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IN *SILICO* STUDIES ON N-AMIDE DERIVATIVES OF INDOLE-BENZIMIDAZOLE-ISOXAZOLE FOR BIOLOGICAL ACTIVITY

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Abstract:
The molecular docking studies of new library of amide derivatives of Indole-benzimidazole-Isoxazole were performed for and have shown prominent binding affinity. *In silico*Molecular docking results indicated that the hybrids**1a** and **1b** had good binding affinity towards HDAC6 and established H-bond interaction with crucial residues and binding was extended with Zn^{2+} binding site.

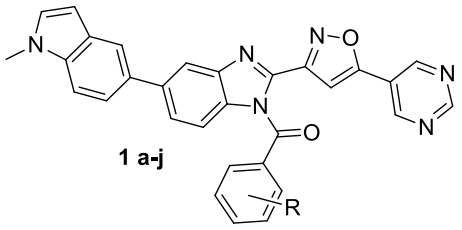
Keywords*:* Nocodazole, benzimidazole, Panobinostat, indole and anticancer activity.

Introduction

Histone deacetylase (HDAC)[i-iv]isresponsible for several types of cancers. Some homologs can cause cancer, neurological problems and inflammatory conditions.Histonedeacetylase inhibitors(HDACi)[v-viii]are used to treat cancer by a mechanism of acetylation of non-histone proteins and histones. The results ofclinical trials and pre-clinical research showed that histone deacetylase inhibitors (HDACi) and Panobinostat (PAN)[ix-xiii] combination can cure the cancer, the results proved in. Panobinostat was approved by US FDA in 2015 to treat multiple myeloma.[xiv]Panobinostat contains Indole core moiety is indole. Indoles possess wide range of biological properties[xv-xvii]includingantituberculosis[xviii, xix], antiviral[xx]and anticancer activities, etc.[xxi-xxix]

In view of the above side effects, we have incorporated Benzimidazole moiety.Benzimidazole contains the five-member imidazole fused with six-member benzene ring system, which exhibits good biological properties.Benzimidazole and indole rings, are bioavailable compounds, these moieties carry pyrimidine and purine nitrogen bases, Benzimidazole-indoletype of scaffolds possess various biological properties.[xxx-xxxvii]

Results and Discussion



1a; R = 3,4,5-trimethoxy
1b; R = 3,5-dimethoxy
1c; R = 4-methoxy
1d; R = 4-nitro
1e; R = 3,5-dinitro
1f; R = 4-chloro
1g; R = 4-bromo
1h; R = 4-cyano
1i; R = 4-methyl
1j; R = 4-(dimethylamino)

Molecular Docking Studies

Molecular Docking Studies with HDAC6 (PDB ID: 5EF8)

The aim of molecular docking study is to predict the binding affinity against HDAC6 as title compounds (**1a-j**) in structure which are similar to Panobinostat, an US FDA drug for multiple myeloma.[xxxviii]The most likely target for these compounds is HDAC6, which is reported to be overexpressed in different types of cancers in human beings. The 3D protein structure of HDAC6 catalytic domain 2 is complex with panobinostat (PDB ID: 5EF8) obtained from Protein Data Bank (RCSB PDB) (https://www.rcsb.org/structure/5EF8).[xxxix]Autodock 4 was used for molecular docking studies, binding energy calculations and best poses were extracted.As shown in **Table 1**, the dock scores in kcal/mol of all the docked ligands were found to be in negative values and are within similar range. The negative values of the docking scores suggest that binding of the compounds in the binding site of HDAC6 (PDB ID: 5EF8) are thermodynamically favourable.

Top scored molecular poses of the docked ligandswith 3D and 2D interactions are shown in Figure 2. The docking studies were initially validated by performing redocking with co-crystallized ligand (Panobinostat) and were shown as RMSD value within the acceptable limit. The binding poses of redocking have matched with native pose with same binding interaction pattern. The key amino acid residues in the binding site that participate in interaction with the Panobinostat are found to be H-bonding with His573, Ser531 amino acid residues and hydrophobic interactions with His463, Pro464, Phe643 and π-π interactions with Phe643 amino acid residue.

The docking of compound **1a** substituted with 3,4,5-trimethoxy group on phenyl ring coordinates to Zn^{2+} and interacts with Tyr745 residue. The 'N' atom of benzimidazole ring makes H-bond interaction with Ser531. The indole ring is placed in hydrophobic pocket with Phe643 and π-π interactions are exhibited with Phe643, Phe583 residues, which is seen similar in Panobinostat. The docking of compound **1b** substituted with 3,5-dimethoxy group on phenyl ringexhibited two H-bond interactions with Ser531 and Tyr754 and hydrophobic interactions with Phe583 and Phe643 and aromatic rings comprising benzimidazole, pyrimidine and phenyl rings makes π-π interactions with Phe643, Phe583 and Phe642 residues respectively, which is similar to Panobinostatligand. The dock score of HDAC6 with compound **1a** and **1b** was found to be -9.71 and -9.39 kcal/mol, respectively. The designed compounds had strong interaction with HDAC6 as binding was extended from the hydrophobic cap region to Zinc binding region.

Molecular Docking Studies with EGFR in Complex with Erlotinib (PDB ID:4HJO):

The target compounds (**1a-j**) are also performed molecular docking studies with EGFR a tyrosine kinase in complex with erlotinib (PDB ID: 4HJO).[xxxx]The binding affinity for the docked ligands were ranges from -8.9 and -10.70 kcal/mol. The binding affinity values of best poses that bind to the binding site of the EGFR are illustrated in Table 2. Thecrucial amino acid residues in the active site of the EGFRimportant in catalytic effect are Ala719, Met769, Cys773, and Leu694 (Figure 3). Initially, the docking was validated by performing redocking

with co-crystallized ligand (erlotinib) against EGFR and found to have RMSD value 0.58 A^{o}. The N1 of quinazoline ring is anchored into the adenine binding region and exhibited H-bond interaction with Met769. The hydrophobic interactions are exhibited with Leu768, Leu820, Leu694 and Gly772 amino acid residues with binding affinity value -13.48 kcal/mol.

Compound**1a**exhibits good binding affinity value with ΔG =-10.7 kcal/mol and interacted with the active site residues in a similar way to erlotinib. It formed H-bonds with Gly772, Asp831, Leu694 and Lys721 at distances of 3.07, 3.68, 3.66 and 2.73 Å respectively. In addition, π-H binding with Leu694 and Cys773 were formed. The benzimidazole moiety occupies the adenine pocket and the indole ring interact with hydrophobic pocket.

Similarly, Compound **1b**also involves H-bond interactions with Gly772, Ala719, and Leu764 at distances 3.16, 3.20 and 2.95 Å, respectively. Besides, π-H binding with Leu694Val702 and Cys773 were formed.

Thus the docking studies revealed that the compounds could synergistically inhibit two protein targets involved in cancer HDAC and tyrosine kinase protein EGFR.

***In silico* Bioavailabilityand Toxicity Prediction:**

To explore the bioavailability and toxicity prediction of all compounds the *in silico* performed by using RDkit and Data warrior software. Different descriptors were measured to explore the drug likeliness of the synthesized Indole-benzimidazole-Isoxazole derivatives. All the compounds have obeyed the Lipinski's rule of 5 which meet the criteria for oral bioavailability. All compounds were predicted to have molecular weight in the range of 510.56-586.61,AlogP from 5.81-6.65, H-bond acceptor (HBA) ≥ 10, H-bond donor (HBD) ≥ 0, PSA ≥ 177.91 Å, rotatable bonds (ROTB) ≥ 7, and QED values between0.20-0.36. Compounds solubility was in the range of -7.98 to -8.94 (Table-2). Data Warrior software was used for toxicity prediction by measuring mutagenic, tumorigenic, reproductive and irritant effects. All the compounds exhibit high value for mutagenic, tumorigenic and irritant.

Conclusion

*In silico*Molecular docking results demonstrated that potent hybrids**1a** and **1b** had good binding affinity with HDAC6 and established H-bond interaction with crucial residues and binding was extended with Zn^{2+}binding site.Moreover, compound **1a** and **1b** also anchored well in the EGFR binding pocketand established interactions with crucial amino acid residues.

Table-1: Binding affinity values for all synthesized compounds against the HDAC6 and EGFR targets:

Compound	HDAC (PDB ID: 5EF8)	EGFR (PDB ID: 4HJO)
10a	-9.71	-10.70
10b	-9.31	-10.54
10c	-8.77	-10.00
10d	-8.92	-9.8
10e	-8.20	-9.7
10f	-8.97	-8.9
10g	-7.47	-10.09
10h	-8.07	-9.9
10i	-9.71	-8.18
10j	-8.37	-9.7
Co-crystallized Ligand	-10.47	-13.48

Table-2: Drug-likeness quantitative estimation (QED) with RDKit and Toxicity with Datawarrior Software N-Amide Derivatives of Indole-benzimidazole-Isoxazole (10a-j)

Index	Compd	MW	ALOGP	HBA	HBD	PSA	ROTB	AROM	ALERTS	LRo5	QED	Drugliketiness	Mutagenic	Tumorigenic	Reproductive Effective	Irritant
1	10a	586.61	6.02	9	0	119.32	7	7	0	2	0.29	4.07	high	high	none	high
2	10b	556.58	6.01	8	0	110.09	6	7	0	2	0.32	4.07	high	high	none	high
3	10c	526.56	6.00	7	0	100.86	5	7	0	2	0.35	4.07	high	high	none	high
4	10d	541.53	5.90	8	0	134.77	5	7	2	2	0.22	-1.03	high	high	none	high
5	10e	586.52	5.81	10	0	177.91	6	7	2	2	0.20	-1.03	high	high	none	high
6	10f	530.98	6.65	6	0	91.63	4	7	0	2	0.33	4.07	high	high	none	high
7	10g	575.43	6.76	6	0	91.63	4	7	0	2	0.30	2.23	high	high	none	high
8	10h	521.54	5.87	7	0	115.42	4	7	0	2	0.36	-0.26	high	high	none	high
9	10i	510.56	6.30	6	0	91.63	4	7	0	2	0.36	3.98	high	high	none	high
10	10j	539.6	6.06	7	0	94.87	5	7	0	2	0.34	4.34	high	high	none	high

molecular weight (MW), octanol–water partition coefficient (ALOGP), number of hydrogen bond donors (HBDs), number of hydrogen bond acceptors (HBAs), molecular polar surface area (PSA), number of rotatable bonds (ROTBs), number of aromatic rings (AROMs) and number of structural alerts (ALERTS).; NPL: Natural Product likeness calculator

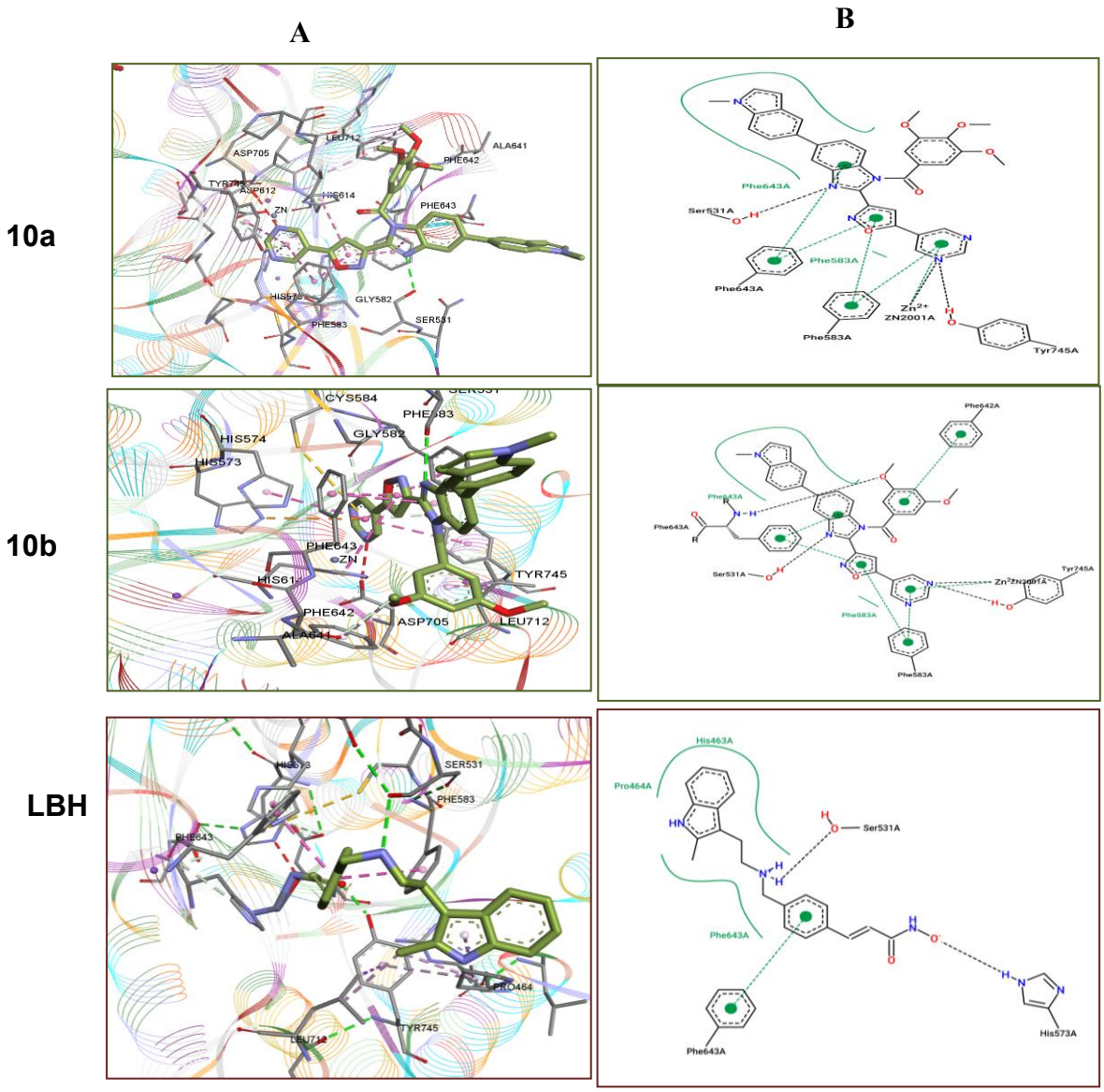


Fig-2: Molecular Docking Studies of compound 10a, 10b and LBH with HDAC6 (PDB ID: 5EF8) A) 3D Interactions B) 2D Interactions

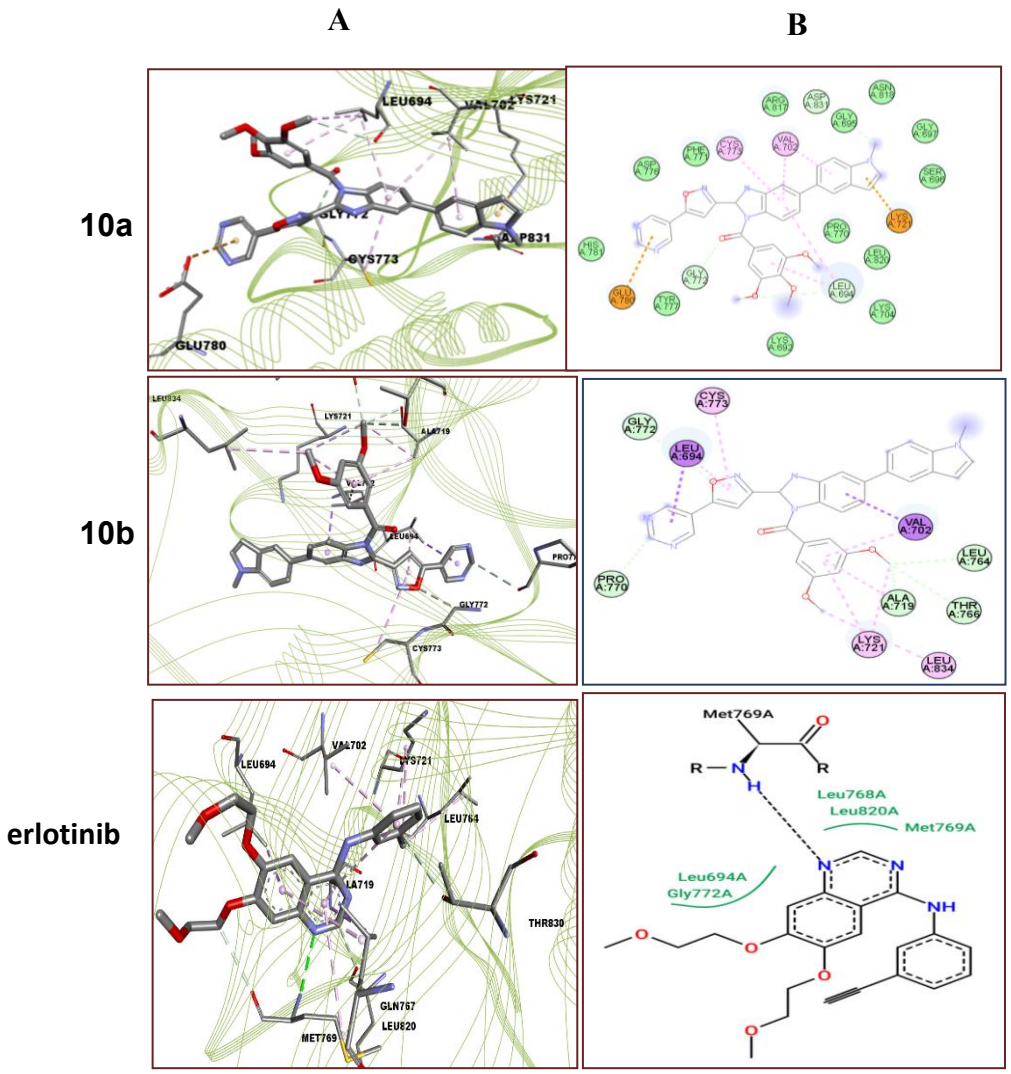


Fig-3: Molecular Docking Studies of compound 10a, 10b and erlotinib with EGFR (PDB ID: 4HJO) A) 3D Interactions B) 2D Interactions

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