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In Silico Investigation of Anticancer Potential of Polysubstituted Thiophenes Through Molecular Docking Tools

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Abstract
Development of new drug candidates for the treatment of cancer is under keen investigation since last few decades. Overexpression of carbonic anhydrase enzyme leads to development of hypoxic cancer. Many drug candidates have been already reported against carbonic anhydrase, but they are associated with toxicity and drug resistance issues. Therefore, in order to develop new drug candidates as carbonic anhydrase inhibitors, we have designed a libarary of 96 compounds possessing two heterocyclic moieties (thiophene and triazole).The designed molecules were further subjected to molecular docking against carbonic anhydrase enzyme (PDB ID 1XPZ) using GOLD and MOE software. The Gold and MOE scores of all the compounds were calculated among which 6 best compounds were screened and their binding patterns with the receptor were observed. Further, ADME, drug likeliness and toxicity characteristics have been predicted by using Swiss ADME predictor and preAdme tool, Lazar and protox. All the parameters were in the specified limits and followed the Lipnski's rule. However, compound 67 has showed best interactions when compared with internal ligand by forming hydrogen bond interactions with THR 200, HIS 94, THR 199 and metal interaction with Zn 262. Validation of docking procedure was done and RMSD value was found to be 1.76Å. Interactions of best 6 compounds were predicted on MOE software which showed similarity in the binding pattern as predicted by GOLD software

Keywords: Hypoxic cancer, carbonic anhydrase, Lipinski's rule, hydrogen bonding, validation, RMSD.

1. INTRODUCTION

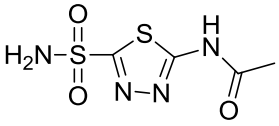
Cancer is one of the leading cause of death worldwide and its global burden has constantly been increasing [1,2]. Since the last few decades cancer chemotherapy has been the major advancement but due to unwanted side effects and narrow therapeutic index, there is a need for the development of newer anticancer agents [3,4].

Heterocyclic chemistry has already paid a tremendous role towards producing anti-cancer compounds with promising activity. In the drug discovery polysubstituted thiophenes

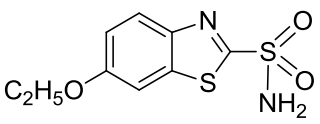
have emerged as significant heterocycles due to its diverse pharmacological profile which include anticonvulsant [5], antimicrobial [6], anti-proliferative [7], anti-allergic [8], anti-diabetic [9], analgesic [10], anti-inflammatory[11] and hypotensive[12] properties. Moreover, nitrogen containing heterocyclic derivativesi.e 1,2,4-triazole has been found in numerous bioactive molecules as an efficient antimicrobial, antioxidant and antitumour agent [13-17]. Literature reports also revealed that Schiff bases of heterocyclic moiety have significant biological activities including antifungal, antimalarial, antituberculosis, antimicrobial, antitumour, antiviral, antiinflamatory etc [18-20]. Combining thiophene with other heterocyclic moiety may results in a bioactive heterocyclic compound with enhanced anticancer activities [21].

Cancer develops due to overexpression of various enzymes in the body. Carbonic anhydrase inhibitors have attracted keen attention of researchers towards design of therapeutic candidates against many types of cancer. Generally, overexpression of carbonic anhydrase(CA IX, CA XII) leads to development of cancer. It has been reported that 25 drugs are used clinically as carbonic anhydrase inhibitors [22]. In the literature various reports have suggested the role of carbonic anhydrase in cancer [23-25]. Drugs available as carbonic anhydrase inhibitors for the treatment of cancer have serious side effects due to off target binding of drugs. Reported inhibitors of CA IX and CA XII (memberane bound) unable to differentiate between different isomeric forms of CA and off target inhibit the CA II (cytoplasm) expressed in normal cells leading to toxicity [26,27]. Considering this problem there is an urgent need for the development of newer drugs having targeted delivery of drug. SLC-0111 (Phase I completed) [28] and E7070/indisulam (Phase I Completed) [29], Girentuximab cG250 (Phase III Completed) [30], BAY 79–4620 (Phase I completed) [31] are under clinical trials as CA IX inhibitors for the treatment of cancer. Bulut et al [32], Vats et al [33] and Kumar et [34] have recently reported significant carbonic anhydrase inhibitory activity of triazole derivatives. Various reported CA IX inhibitors (1-4) substituted triazole derivatives (5-7) against cancer have been presented in **figure 1**.

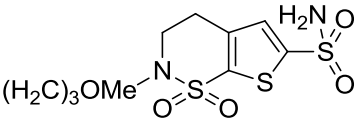
By the Encouragement of our aforementioned findings and in continuation of our search for polysubstituted thiophene as anticancer agents [35,36], we have designed 96 hit compounds having polysubstituted thiophenes schiff bases with 1,2,4-triazole at the 3rd position. Molecular docking studies are helpful in studying the drug receptor interactions and in predicting the most energetically favoured binding pose of a ligand to its receptor. In the present in silico study, molecular docking studies were performed to check the interaction of designed molecules towards carbonic anhydrase (PDB ID 1XPZ). Further, drug likeliness, ADME and safety profile of the designed molecules has been also evaluated.



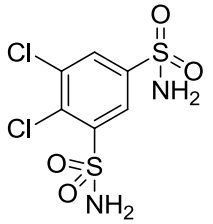
Acetazolamide
(1)



Ethoxazolamide
(2)

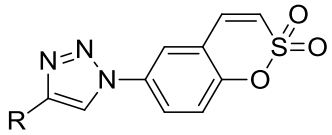


Brinzolamide
(3)

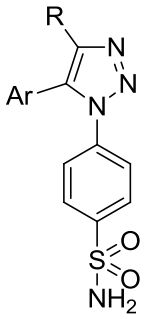


Dichlorophenamide

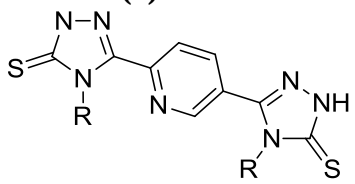
(4)



(5)



(6)



(7)

Fig 1. Reported Carbonic anhydrase inhibitors

2. MATERIALS AND METHOD

2.1 Molecular Docking

A series of 96 derivatives were designed by implementing six different kinds of substitution. All the designed molecules were then docked against carbonic anhydrase enzyme. The various steps involved in docking process are as follows

2.1.1. Preparation of protein Structure

The 3D crystal structure of human carbonic anhydrase (PDB ID 1XPZ) was obtained from RCSB-PDB (http:/www.rcsb.org/pdb) [38] pdb format with resolution of 2.02 Å. The protein cavity was created by using Molecular Operating Environment (MOE) software by selecting the ligand nearby area upto 10Å.This protein cavity was prepared shown in **Figure 2** via GOLD 5.0 by addition of hydrogens and extracting the internal ligand as mol file.

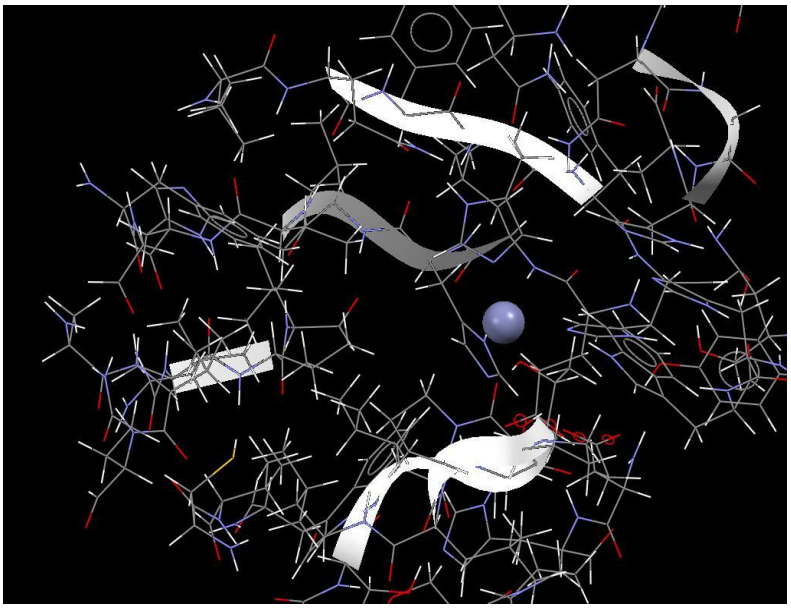


Fig. 2. 3D-Crystal Structure of Human Carbonic anhydrase (PDB ID 1XPZ)

2.1.2. Preparation of ligand Structure

2D structures of the Designed ligands were prepared with the help of Chem draw ultra 12.0 tool in MDL mol files. The designed 96 ligands were energy minimized in MOE software.

2.1.3. Ligand docking and validation protocol

Ligand docking was carried out by using GOLD 1.6.2 softwareon the energy minimized molecules. The 3D X-ray crystal structure of carbonic anhydrase was further refined by addition of hydrogens to the PDB. Validation of the docking procedure was done by reproducing the confirmation of co-crystallized ligand and after this root mean square deviation (RMSD) value was calculated between co-crystallized and re-docked conformations. In the first step, various conformations of the ligand inside the receptor pocket were generated by GOLD using genetic algorithm based search functions. This analysis was done by carrying out approximately 10000 operations on a population size of 100 along with fixing the values of crossover, mutation and migration frequencies to 95, 90 and 10 respectively. In the second step ranking based upon Gold score was assigned to various conformations. Gold scores of all the 96 compounds were analysed and 6 compounds with best Gold scores were selected. These six compounds were further checked for finding out various drug-receptor interactions in the best orientation and lowest energy state. Various interactions were calculated and best orientation poses were selected.

A comparative study of drug-receptor interactions was carried out between 3D-interactions of GOLD and 2-D interactions obtained after docking in MOE software to obtain comparision between the docking by two softwares.

2.2. In silico Drug likeliness Predictions

On the basis of structural and physicochemical properties drug likeliness predicts the oral bioavailabilty of the designed molecules. In the present study Lipinski's rule of five was used to predict the drug like properties [38].

2.3. In silico ADME Predictions

For a drug to be a potent therapeutic candidate, it must reach the target site in sufficient concentration before start of its action. Therefore, ADME properties play a crucial role in the development of new drug candidates. The ADME properties of the designed molecules were calculated using pre-ADMET tool version 2.0 software . It helps in comparing the properties of designed with that of 95% known drugs. The compounds were evaluated for prediction of absorption through HIA (human intestinal absorption), Caco-2 cell, MDCK (Maden Darby Canine Kidney), BBB (blood brain barrier) and plasma protein binding [39].

2.4. In silico Toxicity Prediction

The prediction of Toxicity is important for studying the harmful effects of a drug candidate on normal cells and tissues. in silico toxicity prediction of best six compounds was carried out using PreADME and protox two online softwares, having different parameters to determine the toxicity of designed molecules.

2.4.1. PreADME

PreADMEpredict toxicity on a online web server (http://preadmet.bmdrc.org/). It predict safety profile by predicting toxicity on carcino rats, carcino mice models and Human Ether Related Gene factor (hERG).This HERG is associated with fatal cardiotoxicity [39].

2.4.2. Protox

Protox is a online web server (http://tox.charite.de/tox) predict the toxic interactions of the drugs to various targets by using various targets. It is also helpful in predicting in minimum lethal dose of a drug [40].

These computational predictions are helpful to minimize animals experiments, cost and time.

3. RESULTS AND DISCUSSION

2.1. Interaction of designed triazole derivatives toward carbonic anhydrase enzyme

Molecular docking was performed to check the interaction of designed analogues towards carbonic anhydrase enzyme (PDB 1XPZ). The re-docking of the internal ligand showed good interaction with carbonic anhydrase enzyme with gold score 58.1 through hydrogen bonding interactions with THR199, HIS94 given in **figure 3**. The docking was validated with RMSD value of 1.76 Å between redocked confirmation of internal ligand in the carbonic anhydrase enzyme pocket and co-crystallized ligand. All the 96 designed analogues have been docked with carbonic anhydrase enzyme. Among 96 designed triazole derivatives, 6 designed analogues have shown good binding affinity towards carbonic anhydrase enzyme as that of internal ligand as shown in **table 1.** In literature, various reports are available which indicated the interactions of the heterocyclic derivatives (1,3,4-thiadiazole) towards carbonic anhydrase enzyme. Gomha et al [30] and ghorab et al [31] have reported good interactions towards carbonic anhydrase enzyme. To best of our knowledge, in literature, no study is reported regarding interaction of triazole analogs towards carbonic anhydrase enzyme.

Among the designed analogues 6 best derivatives were analysed and it was revealed that compound no.**67** has shown best interaction in compare to internal ligand. Further, the best 6 compounds were subjected to docking using MOE software as shown in **table 2** and

the binding interactions poses have been depicted in **figure 7** and **Figure 8a-8f**. The finding obtained using MOE revealed almost similar results as depicted in GOLD. The important amino acids found in the binding pocket of the active site are HIS94, HIS96, ASN62, GLN92, VAL121, HIS119, THR199, LEU198, PRO202, PHE131, TRP 209 and LEU204.

The binding interaction poses of best 6 designed analogues by GOLD software have been shown in **figure 4a-4f**. The compound no 67 has shown good hydrogen bonding interactions towards various amino acids (THR 199 (3.4 Å) and THR 200 (3.9 Å)), pie pie stacking (Phe 131) and metal interactions (HIS 119 (2.0 Å) and HIS 94 (2.0 Å)) respectively.

On qualitative evaluation is was observed that binding mode of co-crystallized ligand and best confirmation and potent designed analogue 67 suggest that the orientation of both is almost parallel shown in **figure 5.** Cluster of all the potent designed analogues revealed that all the designed analogues occupy the same binding pocket in the active site of carbonic anhydrase enzyme as shown in **figure 6.**

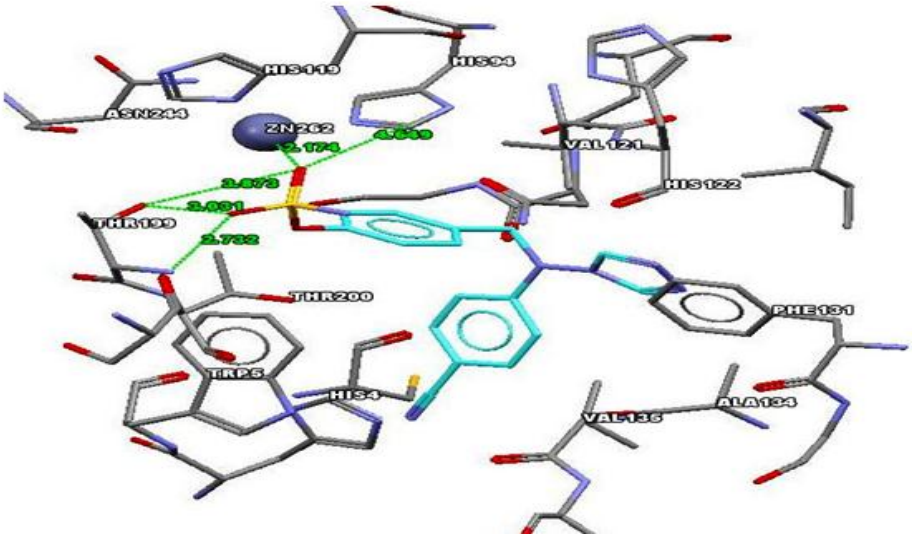


Fig 3: Binding interaction of co-crystallized ligand toward carbonic anhydrase

Table 1. Gold Scores and binding interactions of best six compounds and internal ligand

Chemical Entity	**Gold score**	**No of interactions**	**Type of interactions**
4	71.58	4	Ser 29 (3.6 Å) (H-Bond) Phe 131 (3.6, 4.6 Å) (Hydrophobic bond) THR 200 (3.8 Å) (H-Bond) ZN 262 (1.9, 4.0 Å) (metal)
19	69.83	5	THR 199 (2.8 Å) (H-Bond) ZN 262 (2.6 Å) (metal)
35	67.91	4	His 119 (3.4 Å) (H-Bond) Phe 131 (pie pie stacking) THR 200 (3.9 Å) (H-Bond) ZN 262 (2.61, 1.68 Å) (H-Bond)
51	69.23	4	Phe 131 (pie pie stacking) THR 199 (3.2 Å) (H-Bond) ZN 262 (2.3 Å) (metal)

67	**73.08**	6	THR 200 (4.22 Å) (H-Bond) HIS 94 (4.87 Å) (H-Bond) HIS 94 (3.76 Å) (H-Bond) ZN 262 (2.73, 1.71 Å) (metal) THR 199 (2.66 Å) (H-Bond)
83	66.65	3	ZN 262 (2.2 Å) (metal) THR 199 (3.9 Å) (H-Bond) Phe 131 (4.2) (Hydrophobic bond)
Internal Ligand	58.01	4	THR 199 (2.7 Å) (H-Bond) THR 199 (3.9 Å) (H-Bond) Gln 92 (4.2 Å) (H-Bond) Phe 131 (pie pie stacking) Zn 262(2.9, 2.5 Å)(H-Bond)

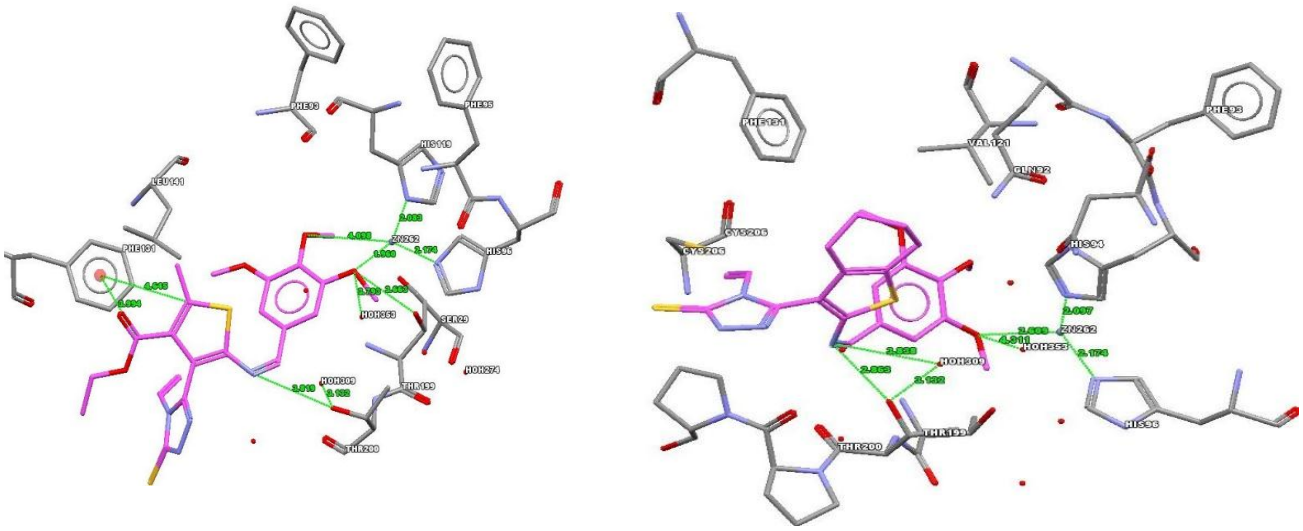


Fig.4 (a) Ligand receptor interactions of Compound 4 **Fig.4 (b)** Ligand receptor interaction of compound 19

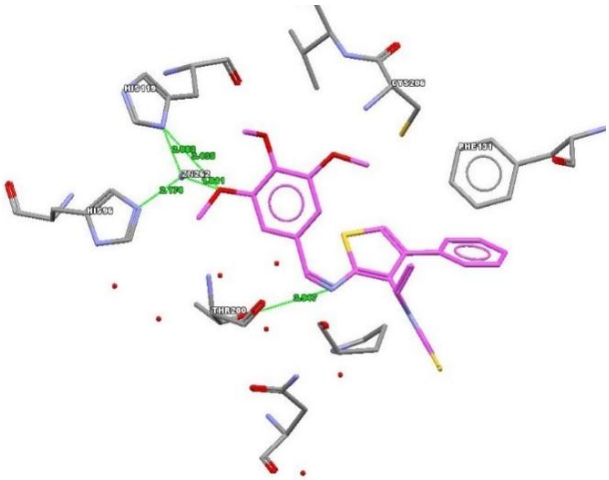


Fig 4 (c): Ligand receptor interaction of compound 35

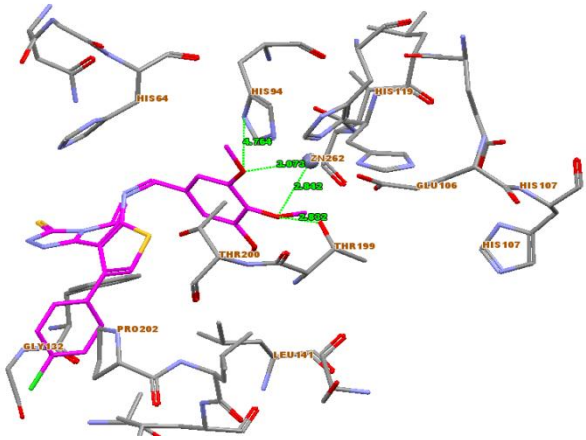


Fig 4 (d): Ligand receptor interaction o compound 51

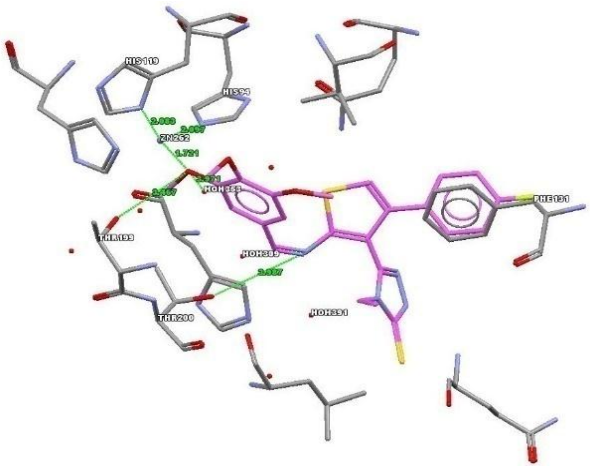


Fig 4 (e): Ligand receptor interaction of of compound 67

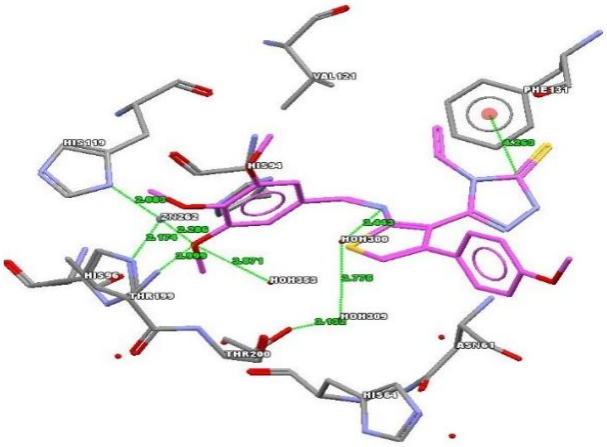


Fig 4 (f): Ligand receptor interaction compound 83

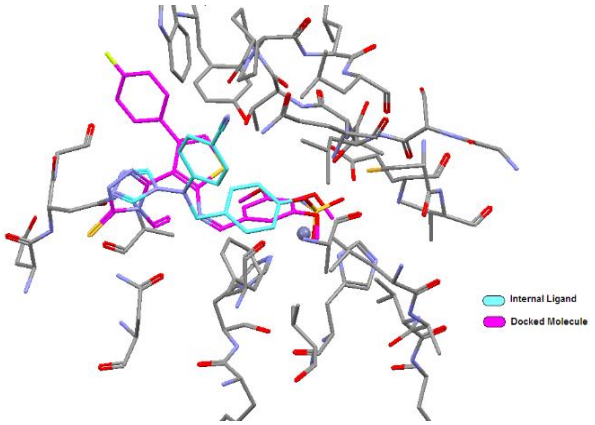


Fig 5: Superimposition of internal ligand and docked compounds in molecule at the active site

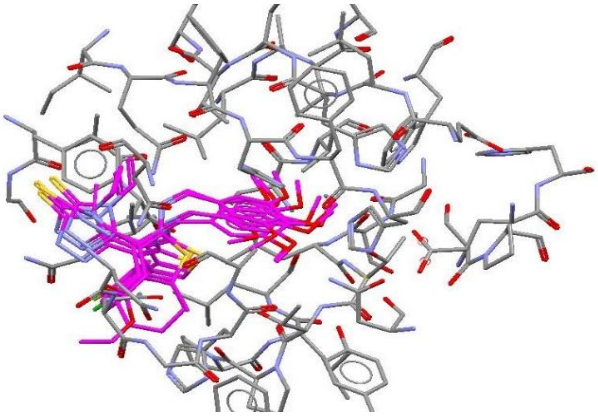


Fig 6: Cluster structure potent at the active site

Table 2. Interactions of potent 6 compounds using MOE software

Chemical entity	**Score**	**Interaction**	**Type of interaction**
4	-11.75	4	THR 199 (H Bond) HIS 119 (H Bond) HIS 96 (H Bond) Zn 262 (Metal)
19	-11.61	2	THR 199 (H Bond) THR 200 (H Bond)
35	-11.78	1	HIS 94 (arene-arene)
51	-12.31	4	HIS 94 (H Bond) HIS 119 (H Bond) HIS 96 (H Bond) THR 199 (H Bond)
67	-11.05	4	THR 200 (H Bond) HIS 64 (H Bond) Trp 5 (H Bond) HIS 4 (H Bond) Zn 262 (Metal)
83	-11.19	5	Arg 58 (H Bond) Gln 92 (H Bond) HIS 94 (H Bond) HIS 119 (H Bond) Zn 262 (Metal)
Internal Ligand	-12.81	3	THR 199 (H Bond) HIS 119 (H Bond) Zn 262 (H Bond)

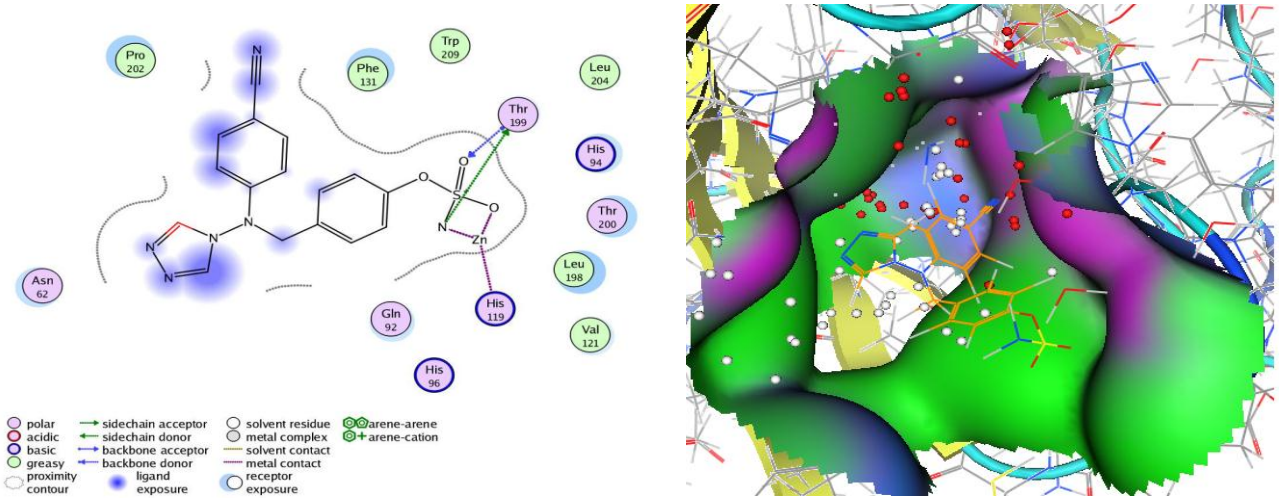


Fig 7: 2D and 3D interactions of internal ligand with the active site amino acids of CA

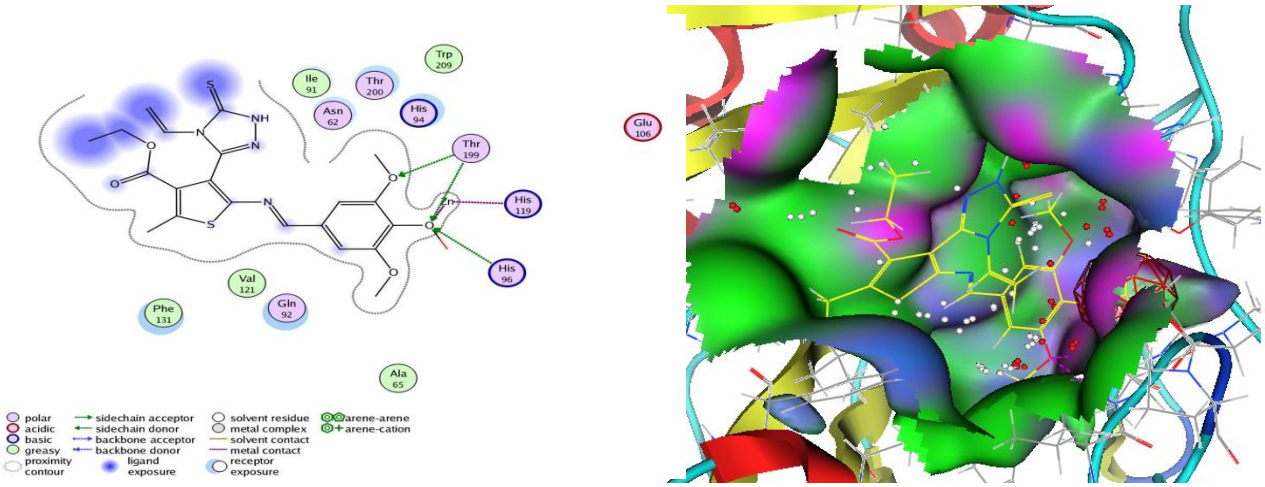


Fig 8 (a): 2D and 3D interactions of Compound 4 with the active site amino acids of CA

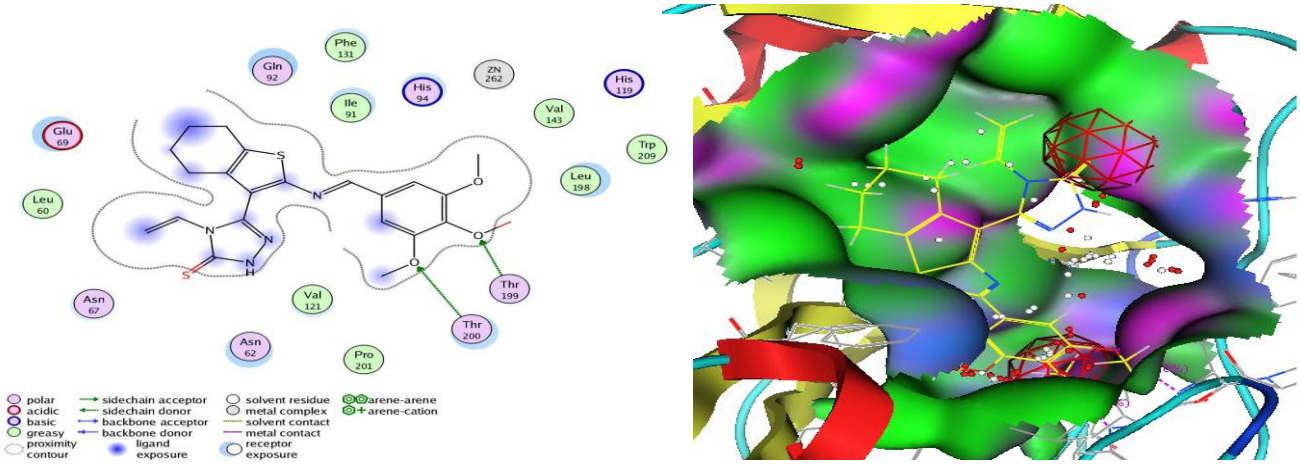


Fig 8 (b): 2D and 3D interactions of Compound 19 with the active site amino acids of CA

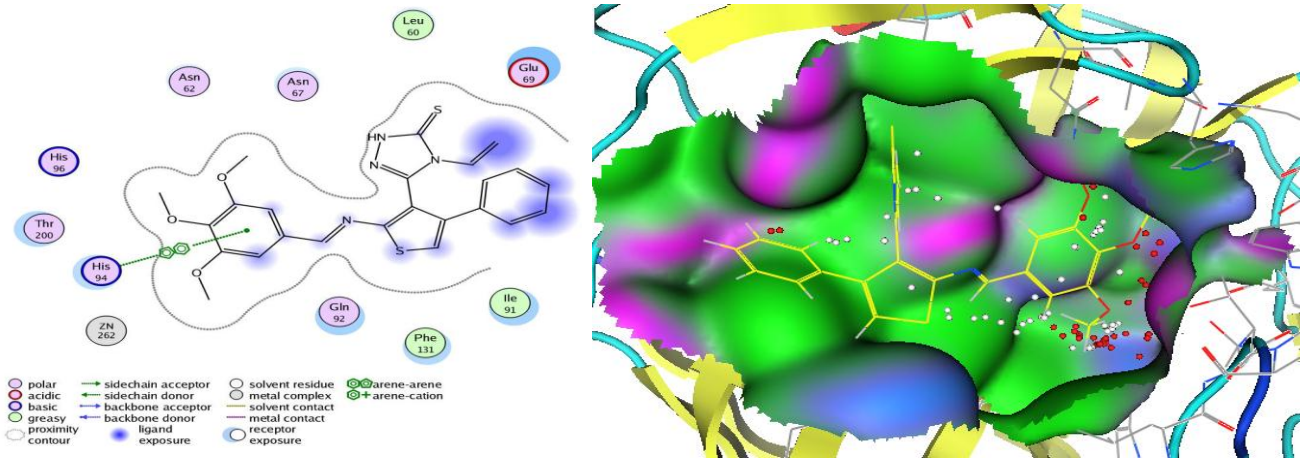


Fig 8 (c): 2D and 3D interactions of Compound 35 with the active site amino acids of CA

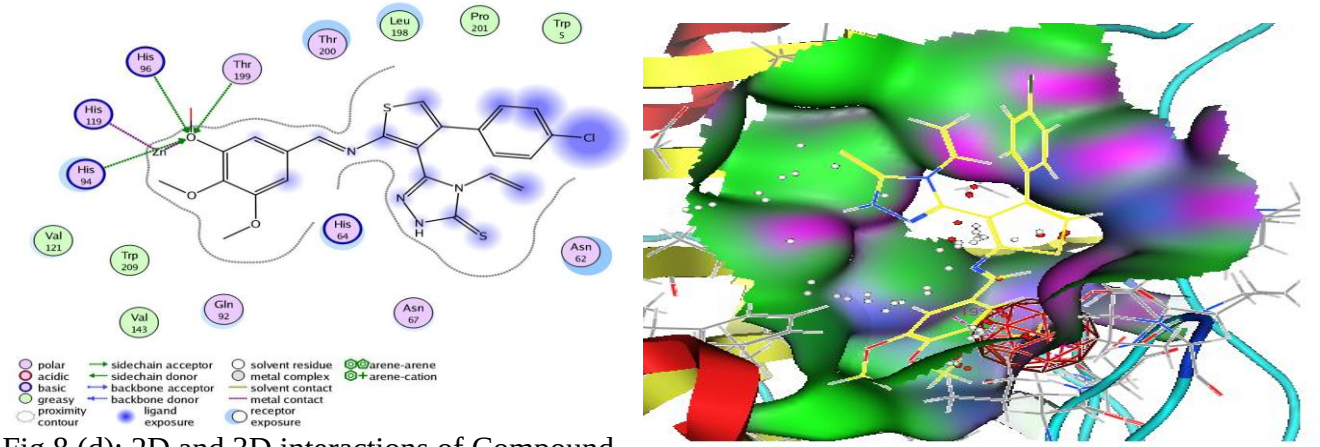


Fig 8 (d): 2D and 3D interactions of Compound 51 with the active site amino acids of CA

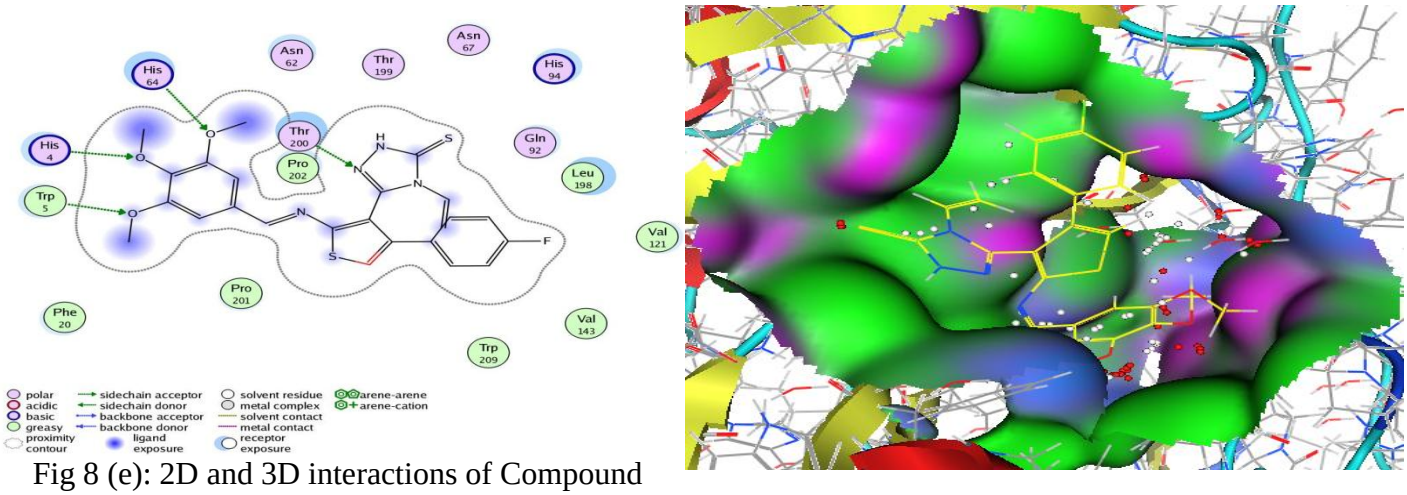


Fig 8 (e): 2D and 3D interactions of Compound 67 with the active site amino acids of CA

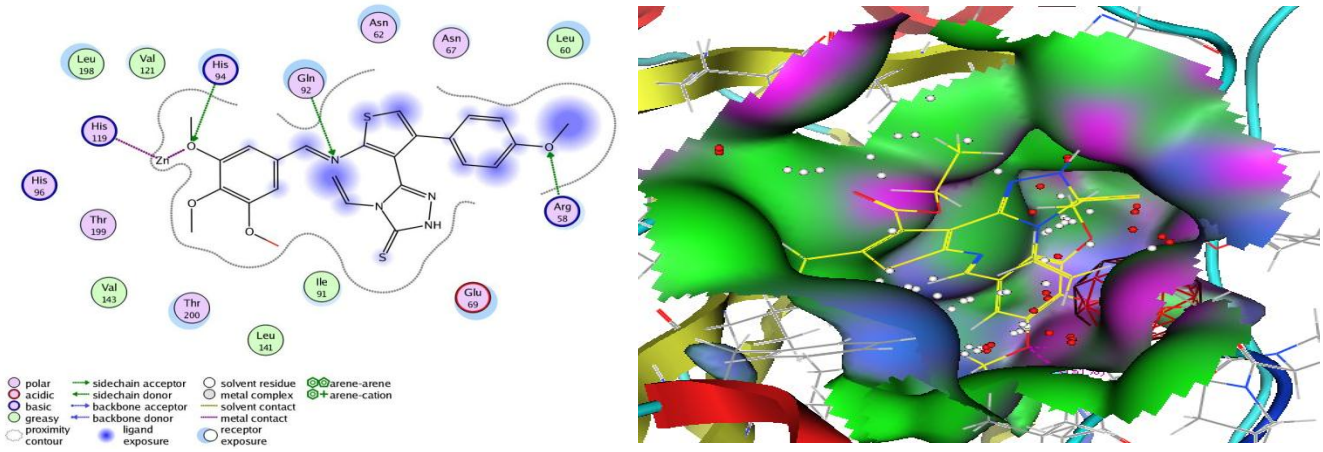


Fig 8 (f): 2D and 3D interactions of Compound 83 with the active site amino acids of CA

3.2. Drug likeliness Predictions

Drug likeliness predictions are helpful to envisage the drug like belongings of a compound to predict how "drug like" a compound is. In silico Drug likeliness prediction of designed analogues were projected using Swiss ADME predictor tool shown in **table 3**. Percentage absorption (% ABS) was planned by using method %ABS = 109- (0.345 X TPSA). The designed potent analogues displayed modest absorption in the range of 57.5-62.77. Results revealed that designed potent analogues showed no violation of Lipinski rule of five. These calculations revealed that the designed analogues may be utilized to develop drug like candidates.

Table 3. Drug likeliness parameters (Lipnski's rule of five)

Cd	**TPSA[a]**	**MW[b]**	**RoB[c]**	**HBD[d]**	**HBA[e]**	**IlogP (MlogP)[f]**	**logS[g]**	**% ABS[h]**
Rule	**≤140**	**>500**	**>10**	**>5**	**>10**	**<5**	**>-4**	-
67	133.99	496.58	8	1	6	2.64	-7.64	62.77
4	150.29	488.58	10	1	7	1.76	-7.04	57.15
19	133.99	456.58	7	1	5	2.05	-7.12	62.77
51	133.99	513.03	8	1	5	3.01	-8.19	62.77
35	133.99	478.59	8	1	5	2.54	-7.53	62.77
83	133.99	508.61	9	1	6	2.22	-7.70	62.77

Abbreviations:[a]Topological polar surface area; [b]Molecular weight; [c]Number of rotatable bonds; [d] Number of hydrogen bond donors; [e]Number of hydrogen bonds acceptors; [f] Logarithm of compound partition coefficient between n-octanol and water; [g]Logarithm of water solubility; [h]Percentage absorption

2.3 In silico ADME Predictions

Most of the drugs under clinical trials fail to reach the clinic due to their unfavourable pharmacokinetic profile. Initial screening before clinical trials will not only reduce the cost

but also reduces the risk of failure. Preliminary In silico ADME characteristics of best 6 compounds were calculated using preADME tool version 2.0 software (preadmet.bmdrc.kr) shown in **table 4**. As a part of drug designing such tools screen whether the designed molecules exhibit suitable ADME properties. All the calculated values were within the standard limits. Among the designed analogues the potent compounds (4,19,35,51,67,83) exhibit human intestinal absorption in the range of 97.47 to 98.90. Lower value of BBB from 0.24-0.90 indicate compounds cannot cross the blood brain barrier. Caco 2 with Value (47.49-53.60 nm/sec) greater than 25 nm/sec of the potent compounds is an indicator of greater intestinal absorption of drugs. The potent compounds with MDCK value lower than 0.06 nm indicate lower absorption towards kidney cells. Plasma protein binding (89.25-90.22) greater than 85 indicate distribution properties of potent compounds.

Table 4. ADME Characteristics of best 6 compounds

Cd	**HIA%**	**Caco-2(mm/sec)**	**MDCK**	**BBB (log PS)**	**Plasma protein binding**
	>80-100%	**47.32-54.33 nm/sec**	**0.06-0.61nm**		**>85**
67	98.09	53.60	0.043	0.75	89.31
4	97.97	47.49	0.043	0.24	89.25
19	98.91	53.36	0.043	0.47	90.22
51	97.47	53.36	0.043	0.90	89.49
35	98.10	53.23	0.043	0.67	89.54
83	98.71	53.25	0.43	0.61	89.83

2.4. Toxicity Prediction:

Toxicity prediction predict whether the designed analogues are safe. Safety profile of designed analogues has been predicted using PreADME and protoxweb based tools. As projected by protox Minimum lethal dose (LD50) of the designed referents were in the range of 500-1500 mg/kg. Predicted data revealed that the designed analogues are non-carcinogenic as revealed by their negative values on predicted models. Medium risk for hERG inhibition indicates that designed analogues have minimum risk on cardiac action potential given in **Table 5**. From the toxicity estimates it is evident that the premeditated analogues are safer for upcoming studies.

Table 5. Toxicity analysis of best 6 compounds

Cd	**Carcino-Mouse**	**Carcino-Rat**	**HERG-inhibition**	**Protox Predicted LD50**	**Protox Predicted Class**
4	Negative	Negative	Medium risk	1500 mg/kg	Class 4
19	Negative	Negative	Medium risk	1500 mg/kg	Class 4
35	Negative	Negative	Medium risk	1000 mg/kg	Class 4
51	Negative	Negative	Medium risk	500 mg/kg	Class 4
67	Negative	Negative	Medium risk	1000 mg/kg	Class 4
83	Negative	Negative	Medium risk	1000 mg/kg	Class 4

It was evaluated that among 96 designed compounds 6 compounds (4,19,35,51,67,83) showed best gold score and score was found to be more than the observed Gold score of internal ligand. Results revealed that designed compound are stabilized by hydrogen bonding, hydrophobic and some polar interactions interactions. The compound number 67 binding found most suitable with pocketof internal ligand and showed greater interactions with the amino acids as compared to internal ligand. Potent designed analogues also revealed good pharmacokinetic (ADME), drug like properties and moderate safety profile. Results revealed that Schiff base with 3,4,5-trimethoxy benzyl substation on 2^{nd} position and electron withdrawing substitution (4-Cl, 4-F) on 4^{th} position of thiophene gives best gold scores as compared to the compounds with electron donating substitutions (4-OCH_3) and fits well in the binding pocket of active site amino acids.

4. CONCLUSION

Current in silico study on the hypothesized 96 hit compounds were carried out to predict binding pattern of triazole derivatives to carbonic anhydrase enzyme. Gold scores and hydrogen bond interactions were predicted through molecular docking. Best 6 hit compounds were screened and further studied for ADME, drug-likeliness and toxicity characteristics. All the 6 hit compounds followed the Lipinski's rule of five and were found non-toxic as per the software predictions. Among these, hit compound no 67 was found best with Gold score 73.08 and % absorption 62.77.

The main goal of the current study was to predict new potential candidates as inhibitor of carbonic anhydrase enzyme as a mean to suppress the growth of cancer cells. Best hit compounds further synthesized and characterized by analytical techniques. It is anticipated that in the future research in vitro and in vivo testing is desirable to experimentally validate the carbonic anhydrase inhibitory activity of best hit compounds.

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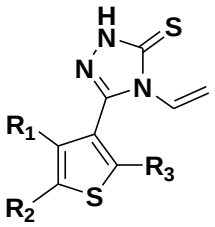

Substituted triazole derivatives (1-96)

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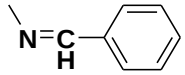
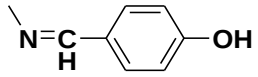
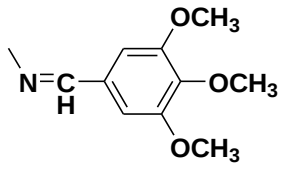
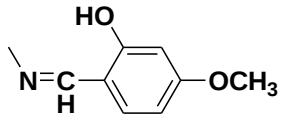
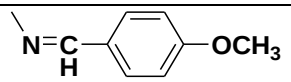
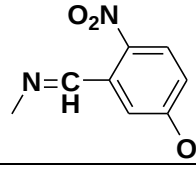
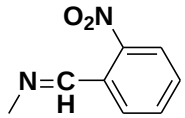
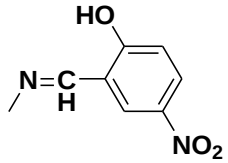
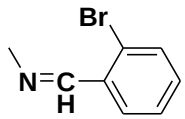
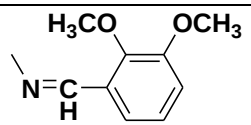
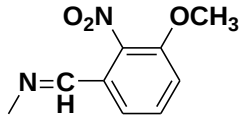
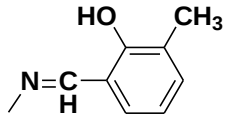
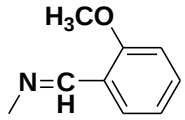
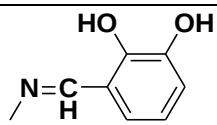
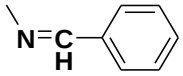
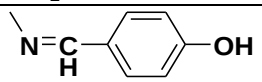
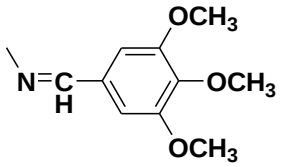
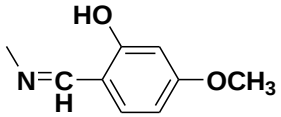
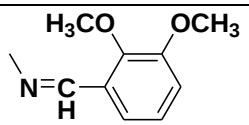
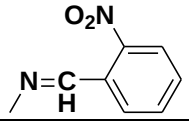
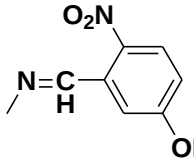
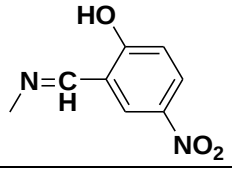
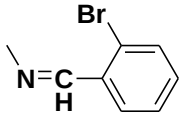
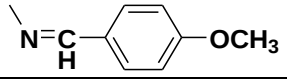
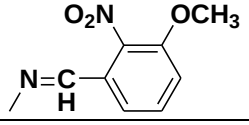
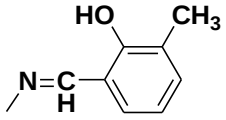
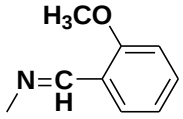
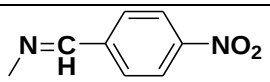
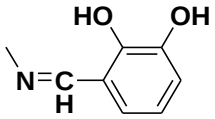
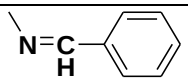
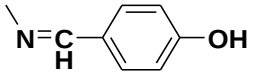
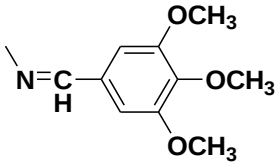
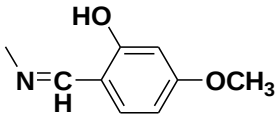
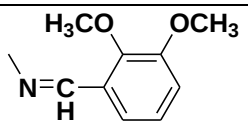
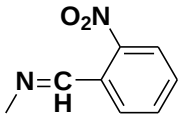
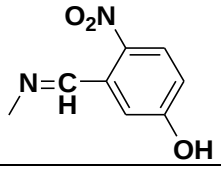
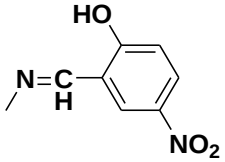
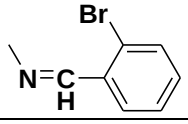
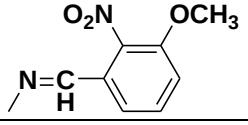
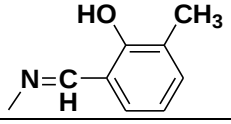
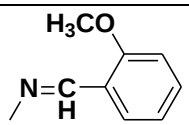
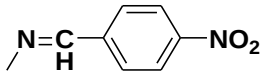
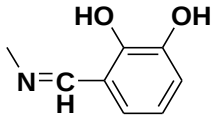
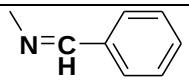
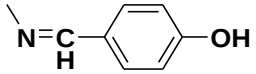
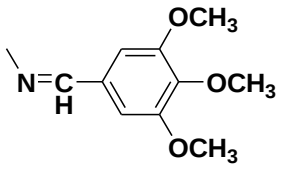
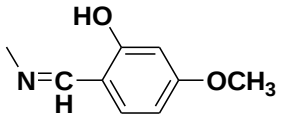
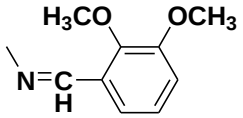
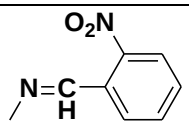
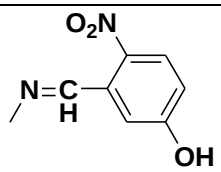
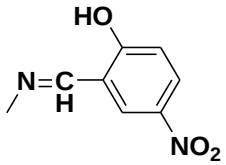
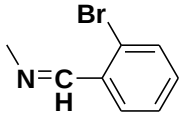
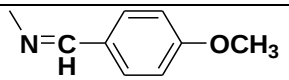
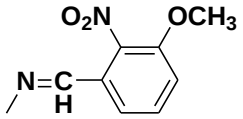
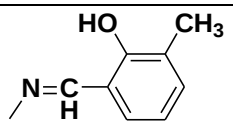
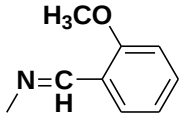
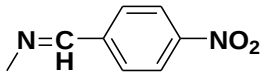
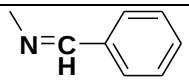
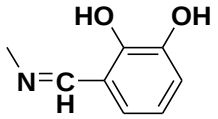
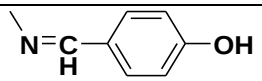
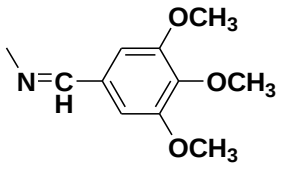
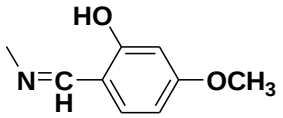
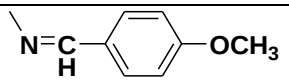
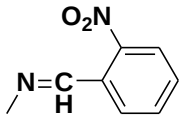
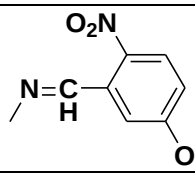
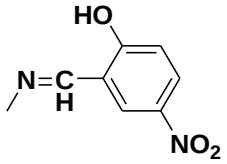
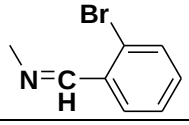
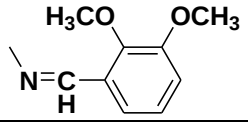
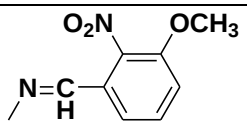
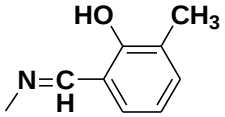
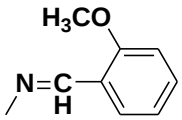
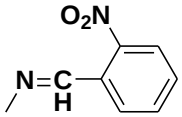
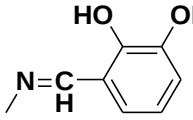
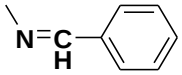
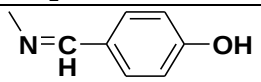
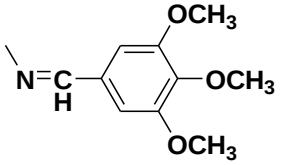
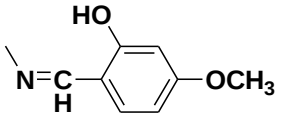
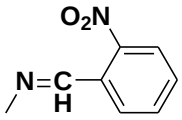
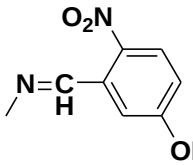
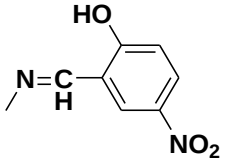
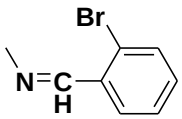
CD	R^1	R^2	R^3	Gold score (1XPZ)
1	$-OCOC_2H_5$	CH_3	NH_2	53.36
2	$-OCOC_2H_5$	CH_3		61.96
3	$-OCOC_2H_5$	CH_3		55.57
4	$-OCOC_2H_5$	CH_3		**71.58**
5	$-OCOC_2H_5$	CH_3		55.37
6	$-OCOC_2H_5$	CH_3		58.34
7	$-OCOC_2H_5$	CH_3		63.77
8	$-OCOC_2H_5$	CH_3		55.52
9	$-OCOC_2H_5$	CH_3		56.80
10	$-OCOC_2H_5$	CH_3		60.95
11	$-OCOC_2H_5$	CH_3		58.74
12	$-OCOC_2H_5$	CH_3		59.63
13	$-OCOC_2H_5$	CH_3		55.36
14	$-OCOC_2H_5$	CH_3		54.88

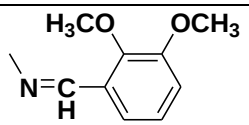
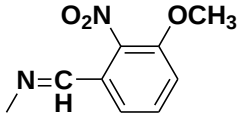
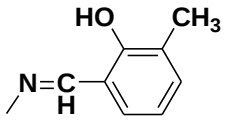
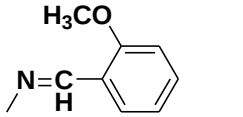
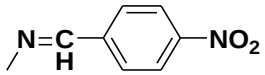
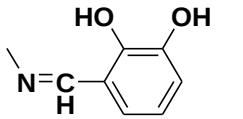
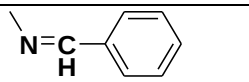
15	$-OCOC_2H_5$	CH_3		66.28
16	$-OCOC_2H_5$	CH_3		65.34
17	$R_1=R_2=(CH_2)_4$		NH_2	50.97
18	$R_1=R_2=(CH_2)_4$			51.51
19	$R_1=R_2=(CH_2)_4$			**69.83**
20	$R_1=R_2=(CH_2)_4$			64.80
21	$R_1=R_2=(CH_2)_4$			63.31
22	$R_1=R_2=(CH_2)_4$			56.37
23	$R_1=R_2=(CH_2)_4$			65.34
24	$R_1=R_2=(CH_2)_4$			64.93
25	$R_1=R_2=(CH_2)_4$			64.42
26	$R_1=R_2=(CH_2)_4$			60.88
27	$R_1=R_2=(CH_2)_4$			55.35
28	$R_1=R_2=(CH_2)_4$			54.05
29	$R_1=R_2=(CH_2)_4$			50.82

30	$R_1=R_2=(CH_2)_4$			54.22
31	$R_1=R_2=(CH_2)_4$			64.76
32	$R_1=R_2=(CH_2)_4$			49.89
33	C_6H_5	H	NH_2	45.59
34	C_6H_5	H		64.83
35	C_6H_5	H		**67.91**
36	C_6H_5	H		57.78
37	C_6H_5	H		58.84
38	C_6H_5	H		62.24
39	C_6H_5	H		59.10
40	C_6H_5	H		55.36
41	C_6H_5	H		56.26
42	C_6H_5	H		59.72
43	C_6H_5	H		55.81
44	C_6H_5	H		57.20

45	C_6H_5	H		58.80
46	C_6H_5	H		63.11
47	C_6H_5	H		61.17
48	C_6H_5	H		58.28
49	4-ClC_6H_5	H	NH_2	49.28
50	4ClC_6H_5	H		61.39
51	4ClC_6H_5	H		**69.23**
52	4ClC_6H_5	H		58.06
53	4ClC_6H_5	H		62.68
54	4ClC_6H_5	H		57.85
55	4ClC_6H_5	H		62.93
56	4ClC_6H_5	H		57.55
57	4ClC_6H_5	H		60.30
58	4ClC_6H_5	H		65.10
59	4ClC_6H_5	H		60.38

60	$4ClC_6H_5$	H		55.30
61	$4ClC_6H_5$	H		55.01
62	$4ClC_6H_5$	H		58.66
63	$4ClC_6H_5$	H		56.48
64	$4ClC_6H_5$	H		60.87
65	$4FC_6H_5$	H	NH_2	40.32
66	$4FC_6H_5$	H		56.91
67	$4FC_6H_5$	H		**73.08**
68	$4FC_6H_5$	H		60.34
69	$4FC_6H_5$	H		59.17
70	$4FC_6H_5$	H		54.60
71	$4FC_6H_5$	H		62.53
72	$4FC_6H_5$	H		57.68
73	$4FC_6H_5$	H		54.77
74	$4FC_6H_5$	H		64.99

75	$4FC_6H_5$	H		55.73
76	$4FC_6H_5$	H		54.62
77	$4FC_6H_5$	H		60.09
78	$4FC_6H_5$	H		62.48
79	$4FC_6H_5$	H		62.95
80	$4FC_6H_5$	H		58.99
81	$4OCH_3C_6H_5$	H	NH_2	59.46
82	$4OCH_3C_6H_5$	H		60.02
83	$4OCH_3C_6H_5$	H		**66.65**
84	$4OCH_3C_6H_5$	H		55.65
85	$4OCH_3C_6H_5$	H		57.55
86	$4OCH_3C_6H_5$	H		58.55
87	$4OCH_3C_6H_5$	H		63.30
88	$4OCH_3C_6H_5$	H		60.32
89	$4OCH_3C_6H_5$	H		56.36

90	$4OCH_3C_6H_5$	H		65.51
91	$4OCH_3C_6H_5$	H		57.91
92	$4OCH_3C_6H_5$	H		60.97
93	$4OCH_3C_6H_5$	H		60.75
94	$4OCH_3C_6H_5$	H		61.88
95	$4OCH_3C_6H_5$	H		59.96
96	$4OCH_3C_6H_5$	H		54.97
	Internal Ligand			58.01

Library of 96 Molecules with their gold score