Design, Synthesis, and biological Introduction of QUANTA as Potential PROTECTIVE DRUG FOR FUNGAL AND BACTERIAL INFECTIONS

(ZENITH PHARMACEUTICALS)

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B.Pharm, M.Pharm (Medicinal Chemistry)

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Company – Zenith Industry's

NOVEL DRUG SCAFFOLD FOR MULTI DRUG RESSISTANCE AGAINST BACTERIA AND FUNGUS

Flow of Presentation

- Introduction
- Review of Literature
- Design and Rationale
- Aim of Present Work
- Work Done
- Summary of Work Done


INTRODUCTION

Introduction

- Bacterial infections are very common in the animal husbandry due to huge population and overcrowding.
- Poor sanitation
- Increase community acquired resistance
- Ineffective infection control program
- Increasing antibiotic use which shows resistance to the bacterial and fungal infections
- Constant use of same biotic and same molecules
- We need to have novel molecules which shows good activity on resistance bacteria.

World Health Organization (WHO). Global Report 2019
https://apps.who.int/iris/bitstream/handle/10665/329368/9789241565714-eng.pdf?ua=1 (accessed July 26, 2020)

Introduction – Need of New antibiotics in the animal husbandry

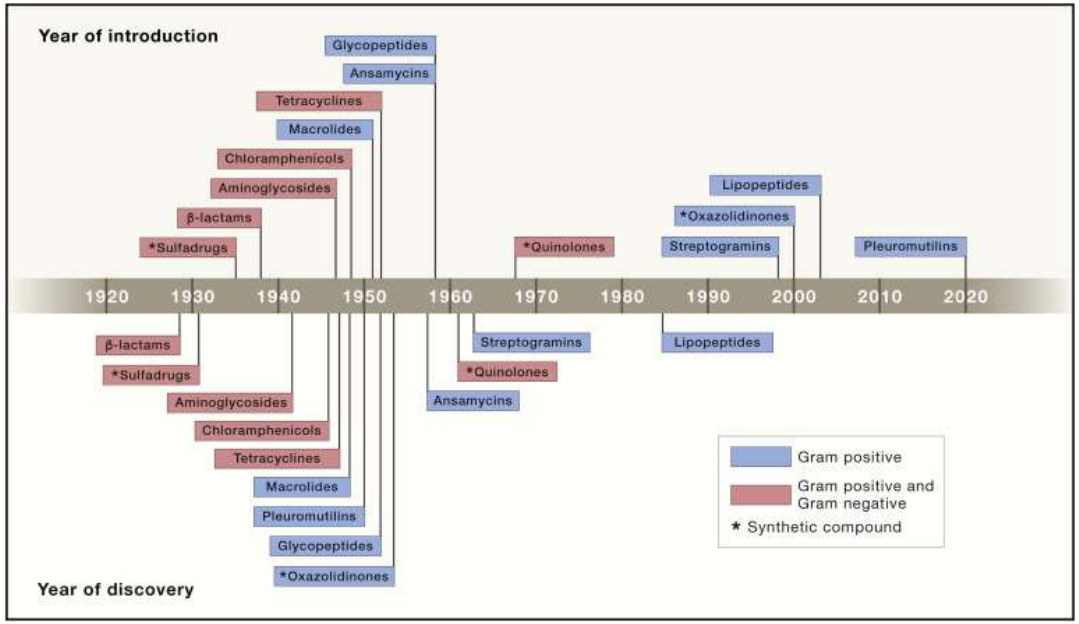
With the time running the bacterial infections are showing the good resistance to the existing Antibiotics

The major reason is the lack of new and novel drug molecules on the veterinary side

At every 15 years a new antibiotic combination with the delivery dose and novel target is found for humans and fight against the resistance bacterial infection

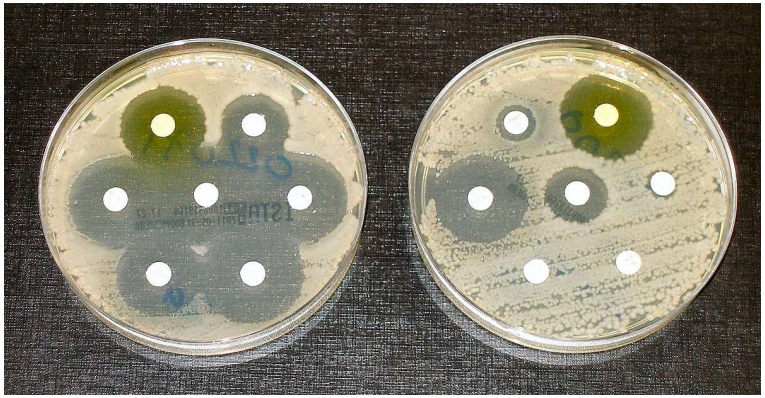
The infections on the veterinary side are far more complex then the human pathogens, as the personal hygiene and intellect humans have regarding pain and illness.

The researcher focus on human pathogen by taking trials and remarks from animals, still new formulations due to cost and synthetic controls the drugs are not delivered to the animal husbandry's.



Generation 2nd Bacterial infection

Generation 3rd Bacterial infection



Introduction – Why Quanta

The anti microbial Disc diffusion method as per NCCLS Guideline is conducted by the authorized lab.
The test inoculum contains bacteria and fungus spores for testing.

Results for bacteria.

1. Staphylococcus aurous ATCC 6538
2. Escherichia Coli ATCC 11229
3. Aspergillums Niger ATCC 16404.

Test Procedure:

The test organisms diluted to approximately 10-10 CFU/ ml was individually spread by a sterile swab evenly over the face of Soyabean Casein digest agar. Test preparation equivalent to 30 ul was smeared on sterile disc. The disc was then placed on seeded plate. Control plate comprised of distilled water solution on disc. The plates incubated at 37 CI 28'C for 24/ 72 hrs. Zone of inhibition were measured by calibrated ruler.

Sample Identification	Zone of inhibition in mm (Average)		
	Staphylococcus aurous	Escherichia Coli	Aspergillums Niger
Quanta	52mm	50mm	60mm

Introduction – Why Quanta

Test Method and Name.

Minimum Inhibitory Concentration (MIC) by Plate Method.

Test Method – ASM Manual of Microbiology Methods.

Purpose.

The MIC of the of an antifungal is the maximum dilution of the product Quanta that will still inhibit the growth of a test microorganism. In Plate dilution method, serial dilution of the products are made in fungal growth media. The test organisms are then spread on the plate containing dilution of the products, incubated and scored for the growth.

Concentrations of MIC.

0.001%, 0.01%, 0.03%, 0.05%, 0.08%, 0.1%, 0.5%, 0.8%, 1%, and 2%

Results for Fungus

Test Organism	Concentration of Product: QUANTA > REF 5689												MIC (%)
	0.001	0.005	0.01	0.03	0.05	0.03	0.3	0.5	0.8	1.0	1.5	2.0	
Candida albicans ATCC 10231	G	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	0.005
Aspergillus niger ATCC 6275	G	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	0.005
Penicillum Funicolosum A-11797	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	0.001
Trichoderma Virens MTCC 1372	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	0.001

Antibiotic -Introduction

- General data As Certificate of Analysis

ITEMS	Specification
Description	Ceramic yellow powder
Solubility	DMSO, Acetone, Toluene, Acidic and basic medias
Acidity	4.5-5.0
Powder PH	7.1
Iron	NMT 50ppm
Assay by GC/HPLC/MS	99.6% Purity
Product name	Quanta
100% crude price	1350 - 1700 Rs/KG
Manufacturing Capacity	100MT/Month

Introduction – Clinical Data

ACUTE TOXICITY – QUANTA

CHEMICAL NAME – PROTECTED
ORAL : RAT: LD50 (mg/kg) – 955
Dermal : RAT : - 10%EC
INHALATION – DATA TO BE IDENTIFIED

REPRODUCTIVE TOXICITY

On RATS
360mg/kg/day – Reproductive test – no adverse effect on futility
50mg/kg/day - Reproductive test – no adverse effect on futility
810mg/kg/day - Reproductive test – no adverse effect on futility

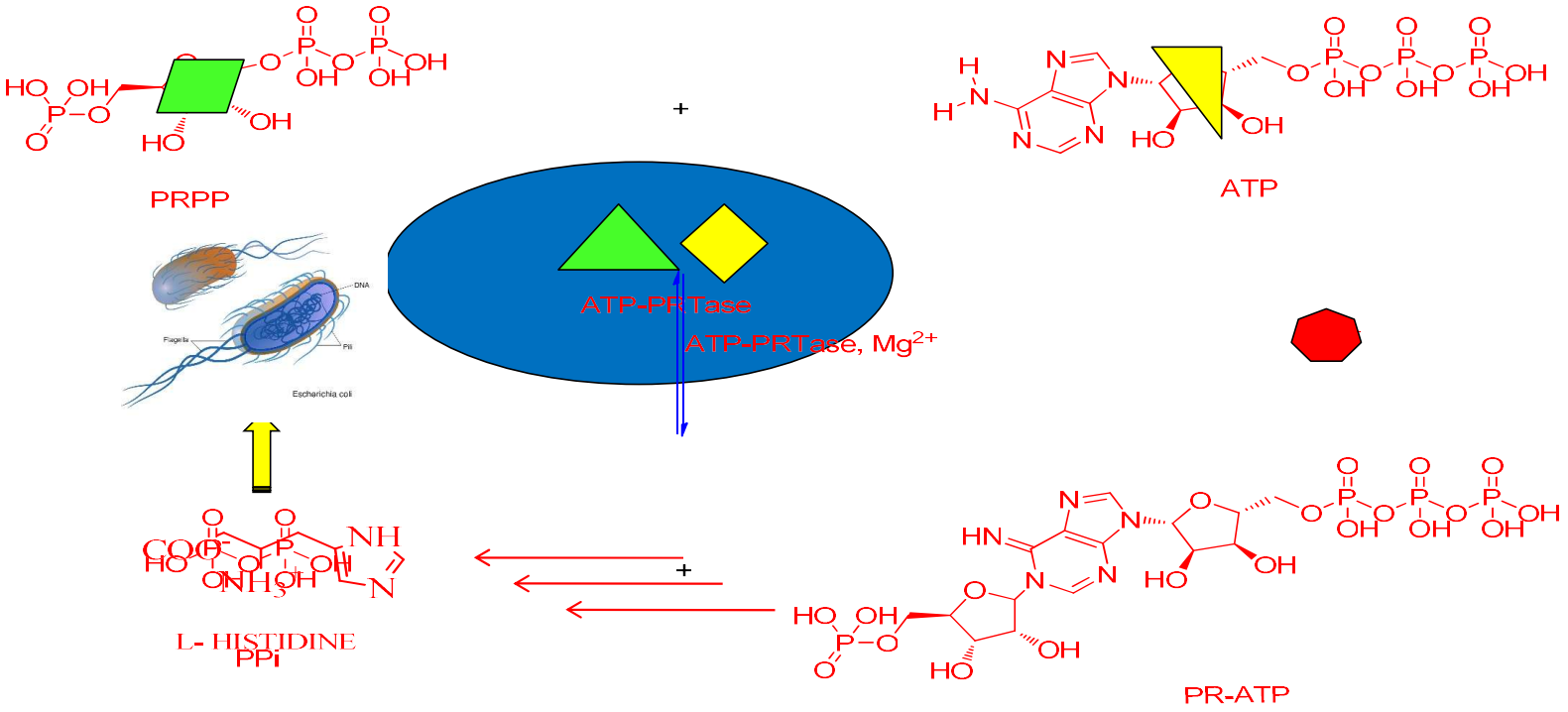
Dose
30-100gm/ton feed.

DURATION
9-16 Weeks in the later laying cycle.

Novel Targets for Bacteria

- Targeting cell wall biosynthesis
- Peptidoglycan biosynthesis
- Arabinogalactan biosynthesis
- Fatty acid biosynthesis
- Targeting amino acid biosynthesis
- Targeting cofactor biosynthesis
- Targeting mycothiol biosynthesis
- Targeting terpenoid biosynthesis
- Targeting DNA synthesis
- Targeting the glyoxylate shunt
- Targeting regulatory proteins
- Inhibiting menaquinone biosynthesis
- Targeting the stringent response enzyme
- **Targeting ATPPRTase**

Role of HisG (ATP-PRTase) Enzyme

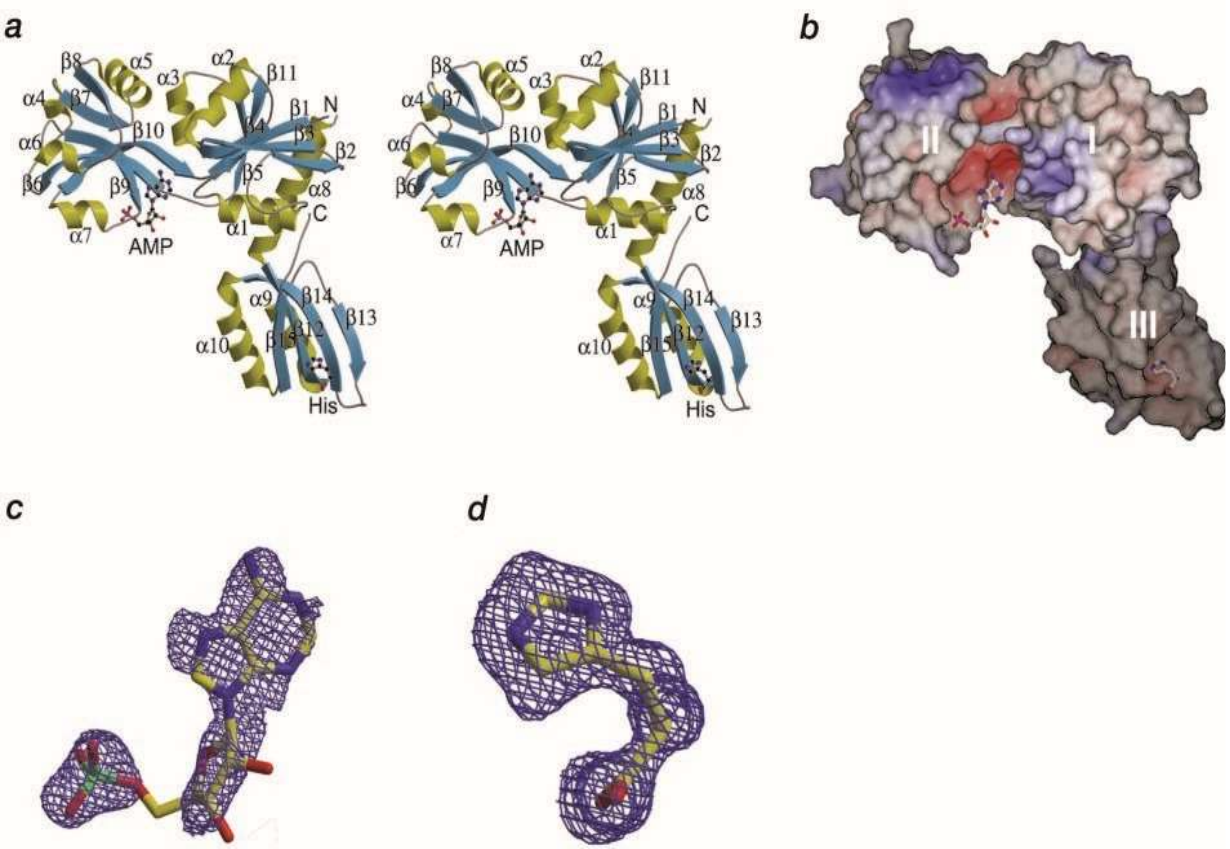


HisG: ATPPRTase as Novel Target for anti-bacterial activity

- The crystal structures of the *N*-1-(5-phosphoribosyl)-ATP transferase from *bacteria* in complex with inhibitor histidine and AMP has been determined to 1.8 Å resolution and without ligands to 2.7 Å resolution

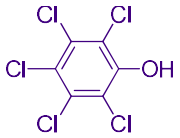
- The active enzyme exists primarily as a dimer, and the histidine-inhibited form is a hexamer. The structure represents a new fold for a phosphoribosyltransferase, consisting of three continuous domains. The inhibitor AMP binds in the active site cavity formed between the two catalytic domains

Crystal Structure of ATP Phosphoribosyltransferase

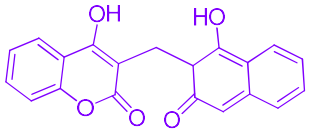


The overall fold of the *mt*ATP-PRTase. *a*, stereo view of the ribbon representation of the *mt*ATP-PRTase protomer. Ribbon is colored by secondary structure with *yellow* for helices, *cyan* for sheets, and *gray* for coils. The ligand AMP and His are shown in *ball-and-stick* representation colored by type of atom. *b*, molecular surface of an ATP-PRTase protomer colored by electrostatic potential. AMP was located in the cleft between domains I and II and the histidine on the allosteric regulatory domain. *c* and *d*, electron density of bound AMP (*c*) and histidine (*d*). Shake & Warp electron density map was averaged from six independent refinements of a composite model

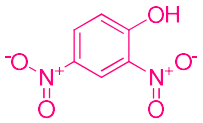
HisG Inhibitors



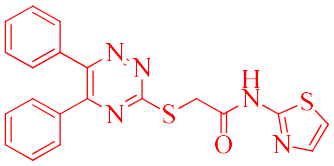
1976
Pentachlorophenol
40 µM



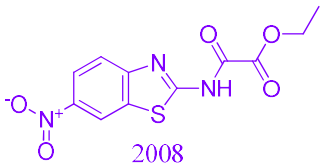
1976
Dicoumerol
60 µM



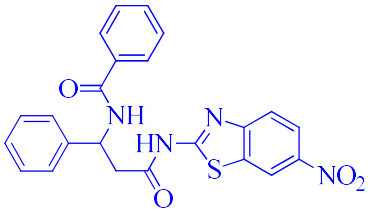
1976
2,4-dinitrophenol
IC_{50} - 0.4 mM *E. coli HisG*



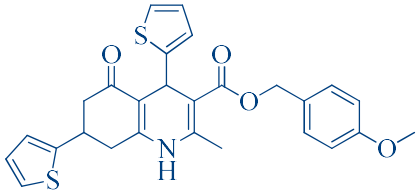
2008
IC_{50} 4µM



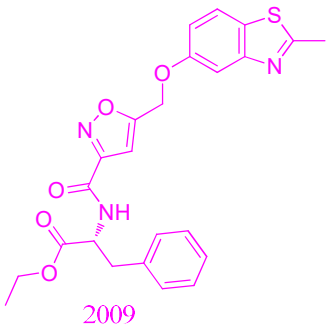
2008
IC_{50} - 13.9 mM *Mtb HisG*



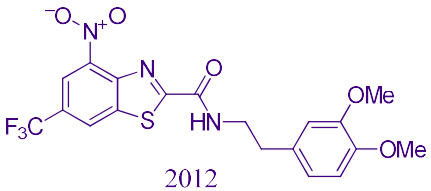
2008
IC_{50} - 6 mM *Mtb HisG*



2008
IC_{50} 10 µM

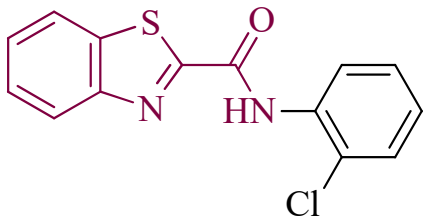


2009
MIC - 1.4 mM *Mtb*



2012
MIC - 35 nM *Mtb*
Sanoffi Aventis

HisG Inhibitors



MIC : 2.70 μM

Molecular docking suggests that these compounds to the catalytic site of the enzyme ATP Phosphoribosyltransferase (HisG) and might be attributed to their anti-bacterial potential. Only docking has been reported in absence of target identification


REVIEW
of
LITERATURE

Benzimidazoles as Agents

Various 6-5 membered rings such as benzimidazoles have been reported for potential anti-bacterial Activity

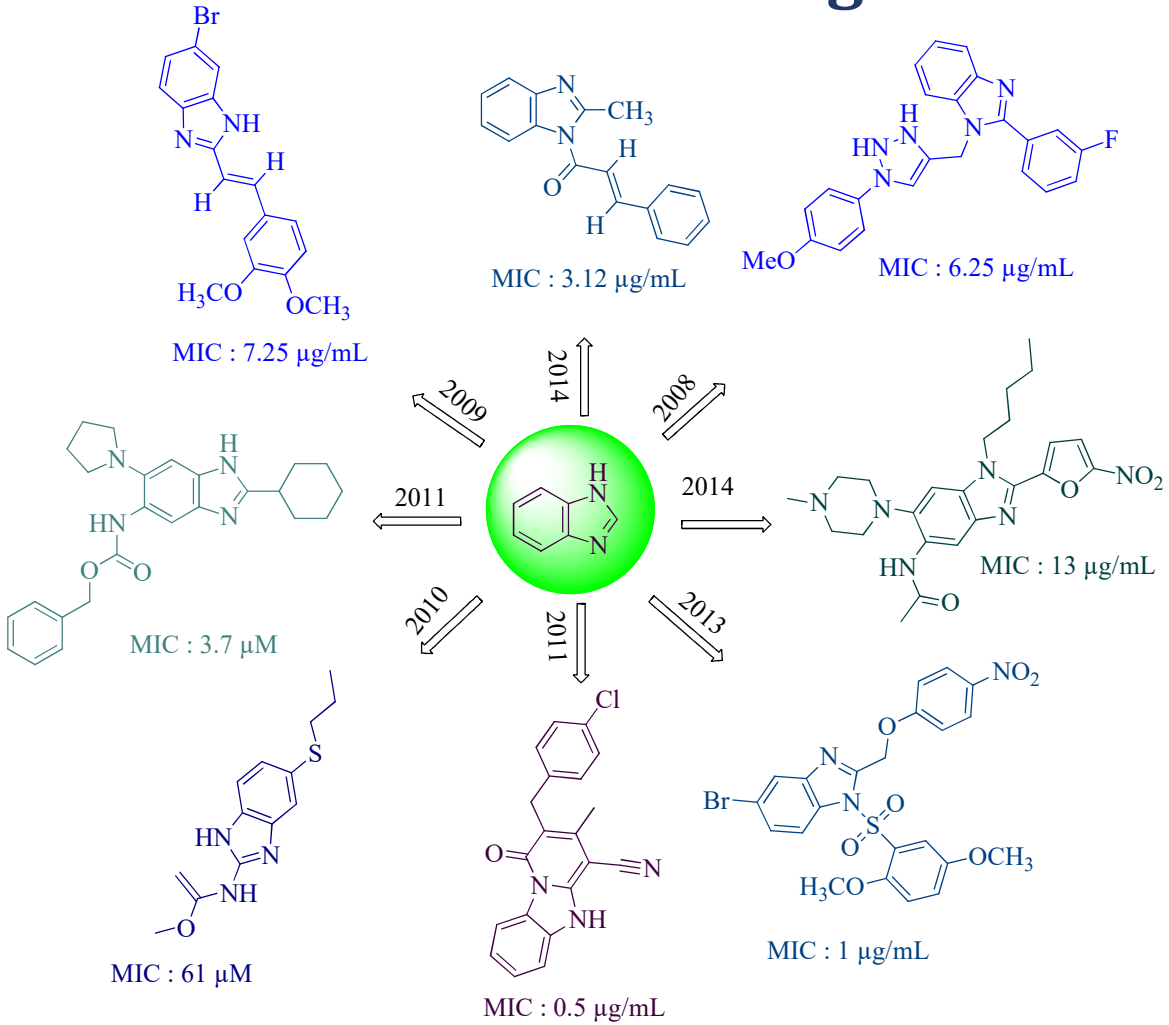


Figure. Various benzo[*d*]imidazole scaffolds with anti-microbial activity

Benzthiazoles as Agents

Various 6-5 membered rings such as benzthiazoles have been reported for potential anti-bacterial Activity

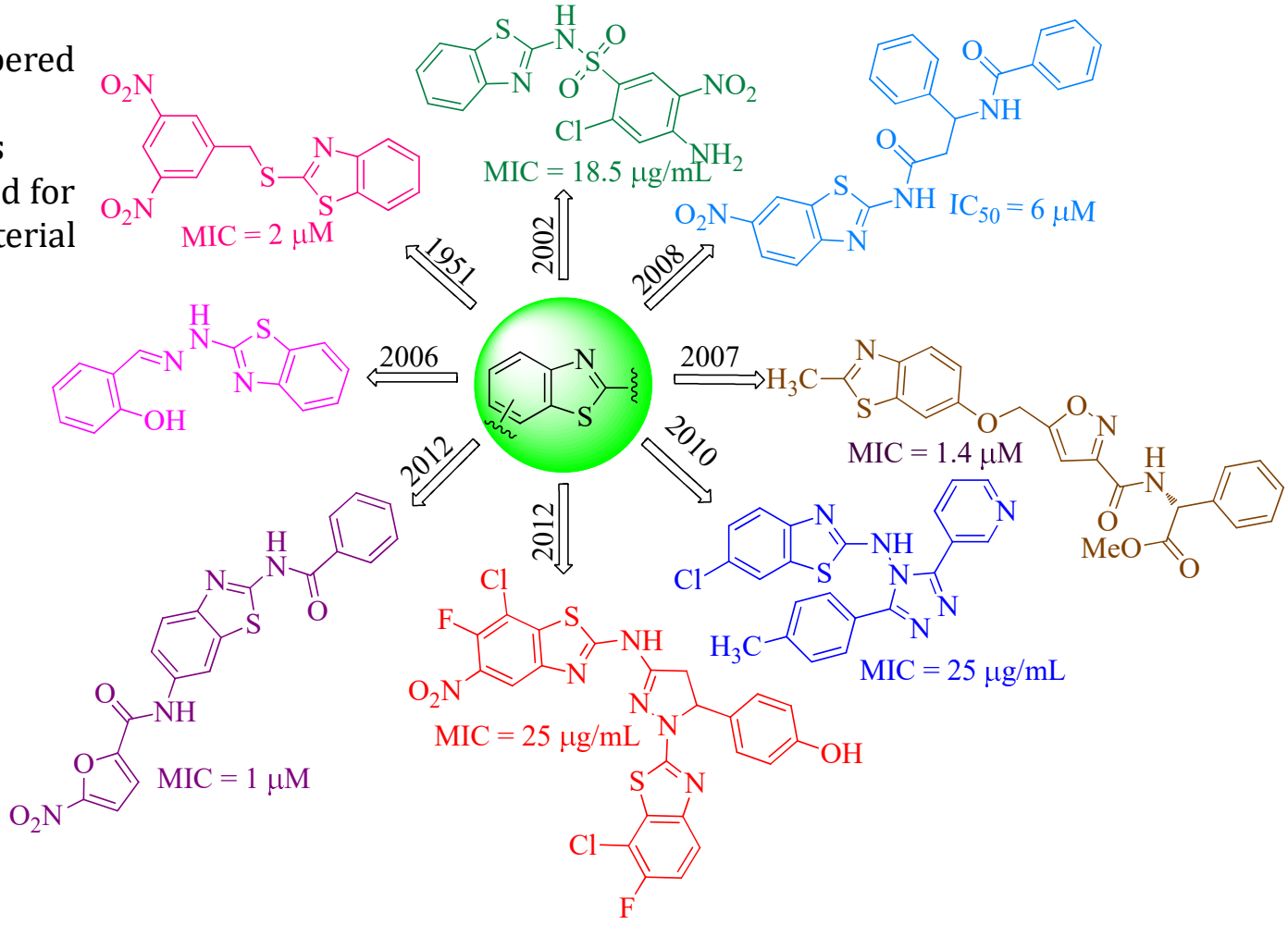


Figure. Various benzhiazole scaffolds with anti-microbial activity

Benzoxazoles as Agents

Various 6-5 membered rings such as benzoxazoles have been reported for potential anti-bacterial Activity

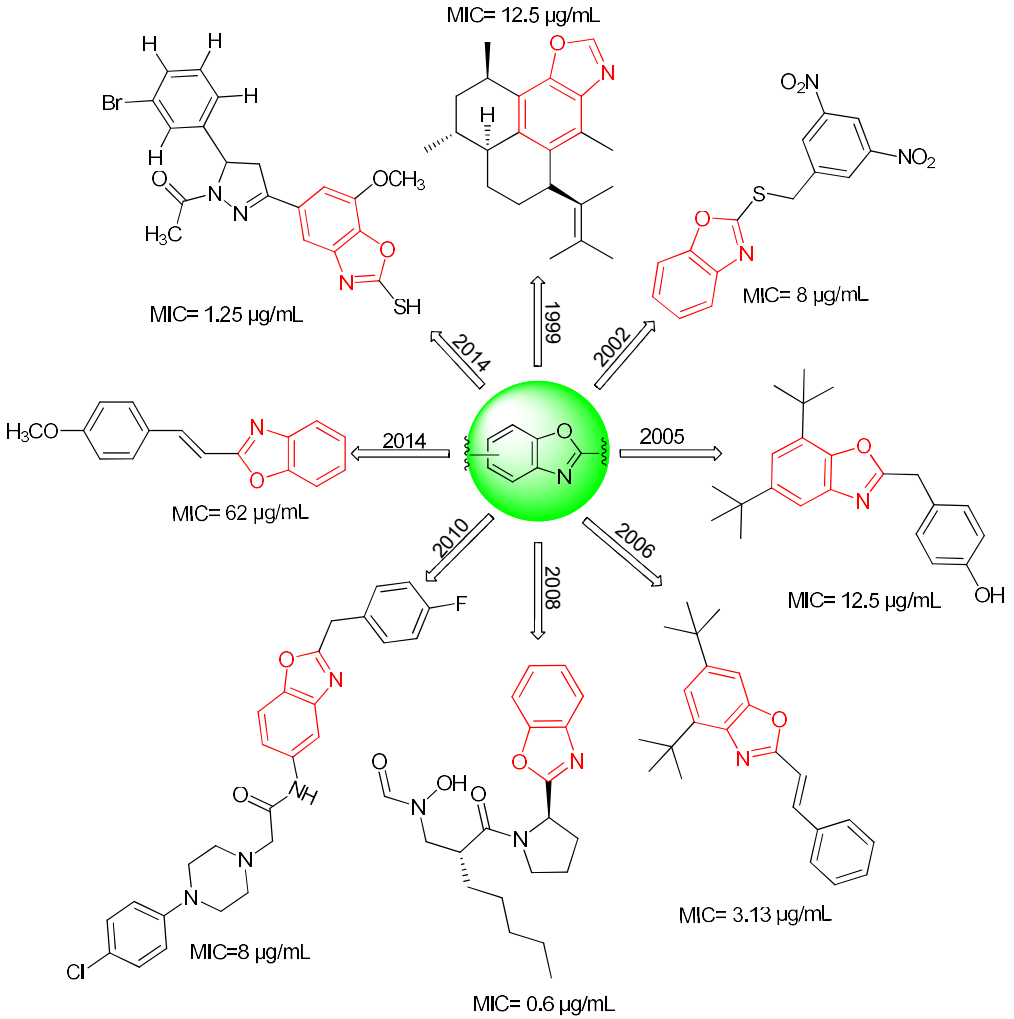
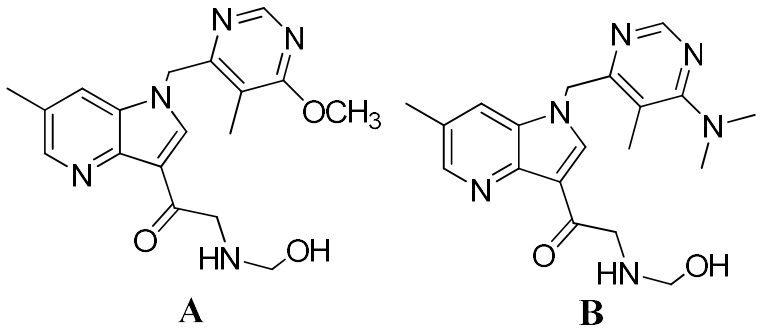


Figure. Various benzoxazole scaffolds with anti-microbial activity

Azaindoles as Agents

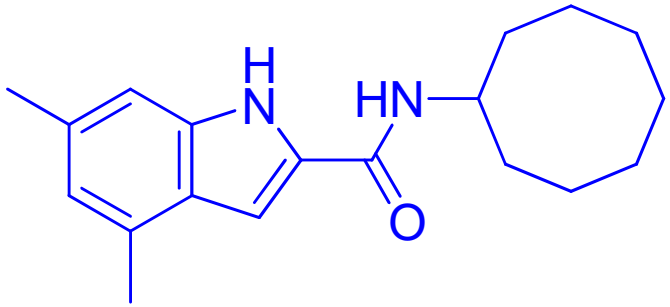
- Chatterji *et al.* have identified two potent inhibitors of bacterial from a series of compounds containing 1,4-azaindole ring as the central moiety
- It demonstrated potent inhibition of decaprenylphosphoryl--D-ribose oxidase (DprE1) with IC_{50} values of 0.010 M and 0.032 M, respectively.



1,4-Azaindole as anti-bacterial agents

Indoles as Agents

- Franz *et al*. reported *N*-cyclooctyl-4,6-dimethyl-1*H*-indole-2-carboxamide as anti-bacterial agents having MIC 0.0195 μm.

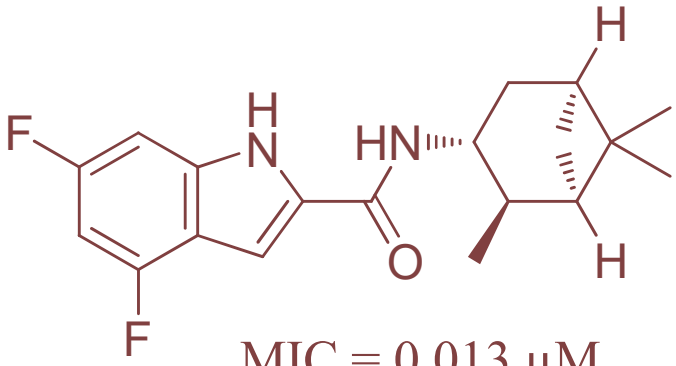


MIC = 0.0195 μM

Franz, N. D.; Belardinelli, J. M.; Kaminski, M. A.; Dunn, L. C.; Calado Nogueira de Moura, V.; Blaha, M. A.; Truong, D. D.; Li, W.; Jackson, M.; North, E. J. Design, Synthesis and Evaluation of Indole-2-carboxamides with Pan Anti-microbacterial Activity. *Bioorg. Med. Chem.* 2017, *25*, 3746–3755

Indoles as Agents

- Stec *et al.* reported 4,6-difluoro-*N*-((1*R*,2*R*,3*R*,5*S*)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-yl)-1*H*-indole-2-carboxamide as anti-bacterial agents



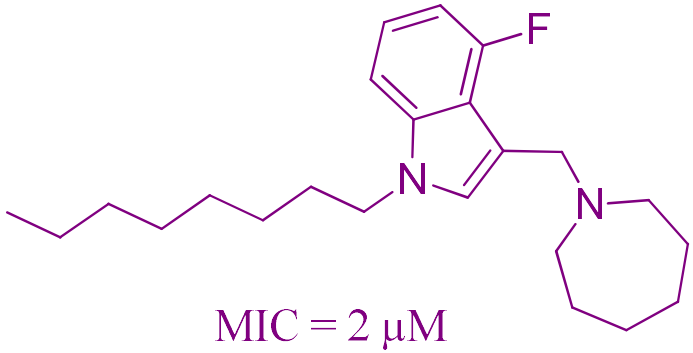
MIC = 0.013 μM

Stec, J., Onajole, O. K., Lun, S., Guo, H., Vistoli, G.; Bishai, W. R.; Kozikowski, A. P. Indole-2-Carboxamide based MmpL3 Inhibitors Show Exceptional Antimicrobial Activity in an Animal Model

J. Med. Chem. **2016**, *59*, 6232–6247

Indoles as Agents

- Yang *et al.* reported 3-(azepan-1-ylmethyl)-4-fluoro-1-octyl-*1H*-indole as anti-bacterial agents.

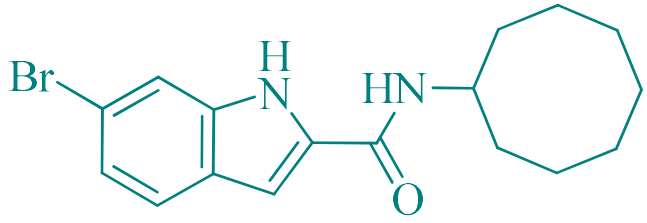


MIC = 2 μM

Yang, T.; Moreira, W.; Nyantakyi, S. A.; Chen, H.; Aziz, D. binte; Go, M. L.; Dick, T. Amphiphilic Indole Derivatives as Antimicrobacterial Agents: Structure-Activity Relationships and Membrane Targeting Properties. *J. Med. Chem.* **2017**, *60*, 2745–2763

Indoles as Agents

- Kozokowski *et al.* reported 6-bromo-*N*-cyclooctyl-1*H*-indole-2-carboxamides as anti-bacterial agents.

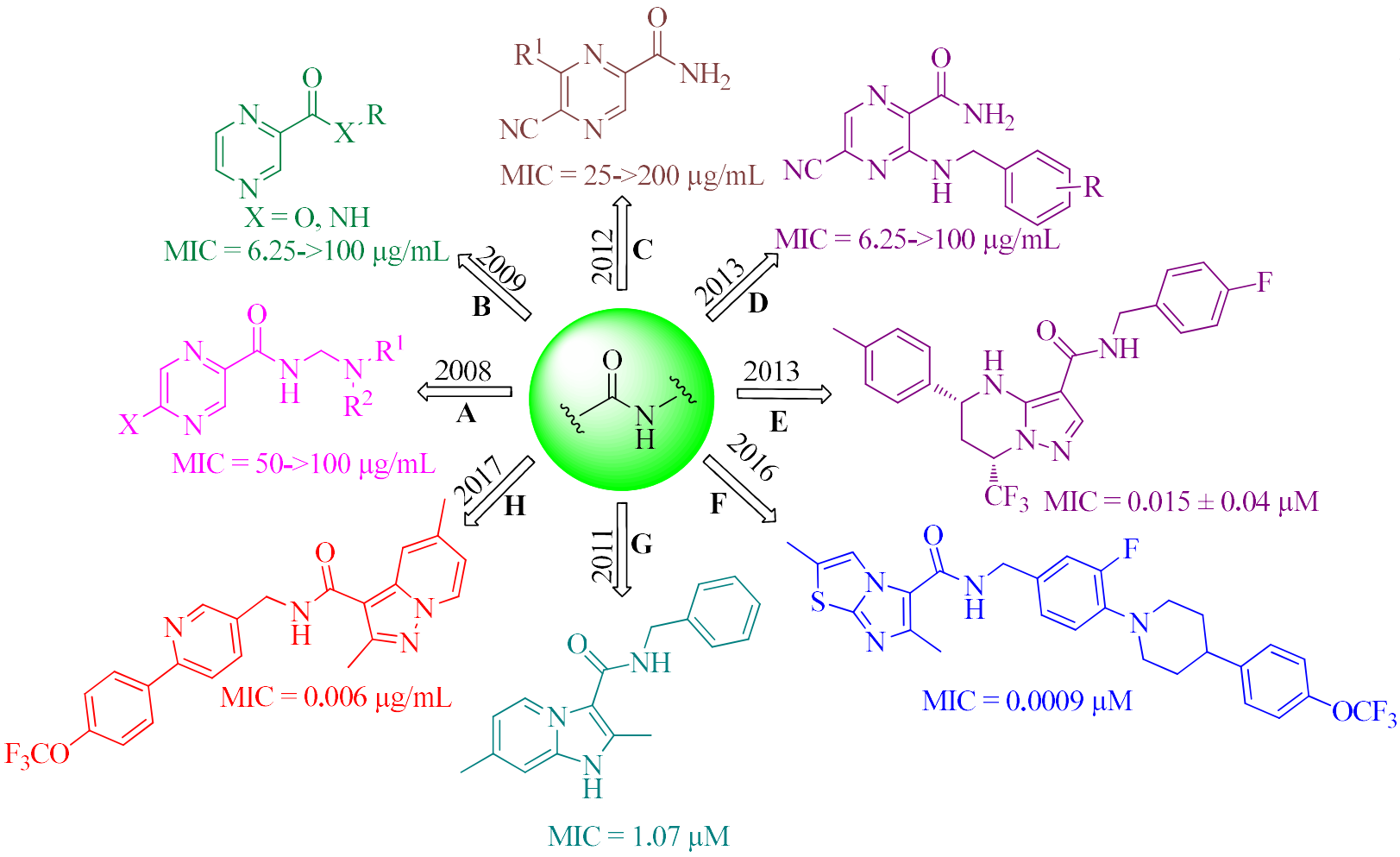


MIC = 0.12 μg/mL
M. abscessus

Kozikowski, A. P.; Onajole, O. K.; Stec, J.; Dupont, C.; Viljoen, A.; Richard, M.; Chaira, T.; Lun, S.; Bishai, W. R.; Raj, V. S.; Ordway, D., Kremer, L. Targeting Mycolic Acid Transport by Indole-2-Carboxamides for the Treatment of *erium abscessus* Infections Targeting Mycolic Acid Transport by Indole-2-carboxamides for the Treatment of *bacterial* Infections. *J. Med. Chem.* **2017**, *60*, 5876–5888

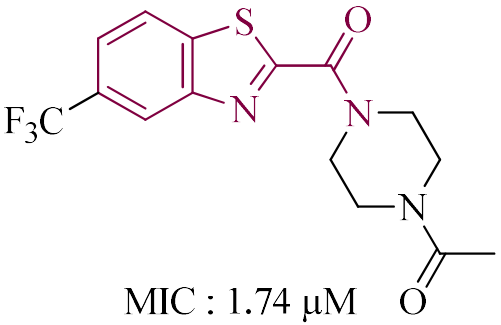

Design
and
Rationale

Various Carboxamides with Anti-Microbial Activity



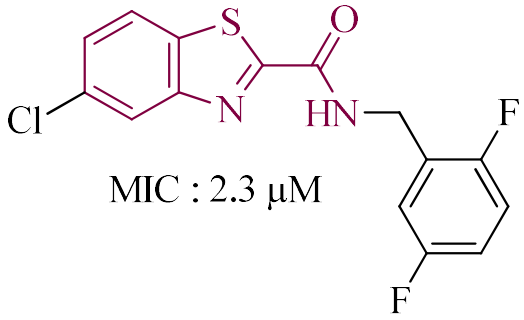
A numbers of compounds bearing amide or carboxamides moieties have been reported for anti-microbial activity. These include aminomethylene pyrazinamides (**A**), pyrazinoic acid amides (**B**), *N*,*N*′-alkyl-diylpyrazine-2-carboxamides(**C**), pyrazinamide derivatives with benzylamino substitutions (**D**), tetrahydropyrazolopyrimidines (**E**), imidazo [2,1-*b*] thiazole-5-carboxamides (**F**), imidazo[1,2-a]pyridine-3-carboxamides (**G**), and pyrazolo [1,5-*a*] pyridine-3-carboxamides (**H**).

Benzo[*d*]thiazole-2-carboxaimdes scaffolds reported for anti-microbial activity



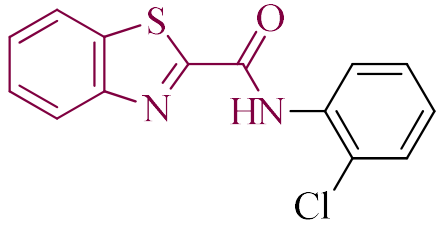
MIC : 1.74 μM

Eur. J. Med. Chem. **2016**, *116*, 187–199



MIC : 2.3 μM

Med. Chem. Commun. **2014**, *5*, 1489-1495

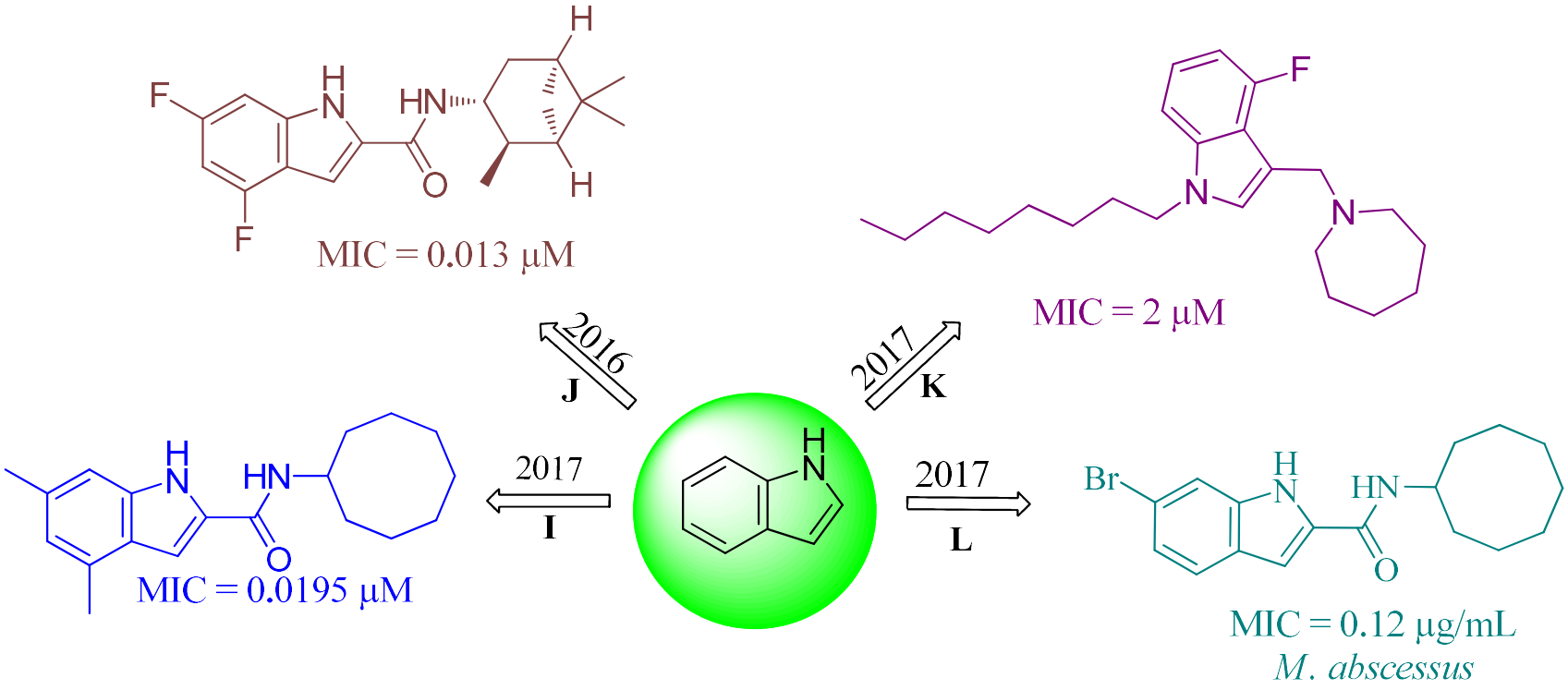


MIC : 2.70 μM

Eur. J. Med. Chem. **2018**, *155*, 364-380

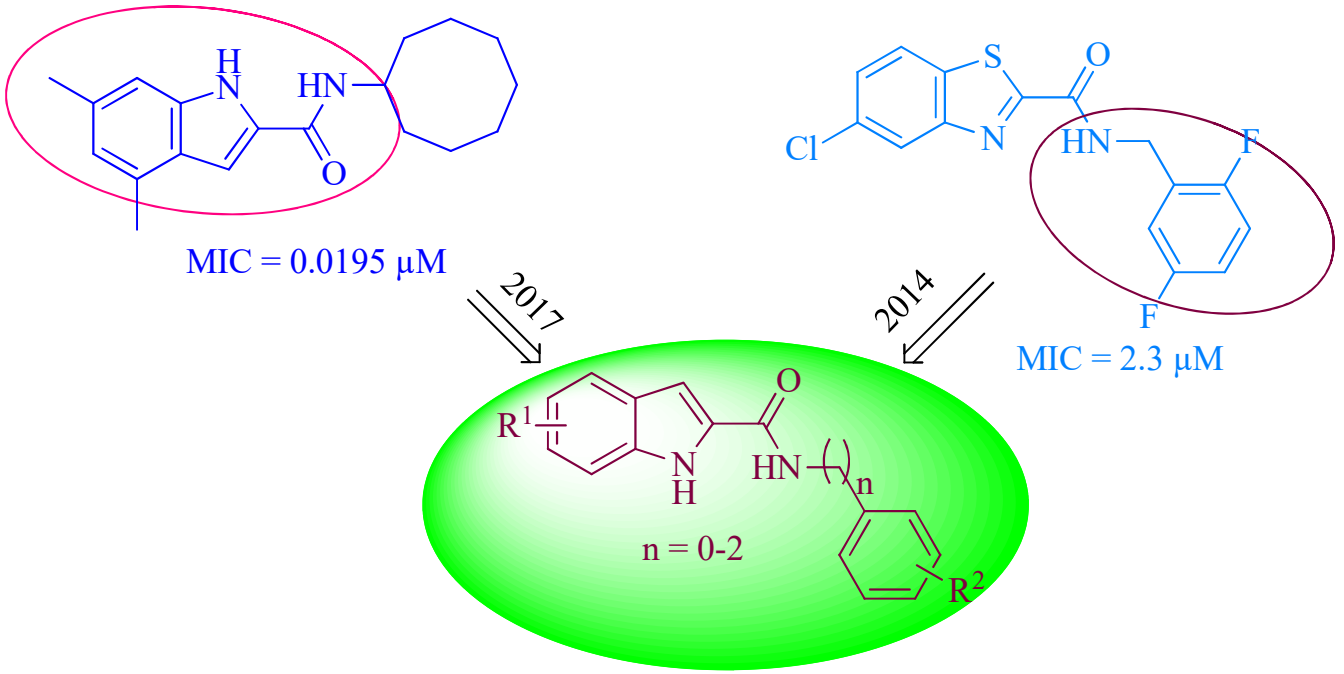
Recently, benzo[*d*]thiazole-2-caboxamides have been identified as key pharmacophoric scaffold for anti-microbial activity

Literature reported indole derivatives as anti-microbial agents



Various substituted indoles have been identified as the potent anti-bacterial agents such as 4,6-dimethyl-1*H*-indole-2-carboxamide (**I**), 4,6-difluoro-*N*-((1*R*,2*R*,3*R*,5*S*)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-yl)-1*H*-indole-2-carboxamide (**J**), 4-fluoro-1-octyl-1*H*-indoles (**K**), and 6-bromo-1*H*-indole-2-carboxamide (**L**)

New indole scaffold with amide substitution



***N*-Benzyl-1*H*-indole-2-carboxamides**

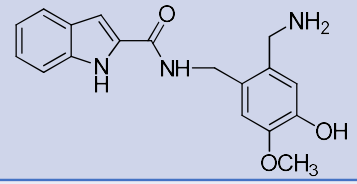
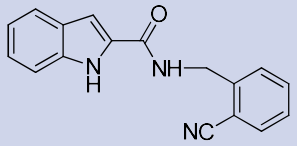
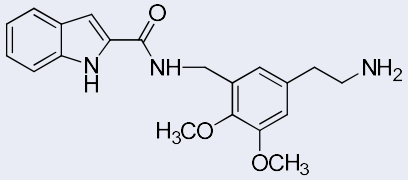
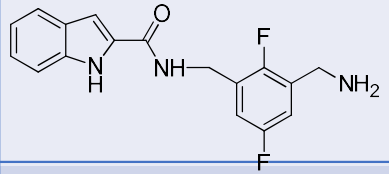
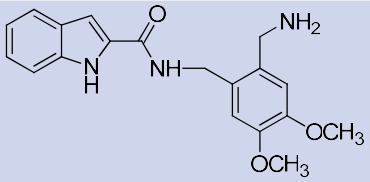
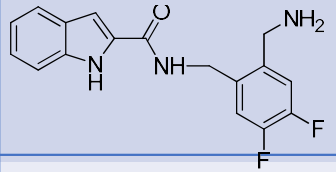
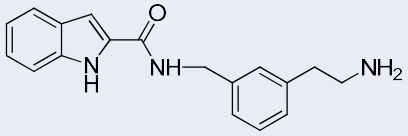
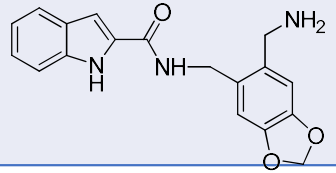
New indole-2-carboxamide scaffold (**I**) with C-2 reverse amide substitution, R^1, R^2 may be various substituents such as alkyl, halide, etc

With this background, compounds belonging to *N*-benzyl-1*H*-indole-2-carboxamide scaffold are proposed for synthesis and biological evaluation of anti-bacterial potential

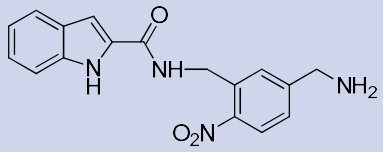
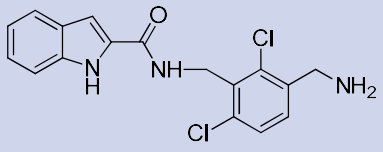
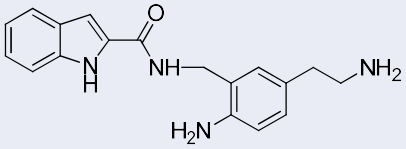
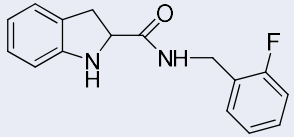
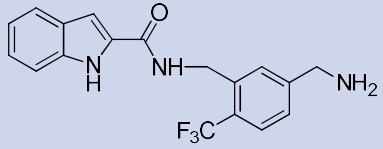
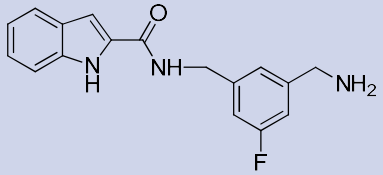
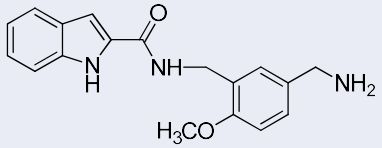
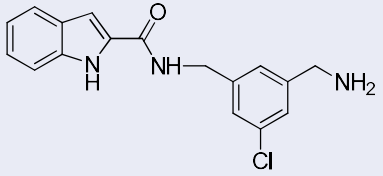
Molecular Docking

- To determine the probable mode of action of the compound, the designed compounds were docked against adenosine triphosphate phosphoribosyl transferase (ATPPRTase) HisG (PDB ID: 1NH8) which is the probable and newly emerging target in bacteria and fungus.
- The designed compounds have been docked against this protein using Maestro 12.1 (Schrodinger) and compared with reported inhibitor of HisG.

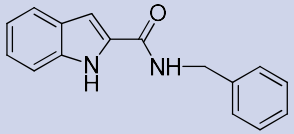
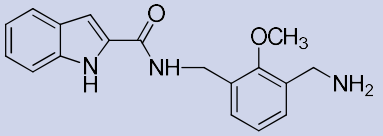
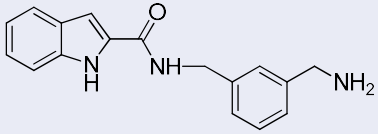
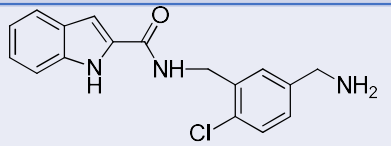
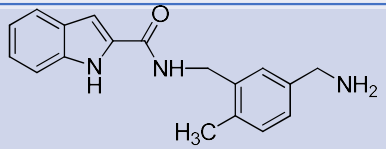
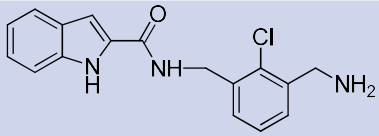
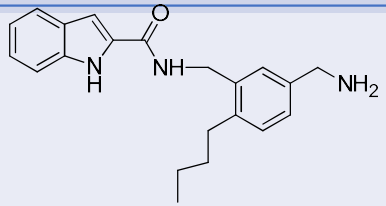
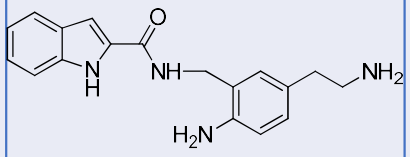
Molecular Docking

Sr. No.	Structure	Docking score	Amino acids Interactions	Sr. No.	Structure	Docking score	Amino acids Interactions
1		-9.313	Phe87, Gly88, Ser89, Ser90, Val155, Val156, Gly157, Ser158, Gly159, Arg160, Thr161, Leu165, Pro15, Ala11	5		-7.850	Leu12, Ala11, Gly157, Val155, Ser90,ser89, Gly88, Phe87,leu12, Ala11
2		-8.305	Asp154, Val155,156, Gly157, Ser158, Gly159, Arg160, Thr161, Ala139, Gly138, Ser90	6		-6.823	Ala139, Gly138, Val140, Glu141, Val156, Val155, Asp154, Leu71, Tyr116, Pro50, Lys51
3		-8.173	Val155, Val156, Gly157, Ser158, Gly159, Arg160, Thr161, Leu162, Ser90, Ser89, Gly88, Phe87, Ala11, Leu12, Pro15	7		-6.788	Val155, Val156, Gly157, Ser168, Gly150, Arg160, Thr161, Ser90, Ser89, Gly88, Phe87, Ala11, Leu12, ```Pro15.
4		-7.921	Gly138, Ala139, Val140, Glu141, Thr161, Asp154, Lys51, Pro50, Tyr116, Leu71	8		-6.722	Gly157, Pyr116, Asp154, Val155, Val156, Gly138, Ala138, Val140, Glu141, Thr161

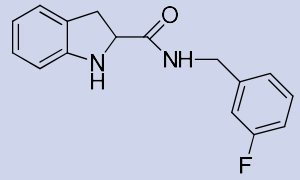
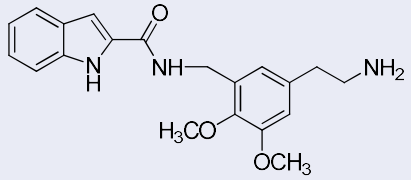
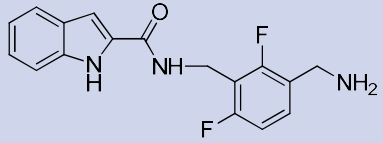
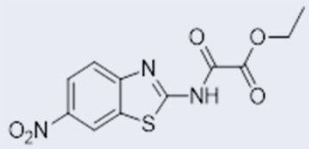
Molecular Docking

Sr. No.	Structure	Docking score	Amino acids Interactions	Sr. No.	Structure	Docking score	Amino acids Interactions
9		-6.611	Gly86, Phe87, Gly88, Ser89, Ser90, Ala11, Leu12, Bal155, Gly157	13		-6.533	Ala11, Leu12, Phe87, Gly88, Ser89, Ser90, Val155, Gly157
10		-6.577	Pro50, Lys51, Leu71, Tyr116, Ase154, Bal155, Glu141, Bal140, Ala139, Gly138, Thr161	14		-6.516	Tro15, Leu71,lys51, Val155, Ser90, Gly88, Leu12, Ala11, Tyr116, Ala115, Gly138, Asp157
11		-6.564	Pro50, Lys51, Leu71, Pyr116, Asp154, Val155, Val156, Gly138, Ala138, Val140, Glu141, Thr161	15		-6.504	Ala11, Leu12, Pro15, The87, Gly88, Ser89, Ser90, Thr161, Arg116, Gly159, Ser158, Gly157, Val156
12		-6.564	Pro50, Lys51, Leu71, Tyr116, Gly136, Ala139, Val140, Glu141, Ase154, Val155, Thr161	16		-6.504	Ala11, Leu12, Pro15, Phe57, Gly88, Ser89, Ser90, Val155, Val156, Gly157, Ser158, Gly159, Arg160, Thr161

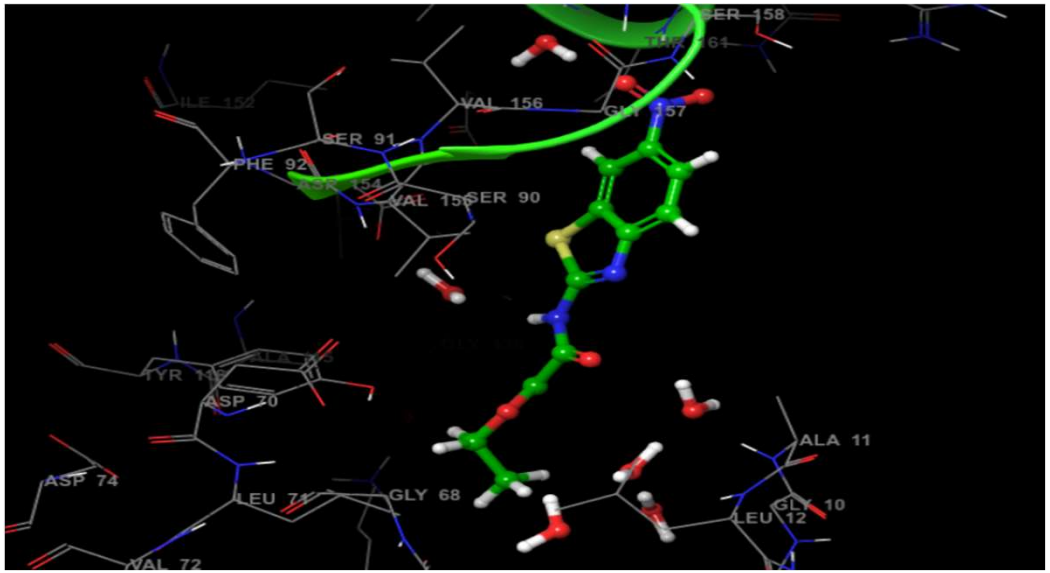
Molecular Docking

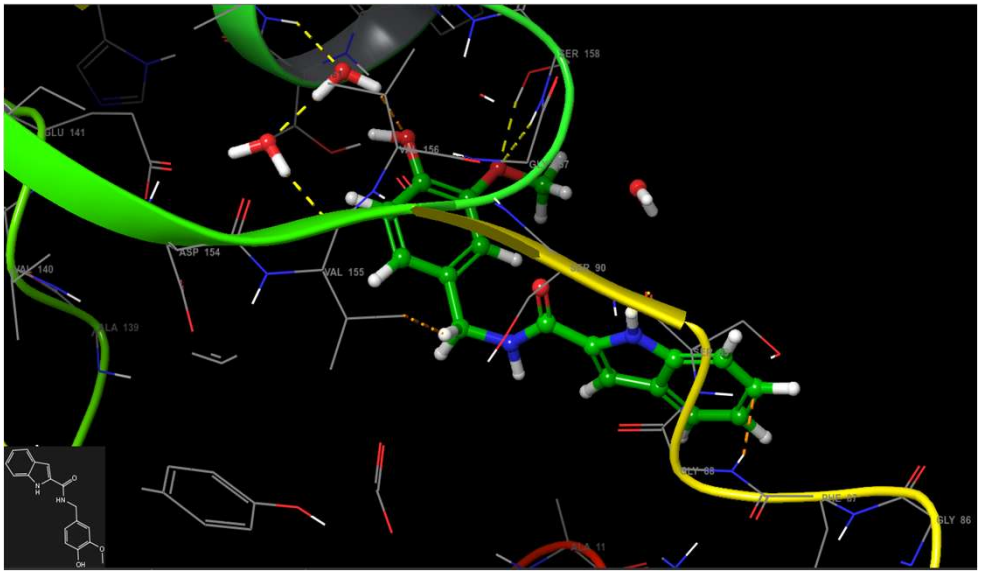
Sr. No.	Structure	Docking score	Amino acids Interactions	Sr. No.	Structure	Docking score	Amino acids Interactions
17		-6.461	Ala11, Tro15, The87, Gly88, Ser89, Ser90, Val155, Pla156, Gly157, Ser158, Gly159, Arg160, Thr161	21		-6.211	ALA11, LEU12, GLY157, VAL155, TYR116, LEU171, SER180, GLY188, PHA187
18		-6.39	Gly138, Ala139, Val140, Glu141, Asp154, Val155, Gly157, Ser158, Arg160, Thr161	22		-6.189	Ala11, Ala139, Val 140, Glu 141, Asp154, Val 155,val156, Gly157, Ser158, Thr161, Ser90, Ser89, Gly88, Ala11, Leu12
19		-6.378	Gly138, Ala139, Val140, Glu141, Asp154, Val155, Gly157, Ser158, Arg160, Thr161	23		-6.177	Ala139, Ala140, Val140, Glu141, Asp154, Val155, Val156, Gly157, Ser158, Thr161, Ser90, Ser89, Gly88, Ala11, Leu12
20		-6.314	Ala11, Leu12, Gly86, The87, Ser89, Ser90, Val155, Val156, Gly157, Ser158, Thr161	24		-6.16	Glu141, Val140, Ala139, Thr161, Ser158, Gly157, Val156, Val155, Asp154, Tyr116, Ala115, Leu71, Lys51, Pro50

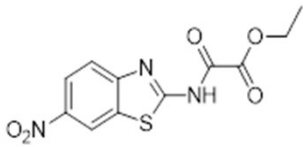
Molecular Docking

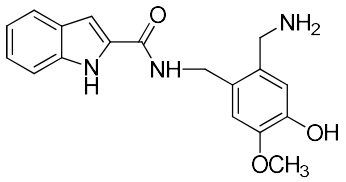
Sr. No.	Structure	Docking score	Amino acids Interactions
25		-6.152	Val155, Val156, Gly157, Ser158, Gly159,arg156,thr161, Ser90, Ser89, Gly88, The87, Tro15, Leu12, Ala 11
26		-6.086	Thr161, Ser158, Gly157, Val156, Val155, Asp154, Tyr116, Ala115, Leu71, Lys51, Pro50, Glu141, Val140, Ala139
27		-6.55	Val155, Val156, Gly157, Ser158, Gly159, Arg160, Thr161, Ser90, Ser89, Gly88, Phe87, Pro15, Leu12, Ala11
28		-8.841 **Standard**	Ser91, Phe92, Val15, Ser90, Val156, The161, Ser158, Tyr116, Asp74, Asp70, Leu71, Gly68, Asp74

Molecular Docking





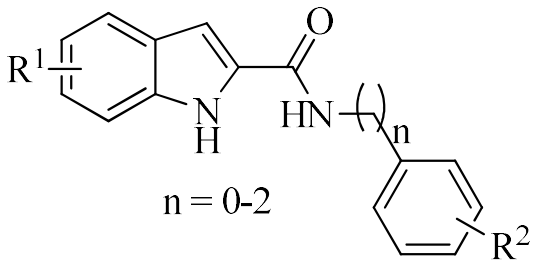




Docking pose of HisG inhibitor (nitrobenzothiazole derivative) with HisG (ATPPRTase)
Interacting aminoacid residues: Ser91, Phe92, Val15, Ser90, Val156, The161, Ser158, Tyr116, Asp74, Asp70, Leu71, Gly68, Asp74

Docking pose of designed compound with HisG (ATPPRTase)
Interacting aminoacid residues: Phe87, Gly88, Ser89, Ser90, Val155, Val156, Gly157, Ser158, Gly159, Arg160, Thr161, Leu165, Pro15, Ala11

Objectives

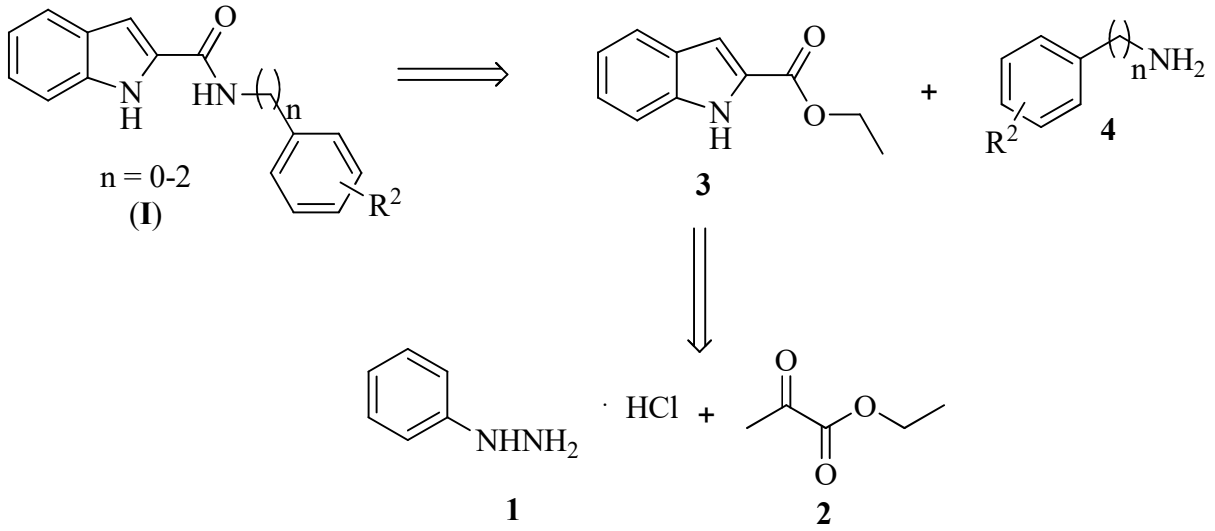


***N*-Arylalkyl-1*H*-indole-2-carboxamides**

New indole-2-carboxamide scaffold (**I**) with C-2 reverse amide substitution, R^1, R^2, may be various substituents such as alkyl, halide, etc

1. Design of the proposed compounds (**I**)
2. Synthesis of the derivatives of indole-2-carboxamides and their characterization
3. Submission of synthesized compounds for anti-bacterial evaluation

Synthetic Strategy



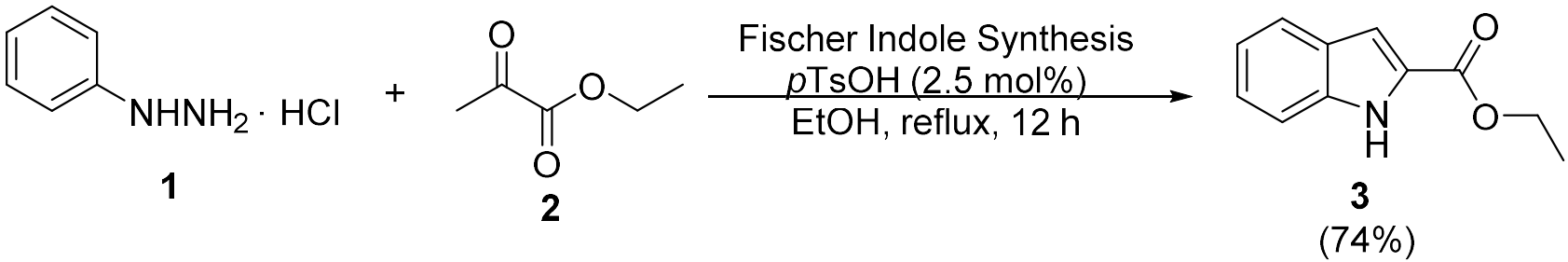
Scheme. Retro-synthesis of the indole-2-carboxamides.

The key intermediate, ethyl indole-2-carboxylate (**3**) required for the synthesis of the target compounds (**I-III**) has been thought to synthesize using Fischer indole synthesis from cyclocondensation reaction between phenyl hydrazine hydrochloride (**1**) and ethyl pyruvate (**2**) using of *p*-tosylic acid as catalyst and ethanol as solvent.
To synthesize the target compounds of series **I**, **3** would be subjected to amidation reaction using ammonium chloride under neat conditions with varieties of commercially available and substituted amines such as anilines (n = 0), benzylamines (n = 1) and phenethyl amines (n = 2)

Franz N.D., Belardinelli J.M., Kaminski M.A., Dunn L.C., de Moura V. C. N., Blaha M.A., "Design, synthesis and evaluation of indole-2-carboxamides with pan anti-microbacterial activity" *Bioorg. Med. Chem.* **2017,** *25,* 3746–55.


WORK DONE

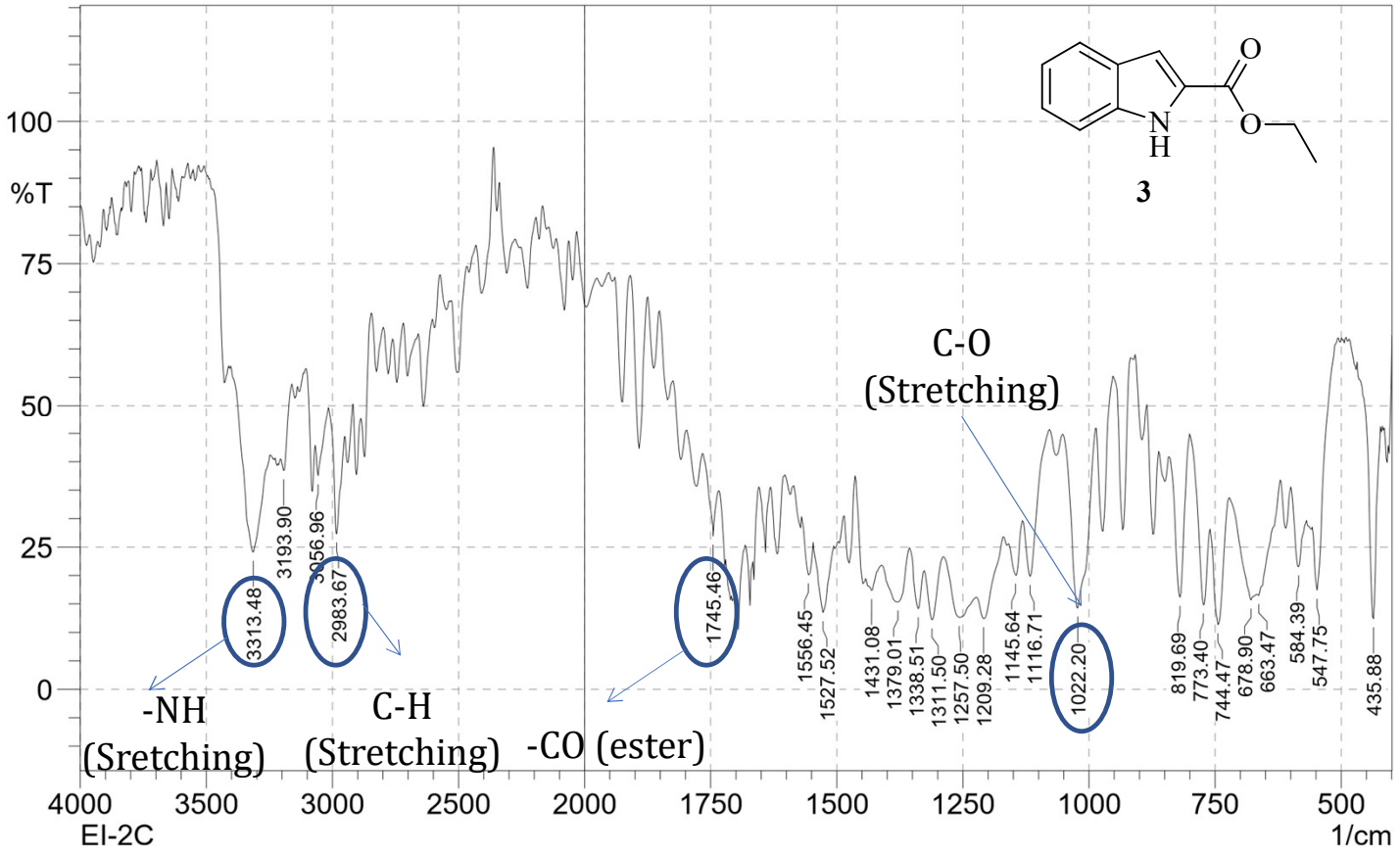
Work Done



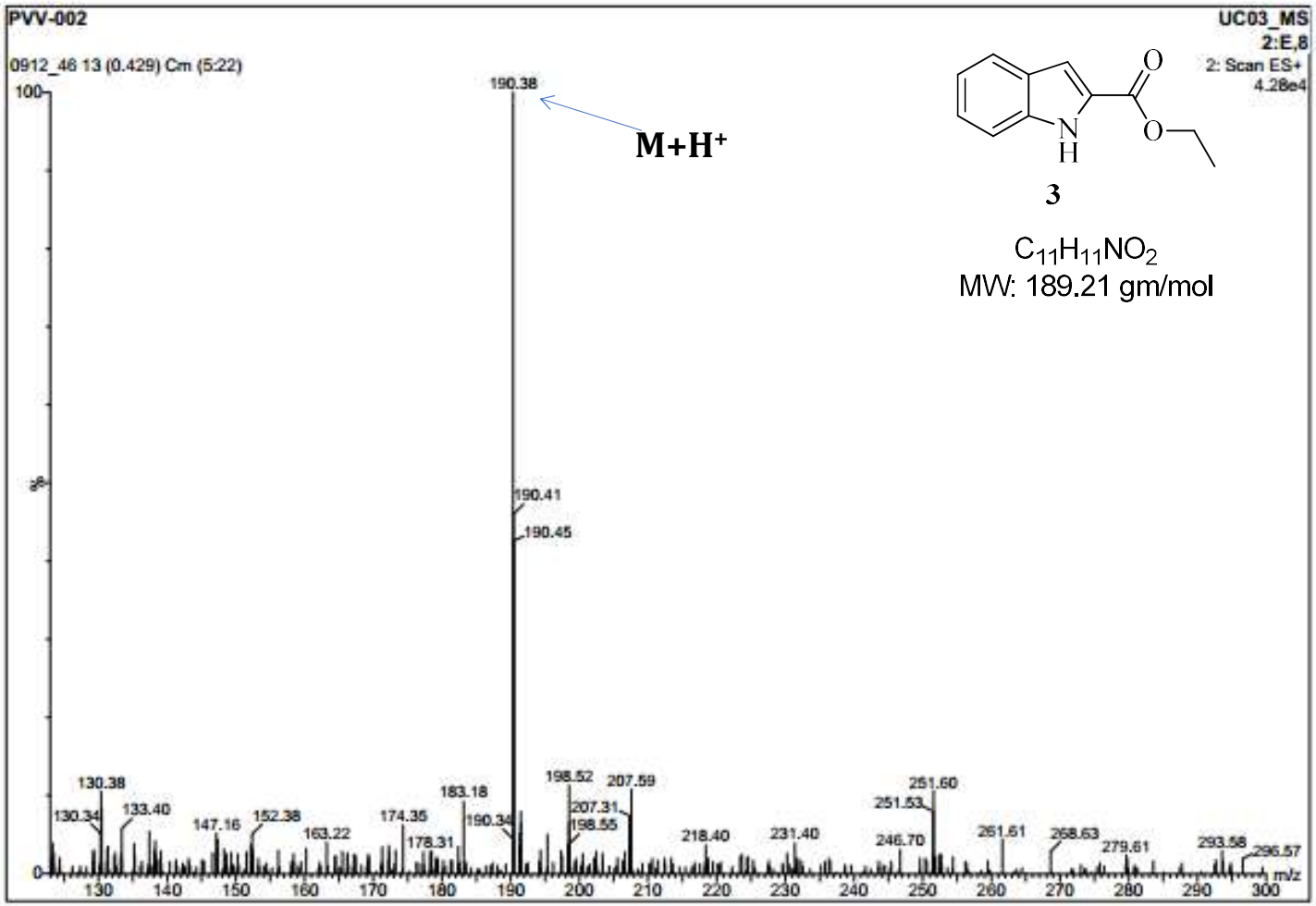
White solid, 74%, mp: 125-128 ˚C (reported value: 128-130), R_f: 0.57 (*n*-Hexane: EtOAc = 7:3); IR (KBr, cm^{-1}) 3313 (NH), 1745 (C=O), 2983 (C-H), 3056 (Aromatic C-H); ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.97 (s, 1H), 7.72 (d, *J* = 8.0; 1H), 7.45 (d, *J* = 8.0; 1H), 7.35 (t, *J* = 7.4; 1H), 7.28-7.25 (m, 1H); 7.20-7.16 (m, 1H); 4.44 (q, *J* = 7.1 Hz, 2H), 1.44 (t, *J* = 7.2 Hz, 3H); MS (ESI): 190 $(M+H)^+$

Franz N.D., Belardinelli J.M., Kaminski M.A., Dunn L.C., de Moura V. C. N., Blaha M.A., "Design, synthesis and evaluation of indole-2-carboxamides with pan anti-microbacterial activity" *Bioorg. Med. Chem.* **2017,** *25,* 3746–3755

FT-IR of 3

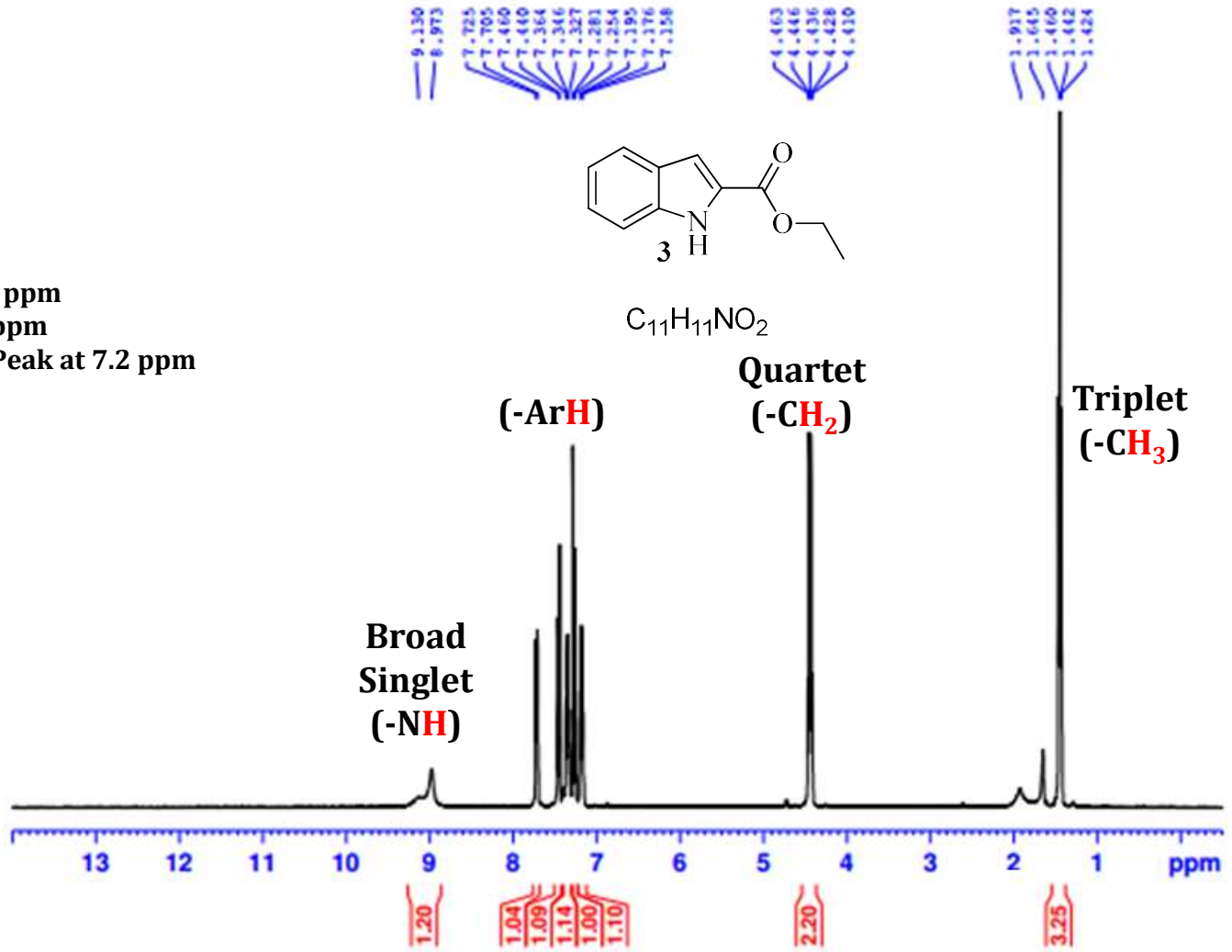

100
%T
75
50
25
0
4000
3500
3000
2500
2000
1750
1500
1250
1000
750
500
1/cm
EI-2C
3313.48
3193.90
3056.96
2983.67
1745.46
1556.45
1527.52
1431.08
1379.01
1338.51
1311.50
1257.50
1209.28
1145.64
1116.71
1022.20
819.69
773.40
744.47
678.90
663.47
584.39
547.75
435.88
-NH
(Sretching)
C-H
(Stretching)
-CO (ester)
C-O
(Stretching)
O
N
H
O
3

MS of 3

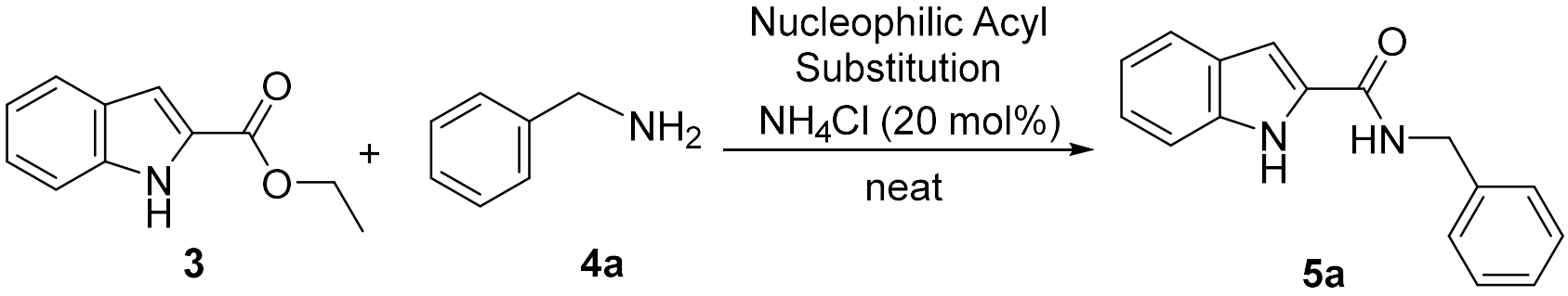

PVV-002
0912_46 13 (0.429) Cm (5:22)
UC03_MS
2:E,8
2: Scan ES+
4.28e4
100
%
0
190.38
M+H+
190.41
190.45
130.38
130.34
133.40
147.16
152.38
163.22
174.35
178.31
183.18
190.34
198.52
207.59
207.31
198.55
218.40
231.40
246.70
251.60
251.53
261.61
268.63
279.61
293.58
296.57
m/z
130
140
150
160
170
180
190
200
210
220
230
240
250
260
270
280
290
300
O
O
N
H
3
C11H11NO2
MW: 189.21 gm/mol

^{1}H NMR of 3

Moisture at 1.6 ppm
Grease at 1.9 ppm
Solvent ($CDCl_3$) Residual Peak at 7.2 ppm



Work Done

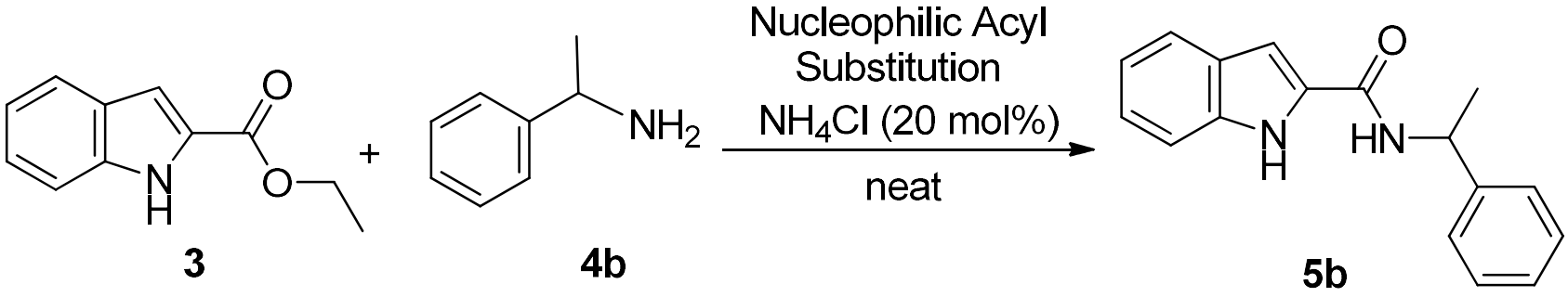


Sr. No.	Time (h)	Temp. (°C)	Yield 5a (%)	Remark
1	12	rt	0	**3** recovered
2	12	100	0	**3** recovered

Shah, P.; Dhameliya, T. M.; Bansal, R.; Nautiyal, M.; Kommi, D. N.; Jadhavar, P. S.; Sridevi, J. P.; Yogeeswari, P.; Sriram D.; Chakraborti, A. K. "*N*-Arylalkylbenzo[*d*]thiazole-2-carboxamides as Anti-mycobacterial Agents: Design, New Methods of Synthesis and Biological Evaluation," *Med. Chem. Commun.* **2014**, *5*, 1489-1495

Work Done

Changing benzyl amine to (+)α-methyl benzyl amine

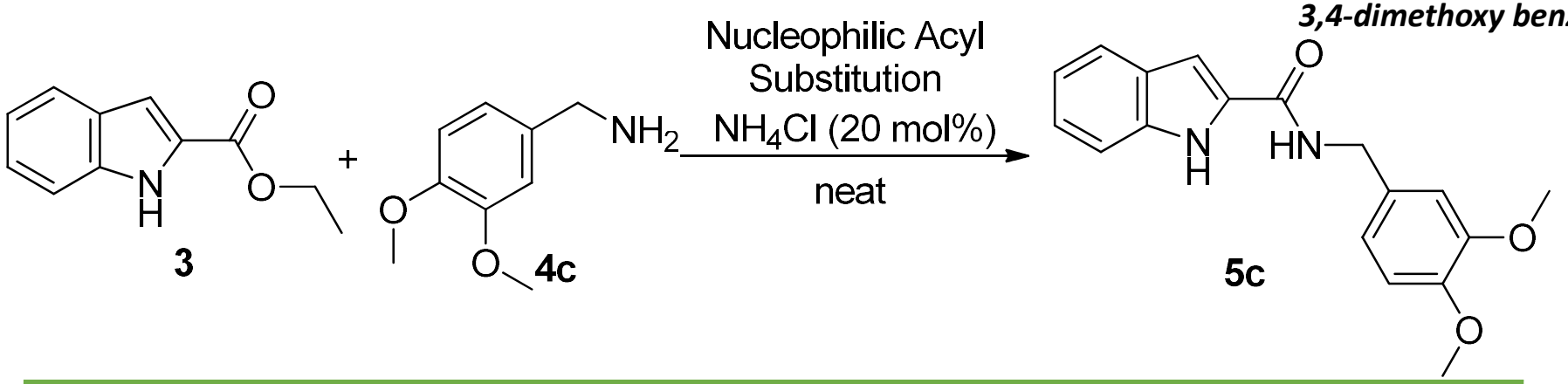


3 **4b** **5b**

Sr. No.	Time (h)	Temp. (°C)	Yield 5b (%)	Remark
3	12	rt	0	**3** recovered
4	12	100	0	**3** recovered

Work Done

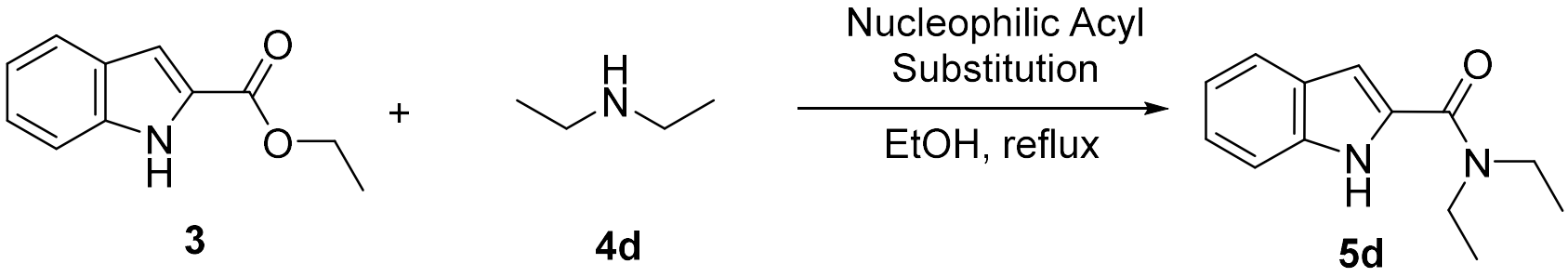
Changing benzyl amine to 3,4-dimethoxy benzylamine



Sr. No.	Time (h)	Temp. (°C)	Yield 5c (%)	Remark
5	12	rt	0	**3** recovered
6	26	100	0	**3** recovered

Work Done

Changing benzyl amine to diethyl amine



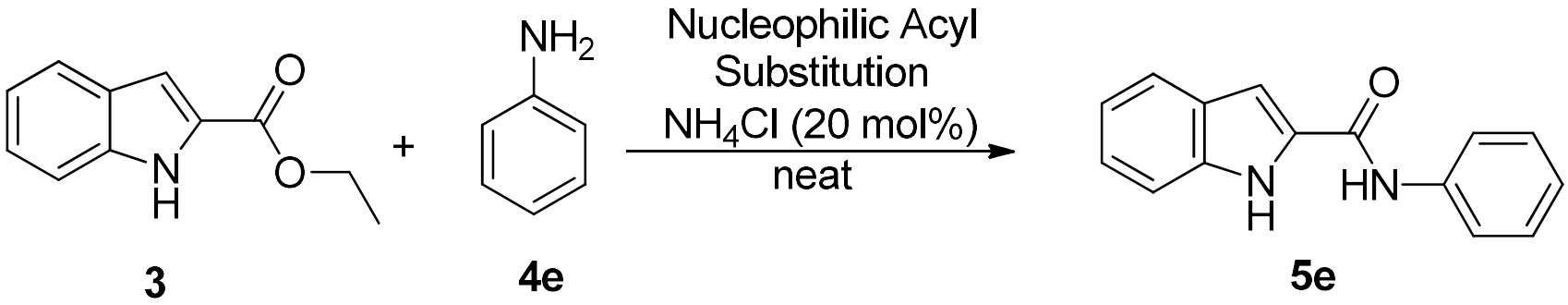
Sr. No.	Time (h)	Temp. (°C)	Yield 5d (%)	Remark
7	12	rt	0	**3** recovered
8	26	78	0	**3** recovered

Although the reaction is reported methyl indole-2-carboxylate with ethyl amine, but we failed to get **5d** using the reported conditions between the reaction of **3** and diethyl amine (**4d**)

Mistry S. N., Shonberg J., Draper-Joyce C. J., Herenbrink C. K., Michino M., Shi L., Christopoulos A., Capuano B., Scammells P. J., Lane J. R. Discovery of a Novel Class of Negative Allosteric Modulator of the Dopamine D2 Receptor Through Fragmentation of a Bitopic Ligand, *J. Med. Chem.* **2015**, *58*, 6819-6843

Work Done

Changing benzyl amine to aniline



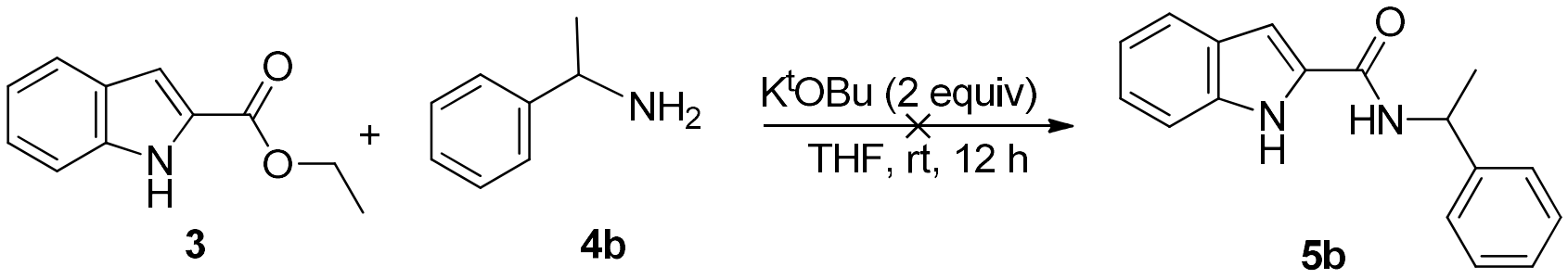
3 **4e** **5e**

Sr. No.	Time (h)	Temp. (°C)	Yield 5e (%)	Remark
9	12	rt	0	**3** recovered
10	26	100	0	**3** recovered

The reaction didn't undergo with aromatic amine

Work Done

***tert*-Butoxide-Assisted Amidation of Esters under Green Conditions**



3 **4b** **5b**

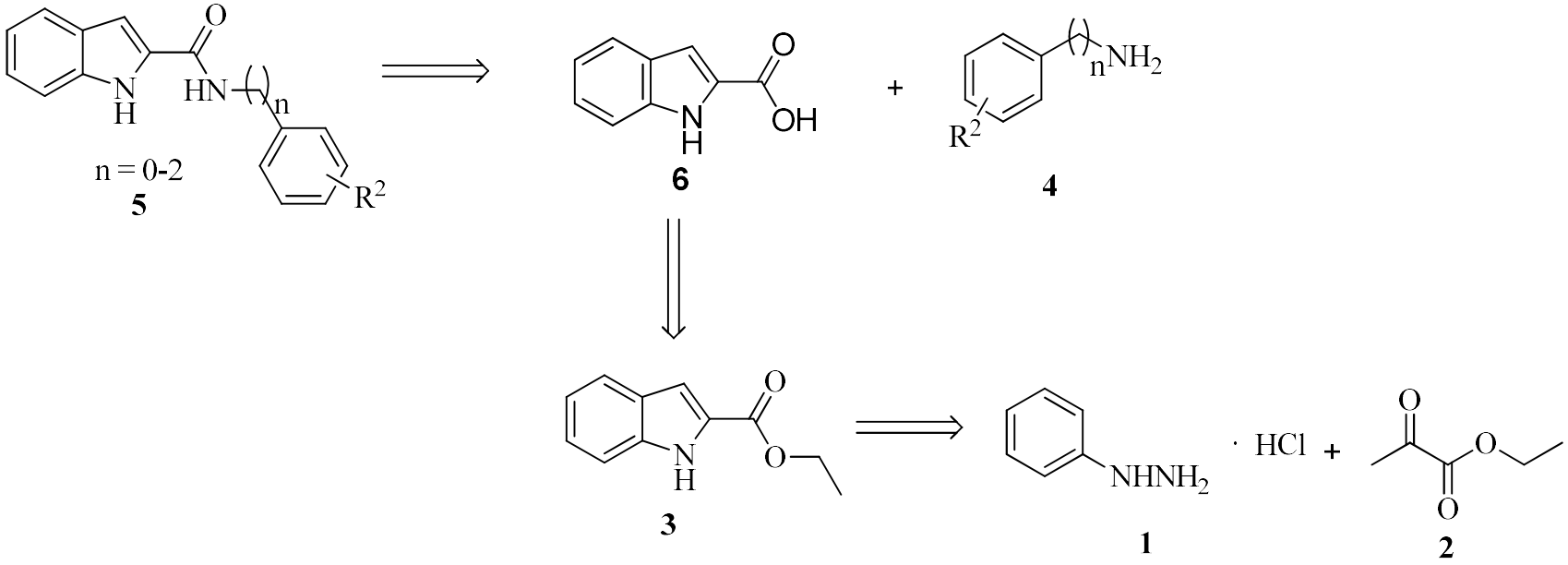
Stating material recovered

The reaction didn't undergo with aryl alkyl amine

Kim, B. R.; Lee, H.-G.; Kang, S.-B.; Sung, G. H.; Kim, J.-J.; Park, J. K.; Lee, S.-G.; Yoon Y.-J. "*tert*-Butoxide-Assisted Amidation of Esters under Green Conditions" *Synthesis* **2012**, *44*, 42-50

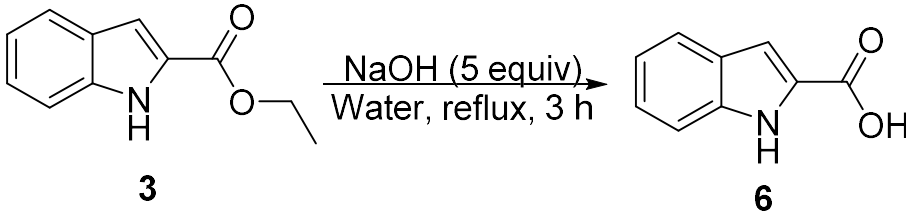
Work Done

- The free -NH of indole might be hampering the amidation of ester (**3**) with amines (**4**)
- Failure of these strategies led us to opt for alternative strategy of CDI-mediated amidation of carboxylic acids with amines for the synthesis of target compounds



Work Done

To synthesize acid from ester by using NaOH (5 equiv) by using water



3 **6**

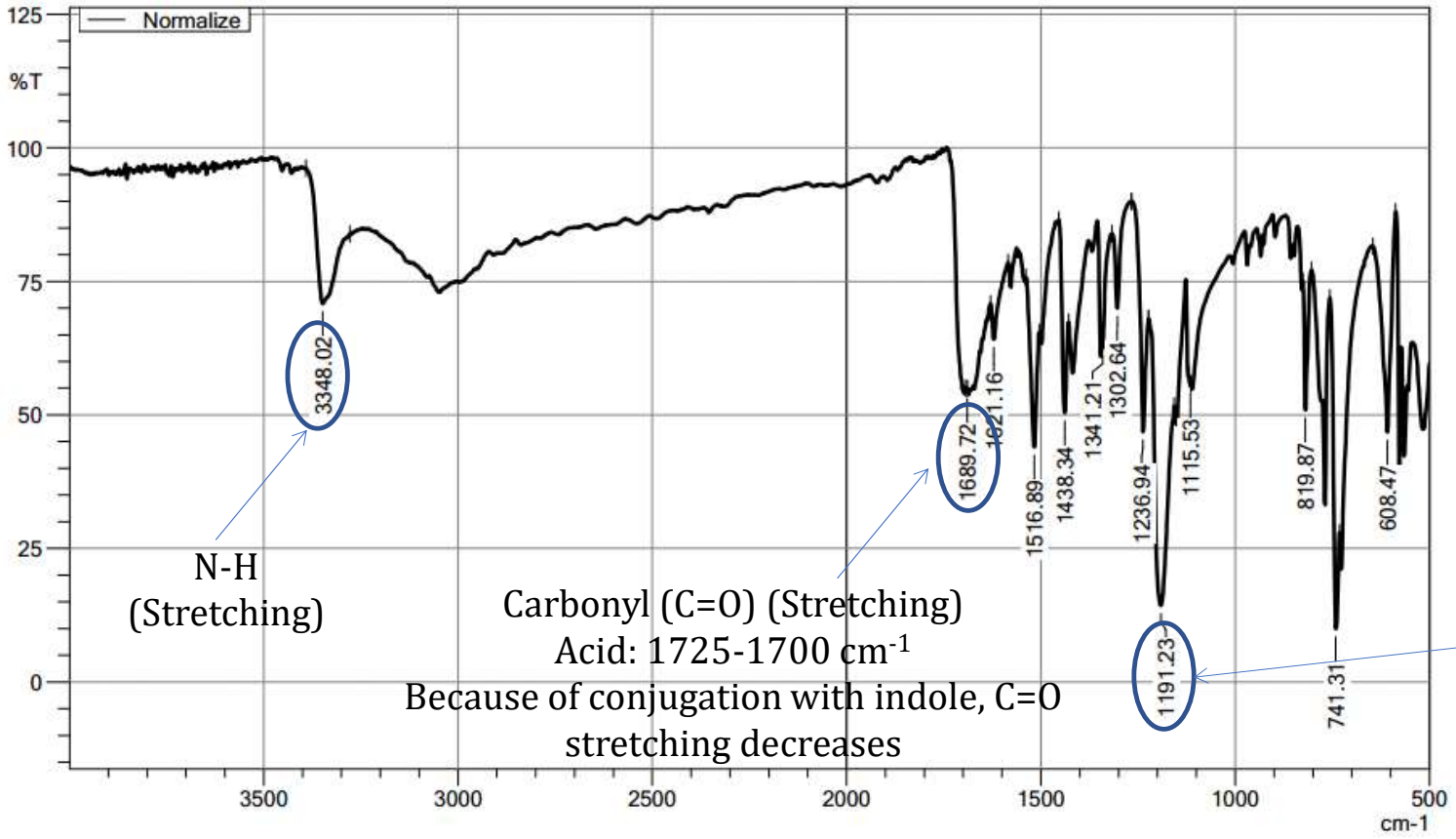
(63% yield)

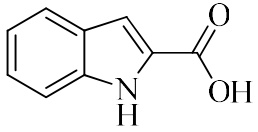
mp: 230-233 °C (observed), 232-234 (lit.)

Cofirmed by FT-IR

Shi Y, Duan Y-H, Ji Y-Y, Wang Z-L, Wu Y-R, Gunosewoyo H, Xie X. Y., Chen J. X., Yang F., Li J., Tang J., Xie X., and Yu L.-F. "Amidoalkylindoles as Potent and Selective Cannabinoid Type 2 Receptor Agonists with in Vivo Efficacy in a Mouse Model of Multiple Sclerosis." *J. Med. Chem.* **2017**, *60*, 7067-7083

FT-IR of 3

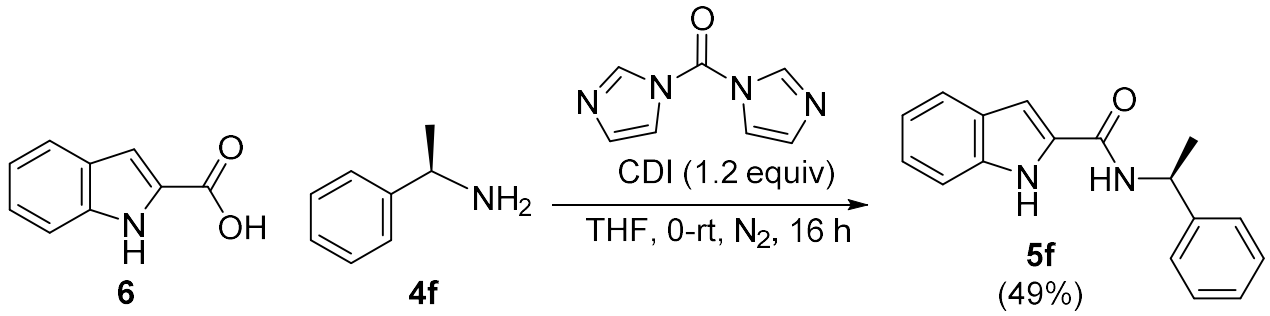

Normalize
125
%T
100
75
50
25
0
3500
3000
2500
2000
1500
1000
500
cm-1
3348.02
1689.72
1621.16
1516.89
1438.34
1341.21
1302.64
1236.94
1191.23
1115.53
819.87
741.31
608.47
N-H
(Stretching)
Carbonyl (C=O) (Stretching)
Acid: 1725-1700 cm-1
Because of conjugation with indole, C=O
stretching decreases
C-O
(Stretching)


O
OH
N
H

6

Work Done

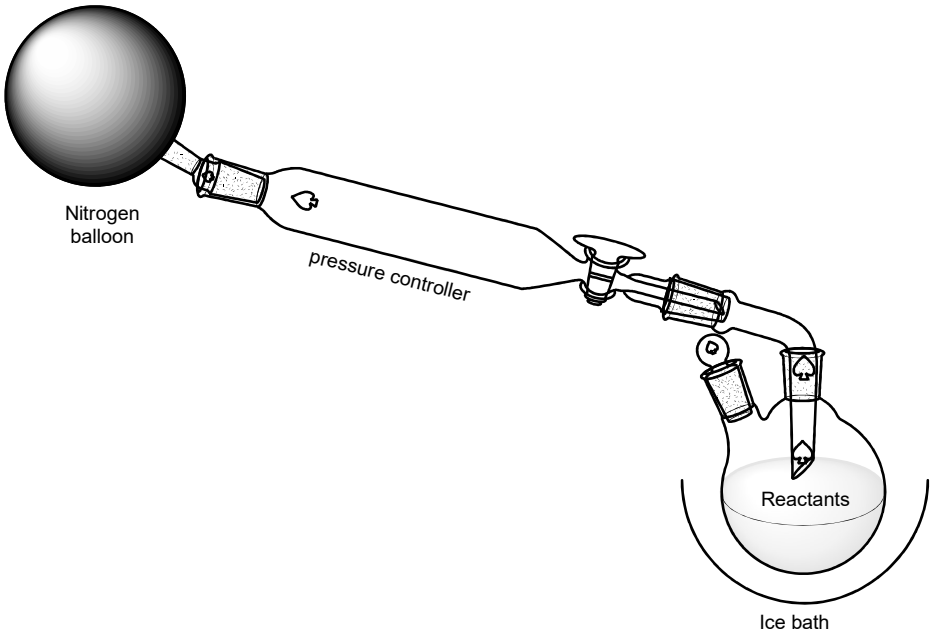
CDI Mediated Amidation for (*S*)-*N*-(1-phenylethyl)-1*H*-indole-2-carboxamide) **(5f)**



Yellow solid, 49%, mp: 187-189˚C; R_f: 0.68 (*n*-Hexane: EtOAc = 7:3); IR (KBr, cm^{-1}): 3377(NH stretch), 1643(CO stretch), 2923 (CH_3) ; MS (ESI): 265 $(M+H)^+$.

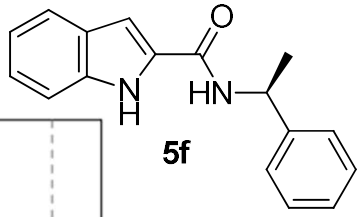
Kumar V., Moritz A. E., Keck T. M., Bonifazi A., Ellenberger M. P., Sibley C. D., Free R. B., Shi L., Lane J. R., Sibley D. R., and Newman A. H. Synthesis and Pharmacological Characterization of Novel trans Cyclopropylmethyl-Linked Bivalent Ligands That Exhibit Selectivity and Allosteric Pharmacology at the Dopamine D_3 Receptor (D_3R). *J. Med. Chem.* **2017**, *60*, 1478-1494

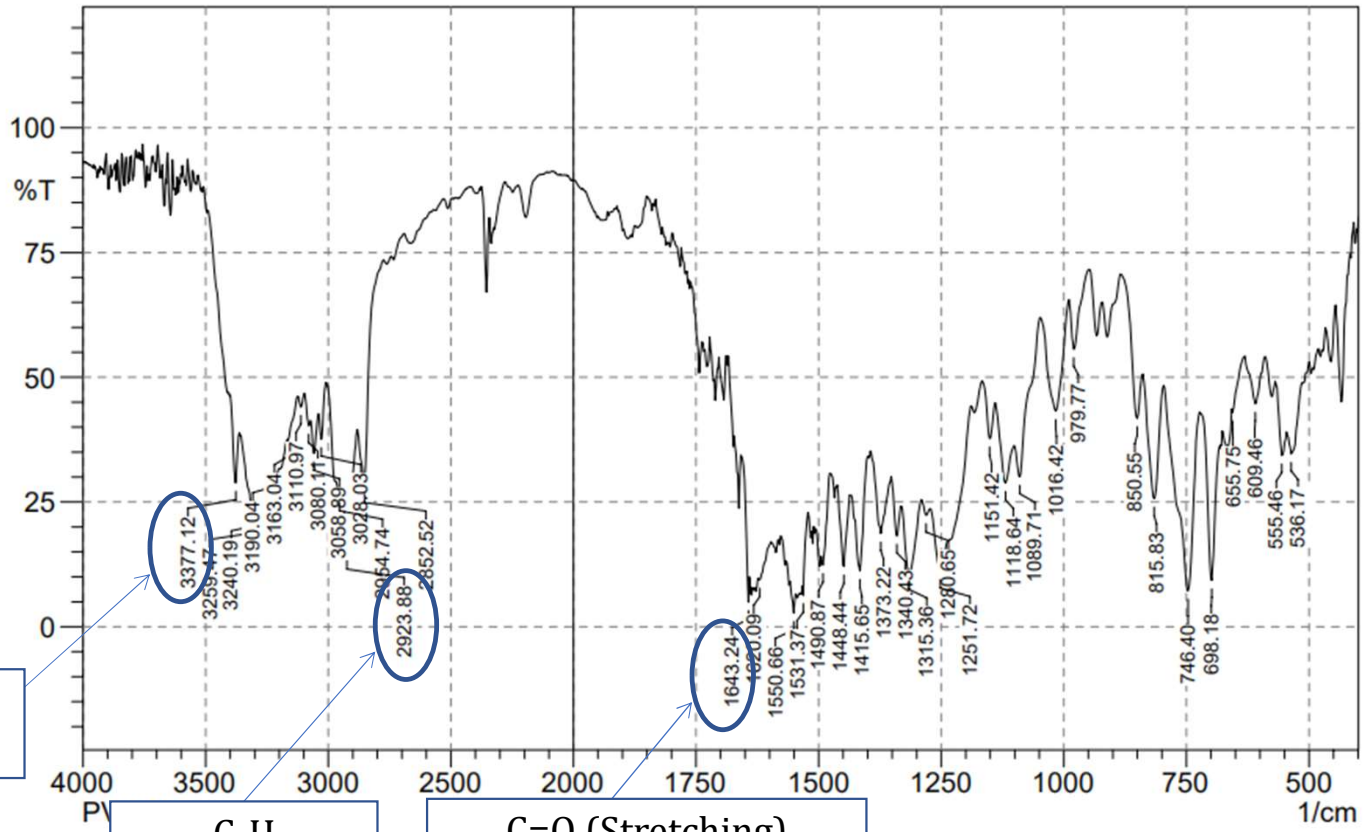
Procedure for synthesis of indole-2-carboxamides



Indole-2-carboxylic acids (**6**, 1 mmol) in THF taken into the round bottom purging flask and allowed to stir for 5 minutes followed by addition of CDI (1.2 equiv) and amine (**4**, 2 equiv). The inert atmosphere has been created by purging nitrogen gas into the vessel followed by stirring of the reaction mixture at 0 °C to rt for 24 h. After completion of the reaction, THF have been evaporated and final compounds have been purified using recrystallization in ethanol.

FT-IR of 5f



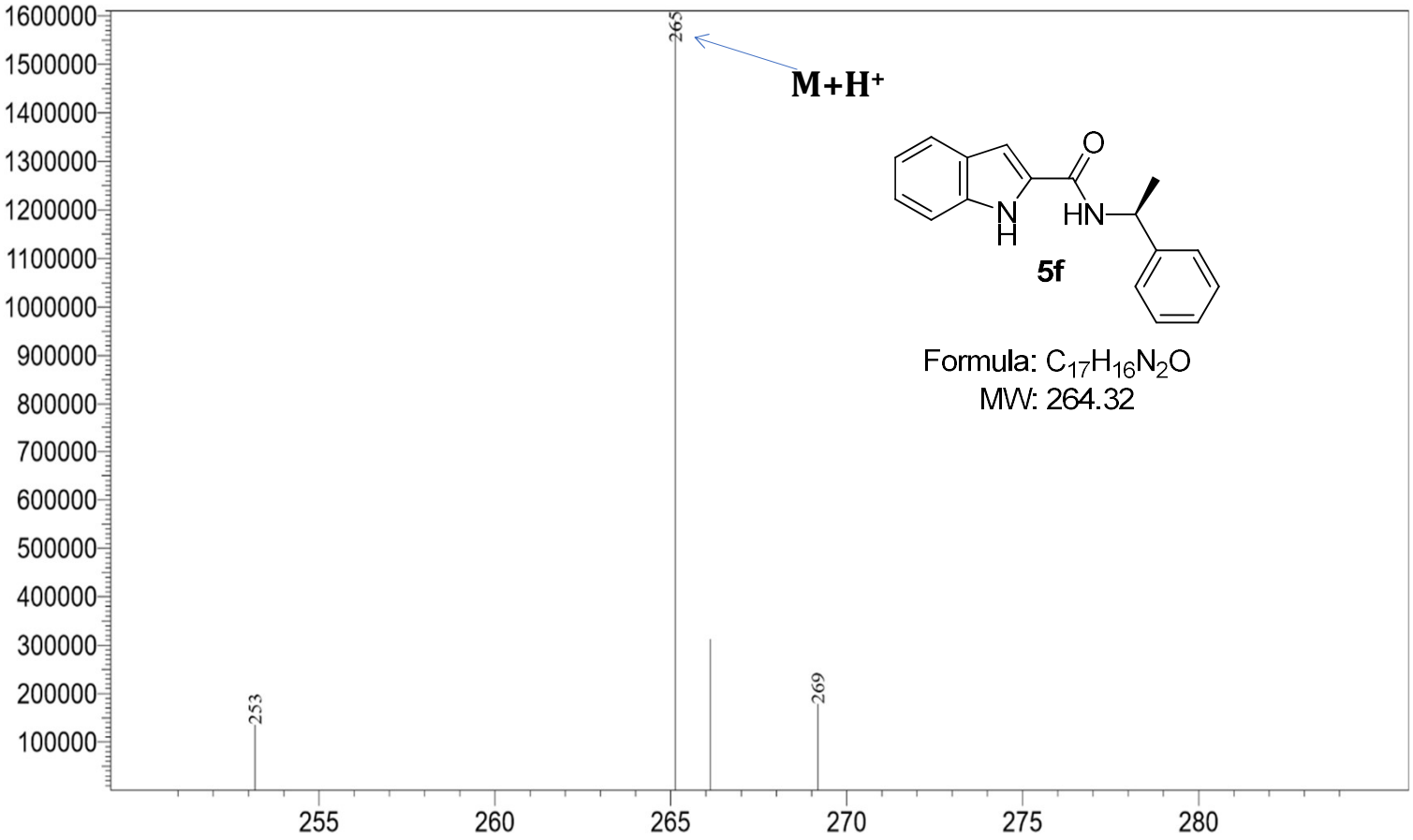


N-H (Stretching)

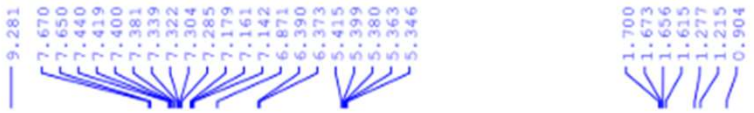
C-H (Stretching)

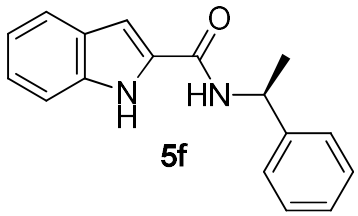
C=O (Stretching)
Amide: 1650-1680 cm^{-1}

MS of 5f


1600000
1500000
1400000
1300000
1200000
1100000
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
265
M+H+
253
269
255
260
265
270
275
280
O
N
H
HN
5f
Formula: C17H16N2O
MW: 264.32

^{1}H NMR of 5f

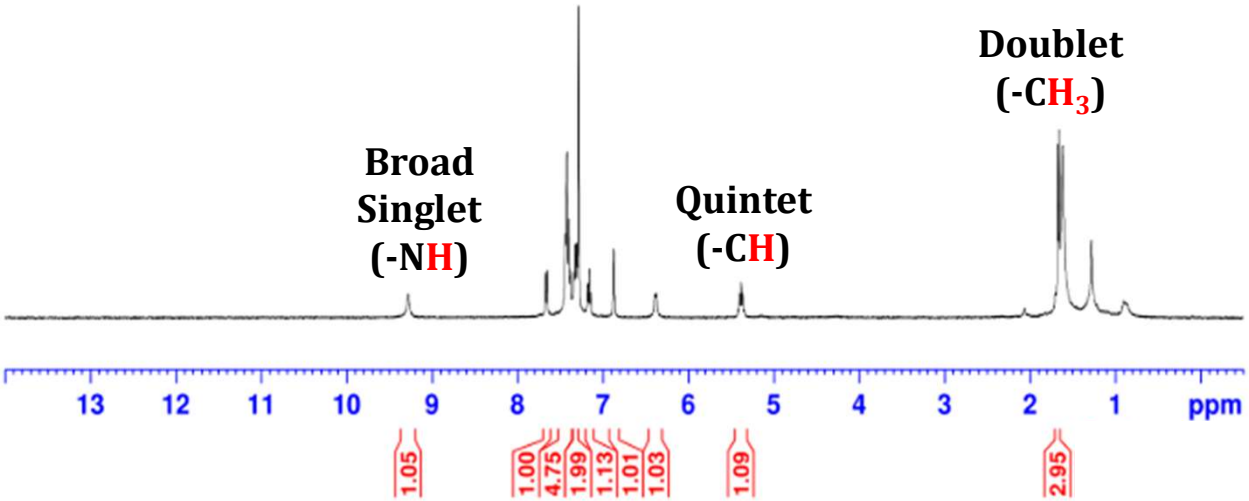


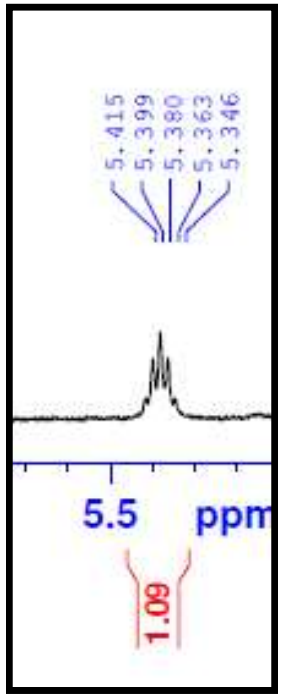


5f

Formula: $C_{17}H_{16}N_2O$

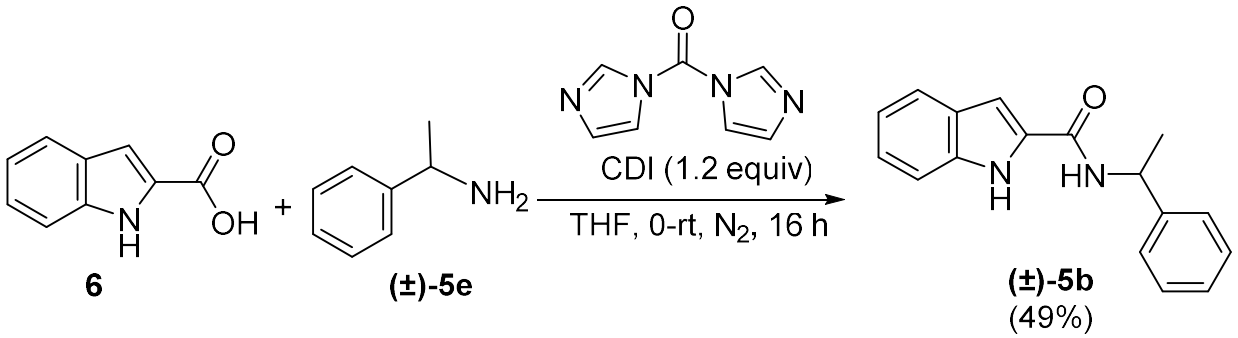
TMS at 0 ppm
Moisture at 1.2 ppm
Grease at 1.9 ppm
Solvent ($CDCl_3$) Residual Peak at 7.2 ppm





Quintet (-CH) protons on a carbon atom adjacent to the nitrogen split by the NH proton (3*J:* H-C-N-H)

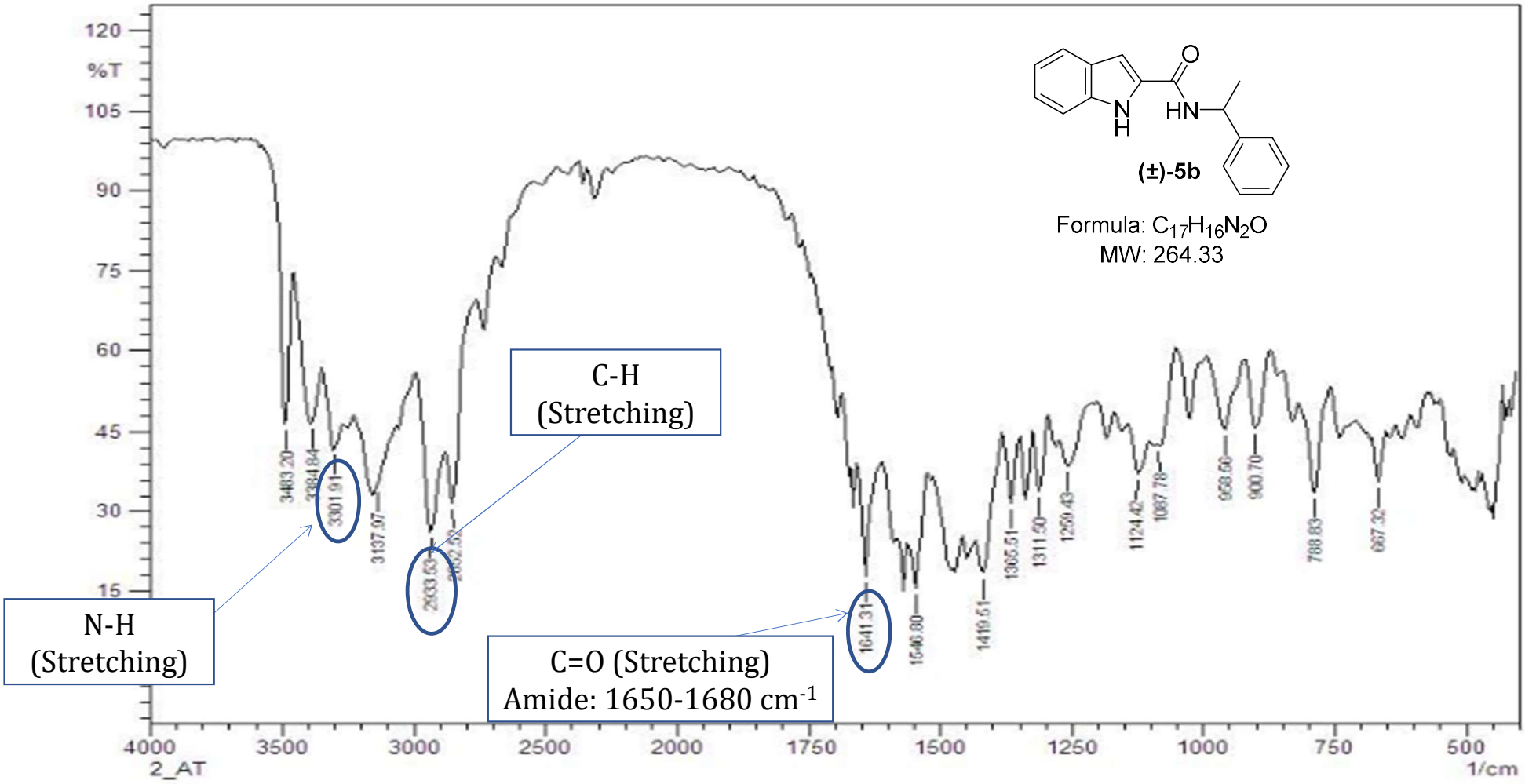
CDI Mediated Amidation for (±)-*N*-(1-phenylethyl)-1*H*-indole-2-carboxamide (5b)



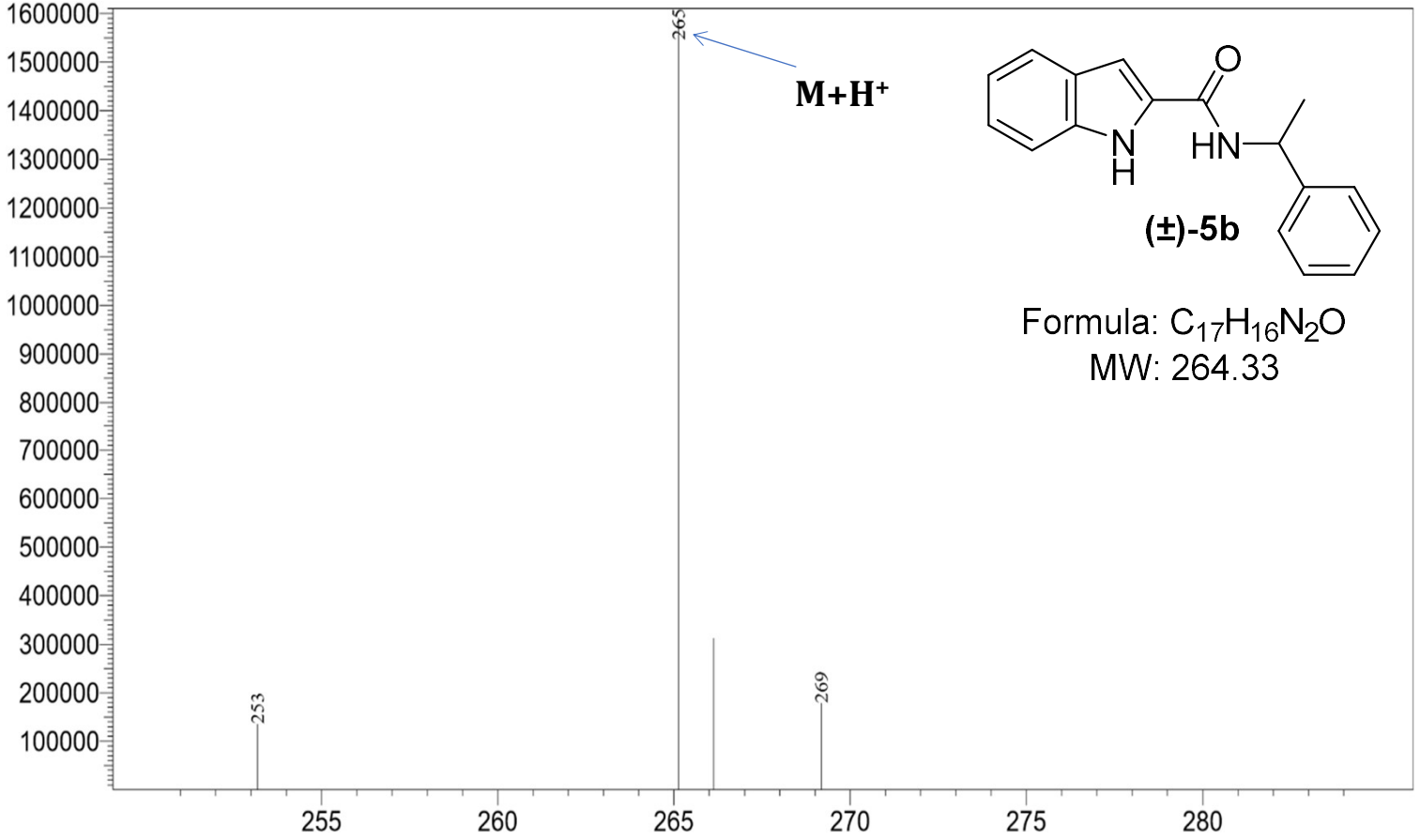
Brown solid, 49%, mp: 187-189 ˚C; R_f: 0.68 (*n*-Hexane: EtOAc = 7:3) IR (KBr, cm^{-1}): 3301(N-H stretching), 1652(amide stretching); MS (ESI): 265 $(M+H)^+$.

Kumar V., Moritz A. E., Keck T. M., Bonifazi A., Ellenberger M. P., Sibley C. D., Free R. B., Shi L., Lane J. R., Sibley D. R., and Newman A. H. Synthesis and Pharmacological Characterization of Novel trans Cyclopropylmethyl-Linked Bivalent Ligands That Exhibit Selectivity and Allosteric Pharmacology at the Dopamine D_3 Receptor (D_3R). *J. Med. Chem.* **2017**, *60*, 1478-1494

FT-IR of 5b

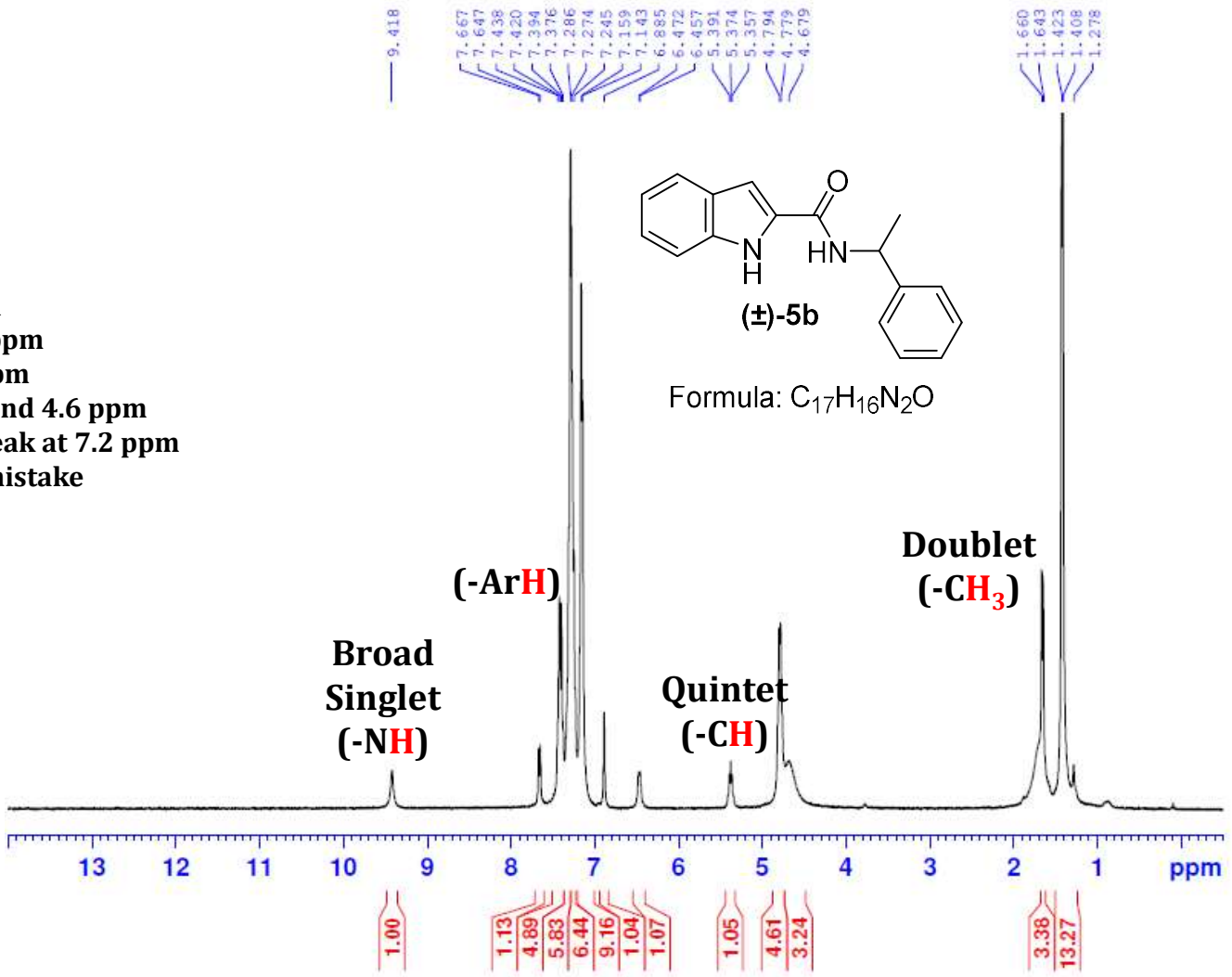

120
%T
105
90
75
60
45
30
15
4000
3500
3000
2500
2000
1750
1500
1250
1000
750
500
1/cm
2_AT
3483.20
3384.84
3301.91
3137.97
2933.53
2862.52
1641.31
1540.80
1419.51
1365.51
1311.50
1269.43
1124.42
1087.78
958.60
900.70
788.83
667.32
C-H
(Stretching)
N-H
(Stretching)
C=O (Stretching)
Amide: 1650-1680 cm-1
O
N
H
HN
(±)-5b
Formula: C17H16N2O
MW: 264.33

MS of 5b

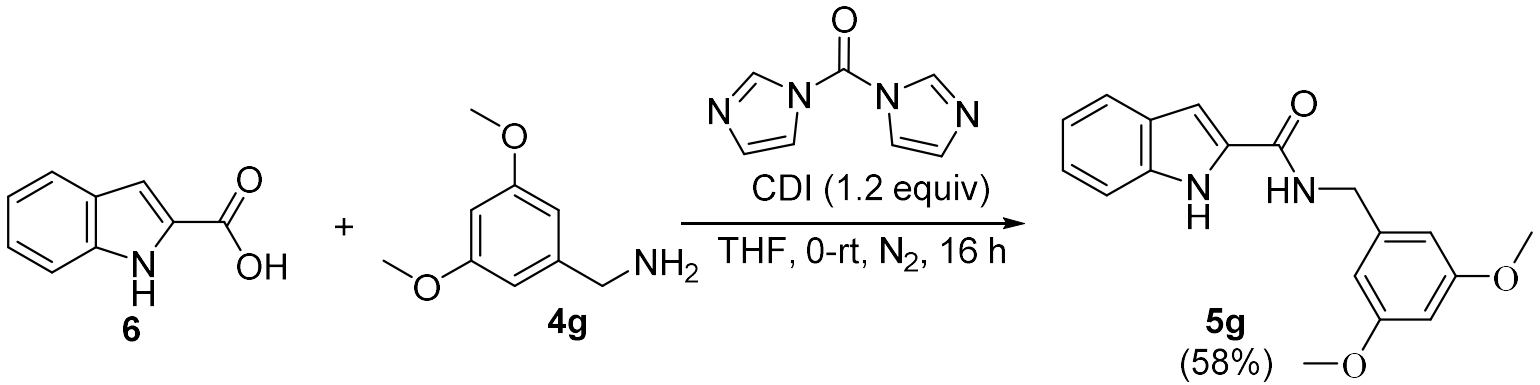

1600000
1500000
1400000
1300000
1200000
1100000
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
265
M+H+
253
269
255
260
265
270
275
280
O
N
H
HN
(±)-5b
Formula: C17H16N2O
MW: 264.33

^{1}H NMR of 5b

TMS at 0 ppm
Moisture at 1.2 ppm
Grease at 1.6 ppm
Other Impurities at 3.2 and 4.6 ppm
Solvent ($CDCl_3$) Residual Peak at 7.2 ppm also calibrated by mistake



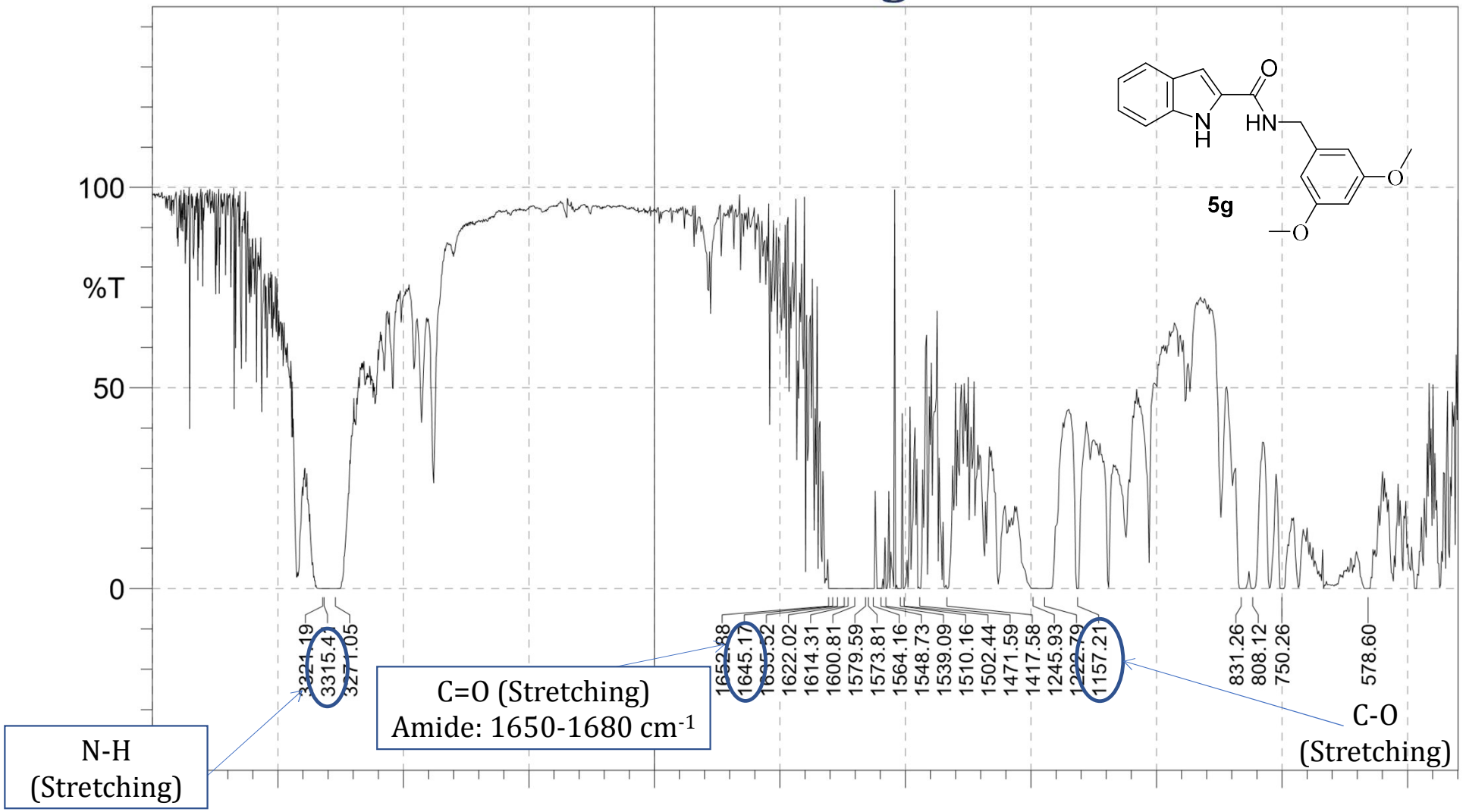
CDI Mediated Amidation for *N*-(3,5-dimethoxybenzyl)-1*H*-indole-2-carboxamide (5g)



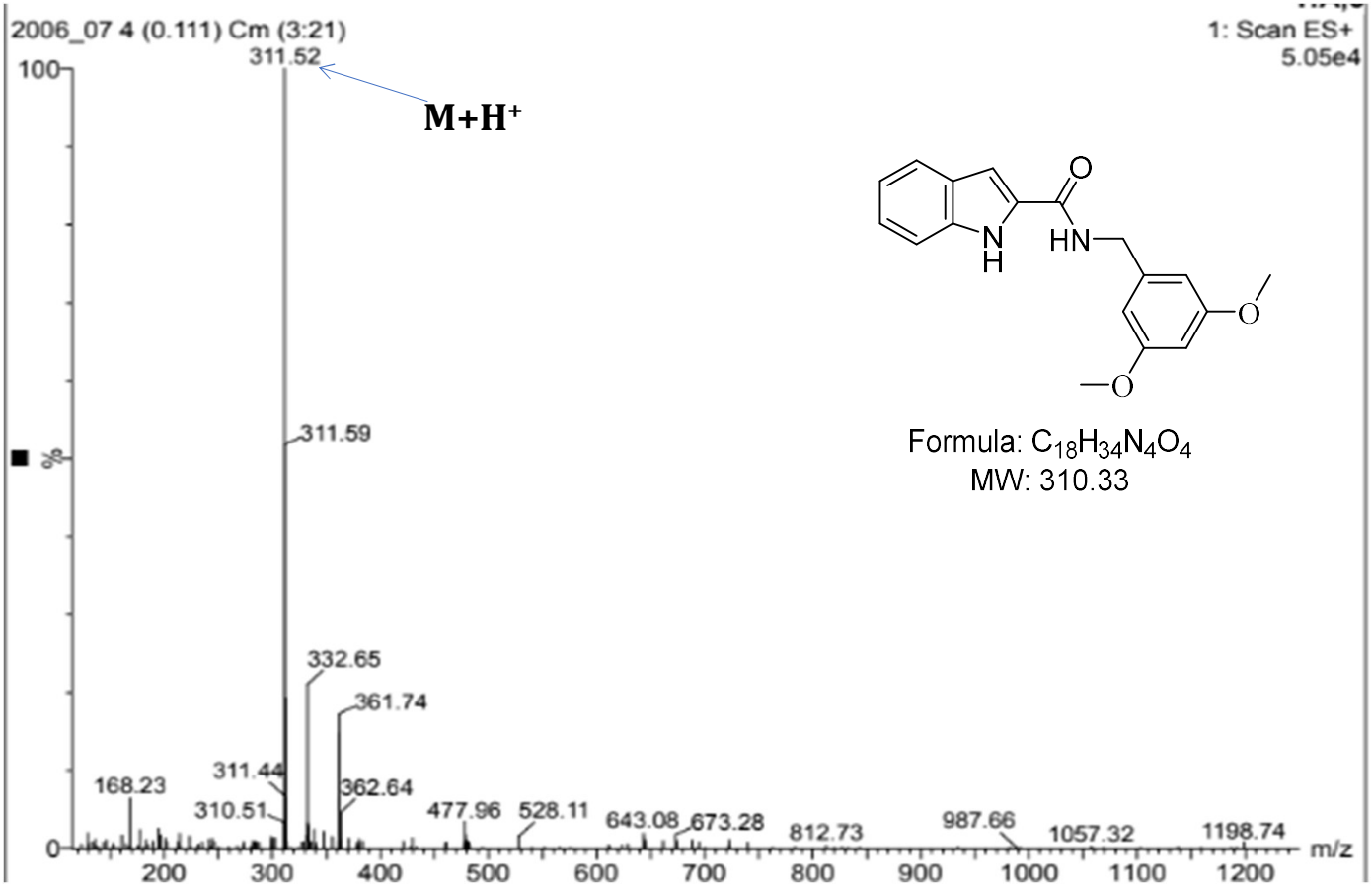
Yellow solid, 58%, mp: 235-240 ˚C; IR (KBr, cm^{-1}): 3315 (N-H stretching), 1642 (C=O stretching); R_f: 0.58 (*n*-Hexane: EtOAc = 7:3); MS (ESI): 311 $(M+H)^+$.

Kumar V., Moritz A. E., Keck T. M., Bonifazi A., Ellenberger M. P., Sibley C. D., Free R. B., Shi L., Lane J. R., Sibley D. R., and Newman A. H. Synthesis and Pharmacological Characterization of Novel trans Cyclopropylmethyl-Linked Bivalent Ligands That Exhibit Selectivity and Allosteric Pharmacology at the Dopamine D_3 Receptor (D_3R). *J. Med. Chem.* **2017**, *60*, 1478-1494

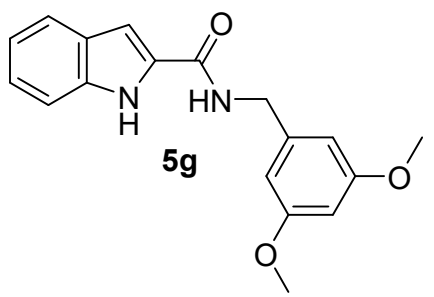
FT-IR of 5g


100
%T
50
0
3321.19
3315.41
3271.05
1652.88
1645.17
1635.52
1622.02
1614.31
1600.81
1579.59
1573.81
1564.16
1548.73
1539.09
1510.16
1502.44
1471.59
1417.58
1245.93
1222.79
1157.21
831.26
808.12
750.26
578.60
O
N
H
HN
O
O
5g
N-H
(Stretching)
C=O (Stretching)
Amide: 1650-1680 cm-1
C-O
(Stretching)

MS of 5g

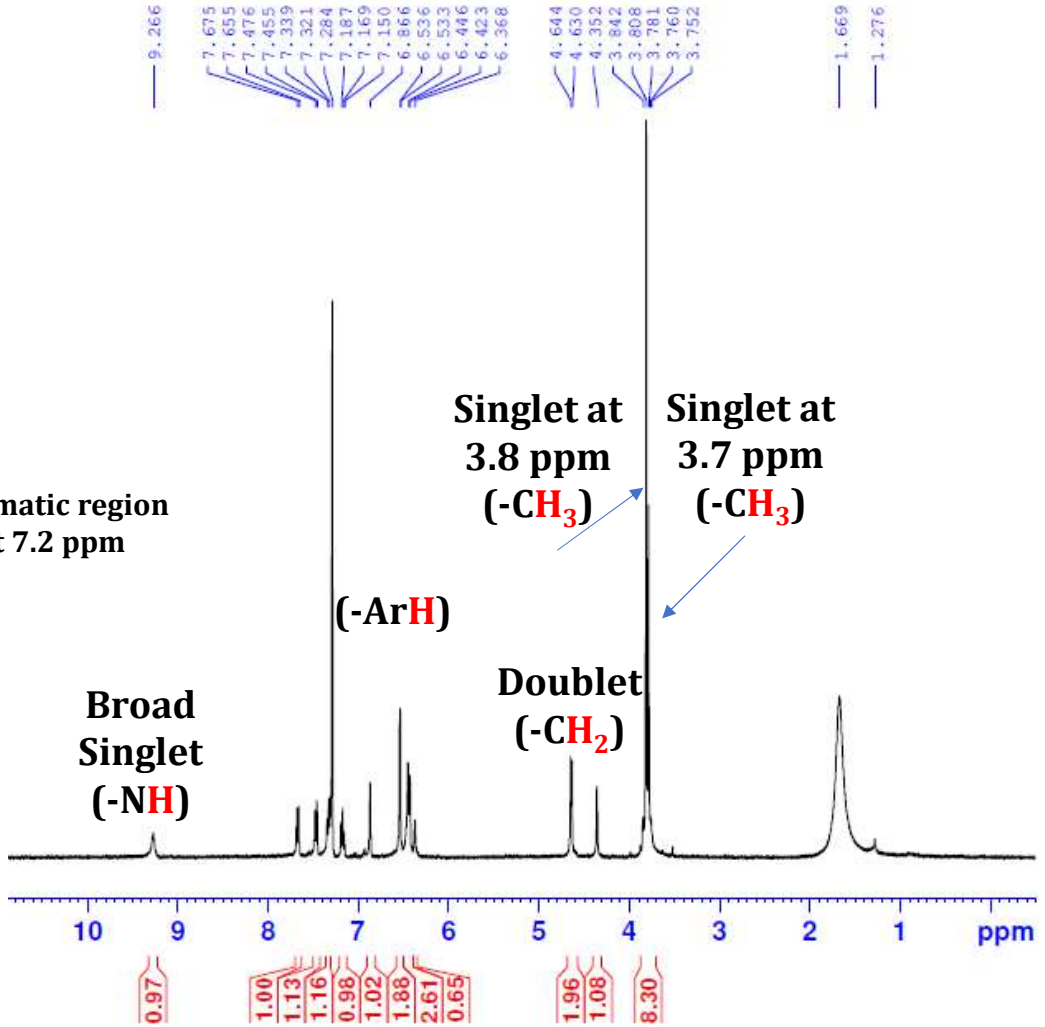

2006_07 4 (0.111) Cm (3:21)
1: Scan ES+
5.05e4
100
%
0
311.52
M+H+
311.59
332.65
361.74
311.44
362.64
168.23
310.51
477.96
528.11
643.08
673.28
812.73
987.66
1057.32
1198.74
200
300
400
500
600
700
800
900
1000
1100
1200
m/z
O
HN
N
H
O
O
Formula: C18H34N4O4
MW: 310.33

^{1}H NMR of 5g



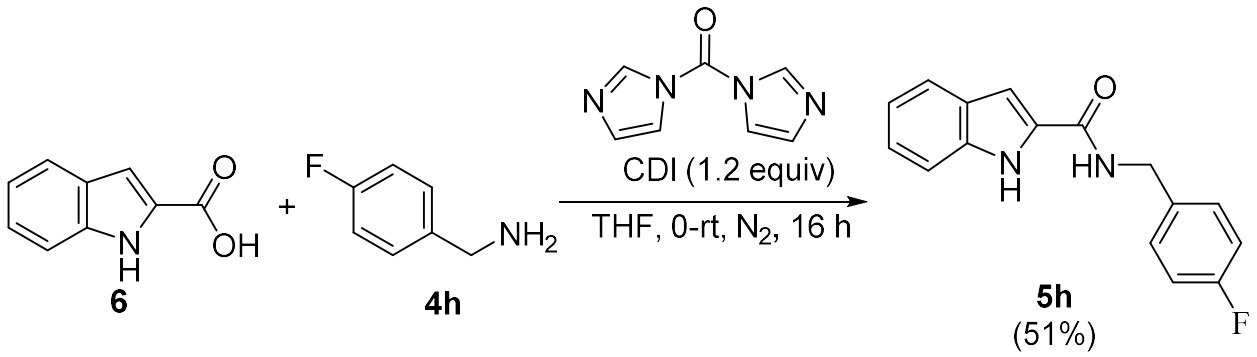
Formula: $C_{17}H_{16}N_2O$

TMS at 0 ppm
Moisture at 1.2 ppm
Grease at 1.6 ppm
Other Impurities at 4.3 ppm in aromatic region
Solvent ($CDCl_3$) Residual Peak at 7.2 ppm



Doublet (-CH_2)
Protons on -CH_2 atom adjacent to -NH split by the NH proton (3*J*: H-C-N-H)

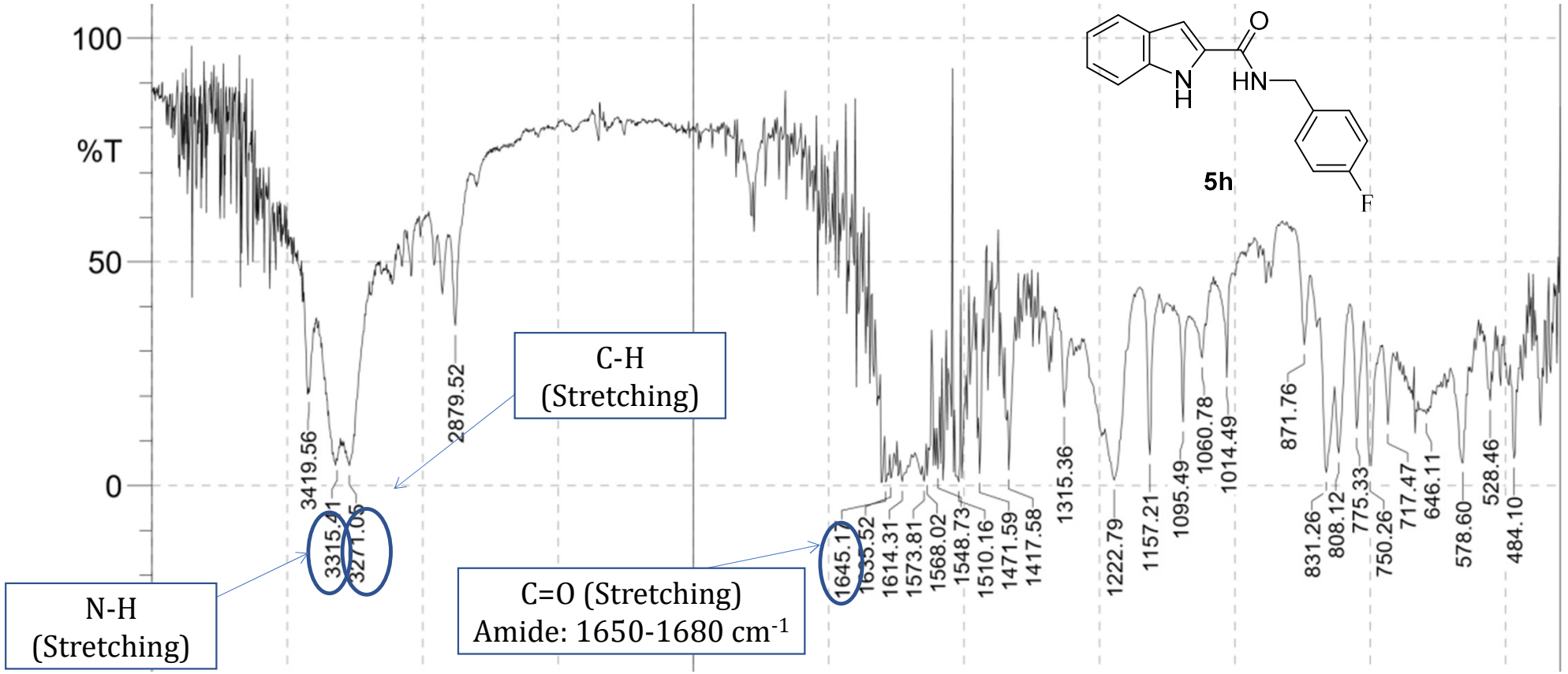
CDI Mediated Amidation for *N*-(4-fluorobenzyl)-1*H*-indole-2-carboxamide (5h)



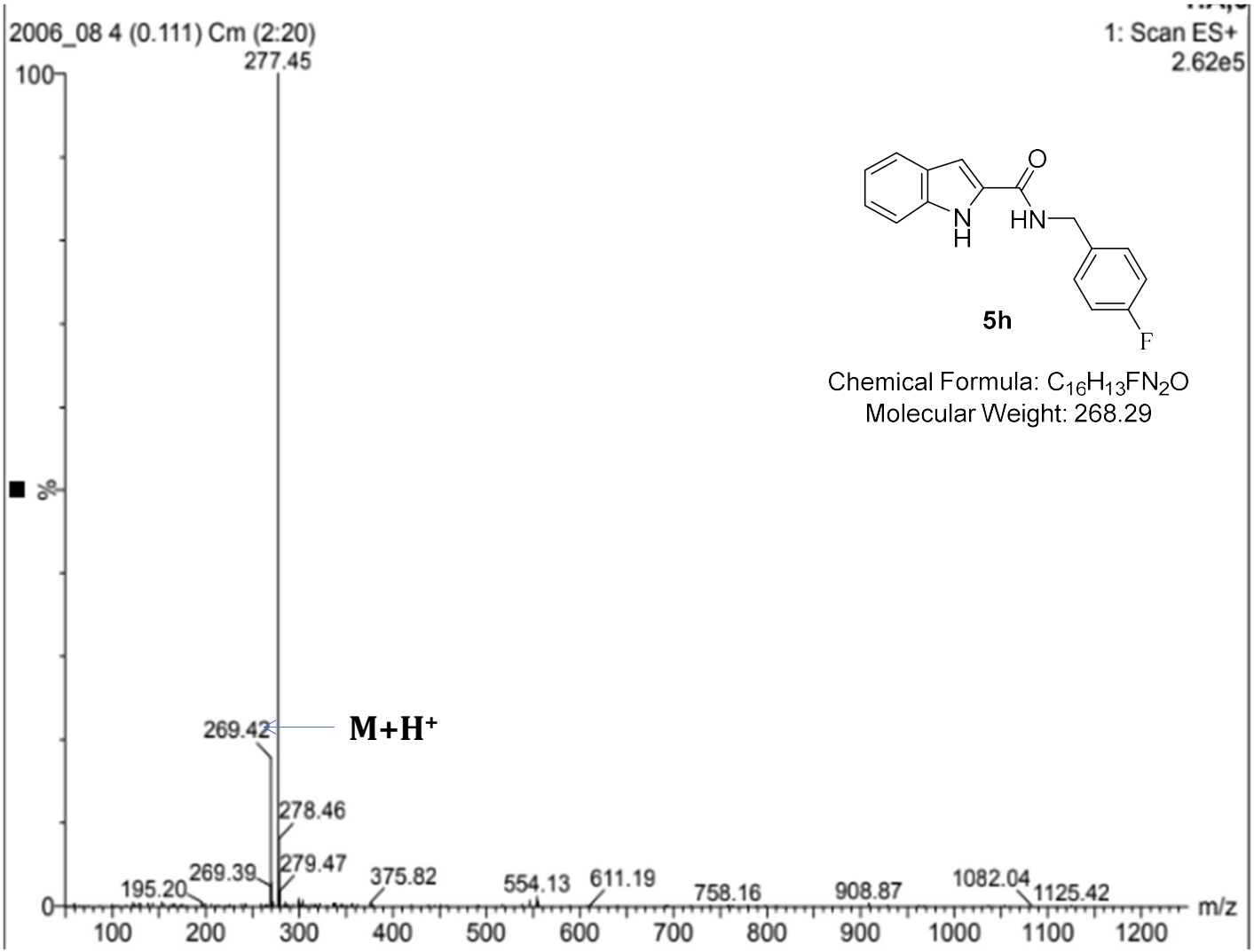
Brown solid, 51%, mp: 228-230 ˚C; R_f: 0.61 (*n*-Hexane: EtOAc = 7:3); IR (KBr, cm^{-1}): 3315(N-H stretching), 1635(C=O stretching) ; MS (ESI): 269 $(M+H)^+$.

Kumar V., Moritz A. E., Keck T. M., Bonifazi A., Ellenberger M. P., Sibley C. D., Free R. B., Shi L., Lane J. R., Sibley D. R., and Newman A. H. Synthesis and Pharmacological Characterization of Novel trans Cyclopropylmethyl-Linked Bivalent Ligands That Exhibit Selectivity and Allosteric Pharmacology at the Dopamine D_3 Receptor (D_3R). *J. Med. Chem.* **2017**, *60*, 1478-1494

FT-IR of 5h


100
%T
50
0
3419.56
3315.41
3271.05
2879.52
1645.17
1635.52
1614.31
1573.81
1568.02
1548.73
1510.16
1471.59
1417.58
1315.36
1222.79
1157.21
1095.49
1060.78
1014.49
871.76
831.26
808.12
775.33
750.26
717.47
646.11
578.60
528.46
484.10
O
N
H
HN
F
5h
C-H
(Stretching)
N-H
(Stretching)
C=O (Stretching)
Amide: 1650-1680 cm-1

MS of 5h



5h

Chemical Formula: $C_{16}H_{13}FN_2O$
Molecular Weight: 268.29

Summary of Work Done

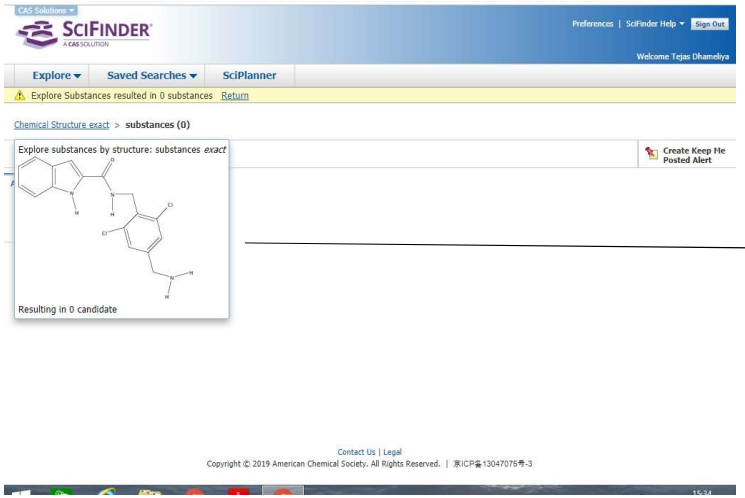
- Previously identified substituted indoles and carboxamides have been reviewed and studied as the potent anti-bacterial agents.
- Docking study have been performed using Maestro 3.0 (Schrödinger) in search of novel ATPPRTase (HisG) inhibitor, which may lead to inhibition of essential amino acid for bacterial strains.
- Tried the synthesis of amide from ester with several amines but no successful results were obtained
- Alternative route for synthesis of amide via CDI mediated amidation of indole-2-carboxylic acid has been planned and executed

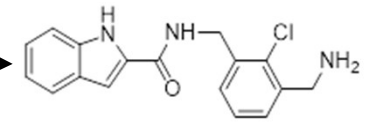
Summary of Work Done

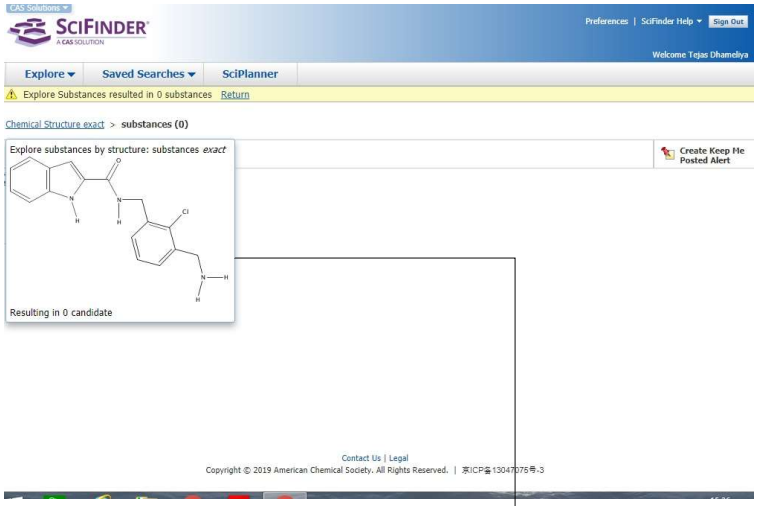
- The key intermediate, indole-2-carboxylic acid required for the synthesis of indole-2-carboxamides have been obtained by hydrolysis of ethyl indole-2-carboxylate. The synthesis of four target compounds (**5b**, **5f**, **5g**, **5h**) have been achieved in moderate yields via CDI mediated amidation of indole-2-carboxylic acid with benzyl amines in THF at 0 ˚C-rt and characterized it using FT-IR, MS, and ^{1}H NMR

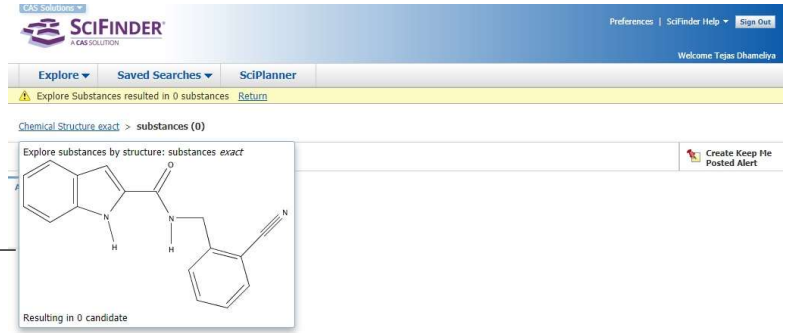
- Samples have been submitted for the biological evaluation and results are awaited.

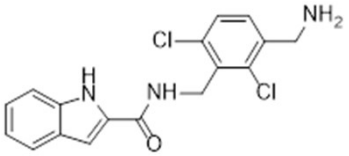
Novelty Status: Sci-Finder Report

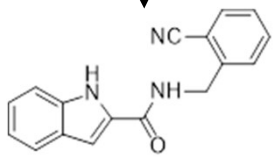




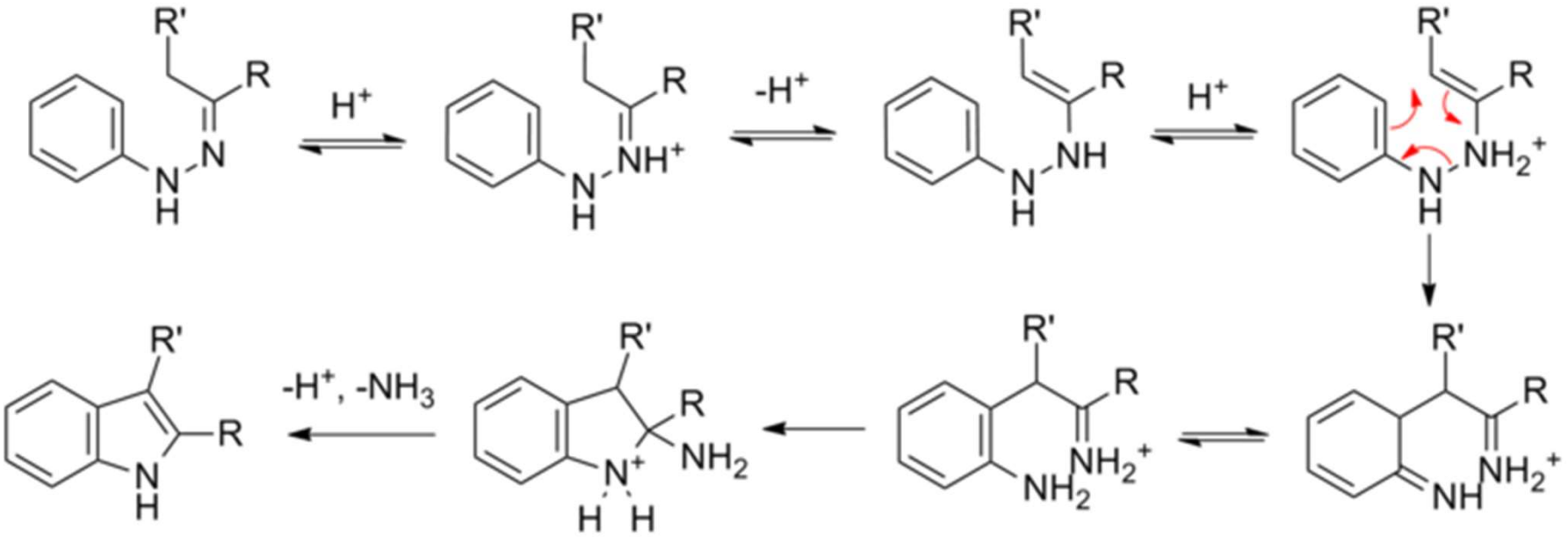




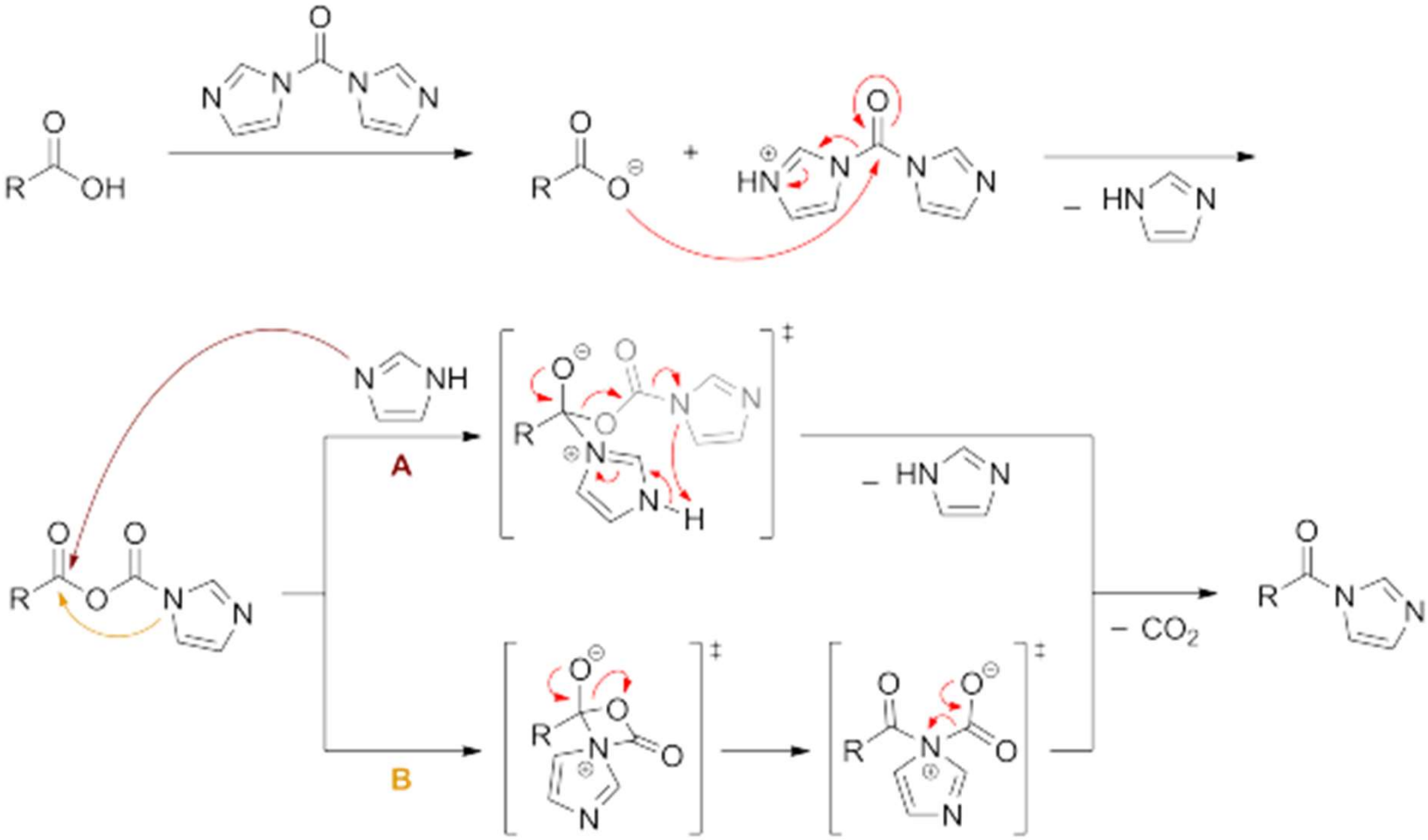




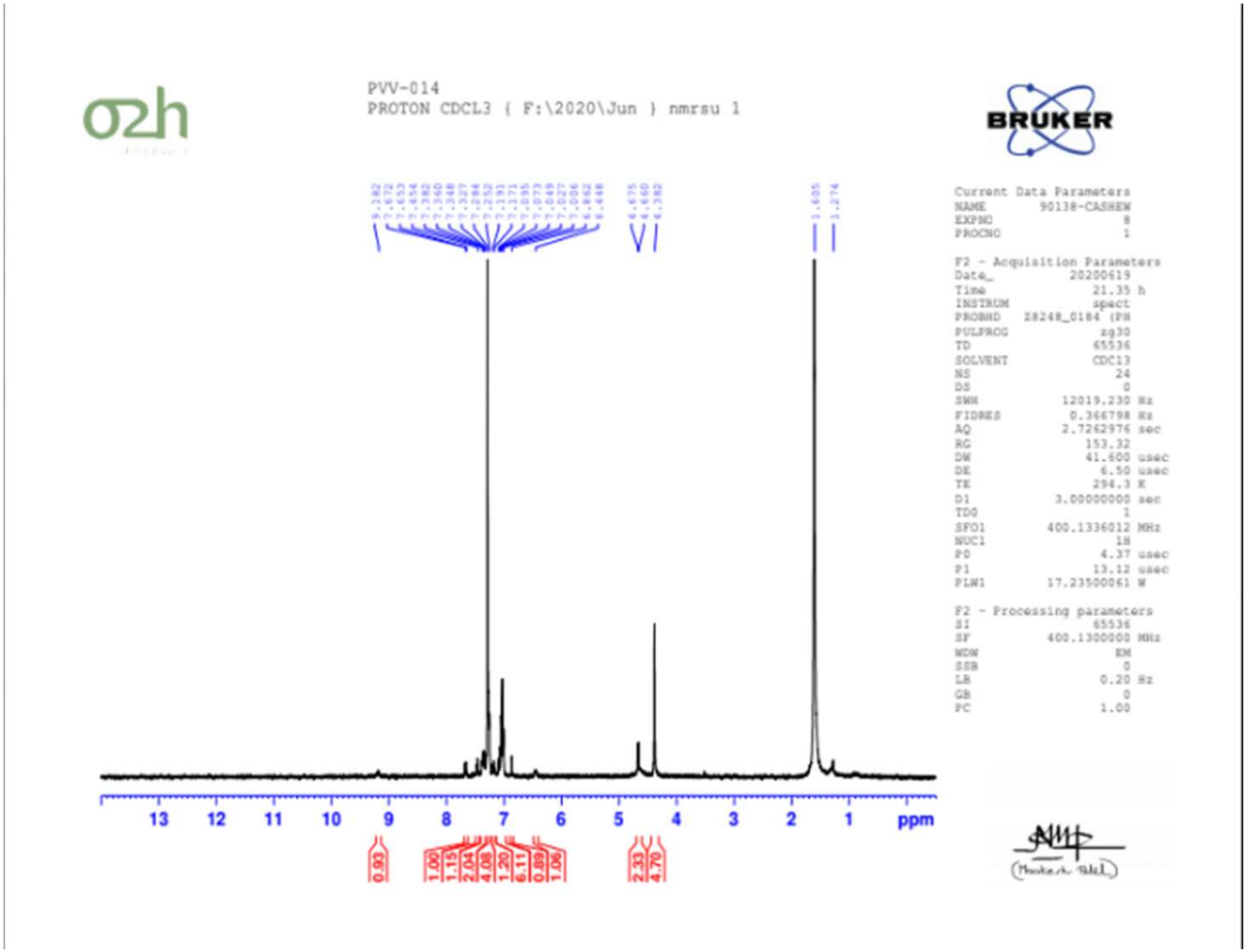
Fischer Indole Synthesis



The reaction of a (substituted) phenylhydrazine with a carbonyl (aldehyde or ketone) initially forms a phenylhydrazone which isomerizes to the respective enamine (or 'ene-hydrazine'). After protonation, a cyclic [3,3]-sigmatropic rearrangement occurs producing an imine. The resulting imine forms a cyclic aminoacetal (or aminal), which under acid catalysis eliminates NH3, resulting in the energetically favorable aromatic indole.


A
B
– HN N
– CO2

^{1}H NMR of 5h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2.04
4.08
1.20
6.11
0.89
1.06
2.33
4.70

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