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DESIGN, MOLECULAR DOCKING STUDIES, *IN SILICO* DRUG LIKELINESS PREDICTION AND SYNTHESIS OF SOME BENZIMIDAZOLE DERIVATIVES AS ANTIHYPERTENSIVE AGENTS

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Abstract:

*Hypertension is a major public problem in the Kingdom of Saudi Arabia. Many ACE inhibitors are in clinical use as antihypertensive agents. However, theses ACE inhibitors possess many undesirable side effects. Recently, benzimidazole derivatives have been reported to possess ACE inhibitory activity. Therefore, it was aimed to provide some benzimidazole derivatives as ACE inhibitors with high potency and low toxicity. A series of some benzimidazole derivatives was designed as ACE inhibitor based on the literature. Molecular docking studies were carried out by using AutoDock vina software to identify the potent compounds. The compounds with predicted high potency were subjected for their toxicity prediction by osiris property calculator and the drug likeliness studies were carried out using available online softwares. The selected compounds were synthesized and evaluated for their ACE inhibitory activity using lisinopril as a standard drug. The compound 2-(2-(butylthio)-5-methoxy-1H-indol-1-yl)-1-(2-nitrophenyl)ethan-1-one **(17)** was identified as an equipotent ACE inhibitor with respect to lisinopril. The in silico toxicity studies revealed that this compound was safe with respect to tumorogenecity, irritation effect, and reproductive effect. The application of the Lipinski's Rule of 5 and drug likeliness studies also revealed that this compound had an acceptable level of drug likeliness property with a potential to become a good orally active candidate. The in vitro ACE inhibitory assay of this compound also revealed that it was almost equipotent with respect to lisinopril. The compound **(17)** has required attributes to become a potential candidate as an ACE inhibitor. However, further studies are recommended to ensure its efficacy and safety in different animal models.*

Keywords: *Molecular modelling, benzimidazole derivatives, ACE inhibitor, drug likeliness, toxicity.*

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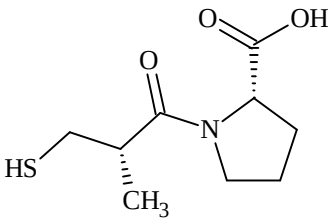
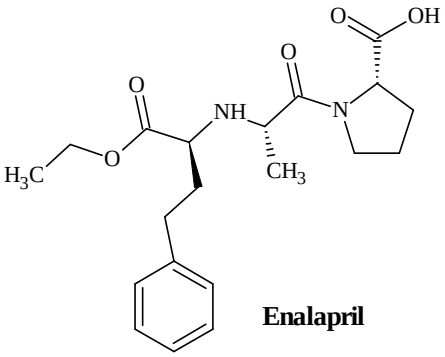
INTRODUCTION:

Hypertension has become a leading cause of human morbidity and mortality. According to World Health Organization [1], about 40% of people aged 25 and above are suffering from hypertension worldwide. According to recent studies [2, 3], the burden of hypertension is increasing at an alarming rate in Gulf region including in the Kingdom of Saudi Arabia. These studies have also stated that if the burden of hypertension remains uncontrolled, it will lead to major challenges to the health care system. Accordingly, efforts should be made to reduce the burden of hypertension worldwide.

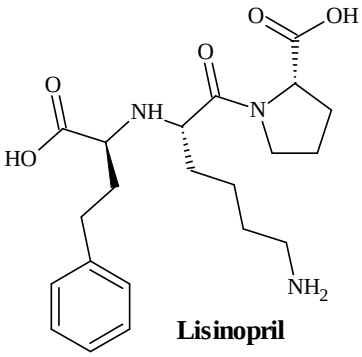
Angiotensin Converting Enzyme (ACE) [4] is an enzyme that converts Angiotensin I to Angiotensin II [4]. Angiotensin II is a potent vasoconstrictor and is implicated in the development of hypertension. Therefore, drugs that prevent the generation of Angiotensin II from Angiotensin I by inhibiting the Angiotensin Converting Enzyme (ACE) as well as the drugs that are Angiotensin II receptor antagonists are in clinical use as antihypertensive agents, for example, captopril [5], enalapril [6], lisinopril [7], telmisartan [8], candesartan [9], and azilsartan [10].



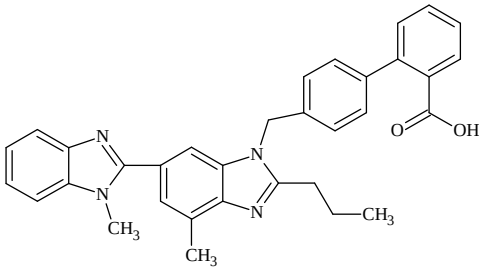
Captopril



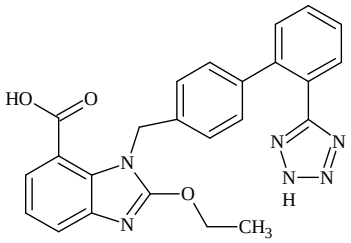
Enalapril



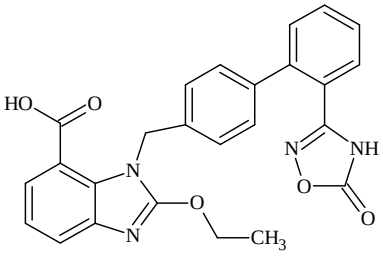
Lisinopril



Telmisartan



Candesartan



Azilsartan

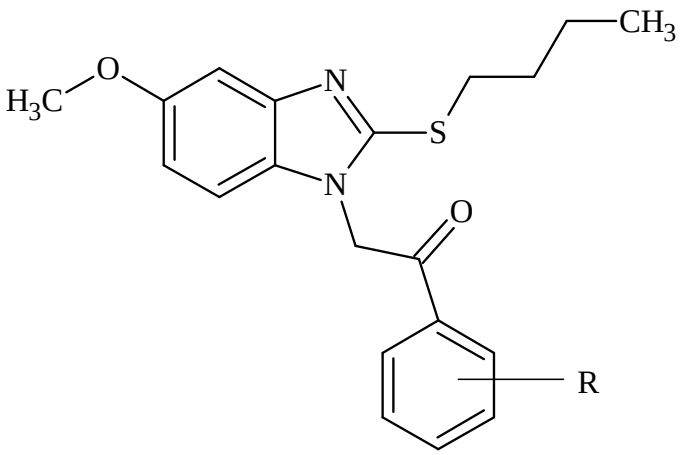
However, many side effects are also associated with these drugs, for example, proteinurea, rashes, bad mouth odor, arrhythmia, allergic reactions, pancreatitis, alopecia, angioedema, and gastrointestinal disorders. Therefore, scientists are working to develop new ACE inhibitors as well as Angiotensine II receptor antagonists that are safer and effective than these existing drugs. Benzimidazole derivatives are reported to possess diverse biological activities [11,12]. Benzimidazole derivatives have also been reviewed as antihypertensive agents including as inhibitors of the Angiotensin Converting Enzyme (ACE) [13-15]. Therefore, it was aimed to perform molecular modelling studies and to synthesize some benzimidazole derivatives as angiotensin converting enzyme inhibitors.

MATERIALS AND METHODS:

Molecular Docking Methodology

Docking studies were carried out by using AutoDock Vina software [16], running on Linux Ubuntu 12.0, installed on Pentium i3 workstation. The program AutoDock Tools (ADT) released as an extension suite to the Python Molecular Viewer was used to prepare the protein and the ligand to convert the molecules into Autodock type, which is a prerequisite for the docking [17, 18]. Discovery studio 4 [19] was used for visualizing the resolutions of docked conformations. ChemDraw ultra 8.0 software [Chemical Structure Drawing Standard; Cambridge Soft corporation, USA (2003)] was used for construction of compounds which were transformed to 3D structures using Chem 3D ultra 8.0 software and the constructed 3D structures were energetically minimized by using MOPAC (semiempirical quantum mechanics) with the AM1 mozyme geometry, 100 iterations and minimum RMS gradient of 0.10. For each ligand, corresponding ATOM/HETATM and CONECT records were extracted from protein complex in the pdb file. After assigning bond orders, missing hydrogen atoms were added. Then in the AutoDock tools package, the partial atomic charges were calculated using Gasteiger-Marsili method [20] and after merging non-polar hydrogens, rotatable bonds were assigned. For receptor, the ligand, as well as any additional chains and all the heteroatoms including water molecules were removed. By the use of AutoDock Tools all missing hydrogens were added. Input molecule files for an AutoDock experiments must conform to the set of atom types supported by it. Therefore, pdbqt format was used to write ligands, recognized by AutoDock. The grid maps were calculated using AutoGrid [21]. In all dockings, a grid map with 60 x 60 x 60 points, a grid spacing of 0.503 A° were used, and the maps were centered on the ligand binding site. All the compounds taken under study were modeled by positioning them in the LPR (PDB ID: 1O86) binding site in the active domain of ACE protein as accorded by the published crystal structure. From the comparative docking study of our compounds with standard binding compound (LPR) we could observe how our compounds might bind to the ACE inhibition site, based on the knowledge of the structure of similar active sites. We redocked LPR into the active site of the protein and then we docked with our compounds in order to compare the binding affinity of both ligand and the test compounds. The docking score of the targeted compounds is also provided in Table 1.

Table 1: Docking scores of the selected compounds and the physical constants of the synthesized compounds



Compound Number	R	Molecular Formula	M.P. (±2°C)	Yield (%)	R_f Value	Docking Score
1	H	$C_{20}H_{22}N_2O_2S$	-	-	-	-8.1
2	4-Br	$C_{20}H_{21}BrN_2O_2S$	-	-	-	-7.8
3	3-Br	$C_{20}H_{21}BrN_2O_2S$	-	-	-	-7.8
4	2-Br	$C_{20}H_{21}BrN_2O_2S$	-	-	-	-7.9
5	2-Cl	$C_{20}H_{21}ClN_2O_2S$	-	-	-	-8.1
6	3-Cl	$C_{20}H_{21}ClN_2O_2S$	-	-	-	-7.9
7	4-Cl	$C_{20}H_{21}ClN_2O_2S$	-	-	-	-7.7
8	4-F	$C_{20}H_{21}FN_2O_2S$	-	-	-	-8
9	3-F	$C_{20}H_{21}FN_2O_2S$	-	-	-	-8.1
10	2-F	$C_{20}H_{21}FN_2O_2S$	141	70	0.81	-8.3
11	2-OCH_3	$C_{21}H_{24}N_2O_3S$	-	-	-	-7.7
12	3-OCH_3	$C_{21}H_{24}N_2O_3S$	-	-	-	-7.9
13	4-OCH_3	$C_{21}H_{24}N_2O_3S$	-	-	-	-7.4
14	4-CH_3	$C_{21}H_{24}N_2O_2S$	-	-	-	-7.8
15	3-CH_3	$C_{21}H_{24}N_2O_2S$	-	-	-	-7.9
16	2-CH_3	$C_{21}H_{24}N_2O_2S$	147	60	0.83	-8.2
17	2-NO_2	$C_{20}H_{21}N_3O_4S$	155	55	0.88	-8.3
18	3-NO_2	$C_{20}H_{21}N_3O_4S$	-	-	-	-8.1
19	4-NO_2	$C_{20}H_{21}N_3O_4S$	-	-	-	-8.1
Lisinopril	-	$C_{21}H_{31}O_5N_3 \cdot 2H_2O$	-	-	-	-8.3

***In Silico* Drug Likeliness Studies**

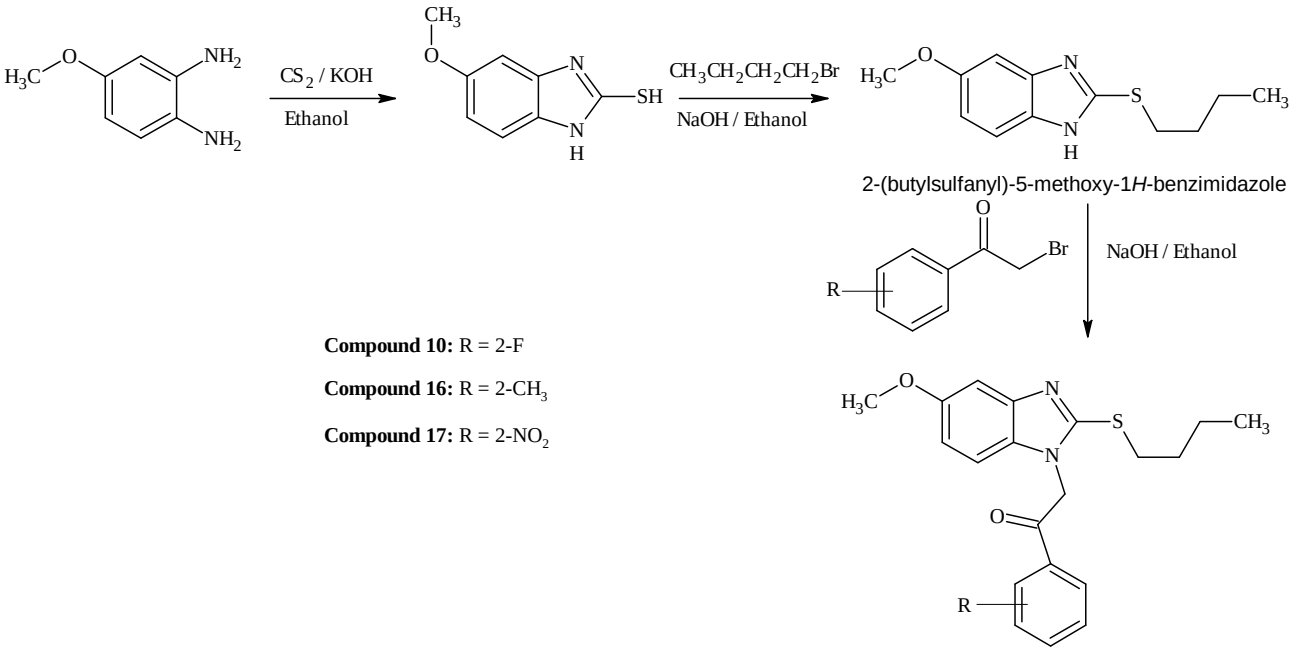
To obtain the efficacious bioavailable drugs the successful compounds (10, 16, and 17) were subjected for the prediction of some basic pharmacokinetic properties such as Molecular weight, Lipophilicity, TPSA (total polar surface area), volume, Drug likeness, Hydrogen bond donors(HBD) and Hydrogen bond acceptors (HBA) etc. as shown in Table-1. An online molecular property prediction tool was used for this purpose found at www.molsoft.com.

***In Silico* Toxicity Studies**

The compounds (10, 16, 1nd 17) were subjected for toxicity prediction by the osiris property calculator. Structure based drug design is now very routine work as many drugs fail to reach clinical phases because of ADME/TOX problems encountered. Therefore prediction of these problems before synthesis is a rational approach to minimize cost production of expensive chemicals. The Osiris calculations are tabulated in Table 2. Toxicity risks (mutagenicity, tumorogenicity, irritation, reproduction) and some physical properties of compounds (10-44) were calculated by Osiris methodology.

Chemistry

Melting points were recorded in open capillary tubes and are uncorrected. IR (KBr) spectra were recorded on a JASCO, FTIR-4100 spectrophotometer. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker Ultrashield 500 Plus MHz spectrophotometer. All reagents used in the present work were of analytical grade. The standard drug Lisinopril used for the assessment of *in vitro* ACE inhibitory activity was procured from Sigma Aldrich, USA. The purity of the compounds was checked on silica gel G plates using iodine vapours as visualizing agent. The R_f value of the compounds was determined by using a mixture of benzene and acetone (9:1). The synthetic pathway for the preparation of the benzimidazole derivatives is provided in Scheme 1.



Scheme 1

Synthesis of 2-(butylsulfanyl)-5-methoxy-1*H*-benzimidazole

5-methoxy-1*H*-benzimidazole-2-thiol (0.01 mol) and sodium hydroxide (0.01 mol) were mixed in 20 ml of ethanol and the mixture was heated for 1 hour. Butyl bromide (0.02 mol) was added to the mixture and the resulting mixture was refluxed for 8 hours. The contents were reduced to half of its volume and then poured on crushed ice. The solid was filtered, washed with water and recrystallized from ethanol.

General procedure for the synthesis of the targeted compounds

2-(butylsulfanyl)-5-methoxy-1*H*-benzimidazole (0.01 mol) was dissolved in 25 ml of acetone. Potassium carbonate (0.015 mol) was added to the mixture and the mixture was stirred for about 30 minutes. Appropriate phenacyl bromide (0.01 mole) was added to the resulting mixture and it was stirred for 20 hours. The contents of the flask were reduced to half of its volume and it was poured on crushed ice. The solid separated was filtered, washed with water and recrystallized from ethanol.

Angiotensin Converting Enzyme Inhibitory Assay

The compounds (**10, 16** and **17**) that showed highest ACE inhibitory activity, according to the molecular modelling studies were subjected for the ACE inhibitory assay using Dojindo ACE Kit-WST test kit, Dojindo Laboratories, Kumamoto, Japan [22]. The enzymatic reaction was initiated by the ACE and aminoacylase in the mixture containing 3HB-GGG (3-hydroxybutyrate glycylglycylglycine) and the ACE-inhibitor. The mixture was then incubated at 37ºC for 60 minutes. During this incubation, the substrate, 3HB-GGG, was enzymatically cut into 3HB-G and G-G and then 3HB and G. The yield of 3HB was monitored indirectly through formazan concentration, which was measured at 450 nm after 10 minute reaction at 25ºC. Testing procedures were run according to the manufacturer's instructions using a 96-well plate without modification, and the inhibition rate was calculated based on a comparison of the optical absorbance of samples-treated wells (*As*), control wells (*Ac*), and blank wells (*Ab*). Absorbance was measured at 450 nm using the microplate reader Biotek-ELX800 (BioTek, Vermont, USA). Inhibition rates were calculated using the following equation.

$$\text{Inhibition rate (\%)} = [Ac - As \,/\, Ac - Ab] \times 100$$

Samples were suspected to inhibit the ACE activity, and therefore inhibit the formation of formazan. The more strongly inhibitory the activity of the samples, the less color appeared in the final solution.

Statistical Analysis

All ACE inhibitory activity data are presented as mean ± standard deviation (SD, n = 3). The data were analyzed by one-way analysis of variance (ANOVA) with Dunnett's Multiple Comparison Test with respect to control group and standard groups using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA). The results were considered significantly different at $p < 0.05$. The IC_{50} values

were determined by linear regression calculator of GraphPad software.

RESULTS:

Molecular Docking Study

The docking data of Lisinopril with the ACE protein revealed that the original co-crystallized and docked ligand overlapped with ACE protein elegantly, thereby validating our docking study. The results of docking scores in terms of binding affinity have been summarized in Table 1. The lisinopril exhibited binding affinity of -8.3 kcal/mol with the ACE protein and interacted with the Glu-162, Asn-277, Asn-281, His-383 and Tyr-523 by forming conventional H-bond as shown in 2D-diagram of ligand protein interaction (Figure 1). Interestingly, compounds **10** (Figure 2)**, 16** (Figure 3)**,** and **17** (Figure 4) also showed binding affinity close to Lisinopril. The compound **10** exhibited binding affinity of -8.3 kcal/mol; the compound **16** exhibited binding affinity of -8.2 kcal/mol; and the compound **17** exhibited binding affinity of -8.3 kcal/mol.

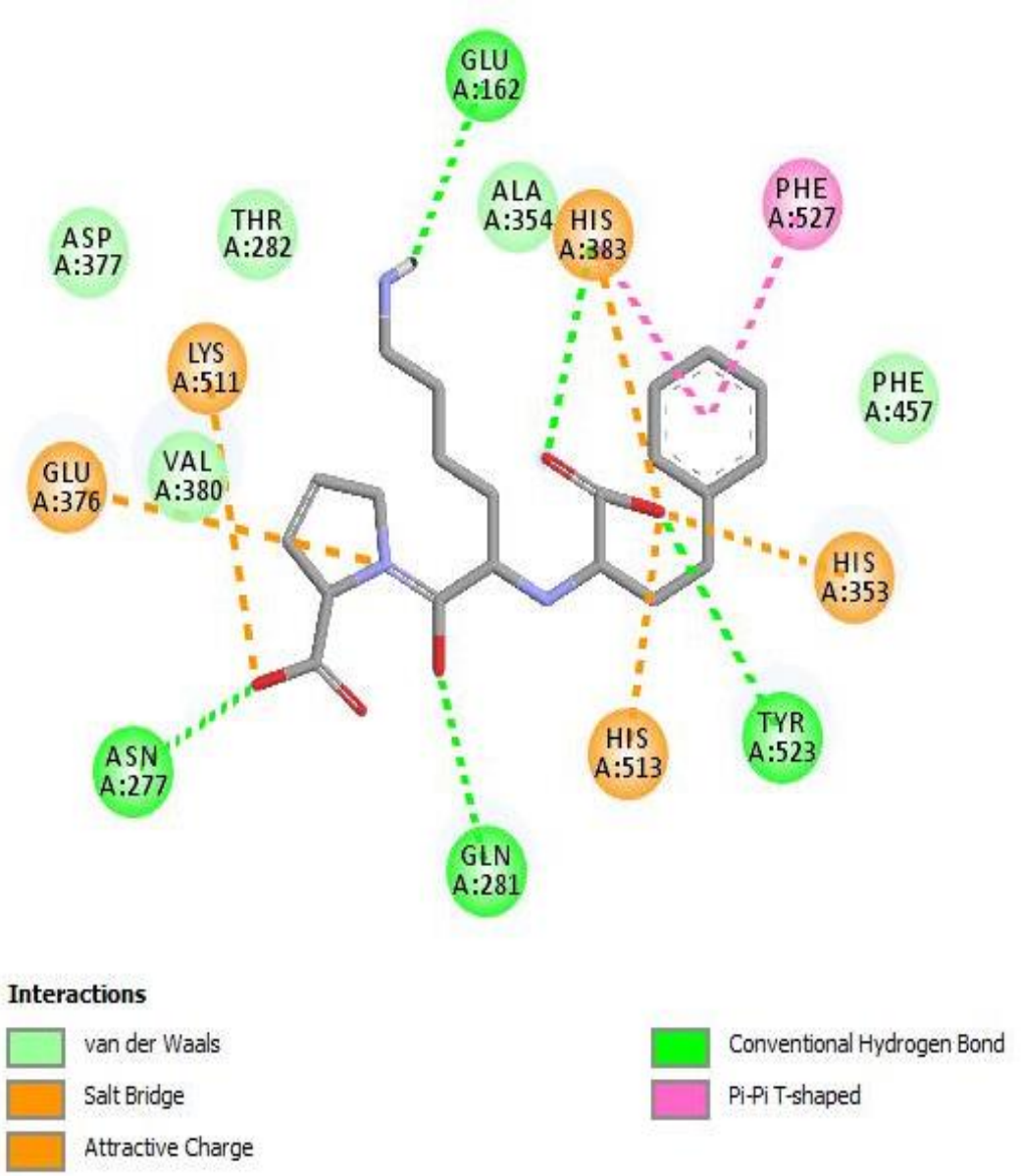


Fig 1: 2D interaction of lisinopril with the active sites of the ACE

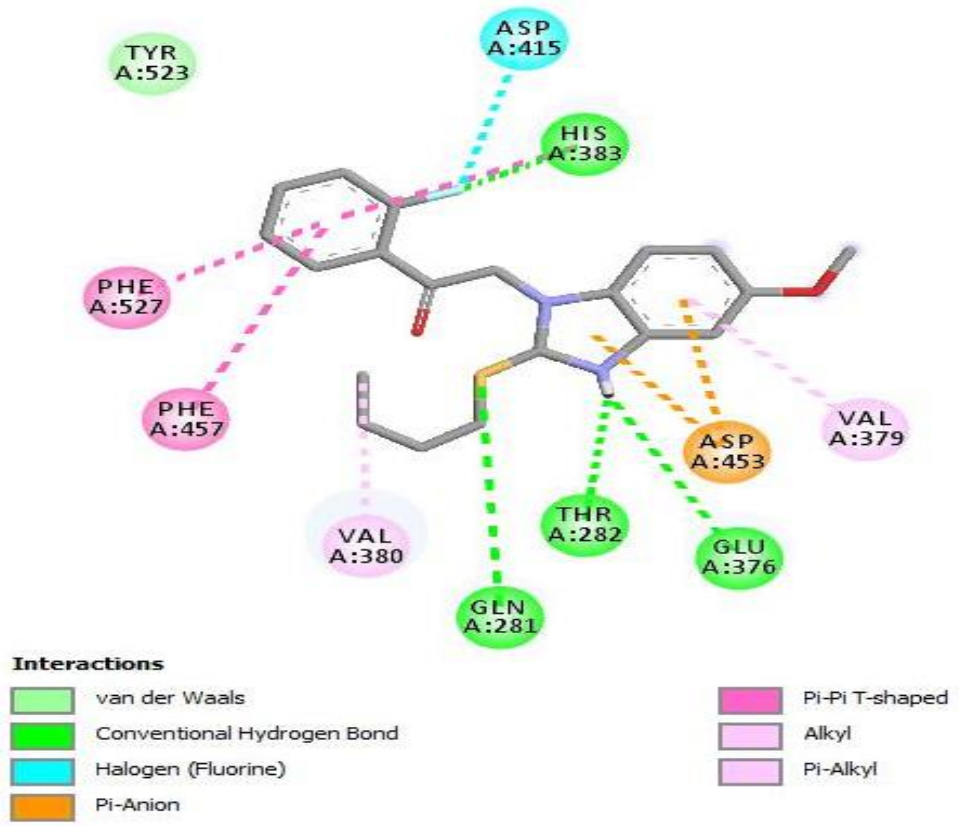


Fig 2: 2D interaction of compound 10 with the active sites of the ACE

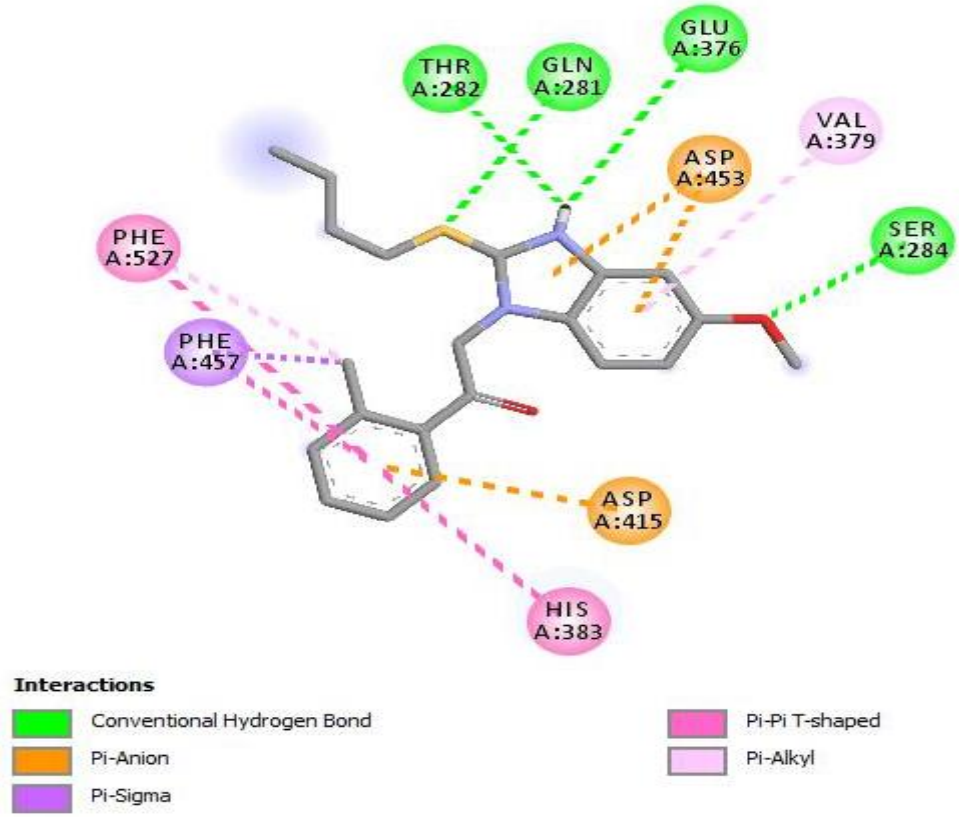


Fig 3: 2D interaction of compound 16 with the active sites of the ACE

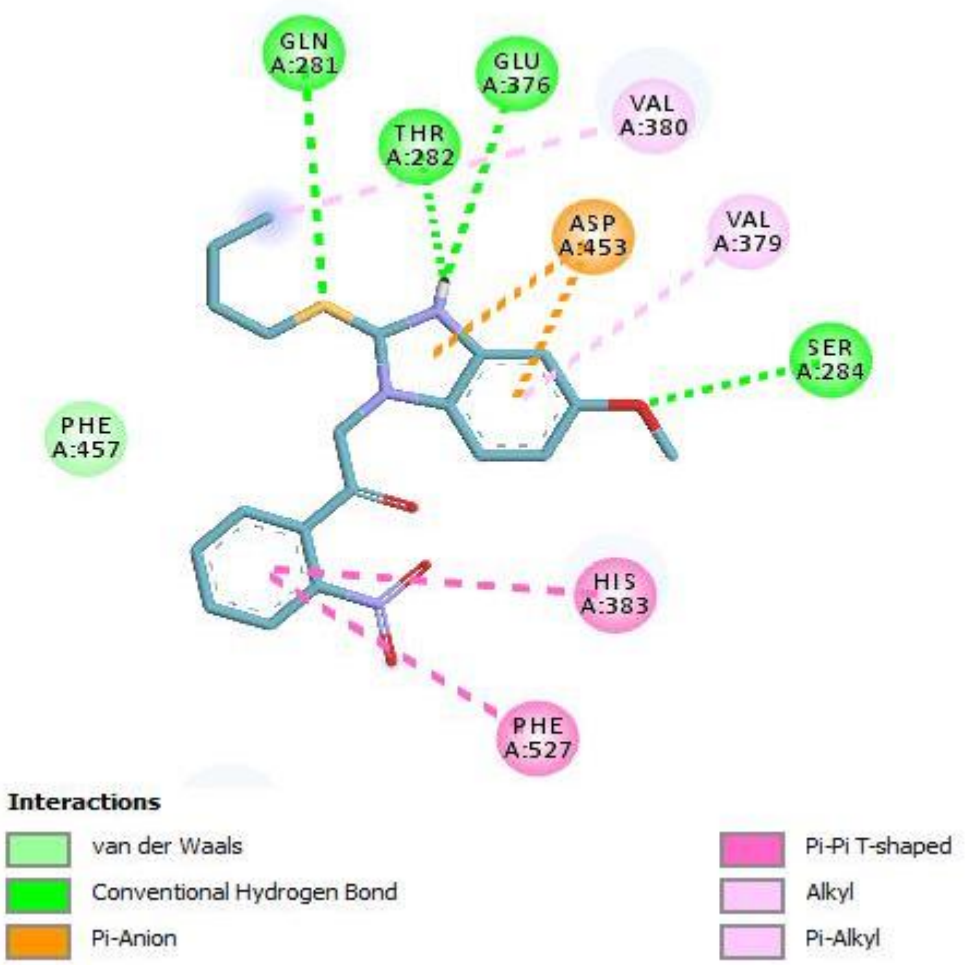


Fig 4: 2D interaction of compound 17 with the active sites of the ACE

In silico Drug Toxicity and Drug Likeliness Studies

Hydrophilicity and Lipophilicity balance plays a very important role in the absorption or permeation of drugs into the systemic circulation. For this reason solubility and log P values were calculated and all the compounds under consideration were subjected to screen by Lipinski's Rule of 5.

It is evident from Table 2 that the compound **17** did not violate any Lipinski's Rule. Therefore, compound **17** can be regarded as to be good oral candidates. Further the compounds were subjected for prediction of drug score and drug likeness along with the prediction of toxicity risk evaluation.

Table 2: Pharmacokinetic Properties important for good oral bioavailability for the promising compounds

Compound No.	logP	HBD	HBA	NRTB	MW	Drug likeness	Lipinski's violations
Rule	< 5	< 5	< 10	<10	<5	--	>/= 1
10	5.15	0	4	8	372	0.42	1
16	5.43	0	4	8	368	0.4	1
17	4.94	0	7	9	399	-0.06	0

NROTB, number of rotatable bonds; MW, molecular weight; LogP, logarithm of compound partition coefficient between n-octanol and water; HBA, number of hydrogen bond acceptors; HBD, number of hydrogen bond donors.

Table 3: Toxicity and Molecular properties Predicted by Osiris Calculator

Cmpd No.	Tumorigenic	Mutagenic	Irritation	Reproductive Effect	Vol	TPSA	Solubility	DS
10					332	44.13	-4.53	0.47
16					343	44.13	-4.56	0.53
17					350	89.95	-4.68	0.32

Colour of circle indicates level of toxicity; Green: low, Yellow: medium, Red: Highly toxic.
TPSA, topological polar surface area; DS, Drug score.

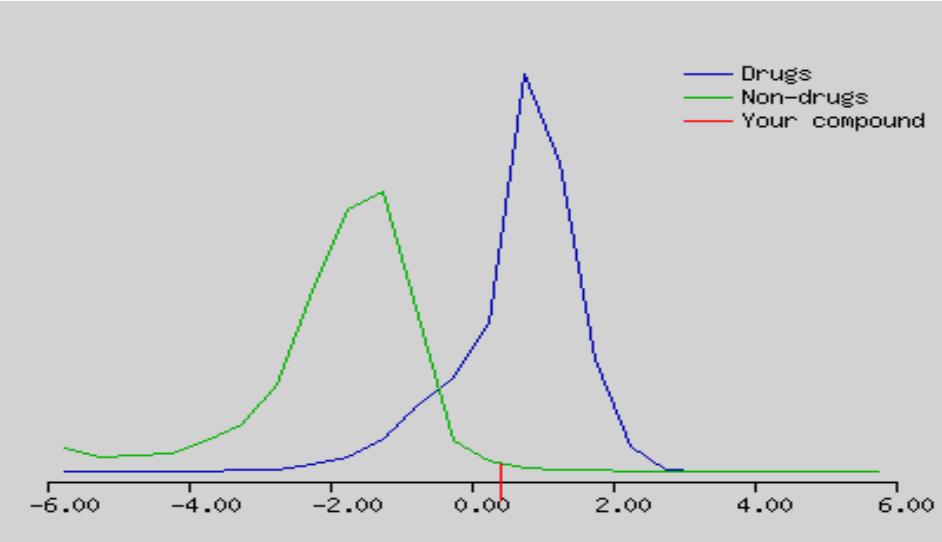


Fig 5: Drug likeness model score predicting graph

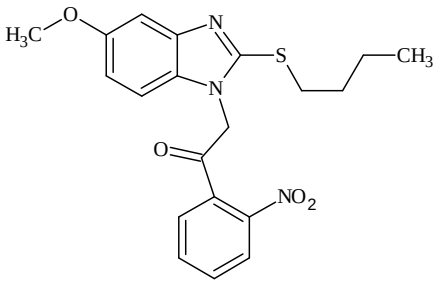
The data of the Table 3 revealed that the selected compounds **10, 16,** and **17** were safe with respect to Tumorogenecity, Irritation and reproductive effect.

Drug Likeness can be defined as balance of various molecular properties and structure features which determine whether particular molecule is similar to the known drugs. Aptness of drug likeness can be judged by matching the graph of drug like compounds as shown in Figure 5. The compound **17** further showed good level of drug likeness prediction.

Chemistry

The benzimidazole derivatives were prepared according to the method provided in Scheme 1. The compound 5-methoxy-1*H*-benzimidazole-2-thiol was reacted with carbon disulfide in the presence of potassium hydroxide to obtain 5-methoxy-1*H*-benzimidazole-2-thiol. The 5-methoxy-1*H*-benzimidazole-2-thiol was treated with butyl bromide in the presence of sodium hydroxide to provide 2-(butylsulfanyl)-5-methoxy-1*H*-benzimidazole.. The 2-(butylsulfanyl)-5-methoxy-1*H*-benzimidazole was treated with 2-fluorophenacylbromide, 2-methylphenacylbromide, and 2-nitrophenacylbromide to obtain the targeted compounds **10, 16** and **17,** respectively. The structure of the compound 17 was confirmed on the basis of following data.

2-[2-(butylsulfanyl)-5-methoxy-1*H*-benzimidazol-1-yl]-1-(2-nitrophenyl)ethanone (17): IR (KBr) cm-1: 2950, 1685 (C=O), 1335, 1390; ^{1}H-NMR (DMSO-d6, δ ppm): 0.88 (t, 3H), 1.31-1.44 (m, 2H), 1.60-1.72 (m, 2H), 3.21 (t, 2H), 3.88 (s, 3H), 5.97 (s, 2H), 7.15-7.41- (m, 3H), 7.53-7.84 (m, 4H); ^{13}C-NMR (DMSO-d6, δ ppm): 12.8, 14.4, 21.6, 32.4, 36.4, 55.1, 55.8, 110.5, 112.1, 121.8, 126.5 (2C), 133.0, 133.7, 134.4, 138.9, 143.7, 151.6, 155.1, 163.1.



Compound 17

Table 4: *In vitro* ACE inhibitory activity of Compound 17 and the standard drug lisinopril

Compound	Concentration (µg/mL)	%ACE Inhibition (Mean ± SD)	IC_{50} (µg/mL)	Docking Score
Lisinopril	1	42.10 ± 0.10*	0.86	-8.3
	2	59.99 ± 0.13*		
	4	75.16 ± 0.16*		
	8	87.11 ± 0.18*		
Compound 17	1	41.98 ± 0.19*	0.81	-8.3
	2	60.12 ± 0.18*		
	4	74.70 ± 0.22*		
	8	85.06 ± 0.35*		

*$p < 0.0001$; n = 3.

ACE inhibitory Assay

Based on the molecular modelling results, In silico toxcicity data, and the drug likeliness prediction, the compound **17** was selected for further *in vitro* ACE inhibitory assay using Dojindo ACE Kit-WST test kit, Dojindo Laboratories, Kumamoto, Japan. The ACE inhibitory activity data of these compounds are provided in Table 4.

DISCUSSION:

The compound 17 was identified as an equipotent ACE inhibitor with respect to standard drug lisinopril having a docking score of -8.3. The *In Silico* toxicity studies revealed that this compound was safe with respect to its tumorogenecity, irritation effect, and reproductive effect. The application of the Lipinski's Rule of 5 and drug likeliness studies also revealed that this compound has an acceptable level of drug likeliness property with a potential to become a good orally active candidate. The *in vitro* ACE inhibitory assay of this compound also revealed that it was almost equipotent with respect to standard drug lisinopril.

CONCLUSION:

It is evident from the results that the compound **17** has required attributes to become a potential candidate as an ACE inhibitor. However, further studies are recommended to ensure its efficacy and safety in different animal models.

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