Journal of Advanced Zoology

ISSN: 0253-7214
*Volume **44** Issue **03 Year 2023** Page **1521:1528***

In-Silico Pharmacokinetics and Molecular Docking Study of The Newly Designed Benzimidazole Derivatives as An Antiepileptic Agent

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Article History

Received: 06 June 2023
Revised: 05 Sept 2023
Accepted: 29 Nov 2023

Abstract

*The moiety benzimidazole exhibited a broad range of pharmacological activity. A molecular docking study is a valuable tool used in recent drug discovery to understand the possible interaction between medicines and receptors. Several benzimidazole derivatives are docked with the active site of human carbonic anhydrase isozyme-II (PDB code: 5LL4) to examine its anticonvulsant properties. The auto-dock Vina platform is used for molecular docking. The outcomes of molecular docking showed that compounds **1a, 1b, 1c, 1f, 1h, 1j, 2f,** and **2g** had excellent scores for docking and improved interactions with vital amino acids. Furthermore, SwissADME and pkCSM software are used to perform ADMET investigations and calculate physiochemical parameter values. The findings showed that all other study parameters including Lipinski's rule of five requirements are significantly satisfied by designed substituted benzimidazoles. According to the in-silico study's findings, all newly framed benzimidazole derivatives would be regarded as leads for the discovery of novel antiepileptic medications.*

Keywords: *Molecular docking, in-silico, Antiepileptic, Substituted benzimidazole, Lead discovery, Carbonic anhydrase isozyme-II*

1. Introduction

Epilepsy is the most prevalent brain illness which affects fifty million individuals globally. Since about 60–80% of epileptic individuals respond to the currently available medications for the treatment of seizures so there is now a need for better agents.[1] Drugs that inhibit carbonic anhydrase (CA) enzymes play a significant role in the prevention of seizures. [2] CAs are involved in the reversible hydration of carbon dioxide to protons and bicarbonate ions. Various such drugs are available in the market such as Acetazolamide, Ethoxzolamide, Sulthiame, and benzene sulphonamide but most of these drugs are sulphonamides and cause undesired side effects like a sulpha allergy.[3] Consequently, it is necessary to find antiepileptic drugs that are more potent and secure. Synthesized benzimidazoles are very influential in numerous pathological problems as they have great biological functions and extensive clinical applications.[4-8] Various substituted benzimidazoles as carbonic anhydrase inhibitors have been reported previously so these results provided the framework for further research on this motif.[9] Molecular modeling is a tool employed in contemporary drug discovery and pharmaceutical product development.[10] The toxicity of drugs is one of the primary causes of drug recalls. Consequently, ADME characteristics are essential factors in determining the drug's clinical success.[11] These days, computer-based approaches are used to estimate the ADMET of the drug and have gained a lot of interest from medicinal scientists as they are inexpensive and have high output. Molecular docking of the substituted benzimidazoles was performed against Human carbonic anhydrase inhibitor isozyme-II (5LL4 protein).

Physiochemical parameters and drug Likeliness estimation

SwissADME software was employed to evaluate the molecular weight, number of hydrogen bond acceptors, hydrogen bond donors, and rotatable bonds. These factors aid in determining drug likelihood by Lipinski's Rule of Five. As per this rule, any good drug candidate should have a molecular weight

(MW) ≤500 Daltons, H-bond donor ≤5, H-bond acceptors ≤10, LogP value ≤5, and total rotatable bond should be ≤10.[12] Physiochemical properties and drug likeliness of designed ligands are given in **Table 1**. This indicated that all the ligand compounds are fulfilling Lipinski's rule of five criteria such as no violation. The estimated values of WlogP for all the compounds are below 5. The topological polar surface area (Å²) of all the ligands was estimated and found within the range (61.19Å²-81.42Å²)). Further, the Molecular refractivity of all the compounds was estimated and results were found inside the range (87.32-99.87) [13, 14].

Table 1: Predicted Physiochemical Properties and Drug Likeliness of the Ligands

S. No.	Ligand	MW	No. of H-bond acceptor	No. of H-bond donor	No. of rotatable bonds	No. of violations (Lipinski)	Wlog P*	TPSA (Å²)	MR
1	***1a***	308.33	4	0	6	0	3.30	61.19	87.37
2	***1b***	322.36	4	0	6	0	3.61	61.19	92.33
3	***1c***	322.36	4	0	6	0	3.61	61.19	92.33
4	***1d***	338.36	5	0	7	0	3.31	70.42	93.86
5	***1e***	338.36	5	0	7	0	3.31	70.42	93.86
3.	***1f***	324.33	5	1	6	0	3.00	81.42	89.39
7	***1g***	324.33	5	1	6	0	3.00	81.42	89.39
8	***1h***	342.78	4	0	6	0	3.95	61.19	92.38
9	***1i***	387.23	4	0	6	0	4.06	61.19	95.07
10	***1j***	326.32	5	0	6	0	3.86	61.19	87.32
11	***2a***	322.36	4	0	7	0	3.69	61.19	92.17
12	***2b***	336.38	4	0	7	0	4.00	61.19	97.14
13	***2c***	336.38	4	0	7	0	4.00	61.19	97.14
14	***2d***	352.38	5	0	8	0	3.70	70.42	98.66
15	***2e***	352.38	5	0	8	0	3.70	70.42	98.66
16	***2f***	338.36	5	1	7	0	3.39	81.42	94.20
17	***2g***	338.36	5	1	7	0	3.39	81.42	94.20
18	***2h***	356.80	4	0	7	0	4.34	61.19	97.18
19	***2i***	401.25	4	0	7	0	4.45	61.19	99.87
20	***2j***	340.35	5	0	7	0	4.25	61.19	92.13

Wlog P= $LogP_{o/w}$, MR= Molecular Refractivity, TPSA= Topological Polar surface area, MW= Molecular weight*

ADMET prediction [15]

ADME study of the designed benzimidazole compounds was performed using the pkCSM signature server. This platform aids in determining the drugs' five primary pharmacokinetic features (absorption, distribution, metabolism, excretion, and toxicity). The ADMET outcomes are displayed in **Table 2**. Caco-2 permeability indicates the ability of the human intestinal mucosa to absorb medications taken orally. All the designed ligands show high Caco-2 permeability except two ligands (***2h*** and ***2i***) which is less than 0.90. The intestine is the principal location of medication absorption and all the compounds predict high intestinal absorption (89.096-92.901). The volume of distribution is deemed low if log VDss is less than -0.15 and high if it is greater than 0.45. Predicted VDss of synthesized compounds is found in the range of -0.06 to -0.345. Every ligand has excellent central nervous system permeability (-2.193 to -2.529). Further, all the ligands are the substrates of Renal OCT2 except one (**Ligand *1f***). All the ligand molecules exhibit comparable overall clearance. Lethal dosage values (LD50) are a commonly used method of determining immediate toxicity that is used to determine the relative toxicity of various compounds and results indicated comparable LD50 values. The physicochemical and ADMET data indicated that these compounds can be employed as medications because all the attributes for the designed molecules fell inside the range.

Table 2: Pharmacokinetic properties of the active benzimidazoles

Ligand	Caco-2 permeability	Intestinal absorption	VDss (Human)	CNS permeability	Renal OCT2 substrate	Total clearance	Rat LD50
1a	1.354	91.929	-0.185	-2.335	Yes	0.778	2.328
1b	1.373	91.727	-0.18	-2.26	Yes	0.786	2.331
1c	1.354	91.885	-0.191	-2.256	Yes	0.779	2.321

1d	1.209	91.934	-0.214	-2.488	Yes	0.849	2.326
1e	1.276	92.901	-0.345	-2.5	Yes	0.849	2.327
1f	1.298	89.515	-0.06	-2.513	No	0.837	2.182
1g	1.223	89.637	-0.21	-2.52	Yes	0.829	2.249
1h	1.381	90.426	-0.203	-2.215	Yes	0.816	2.325
1i	1.386	90.359	-0.201	-2.193	Yes	0.748	2.326
1j	1.345	91.495	-0.323	-2.378	Yes	0.778	2.34
2a	1.375	91.51	-0.178	-2.343	Yes	0.827	2.33
2b	1.394	91.307	-0.173	-2.268	Yes	0.835	2.334
2c	1.375	91.466	-0.183	-2.264	Yes	0.827	2.323
2d	1.291	91.515	-0.206	-2.496	Yes	0.897	2.328
2e	1.259	92.482	-0.337	-2.508	Yes	0.895	2.329
2f	1.28	89.096	-0.053	-2.522	Yes	0.886	2.184
2g	1.206	89.218	-0.203	-2.529	Yes	0.877	2.25
2h	0.399	90.007	-0.195	-2.223	Yes	0.864	2.327
2i	0.396	89.94	-0.193	-2.201	Yes	0.796	2.328
2j	1.366	91.076	-0.316	-2.386	Yes	0.826	2.342

**Caco-2= Colorectal adenocarcinoma cells, VDss= Volume of distributions, CNS= Central Nervous System, OCT2= Organic cation Transporter, LD-50= Lethal Dosage*

Molecular docking evaluation

Building of ligand structure

The structures of newly designed benzimidazoles were drawn in Chem-Draw Professional 16.0. The docked molecule must accurately reflect the real ligand structures as they would look in a protein-ligand interaction to yield the best outcomes. To do this, Chem3D Ultra software is used to organize all the ligands in three dimensions and to minimize their combined energy (MM2) to the lowest possible level.[16] All ligand structures are saved as individual PDB files.

Preparation of Protein structure

The protein, known as human carbonic anhydrase isozyme-II (PDB code: 5LL4), was chosen and obtained from the protein data bank (https://www.rcsb.org). The downloaded protein must be modified before using it in docking studies as it may contain a co-crystallized ligand, metal ions extra chains, or heavy ions. Modify the protein structure in Discovery Studio 2017 R2 Client by removing Chain B, water molecules, adding Polar hydrogen, and then saving the protein in PDB format.

Docking

A study using molecular docking was conducted to determine how the new drug entity binds to 5LL4 protein to show its antiepileptic activity.[17] Docking evaluation was done by using AutoDockTools-1.5.7 as well as Discovery Studio 2017 R2 Client. [18] All the necessary modifications such as removing water, adding polar hydrogen, Kollman charges, and Assigning AD4-type atoms were performed in the AutoDock software. After that, save the protein structure and ligand in the pdbqt file format. Now for blind docking, create the grid box with dimensions x=60, y=60, and z=66, spacing (angstrom) 0.864, and spacing of center grid box were x=0.921, y=-0.289, and z=-0.051. At the end of the docking find out the binding affinity of every ligand and create output files of possible docked poses in pdbqt file format by using the command prompt tool. The software discovery studio was used to investigate potential ligand protein interactions and save the 2D schematics of ligand interactions.

According to computational analyses, all substances that bind with the 5LL4 protein (carbonic anhydrase enzyme) have high binding affinities. The results revealed that Ligands ***1b*** and ***1j*** have a maximum affinity of -9.5 Kcal/mole owing to the greatest number of interactions (GluA:239, GlyA:8, AsnA:11, TrpA:5, HisA:64, TyrA:7, ValA:242, PheA:231, LeuA:240) with the protein that include hydrogen bonds. Compounds ***1a, 1c, 1h,*** and ***2f*** also exhibited good affinities i.e., -9.3 kcal/mole whereas the binding affinity (-8.6 and -8.7) is lower for the molecules ***2d*** and ***2c*** and shows only a few interactions with protein residues such as LysA:9, AsnA:11, PheA:231, GluA:239. The standard drug (Acetazolamide) has a good binding score (-6.9) and the formation of hydrogen bonds with amino acids GlyA:8, AsnA:11, TyrA:7, HisA:64, LysA:170, TrpA:5 with oxygen and nitrogen atoms of Acetazolamide. These substances outperformed the marketplace's standard medication in docking scores. **Table 3** displays the docking scores and interfacial residues of all the ligands and standard drugs. **Figure 1** shows a 3D representation of the protein 5LL4 with its ligand ***2e*** bound at the active

site. Further, the 3D Binding surface and 2D ligand interactions of the best-docked ligands (***1a, 1b, 1c, 1f, 1h, 1j, 2f, 2g***) and acetazolamide are depicted in **Figures 2, 3, 4, 5, 6, 7, 8, 9, 10** respectively.

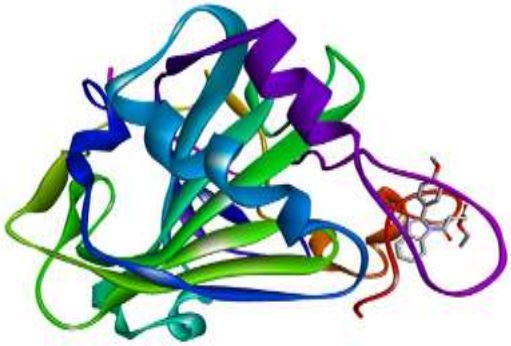

Figure 1. 3D diagram of 5LL4 protein with ligand ***2e***

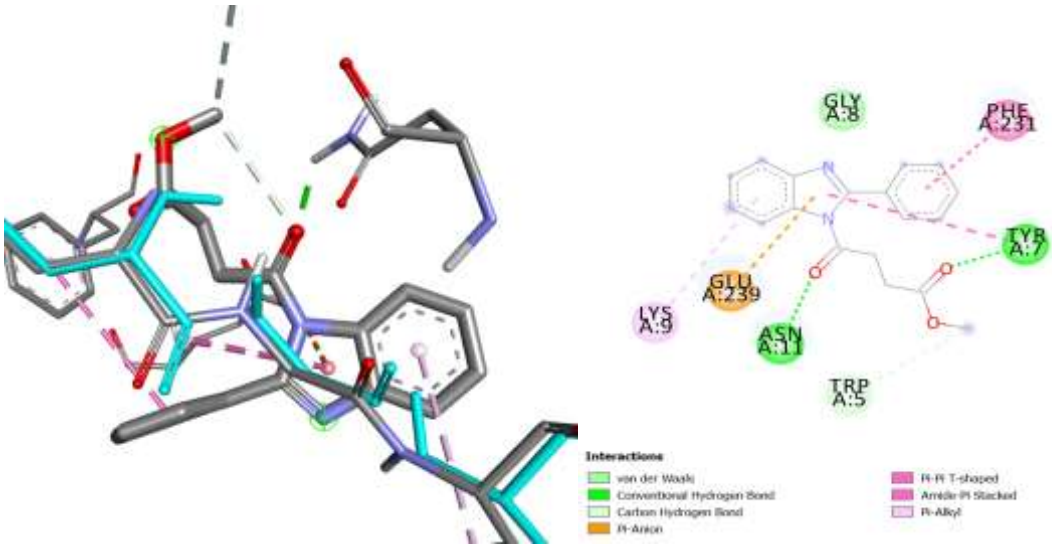

Figure 2: 3D binding surface of 5LL4 and 2D interaction diagram of ligand ***1a***

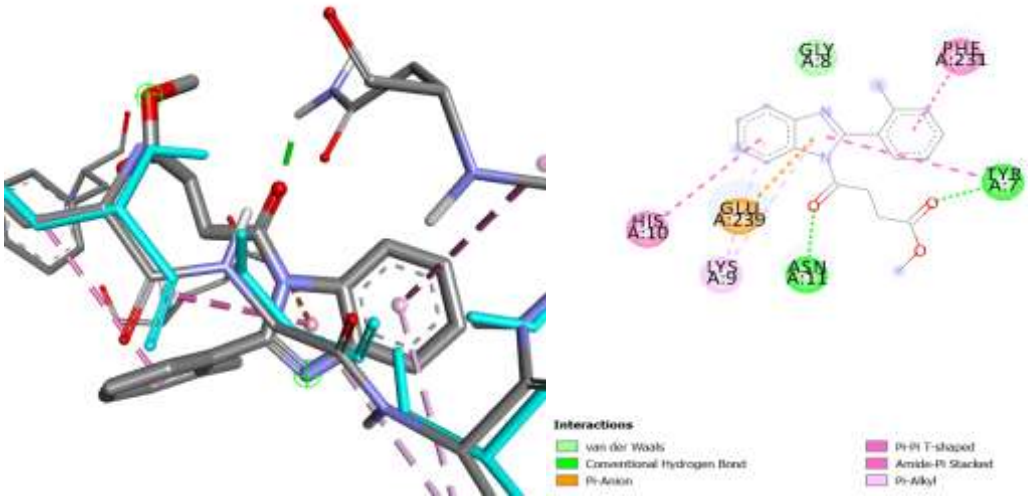

Figure 3: 3D binding surface of 5LL4 and 2D interaction diagram of ligand ***1b***

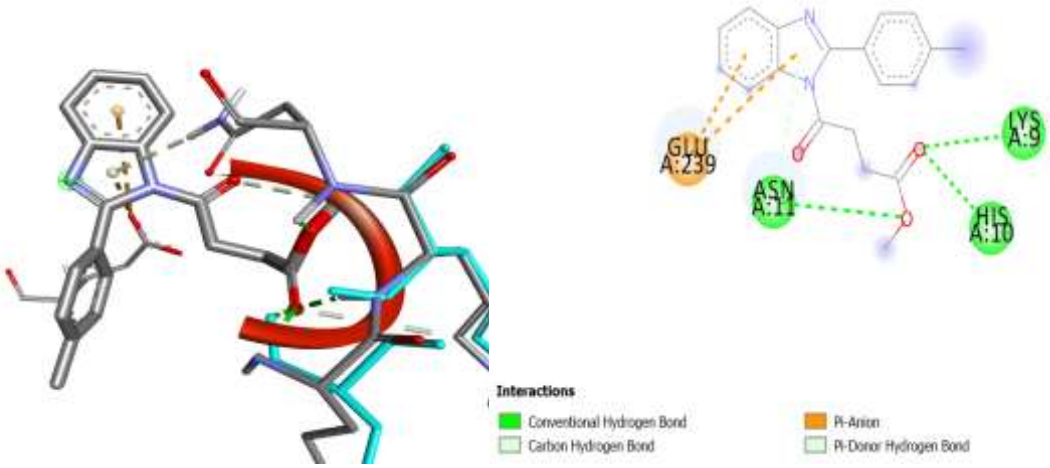

Figure 4: 3D binding surface of 5LL4 and 2D interaction diagram of ligand ***1c***

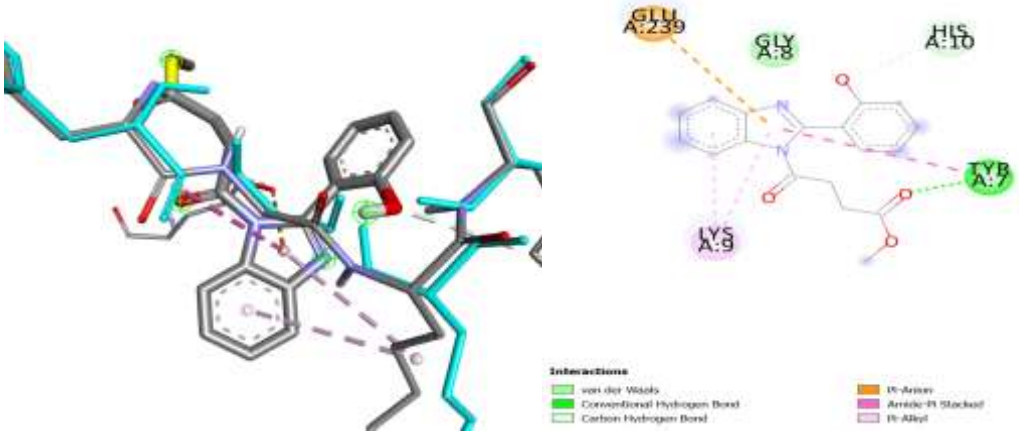

Figure 5: 3D binding surface of 5LL4 and 2D interaction diagram of ligand ***1f***

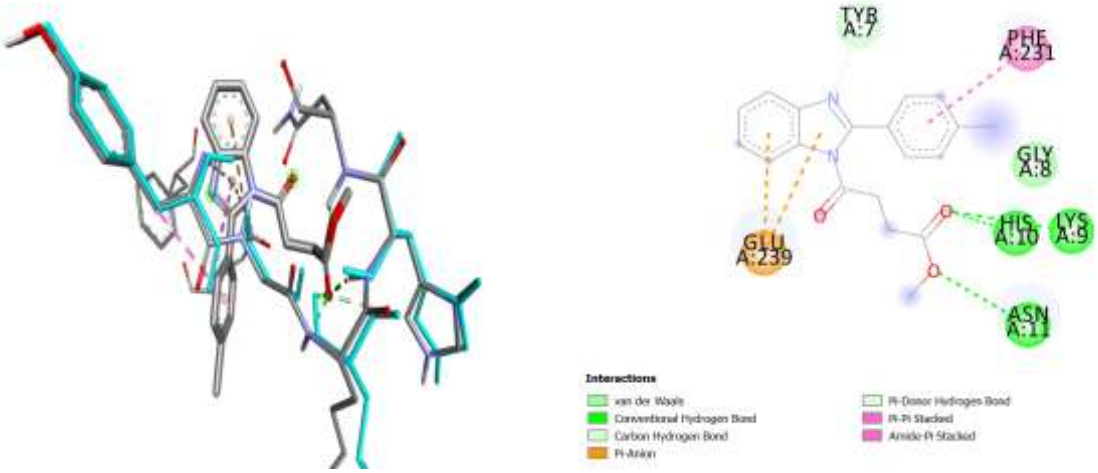

Figure 6: 3D binding surface of 5LL4 and 2D interaction diagram of ligand ***1h***

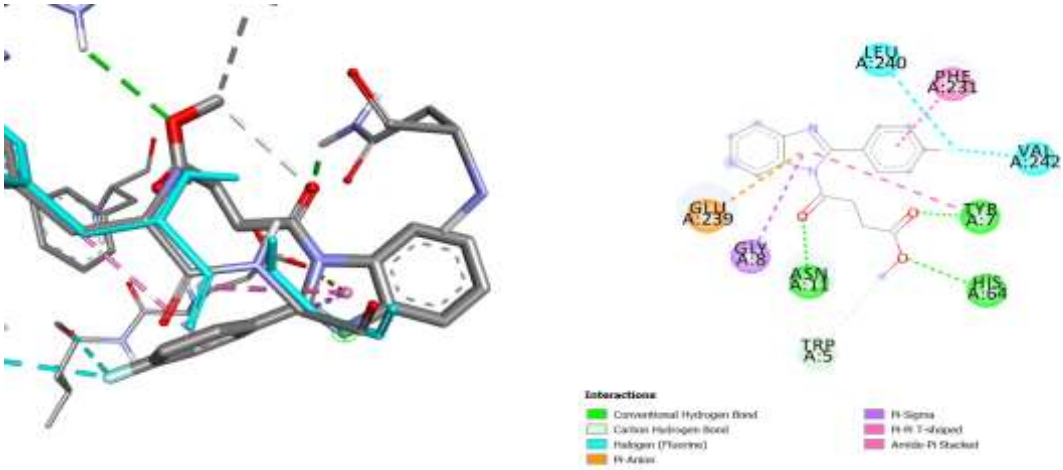

Figure 7: 3D binding surface of 5LL4 and 2D interaction diagram of ligand ***1j***

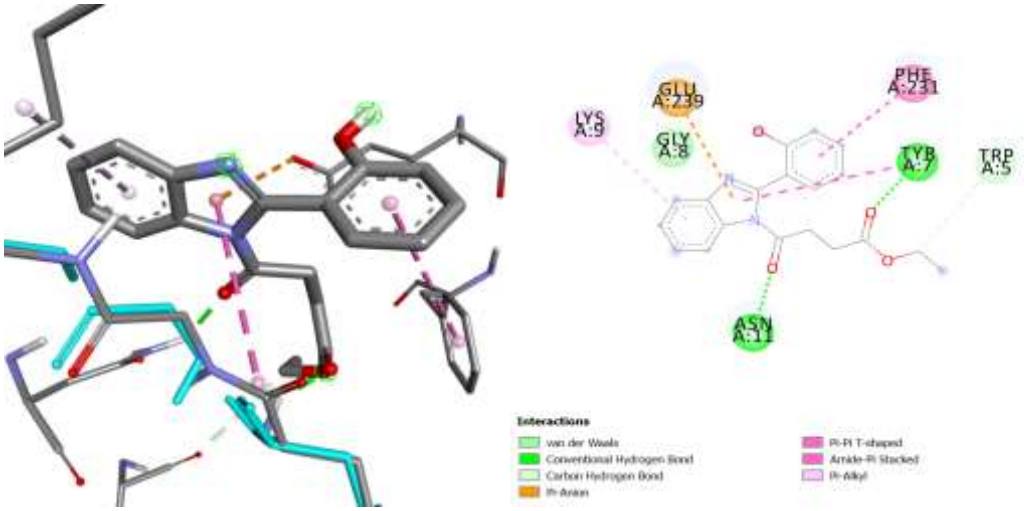

Figure 8: 3D binding surface of 5LL4 and 2D interaction diagram of ligand ***2f***

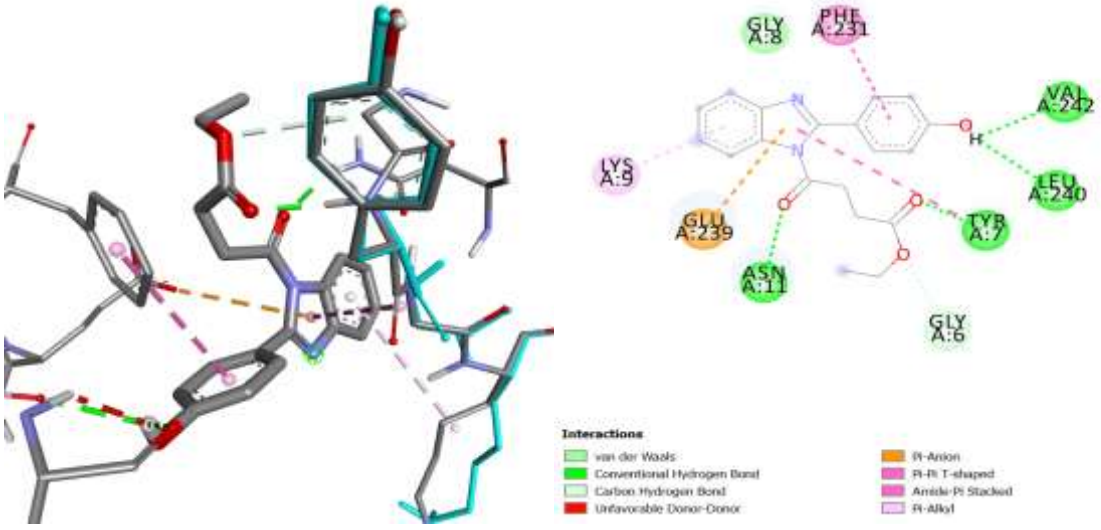

Figure 9: 3D binding surface of 5LL4 and 2D interaction diagram of ligand ***2g***

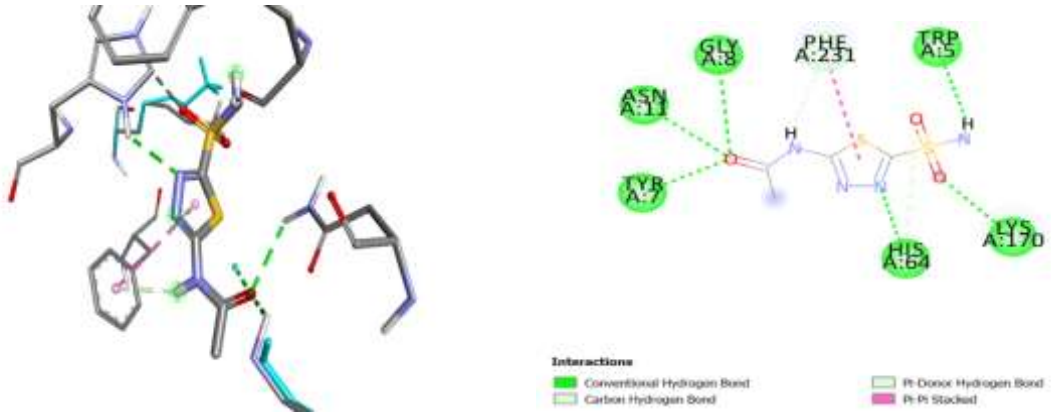

Figure 10: 3D binding surface of 5LL4 and 2D interaction diagram of Acetazolamide

Table 3: Molecular docking score of substituted benzimidazoles

S. No.	Ligand	Binding affinity (Kcal/mol)	Interacting residues
1	***1a***	-9.3	GlyA:8, LysA:9, GluA:239, AsnA:11, TrpA:5, TyrA:7, PheA:231
2	***1b***	-9.5	GlyA:8, HisA:10, GluA:239, LysA:9, AsnA:11, TyrA:7, PheA:231
3	***1c***	-9.3	GluA:239, AsnA:11, hisA:10, LysA:9
4	***1d***	-9.0	LysA:9, GlyA:8, AsnA:11, TyrA:7, PheA:231, GluA:239
5	***1e***	-9.0	TyrA:7, GluA:239, AsnA:11, HisA:10, GlyA:8, LysA:9, PheA:231
6	***1f***	-9.2	GluA:239, LysA:9, TyrA:7, HisA:10, GlyA:8
7	***1g***	-8.5	AsnA:11, PheA:231, GluA:239, HisA:10, LysA:9
8	***1h***	-9.3	TyrA:7, GluA:239, AsnA:11, HisA:10, LysA:9, GlyA:8, PheA:231
10	***1j***	-9.5	GluA:239, GlyA:8, AsnA:11, TrpA:5, HisA:64, TyrA:7, ValA:242, PheA:231, LeuA:240
11	***2a***	-9.1	GluA:239, LysA:9, GlyA:8, TrpA:5, TyrA:7, PheA:231
12	***2b***	-9.0	TyrA:7, LysA:9, AsnA:11, PheA:231, GlyA:8, GluA:239
13	***2c***	-8.7	GluA:239, TyrA:7, GlyA:8, HisA:10, LysA:9, AsnA:11
14	***2d***	-8.6	LysA:9, AsnA:11, PheA:231, GluA:239
15	***2e***	-8.9	PheA:231, GluA:239, AsnA:11, LysA:9, HisA:10
16	***2f***	-9.3	GluA:239, LysA:9, GlyA:8, AsnA:11, TyrA:7, TrpA:5, PheA:231
17	***2g***	-9.2	LysA:9, GluA:239, AsnA:11, GlyA:6, TyrA:7, LeuA:240, valA:242, PheA:231, GlyA:8
18	***2h***	-8.9	GluA:239, AsnA:11, LysA:9, HisA:10
20	***2j***	-8.9	GluA:239, asnA:11, LysA:9, HisA:10, PheA:231
21	***Acetazolamide***	-6.9	GlyA:8, AsnA:11, TyrA:7, HisA:64, LysA:170, TrpA:5, PheA:231

4. Conclusion

In the current work, the computational docking of the disubstituted benzimidazoles was carried out by AutoDock Vina and Discovery Studio. We employed carbonic anhydrase isozyme-II (5LL4) for the antiepileptic effect in this investigation and found the best antiepileptic drug. The results of the docking predicted that compounds***1a, 1b, 1c, 1f, 1h, 1j, 2f,*** and ***2g*** displayed excellent binding affinity towards the target protein, and docking scores were better than the standard drug (Acetazolamide). All the selected benzimidazole compounds have been studied physiochemically and pharmacokinetically, and the results closely match Lipinski's rule of five. The study concludes that these created compounds can offer a novel category of lead compounds for developing drugs such as carbonic anhydrase inhibitors used to treat epilepsy.

Conflict of interest

The authors declare no conflict of interest.

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