysinopsins:

Indole-3-carbaldehyde derivatives (1.0 equiv) and substituted hydantoin (1.0 equiv) were stirred in the presence of ethanolamine (1.5 equiv) in absolute ethanol at 60 °C for 12 h. Compounds were precipitated out, filtered, and crystallized from acetone to get pure product.

(*Z*)-5-((1H-indol-3-yl)methylene)imidazolidine-2,4-dione (66)



The compound **66** was synthesized by following general procedure for synthesis of olefinic oxoaplysinopsin obtained as yellow solid

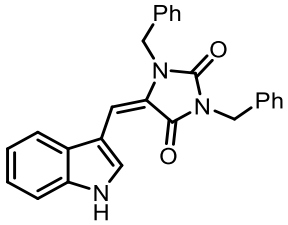
Yield= 72%

IRυ_{max}(film): 3631, 3182, 2487, 2359, 1649 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6) = δ 11.80 (br. s., 1 H), 10.23 (br. s., 2 H), 8.13 (s, 1 H), 7.75 (d, *J* = 7.8 Hz, 1 H), 7.51 - 7.36 (m, 1 H), 7.26 - 7.05 (m, 2 H), 6.81 - 6.70 (m, 1 H)

^{13}C NMR (100 MHz, DMSO-d_6) = δ 165.9, 155.8, 136.3, 127.4, 127.2, 124.1, 122.8, 120.6, 118.5, 112.3, 108.8, 102.2, 40.0

(*E*)-5-((1H-indol-3-yl)methylene)-1,3-dibenzylimidazolidine-2,4-dione (68)



The compound **68** was synthesized by following general procedure for synthesis of olefinic oxoaplysinopsin obtained as yellow solid

Yield= 54%

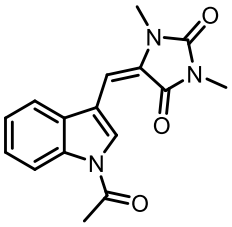
IRυ_{max}(film): 3686, 2887, 2352, 1718, 1644 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6) = δ 11.73 (br. s., 1 H), 8.79 (s, 1 H), 7.67 (d, *J* = 7.4 Hz, 1 H), 7.48 - 7.41 (m, 3 H), 7.41 - 7.33 (m, 6 H), 7.33 - 7.21 (m, 2 H), 7.21 - 7.05 (m, 2 H), 6.80 (s, 1 H), 5.10 (s, 2 H), 4.80 (s, 2 H)

^{13}C NMR (100 MHz, DMSO-d_6) = δ 161.5, 152.8, 136.8, 136.6, 135.6, 129.0, 128.7, 128.6, 127.6, 127.5, 127.4, 127.2, 122.2, 122.2, 120.1, 117.7, 112.1, 110.0, 108.2, 42.5, 41.6

HRMS (ESI): *m/z* calculated for $C_{26}H_{21}O_2N_3Na$ $[M+Na]^+$= 430.1526, Observed = 430.1517.

(*E*)-5-((1-acetyl-1H-indol-3-yl)methylene)-1,3-dimethylimidazolidine-2,4-dione (69)

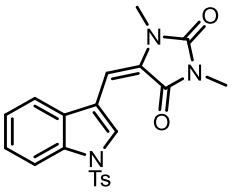


The compound **69** was synthesized from compound **67** by known literature procedure and obtained as yellow solid

Yield= 63%

^{1}H NMR (400 MHz, DMSO-d_6) = δ 9.17 (s, 1 H), 8.36 (d, *J* = 7.9 Hz, 1 H), 8.06 (d, *J* = 6.6 Hz, 1 H), 7.40 (br. s., 2 H), 6.67 (s, 1 H), 3.27 (s, 3 H), 3.03 (s, 3 H), 2.71 (s, 3 H)

(*E*)-1,3-dimethyl-5-((1-tosyl-1H-indol-3-yl)methylene)imidazolidine-2,4-dione (70)

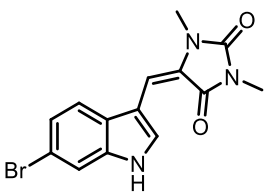


The compound **70** was synthesized from compound **67** by known literature procedure and obtained as yellow solid

Yield= 66%

^{1}H NMR (400 MHz, DMSO-d_6) = δ 9.12 - 9.05 (m, 1 H), 8.03 (d, *J* = 7.4 Hz, 1 H), 7.98 - 7.93 (m, 1 H), 7.88 - 7.82 (m, 2 H), 7.44 - 7.33 (m, 4 H), 6.58 (s, 1 H), 3.23 (s, 3 H), 3.03 (s, 3 H), 2.30 (s, 3 H)

(*E*)-5-((6-bromo-1H-indol-3-yl)methylene)-1,3-dimethylimidazolidine-2,4-dione (71)



The compound **71** was synthesized by following general procedure for synthesis of olefinic oxoaplysinopsin obtained as yellow solid

Yield= 68%

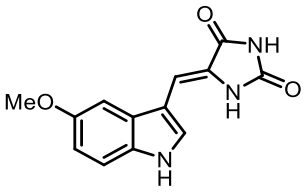
IRυ_{max}(film): 3269, 2889, 2352, 1682, 1436 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 11.74 (br. s., 1 H), 8.81 (d, *J* = 2.5 Hz, 1 H), 7.92 (d, *J* = 8.6 Hz, 1 H), 7.64 (d, *J* = 1.6 Hz, 1 H), 7.26 (dd, *J* = 1.8, 8.5 Hz, 1 H), 6.71 (s, 1 H), 3.22 (s, 3 H), 2.98 (s, 3 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 161.9, 152.8, 136.5, 129.3, 126.7, 125.1, 122.6, 120.2, 114.7, 114.5, 108.8, 107.4, 26.2, 24.3

HRMS (ESI): *m/z* calculated for $C_{14}H_{12}O_2N_3Na$ $[M+Na]^+$= 356.0005, Observed = 356.0001.

(*Z*)-5-((5-methoxy-1H-indol-3-yl)methylene)imidazolidine-2,4-dione (72)



The compound **72** was synthesized by following general procedure for synthesis of olefinic oxoaplysinopsin obtained as yellow solid

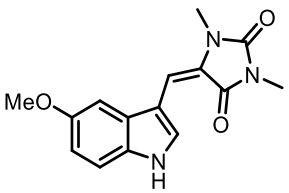
Yield= 58%

IRυ_{max}(film): 3677, 2890, 2488, 2352, 1447 cm^{-1}

^{1}H NMR (400 MHz, DMSO-*d*$_6$) = δ 11.66 (br. s., 1 H), 10.99 (br. s., 1 H), 10.06 (br. s., 1 H), 8.08 (br. s., 1 H), 7.37 - 7.21 (m, 2 H), 6.87 - 6.71 (m, 2 H), 3.81 (s, 3 H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$) = δ 165.4, 155.3, 154.4, 130.7, 127.6, 127.2, 123.1, 112.6, 108.3, 102.4, 99.7, 55.4

(*E*)-5-((5-methoxy-1H-indol-3-yl)methylene)-1,3-dimethylimidazolidine-2,4-dione (73)



The compound **73** was synthesized by following general procedure for synthesis of olefinic oxoaplysinopsin obtained as yellow solid

Yield= 54%

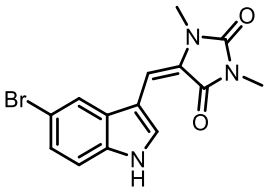
IRυ_{max}(film): 3684, 3287, 2367, 1692, 1446 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6) = δ 11.54 (br. s., 1 H), 8.82 (d, *J* = 2.9 Hz, 1 H), 7.47 (d, *J* = 2.4 Hz, 1 H), 7.34 (d, *J* = 8.8 Hz, 1 H), 6.81 (dd, *J* = 2.4, 8.8 Hz, 1 H), 6.75 (s, 1 H), 3.83 (s, 3 H), 3.25 (s, 3 H), 2.99 (s, 3 H)

^{13}C NMR (100 MHz, DMSO-d_6) = δ 161.9, 154.3, 152.7, 130.6, 129.2, 128.4, 123.9, 112.6, 112.0, 108.7, 108.5, 100.4, 55.6, 26.3, 24.3

HRMS (ESI): *m/z* calculated for $C_{15}H_{16}O_3N_3$ $[M+H]^+$= 286.1186, Observed = 286.1181.

(*E*)-5-((5-bromo-1H-indol-3-yl)methylene)-1,3-dimethylimidazolidine-2,4-dione (74):



The compound **74** was synthesized by following general procedure for synthesis of olefinic oxoaplysinopsin obtained as yellow solid

Yield= 60%

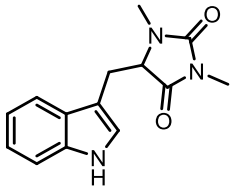
IRυ_{max}(film):

^{1}H NMR (400 MHz, DMSO-d_6) = δ 11.81 (br. s, 1 H), 8.85 (d, *J* = 2.8 Hz, 1 H), 8.24 (d, *J* = 1.8 Hz, 1 H), 7.41 (d, *J* = 8.6 Hz, 1 H), 7.28 (dd, *J* = 1.8, 8.6 Hz, 1 H), 6.76 (s, 1 H), 3.23 (s, 3 H), 2.98 (s, 3 H)

^{13}C NMR (100 MHz, DMSO-d_6) = δ 161.9, 152.8, 134.3, 129.7, 129.6, 125.0, 124.5, 120.9, 113.9, 112.8, 108.4, 107.7, 26.3, 24.3.

HRMS (ESI): *m/z* calculated for $C_{14}H_{12}O_2N_3BrNa$ $[M+Na]^+$= 356.0005, Observed = 356.0000.

5-((1H-indol-3-yl)methyl)-1,3-dimethylimidazolidine-2,4-dione (75)



To a solution of (*E*)-5-((1H-indol-3-yl)methylene)-1,3-dimethylimidazolidine-2,4-dione **67** in methanol (30 mL) was transferred into parr reactor by cannula Pd/C (10 mol%) was added. The reactor was closed and filled with hydrogen gas (300 psi pressure). The reaction was stirred at room temperature for 12 h, after completion of reaction, it was filtered through celite pad and concentrated in *vaccuo* to afford compound **75** as pure product.

Yield= 54%

IRυ_{max}(film): 3335, 2900, 2356, 1700, 1461 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6) = δ 10.90 (br. s., 1 H), 7.50 (d, *J* = 7.9 Hz, 1 H), 7.31 (d, *J* = 8.1 Hz, 1 H), 7.09 - 7.02 (m, 2 H), 7.00 - 6.93 (m, 1 H), 4.29 (t, *J* = 4.5 Hz, 1 H), 3.27 (d, *J* = 4.9 Hz, 1 H), 3.23 - 3.15 (m, 1 H), 2.82 (s, 3 H), 2.65 (s, 3 H)

^{13}C NMR (100 MHz, DMSO-d_6) = δ 173.1, 156.4, 135.8, 127.2, 123.9, 120.9, 118.4, 118.3, 111.4, 107.5, 61.4, 27.8, 24.3, 24.1

2.3.6 References

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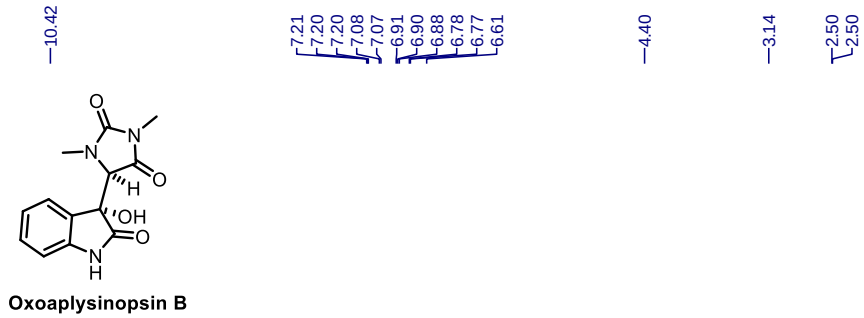
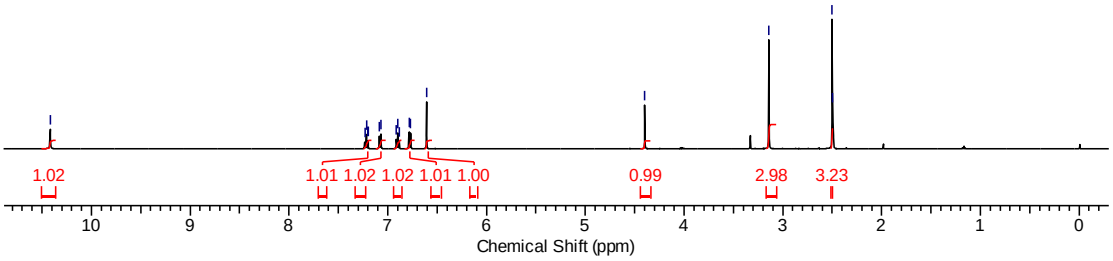
2.3.7 Copies of NMR spectra

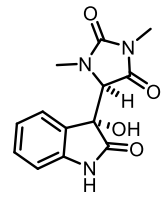
^{1}H NMR of Compound 25 in DMSO-d_6 at 500 MHz



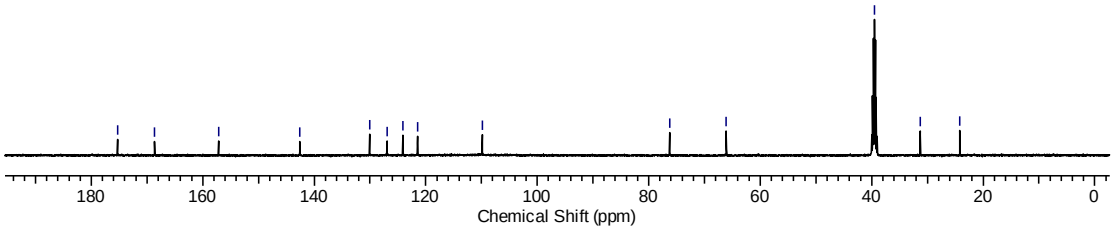
Oxoaplysinopsin B



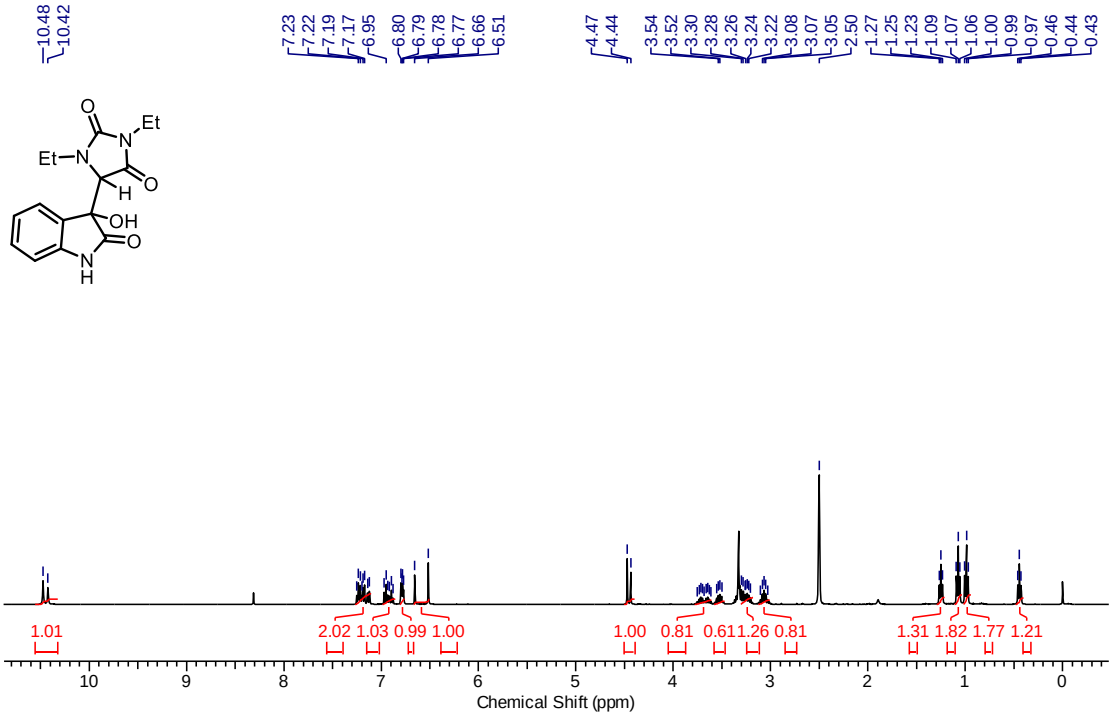
^{13}C NMR of Compound 25 in DMSO-d_6 at 125 MHz



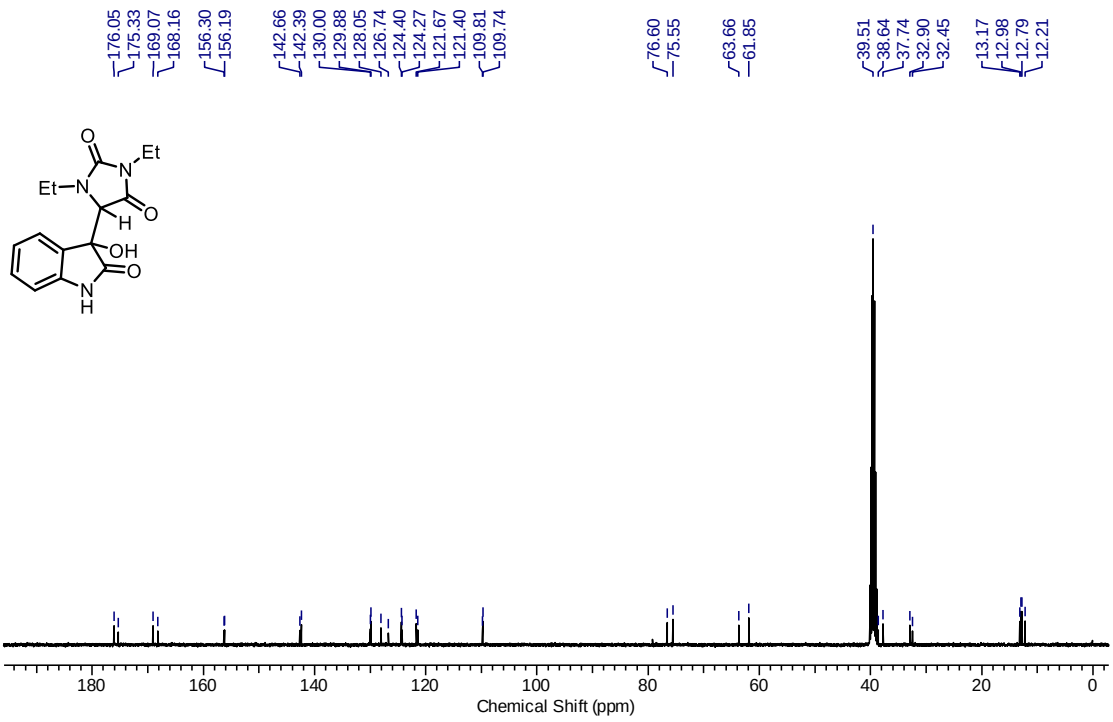
Oxoaplysinopsin B



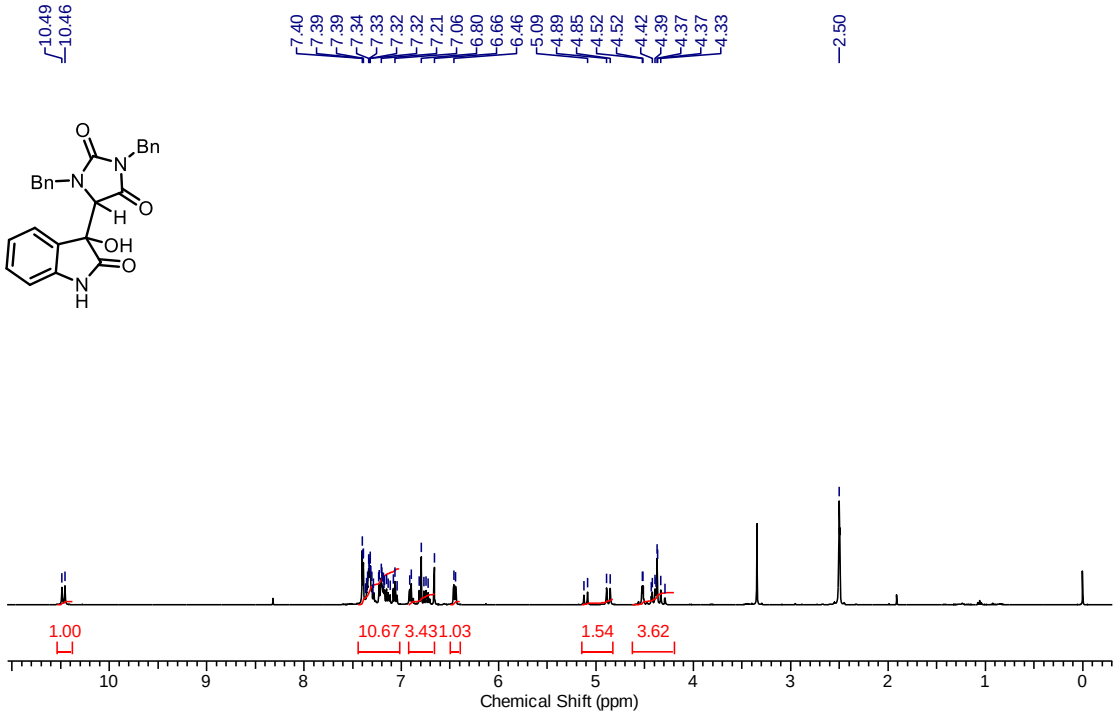
^{1}H NMR of Compound 25a in DMSO-d_6 at 400 MHz



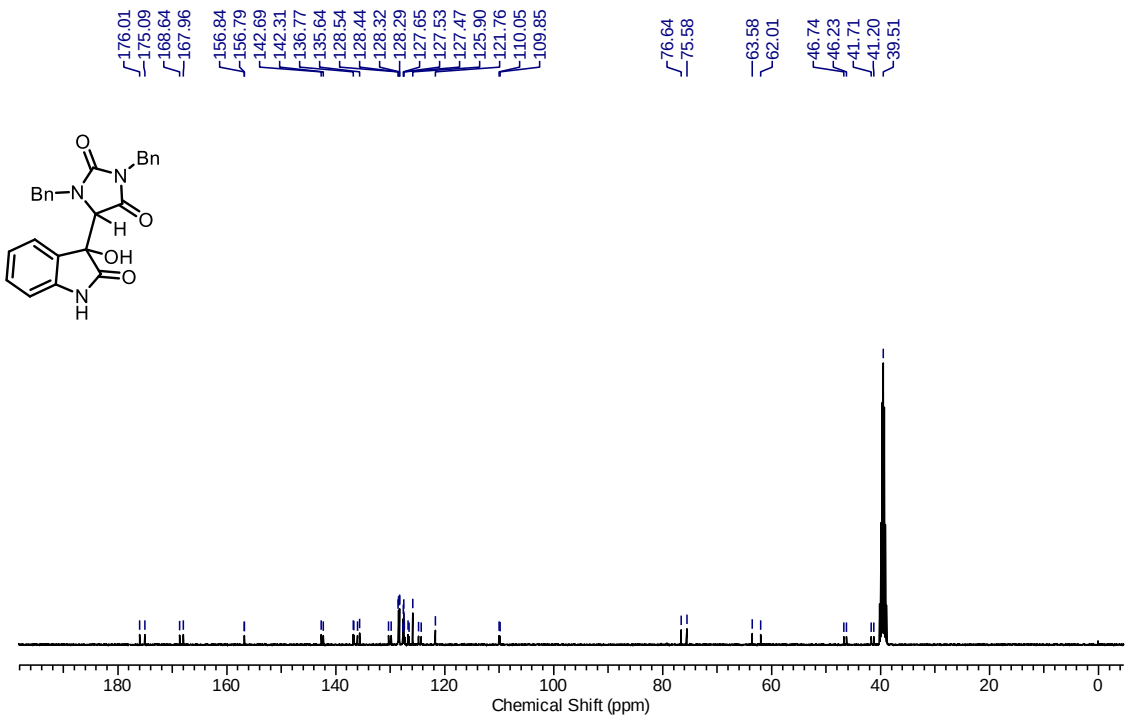
^{13}C NMR of Compound 25a in DMSO-d_6 at 100 MHz



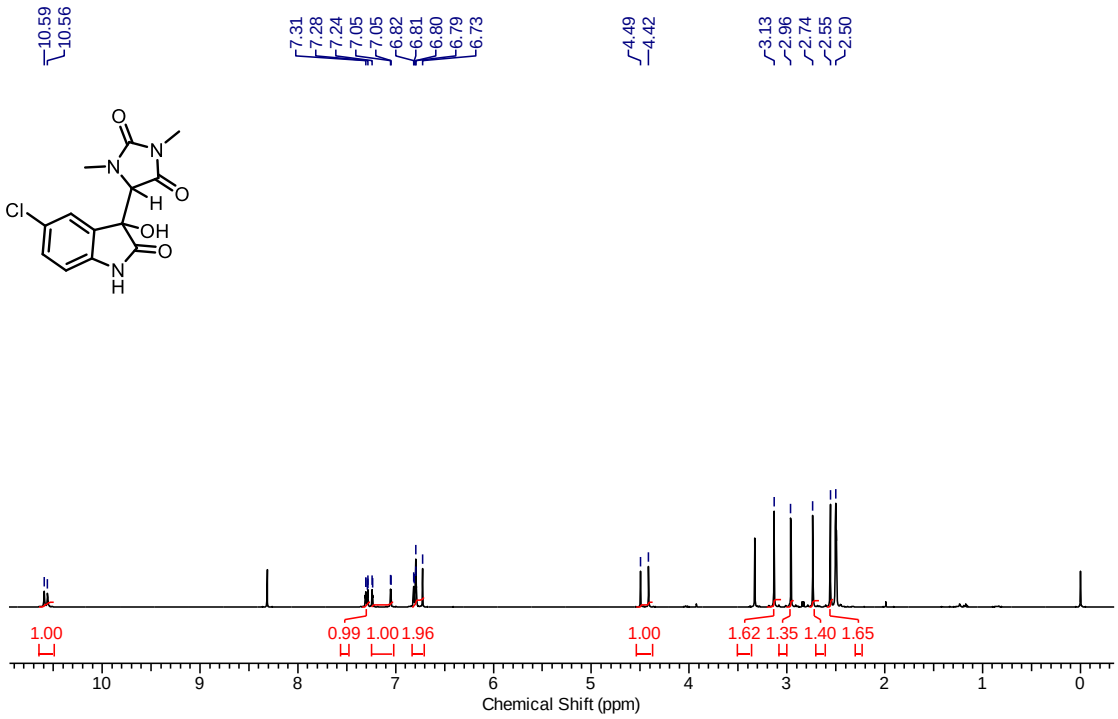
^{1}H NMR of Compound 25b in DMSO-d_6 at 400 MHz



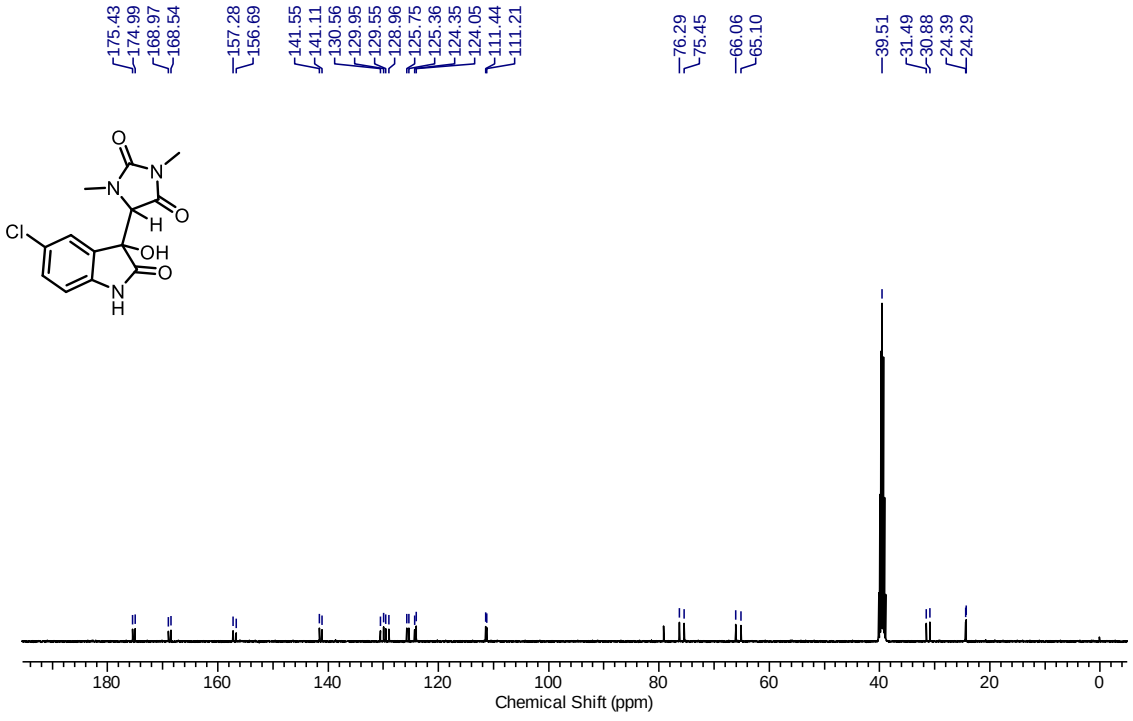
^{13}C NMR of Compound 25b in DMSO-d_6 at 100 MHz



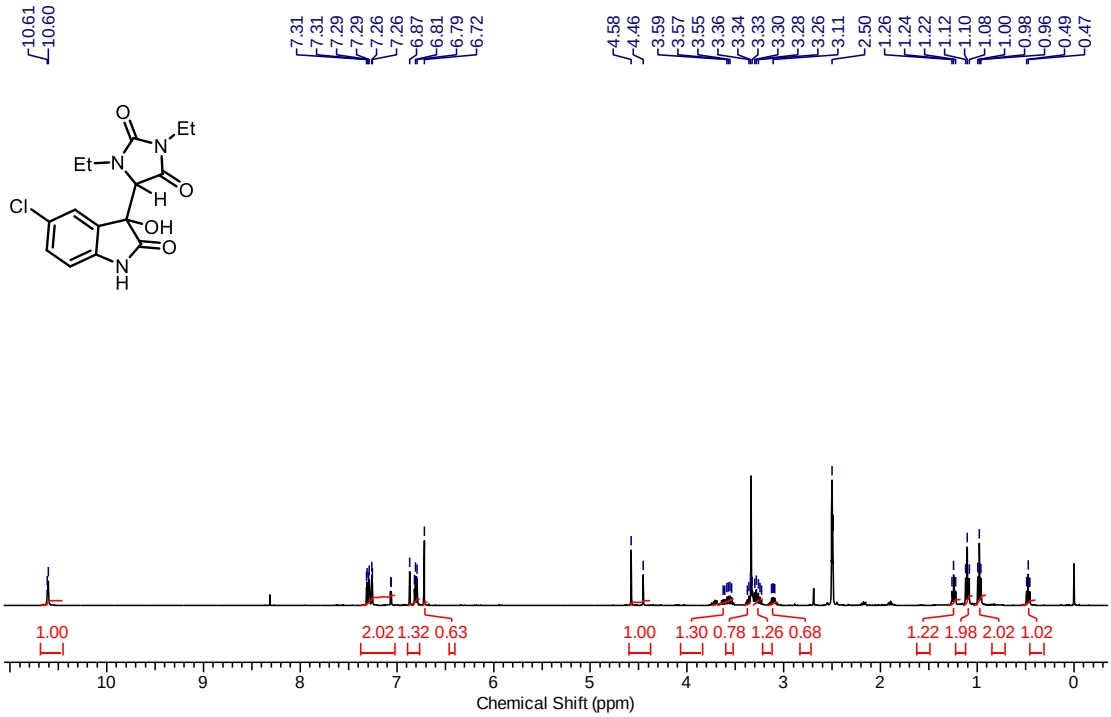
^{1}H NMR of Compound 25c in DMSO-d_6 at 400 MHz



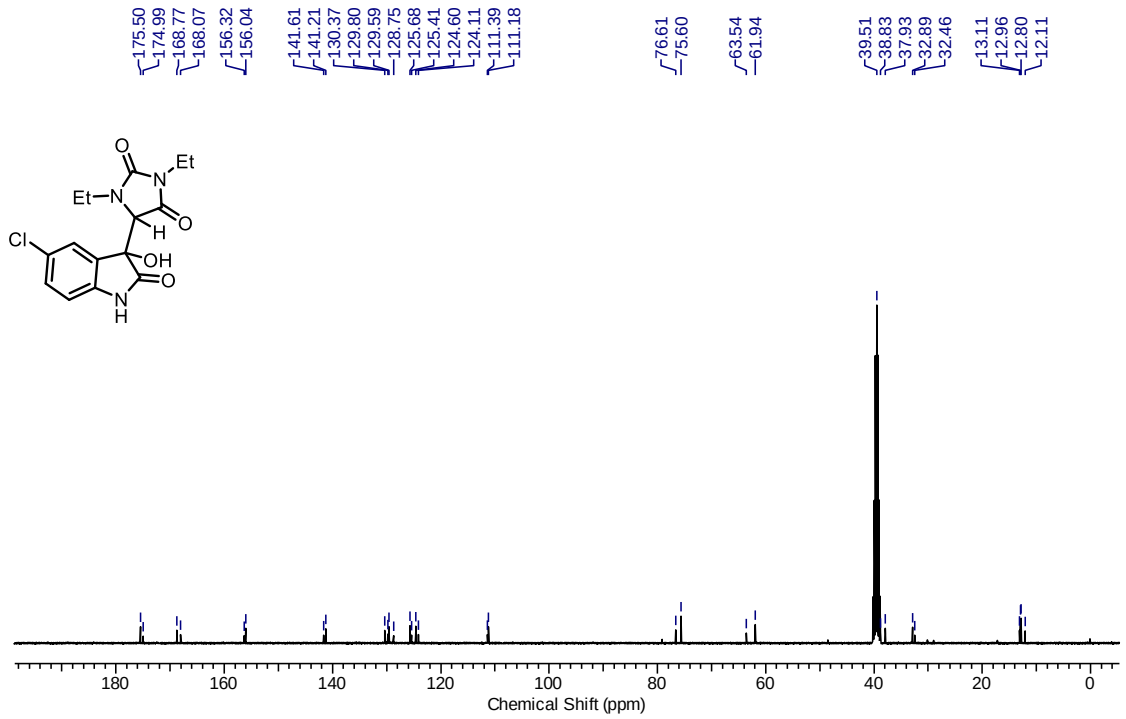
^{13}C NMR of Compound 25c in DMSO-d_6 at 100 MHz



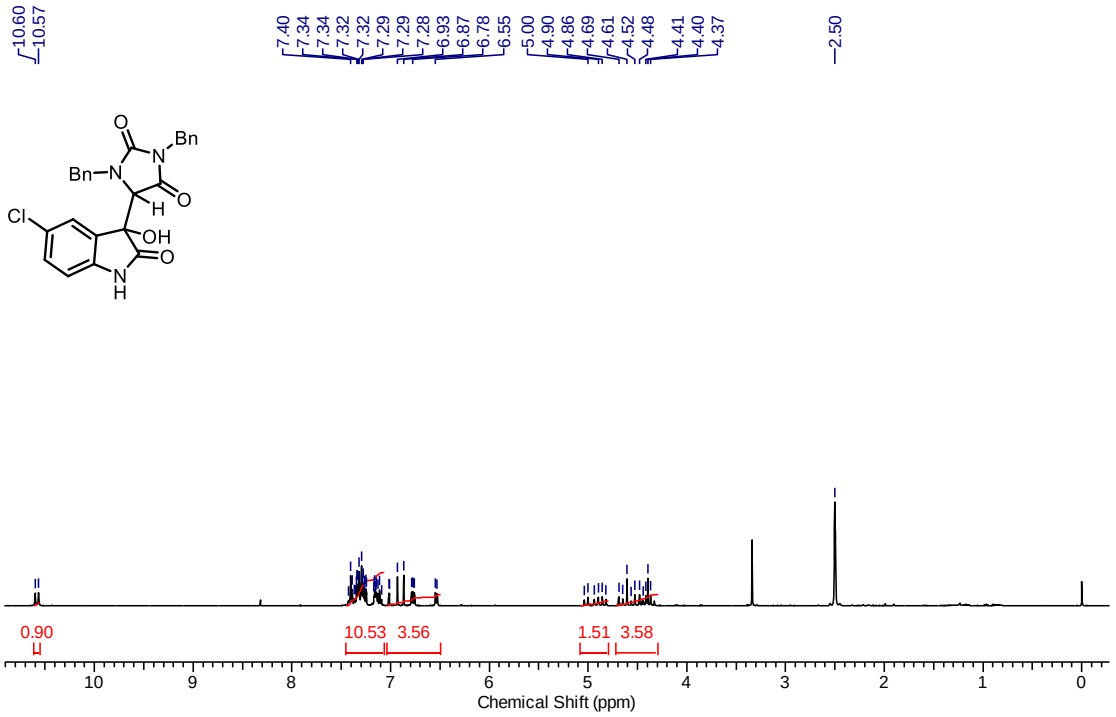
^{1}H NMR of Compound 25d in DMSO-d_6 at 400 MHz



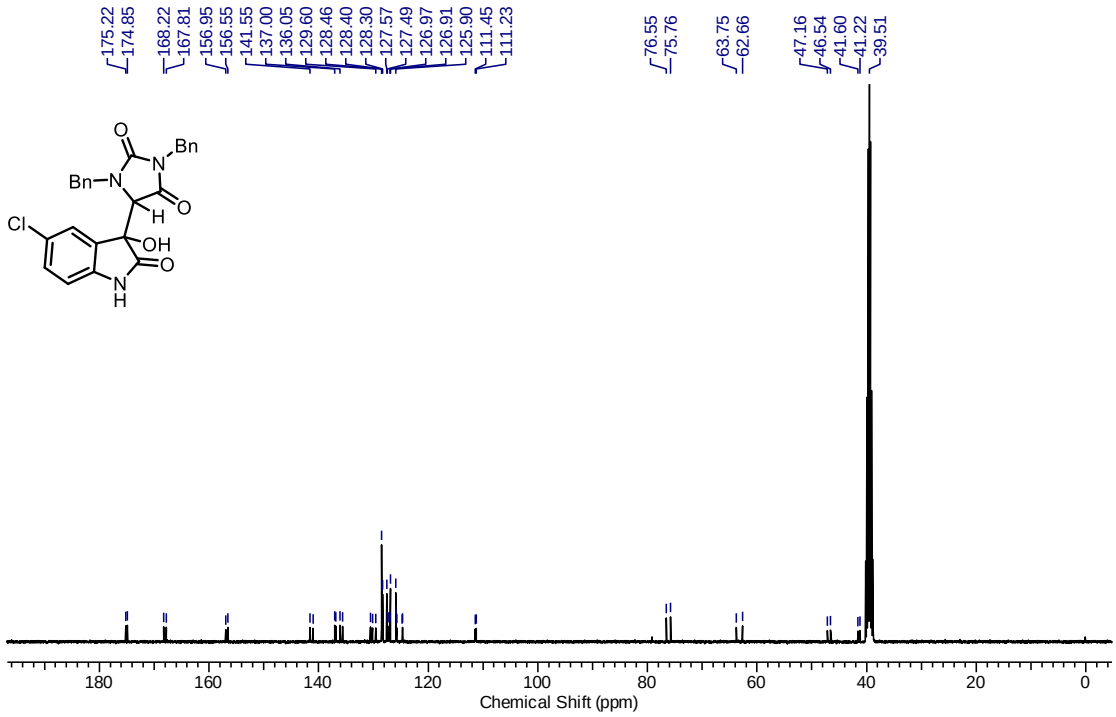
^{13}C NMR of Compound 25d in DMSO-d_6 at 100 MHz



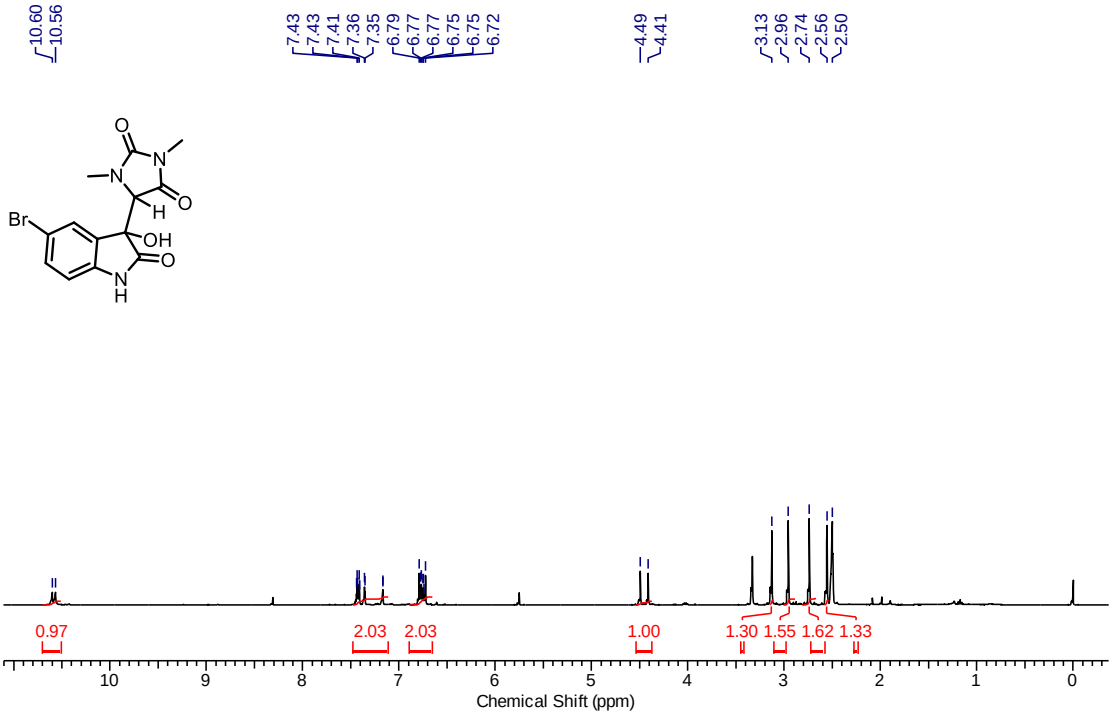
^{1}H NMR of Compound 25e in DMSO-d_6 at 400 MHz



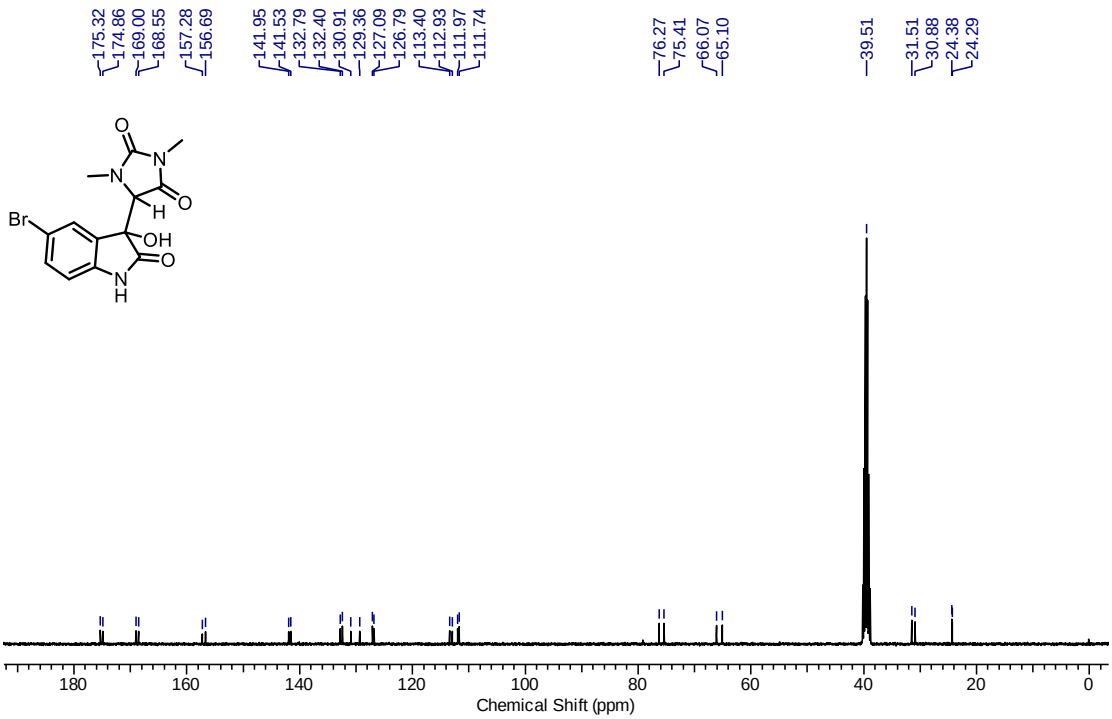
^{13}C NMR of Compound 25e in DMSO-d_6 at 100 MHz



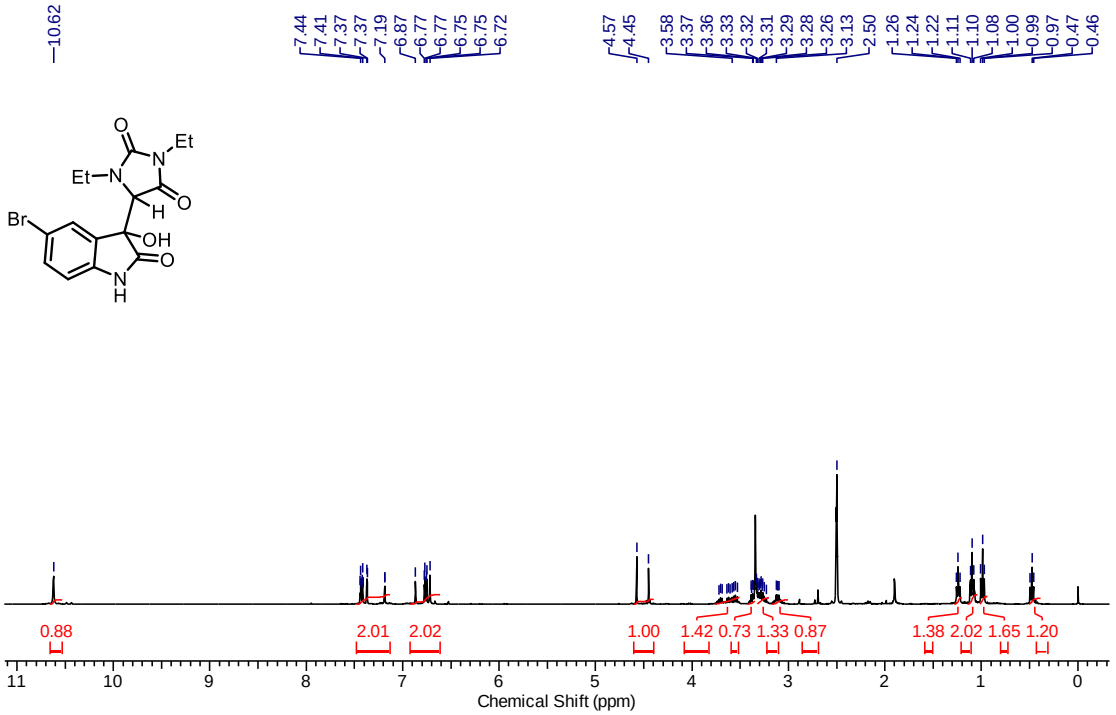
^{1}H NMR of Compound 25f in DMSO-d_6 at 400 MHz



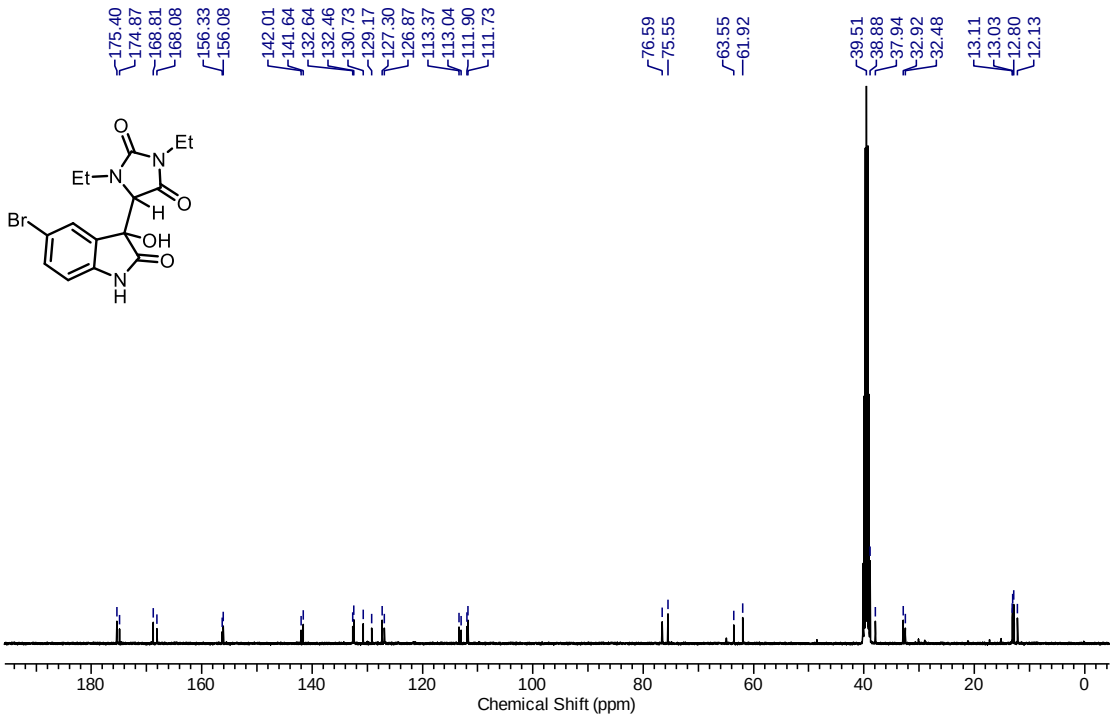
^{13}C NMR of Compound 25f in DMSO-d_6 at 100 MHz



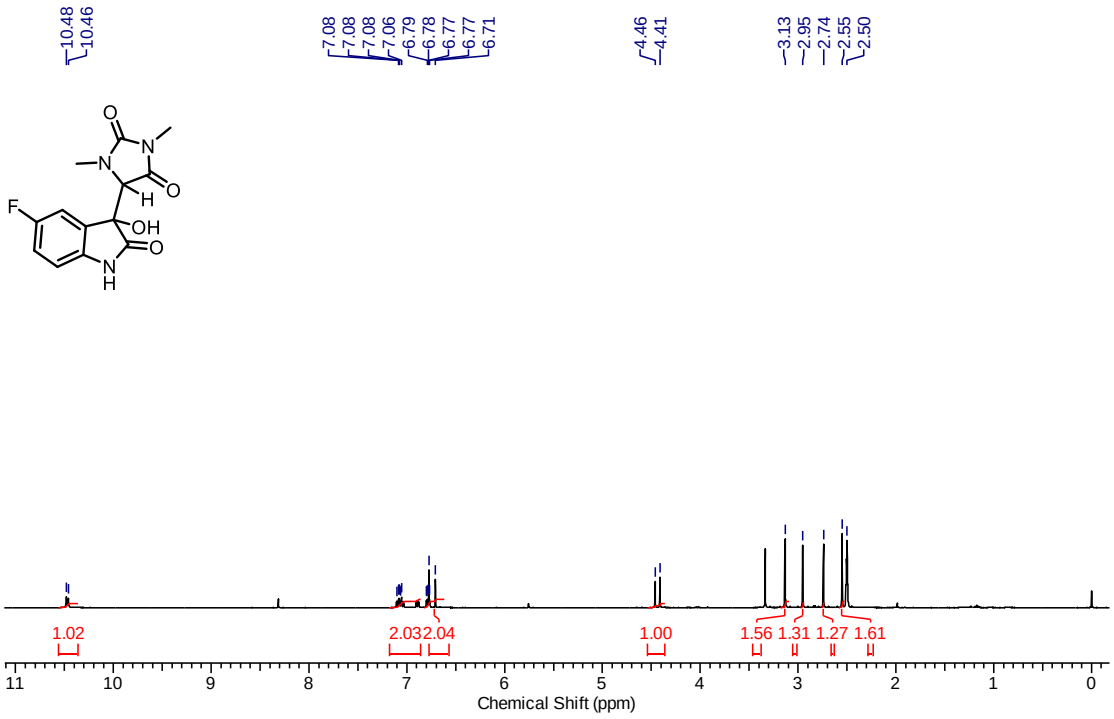
^{1}H NMR of Compound 25g in DMSO-d_6 at 400 MHz



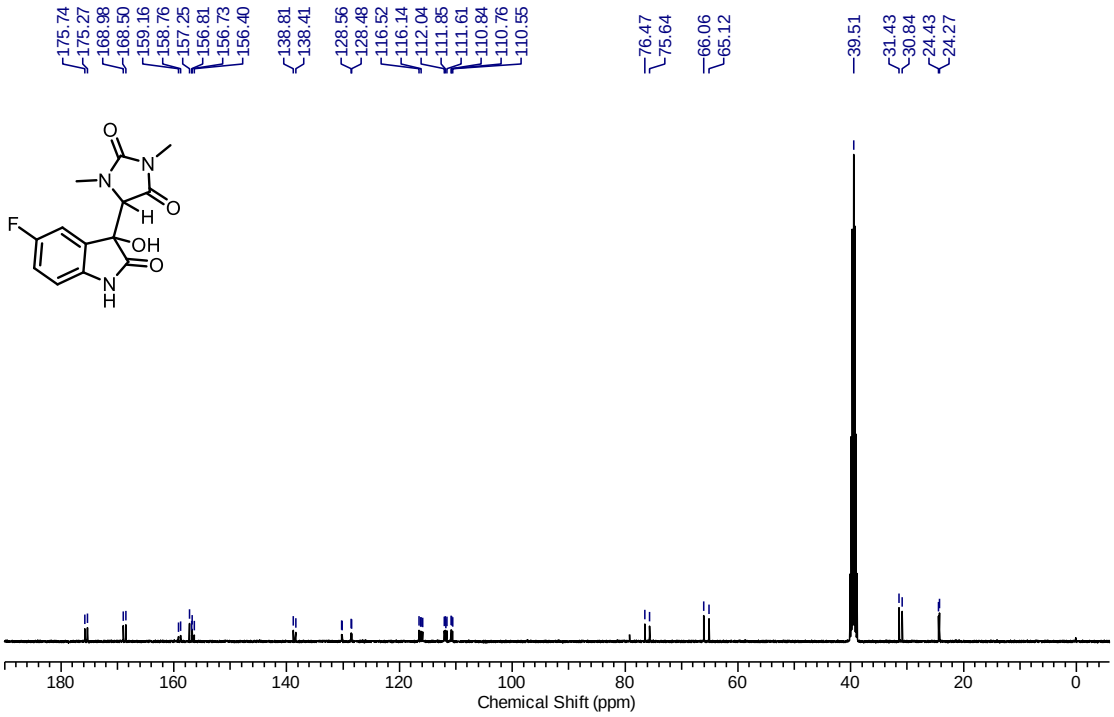
^{13}C NMR of Compound 25g in DMSO-d_6 at 100 MHz



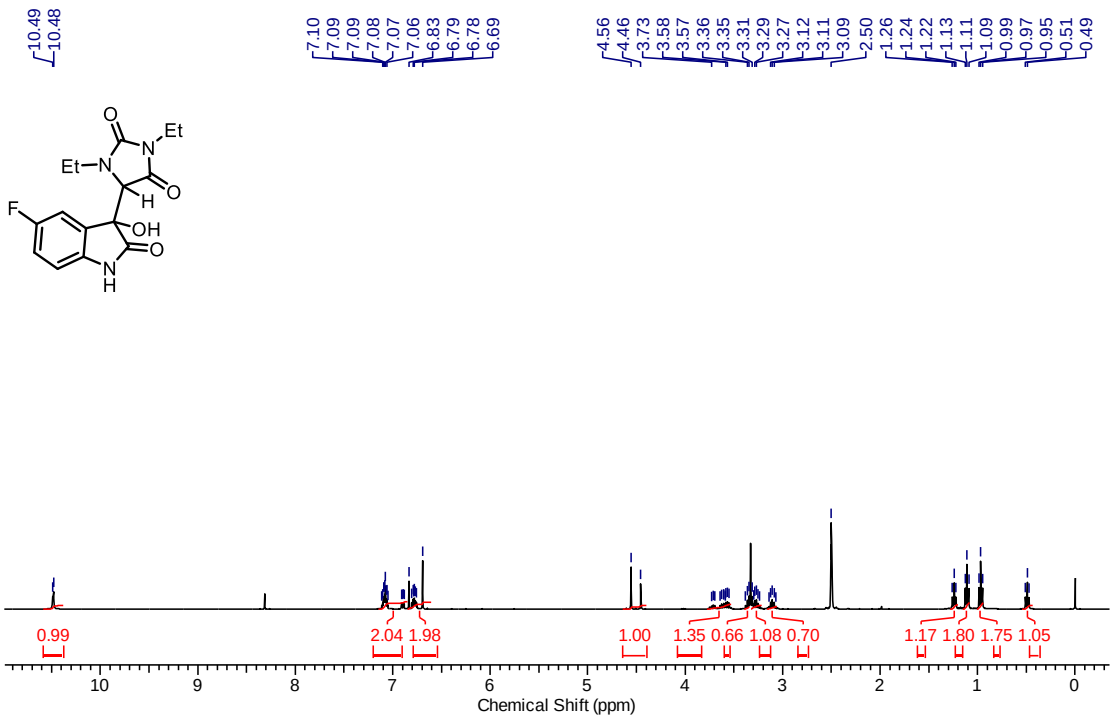
^{1}H NMR of Compound 25h in DMSO-d_6 at 400 MHz



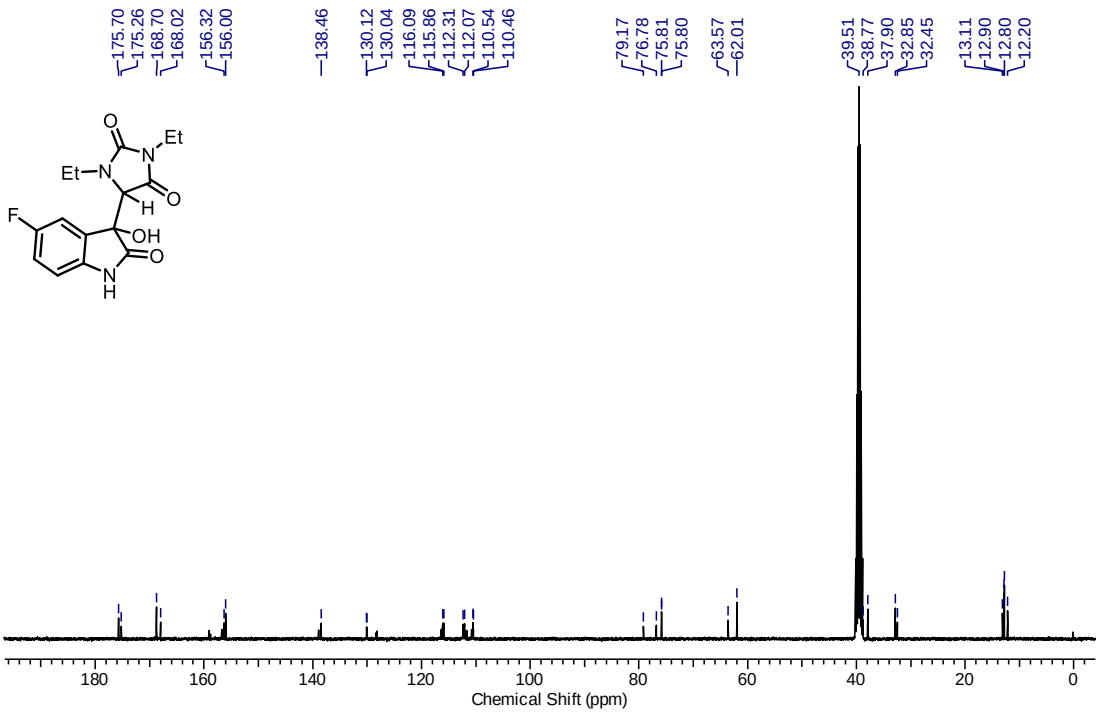
^{13}C NMR of Compound 25h in DMSO-d_6 at 100 MHz



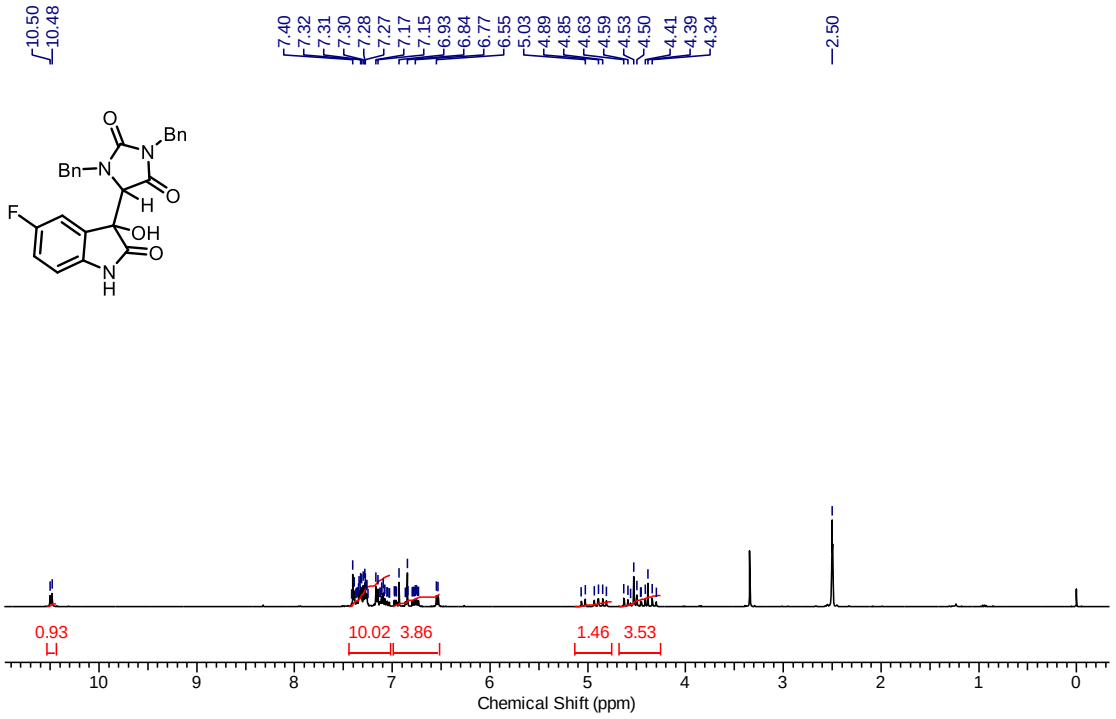
^{1}H NMR of Compound 25i in DMSO-d_6 at 400 MHz



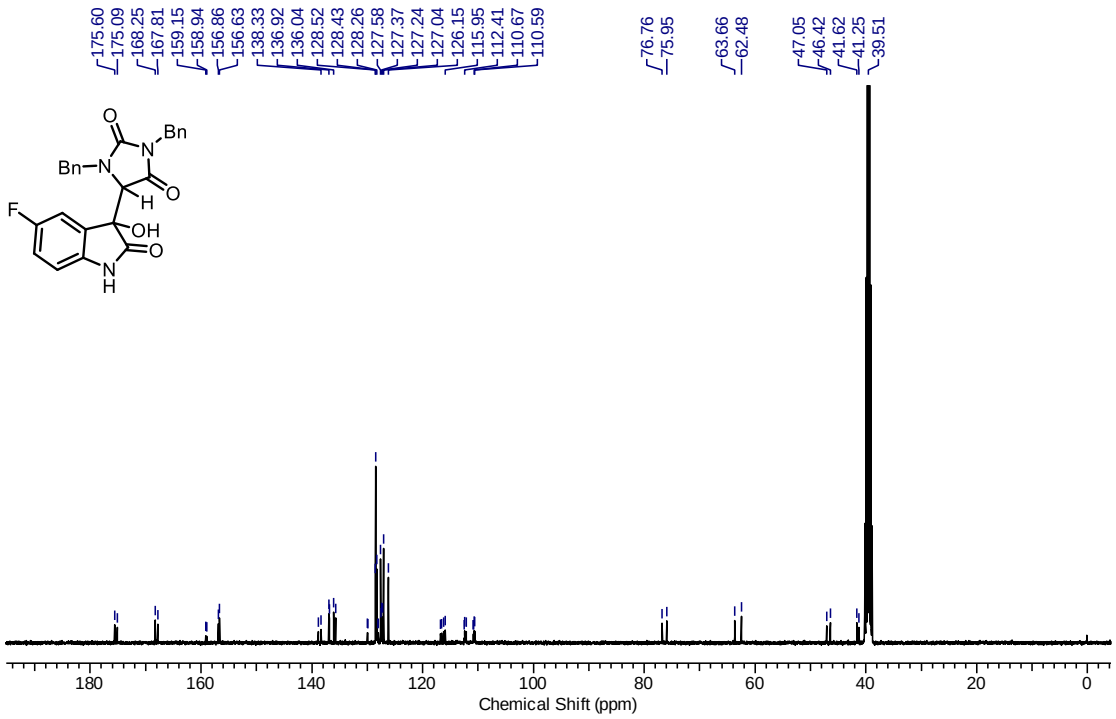
^{13}C NMR of Compound 25i in DMSO-d_6 at 100 MHz



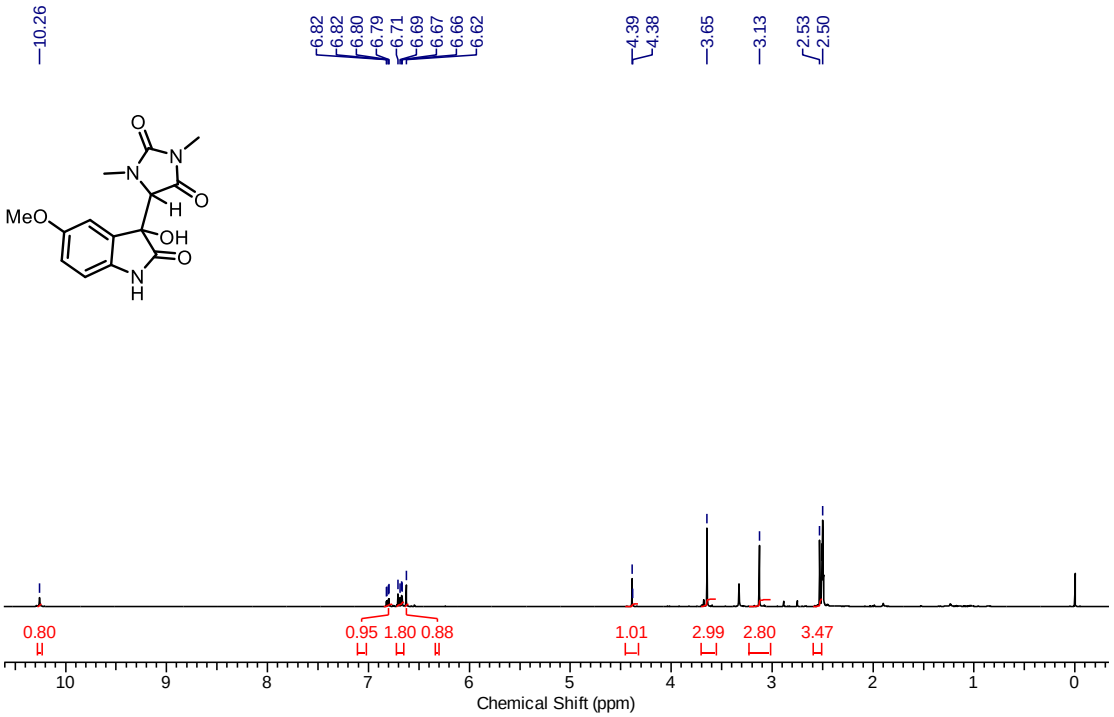
^{1}H NMR of Compound 25j in DMSO-d_6 at 400 MHz



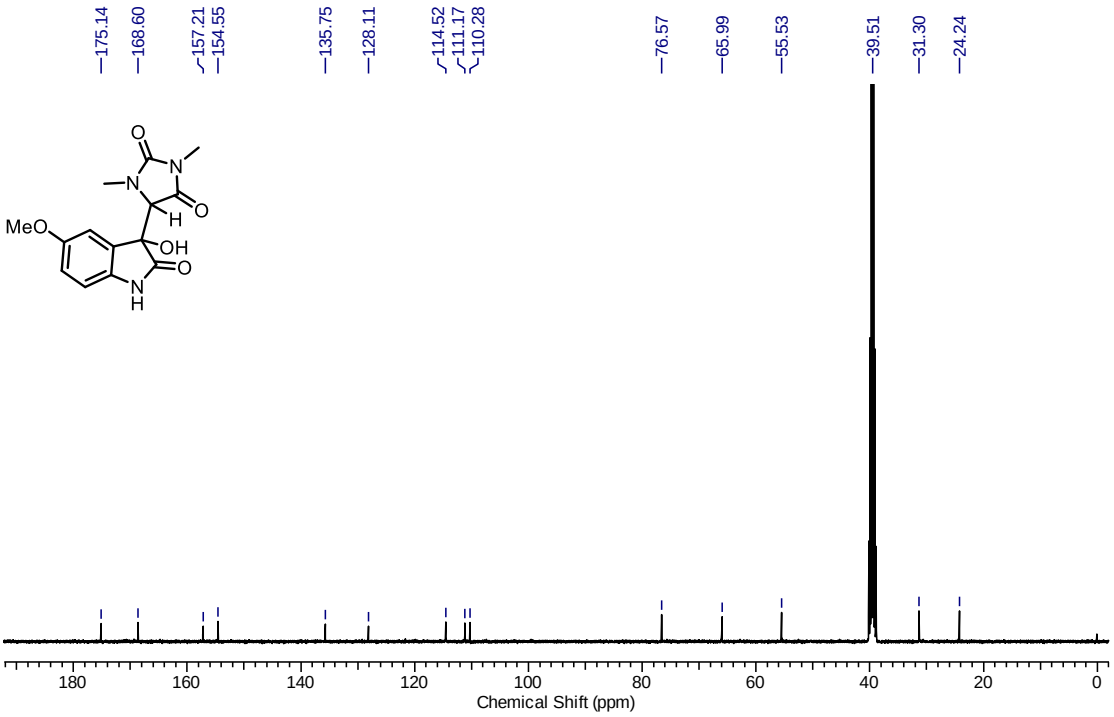
^{13}C NMR of Compound 25j in DMSO-d_6 at 100 MHz



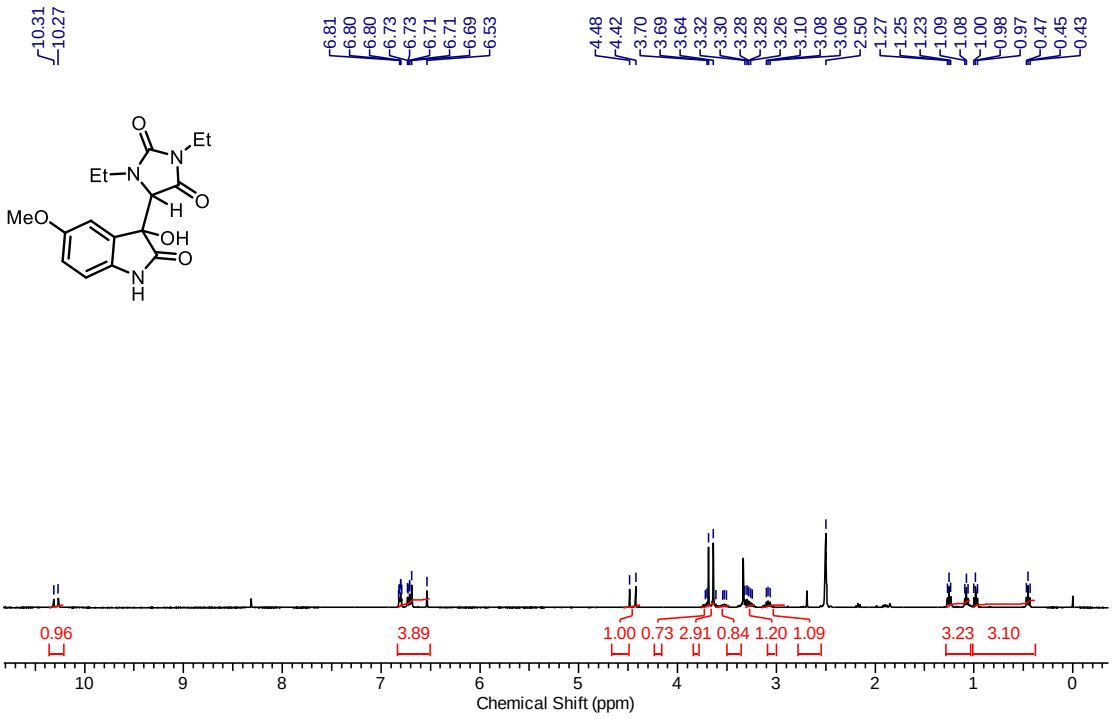
^{1}H NMR of Compound 25k in DMSO-d_6 at 400 MHz



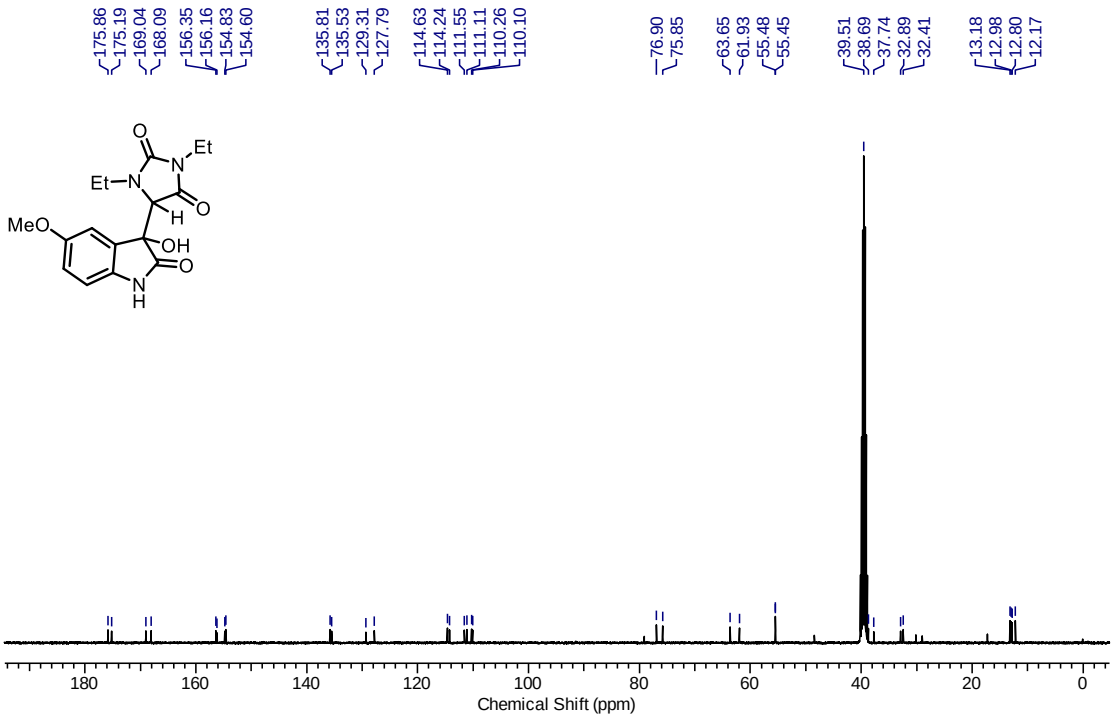
^{13}C NMR of Compound 25k in DMSO-d_6 at 100 MHz



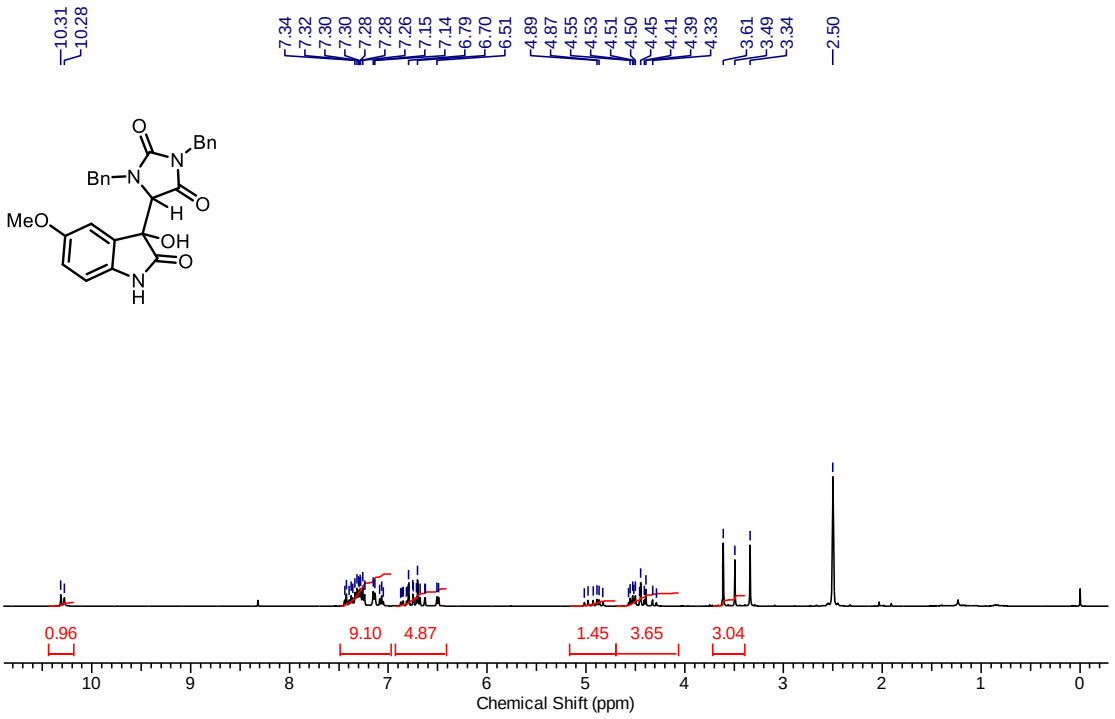
^{1}H NMR of Compound 25l in DMSO-d_6 at 400 MHz



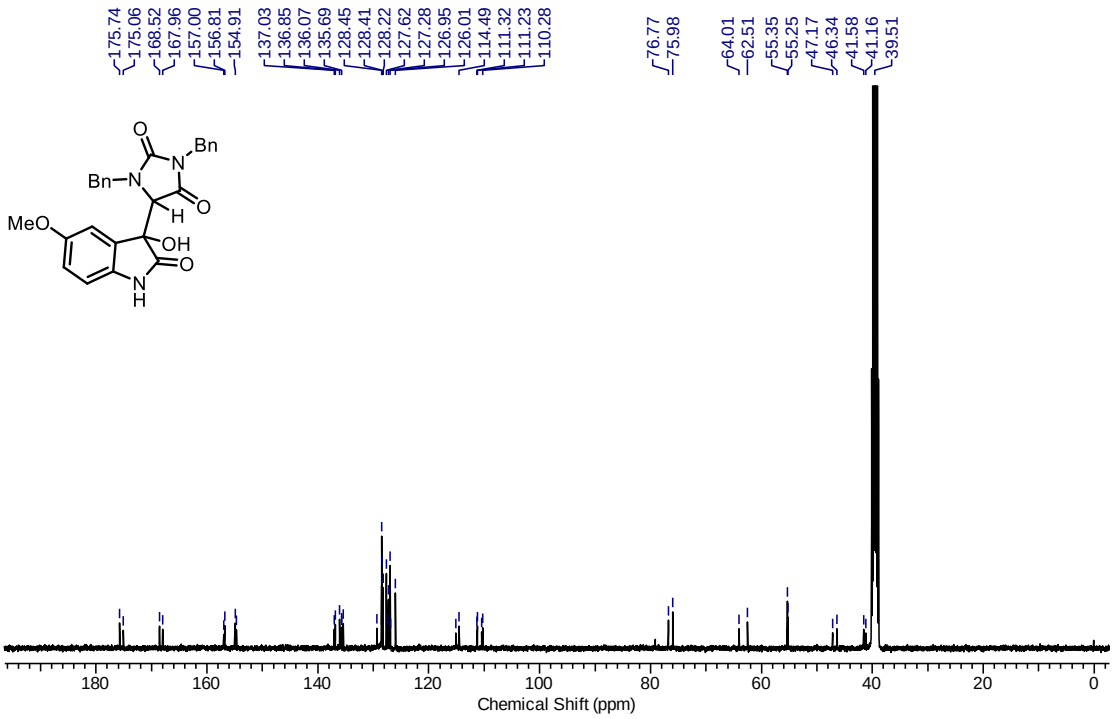
^{13}C NMR of Compound 25l in DMSO-d_6 at 100 MHz



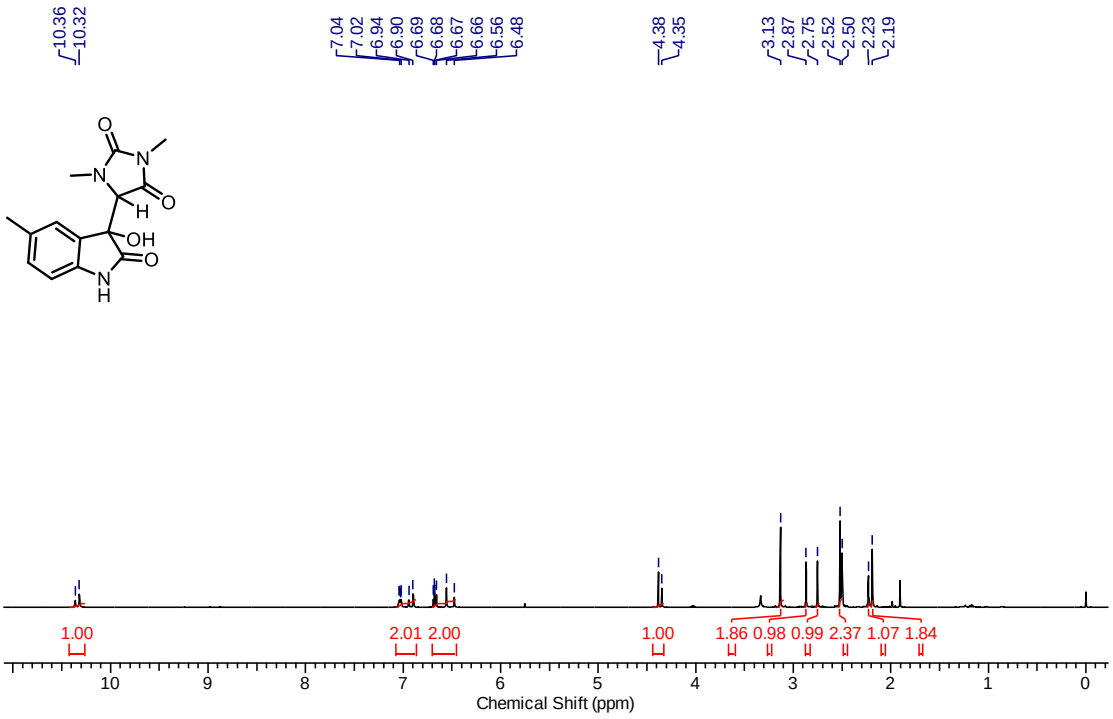
^{1}H NMR of Compound 25m in DMSO-d_6 at 400 MHz



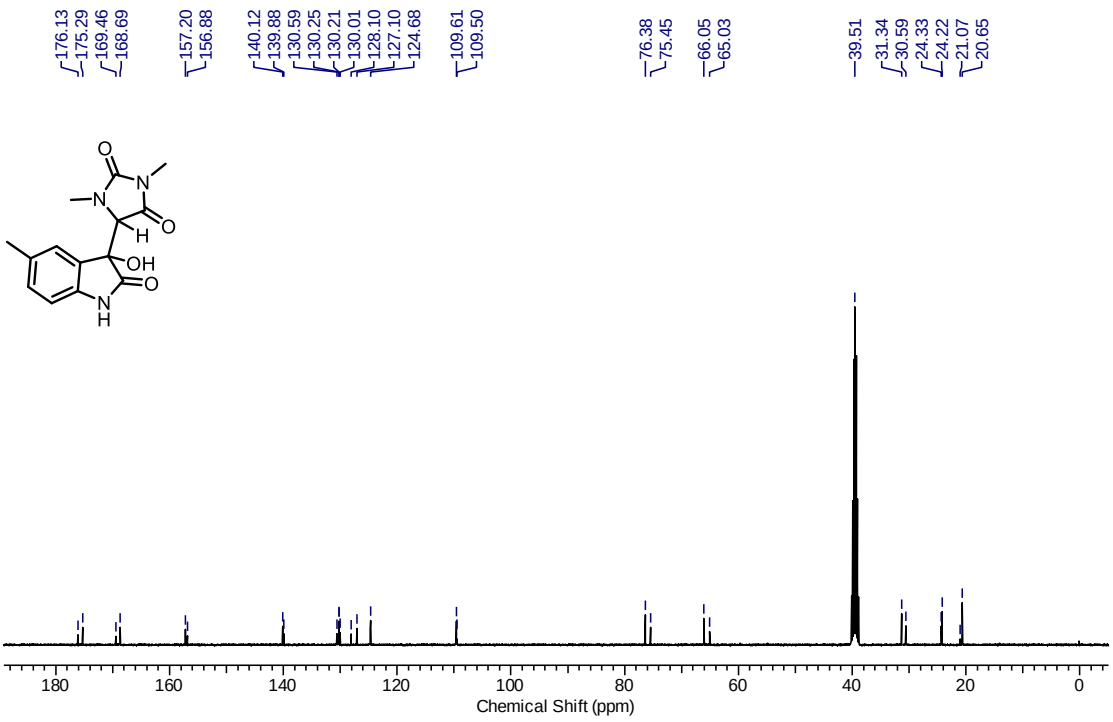
^{13}C NMR of Compound 25m in DMSO-d_6 at 100 MHz



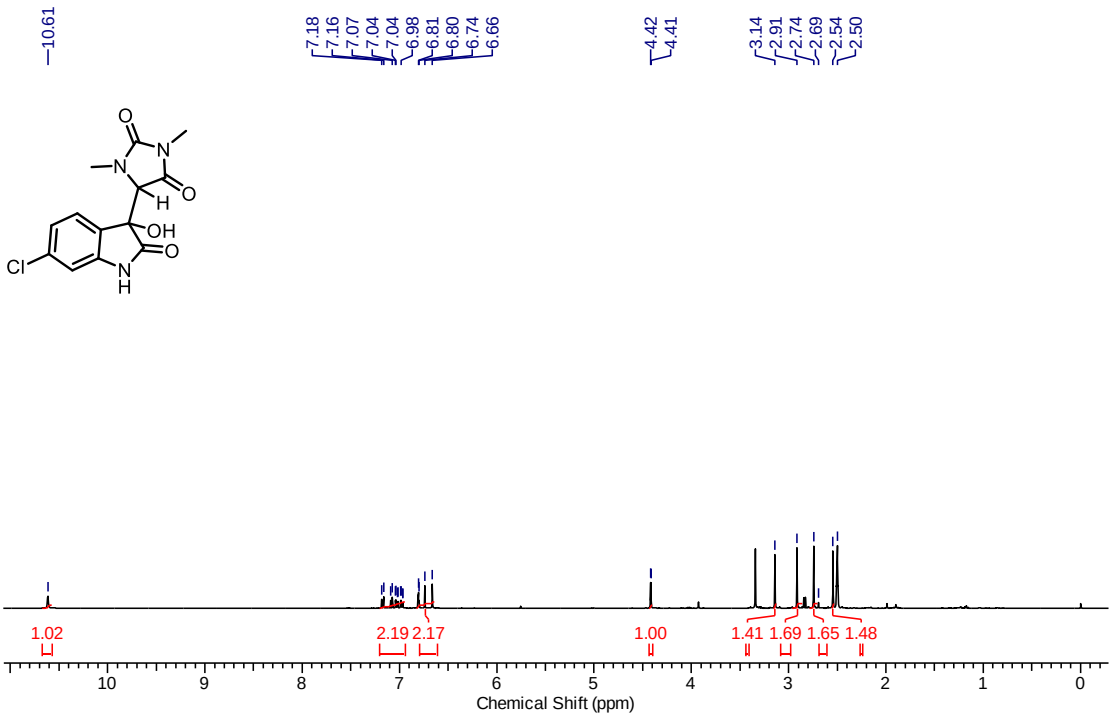
^{1}H NMR of Compound 25n in DMSO-d_6 at 400 MHz



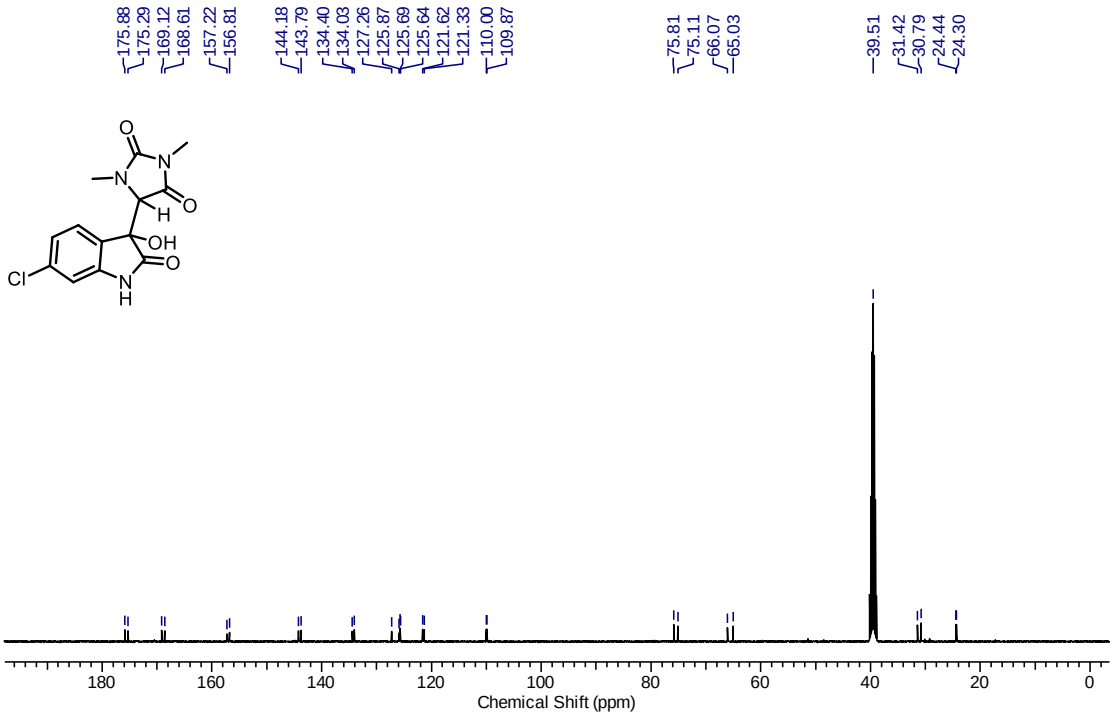
^{13}C NMR of Compound 25n in DMSO-d_6 at 100 MHz



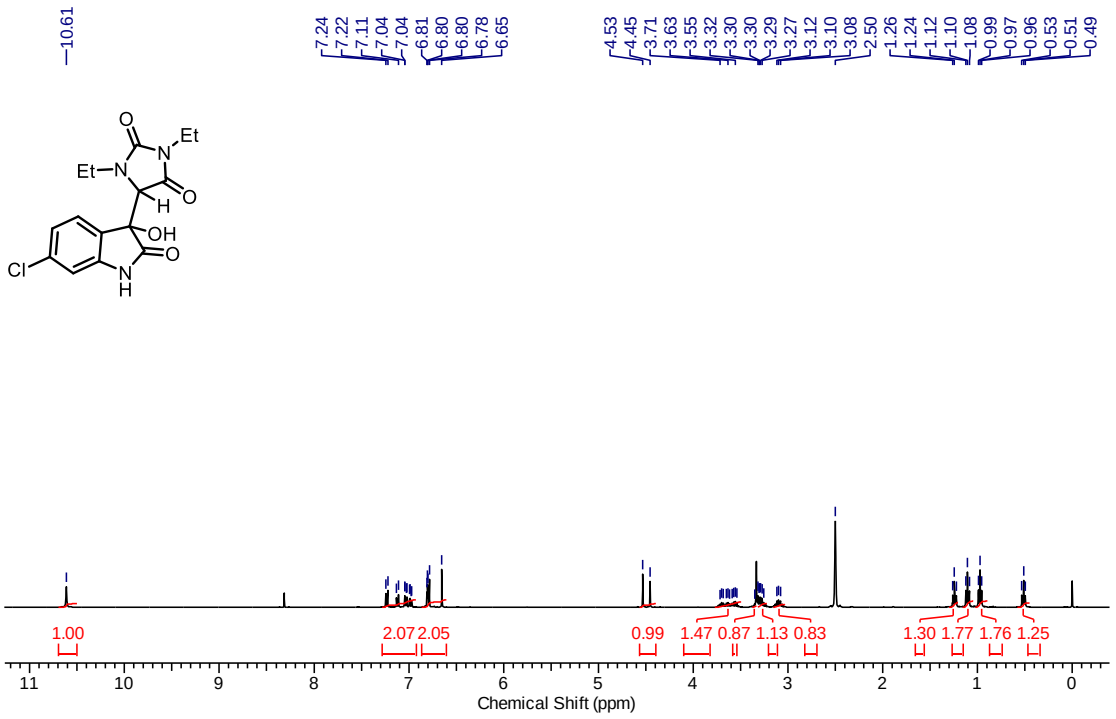
^{1}H NMR of Compound 25o in DMSO-d_6 at 400 MHz



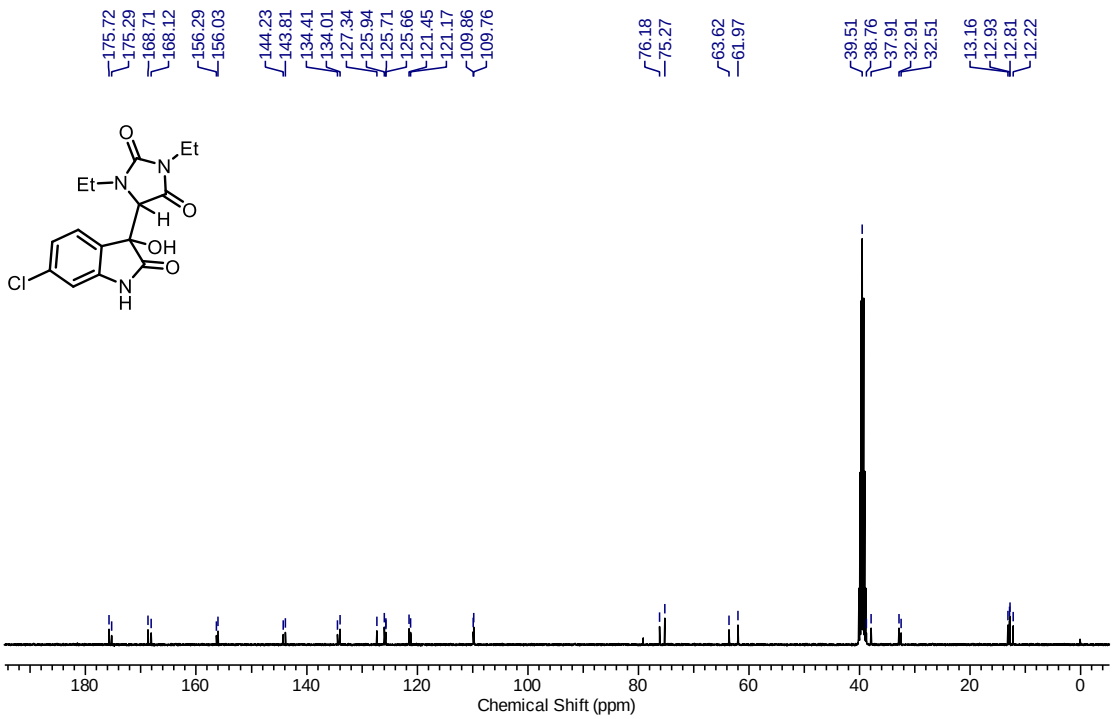
^{13}C NMR of Compound 25o in DMSO-d_6 at 100 MHz



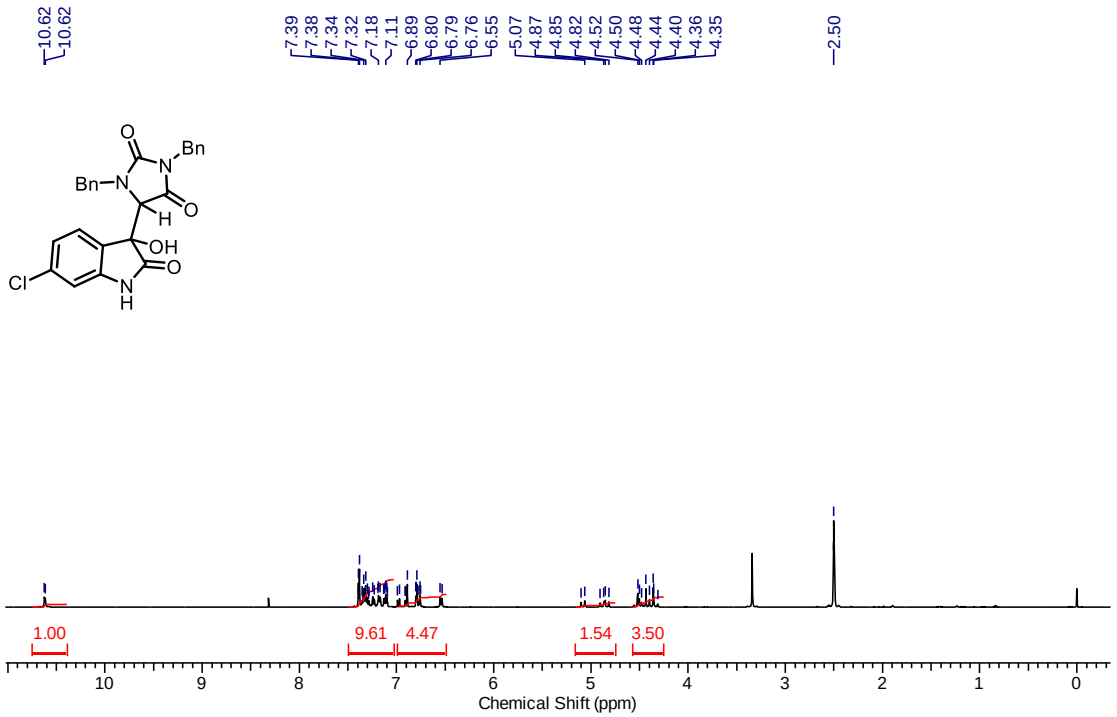
^{1}H NMR of Compound 25p in DMSO-d_6 at 400 MHz



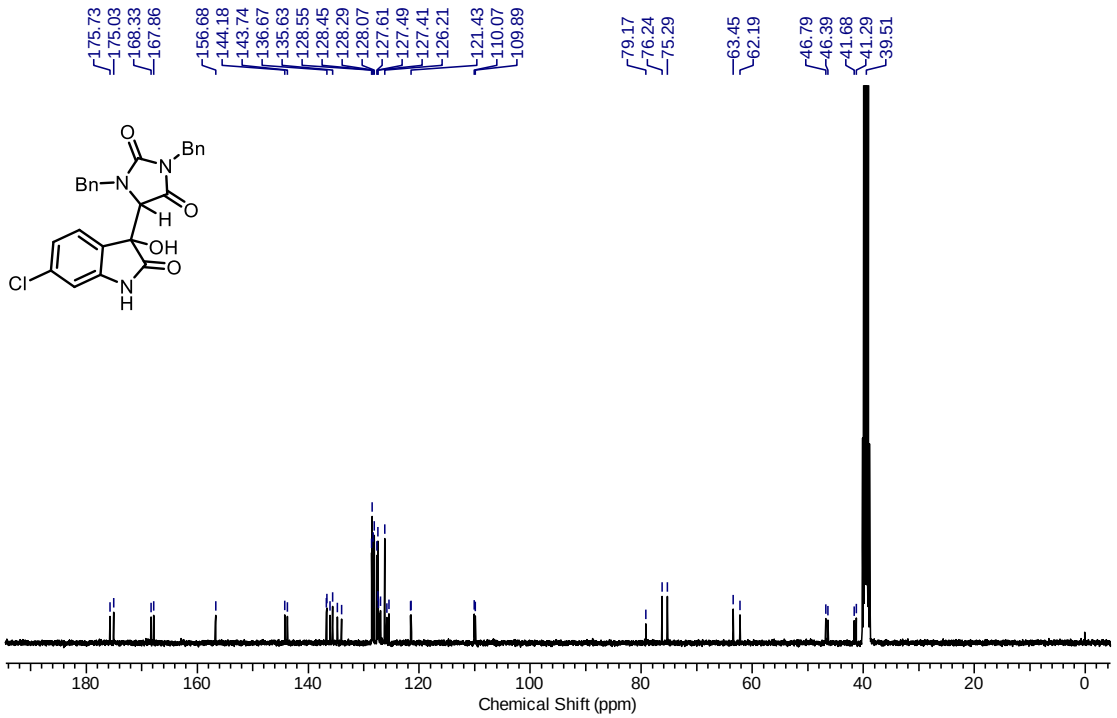
^{13}C NMR of Compound 25p in DMSO-d_6 at 100 MHz



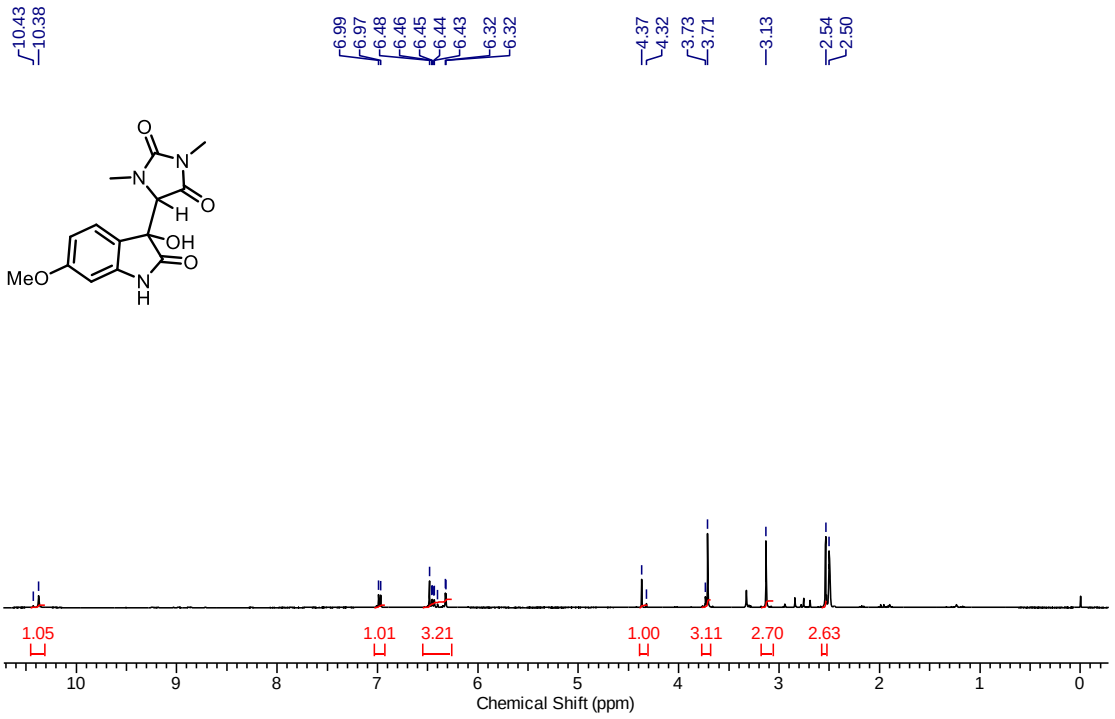
^{1}H NMR of Compound 25q in DMSO-d_6 at 400 MHz



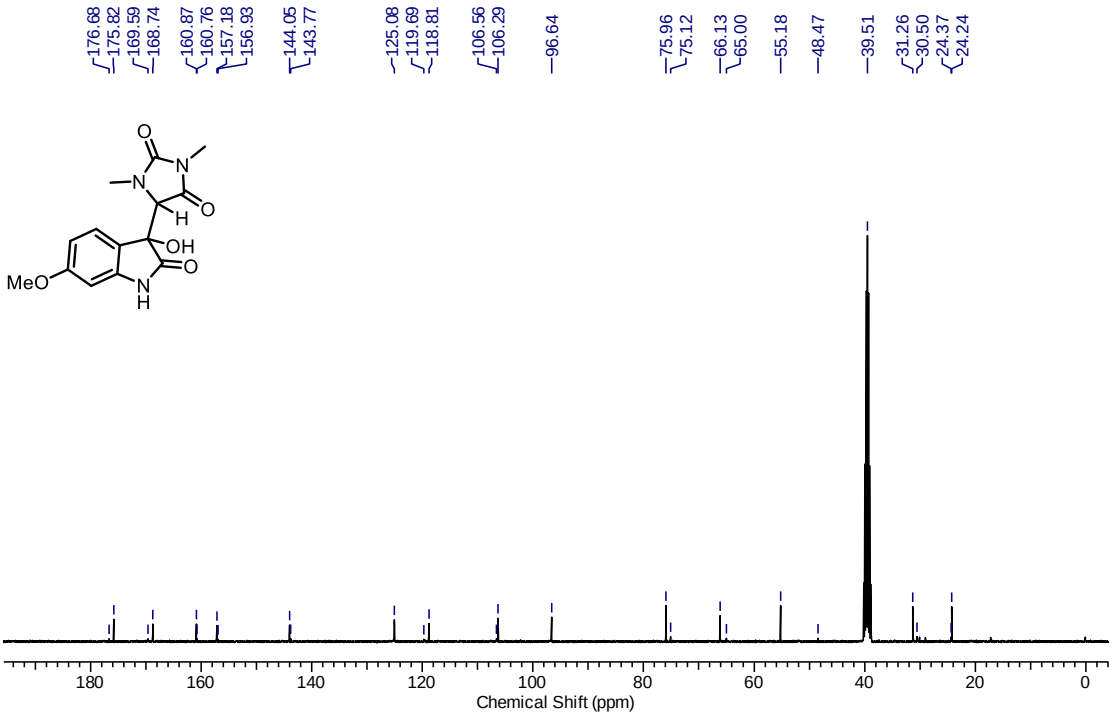
^{13}C NMR of Compound 25q in DMSO-d_6 at 100 MHz



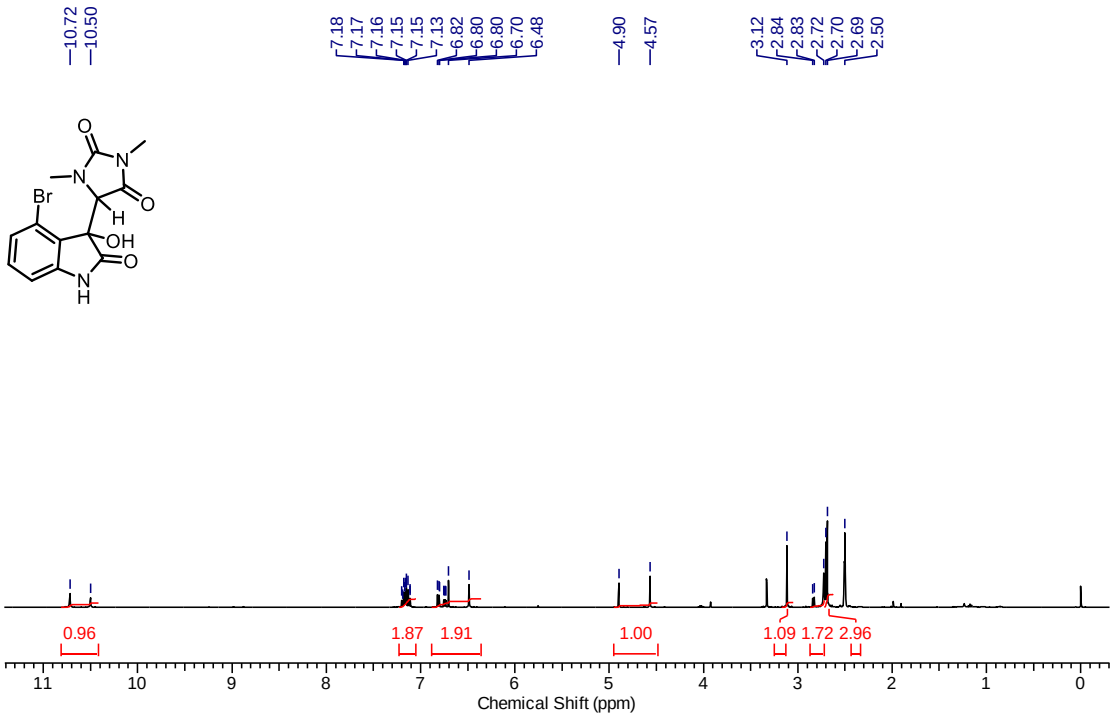
^{1}H NMR of Compound 25r in DMSO-d_6 at 400 MHz



^{13}C NMR of Compound 25r in DMSO-d_6 at 100 MHz



^{1}H NMR of Compound 25s in DMSO-d_6 at 400 MHz



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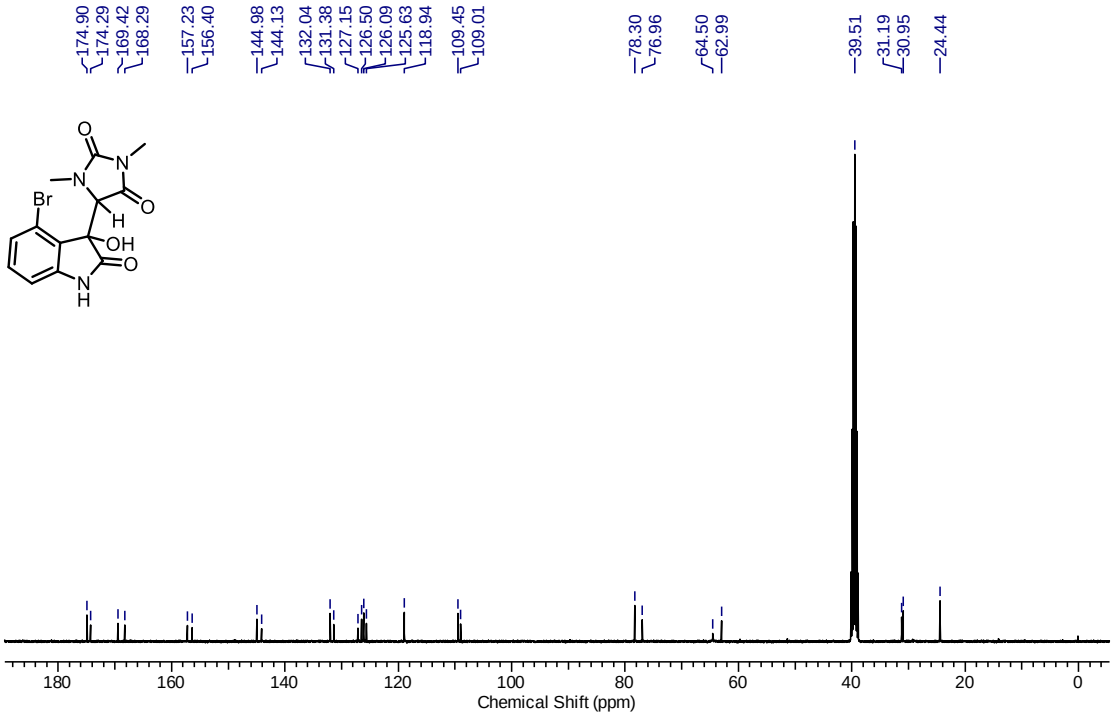
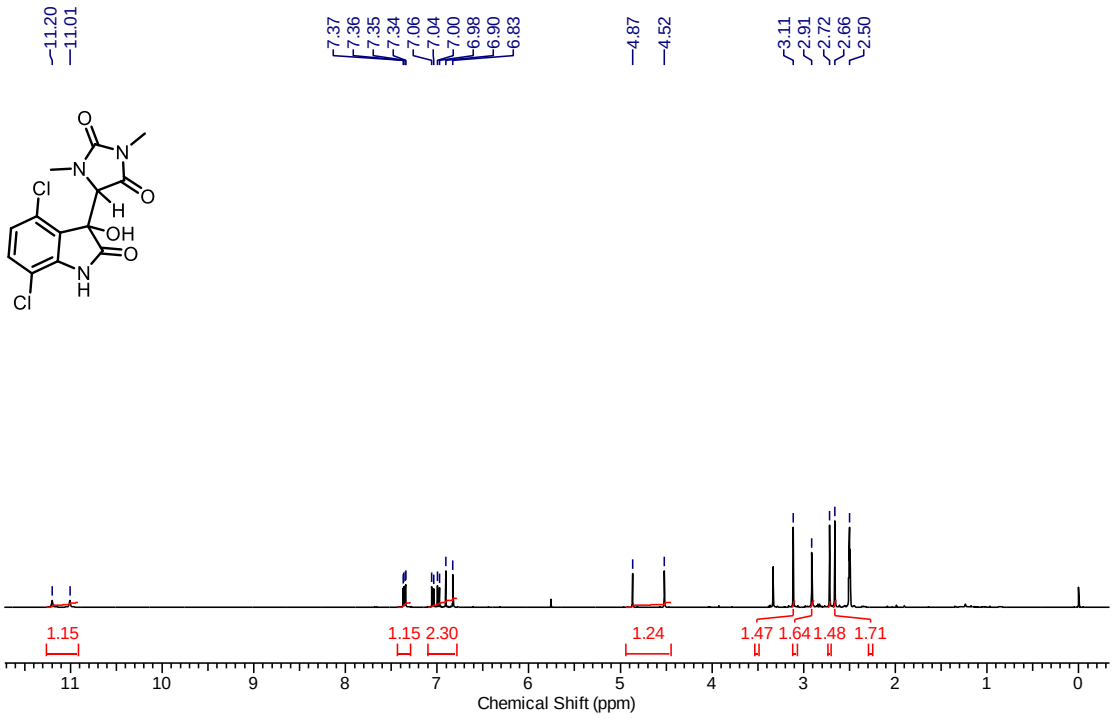
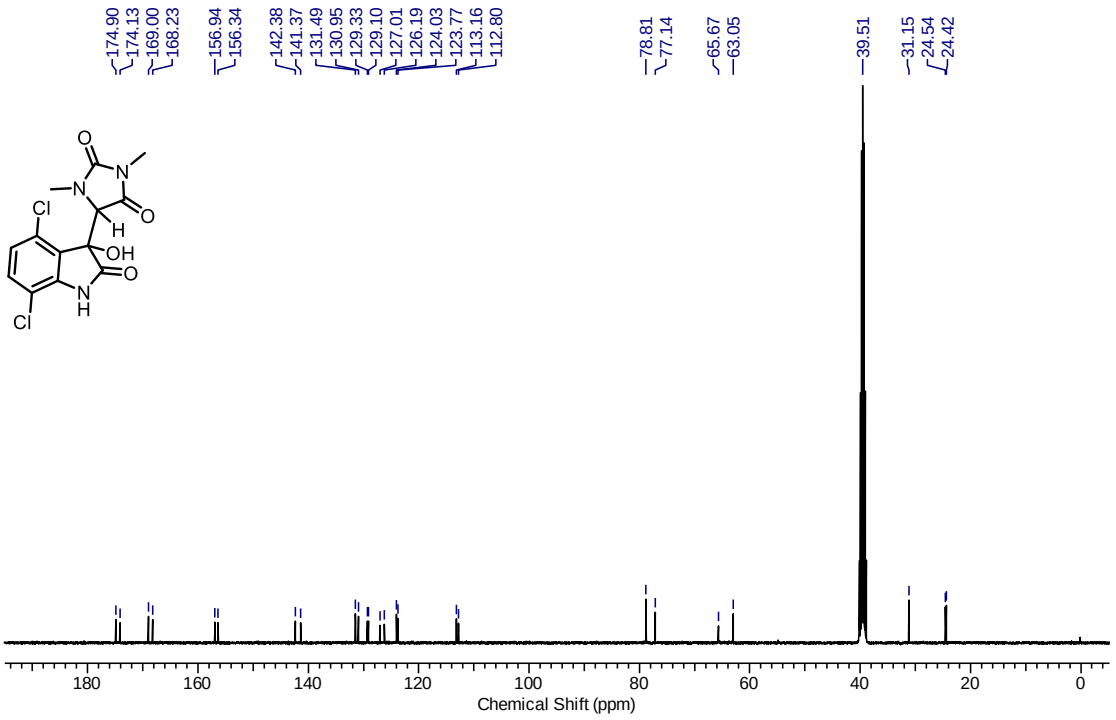


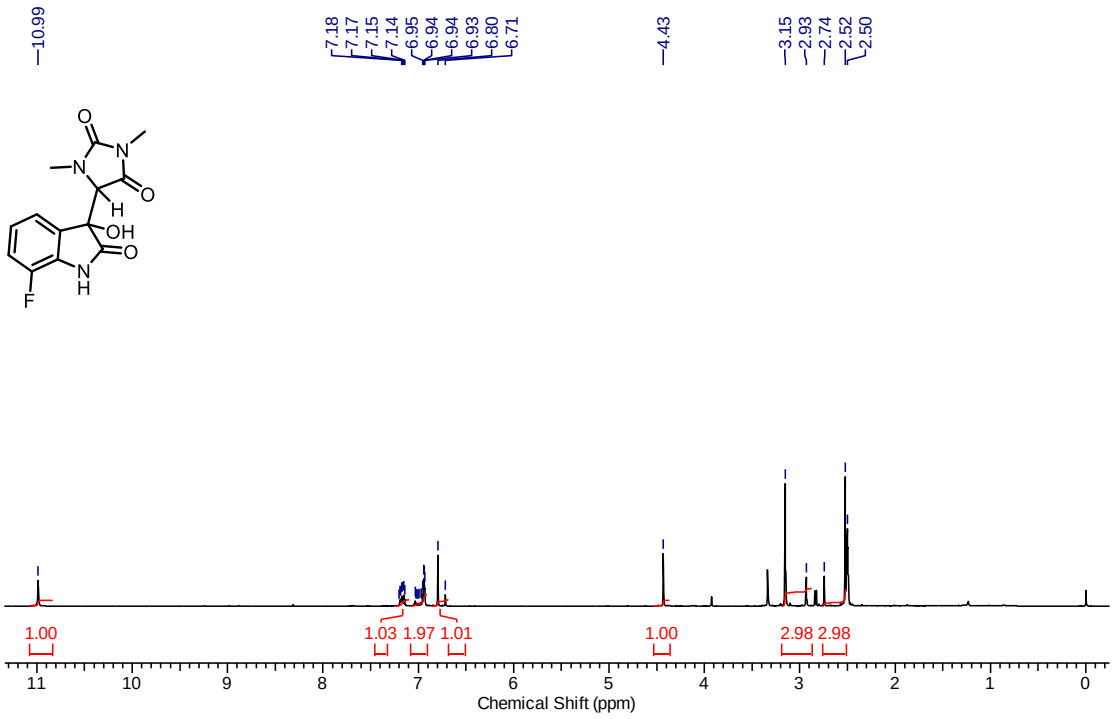
^{1}H NMR of Compound 25t in DMSO-d_6 at 400 MHz



^{13}C NMR of Compound 25t in DMSO-d_6 at 100 MHz



^{1}H NMR of Compound 25u in DMSO-d_6 at 400 MHz



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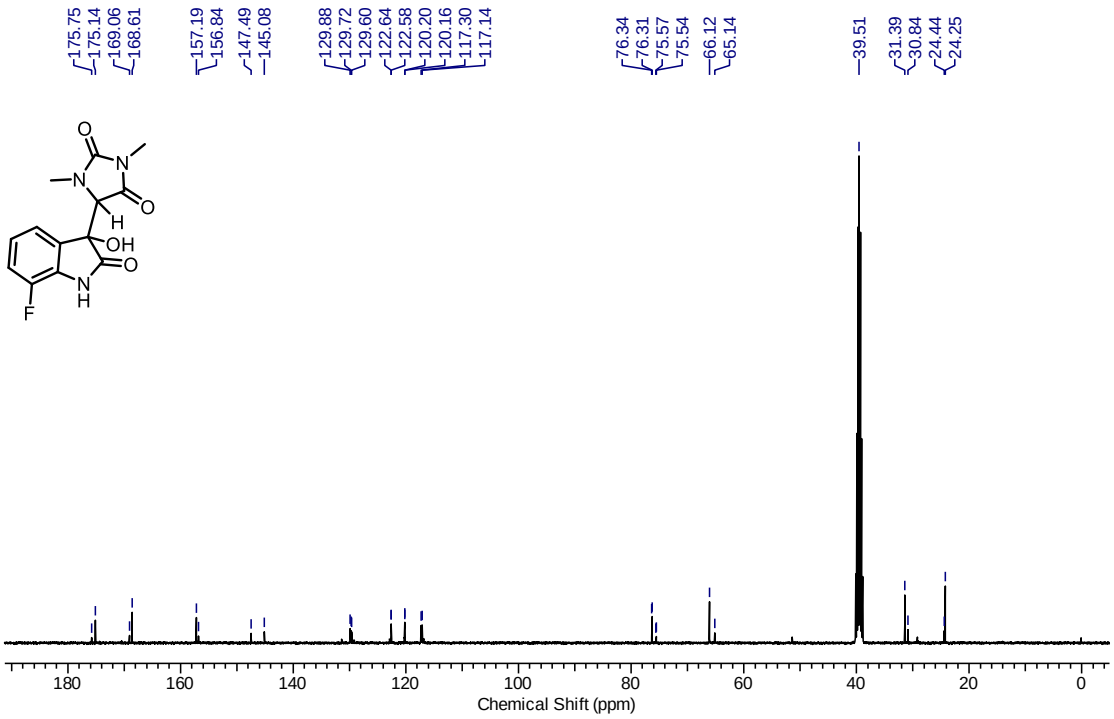

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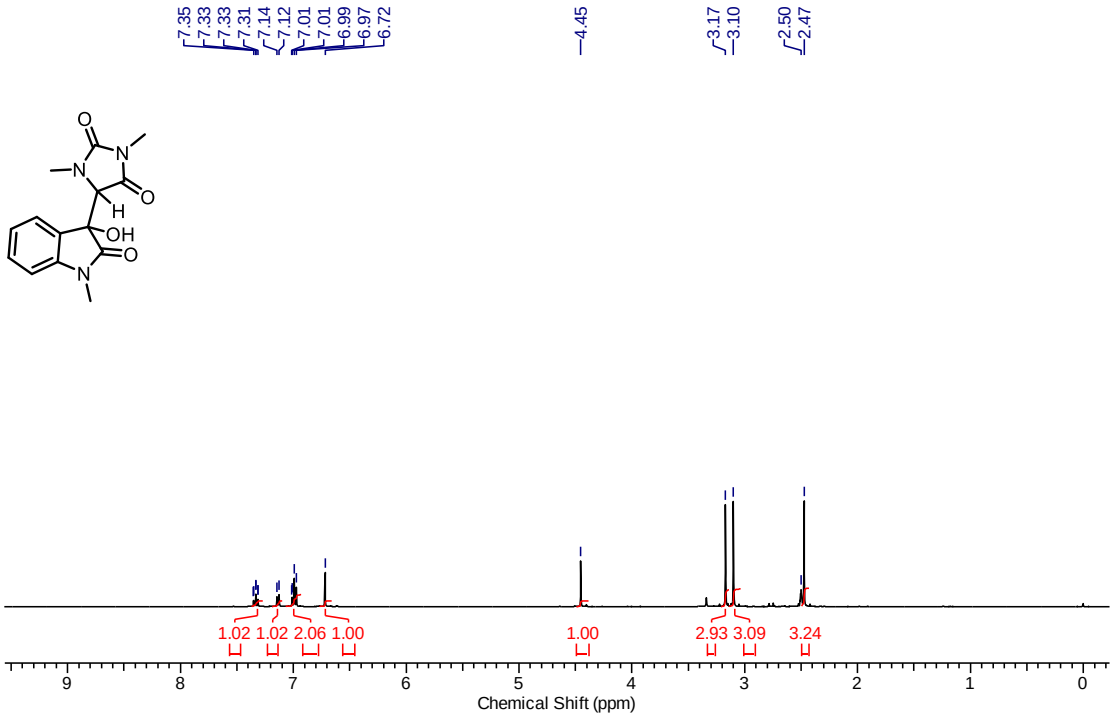

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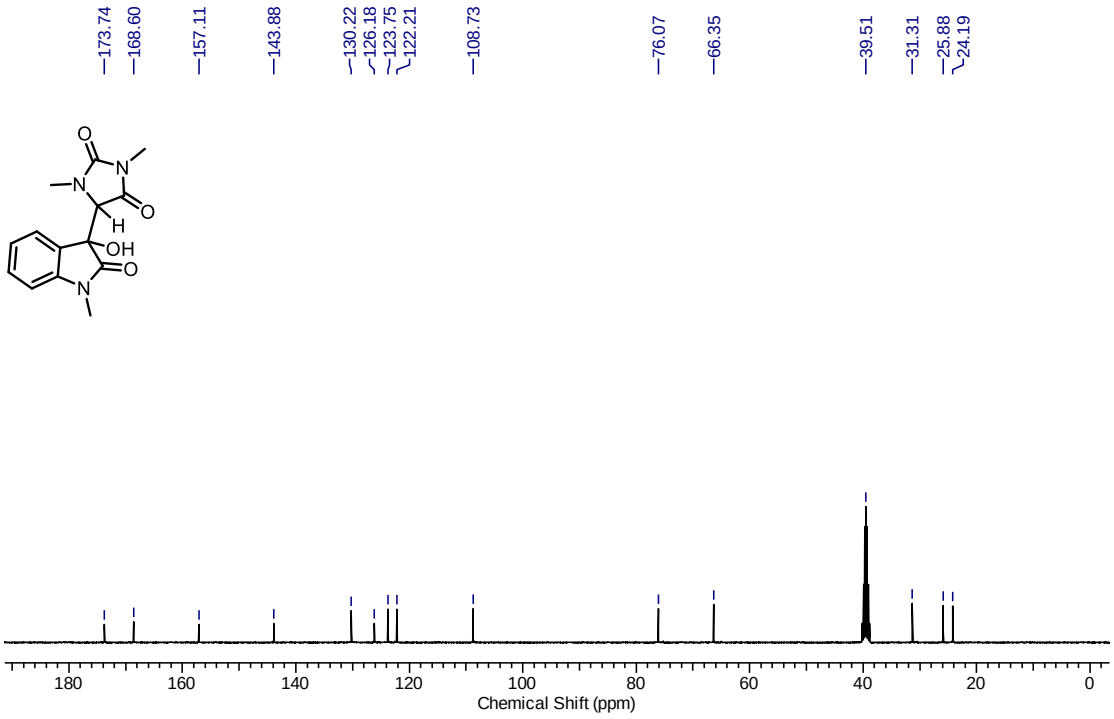

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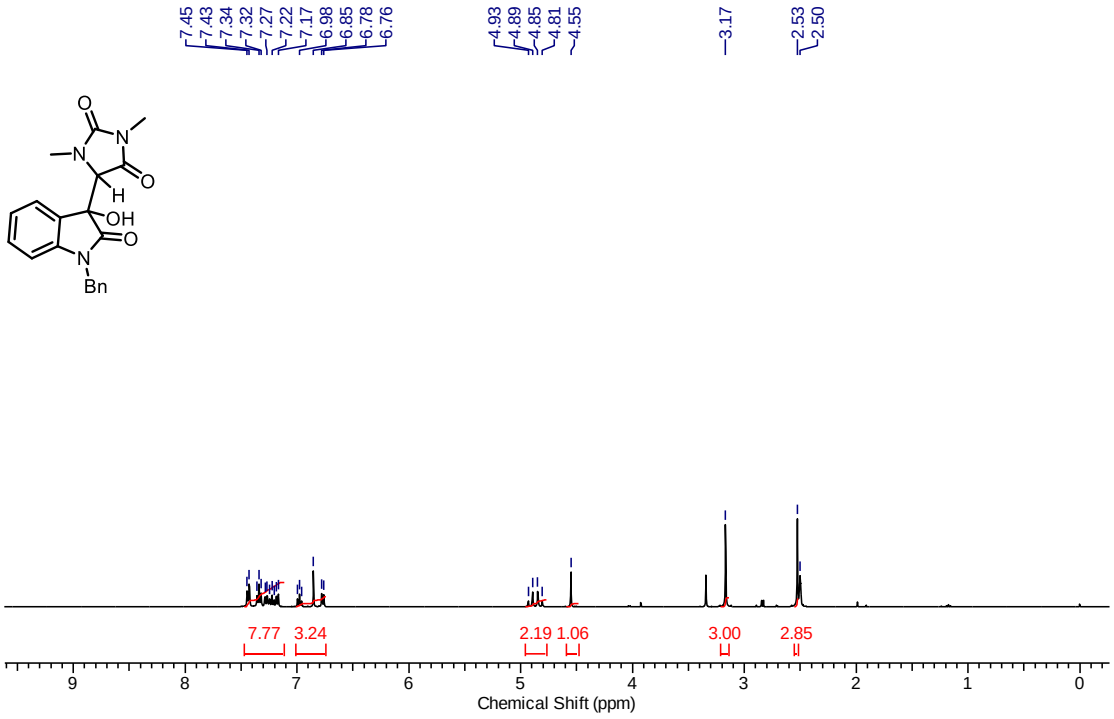

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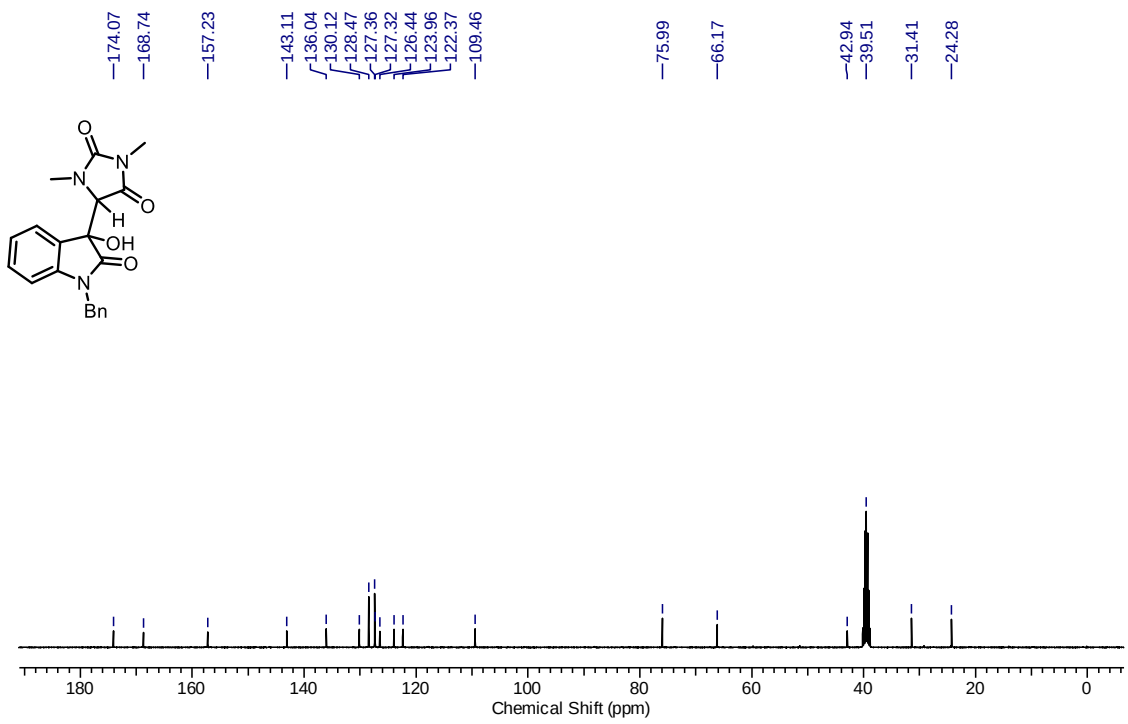

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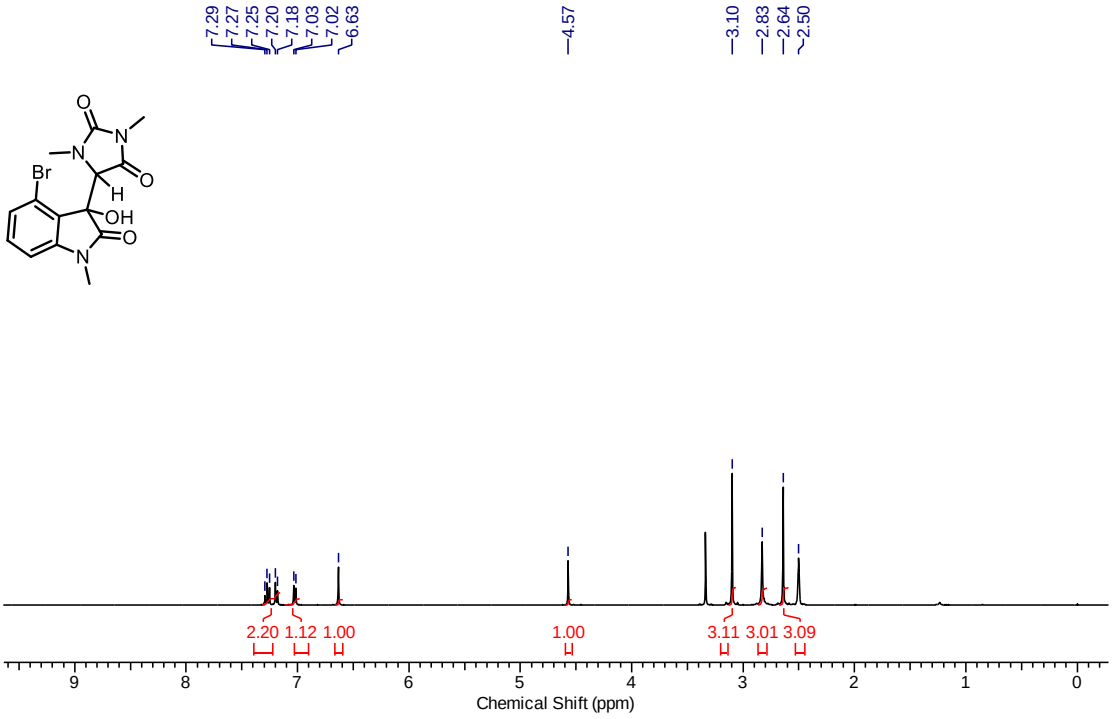

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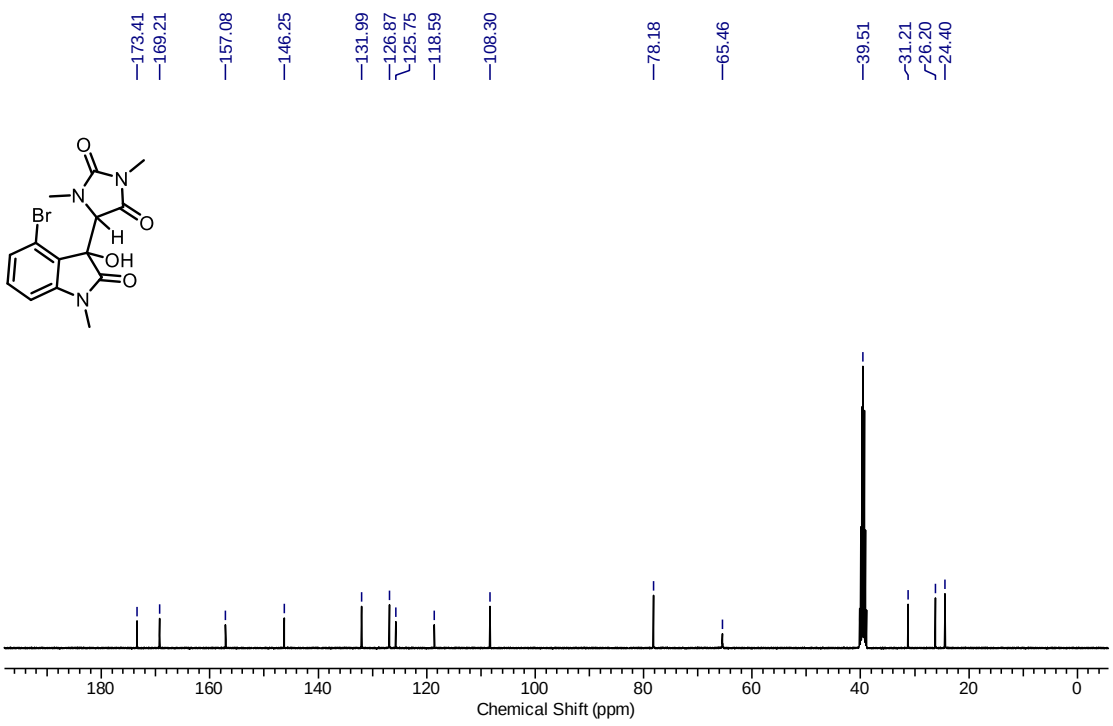

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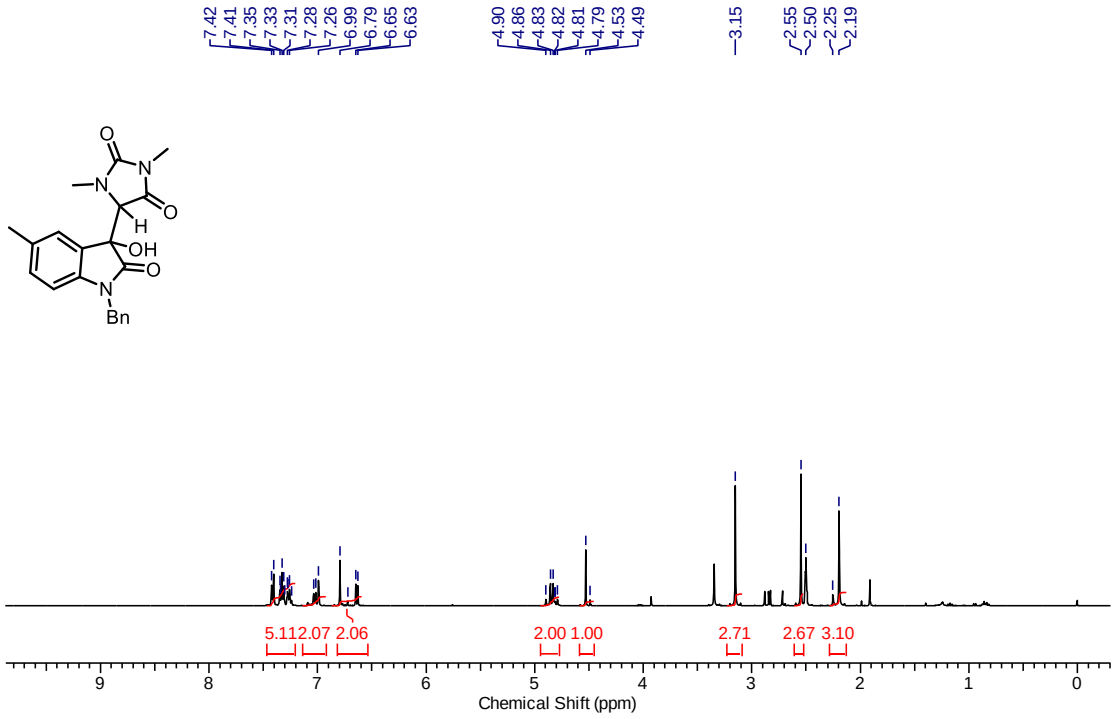
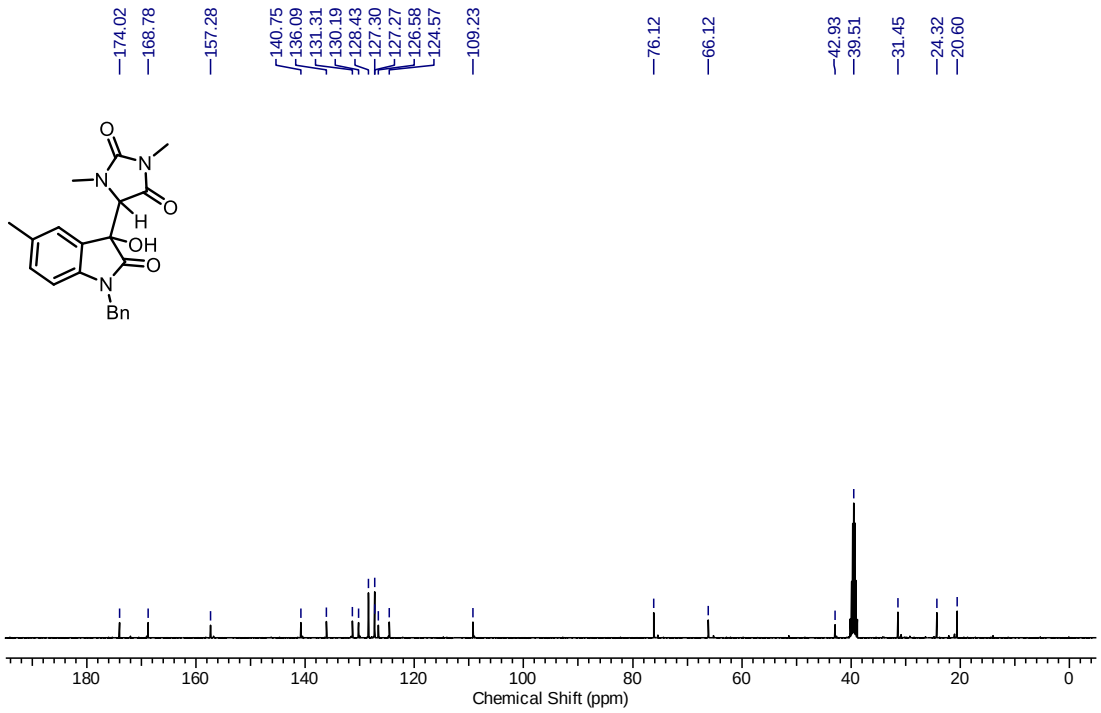


^{13}C NMR of Compound 25y in DMSO-d_6 at 100 MHz



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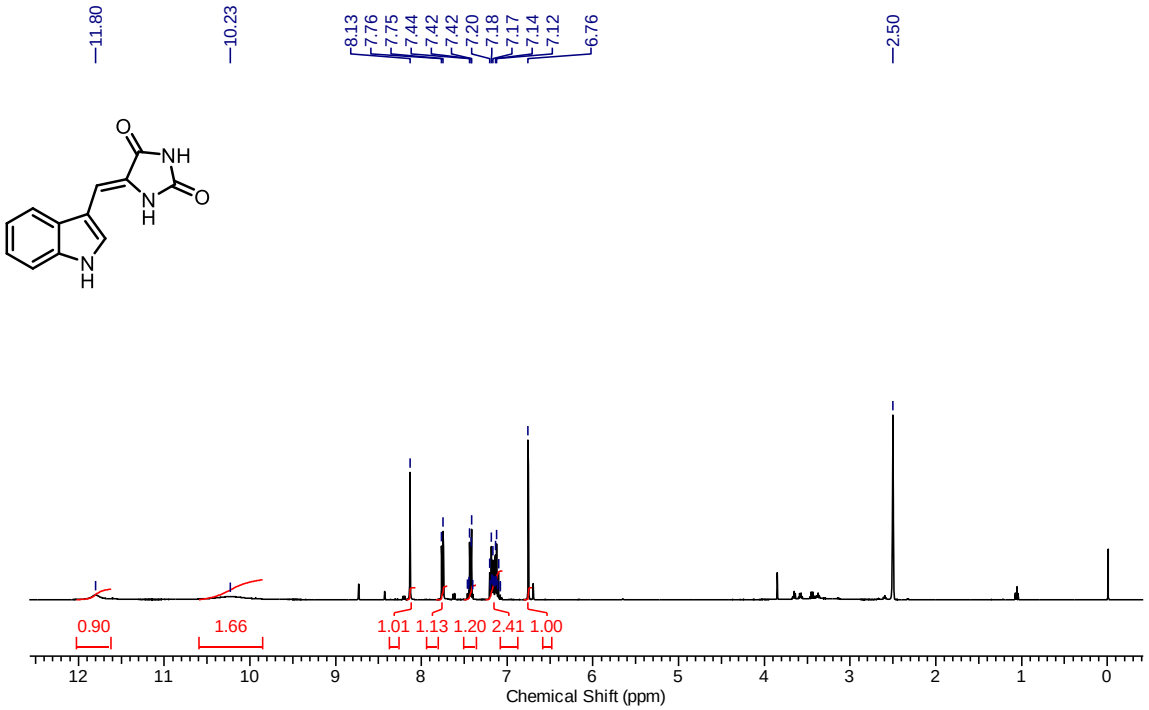

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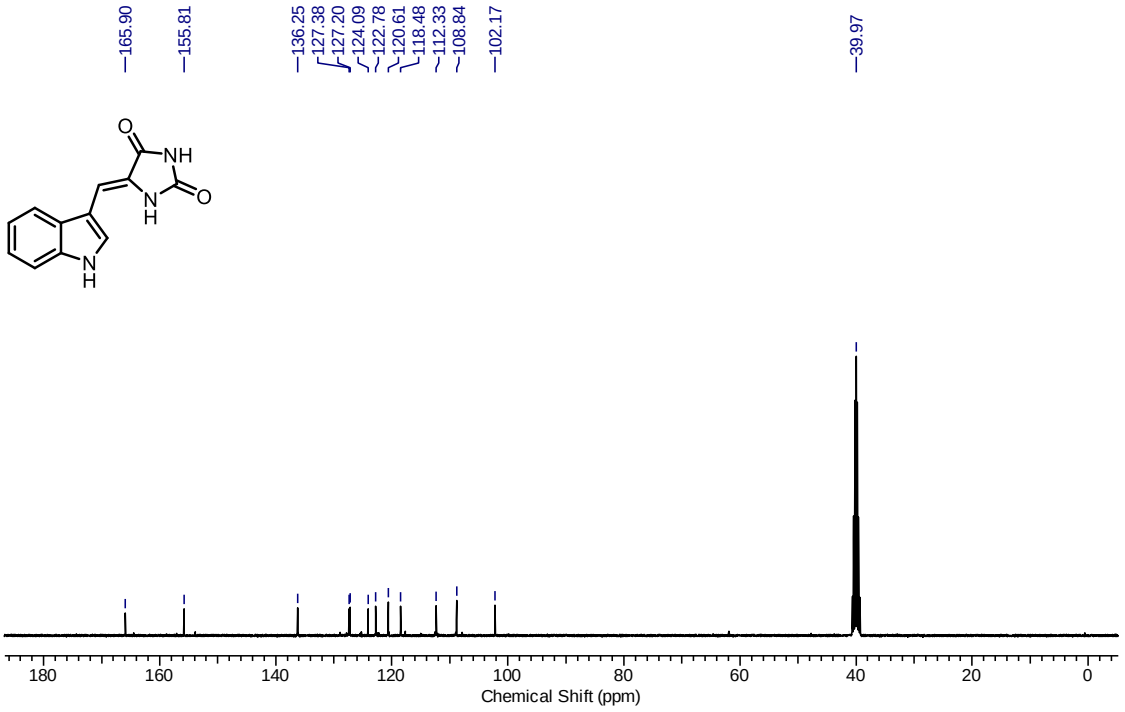

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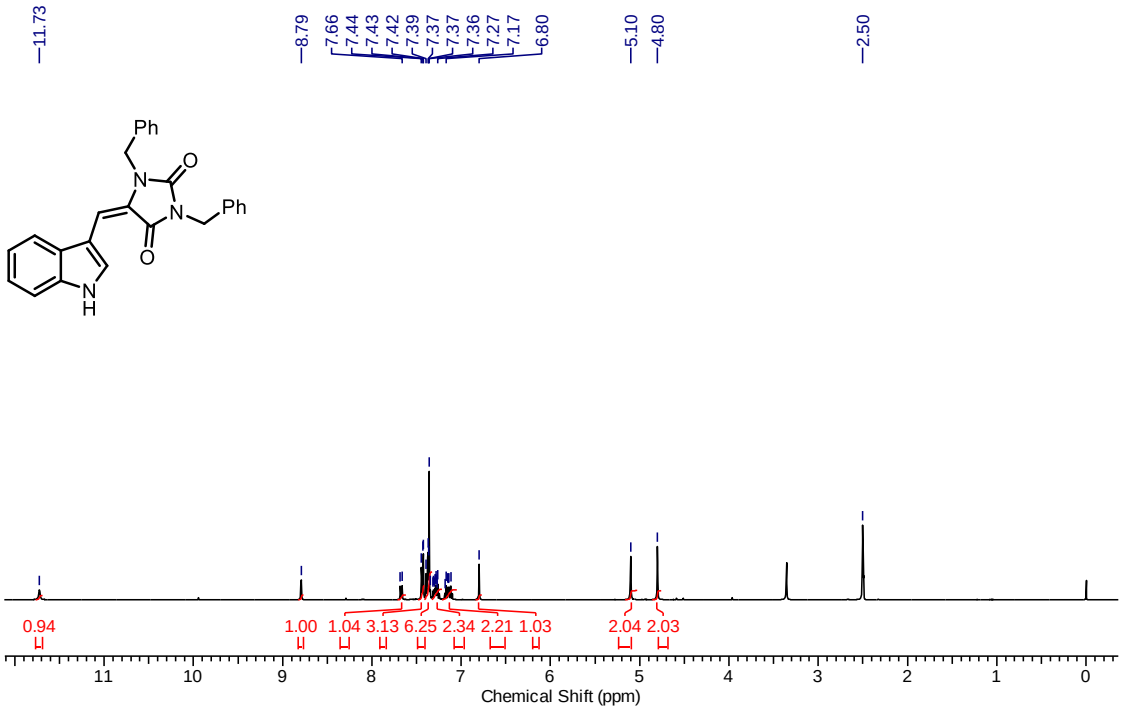
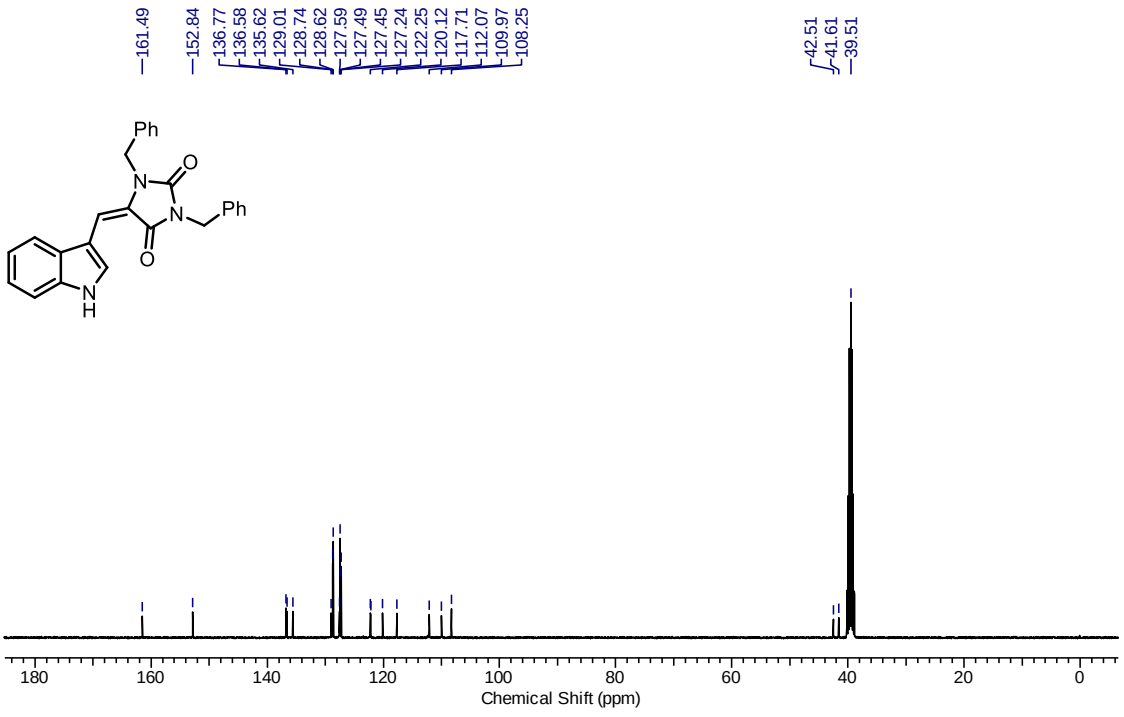
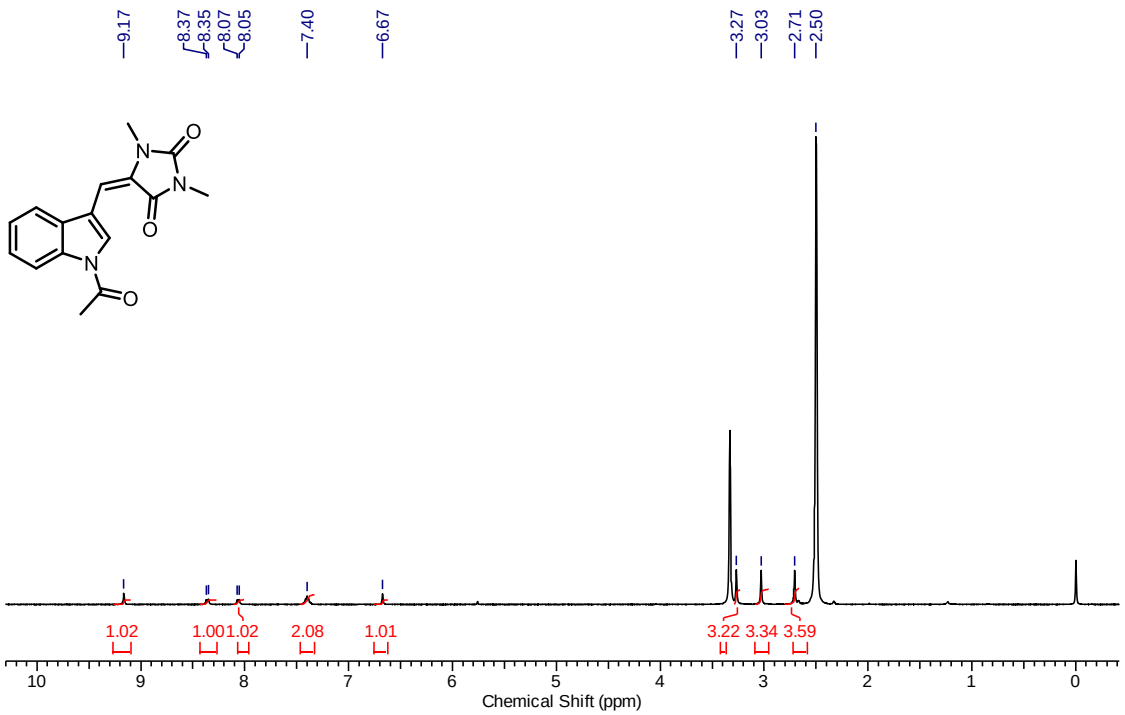


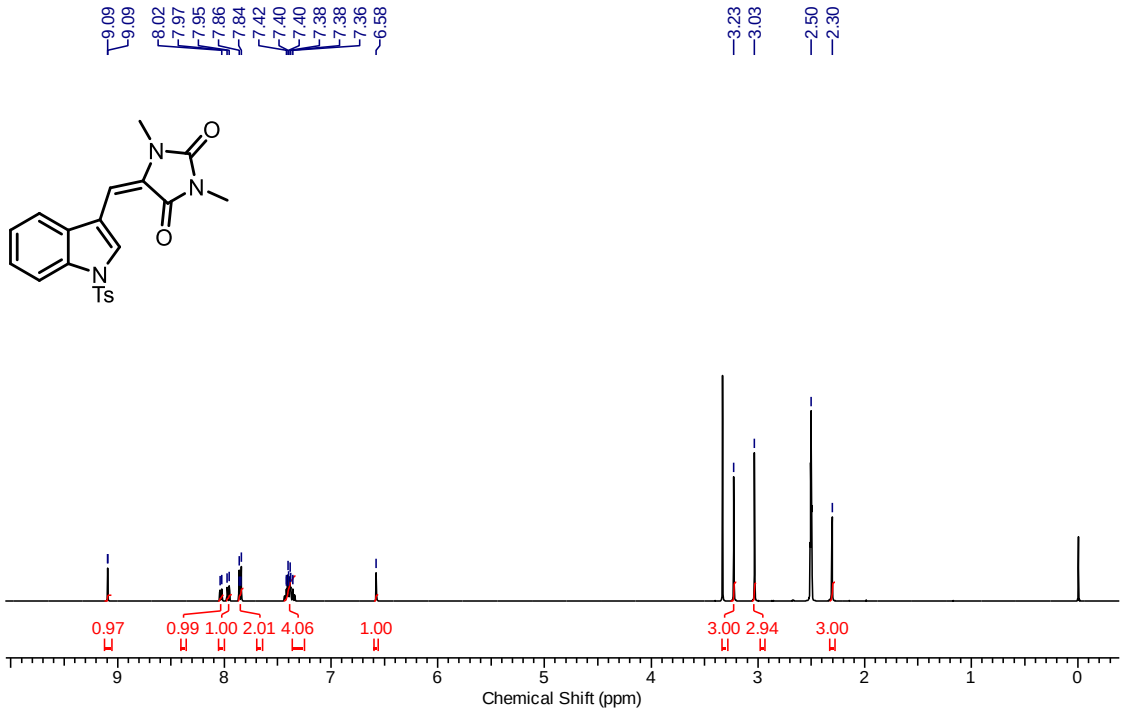
^{13}C NMR of Compound 68 in DMSO-d_6 at 100 MHz



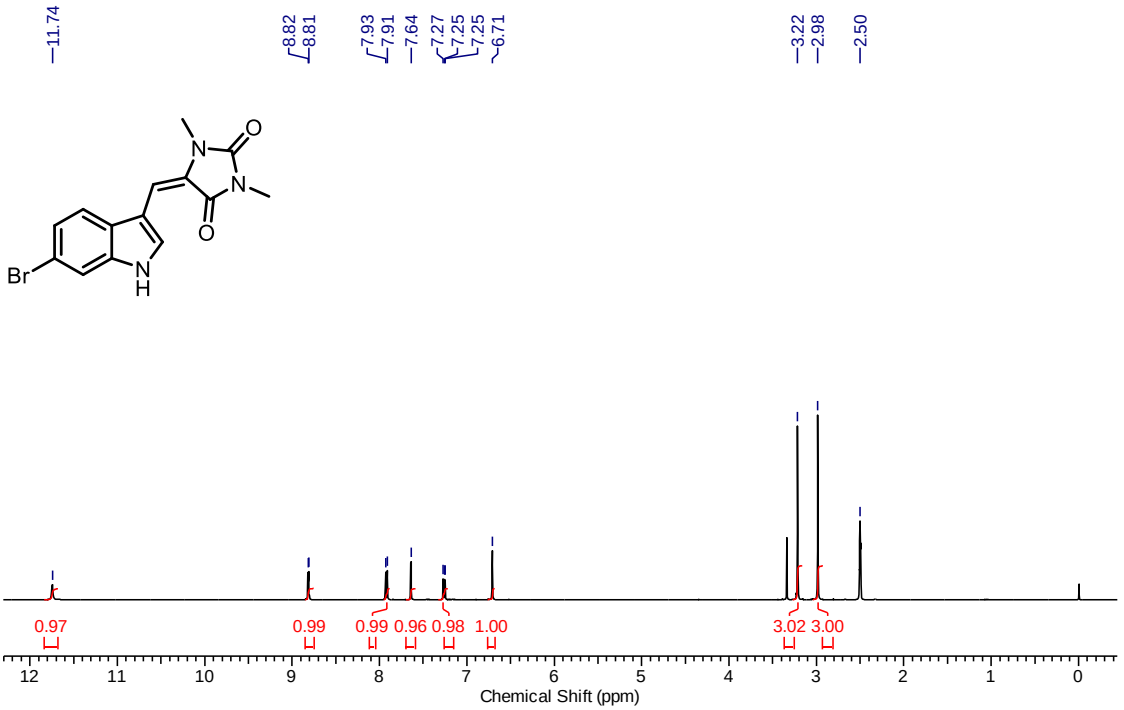
^{1}H NMR of Compound 69 in DMSO-d_6 at 400 MHz



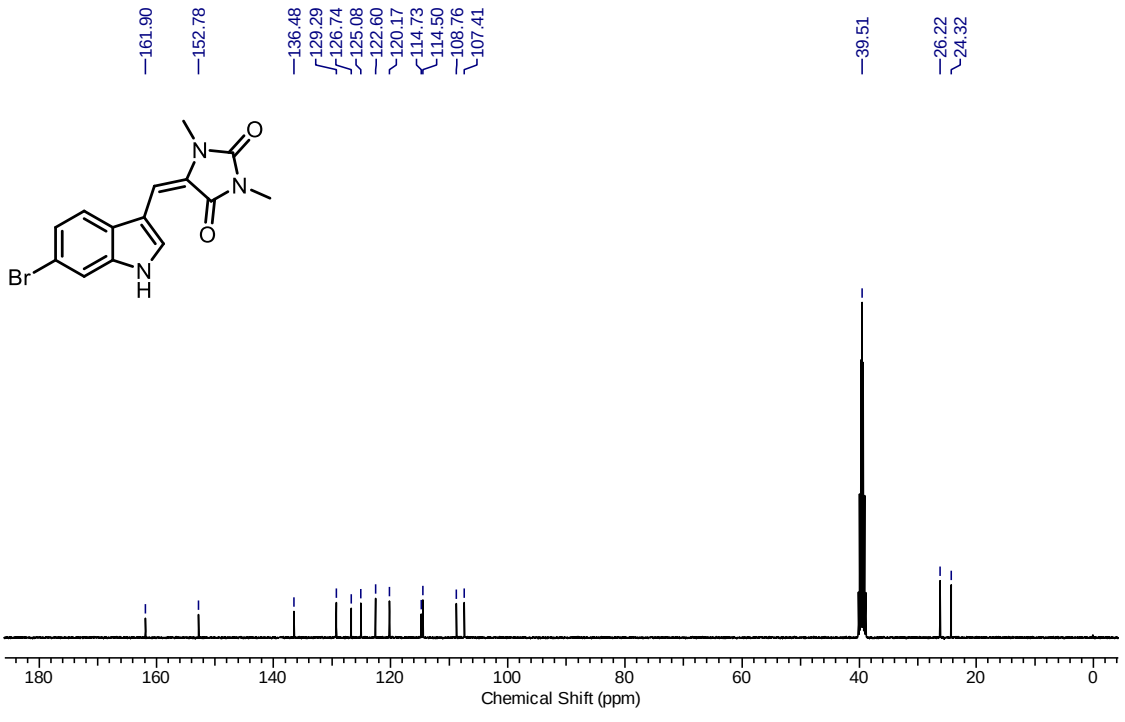
^{1}H NMR of Compound 70 in DMSO-d_6 at 400 MHz



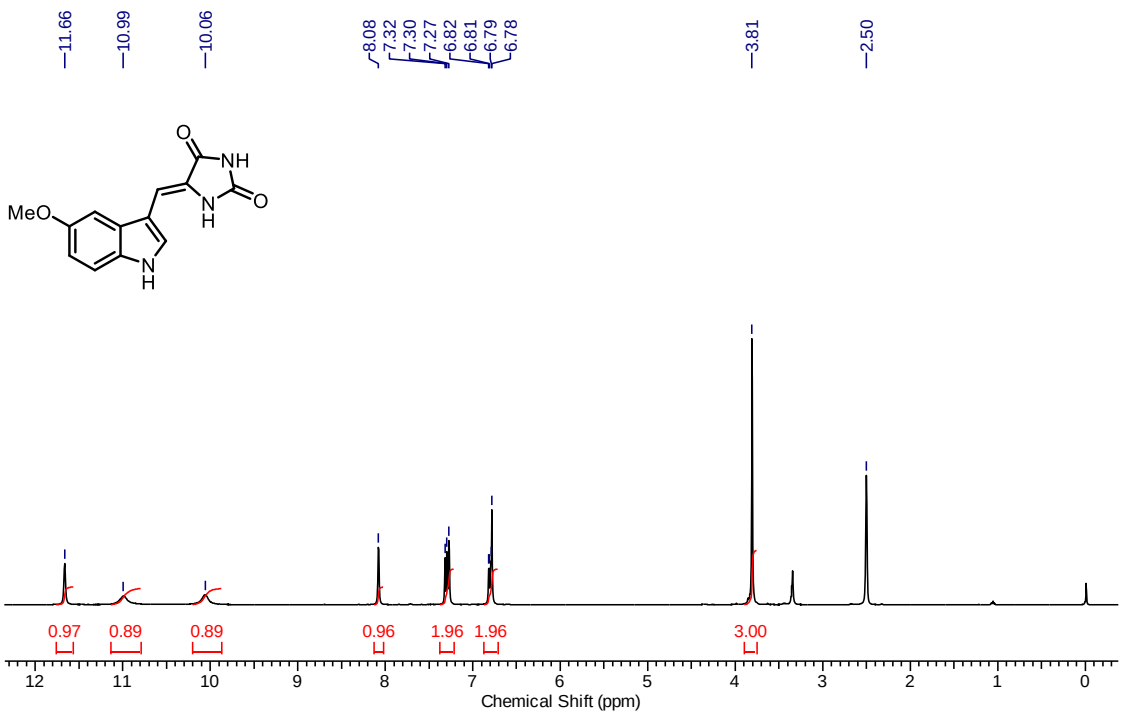
^{1}H NMR of Compound 71 in DMSO-d_6 at 400 MHz



^{13}C NMR of Compound 71 in DMSO-d_6 at 100 MHz



^{1}H NMR of Compound 72 in DMSO-d_6 at 400 MHz



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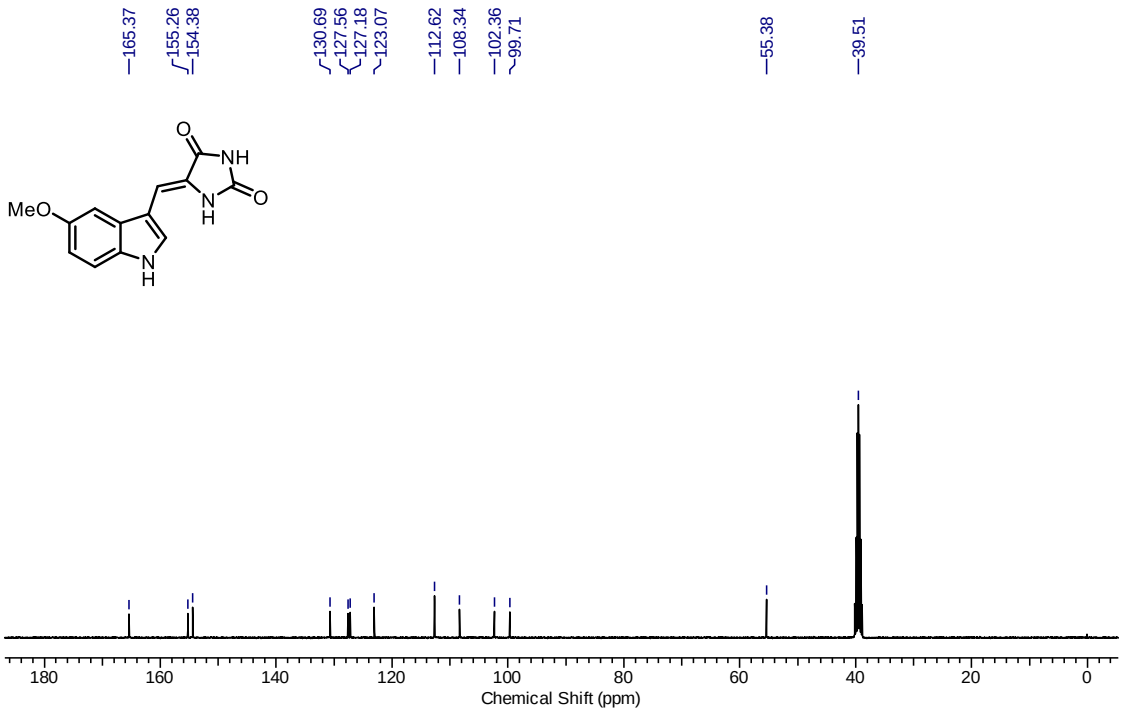

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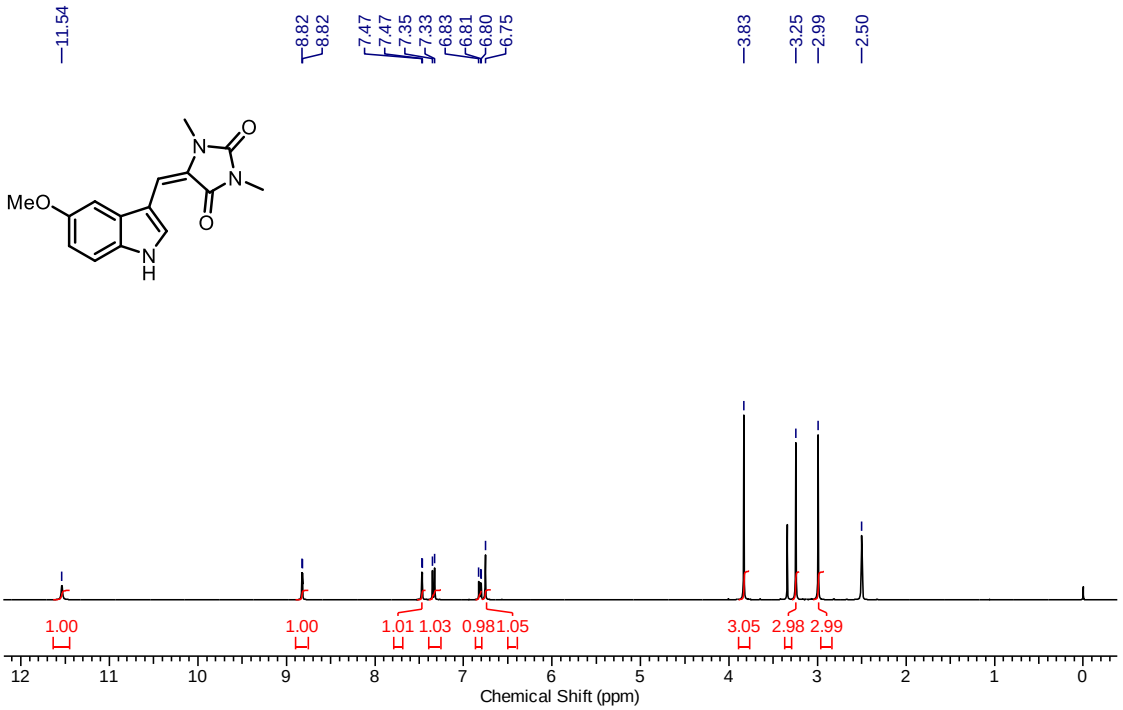

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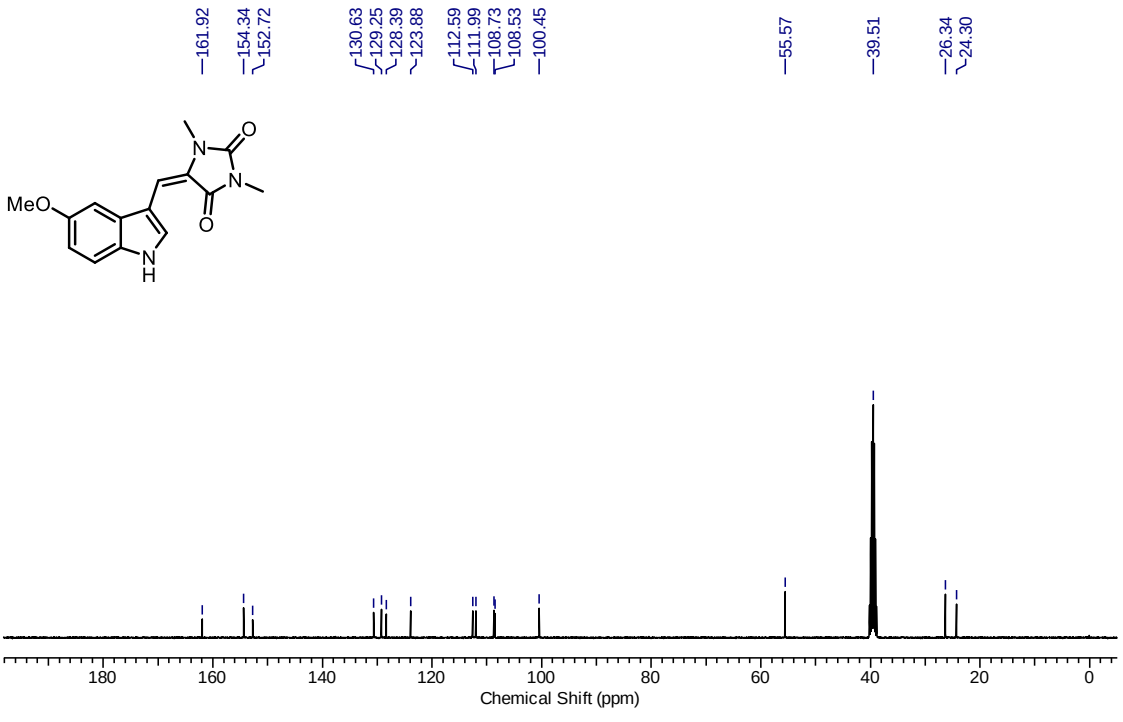

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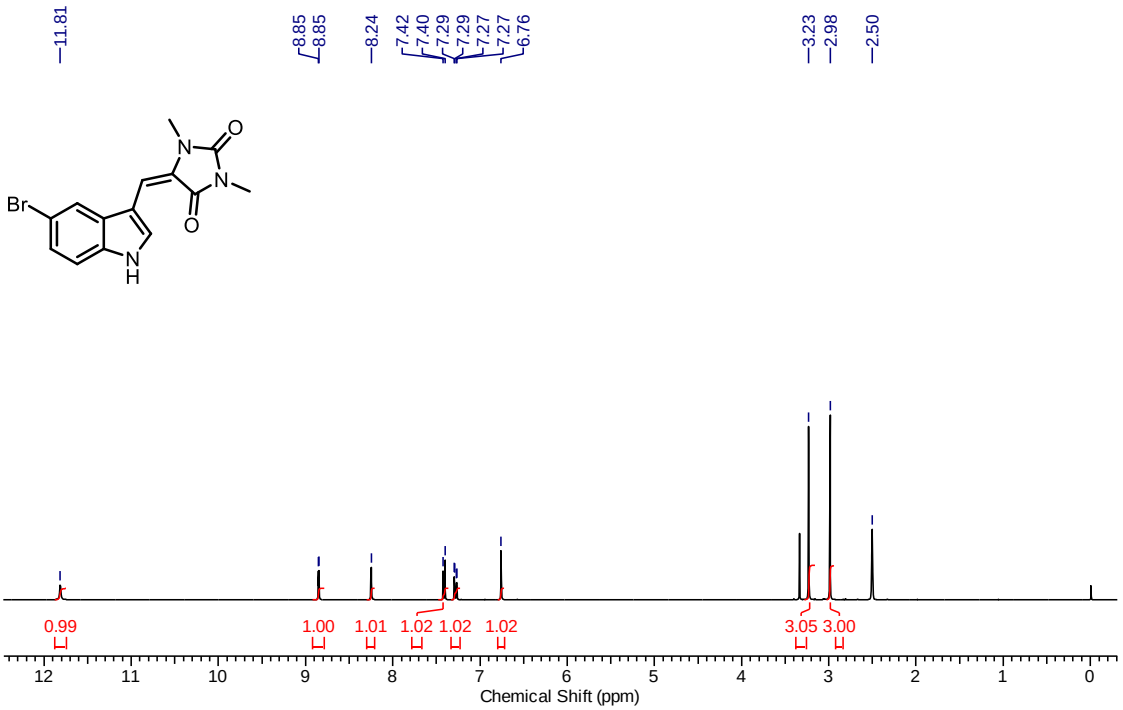
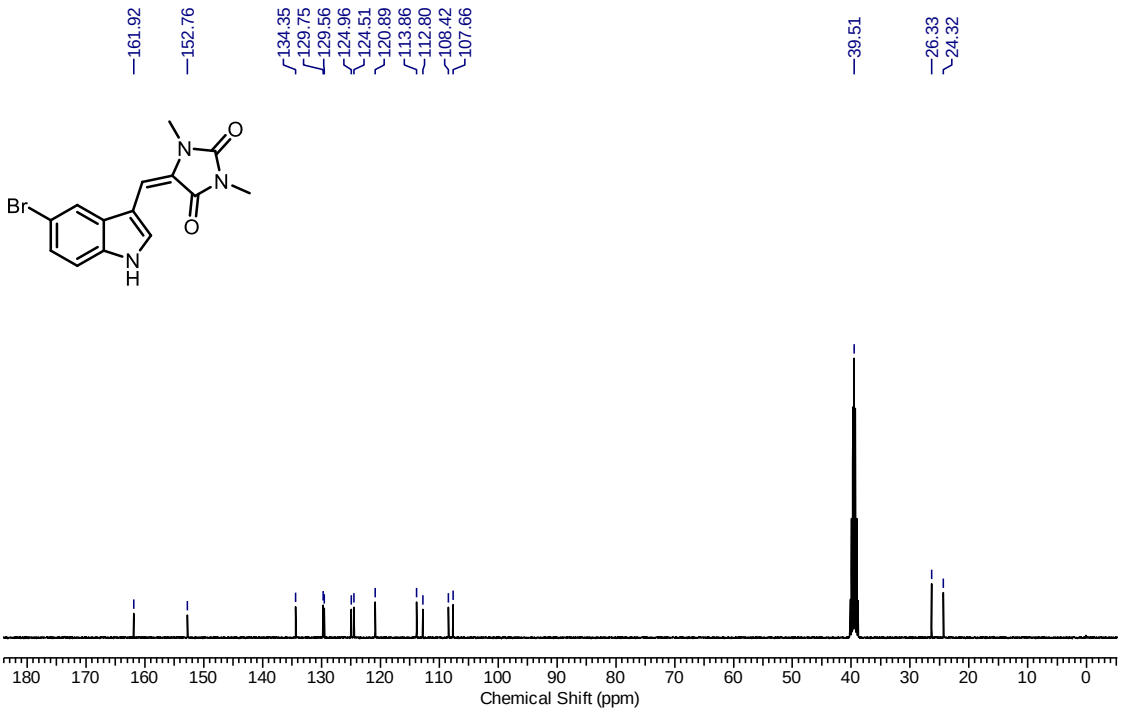
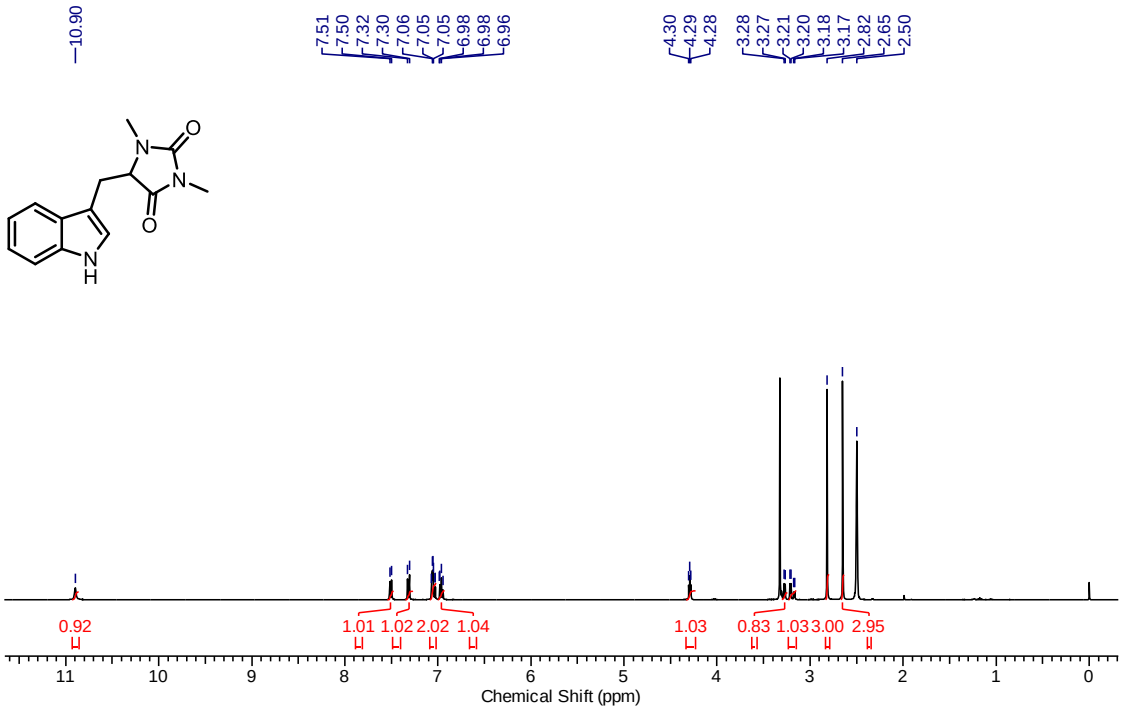


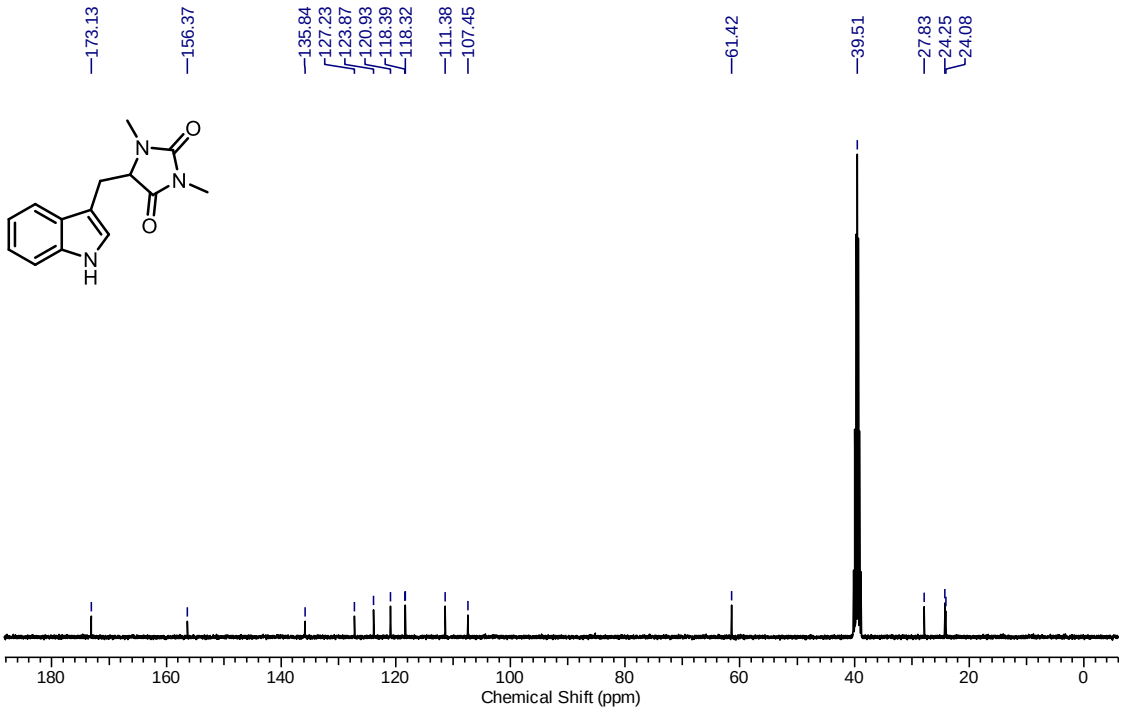
^{13}C NMR of Compound 74 in DMSO-d_6 at 100 MHz



^{1}H NMR of Compound 75 in DMSO-d_6 at 400 MHz



^{13}C NMR of Compound 75 in DMSO-d_6 at 100 MHz



ABSTRACT

Name of the Student: Akshay S. Kulkarni **Registration No.:** 10CC19J26004

Faculty of Study: Chemical Science **Year of Submission:** 2022

AcSIR academic centre/CSIR Lab: **Name of the Supervisor:** Dr. D. Srinivasa Reddy

CSIR-National Chemical Laboratory, Pune

Title of the thesis: Natural Products Peharmaline A and Oxoaplysinopsins: Synthesis, Analogues and their Biological Evaluation

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The work included in this thesis is mainly based on the total synthsis of the natural products, synthesis of analogues and their biological evaluation. Herein, we have developed first synthetic route to access anticancer natural product (±)-peharmaline A. Further developed synthetic route was employed for the synthesis of their analogues and subsequently all analogues were tested against five cancer cell lines which includes HCT-116, MCF-7, A549, HOP-92 and PC-3 using MTT assay. Structure Activity Relationship (SAR) of all the compounds were carried out and identified three lead compounds having simplified structure and higher potency than parent natural product.

Next, we have accomplished synthesis of oxoaplysinopsin D, E, F and G from common dihydroxy intermediate. During their synthesis we have observed one-pot oxidation of secondary alcohol to α-hydroxy ketone using PDC. This method was developed further and substrate scope was identified with >30 examples. Moreover, we have carried out synthesis of oxoaplysinopsin B using scalable one step protocol and synthesized 25 analogues of oxoaplysinopsin B and tested them against two cancer cell lines, however only few of them showed moderate activity. At the end, we have prepared new olefinic analogues of oxoaplysinopsin which raised the number of synthesized analogues to 115. Further, having all new oxoaplysinopin analogues in hand, we have selected 22 best compounds on the basis of their docking score and tested them against acetylcholinesterase .

List of Publications Emanating from the Thesis Work

1. **Kulkarni, A. S.**; Ramesh, E.; Reddy, D. S. One-Pot Oxidation of Secondary Alcohols to *α*-Hydroxy Ketones: Application to Synthesis of Oxoaplysinopsin D, E, F, & G *Eur. J. Org. Chem.* **2021**, 2188.
2. **Kulkarni, A. S.**; Shingare, R. D.; Dandela, R.; Reddy, D. S. Total Synthesis of an Anticancer Natural Product (±)-Peharmaline A and Its Analogues *Eur. J. Org. Chem.* **2018**, 6453.

List of Publications Non-Emanating from the Thesis Work

1. **Kulkarni, A. S.**; Ramesh, R.; Walia, S.; Sayyad, S. I.; Gathalkar, G. B.; Balamkundu, S.; Joshi, M.; Sen, A.; Reddy, D. S. Identification of a Novel Series of Potent Organosilicon Mosquito Repellents *ACS Omega* **2021**, *6*, 31236.
2. Shivapurkar, R.; Hingamire, T.; **Kulkarni, A. S.**; Rajmohan, P. R.; Reddy, D. S.; Shanmugam, D. Evaluating Antimalarial Efficacy by Tracking Glycolysis in *Plasmodium falciparum* Using NMR Spectroscopy *Sci Rep.* **2018**, *8*, 18076.
3. Subramanian, G.; Belekar, M. A. Shukla, A. Tong, J. X.; Sinha, A.; Chu, T. T; **Kulkarni, A. S.**; Preiser, P. R.; Reddy, D. S.; Tan, K. S. Shanmugam, D.; Chandramohanadas, R. Targeted Phenotypic Screening in Plasmodium falciparum and Toxoplasma gondii Reveals Novel Modes of Action of Medicines for Malaria Venture Malaria Box Molecules *mSphere* **2018**, *3*, e00534.
4. Shingare, R. D.; **Kulkarni, A. S.**; Sutar, R. L.; Reddy, D. S. Route to Benzimidazol-2-ones via Decarbonylative Ring Contraction of Quinoxalinediones: Application to the Synthesis of Flibanserin, A Drug for Treating Hypoactive Sexual Desire Disorder in Women and Marine Natural Product Hunanamycin Analogue *ACS Omega* **2017**, *2*, 5137.

List of Posters Presented with Details

1. National Science Day Poster presentation at CSIR-National Chemical Laboratory, Pune (February 25-27, **2020**):

   Title: Synthesis of Oxoaplysinopsin and their Library

   Abstract: Total synthesis of oxoaplysinopsin, a family of anticancer natural products has been accomplished for the first time. We have devised a common dihydroxy intermediate and synthesized four natural products oxoaplysinopsin D, E, F and G. During this process, a simple one-pot transformation of secondary alcohols to α-hydroxy ketones using pyridinium dichromate (PDC) in DMF at room temperature has been developed. In addition, a library of analogues (> 60 nos.) related to the targeted natural products has been created.

List of Conference Attended with Details

1. Poster presentation at JNOST, Department of Chemistry, University of Delhi, (October 18-21, **2019**)

   Title: Design, Synthesis and Evaluation of an Anticancer Natural Product (±)-Peharmaline A and Library of Analogues

   Abstract: (±)-Peharmaline A, a pair of novel β-carboline – vasicinone hybrid alkaloid enantiomers with a unique hybrid dimeric system has been isolated from the seeds of Peganum harmala L. by Wang *et.al* in 2017. (±)-peharmaline A displayed moderate cytotoxic activity against the HL-60, PC-3, and SGC-7901 cancer cell lines with IC_{50} values of 9.2, 21.6, and 25.4 μM, respectively whereas its two biosynthetically related precursors, harmaline and vasicinone were found to be inactive. This clearly implies that the unique dimeric structure is crucial for exhibiting significant cytotoxicity. We have accomplished the first total synthesis of anticancer natural product (±)-peharmaline A in three steps starting from 6-methoxy tryptamine. Stereoselective Pictet-Spengler reaction followed by construction of vasicinone moiety in one pot is the highlight. The developed route is useful in making analogues of the peharmaline scaffold and it was demonstrated by synthesizing several new analogues, which opens up opportunities for systematic structure activity relationships studies.

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EurJOC European Journal of Organic Chemistry

Communication

Natural Product Synthesis

Total Synthesis of an Anticancer Natural Product (±)-Peharmaline A and Its Analogues

Akshay S. Kulkarni,[a] Rahul D. Shingare,[a,b] Rambabu Dandela,[a] and D. Srinivasa Reddy*[a,b]

Dedicated to Professor Kotha Sambasivarao (IIT Bombay) on the occasion of his 60th birthday.

Abstract: First total synthesis of a rare β-carboline–vasicinone hybrid alkaloid (±)-peharmaline A has been accomplished in just 3 steps starting from known compounds. Stereoselective Pictet–Spengler reaction to nitrogenated tertiary carbon center and one-pot construction of the tricyclic skeleton of vasicinone are the highlights of present synthesis. We have also synthesized structurally close analogues of the natural product by following the developed route.

Introduction

Peganum harmala L. is a traditional medicinal plant and rich source of β-carboline alkaloids, which has been used for the treatment of alimentary tract cancers and malaria in Northwest China.[1] β-Carboline alkaloids exhibit a wide range of biological activities such as antitumor, antimalarial, antimicrobial and anti-inflammatory effects and selected examples are shown in Figure 1.[2]

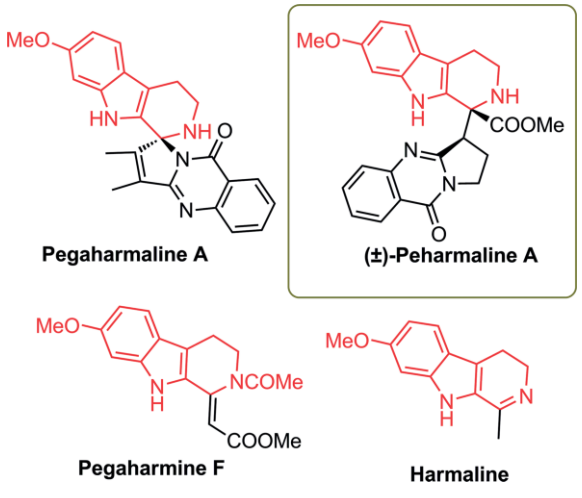


Figure 1. Natural products isolated from *Peganum harmala* containing β-carboline alkaloids.

[a] *Organic Chemistry Division, CSIR-National Chemical Laboratory, Dr. Homi Bhabha Road, Pune, 411008, India*
E-mail: ds.reddy@ncl.res.in (D. S. Reddy)
http://academic.ncl.res.in/ds.reddy
[b] *Academy of Scientific and Innovative Research (AcSIR), New Delhi, 110 025, India*
Supporting information and ORCID(s) from the author(s) for this article are available on the WWW under https://doi.org/10.1002/ejoc.201800949.

(±)-Peharmaline A, a pair of novel β-carboline–vasicinone hybrid alkaloid enantiomers with a unique hybrid dimeric system, was isolated by Wang et al. in 2017 from the seeds of *Peganum harmala* L.[3] Newly discovered (±)-peharmaline A contains an unprecedented linkage of vasicinone and β-carboline units. Structure of the natural product was determined using extensive NMR spectroscopy. The enantiomers were further resolved by chiral-phase HPLC, and their absolute configurations were determined by comparison between the calculated and experimental electronic circular dichroism (ECD) spectra. Interestingly, compound (±)-peharmaline A displayed significant cytotoxic activity against the HL-60, PC-3, and SGC-7901 cancer cell lines with IC_{50} values of 9.2, 21.6, and 25.4 μM, respectively, whereas its two biosynthetically related precursors, harmaline and vasicinone, were found to be inactive. This clearly implies that the unique dimeric structure is crucial for cytotoxicity.

Results and Discussion

Our group has continued interest in the synthesis of biologically important natural products and their analogues towards identifying lead molecules for various diseases.[4] Along these lines, (±)-peharmaline A, owing to its interesting structure, significant bioactivity and material scarcity from natural source motivated us to initiate the project. Our goals in this project are total synthesis to confirm the assigned structure through synthesis to produce sufficient quantities and to access a library of compounds around the target natural product skeleton to understand an in-depth SAR. Towards this now we have made significant progress and the results are disclosed here in this communication. The planned strategy to access the natural product (±)-peharmaline A (**1**) is depicted in Figure 2. We have identified tryptamine (A), pyrrolidinone (B) and anthranilic acid (C) derivatives as three key fragments to construct the desired natural product **1** skeleton. The nitrogenated tertiary carbon center of the target is planned through a Pictet–Spengler (P–S) reaction

between the fragments (A) and (B). Finally, the construction of the linearly fused tricyclic core (deoxyvasicinone) of the peharmaline A using fragment (C) is planned.

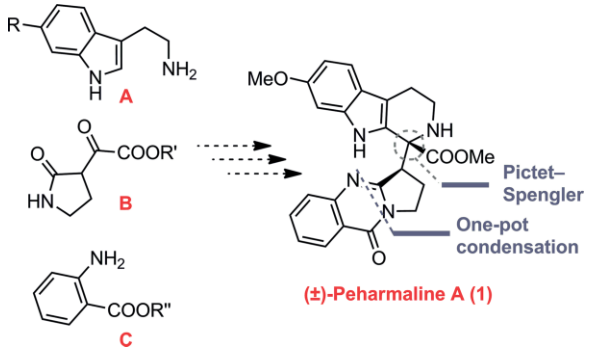
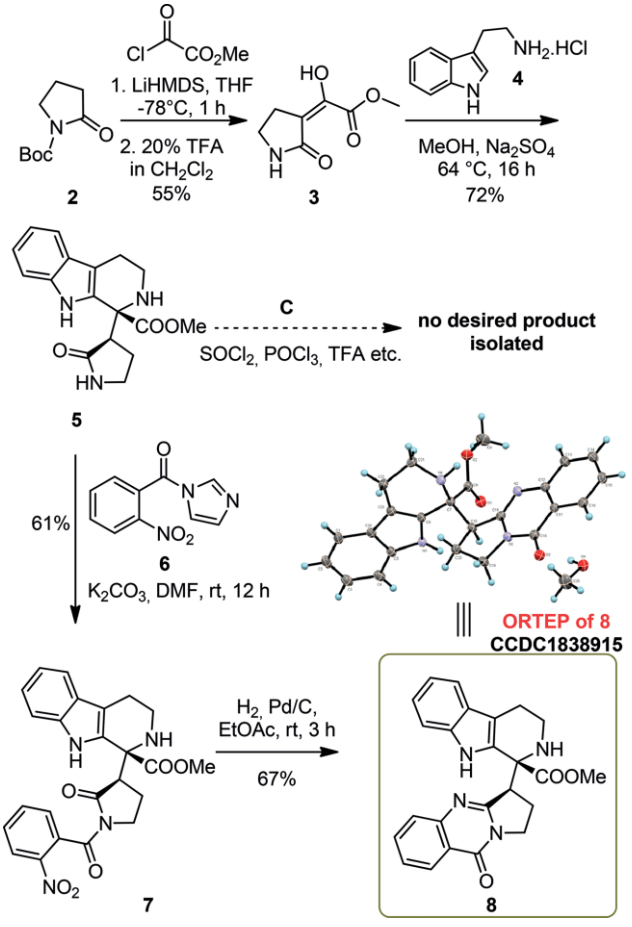


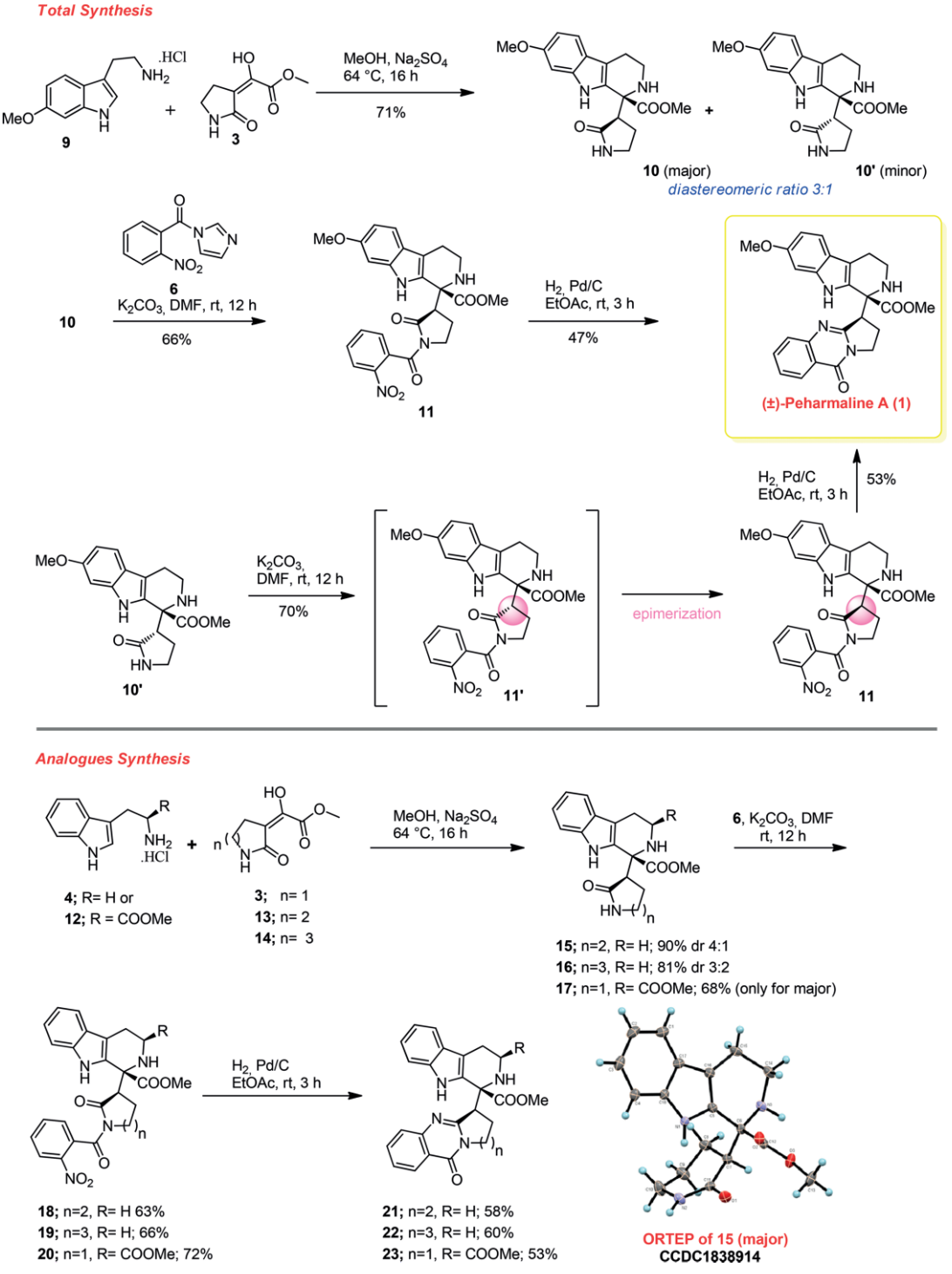
Figure 2. Synthetic plan towards (±)-Peharmaline A.

To optimize the planned strategy and steps, we have chosen readily available tryptamine as a building block A. For second fragment (B), we have utilized a known Boc-protected pyrrolidinone **2** and prepared compound **3** in a gram scale using LHMDS and appropriate acylating agent.[5] The nitrogenated tertiary carbon center present in the tetrahydro-β-carboline unit of the natural product was planned using Pictet–Spengler reaction.[6] Accordingly, the reaction between compound **3** and hydrochloride salt of tryptamine **4** under mild conditions (Na_2SO_4, MeOH, reflux) followed by simple aqueous workup produced the desired compound **5** in 72 % yield. To our pleasant surprise, an excellent distereoseletivity was observed in this reaction to have a ≈ 9:1 mixture (based on crude NMR). We arrived at this optimized procedure after a few experimental conditions. All the spectroscopic data is in agreement with the proposed structures and the details are available in the Supporting Information. The next task was to build the tricyclic core of vasicinone. We have attempted a one-pot condensation of anthranilic acid[7] or its equivalent (C) which did not give any fruitful results (See SI for additional details). These unsuccessful results prompted us to carry out the cyclization reaction in a step-wise manner. In this regard, we have synthesized stable activated ester **6** by the reaction of 2-nitrobenzoic acid and carbonyldiimidazole (CDI).[8] The activated ester **6** on reaction with compound **5** in presence of K_2CO_3 as a base gave chemoselective *N*-acylation at the pyrrolidinone nitrogen to afford **7** in good yield (61 %). The nitro group of the *N*-acylated compound **7** was reduced using 10 % palladium on carbon, which then underwent spontaneous intramolecular condensation with the amide carbonyl to obtain the desired demethoxypeharmaline **8** in 67 % yield. The authenticity of the structure was confirmed by comparing spectroscopic data of compound **8** and with that of the natural (±)-peharmaline A. As the present compound **8** lacks an OMe group, it can be called as demethoxypeharmaline. Towards further confirmation, compound **8** was recrystallized using methanol/dichloromethane and hexane solvents and its structure was unambiguously confirmed by its single-crystal X-ray diffraction (Scheme 1). Having an optimized route of synthesis in hand, we attempted the synthesis of the target molecule (±) peharmaline A. The Pictet–Spengler reaction of 6-methoxytryptamine hydrochloride salt **9** and compound **3** using previously optimized condition to afford a mixture of diastereomers **10** and **10′** in good yield (71 %). Although the observed diastereoselectivity was not impressive (≈ 3:1) in present case, both the compounds were cleanly separable using silica gel column chromatography. We have also screened a few reaction conditions to improve the yield and diastereomeric ratio but with no success. The major diastereomer **10** was *N*-acylated (K_2CO_3, DMF, room temp.) to give **11** (66 %), followed by reductive condensation to obtain the natural product (±)-peharmaline A **1** in 47 % yield (Scheme 2). All the spectroscopic data of the synthetic sample is in good agreement with the literature reports.[3] Next, we wanted to convert the minor diastereomer **10′** obtained in the Pictet–Spengler reaction to the final product following the same procedure (*N*-acylation followed by reductive condensation). To our surprise, ^1H and ^{13}C NMR of the product **11′** obtained after the first step exactly matches with that of compound **11** indicating that possible epimerization at the center next to pyrrolidinone carbonyl functionality. To confirm further, the *N*-acylated compound **11′** prepared from minor diastereomer was subjected to reductive cyclization, analysed the spectroscopic data and found that which was identical to that of natural product (±)-peharmaline A (**1**).



Scheme 1. Synthesis of demethoxypeharmaline A.

At this stage, we became interested in generating close analogues of the natural product by varying structural features, in particular, by changing five-membered pyrrolidine ring size,



Scheme 2. Total synthesis of (±)-Peharmaline A and analogues.

which may adopt different conformation to the molecule by keeping the unique dimeric skeleton intact. Hua's group also has emphasized that synthetic efforts and in-depth biological testing are needed because, (±)-peharmaline A exhibited significant cytotoxic activity, whereas, their two biosynthetically related precursors monomeric structures harmaline and vasicinone were found to be inactive.[3] Accordingly, we have synthesized keto-ester derivative of piperidin-2-one **13** and azepan-2-one **14** for the Pictet–Spengler reaction. All these compounds were treated with tryptamine/L-tryptophan methylester under the same conditions used previously to afford desired β-carboline derivatives **15**–**17** in excellent yields with varying diastereomeric ratios (Scheme 2). Next all three compounds **15**, **16** and **17** were subjected for *N*-acylation followed by reductive cyclization to furnish final dimeric products **21**, **22** and **23**, respectively. All the compounds were well characterized using spectroscopic technique along with a crystal structure of an intermediate **15**.

Conclusions

In conclusion, we have prepared anticancer natural product (±) peharmaline A (**1**) in a short sequence for the first time. Although stereoselectivity in Pictet–Spengler reaction using methoxytryptamine was not great, to our delight, we have observed the inversion of the chiral center present in the minor diastereomer during the course of synthesis to afford the natural product. In addition, we have synthesized four new analogues of peharmaline A which may be useful for further structure–activity relationship study. Developing the enantioselective synthesis of peharmaline A and in-depth biological evaluation of the library of compounds around the skeleton towards finding the optimized anticancer lead will be the future direction of the project.

Experimental Section

CCDC 1838914 (for **15**, major isomer), and 1838915 (for **8**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre.

Supporting Information (see footnote on the first page of this article): including Table S1–S2, Experimental section, NMR and ESI/HRMS spectra, HPLC data.

Conflict of Interest: The authors declare no conflict of interest.

Acknowledgments

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Keywords: Nitrogen heterocyles · Total synthesis · Natural products · Alkaloids · Cytotoxicity

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One-Pot Oxidation of Secondary Alcohols to α-Hydroxy Ketones: Application to Synthesis of Oxoaplysinopsin D, E, F, & G

Akshay S. Kulkarni,[a, b] Eagala Ramesh,[a, c] and D. Srinivasa Reddy*[a, b, c]

A simple one-pot transformation of secondary alcohols to α-hydroxy ketones using pyridinium dichromate (PDC) in DMF has been developed and substrate scope tested with 25 compounds of hydantoin derivatives. Using this method, we have devised a common dihydroxy intermediate and synthesized four natural products oxoaplysinopsins D, E, F, and G for the first time.

Very recently, seven new oxygenated derivatives of aplysinopsin-type alkaloids, oxoaplysinopsins A–G were isolated by Wang *et al.* from *F. reticulata* of the XiSha Islands (Paracel Islands).[1] This oxoaplysinopsin family of natural products showed remarkable stereochemical diversity, which are possibly originated from corresponding olefinic biogenetic precursors. Although structures of these natural products seem to be simple, assigning stereochemistry to individual natural products is very challenging. Commendable efforts from Wang *et al.* using extensive NMR spectroscopy helped in determining stereochemistry assignments to all seven pairs of oxoaplysinopsins A–G, (Figure 1). Closely related aplysinopsins[2] are pharmaceutically important class of natural products with neuromodulation, antineoplastic, antiplasmodial, and antimicrobial properties.[3,4] By considering parent family aplysinopsins were the topic of research interests for biologists and chemists,[4] present class of molecules oxoaplysinopsins with druggable heterocyclic scaffold are expected to attract attention for synthetic and medicinal chemists in the future. As part of on-going activity in our group towards identification of leads based on natural products,[5] we aimed at total synthesis of these natural products which can provide access to sufficient quantities and to generate a library of analogues around the scaffold using the easily accessible materials and methods. Details of our efforts are disclosed in this work.

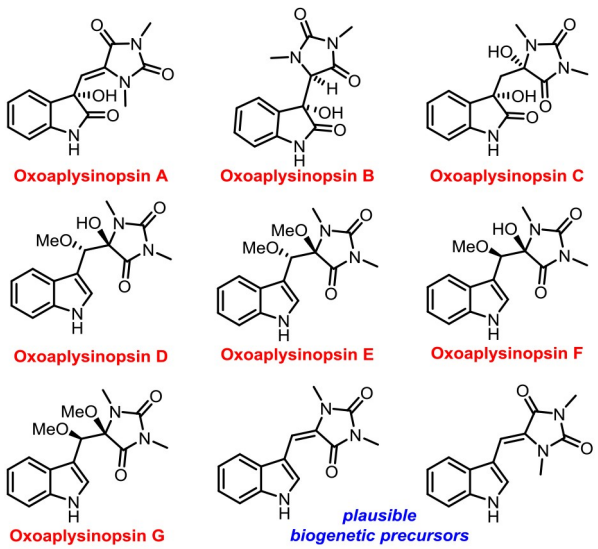
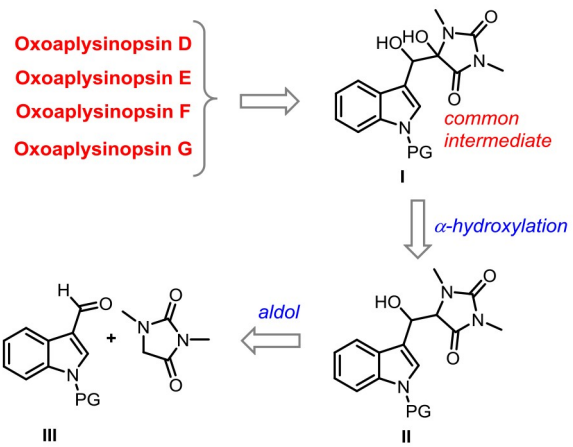


Figure 1. Structures of oxoaplysinopsin natural products.

The planned strategy to access oxoaplysinopsins D, E, F and G is depicted in Scheme 1. All the four natural products could be visualized from a key dihydroxy intermediate (I) through appropriate stereo- and functional-group transformations. The key compound (I) could be obtained from compound (II) through a challenging hydroxyl group installation at the nitro-



Scheme 1. Synthetic plan for oxoaplysinopsins D, E, F & G.

[a] *A. S. Kulkarni, Dr. E. Ramesh, Dr. D. Srinivasa Reddy*
Organic Chemistry Division
CSIR-National Chemical Laboratory
Dr. Homi Bhabha Road, Pune, 411008, India
E-mail: ds.reddy@ncl.res.in
http://academic.ncl.res.in/ds.reddy
[b] *A. S. Kulkarni, Dr. D. Srinivasa Reddy*
Academy of Scientific and Innovative Research (AcSIR)
Ghaziabad, 201002, India
[c] *Dr. E. Ramesh, Dr. D. Srinivasa Reddy*
CSIR-Indian Institute of Integrated Medicine
Canal Road, Jammu, 180001, India

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genated tertiary centre. We planned to introduce tertiary hydroxy group through a direct oxygenation as the centre is next to amide carbonyl or by oxidation of secondary alcohol in (II) followed by α-hydroxylation such as Rubottom oxidation. The two starting materials indole-3-carboxaldehyde (III) and dimethyl hydantoin (IV) could be combined through an aldol reaction to have compound (II).

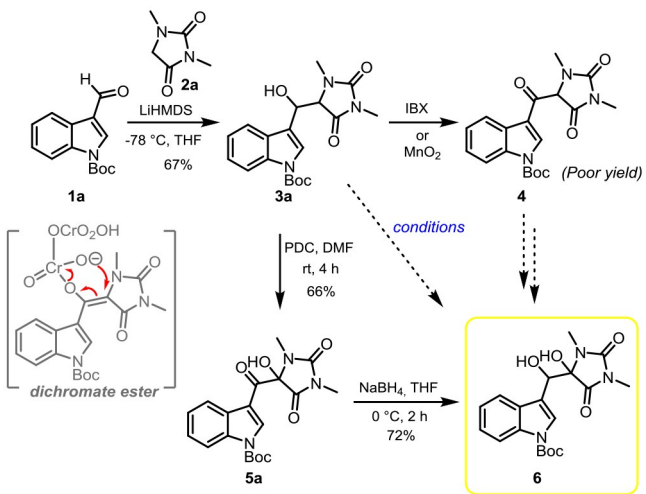
To begin with, we have chosen known compounds Boc-indole-3-carboxaldehyde **1a**[6] and dimethyl hydantoin **2a**[7] as starting points to test the feasibility of planned strategy. The desired compound **3a** was prepared in gram scale using aldol reaction with the help of LiHMDS base. The next challenge was to install the tertiary hydroxyl group, for which we attempted several possible α-hydroxylation conditions,[8] to convert **3a** to **6** directly with no desired outcome, but with these conditions we observed retro-aldol products, (Table 1). These unsuccessful efforts prompted us to prepare activated 1,3-dicarbonyl compound **4** to avoid the retro-aldol reaction followed by α-hydroxylation.[8,9] Accordingly, for oxidation of compound **3a**, different oxidizing reagents and solvents were surveyed and found that IBX or MnO_2 are the better reagents to give required ketone **4** in ~30% yields. To our surprise, while we were working on optimization (Table 1) to improve yields, pyridinium chlorochromate (PCC) mediated oxidation gave interesting results, as an additional prominent spot on TLC was seen. After careful analysis, it was concluded that the additional spot was caused by the desired ketone **5a** with requisite oxygenation at nitrogenated tertiary carbon center while further addition of excess PCC to the reaction mixture resulted in compound **5a** as the sole product. Probably, the reaction is proceeding through a dichromate ester as shown in Scheme 2. Reduction of ketone present in **5a** by using sodium borohydride afforded the required dihydroxy intermediate **6** and the reaction was further scaled up to a gram scale. A literature survey revealed that present methodology was not documented previously. However, a similar kind of transformation under PCC conditions[10] was observed by Mehta *et al.* during the synthesis of seco-prezizaane[10a] and Paterson *et al.* during the synthesis of Jiadifenolide.[10b] We also found a few related transformations using iodine based reagents[11] which were unsuccessful on our substrate to furnish desired product **5a**. As this reaction is interesting and it was not well studied, we decided to test the scope of this method which may be useful to the community and also for the synthesis of related natural products.

Having made this interesting observation, compound **3a** was chosen for optimization for converting to **5a** and for which we subjected **3a** to various conditions (entry 11–19) as shown in Table 1. However, increasing the equivalents of PCC, elongating the reaction time up to 12 h or changing the solvent (entry 10–12) did not affect the transformation to a significant extent. Next, to understand the role of atmospheric oxygen we also used O_2 as well as water, however no effect was observed in the reaction (entry 13 & 14). Furthermore, PDC in DCM showed very little improvement in α-hydroxy ketone formation (entry-15). To our delight, only α-hydroxy ketone was observed after use of PDC in DMF at room temperature for 3 h (entry-16) while changing the solvent to DMSO showed faster reaction to afford α-hydroxy ketone with 58% yield along with 15% ketone formation (entry-17). However, no reaction was observed with the use of CrO_3 (entry 18–19). Finally, we settled with optimized condition as 3 equiv. of PDC in DMF at room temperature which results only **5a** and no ketone formation was observed (entry-16, Table 1). As discussed earlier, our main goal was to synthesize oxoaplysinopsin natural products and their novel library of analogues. Therefore, we have mostly planned substrates similar to the natural product scaffold. The indole carboxaldehydes and hydantoins **2a**, **2b** and **2c** used in the present study were prepared using the literature procedures,[7] which upon aldol reaction generated corresponding aldol adducts (**3a**–**3q**). Under the optimized conditions, **3a**–**3q** were successfully converted to the desired α-hydroxy ketones (**5a**–**5q**) in moderate to good yields (Table 2). We have found that

Table 1. Optimization of reaction conditions.

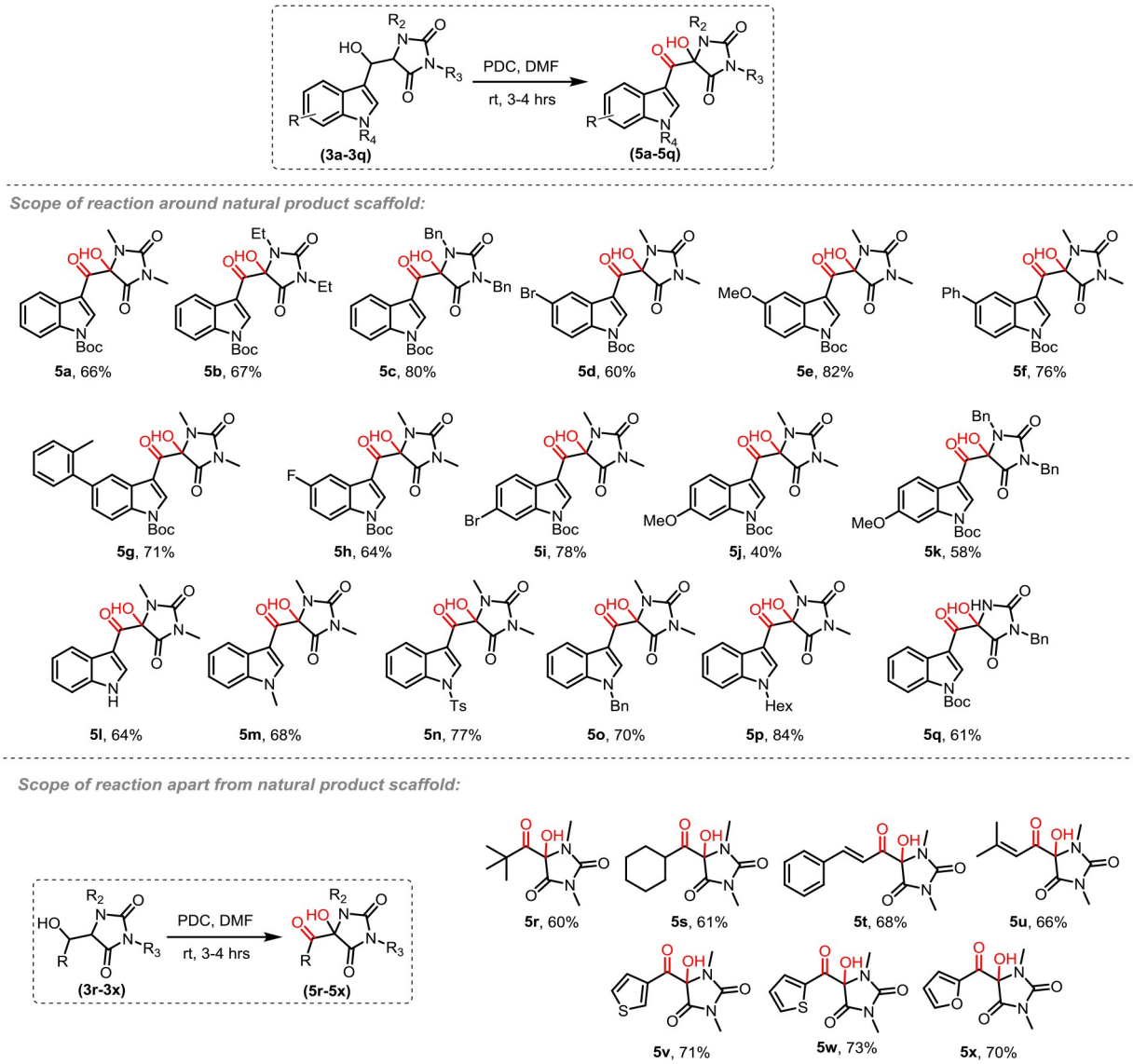
Entry	Conditions[a]	Observation
1	Iodine, DMSO, rt, 12 h	No reaction
2	Iodine, DMSO, 60 °C 12 h	Retro-aldol
3	Iodine, NaOAc, O_2, THF, rt	Retro-aldol
4	Oxone, ACN: H_2O, rt	Retro-aldol
5	NBS, DMSO, rt, 3 h	No reaction
6	DMP, $NaHCO_3$, rt, 6 h	Retro-aldol
7	DMP, $NaHCO_3$, 0 °C, 1 h	Retro-aldol
8	MnO_2, DCM, rt, 4 h	**4** (20%) + retro-aldol
9	IBX, DMSO, rt, 3 h	**4** (31%) + retro-aldol
10	PCC (3 eq.), DCM, rt, 3 h	**4** (37%) + **5a** (30%)
11	PCC (6 eq.), DCM, rt, 12 h	**4** (30%) + **5a** (40%)
12	PCC, DMF, rt, 4 h	**4** (31%) + **5a** (38%)
13	PCC, DMF, H_2O, rt, 4 h	**4** (35%) + **5a** (36%)
14	PCC, DMF, O_2, rt, 4 h	**4** (33%) + **5a** (35%)
15	PDC (3 eq.), DCM, rt, 3 h	**4** (31%) + **5a** (39%)
16	PDC (3 eq.), DMF, rt, 3 h	Only **5a** (66%)
17	PDC (3 eq.), DMSO, rt, 3 h	**4** (15%) + **5a** (58%)
18	CrO_3 (VI), DCM, rt, 12 h	No reaction
19	CrO_3 (VI), DMF, rt, 12 h	No reaction

[a] Conditions 1–5: Selected conditions for **3a** to **6**; Conditions 6–10: Selected conditions for **3a** to **4**; Conditions 11–19: Selected conditions for **3a** to **5a**.



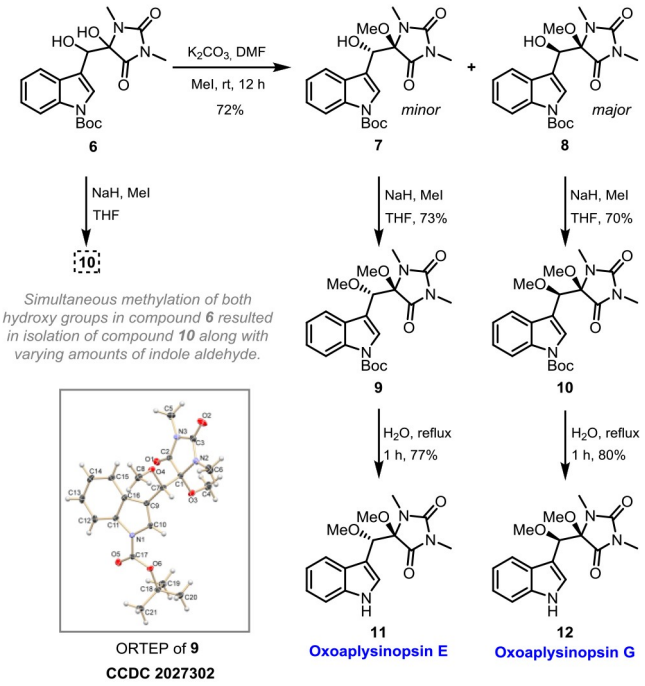
Scheme 2. Synthesis of common dihydroxy intermediate and optimization of α-hydroxylation method.

Table 2. Substrate scope of PDC mediated one pot method.

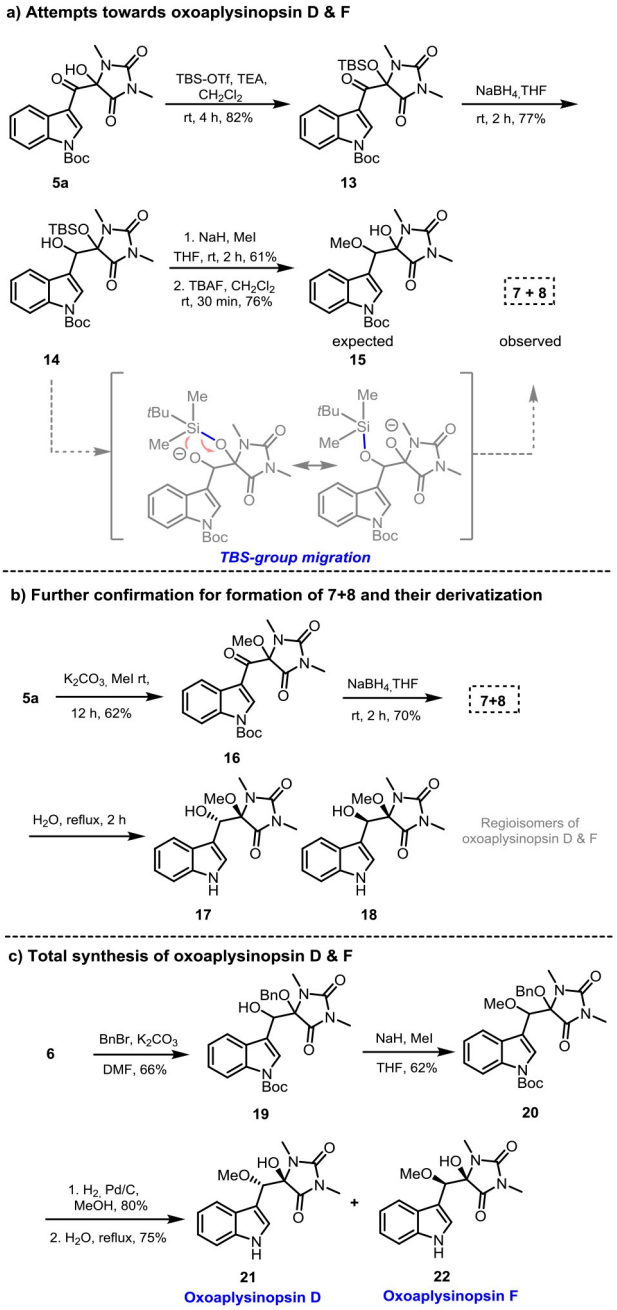


the substituents on the hydantoin moiety have some effect on the yield of reaction. For example, better yield were achieved in case of aldol adducts having *N,N*-dibenzyl hydantoin **5c** followed by the *N,N*-diethyl hydantoin **5b**, and lowest yield was observed with *N,N*-dimethyl hydantoin **5a**. Even in case of a mono-substituted hydantoin, the desired α-hydroxy ketone **5q** was obtained in good yield. On the other hand, substitution on the indole and protecting group on indole NH does not have any considerable effect on the yield of the reaction (**5l** to **5p**). To further expand the scope of this method, we replaced indole moiety with other aromatic moieties such as thiophenes (**5v** & **5w**), furan (**5x**), and styryl group (**5t**) all of which gave good yields. Even in case of non-aromatic moieties such as cyclohexyl (**5s**), *t*-butyl (**5r**) and 3-methyl-2-butenyl (**5u**) group's reaction went with ease. Thus, we have identified the scope of this interesting one-pot oxidation reaction with a variety of substrates and utilized it to create a library of novel analogues around natural product scaffold.

Next, we turned our attention to the synthesis of target natural products, initially towards oxoaplysinopsin E and G. (Scheme 3) For this purpose, alkylation of common dihydroxy intermediate **6** using methyl iodide in presence of NaH led to the formation of a single diastereomer of **10** in poor yields along with indole-3-carboxaldehyde, which was probably result of a retro-aldol reaction. By changing the base to K_2CO_3 gave chemoselective *O*-alkylation at the tertiary centre to afford a mixture of diastereomers (2:3 ratio) **7** and **8** in good yields. Compounds **7** and **8** were cleanly separable using silica gel column chromatography. Then both the diastereomers were taken forward separately for further transformations. The minor diastereomer **7** was methylated to give **9** (73%) which was recrystallized using ethyl acetate and hexane solvents and its



Scheme 3. Synthesis of oxoaplysinopsin E and G.



Scheme 4. a) Attempts towards oxoaplysinopsin D and F; b) Further confirmation for formation of **7** and **8** and their derivatization; c) Total Synthesis of oxoaplysinopsin D and F.

structure was unambiguously confirmed by single-crystal X-ray diffraction (CCDC-2027302), further compound **9** was subjected to Boc-deprotection in water[12] resulted in the natural product oxoaplysinopsin E **11** in 77% yield. The major diastereomer **8** was subjected to same sequence to afford oxoaplysinopsin G **12**. All the spectroscopic data of both the synthesized natural products are in complete agreement with the reported data by Wang's group.[1]

For the synthesis of natural products oxoaplysinopsin D and F, we wanted to protect tertiary hydroxy group as it was more reactive and further methylate the secondary hydroxy group for which the hydroxy ketone **5a** upon reaction with TBS-OTf in presence of TEA in CH_2Cl_2 gave compound **13** with 82% yield. Compound **13** was further subjected to $NaBH_4$ reduction in THF to access required hydroxy compound **14**. Compound **14** on reaction with methyl iodide and NaH gave the required methylated product which on TBS-deprotection using TBAF in CH_2Cl_2 was expected to deliver **15** as a diasteriomeric mixture; but to our surprise, ^1H and ^{13}C NMR of the isolated products were matching with that of previously prepared compounds **7** and **8**, (Scheme 3). Formation of unexpected products could be explained by the TBS-group migration in compound **14** as shown in Scheme 4a. To further confirm this assumption, methylation of compound **5a** was done using K_2CO_3 and methyl iodide to give **16** which on further reduction with $NaBH_4$ yielded compound **7** and **8** for which all the data were identical with previously synthesized compounds from (Scheme 3 and Scheme 3a). The separated diastereomers **7** and **8** deprotection using refluxing water[12] resulted in compounds **17** and **18** respectively. These two compounds **17** and **18** are the regioisomers of oxoaplysinopsin D and F, respectively. To overcome the undesired TBS- migration phenomenon, we decided to go with benzyl protecting group instead of TBS group. (Scheme 3c) Accordingly, dihydroxy intermediate **6** was subjected for chemoselective benzyl protection of tertiary alcohol using benzyl bromide in presence of K_2CO_3 to give compound **19** in 66% yield which on further treatment with NaH and methyl iodide afforded compound **20**. Deprotection of benzyl group followed by Boc-deprotection furnished both

oxoaplysinopsin D **21** and F **22** in good yields. All the spectroscopic data of both the synthesized natural products **21** and **22** were in agreement with the reported data.[1,14]

In summary, we have synthesized four natural products of oxoaplysinopsin family for the first time. All four oxoaplysinopsins D, E, F and G were synthesized from a common dihydroxy intermediate, which in turn was prepared through an aldol reaction between Boc-indole-3-carboxaldehyde and *N,N*-dimethyl hydantoin followed by one pot α-hydroxylation. During this process, a simple and efficient method for the conversion of secondary alcohols to α-hydroxy ketones using PDC has been developed, further scope of the method was tested with a variety of substrates.

Deposition Number 2027302 (for **9**) contains the supplementary crystallographic data for this paper. These data are provided free of charge by the joint Cambridge Crystallographic Data Centre and Fachinformationszentrum Karlsruhe Access Structures service www.ccdc.cam.ac.uk/structures.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: α-Hydroxylation · Hydantoin · Natural product · Oxoaplysinopsin · Retro-aldol

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[13] See electronic supplementary information for further details.
[14] Both natural products 21 and 22 were isolated as a mixture. We could not separate both of them in pure form and compared the spectral data of mixture with that of individual natural products.

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