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Molecular Docking Study of Indole Moiety as an Anti-Cancer, Anti-Inflammatory, And Antitubercular Agent

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ABSTRACT:

Indole or 2,3 benzo-pyrrole is an important compound in the synthesis of various medicinally active compounds and is even included in the synthesis of certain natural compounds (reserpine, ergot, tryptamine, physostigmine, etc.). As the naturally available indole derivatives had shown the diverse biological activity leads to the synthetic development of indole compounds that have a potent activity with targeted action. In the view of synthetic development of medicinal compounds, certain promising compounds were selected from previously existing synthesised compounds, and the current study involves the discovery of the activity of such promising compounds on different receptor proteins with the application of molecular docking.

KEY WORDS: Indole, Molecular docking, Anticancer, Anti-inflammatory, Antitubercular

INTRODUCTION

Indole is a heterocyclic compound consisting of the molecular formula is C8H7N and has a structure of a fused benzene ring along with the pyrrole ring, it is a planar molecule having ten pi electrons and the delocalization of the lone pair electrons on the nitrogen atom makes the molecule weakly basic in nature[1]. Indole derivatives as shown various pharmacological activities such as the NSAIDs (indomethacin, pravadoline, tenidap, melatonin)[2,3], antihypertensive (reserpine, yohimbine, pindolol)[4], antihistamines (zafirlukast, ondansetron, dolasetron)[5–7], anticancer (vincristine, adozelesin, carzelesin, intoplicine)[5,8], antidepressant (binedaline, trandolapril, siramesine, amedalin)[9], antipsychotic (psilocybin)[10], and various other activities are reported with this compound [8,11–13]. Indole derivatives are naturally available as such in alkaloids (strychnine, ajmalicine)[14,15], proteins, or amino acids.

Accidental discoveries, ancestral or traditional knowledge of the plants or the in-depth knowledge of the phytochemical constituents give the lead for new drug discoveries. The new drug discovery in the prior years was included large extensive in-vivo studies and laborious synthetic trials which were non-cost effective. To provide a better efficacious safer drug to the public it's important for a cost-effective way of drug designing, in such mode in-silico drug designing is a breakthrough[16]. These in-silico studies are included with various computational methods such as high-throughput screening (HTS), structure-based (target structure, pharmacophore modeling, ligand docking, de novo design, molecular dynamics), and ligand-based CADD (QSAR, ligand-based virtual screening)[17].

Molecular docking is a structure-based drug design that consists of recognition of binding energy of molecular interaction and conformation of a small molecule with biological proteins[18]. The development of the computational methods that introduced various algorithms that can generate a larger number of sample ligands for the development (matching algorithm, Monte Carlo, molecular dynamics, Dock 4.0), supported by a

suitable scoring function that estimates the binding affinity of the biomolecule with sample ligands and involvement of different docking methodologies (rigid docking and flexible docking) combinedly provide the knowledge of the interaction of a compound at its molecular levels[19].

Various computational methods are used to describe the potential drug compound in the drug discovery such as drug-likeness, fragment-based virtual screening, bioactivity prediction, and toxicity studies that are compelled in several software.

Drug-likeness and toxicity are the most common parameters which are used to predict the properties of the compound such as solubility, logP (partition coefficient), Lipinski rule of five, and the toxicity study with the computed model such as boiled egg and other help known the adverse effect of compound [20].

MATERIAL AND METHOD

Selection of compounds: -

The indole ring containing compounds are selected by reviewing the literatures that are open access in which the in-silico studies that are carried out in the current scheme were not performed, through which the structure activity between the selected compounds can be determined and that are helpful in developing the new compounds also. The compounds were selected based on their highest quantity of yield, toxicity of compounds studied, and highest purity.

The compound structure was drawn using Chemdraw software and were optimized by using Chem3D pro software by setting 0.010 Minimum RMS Gradient. The SMILES notation was generated by using ACD /Chemsketch software.

Drug likenesses of the reported compounds were determined by using MOLINSPIRATION a tools that calculate parameters of the compounds by using the Lipinski rule of Five [21]. The drug like property of the compounds were studied also with the webserver SwissADME tool to determination of various physicochemical and Pharmacokinetics parameters along with five different rules that helps in determination of the drug likeness.[22]

Docking studies of the compounds were performed by using AutoDock Vina software[23] and the proteins (PDB ID; 2J5F, 2ITY, 2OYU, 3LN1, 4KFG, 1OHJ) in the study were retrieved through the RCSB protein data bank[24]. Biovia Discovery studio are used to prepare the ligands and proteins for the study and to visualize the interaction of compounds with receptor proteins.

The webserver ProTox-II which is a virtual lab that computationally estimates the toxicity of the compounds and to determine the LD50 dose of the compounds.

RESULTS AND DISCUSSION

Molinspiration tool are used estimate the molecular properties and predict the bioactivity of compounds against the important receptors like GPCR, nuclear receptors, ion channel modulators, and kinase inhibitors.

Lipinski rule of 5:

It is the set of molecular descriptors that states that, most of the "drug-like" properties of the molecules consider the criteria like log p ≤ 5, molecular weight ≤ 500, number of hydrogen bond acceptors ≤ 10, and hydrogen bond donor's ≤ 5.

Log P(MiLogP): octanol/water partition coefficient.

No. of H Bond acceptors (nON): no. of oxygen and nitrogen atoms.

No. of H Bond donors (nOHNH): no. of –OH and –NH groups.

No. of rotatable bonds (nrotb): It is any single non ring bond bounded to non-terminal none hydrogen atom. This descriptor is useful for oral bioavailability.

Molecular docking studies:

Molecular docking studies was carried out on 6 distinct protein receptor targets using auto dock vina software tool.

- Anti-cancer: Breast cell line: 2J5F & Lung cell line: 2ITY
- Anti-tubercular: 1OHJ
- Anti-bacterial: 4KFG
- Anti-inflammatory: COX 1: 2OYU & COX 2: 3LN1

Protein preparation:

Protein preparation was done in 'Discovery studio v16.1.0.15350 visualizer' software.

Proteins were prepared by deleting water molecules and by adding polar Hydrogen's to the 3D X ray crystallographic structure of the protein receptor target.

Co-ordinates of co-crystallised ligands were separately saved for definition of active site.

Ligand preparation:

All ligands were prepared in 'Discovery studio v16.1.0.15350 visualizer' software employing Merck Molecular Force field (MMFF) optimization method with 10,000 number of cycles and convergence criteria of 0.01Å.

Distance dependent dielectric properties were employed for charge calculations.

Co crystallised ligand and a marketed drug for each protein is prepared for the comparative study.

'Autodock vina' algorithm was run and analyzed for their binding affinity (kcal/mol) and ligand protein interactions (electrostatic and van der Waals interactions).

IN – SILICO TOXICITY STUDIES:

Online Toxicity Prediction by ProTox II, which predict the toxicity with different models and includes various toxicity models to determine acute toxicity, organ toxicity, toxicological pathways and toxicological targets.

Toxic doses are provided LD50 values in mg/kg body weight. The LD50 is the median

lethal dose meaning the dose at which 50% of test subjects die upon exposure to a compound.

Toxicity classified according to the globally harmonized system of classification of

labelling of chemicals (GHS). LD50 values are given in [mg/kg]:

- Class I: fatal if swallowed (LD50 ≤ 5)
- Class II: fatal if swallowed (5 < LD50 ≤ 50)
- Class III: toxic if swallowed (50 < LD50 ≤ 300)
- Class IV: harmful if swallowed (300 < LD50 ≤ 2000)
- Class V: may be harmful if swallowed (2000 < LD50 ≤ 5000)
- Class VI: non-toxic (LD50 > 5000)

CONCLUSION

In the current study, 48 different molecules containing indole nucleus well collected through literature search. All the compounds were subjected to three studies these were –In silico drug likeliness study, In silico molecular docking study, In silico toxicity study. For analysis of each activities result, a marketed drug having indole ring and the reference ligand was also docked to the same receptor site.

Among all 48 screened, indoles containing compounds **AII4(a)** and **AII4(b)** were found to have potent activity as Anti-Breast cancer agents, COX-2 inhibitor, Anti-Lung cancer agents respectively. Both the compounds were found to be non-immunotoxic and non-cytotoxic, carcinogenicity and mutagenicity. Compounds **AII4(b)** exhibited no violation in Lipinski rule of 5 whereas **AII4(a)** showed one violation. Hence both the compounds can be considered as drug candidates with certain modification.

Conflict of Interest: Authors declare no conflict of interest.

Author Contributions

The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript. ‡ Rudresh H M⊥⊥, Arpit Kumar⊥, these authors contributed equally under the guidance of Dr. Monica Arora.

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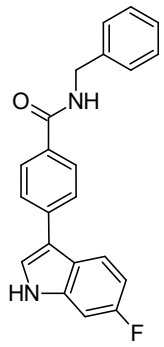
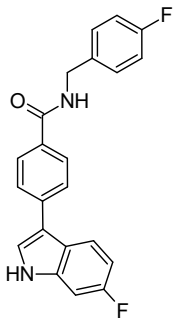
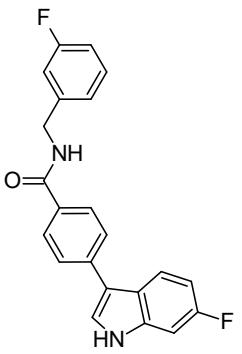
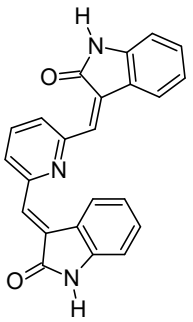
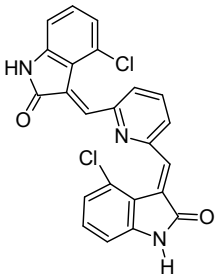
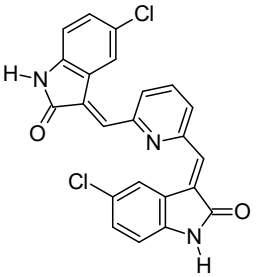
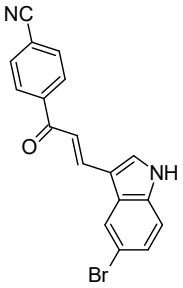
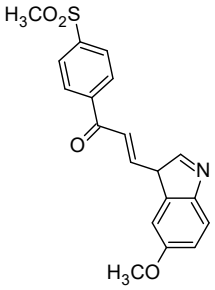
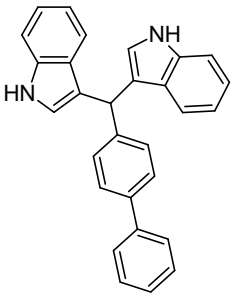
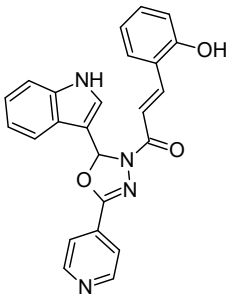
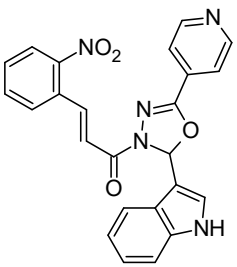
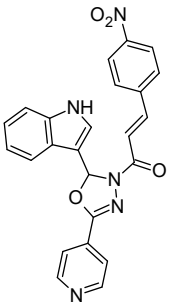
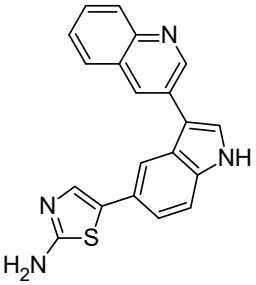
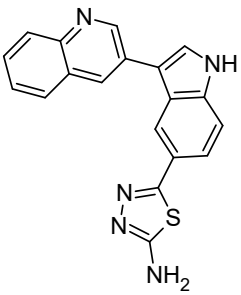
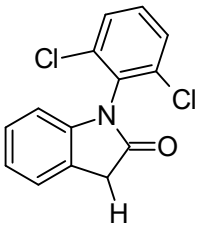
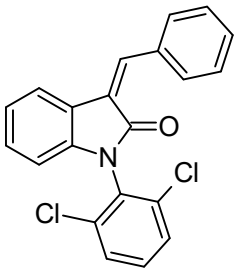
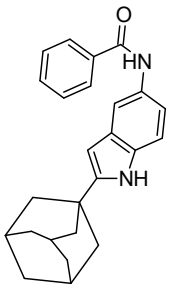
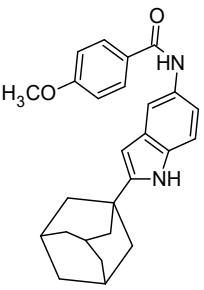
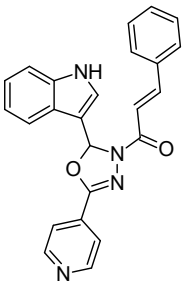
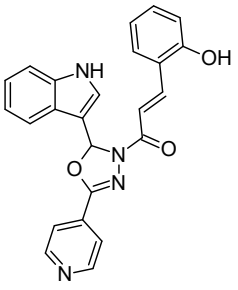
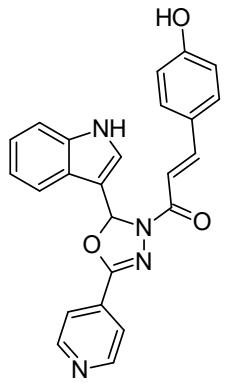
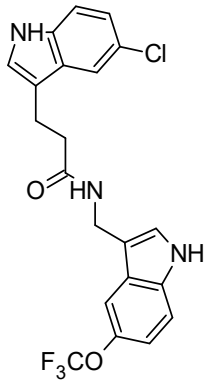
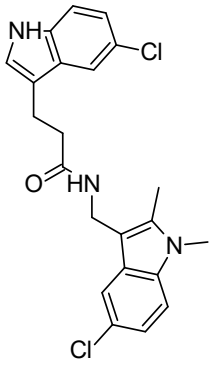
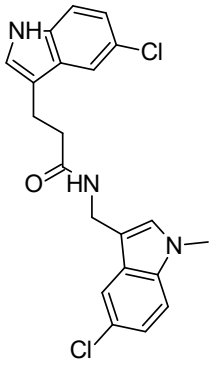
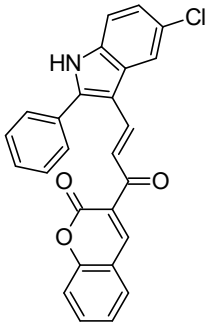
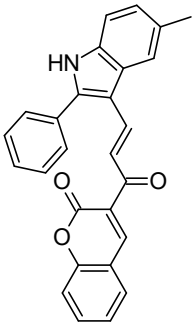
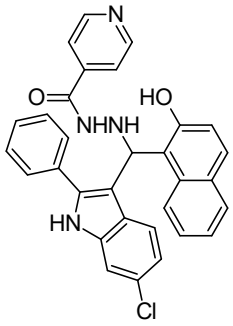
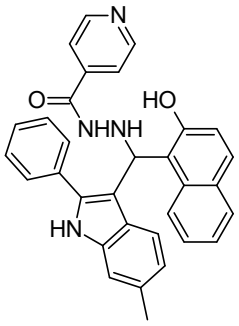
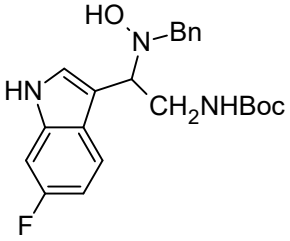
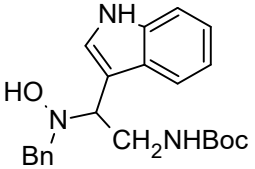
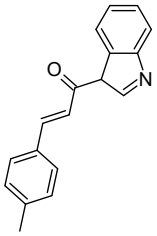
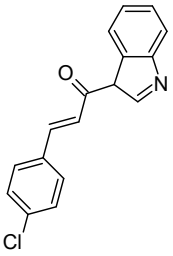
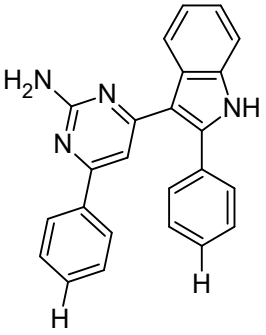
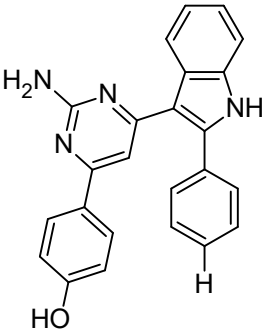
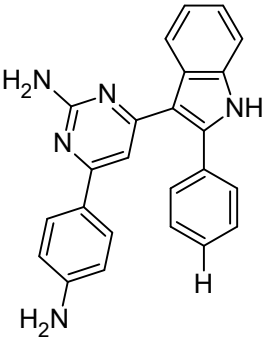
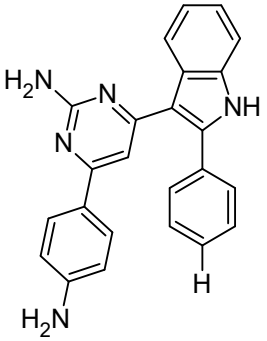
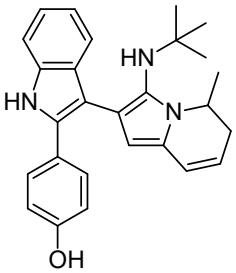
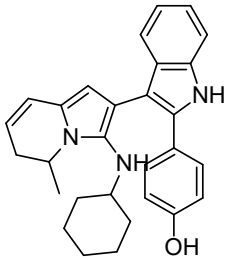
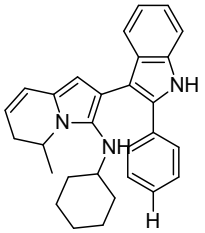
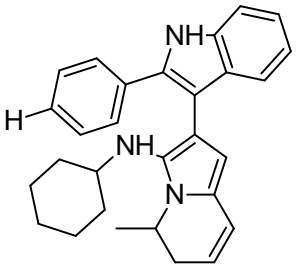
Table 1, Consisting structure of Compounds involved that are in the study

AIC1(a) 	AIC1(b) 	AIC1(c) 	AIC2(a) 
AIC2(b) 	AIC2(c) 	AIGEN(a)I 	AIGEN(b)I 
AIGEN(c)I 	AIGEN(a)T 	AIGEN(b)T 	AIGEN(c)T 
AIC3(a) 	AIC3(b) 	AIC4(a) 	AIC4(b) 
AIC5(a)(i) 	AIC5(b)(i) 	AIT1(a) 	AIT1(b) 

AIT1(c) 	AIT&M1(a)(i) 	AIT&M1(**c**)(i) 	AIT&M1(d)(i) 
AIT&M2(a)(i) 	AIT&M2(b)(i) 	AIT2(a)(i) 	AIT2(b)(i) 
AII1(a)(i) 	AII1(b)(i) 	AII2(a)(i) 	AII2(b)(i) 
AIM(c) 	AII4(a) 	AII4(b) 	AII4(c) 
AIC8(i) 	AIC8(ii) 	AIC8(iii) 	AIC8(iv) 

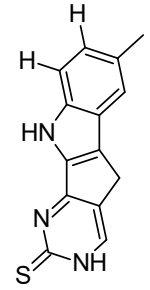
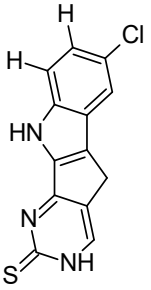
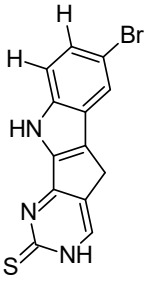
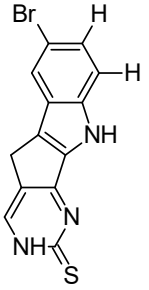
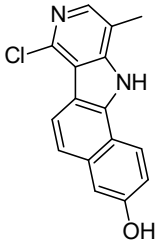
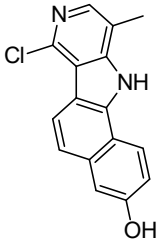
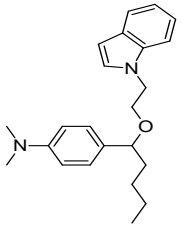
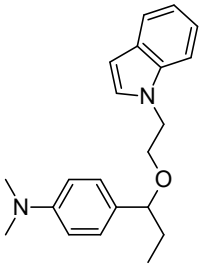
AIC9(i) 	AIC9(ii) 	AIC9(iii) 	AIC9(iv) 
AIC10(i) 	AIC10(ii) 	AIM(a) 	AIM(b) 

Table 2, Drug-likeliness profile of the compounds involved in the study.

Compound Code	MiLogP	natoms	Molecular Weight (g/mol)	nON	nOHNH	nrotb	nviolations
AIC1(a)	4.62	26	344.39	3	2	4	0
AIC1(b)	4.78	27	362.38	3	2	4	0
AIC1(c)	4.75	27	362.38	3	2	4	0
AIC2(a)	3.23	28	365.39	5	2	2	0
AIC2(b)	4.49	30	434.28	5	2	2	0
AIC2(c)	4.53	30	434.28	5	2	2	0
AIGEN(a)I	4.32	22	351.20	3	1	3	0
AIGEN(b)I	2.23	25	355.42	5	0	5	0
AIGEN(c)I	7.19	31	398.51	2	2	4	1
AIGEN(a)T	3.54	31	410.43	7	2	4	0
AIGEN(b)T	3.52	33	439.43	9	1	5	0
AIGEN(c)T	3.74	33	439.43	9	1	5	0
AIC4(a)	4.31	18	278.14	2	0	1	0
AIC4(b)	6.16	25	366.25	2	0	2	1
AIC5(a)(i)	6.17	28	370.50	3	2	3	1
AIC5(b)(i)	6.23	30	400.52	4	2	4	1
AIT1(a)	3.78	30	394.43	6	1	4	0
AIT1(b)	3.54	31	410.43	7	2	4	0
AIT1(c)	3.30	31	410.43	7	2	4	0
AIT&M(a)(i)	5.04	30	435.83	5	3	7	1
AIT&M(c)(i)	5.04	28	414.34	4	2	5	1
AIT&M(d)(i)	4.82	27	400.31	4	2	5	0
AIT&M2(a)(i)	6.11	31	425.87	4	1	4	1
AIT&M2(b)(i)	5.88	31	405.45	4	1	4	1
AIT2(a)(i)	6.36	38	519.00	6	4	6	2
AIT2(b(i)	6.12	38	498.59	6	4	6	1
AII1(a)(i)	4.12	29	399.47	6	3	8	0
AII1(b)(i)	3.98	28	381.48	6	3	8	0
AII2(a)(i)	3.63	20	261.32	2	0	3	0
AII2(b)(i)	3.86	20	281.74	2	0	3	0
AII3(a)	3.90	22	287.32	3	0	2	0
AII3(b)	4.12	23	301.35	3	0	2	0
AIM(a)	5.84	26	350.51	3	0	9	1
AIM(b)	4.78	24	322.45	3	0	7	0
AIM(c)	5.49	28	370.50	3	0	7	1
AII4(a)	5.20	28	362.44	4	3	3	1
AII4(b)	4.72	29	378.24	5	4	3	0
AII4(c)	4.28	29	377.45	5	5	3	0
AIC8(i)	6.55	29	381.52	3	2	4	1
AIC8(ii)	6.43	31	411.55	4	3	4	1
AIC8(iii)	7.16	33	437.59	4	3	4	1
AIC8(iv)	7.63	32	421.59	3	2	4	1
AIC9(i)	1.88	17	239.30	3	2	0	0
AIC9(ii)	2.30	18	253.33	3	2	0	0
AIC9(iii)	2.53	18	273.75	3	2	0	0
AIC9(iv)	2.66	18	318.20	3	2	0	0
AIC10(i)	3.21	27	357.32	7	5	0	0
AIC10(ii)	4.26	20	382.73	3	2	0	0

All the compounds were subjected to drug-likeness profile using the Molinspiration tool. out of 48 compounds

around 33 (68.75%) showed drug-like properties as they not violate the Lipinski rule of five.

Table 3, Molecular docking results on anti-cancer (breast cell line) protein.

LIGAND	BINDING AFFINITY (kcal/mol)	LIGAND	BINDING AFFINITY (kcal/mol)
AII4(a)	-10.5	AIT1(b)	-8.5
AII4(b)	-10.5	AIC4(b)	-8.5
AIC10(i)	-9.9	AIGEN(a)I	-8.4
AIC3(b)	-9.9	AII1(a)	-8.4
AIGEN(c)I	-9.7	AIC2(a)	-8.3
AIC3(a)	-9.4	AII1(b)	-8.3
AITM(b)	-9.4	AIGEN(c)T	-8.2
AIC8(i)	-9.4	AITM(c)(i)	-8.2
AIC1(b)	-9.3	AITM(d)(i)	-8.1
AITM(a)	-9.3	AIM(c)	-7.9
AIC8iv	-9.3	AIGEN(b)I	-7.8
AIC1(c)	-9.2	AIC4(a)	-7.6
AIC5(a)(i)	-9.2	AIC9(ii)	-7.4
AIC6(b)(i)	-9.2	AIC9(iii)	-7.4
AIC8(iii)	-9.1	AIC9(iv)	-7.3
AIC1(a)	-9	AIM(a)	-7.3
AIT1(a)	-9	AIM(b)	-7.2
AIGEN(b)T	-8.9	AIC9(i)	-7.1
AIC8(ii)	-8.9	AIC2(b)	-7
AIGEN(a)T	-8.8	AIC2(c)	-6.2
AIT1(c)	-8.8	AIT2(a)(i)	-5.6
AIC10(ii)	-8.7	AIT2(b)(i)	0.7
AITM(a)(i)	-8.7	Mitomycin	-7.2
AII2(a)(i)	-8.5	Reference ligand	-7.8

Molecular docking score of the compounds (ligands) with the breast cell line protein (PDB ID 2J5F) considering Mitomycin as the standard drug.

Table 4, Molecular docking results on anti-cancer (lung cell line) protein**.**

LIGAND	BINDING AFFINITY (kcal/mol)	LIGAND	BINDING AFFINITY (kcal/mol)
AII4(b)	-11	AITM(a)(i)	-8.5
AIC2(b)	-10.9	AIC1(a)	-8.5
AII4(c)	-10.7	AIGEN(a)I	-8.5
AII4(a)	-10.6	AIT2(b)(i)	-8.5
AITM(b)	-10.5	AIC8(i)	-8.5
AIC5(a)(i)	-10.4	AIC10(ii)	-8.4
AIC5(b)(i)	-10.4	AIC8(iii)	-8.2
AITM(a)	-10.4	AITM(d)(i)	-8.1
AIC10(i)	-10.1	AIC4(b)	-8.1
AIGEN(c)I	-9.7	AII2(a)(i)	-8
AIC3(b)	-9.2	AIGEN(b)I	-8
AIC2(a)	-9.2	AITM(c)(i)	-7.9
AIGEN(b)T	-9.2	AIM(c)	-7.9
AIC8(ii)	-9.2	AII2(b)(i)	-7.8
AIT1(c)	-9.1	AII1(a)	-7.8
AIC3(a)	-8.9	AIC9(ii)	-7.7
AIGEN(c)T	-8.9	AIC9(iii)	-7.5
AIC2(c)	-8.9	AIC9(iv)	-7.5
AIT1(a)	-8.9	AIC9(i)	-7.1
AIC1(c)	-8.8	AIC4(a)	-7.1
AIC1(b)	-8.8	AIM(a)	-7.1
AIT1(b)	-8.8	AII1(b)	-7.1
AIC8(iv)	-8.8	AIM(b)	-6.6
AIGEN(a)T	-8.6	Alectinib	-8.1
AIT2(a)(i)	-8.6	Reference ligand	-8.5

Molecular docking of score ligands with lung cancer cell line protein PDB ID 2ITY considering Alectinib as a standard drug. Activity of the compounds with reference to the standard drug are mentioned in a decreasing order.

Table 5, Molecular docking results of anti-inflammatory activity.

LIGAND	BINDING AFFINITY (kcal/mol)	LIGAND	BINDING AFFINITY (kcal/mol)
AIGEN(c)I	-10.3	AIGEN(b)I	-8.1
AIT1(a)	-10.2	AIC9(i)	-8
AIT1(b)	-10.2	AIC9(ii)	-7.9
AII4(b)	-9.7	AIC9(iii)	-7.8
AII4(c)	-9.7	AII4(a)	-7.8
AIM(c)	-9.5	AIC9(iv)	-7.7
AII1(a)	-9.4	AIC8_iii	-6.6
AII1(b)	-9.2	AIC8_ii	-6.3
AII2(a)(i)	-9.1	AIGEN(b)T	-6.1
AIC1(c)	-9.1	AIC4(a)	-5.8
AITM(a)(i)	-9	AIC8(i)	-5.8
AIC1(b)	-9	AIT2(a)(i)	-5.7
AIC10(ii)	-8.9	AIC10(i)	-5.3
AIC1(a)	-8.9	AIC8(iv)	-5.2
AIT1(c)	-8.9	AIGEN(a)T	-4.9
AII2(b)(i)	-8.8	AAIGEN(c)T	-4.8
AIGEN(a)I	-8.7	AIC2(a)	-4.6
AIM(a)	-8.7	AIC4(b)	-4.4
AIC3(b)	-8.6	AIC5(a)(i)	-4.2
AITM(d)(i)	-8.4	AIC6(b)(i)	-4.2
AIM(b)	-8.4	AIC2(b)	-4
AITM(b)	-8.3	AIC2(c)	-1.8
AIC3(a)	-8.2	AIT2(b)(i)	-1
AITM(a)	-8.2	Indomethacin	-8.3
AITM(c)(i)	-8.1	Reference ligand	-8.2

Molecular docking score of ligands with COX-1 protein PDB ID 2OYU with Indomethacin as a standard drug. Compounds with highest binding affinity have been described in the descending order of their activity.

Table 6, Molecular docking results of anti-inflammatory activity of Cox-2 Co-crystalized enzyme protein.

LIGAND	BINDING AFFINITY (kcal/mol)	LIGAND	BINDING AFFINITY (kcal/mol)
AII4(a)	-11.5	AIGEN(b)I	-8.7
AIC1(c)	-11.1	AIC8(ii)	-8.5
AIC10(ii)	-10.9	AIC10(i)	-8.3
AIC1(a)	-10.8	AIGEN(a)T	-8.3
AIC1(b)	-10.7	AIGEN(b)T	-8.3
AIC3(b)	-10.4	AIC9(iv)	-8.2
AII4(c)	-10.2	AIC4(b)	-8.1
AII4(b)	-10.1	AIC8(iii)	-7.8
AITM(b)	-10	AITM(c)(i)	-7.5
AIGEN(c)I	-9.8	AIT1(a)	-7.3
AIC3(a)	-9.7	AIC8(i)	-7.2
AIM(c)	-9.7	AIC5(a)(i)	-6.8
AII1(b)	-9.5	AIC6(b)(i)	-6.8
AII2(a)(i)	-9.4	AIT1(c)	-6.8
AIM(a)	-9.3	AIT1(b)	-6.7
AIM(b)	-9.3	AIT2(a)(i)	-6.5
AII1(a)	-9.3	AIC4(a)	-6.2
AII2(b)(i)	-9.2	AIC2(a)	-6
AITM(a)	-9.2	AIC8(iv)	-5.8
AITM(d)(i)	-9.1	AIGEN(c)T	-3.2
AIC9(ii)	-9	AIC2(c)	-0.8
AIC9(i)	-8.9	AIC2(b)	-0.4
AIC9(iii)	-8.8	AIT2(b)(i)	-0.5
AITM(a)(i)	-8.8	Rutaecarpine	-10.4
AIGEN(a)I	-8.8	Reference ligand	-12.4

Docking score of the compounds under study with inflammatory COX-2 protein PDB ID 3LN1 with standard drug as Rutaecarpine.

Table 7, Molecular docking results of anti-bacterial activity

LIGAND	BINDING (kcal/mol)	AFFINITYLIGAND	BINDING AFFINITY (kcal/mol)
AITM(b)	-10.7	AIT2(b)(i)	-9
AITM(a)	-10.5	AII2(a)(i)	-8.9
AIGEN(c)I	-10.3	AIC9(iii)	-8.9
AIC3(b)	-9.9	AIC9(iv)	-8.9
AIT1(b)	-9.7	AIC1(b)	-8.9
AITM(c)(i)	-9.5	AIC1(c)	-8.9
AIC5(a)(i)	-9.5	AIC1(a)	-8.8
AIC6(b)(i)	-9.5	AIGEN(b)I	-8.8
AITM(d)(i)	-9.4	AII2(b)(i)	-8.7
AIC2(a)	-9.4	AIC2(c)	-8.7
AIC2(b)	-9.4	AIGEN(a)I	-8.7
AIC3(a)	-9.3	AIC9(i)	-8.2
AIGEN(a)T	-9.3	AIC8(i)	-8.2
AIC4(b)	-9.3	AIGEN(c)(T)	-8.2
AIT1(a)	-9.3	AIT1(c)	-8.2
AII4(a)	-9.3	AIT2(a)(i)	-8
AII4(b)	-9.3	AII1(b)	-8
AII4(c)	-9.3	AII1(a)	-7.9
AIC10(i)	-9.2	AIC10(ii)	-7.8
AITM(a)(i)	-9.2	AIC4(a)	-7.6
AIC8(iii)	-9.2	AIM(c)	-7.4
AIC8(iv)	-9.2	AIM(a)	-6.6
AIC9(ii)	-9.1	AIM(b)	-6.5
AIC8(ii)	-9.1	Indolmycine	-8.4
AIGEN(b)T	-9.1	Reference ligand	-9.6

Docking score compounds with proteins 4KFG a DNA Gyrase-B ATP binding domain of Escherichia coli, for antibacterial activity.

Table 8, Molecular docking results of anti-tubercular activity

LIGAND	BINDING AFFINITY (kcal/mol)	LIGAND	BINDING AFFINITY (kcal/mol)
AIC10(i)	-11.2	AIC8(ii)	-9
AIC6(b)(i)	-11.2	AIC4(b)	-8.9
AIC5(a)(i)	-11.1	AIT2(a)(i)	-8.9
AII4(b)	-10.6	AIC10(ii)	-8.7
AII4(c)	-10.6	AIGEN(b)T	-8.7
AIC3(b)	-10.5	AIT1(b)	-8.7
AII4(a)	-10.4	AIC8(iv)	-8.7
AIC2(a)	-10.3	AIC9(ii)	-8.6
AITM(a)	-10.3	AII1(a)	-8.6
AIC3(a)	-10.1	AII1(b)	-8.6
AITM(c)(i)	-10.1	AIC9(i)	-8.4
AIC1(b)	-9.8	AIT1(a)	-8.4
AIC1(c)	-9.8	AIC4(a)	-8.3
AITM(d)(i)	-9.7	AIC9(iii)	-8.3
AIGEN(b)I	-9.7	AIM(c)	-8.2
AIC1(a)	-9.6	AIC9(iv)	-8.1
AIC2(b)	-9.6	AIGEN(a)T	-8.1
AITM(b)	-9.5	AIT1(c)	-8
AITM(a)(i)	-9.4	AIC8(i)	-7.8
AIC8(iii)	-9.4	AIT2(b)(i)	-7.5
AIC2 (c)	-9.3	AIM(b)	-7.4
AIGEN(a)I	-9.2	AIM(a)	-7.3
AII2(a)(i)	-9	AIGEN(c)T	-4.4
AII2(b)(i)	-9	Reference ligand	-10.7

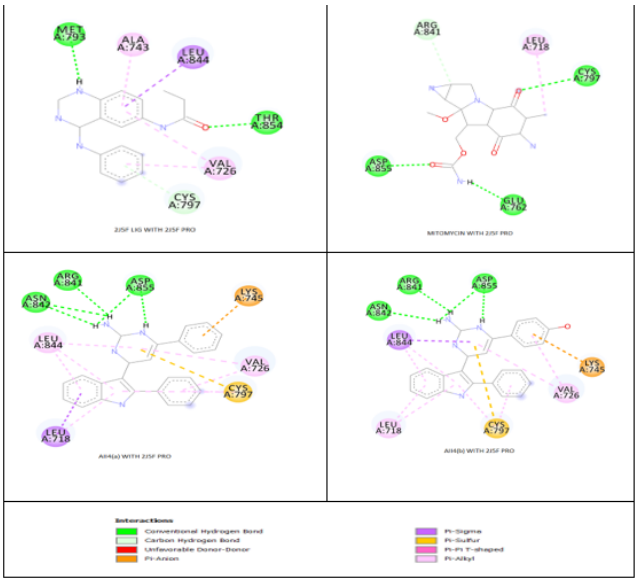
Docking results of compounds with protein 1OHJ (Human Dihydrofolate reductase monoclinic crystal form) for anti-tubercular activity.

Table 9. Online toxicity test of the compounds.

SL.NO.	SAMPLE CODE	PREDICTED LD50 (mg/kg)	PREDICATED TOXICITY CLASS	HEPATOTOXICITY	CARCINOGENICITY	IMMUNOTOXICITY	MUTAGENICITY	CYTOTOXICITY
01.	AIC1 (a)	200	3	Inactive	Inactive	Inactive	Inactive	Inactive
02.	AIC1 (b)	200	3	Inactive	Inactive	Inactive	Inactive	Inactive
03.	AIC1 (c)	200	3	Inactive	Inactive	Inactive	Inactive	Inactive
04.	AIC2 (a)	1000	4	Inactive	Active	Inactive	Active	Inactive
05.	AIC2 (b)	1000	4	Inactive	Active	Active	Inactive	Inactive
06.	AIC2 (c)	1000	4	Inactive	Active	Active	Inactive	Inactive
07.	AIGEN (a)I	1000	4	Active	Inactive	Inactive	Inactive	Inactive
08.	AIGEN (b)I	1000	4	Inactive	Inactive	Active	Inactive	Inactive
09.	AIGEN (c)I	2798	5	Active	Inactive	Inactive	Active	Inactive
10.	AIGEN (a)T	600	4	Active	Active	Active	Inactive	Inactive
11.	AIGEN (b)T	700	4	Active	Active	Active	Active	Inactive
12.	AIGEN (c)T	600	4	Active	Active	Active	Active	Inactive
13.	AIC4 (a)	56	3	Inactive	Active	Inactive	Inactive	Inactive
14.	AIC4 (b)	1000	4	Active	Active	Inactive	Active	Inactive
15.	AIC5(a) (i)	200	3	Active	Inactive	Inactive	Inactive	Inactive
16.	AIC5(b) (i)	200	3	Active	Active	Inactive	Inactive	Inactive
17.	AIT1 (a)	600	4	Active	Active	Inactive	Inactive	Active
18.	AIT1 (b)	600	4	Active	Active	Active	Inactive	Inactive
19.	AIT1 (c)	600	4	Active	Active	Inactive	Inactive	Active
20.	AIT&M (a)(i)	200	3	Inactive	Inactive	Active	Inactive	Inactive
21.	AIT&M (c)(i)	486	4	Inactive	Inactive	Inactive	Inactive	Inactive
22.	AIT&M (d)(i)	500	4	Inactive	Inactive	Inactive	Inactive	Inactive
23.	AIT&M2 (a)(i)	1000	4	Active	Active	Active	Inactive	Inactive
24.	AIT&M2 (b)(i)	1000	4	Inactive	Active	Inactive	Active	Inactive
25.	AIT2(a)i	500	4	Active	Active	Inactive	Inactive	Inactive
26.	AIT2 (b)(i)	1000	4	Active	Active	Inactive	Inactive	Inactive
27.	AII1 (a)(i)	96	3	Inactive	Inactive	Inactive	Inactive	Inactive
28.	AII1 (b)(i)	395	4	Inactive	Inactive	Inactive	Inactive	Inactive
29.	AII2 (a)(i)	430	4	Inactive	Active	Active	Active	Inactive
30.	AII2 (b)(i)	430	4	Active	Active	Active	Inactive	Inactive
31.	AII3 (a)	2400	5	Inactive	Inactive	Inactive	Active	Active
32.	AII3 (b)	2400	5	Inactive	Inactive	Inactive	Active	Active
33.	AIM (a)	450	4	Inactive	Inactive	Inactive	Active	Inactive
34.	AIM (b)	280	3	Inactive	Inactive	Inactive	Active	Inactive
35.	AIM(c)	770	4	Inactive	Inactive	Inactive	Active	Inactive
36.	AII4 (a)	500	4	Active	Active	Inactive	Active	Inactive
37.	AII4(b)	500	4	Active	Active	Inactive	Active	Inactive
38.	AII4(c)	500	4	Active	Active	Inactive	Active	Inactive
39.	AIC8(i)	2000	4	Inactive	Inactive	Inactive	Inactive	Inactive
40.	AIC8(ii)	675	4	Inactive	Inactive	Inactive	Inactive	Inactive
41.	AIC8(iii)	675	4	Inactive	Inactive	Active	Inactive	Inactive
42.	AIC8(iv)	2000	4	Inactive	Inactive	Inactive	Active	Inactive
43.	AIC9(i)	2000	4	Active	Inactive	Inactive	Active	Inactive
44.	AIC9(ii)	2000	4	Active	Inactive	Inactive	Active	Inactive
45.	AIC9(iii)	2000	4	Active	Inactive	Inactive	Inactive	Inactive
46.	AIC9(iv)	2000	4	Active	Inactive	Active	Inactive	Inactive
47.	AIC10 (i)	775	4	Inactive	Inactive	Inactive	Inactive	Inactive
48.	AIC10 (ii)	1000	4	Inactive	Inactive	Active	Inactive	Inactive

Results of online toxicity studies using the webserver for hepatotoxicity, carcinogenicity, immunogenicity, and cytotoxic activity.

Figure 1. Molecular interaction of compounds with 2J5F.



The compounds AII4 (a) and AII4 (a) from the set of selected 48 compounds had shown a highest binding affinity (-10.5kcal/mol) with protein 2J5F, and it was due to the large number of conventional hydrogen bonding that exits due hydrophilic region of the molecule binding to amino acids ARG A-841, ASN A-842, ASP A-855 of the protein in case of both the compounds. It was found that binding of these compounds is much higher with comparison to standard drug Mitomycin (-7.2kcal/mol).

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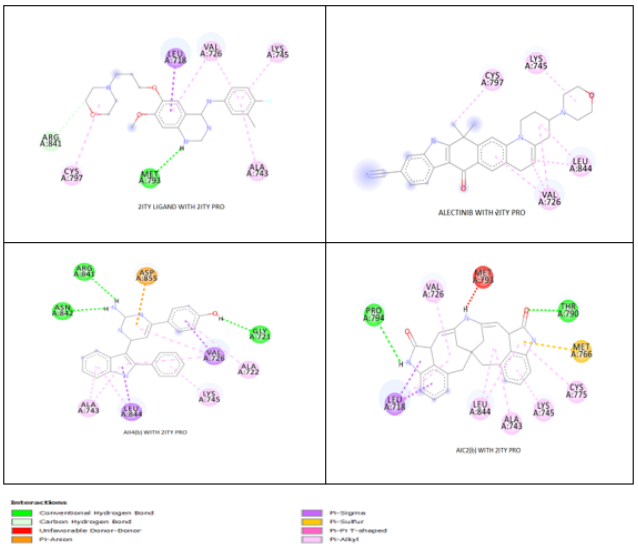
Figure 2. Docking interaction of compounds with receptor 2ITY.



The compound AII4 (b) has shown binding energy of -11kcal/mol with three conventional hydrogen bonding with protein (2ITY) active site amino acids GLY A-721, ARG A-841, and ASN A-842. Molecule AIC2 (b) has shown binding affinity of -10.9kcal/mol with macromolecule active site with two hydrogen bonding with the amino acids THR A-790 and PRO A-794 but also contain highest number of hydrophobic group similar that of the standard compound Alectinib.