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Rational Design, Synthesis And Characterization Of Some Novel Isatin Derivatives As Antitubercular Agents

Namitha K N[1], Velmurugan*

[1*]Associate Professor and Research Scholar , Department of Pharmaceutical Chemistry, SRM College of Pharmacy SRMIST, Chennai, Tamil Nādu, India
[2]Associate Professor, Department of Pharmaceutical Chemistry, SRM College of Pharmacy SRMIST,Chennai, Tamil Nādu, India.

***Corresponding Author:** Namitha K N1
*****Associate Professor and Research Scholar , Department of Pharmaceutical Chemistry, SRM College of Pharmacy SRMIST, Chennai, Tamil Nādu, India

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Abstract
Drugs are small organic molecules compared to their binding target. They stimulate or inhibit the action of biomolecules, like proteins, providing therapeutic benefits. Drugdesign is the process of developing novel drugs based on understanding biological targets. It is an expensive and time-consuming process. Tuberculosis is a contagious bacterial illness marked by the development of small growths (tubercles) in the bodytissues, particularly the lungs. The current investigation indicates that pantothenatesynthetase is a promising focus for developing drugs to treat tuberculosis. Pantothenate synthetase is an enzyme that plays a role in fatty acid synthesis in Mycobacterium tuberculosis (Mtb). 6H-Indolo[2,3-b] quinoxaline is a heterocyclic compound found to be a potential lead for anti-tubercular drug design. Selection of the lead molecule based onvirtual screening using iGEMDOCK v.2 software. Sixteen 6H-Indolo[2,3-b] quinoxaline derivatives were selected according to Lipinski's rule of five and by evaluating the parameters. All the derivatives were docked on the enzyme Mtb Pantothenate synthetase (pdb accession code: 3IMG). All the ligands interact well with the key binding sites GLY 153 and ALA 181. The results showed that the ligands of binding energies in the range of-4.91kcal/mol and -8.06 kcal/mol with Mtb Pantothenate synthetase, indicating that the compounds possess good binding with the active sites and inhibit the enzyme quite prominently. When compared with the standard drug Isoniazid, compounds ISA 2, ISA 5 and ISA 6 of the first series and ISB 1, ISB 2 and ISB 6 of the second series exhibited highbinding energy towards 3IMG.

Keywords: Autodock, iGEMDOCK, Mycobacterium Tuberculosis, Pantothenate synthetase, 6H indolo (2,3b) quinoxaline.

1. Introduction

1.1. Drug Design

The creative process of creating novel drugs using a biological target's information is known as drug design. It's an expensive and time-consuming operation. The most common type of medicine is an organic small molecule that could either stimulate orinhibit the action of biomolecules, like proteins, with the aim of achieving a therapeutic effect. Drug design, in its most basic form, is the creation of tiny molecules that, to interact with a biomolecular target, are complementary to it in structure and charge.

1.2. Tuberculosis

Tuberculosis (TB) is a contagious bacterial infection caused by Mycobacterium tuberculosis. It primarily affects the lungs (pulmonary TB) but can also affect other parts of the body (extrapulmonary

TB). TB is a major global health concern, with an estimated 10 million people falling ill with the disease and 1.4 million dying from it in2019.

Addressing the TB epidemic requires a comprehensive approach, including strengthening health systems, ensuring universal health coverage, promoting research and innovation, and addressing social determinants of health. Collaboration between governments, international organizations, civil society, and the private sector is essential to achieve the goal of ending the TB epidemic by 2030.

1.3. Pantothenate Synthetase

It is also known as D-pantoate: β-alanine ligase, pantothenate synthetase catalyzes the last committed step of pantothenate biosynthesis, the ATP dependent condensation of pantoate and β-alanine to form pantothenate. This is an essentialprecursor for the biosynthesis of CoA and ACP and has therefore been considered asa potential drug target for other bacteria, such as Mycobacterium tuberculosis. It was identified and demonstrated with autotrophic mutants that a functional pantothenatebiosynthesis pathway was necessary for the virulence of tuberculosis.

Pantothenate biosynthesis is essential for the virulence of Mycobacterium tuberculosis, and this pathway thus presents potential drug targets against tuberculosis. Pantothenate Synthetase catalyzes the ATP-dependent condensation of pantoate and beta-alanine to form pantothenate. Its structure reveals a dimer, and each subunit has two domains with tight association between domains. The active-site cavity is on the N-terminal domain, partially covered by the C-terminal domain. One wall of the active site cavity is flexible. When crystals were soaked with, or grown in the presence of, both ATP and pantoate, a reaction intermediate, pantoyl adenylate, is found in the active site. The flexible wall of the active site cavity becomes ordered when the intermediate is in the active site, thus protecting it from being hydrolyzed. Binding of beta-alanine can occur only after pantoyl adenylate is formed inside the active site cavity. The tight binding of the intermediate pantoyl adenylate suggests that nonreactive analogs of pantoyl adenylate may be inhibitors of the PS enzyme withhigh affinity and specificity.

1.4. Isatin

Isatin, also known as 1H-indole-2,3-dione, is a heterocyclic organic compound with the molecular formula C8H5NO2. It is a yellowish-white crystalline solid with a bitter taste and is naturally found in some plants, such as Isatis tinctoria (woad). Isatin is known for its diverse chemical properties and biological activities, making it a valuable compound in various fields, including medicinal chemistry and organic synthesis.

1.4.1. Structure

Isatin consists of a bicyclic structure composed of an indole ring fused to a ketonegroup. It can exist in different tautomeric forms, including the keto and enol forms,depending on the conditions.

1.4.2. Chemical Properties

Isatin is a versatile compound that undergoes various chemical reactions, including electrophilic substitution, nucleophilic addition, and condensation reactions. These reactions allow for the synthesis of a wide range of isatin derivatives with diverseproperties and applications.

1.4.3. Biological Activities

Isatin and its derivatives exhibit a wide range of biological activities, including antimicrobial, anticancer, antiviral, anti-inflammatory, and anticonvulsant properties. Isatin derivatives have also shown potential as enzyme inhibitors andneuroprotective agents.

Due to its diverse biological activities, Isatin and its derivatives have applications in medicinal chemistry, agrochemicals, and material science. They are used as intermediates in the synthesis of pharmaceuticals, dyes, and fluorescent compounds.

2. Aim and Objective

According to recent research, the most recent development in drug discovery is the identification of possible leads that block the target enzyme. Drug discovery methodsare currently being used to create new, safe, and effective entities. One possible pharmacological target for the creation of novel antimicrobial drugs is pantothenateSynthetase. Pantothenate is absent in mammals and needs to be obtained from dietary sources. Hence, the pantothenate biosynthesis pathway is an impending target for emerging new therapeutics to treat Tuberculosis. Pantothenate synthetase inhibitors include triclosan analogues, pyrazole derivatives, indole-5 amides, pyrrolidine carboxamides, etc. It has also been observed that thiadiazoles, oxadiazoles, and oxadiazines exhibit a range of biological activities, including antitubercular, antibacterial, and antifungal properties. The potential of diphenyl ethers and pyrrolidine carboxamides as direct inhibitors of enoyl ACP reductase isbeing investigated in further detail.

3. Materials and Methods

3.1. Insilico

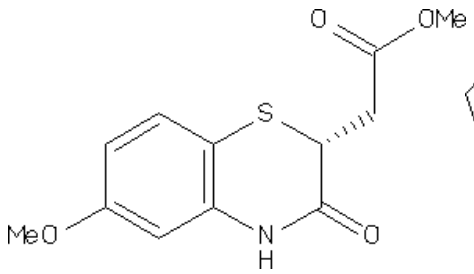
Softwares and Databases used

- iGEMDOCK v.2
- PyRx
- ZINC15 – Compound database
- admetSAR
- Molinspiration server
- Autodock Vina

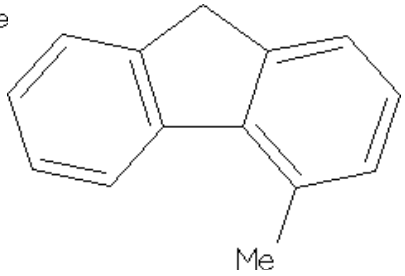
3.2. Virtaul Screening

Identification of the drug target and selection of the lead based on virtual screening using iGEMDOCK v.2 software.

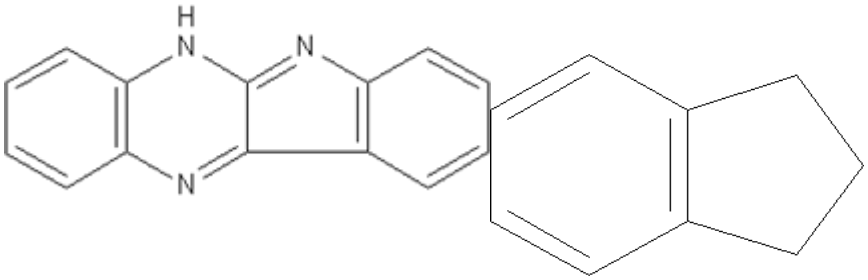
Some of the structures of the compounds containing lead showing highest binding affinity towards the target as follows:



zinc 6182368



zinc 2039459



zinc 1300693

zinc 1680824

3.3. Lead Optimization

3.3.1. ADMET properties

The compounds selected by its optimal absorption, metabolism, and excretion. The ADME studies are performed by using admetSAR. It will help in the computationalevaluation of the ADME tox scores.
The SMILES were introduced into the admetSAR online page. After loading the structure, the datas were generated automatically. The data for descriptors were BBB (blood brain barrier), plasma protein binding, aqueous solubility, and hepatotoxicity,carcinogenicity etc.

3.3.2. Drug Like Properties

For better oral absorption of the ligands the drug likeliness scores were evaluated forgetting information about solubility, diffusion, Log P, molecular weight etc. One of the ideal methods for this is by using Lipinksi's rule of five with the molinspiration server.

3.4. Docking

3.4.1. Target Selecting

From the literature review, we have selected Mycobacterium Tuberculosis Pantothenate Synthetase (3LE8) as a target for the present study. Pantothenate synthetase of Mycobacterium Tuberculosis has been selected from Brookhaven Protein Data Bank where the X-ray crystallographic structures are obtained.
Protein 3IMG downloaded from protein data bank as such cannot directly use fordocking, it should undergo refinement. Refinement of downloaded protein involves the removal of water, heteroatoms, and bound ligands if any.

3.4.2. Docking

Docking was performed using AutoDock Vina. It requires refined protein and ligands in PDB format. The docking results of Mycobacterium Tuberculosis pantothenate synthetase (3LE8.pdb) with the ligands ISA1-8 and ISB1-8 and standard has beenreported below. The best-docked structures should have a binding energy lower to thestandard.

3.5. Synthesis

3.5.1. Reagents Used

Isatin (indoline 2,3b dione), Sulphuric acid, nitric acid, Potassium nitrate, Glacial acetic acid, orthpohenylene diamine, ethyl acetate, chloroacetyl chloride, anhydrous magnesium sulphate, sodium acetate trihydrate, piperidine, morpholine, dimethylamine, 2,4- thiazolidine dione, imidazole, indole, dipropyleamine, dibutylamine, potassium dichromate, dimethyl formamide, Dimethyl sulphoxide diethyl ether, silica gel.

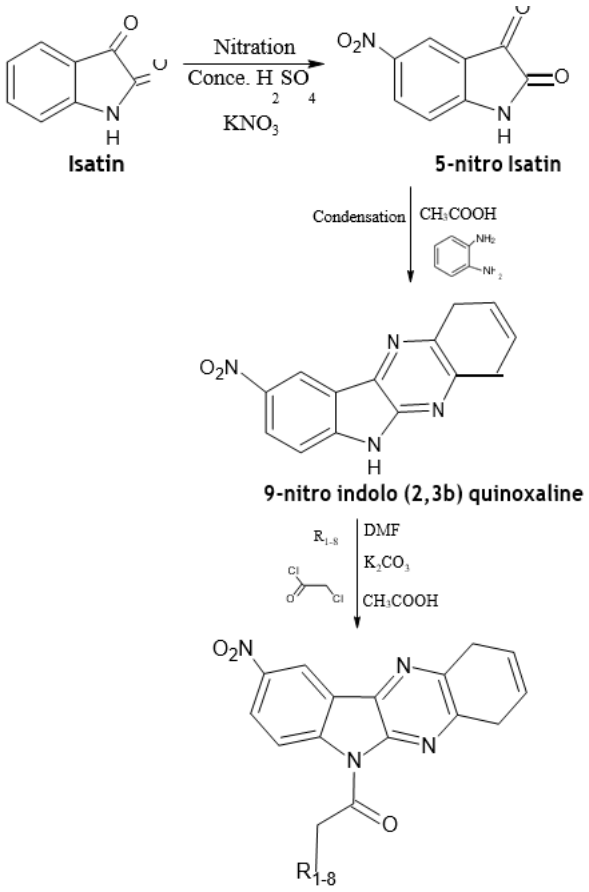
All the reagents and chemicals were obtained from Sigma-Aldrich Co, USA, Hi-media Laboratories Pvt Ltd, Mumbai, India and Merck Specialty Chemicals, Mumbai, India, and Loba Chem. All the compounds procured were purified and dried, whenevernecessary before use

3.5.2. Apparatus Used

Beakers (1000ml,500ml,100ml,50ml), conical flasks, dropping funnels, condenser, pipettes (10ml,5ml, 1ml, micropipettes), round bottom flask, separating funnel, glassrod, magnetic stirrer, digital pH meter, thermometer, heating mantle, melting pointapparatus.

3.5.3. Synthetic Schemes

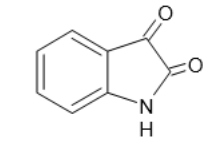
SCHEME I



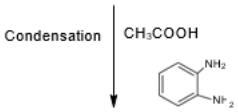
R	Substitution
ISA 1	Piperidine
ISA 2	Morpholine
ISA 3	Dimethylamine

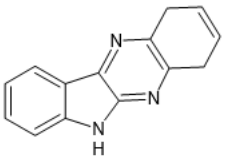
ISA 4	2,4- thiazolidine dione
ISA 5	Imidazole
ISA 6	Indole
ISA 7	Dipropyl amine
ISA 8	Dibutyl amine

SCHEME II

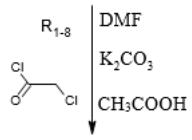


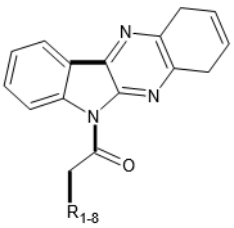
Isatin





Indolo (2,3b) quinoxaline





N-substituted indolo (2,3b) quinoxaline

R	Substitution
ISB 1	Piperidine
ISB 2	Morpholine
ISB 3	Dimethylamine
ISB 4	2,4- thiazolidine dione
ISB 5	Imidazole
ISB 6	Indole
ISB 7	Dipropyl amine
ISB 8	Dibutyl amine

3.6. Spectral Characterization

The characterization and thus the structure of the synthesized compounds were established based on IR, NMR and Mass Spectral datas. The purity of the synthesized compounds was established by single spot-on TLC plate.

3.6.1. Infrared Spectroscopy (KBr Pellet Method)

The IR spectral analysis for selected compounds were performed in the Central Instrumentation lab, Ezhuthachan College of Pharmaceutical Sciences, Neyyattinkara, Thiruvananthapuram, Kerala.

3.6.2. C_{13}NMR and Mass Spectrum

C13NMR and Mass Spectral analysis were performed and reported by the Central Laboratory for Instrumentation and Facilitation (CLIF), University Campus, Kariavattom, Thiruvananthapuram Kerala

4. Results and Discussion

4.1. Virtual Screening

The results were tabulated in Table 1. Among the 1000 direct pantothenate synthetase inhibitors screened, 50 compounds were found to be the lead.

Sl. no	Compound	Energy
1	zinc 291431	-83.445
2	zinc 306478	-93.3862
3	Zinc 968333	-60.7295
4	zinc 984053	-82.7708
5	zinc 1009012	-77.9513
6	zinc 1015874	-68.5287
7	zinc 1023908	-80.787
8	zinc 1038917	-61.5508
9	zinc 1038919	-69.833
10	zinc 1243721	-83.5579
11	**zinc 1300693**	**-46.3161**
12	zinc 1459890	-62.7592
13	zinc 1461304	-83.4189
14	zinc 1580786	-63.8163
15	zinc 1647054	-92.033
16	**zinc 1680824**	**-51.5181**
17	zinc 1709020	-76.553
18	zinc 1721888	-77.0064
19	zinc 1724917	-81.1309
20	zinc 1870211	-61.2032
21	**zinc 2039459**	**-50.5204**
22	zinc 2090503	-73.6476
23	zinc 3130625	-64.6042
24	zinc 3844873	-94.8348
25	zinc 4288680	-74.671
26	zinc 4366102	-68.1797
27	zinc 4532308	-70.4201

28	zinc 4532517	-93.5587
29	zinc 4532518	-93.5696
30	zinc 4990928	-74.2786
31	zinc 5519407	-86.4909
32	**zinc 6182368**	**- 49.2768**
33	zinc 7814784	-80.323
34	zinc 8387018	-76.5727
35	zinc 8616463	-70.5602
36	zinc 12941871	-82.1227
37	zinc 13284396	-77.1845
38	zinc 16982856	-82.5888
39	zinc 17001944	-87.4395
40	zinc 20031600	-82.1732
41	zinc 25490929	-87.0897
42	zinc 37116746	-64.9541
43	zinc 37625174	-66.6922
44	zinc 40162807	-83.7636
45	zinc 45352651	-73.2103
46	zinc 55027869	-80.5327
47	zinc 55161370	-81.1544
48	zinc 63591195	-86.7031
49	zinc 71476918	-57.3364
50	zinc 72416605	-71.1168

Virtual screening was performed by iGEMDOCK v.2 software, which showedgood fitness value for the Indophenazine moiety when screened with *Mycobacterium Tuberculosis Pantothenate synthetase.* Therefore, indophenazine was selected as the lead moiety and was subjected for optimization.

4.2. Lead optimization

Lead optimization was done by performing ADMET studies and evaluating the pharmacokinetic parameters. The data for descriptors were BBB (blood brain barrier), plasma protein binding, aqueous solubility, and hepatotoxicity. Resultswere tabulated in Table 2.

Sl No.	Compound code	FPSA	SOL LOG	SOL LOG LEV	BBB LOG LEV	ALOGP98	HEPATOTOX	HEPATOTOXPROB	FABS LEV	FABS T2	PB LEV
1	ISA 1	63.502	-4.403	2	-100	2.018	1	0.9231	0	2	1

2	ISA 2	84.450	-4.513	2	-100	0.795	1	0.9165	0	1	1
3	ISA 3	82.260	-3.942	3	-100	1.967	1	0.9412	1	1	1
4	ISA 4	94.522	-3.401	3	-100	1.345	1	0.9387	0	1	1
5	ISA 5	118.463	-4.637	2	-100	2.875	1	0.9486	0	1	0
6	ISA 6	122.534	-4.765	2	-100	-1.987	1	0.9457	0	1	1
7	ISA 7	112.452	-3.534	3	-100	2.211	1	0.9198	1	2	1
8	ISA 8	109.522	-4.669	2	-100	2.645	1	0.9273	2	1	2
9	ISB 1	107.654	-3.933	3	-100	2.654	1	0.9219	0	1	1
10	ISB 2	87.402	-3.765	3	-100	2.443	1	0.9377	0	2	1
11	ISB 3	92.533	-3.985	3	-100	2.943	1	0.9511	1	1	2
12	ISB 4	88.616	-4.654	2	-100	0.923	1	0.9419	0	1	1
13	ISB 5	77.874	-3.654	3	-100	2.038	1	0.9271	0	2	1
14	ISB 6	97.506	-4.120	2	-100	2.610	1	0.9427	0	1	1
15	ISB 7	77.598	-3.986	3	-100	2.875	1	0.9199	1	1	1
16	ISB 8	66.716	-4.654	2	-100	2.471	1	0.9374	0	1	1

The lead subjected to ADMET studies using **admetSAR** showed that the ligand of indophenazine possessed excellent pharmacokinetic profile.

4.3. Drug Like properties for oral bioavailability

Sl No	Compound code	Log P	Mol. Wgt	Hydrogen Acceptors	Hydrogen Donors	No. of Violations
1	ISA 1	3.976	403.442	8	0	0
2	ISA 2	2.914	405.414	9	0	0
3	ISA 3	3.068	363.377	8	0	0
4	ISA 4	3.113	434.433	9	0	0

5	ISA 5	2.782	388.387	9	0	0
6	ISA 6	4.758	437.459	8	0	0
7	ISA 7	4.825	419.485	8	0	0
8	ISA 8	5.943	447.539	8	0	1
9	ISB 1	4.443	344.418	5	0	0
10	ISB 2	3.381	346.39	6	0	0
11	ISB 3	3.535	304.353	5	0	0
12	ISB 4	3.108	376.397	7	0	0
13	ISB 5	3.249	329.363	6	0	0
14	ISB 6	5.225	378.435	5	0	1
15	ISB 7	5.292	360.461	5	0	1
16	ISB 8	6.41	388.515	5	0	1

In vivo absorption capabilities of the designed molecules were assessed by means of Lipinski's Rule of Five using molinspiration server. The lead compound satisfied the rule indicating that the ligands ISA_{1-8} and ISB_{1-8} have good oral absorption.

4.4. Docking

The docking results of Mycobacterium Tuberculosis pantothenate synthetase (3LE8.pdb) with the ligands ISA_{1-8} and ISB_{1-8} and standard has been reported below. The best-docked structures should have a binding energy lower to the standard. The binding sites and the active sites are shown in the snap shots and the binding energy in the Table 4.

Sl No	Compound Code	Binding Energy (ΔG_b kcal/mol)
1	ISA 1	-5.92
2	ISA 2	**-6.93**
3	ISA 3	-5.52
4	ISA 4	-5.86
5	ISA 5	-6. 03
6	ISA 6	**-8. 06**
7	ISA 7	-5.1
8	ISA 8	-4.91
9	ISB 1	**-6.28**
10	ISB 2	**-6.43**
11	ISB 3	-5.08
12	ISB 4	-5.19
13	ISB 5	-5.94
14	ISB 6	-6.19
15	ISB 7	-5.29
16	ISB 8	-6.04
17	ISONIAZID	-4.99

Binding snap shots of compounds havin g best docked scores; ISA2, ISA6, ISB1 and ISB2

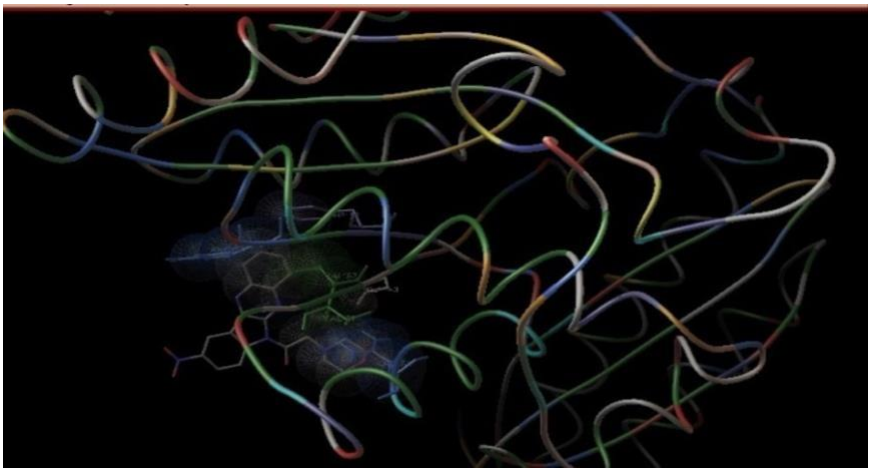


Figure 1:Snap shots of ISA 2 binding with Mtb Pantothenate synthetase (3LE8.pdb)

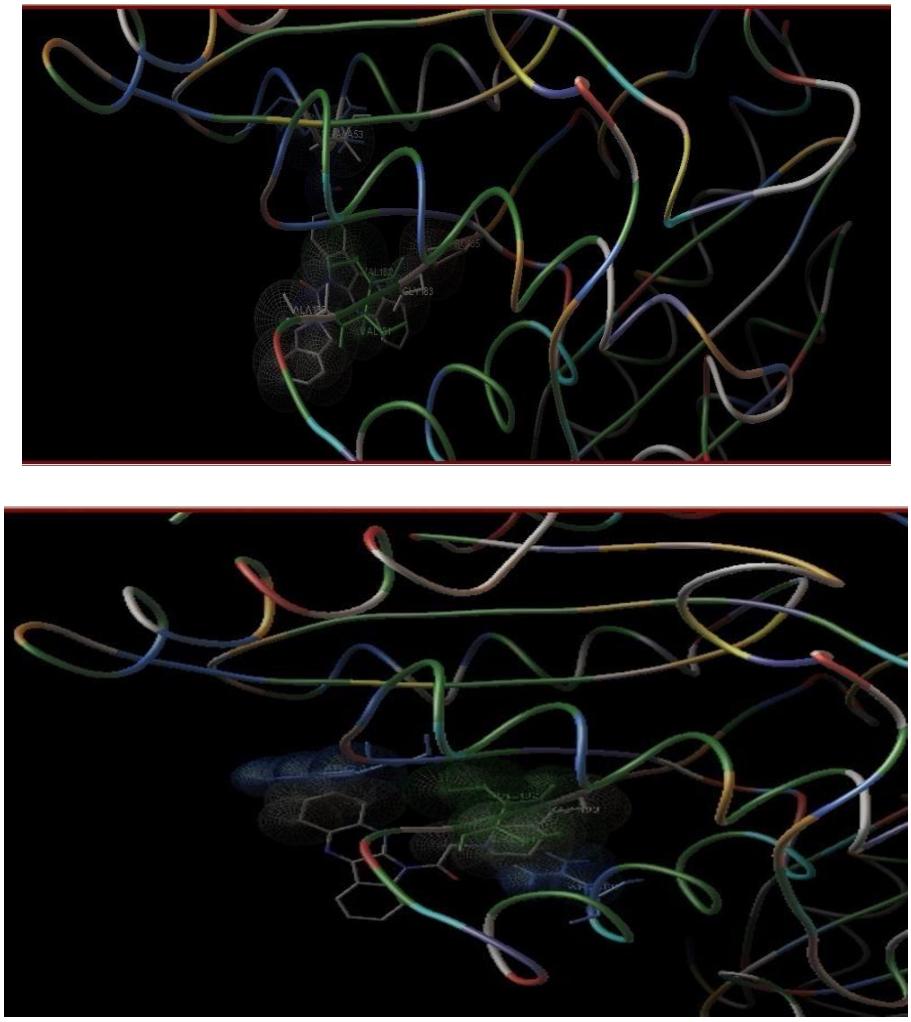


Figure 3: Snap shots of ISB 1 binding with Mtb Pantothenate synthetase (3LE8.pdb)

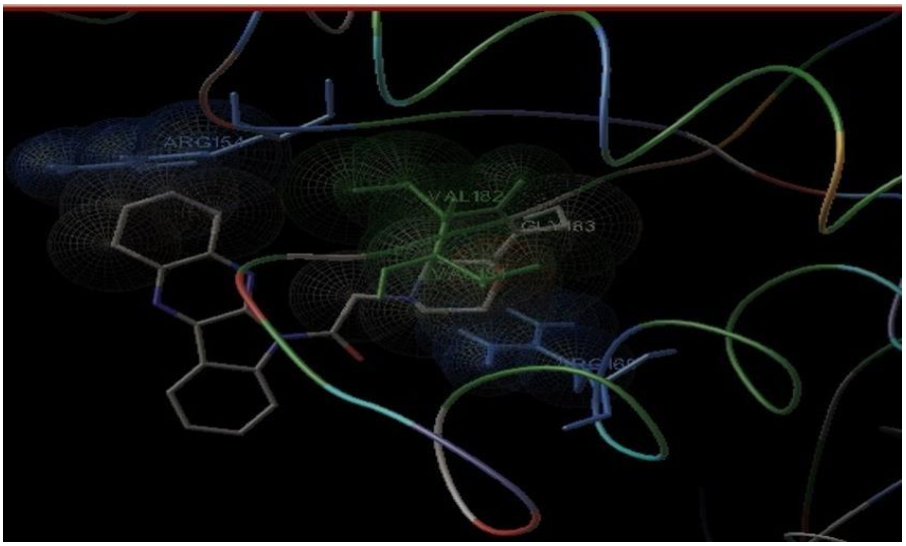


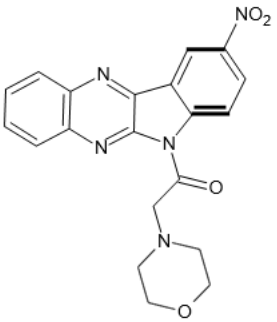
Figure 4: Snap shots of ISB 2 binding with Mtb Pantothenate synthetase (3LE8.pdb)

4.5. Spectral Data

The spectral data for the best docked compounds has been correlated.

4.5.1. IR Spectra (KBr Pellet method)

Compound code: ISA 2

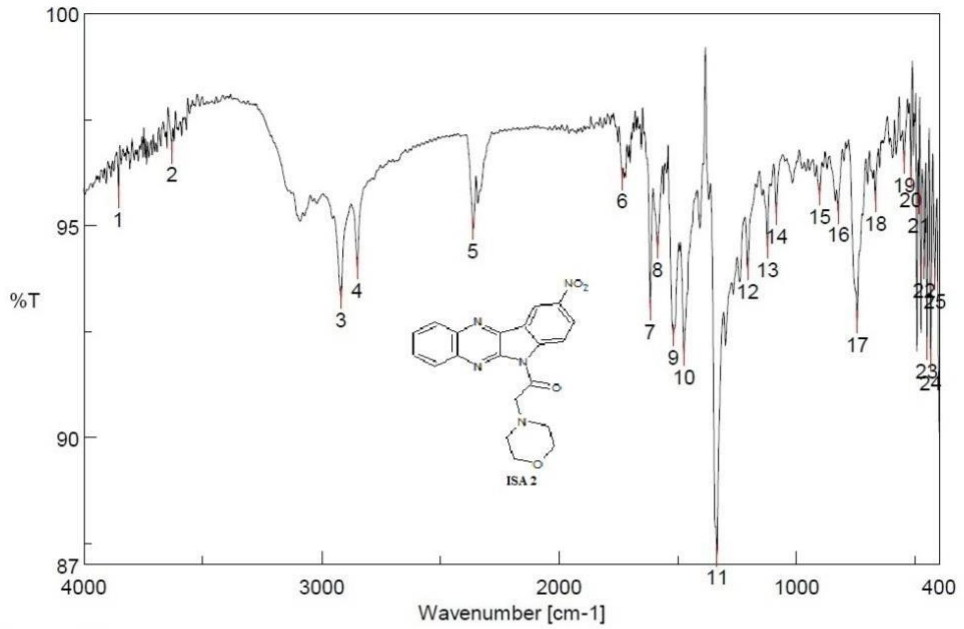


Chemical Name	:	2-(morpholin-4-yl)-1-(5-nitro-2,3-dihydro-1*H*-indophenazine-1- yl)ethanone.
Molecular Formula	:	$C_{20}H_{19}N_5O_4$
Percentage yield	:	68
R_f value	:	0.51

Solubility: Soluble in DMF, DMSO
Melting point: 258.2
Molecular weight: 393.39

Sl No	Type of Vibration	Frequency in cm^{-1}	Peak No
1	C-C stretching	903.487	15
2	N=O stretching	1335.46	11
3	Aromatic C-H bending	745.352	17
4	C-N stretching	1206.26	12
5	C-H bending in plane	1474.31	10

Peak Find - ISA 2

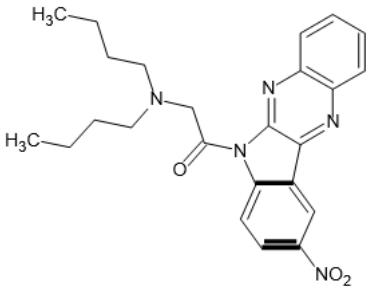


[Comments]
Sample name ISA 2
Comment
User
Division
Company SRIPMS

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
1	3855.01	95.6746	2	3630.34	96.7333
3	2917.77	93.3036	4	2849.31	94.0013
5	2361.41	94.9199	6	1734.66	96.1022
7	1616.06	93.0125	8	1585.2	94.4879
9	1519.63	92.4148	10	1474.31	91.9621
11	1335.46	87.227	12	1206.26	93.9902
13	1123.33	94.487	14	1085.73	95.2779
15	903.487	95.7384	16	825.384	95.3171
17	745.352	92.7258	18	668.214	95.5698
19	547.685	96.4903	20	518.758	96.1432
21	485.974	95.5225	22	461.868	93.9997
23	451.261	92.1007	24	435.834	91.8195
25	417.513	93.7322			

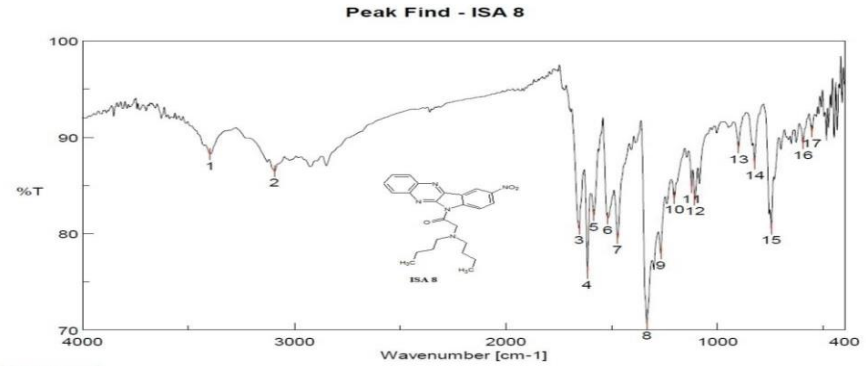
Compound code: ISA 8



Chemical Name : 2-(dibutylamino)-1-(5-nitro 2,3-dihydro-1*H*-indophenazine-1- yl)ethanone

Molecular Formula : $C_{24}H_{29}N_5O_3$
Percentage yield : 68
R_f value : 0.78
Solubility : Soluble in DMF, DMSO
Melting point : 254.6
Molecular weight : 435.51

Sl No	Type of Vibration	Frequency in cm^{-1}	Peak No
1	C-N stretching	1205.29	10
2	N=O stretching	1334.5	8
3	C-C stretching	1615.09	4
4	Aromatic C-H bending	746.317	15
5	C-H stretching	3093.26	2

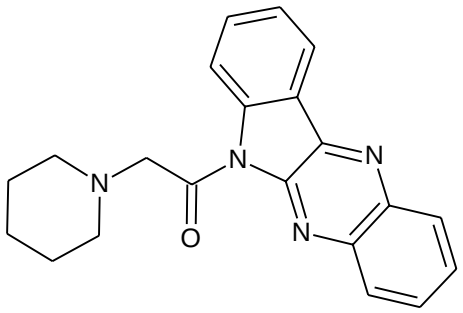


[Comments]
Sample name ISA 8
Comment
User
Division
Company SRIPMS

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
1	3396.99	88.2532	2	3093.26	86.4999
3	1654.62	80.5344	4	1615.09	75.9225
5	1585.2	81.9083	6	1518.67	81.5898
7	1472.38	79.5679	8	1334.5	70.6362
9	1268.93	77.9382	10	1205.29	83.7208
11	1123.33	84.8091	12	1106.94	83.5416
13	901.558	88.94	14	825.384	87.274
15	746.317	80.5521	16	596.861	89.3606
17	555.398	90.6016			

Compound code: ISB 1



Chemical Name: 1-(2,3-dihydro-1*H*-indophenazine-1-yl)-2-(piperidin-1-yl)ethanone
Molecular Formula: $C_{21}H_{22}N_{40}$

Percentage yield	: 72
R_f value	: 0.58
Solubility	: Soluble in DMF, DMSO
Melting point	: 247.4
Molecular weight	: 346.42

Sl No	Type of Vibration	Frequency in cm^{-1}	Peak No
1	C-C stretching	1615.09	5
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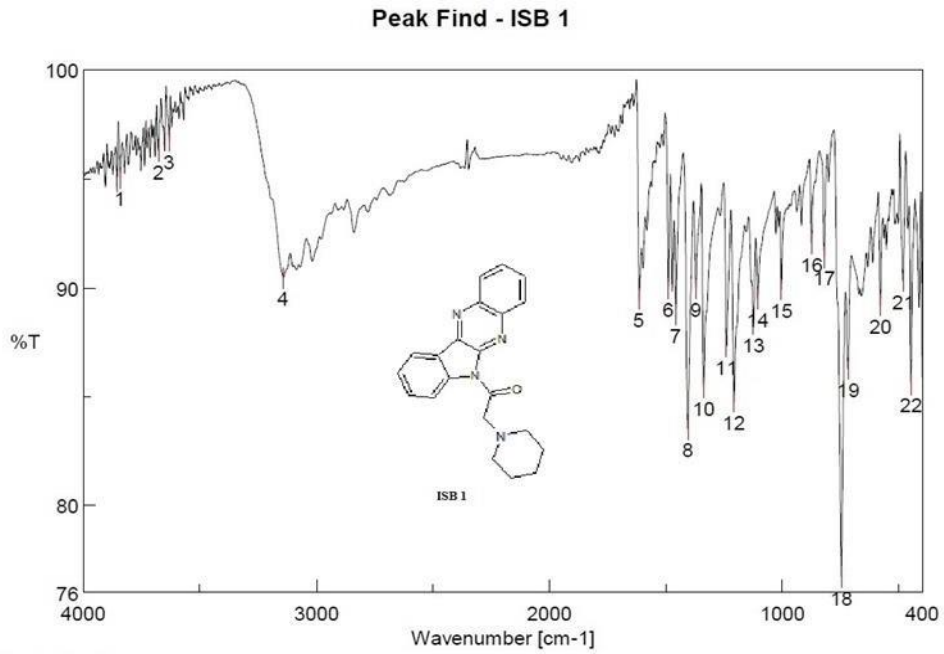
3	C-N stretching	1208.18	12
4	C-H bending	746.317	18
5	C-H rocking	718.354	19

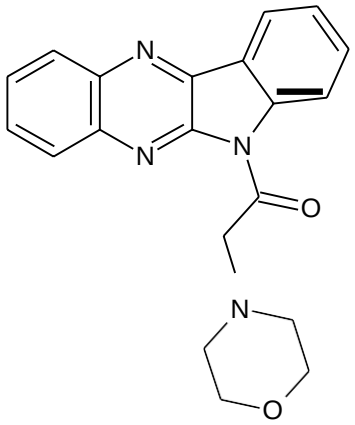


[Comments]
Sample name ISB 1
Comment
User
Division
Company SRIPMS

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
1	3841.51	95.0355	2	3677.59	96.264
3	3631.3	96.7342	4	3141.47	90.4589
5	1615.09	89.4628	6	1489.74	89.9527
7	1457.92	88.7696	8	1405.85	83.4989
9	1371.14	89.9506	10	1338.36	85.4074
11	1240	87.2959	12	1208.18	84.7466
13	1124.3	88.308	14	1105.98	89.4574
15	1004.73	89.9095	16	874.56	92.012
17	820.563	91.3902	18	746.317	76.6958
19	718.354	86.2495	20	579.504	89.1733
21	482.117	90.3011	22	448.369	85.552

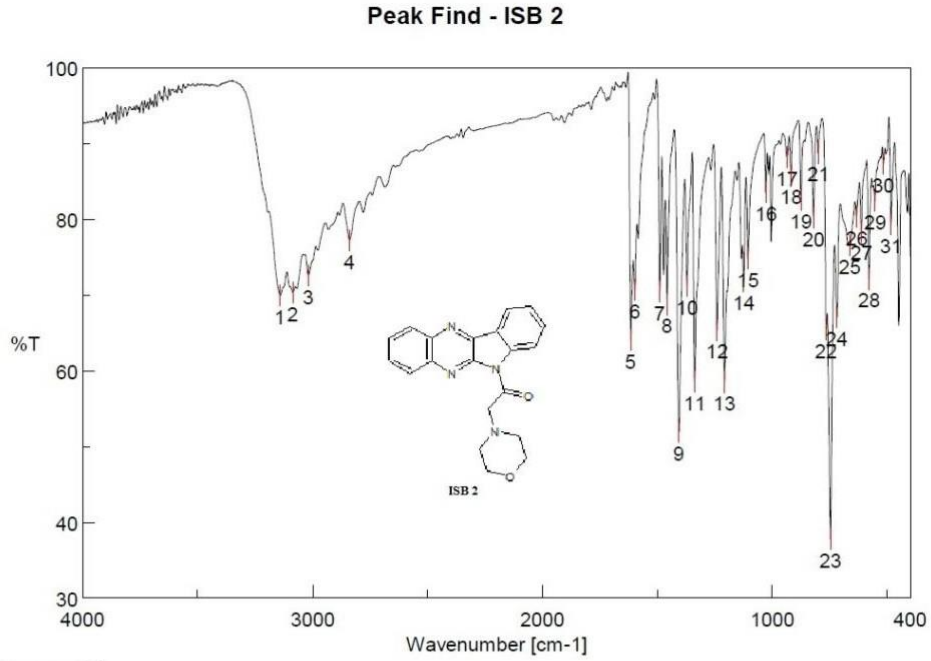
Compound code: ISB 2



Chemical Name: 1-(2,3-dihydro-1*H*-indphenazinel-1-yl)-2-(morpholin-4-yl) ethenone

Molecular Formula: $C_{20}H_{20}N_4O_2$
Percentage yield: 74
R_f value: 0.61
Solubility: Soluble in DMF, DMSO
Melting point: 246.7
Molecular weight: 348.39

Sl No	Type of Vibration	Frequency in cm^{-1}	Peak No
1	C-C stretching	1616.06	5
2	C=C stretching	1489.74	7
3	C-N stretching	1208.18	13
4	C-H rocking	820.563	20
5	C-H bending	747.281	23



[Comments]
Sample name ISB 2
Comment
User
Division
Company SRIPMS

[Result of Peak Picking]

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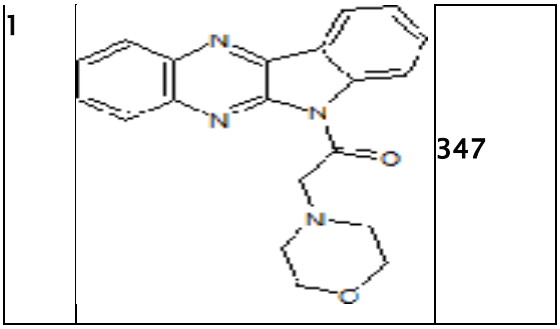
3	3018.05	72.5725	4	2839.67	77.2712
5	1616.06	64.0611	6	1597.73	70.7176
7	1489.74	70.4843	8	1457.92	68.7409
9	1406.82	51.9095	10	1371.14	71.2659
11	1338.36	58.5505	12	1240.97	65.3618
13	1208.18	58.4733	14	1125.26	71.8485
15	1105.98	74.8028	16	1027.87	83.5386
17	936.271	88.242	18	918.914	85.7866
19	875.524	82.5706	20	820.563	80.1491
21	800.314	88.7629	22	765.601	65.4391
23	747.281	37.7556	24	719.318	67.0197
25	663.393	76.5193	26	634.466	80.303
27	613.252	78.3803	28	579.504	72.1333
29	554.434	82.4562	30	516.829	87.3992
31	484.045	79.2985			

4.5.2. Mass Spectra

1) Compound Name: ISB 2

Sl No	Molecular Structure	m/zratio
		350



Report(Report Editor) Status:Temporary 09/27/2023 3:48:23pm

Acquired by	: Admin
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Sample ID	: ISB 2 +VE SIM
Vail #	: 2
Injection Volume	: 15 uL
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Method File Name	: kongunadu.lcm
Batch File Name	:
Report File Name	: DefaultLCMS.lcr

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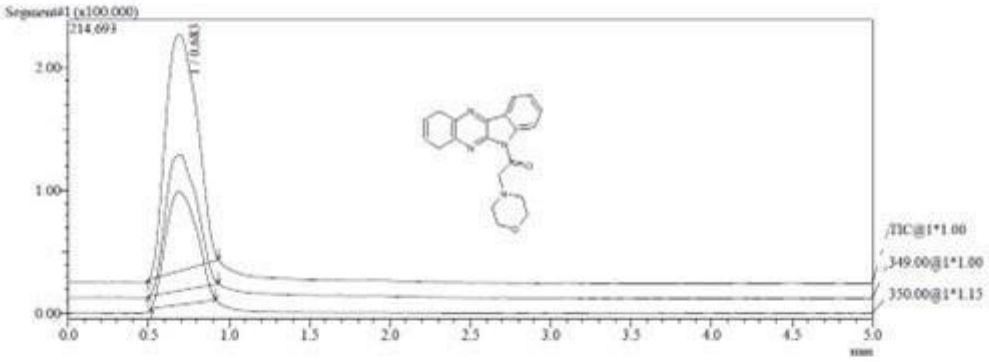

MS Spectrum Graph D:\1309TSS\26.lcd

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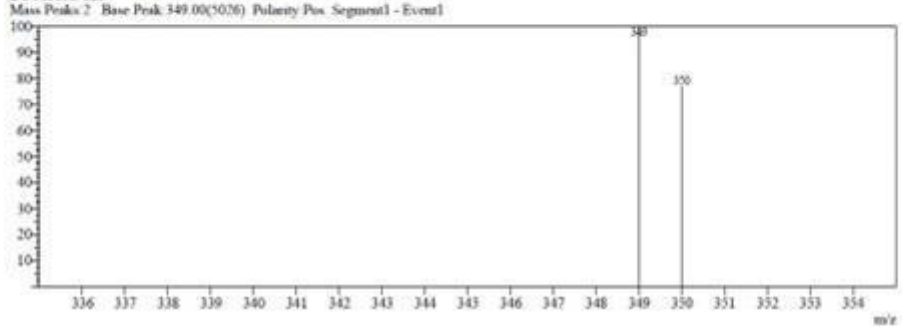
BG Mode:None
Mass Peaks:2 Base Peak:349.00(5026) Polarity:Pos Segment1 - Event1



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Sample ID	: ISA 2 -VE SIM
Vail #	: 1
Injection Volume	: 15 uL
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Method File Name	: kongunadu.lcm
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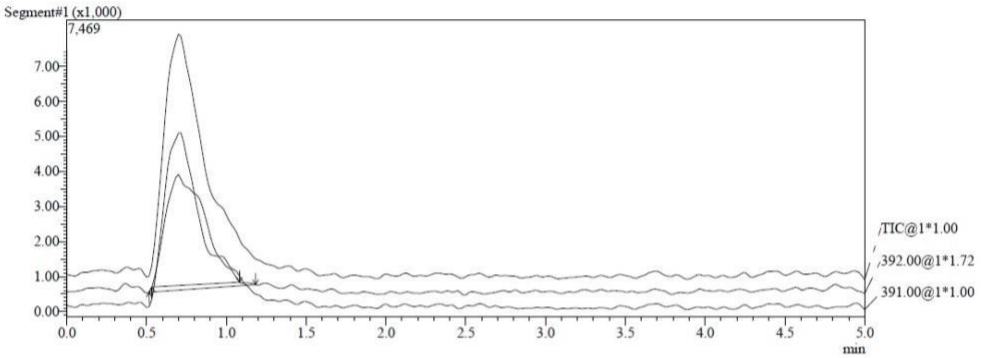
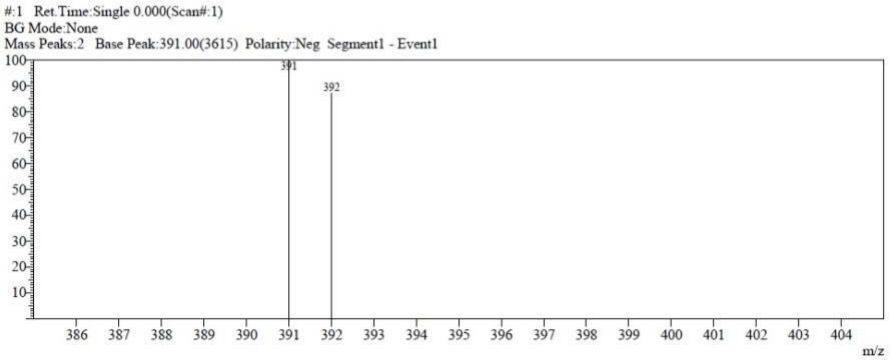
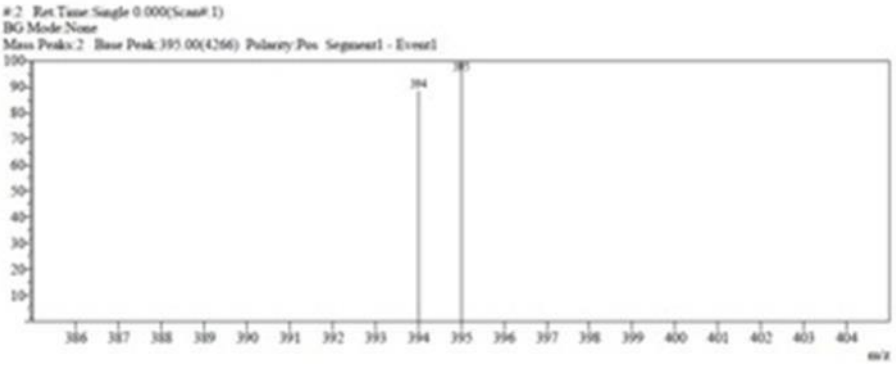
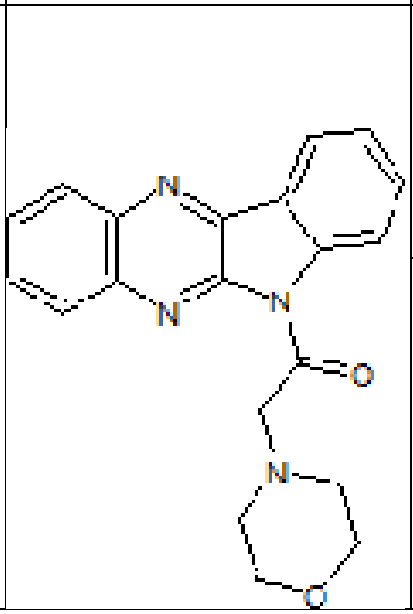


MS Spectrum Graph D:\1309JSS\25.lcd





Compound Name: ISB 2

Sl No	Molecular Structure	m/zratio
1		350 347

Report(Report Editor) Status:Temporary

09/27/2023 6:11:39 1 / 1

Acquired by	: Admin
Sample Name	: ISB 2 +VE SIM
Sample ID	: ISB 2 +VE SIM
Vail #	: 2
Injection Volume	: 15 uL
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Method File Name	: kongunadu.lcm
Batch File Name	:
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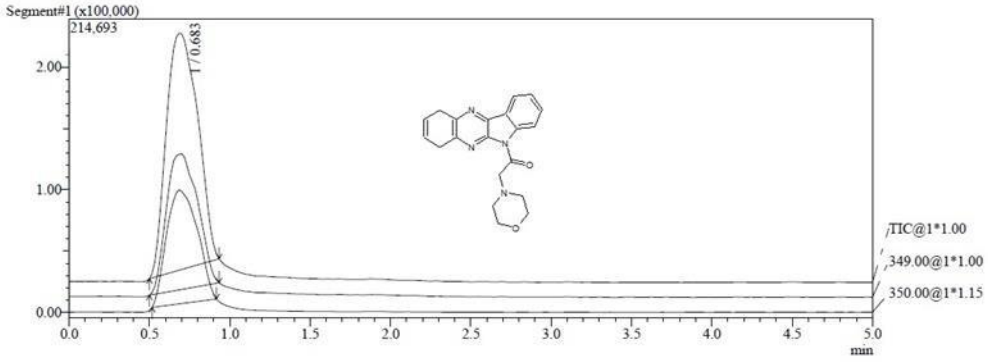
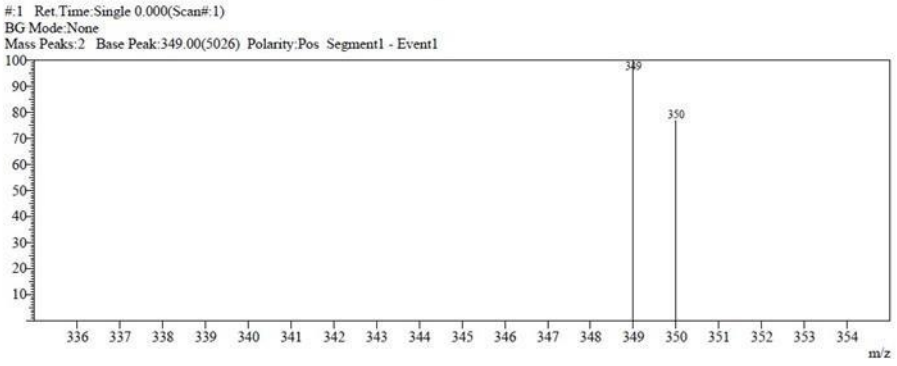
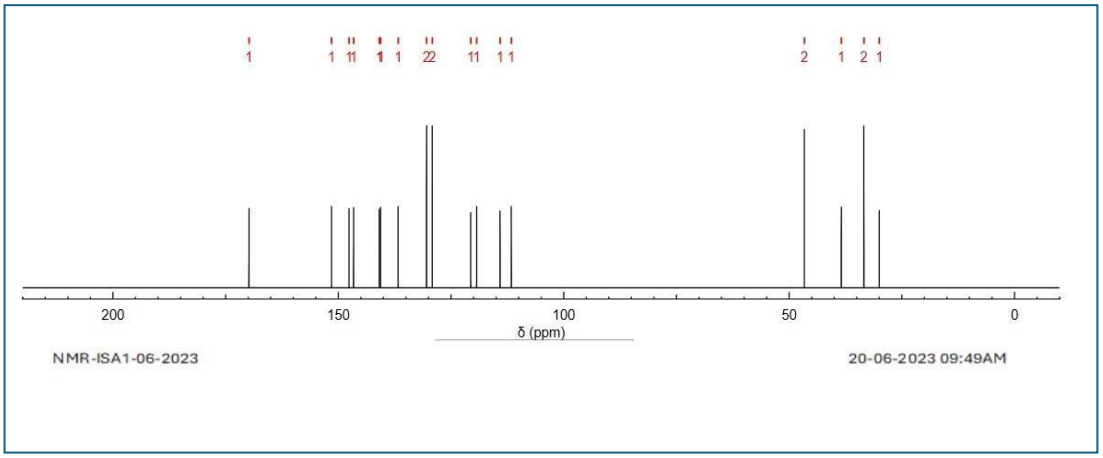


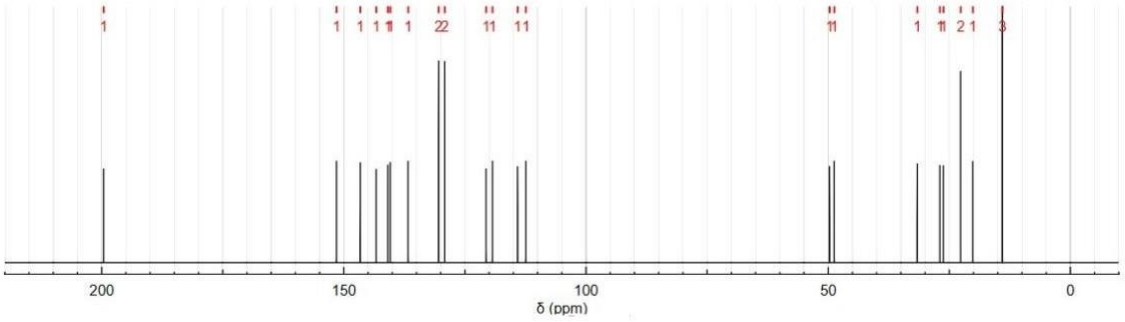
MS Spectrum Graph D:\1309JSS\26.lcd



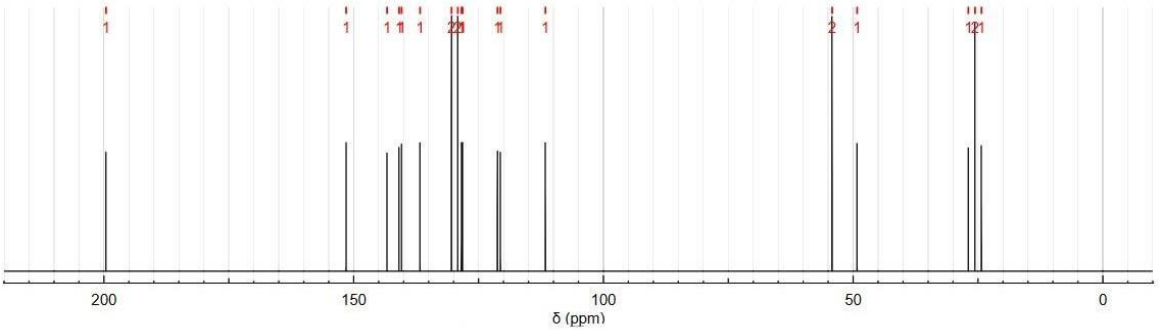
4.5.3. NMR Spectra (C13 NMR)Compound ISA 1



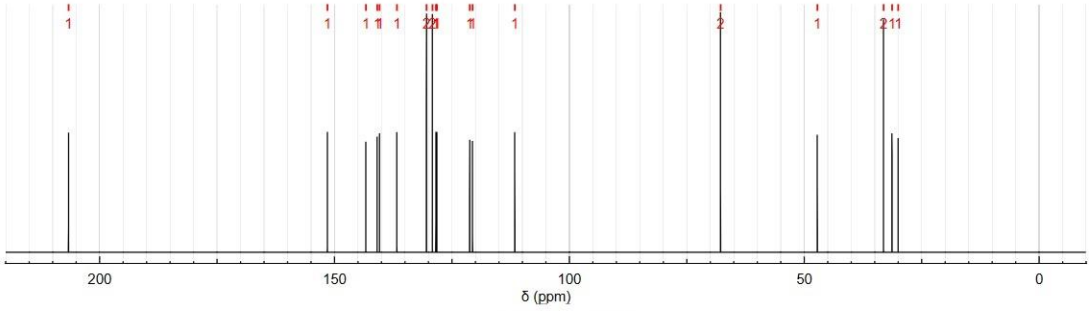
Compound ISA8



Compound ISB1



Compound ISB2



5. Summary and Conclusions

The present work was focused on the design, ADME studies, docking synthesis and evaluation of anti-tubercular activity of indophenazine derivatives as pantothenate synthetase inhibitor. Virtual screening was performed by iGEMDOCK v.2 with a1000 compounds containing molecular library from the ZINC database, which showed 50 lead compounds. From that, the compounds having indophenazine nucleus was having an excellent fitness score. Lead optimization was done by observing the insilico ADME studies and computation of drug like properties. The ligands ISA_{1-8} and ISB_{1-8} showed good scores that they are eligible for the study. Lead optimized compounds were subjected to docking by Autodock Vina for Mycobacterium Tuberculosis pantothenate synthetase (3LE8.pdb). For the tuberculosis enzyme, the best binding energies were shown by ISA 2, ISA 6 of the first series and ISB 1, ISB 2, ISB 6 of the second series. All the ligands were found to interact with the key binding site GLY 183 of Mycobacterium Tuberculosis pantothenate synthetase (3LE8.pdb). By viewing the

docking results, the designed compounds were subjected to synthesis. In the recent study, sixteen novel compounds were synthesized. The first scheme was the synthesis of nitro substituted indophenazine derivatives and second scheme was the synthesis ofindophenazine derivatives without the substitution of nitro group. The melting points of all the compounds were determined. Rf values were determined by TLC. The structure was finally characterized by IR, NMR and Mass spectroscopic methods.

It has been summarized that the structure-based drug design minimizes the tedious drug discovery process. The virtual screening methodology has given the easy way to select the lead compound to with good fitness values. The in-silico ADME studies and drug likeness score confirmed the compounds are pharmacologically active. The binding energies obtained by docking studies were further confirmed possibility of the titled compound as lead pantothenate synthetase enzyme inhibitor in Mycobacterium tuberculosis. High score docked compounds were synthesized based on the developed scheme of synthesis and good yields obtained. Melting points, TLC, IR, NMR and Mass Spectra, confirmed the structures of the synthesized compounds. All the synthesized compounds can be given for their antitubercular activity and correlation study. Sixteen novel indophenazine derivatives were synthesized and it was proved that some of the derivatives are significantly potent as Mycobacterium Tuberculosis pantothenate synthase inhibitor.

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