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in silico Evaluation of 4-Amino-5-substituted-4*H*-1,2,4-triazole-3-thiol Derivatives against DNA Gyrase, COX-2 and Cathepsin B

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Biological activities of 1,2,4-triazoles, in particular, anticancer, anti-inflammatory and antimicrobial activities are potentiated by the presence of thiol group and free amino groups. Enticed by this, a series of 1,2,4-triazole derivatives were designed by introducing different substituent groups at 5th position of 4-amino-4*H*-1,2,4-triazole-3-thiol ring and their binding affinities were determined by molecular docking studies with the targets associated with bacterial infections, inflammation and cancer (DNA gyrase, cyclooxygenase-II (COX-2) and cathepsin B; PBD IDs: 1KZN, 3LN1 and 1SP4). Results revealed that all the compounds displayed good binding affinity towards the selected targets. The designed compounds showed relatively good affinity for cathepsin B and DNA gyrase enzymes when compared to COX-2. In comparison to aromatic groups, substitution with long aliphatic chains at 5th position significantly improved the binding properties of the compounds towards the targets. 1,2,4-Triazole ring was found to be crucial to form hydrogen bonding interactions with the active site amino acid residues. Stearyl and oleayl substituted derivatives (B_6 and B_7) exhibited superior binding properties and thus disclosing their pharmacological significance. Interestingly none of the compound showed affinity for permeability glycoprotein (P-gp), suggesting that their cellular uptake will be good in cancer cells.

Keywords: 1,2,4-Triazoles, Molecular docking, Cathepsin B, Cyclooxygenase-II, DNA gyrase subunit B.

INTRODUCTION

1,2,4-Triazole (pyrrodiazole) is a basic heterocyclic nucleus possessing broad range of biological activities. Good number of anti-inflammatory, analgesic, antifungal, anticonvulsant, antiviral, anticancer, antimicrobial, antimalarial, antidepressant, cardioprotective and antianxiety agents contain 1,2,4-triazole ring as pharmacophoric unit in their structure [1-3]. Being an important pharmacophore that can be used to produce novel functional drug candidates, triazole moiety also provides a very flexible and effective pathway to construct a wide variety of bioactive and therapeutically useful molecules. Broad spectrum of activity, low toxicity and attractive pharmacokinetic cum pharmacodynamic profiles made 1,2,4-triazoles as interesting and challenging candidates, attracted by medicinal chemists [4]. One of the interesting structural feature of 1,2,4-triazole ring is presence of three nitrogen atoms (electron rich), which can participate in a variety of bonded and non-bonded interactions with the active site of receptors *viz.*, hydrogen bonds, ion-dipole bonds and van der Waals interactions [5].

1,2,4-Triazole bearing compounds demonstrate potent anticancer activity by acting on various targets associated with tumor growth such as epithelial growth factor receptor, tumor necrosis factor TNFα, integrin avb3 receptor, cathepsin B *etc*. Anastrozole and letrozole are 1,2,4-triazole based drugs that are clinically recommended for the treatment of breast cancer, which act by aromatase inhibition [6,7]. 4-Amino 1,2,4-triazoles competitively inhibit cathepsin B and cathepsin H and their potency is altered based on nature and position of substituent groups on 1,2,4-triazole ring [8]. N-Bridged heterocyclic derivatives of 1,2,4-triazoles like 1,2,4-triazolo[3,4-b]thiadiazole and 1,3,4-oxadiazoles also possess good anticancer activities [9].

Good anti-inflammatory and analgesic activities have been reported for 3,5-substituted 1,2,4-triazoles. *Bis*-triazoles, benzoxazolinone-substituted triazoles and various substituted triazoles display cyclooxygenase-II inhibitory activity [6,10]. 1,2,4-Triazoles bearing a free thiol group at 5th position and 3,4-dimethoxy phenyl ring at 3rd position exerted anti-inflammatory activity with lower acute toxicity which is a common side effect

of NSAIDs [11]. It has been also reported that 1,2,4-triazole derivatives possessing free thiol group at 5th position disclosed antibacterial, anti-inflammatory and antitubercular activities [12]. The presence of free amino group at 4th position of 1,2,4-triazole nucleus may also impart greater inhibitory effect against one or more types of bacteria [13].

Presence of SH group in triazole moiety seems to improve the biological activities and antioxidant activity [14]. Thiol group (sulfhydryl group) acts as free radical scavenger and potent antioxidant agent, for example several natural thiols such as glutathione, N-acetylcysteine and homocysteine are potent antioxidant agents and also exhibit various important biological activities due to the presence of thiol group in their structures [15].

The survey of literature suggested that 1,2,4-triazole can function as an important scaffold by virtue of its hydrogen bonding ability. In this study, it was planned to design various 1,2,4-triazole derivatives by introducing different substituent groups at 5th position of 4-amino-4*H*-1,2,4-triazole-3-thiol ring. The basic skeleton namely, 4-amino-4*H*-1,2,4-triazole-3-thiol ring includes a free thiol and a free amino group which may impart a notable effect in hydrogen bond formation with target binding site. To explore their binding efficacy towards the targets such as DNA gyrase, COX-2 and cathepsin B, associated with antibacterial, anti-inflammatory and anticancer activities, molecular docking was performed.

EXPERIMENTAL

***in silico* Analysis of drug likeness and pharmacokinetic properties:** Drug likeness explains an integrated equilibrium between molecular properties and structural features that evaluate whether a compound is comparable to already existing drugs. Lipinski rule of five is commonly used to evaluate drug likeness properties of a compound. Molinspiration on-line property calculation tool is applied to determine Lipinski's molecular properties, number of rotatable bonds, together with topological polar surface area (TPSA) and molecular volume (a determinant of transport characteristics) and some other molecular descriptors (by http://www.molinspiration.com/cgi-bin/properties) [16-20].

Important pharmacokinetic properties such as gastro intestinal absorption, brain permeability *etc*. were calculated by using Swiss ADME web service (http://www.swissadme.ch/). Probable targets for the title compounds were predicted by Swiss target prediction analysis, using http://www.swiss target prediction.ch, a web server, based on the two and three dimensional measures matching with known ligands [21-23].

Molecular docking studies: Molecular docking studies were performed on a series of 3-amino-5-substituted-4*H*-1,2,4-triazole-3-thiol derivatives to predict their binding modes and interactions with target proteins, associated with the pharmacological potentiality of title compounds. Target proteins were obtained from the Protein Data Bank (PBD ID: 1KZN, DNA gyrase; PBD ID: 3LN1, COX-2; PBD ID: 1SP4, cathepsin B). Chemical structures of the title compounds were drawn using Chem and Bio Draw 12.0, a flexible tool and are saved as mol2 files using Chem and Bio 3D version12.0.

Molecular docking programme is an essential computational technique, useful to identify the best possible conformation of the ligand in a target protein that plays a major role in drug design. Swiss Dock, a computational programme was developed for carrying out molecular docking, fragment based drug design and lead optimization [24,25].

RESULTS AND DISCUSSION

***in silico* Analysis of drug likeness and pharmacokinetic properties:** The title compounds complied with Lipinski's rule of five, indicating good oral bioavailability (Table-1) and showed good bioactivity scores for kinase inhibitor (-0.28 to -1.22) and as enzyme inhibitors (-0.33 to -1.03). Swiss ADME results indicated good oral bioavailability and gastrointestinal absorption for all compounds except for B_8, B_{13} and B_{17} which showed low gastrointestinal absorption. 3,5-Dinitro substituted derivative (B_{17}) was resulted as a P-glycoprotein (Permeability glycoprotein) substrate and displayed relatively low GI absorption as per Swiss ADME that possesses drug likeness property [26].

TABLE-1
ANALYSIS OF DRUG LIKENESS AND BIOACTIVITY SCORES

Compound	R_1	Lipinski rule	GPCR ligand	Kinase inhibitor	Protease inhibitor	Enzyme inhibitor
B_1	C_6H_5	YES	-1.97	-1.22	-2.15	-1.03
B_2	4-ClC_6H_4	YES	-1.77	-1.09	-2.02	-0.97
B_3	C_6H_5-CH=CH	YES	-1.27	-0.67	-1.59	-0.80
B_4	2-Pyridyl	YES	-1.89	-0.98	-2.12	-0.93
B_5	2-OH, 5-NH_2-C_6H_3	YES	-1.71	-1.02	-1.92	-0.91
B_6	$C_{18}H_{36}O_2$ (stearyl)	YES	-0.48	-0.28	-0.54	-0.39
B_7	$C_{18}H_{34}O_2$ (oleayl)	YES	-0.43	-0.30	-0.54	-0.33
B_8	2-OH, 3,5-$(NO_2)_2C_6H_3$	YES	-1.19	-0.57	-1.27	-0.52
B_9	2-OH C_6H_5	YES	-1.76	-1.07	-1.96	-0.85
B_{10}	2,4-$(Cl)_2C_6H_4$	YES	-1.52	-0.93	-1.81	-0.89
B_{11}	4-$NO_2C_6H_5$	YES	-1.67	-1.02	-1.81	-0.89
B_{12}	4-$CH_3C_6H_5$	YES	-1.84	-1.15	-2.05	-1.02
B_{13}	4-Cl, 3-NO_2-C_6H_3	YES	-1.50	-0.88	-1.79	-0.84
B_{14}	3,5-$(OCH_3)_2C_6H_3$	YES	-1.37	-0.68	-1.57	-0.69
B_{15}	2-Cl C_6H_5	YES	-1.77	-1.09	-2.05	-0.95
B_{16}	4-$NH_2C_6H_5$	YES	-1.72	-0.92	-1.86	-0.80
B_{17}	3,5-$(NO_2)_2C_6H_4$	YES	-1.26	-0.67	-1.37	-0.66
B_{18}	4-$OCH_3C_6H_5$	YES	-1.66	-0.99	-1.85	-0.88

No single compound of the series displayed BBB penetration, showing that they are devoid of CNS side effects. Predicted pharmacokinetic properties (ADME) of title compounds suggested that they can act as orally active and potential drug candidates with a predicted high safety profile (Table-2). P-Glycoprotein is an ATP-binding cassette transporter that selectively pumps out drug molecules into gastrointestinal tract and reduces their therapeutic efficacy. It is highly expressed in cancer cells and if a drug molecule is a P-glycoprotein substrate then it will be expelled out of the cell, its absorption and bioavailability are significantly reduced. P-glycoprotein has potential role in cellular uptake of drugs and seriously limits drug effectiveness and in case of cancer, it is a major hurdle leading to multi resistant phenomenon. Theoretically it can be assumed that P-glycoprotein substrates are ineffective in cancer treatment [27,28]. The designed compounds do not have affinity for P-glycoprotein, so it can be predicted that cellular uptake will not be an issue for the compounds in the cancer cells.

Molecular docking studies: The designed compounds were docked within the crystal structure of targets and were tested for the binding energies and hydrogen bonding interactions with active site. Docking studies provide the information to find out the crucial residues at the active site of target. Ranking or grading of the compounds is based mainly on the binding modes with high binding energies and better number of hydrogen bonds. Output of all compounds was shown in energy terms in kcal/mol as shown in Table-3 and their interactions were predicted in Table-4.

TABLE-2
SWISS ADME AND SWISS TARGET PREDICTION DATA FOR TITLE COMPOUNDS

Compound	Oral bioavailability	BBB penetration	P-gp substrate	Drug likeness and bioavailability score	Target class
B_1	High	No	No	Yes (0.55)	Enzyme
B_2	High	No	No	Yes (0.55)	Membrane receptor
B_3	High	No	No	Yes (0.55)	Enzyme
B_4	High	No	No	Yes (0.55)	Membrane receptor
B_5	High	No	No	Yes (0.55)	Enzyme
B_6	High	No	No	Yes (0.55)	Enzyme
B_7	High	No	No	Yes (0.55)	Enzyme
B_8	Low	Low	No	Yes (0.55)	Enzyme
B_9	High	No	No	Yes (0.55)	Enzyme
B_{10}	High	No	No	Yes (0.55)	Enzyme
B_{11}	High	No	No	Yes (0.55)	Enzyme
B_{12}	High	No	No	Yes (0.55)	Enzyme
B_{13}	Low	Low	No	Yes (0.55)	Enzyme
B_{14}	High	No	No	Yes (0.55)	Enzyme
B_{15}	High	No	No	Yes (0.55)	Enzyme
B_{16}	High	No	No	Yes (0.55)	Kinase
B_{17}	low	Low	yes	Yes (0.55)	Enzyme
B_{18}	High	No	No	Yes (0.55)	Enzyme

TABLE-3
DOCKING SCORES OF TITLE COMPOUNDS OBTAINED FROM SWISS DOCK STUDIES

Compound	DNA gyrase subunit B		COX-2		Cathepsin B	
	ΔG (Kcal/mol)	Full fitness	ΔG (Kcal/mol)	Full fitness	ΔG (Kcal/mol)	Full fitness
B_1	-7.55	-1311.81	-7.55	-2257.16	-7.23	-1184.77
B_2	-7.69	-1312.84	-7.59	-2258.96	-7.33	-1186.57
B_3	-7.95	-1311.72	-8.11	-2259.11	-7.75	-1175.70
B_4	-7.60	-1312.65	-7.45	-2257.81	-7.55	-1190.81
B_5	-7.90	-1307.75	-7.61	-2253.39	-7.56	-1183.44
B_6	-9.09	-1368.66	-8.83	-2314.72	-9.11	-1240.77
B_7	-9.04	-1362.11	-8.88	-2307.14	-9.39	-1236.81
B_8	-8.28	-1281.27	-8.75	-2229.57	-8.10	-1162.13
B_9	-7.99	-1311.72	-7.55	-2262.58	-7.53	-1188.10
B_{10}	-8.04	-1315.91	-7.74	-2261.70	-7.50	-1192.64
B_{11}	-8.10	-1295.34	-7.87	-2238.14	-7.42	-1167.00
B_{12}	-8.00	-1315.15	-7.68	-2260.28	-7.28	-1185.71
B_{13}	-8.32	-1308.78	-8.12	-2247.35	-7.68	-1180.50
B_{14}	-7.95	-1297.65	-8.21	-2248.96	-7.66	-1173.70
B_{15}	-7.89	-1316.76	-7.84	-2266.83	-7.35	-1190.63
B_{16}	-7.99	-1320.72	-7.60	-2260.27	-7.29	-1194.10
B_{17}	-8.30	-1287.81	-8.45	-2229.78	-7.92	-1157.05
B_{18}	-8.02	-1312.98	-7.93	-2259.97	-7.39	-1184.14
Clorobiocin	-10.08	-1450.25	–	–	–	–
Celecoxib	–	–	-10.17	-2275.86	–	–
NS-134	–	–	–	–	-10.35	-1164.35

TABLE-4
DEPICTION OF INTERACTING AMINO ACIDS AND CORRESPONDING BOND LENGTHS BETWEEN THE LIGAND AND STUDIED TARGETS

Compound	DNA gyrase subunit B		COX-2		Cathepsin B	
	Interacting amino acids	Bond length	Interacting amino acids	Bond length	Interacting amino acids	Bond length
B_1	LIG 1H6-ASP 73 OD1	1.879	LIG 1H-GLU 222 O	2.498	LIG 1H-CYS 100 O	2.602
B_2	LIG 1H1-ASP 73 OD1	1.866	LIG 1H2-HEME 605 O	2.054	LIG 1 H1-GLU 122 O	2.018
B_3	LIG 1H7-ASP-73-OD1	1.895	LIG 1H6-HEME 605 O	2.101	LIG 1 H7-GLU 122 O	2.100
B_4	LIG 1H2-ASP-73 OD1	1.858	LIG 1H-GLU 222 O	2.176	LIG 1 H2-CYS 252 O	2.450
B_5	LIG 1H1-ASP-73-OD1	1.900	LIG 1H-THR 198 O	2.537	LIG 1 H2-HSD 111 N	2.239
B_6	LIG 1H-GLY 117 O	2.879	LIG 1H1-GLY 221 O	2.140	LIG 1 H2-GLU 122 O	1.977
	LIG 1H-GLU 42 O	1.968	LIG 1H2-ASP 215 O	1.921	LIG 1N1-TRP 221 H	2.378
	LIG 1H1-GLU 42 O	2.151			LIG 1H2-GLU 122 O	2.336
	LIG 1H1-ASN 46 O	2.541			LIG 1H-HSE 110 N	2.546
	LIG 1H-ASN 46 O	2.314			LIG 1H1-HSE 119 N	1.189
B_7	LIG 1H1-GLU 42 OE1	2.252	LIG 1H1-THR 198 O	2.107	LIG 1 H2-GLU 122	2.072
	LIG 1H1-GLN 72 O	2.403			LIG 1H2-GLU 122 O	2.155
	LIG 1H-GLU 58 O	2.742	LIG 1H-HEME 605 O	2.075	LIG 1H-HSE 110 N	2.132
	LIG 1H2-ASP 73 O	2.261	LIG 1H1-HEME 605 O	2.360	LIG 1N1-TRP 221 H	2.293
B_8	LIG 1 O3-ASP-49 O	3.409	LIG 1N1-GLN 275 H		LIG 1 H-HSE 110 N	2.096
B_9	LIG 1H-ASP 73 OD1	2.093	LIG1N1-GLN 275 H	2.225	LIG 1 H-GLY 198 O	2.026
B_{10}	LIG 1H-ASP 73 OD1	2.177	LIG 1H1-ASN 86 O	1.963	LIG 1H2-GLU 122 O	2.083
B_{11}	LIG 1H2-ASP 73 OD1	1.826	LIG 1N1-PHE 566 H	2.441	LIG 1 O-GLY 198 H	2.241
B_{12}	LIG 1H2-ASP 73 OD1	1.845	LIG 1H-GLU 222 O	2.361	LIG 1N-HSD 145 H	2.112
B_{13}	LIG 1H2-ASP 73 OD1	2.064	LIG 1O1-GLN 189 H	1.951	LIG 1 H2-GLU 122 O	2.141
B_{14}	LIG 1H1-ASP 73 OD1	1.885	LIG 1O-GLN 275 H	2.069	LIG 1 O1-ARG 101 H	2.534
B_{15}	LIG 1H2-ASP 73 O	1.881	LIG 1N1-GLN 275 H	2.402	LIG 1 N1-TRP 221 H	2.327
B_{16}	LIG 1H-ASP 73 OD1	2.076	LIG 1H8-ARG 29 O	2.386	LIG 1 H7-GLY 198 O	2.075
B_{17}	LIG 1H1-ASN 46 O	2.517	LIG 1O3-ALA 142 H	1.997	LIG 1 H-HSE 110 N	2.310
B_{18}	LIG 1H1-ASP 73 O	1.840	LIG 1H-PHE 196 O	2.417	LIG 1 H-HSE 45 O	1.991

Molecular docking studies of designed compounds with DNA gyrase subunit B (PDB ID: 1KZN): DNA gyrase is a type II topoisomerase, present in all bacteria and plays a vital role in the regulation of topological state of DNA. Relaxation of supercoiled DNA is important requirement for the bacterial transcription, replication and inhibition of DNA gyrase blocks this relaxation step. DNA gyrase is a specific, selective and interesting target for antibacterial agents such as widely studied coumarin antibiotics, *e.g*. clorobiocin [29].

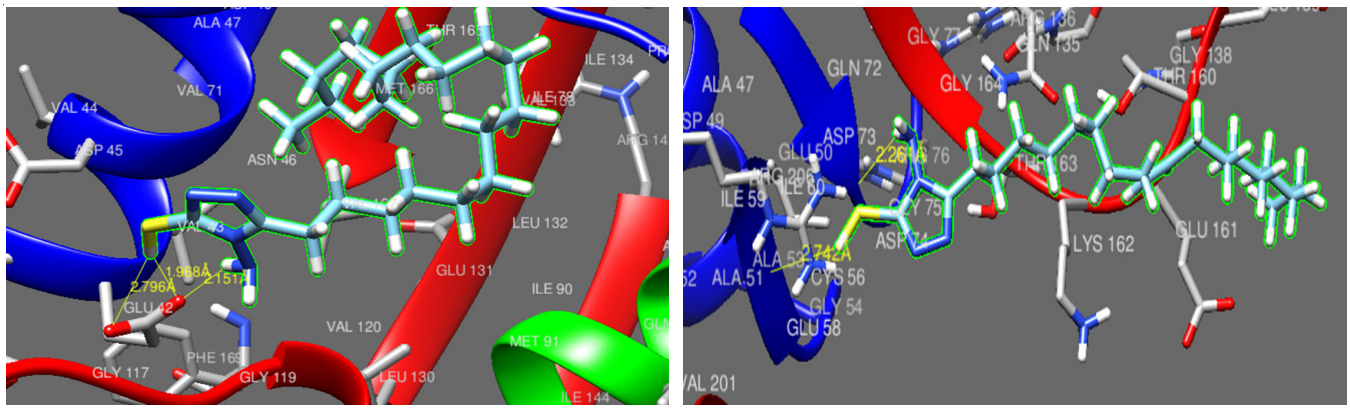
Molecular docking results showed that except few (B_1, B_2 and B_4), all the designed compounds displayed moderate to good binding affinity towards DNA gyrase (Table-3). It can be suggested that substituted phenyl ring is more interactive with the enzyme than the unsubstituted phenyl ring (B_1; ΔG= -7.55 kcal/mol), as the phenyl substituted derivatives showed improved affinity towards the active site. It was interesting to observe that when the phenyl ring is substituted with electron withdrawing groups, binding affinity was significantly improved. In case of 4-chloro-3-nitro derivative (B_{13}) and 3,5-dinitro derivative (B_{17}), ΔG scores were -8.32 and -8.30 kcal/mol, respectively, suggesting that they can interact well with the active site.

Binding modes of the title compounds were compared with the binding mode of coumarin class of drug, clorobiocin. Clorobiocin formed hydrogen bonding interactions with critical amino acids such as ASP 73, ASN 46, GLY 77 and ARG 136. All the compounds displayed effective binding affinity towards the target enzyme and interactions were noted with GLU 42, GLU 58, ASN 46, ASP 49, ASP 73, GLN 72and GLY 117. These observations indicated that designed compounds acquired similar binding pose as that of clorobiocin.

Among all the derivatives, stearyl derivative (B_6) exhibited the maximum binding affinity (-9.09 kcal/mol), followed by the oleayl derivative B_7 with good binding affinity -9.04 kcal/mol, which can be compared with the binding affinity of standard inhibitor, clorobiocin (ΔG = -10.08 kcal/mol) (Table-3). Binding modes of stearyl, oleayl substituted derivatives and the standard drug clorobiocin are shown in Figs. 1 and 2, respectively.

Highly interactive stearyl derivative (B_6) formed hydrogen bonds with GLU 42 and ASN 46. In the preferred binding pose, hydrogens attached to the free amino group substituted at 4th position of 1,2,4-triazole ring formed hydrogen bonds with oxygen of GLU 42 and ASN 46 with bond lengths of 2.151 Å and 2.541 Å, respectively and hydrogens attached to the free thiol group substituted at 3rd position of 1,2,4-triazole ring formed hydrogen bonds with oxygen of GLU 42 with bond lengths of 2.796 and 1.968 Å. Similarly for the oleayl derivative B_7 hydrogen bonds were found with GLU 58, GLN 72 and ASP 73. In this case, hydrogens attached to the free amino group substituted at 4th position of 1,2,4-triazole ring formed hydrogen bonds with oxygen of GLN 72 and ASP 73 with bond lengths of 2.403 Å and 2.261 Å, respectively and hydrogens attached to the free thiol group substituted at 3rd position of 1,2,4-triazole ring formed hydrogen bonds with oxygen of GLU 58 with bond length of 2.742 Å.

Lipophilic and unsaturated fatty acid substitution on 1,2,4-triazole scaffold has resulted in the potent compounds which can effectively bind with the DNA gyrase enzyme. With these results, it can be predicted that the active site of enzyme can accommodate bulky and lipophilic moieties.



(a) (b)

Fig. 1. Binding interactions of B_6 and B_7 with DNA gyrase subunit B (presentation in ribbon style)

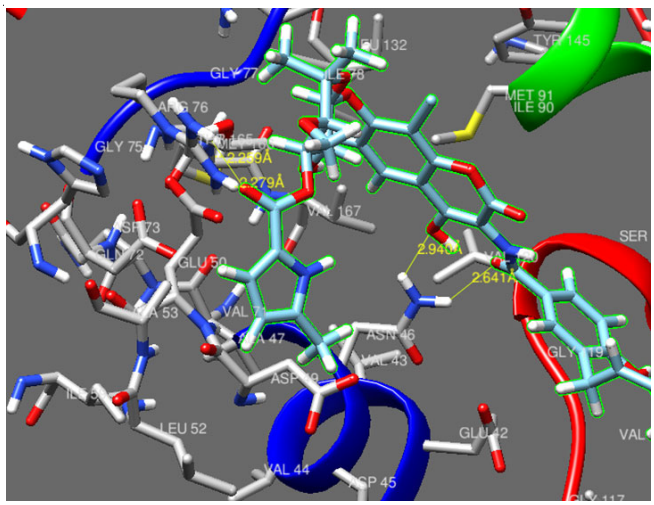
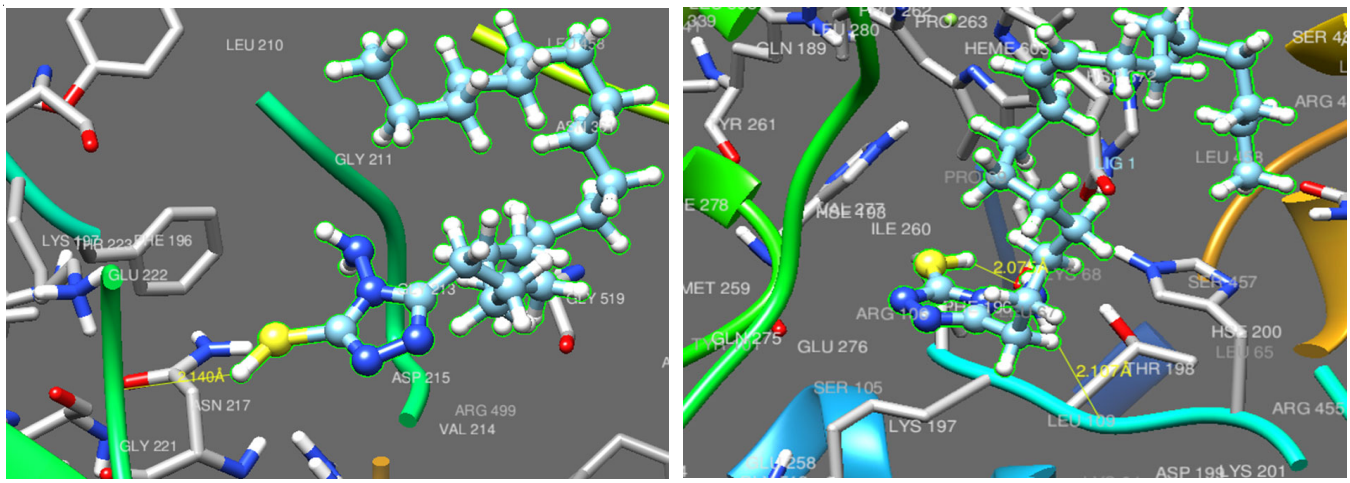


Fig. 2. Interactive ribbon representation of clorobiocin with DNA gyrase subunit B

Molecular docking studies of designed compounds with COX-2 (PBD ID: 3LN1): Cyclooxygenases (COXs) or prostaglandin endoperoxide synthases are important enzymes for the synthesis of prostaglandins which are the key mediators of pain, inflammation and hyperpyrexia. Out of two isoforms of COX enzymes, COX-2 is the specific one and it selectively involved in pain and inflammation and plays a grand role in prostaglandin biosynthesis in inflammatory cells and CNS. COX-2 inhibition has two advantages over COX-1 inhibition: very less gastric irritation and reduced risk of peptic ulceration. Celecoxib is the most effective COX-2 inhibitor [30].

Most of the designed compounds displayed moderate to good binding affinity towards the target enzyme. The highest binding affinity was observed with stearyl and oleayl derivatives (B_6 and B_7) with binding scores of -8.83 and -8.88 kcal/mol, respectively comparable to celecoxib (-10.17 kcal/mol), a good anti-inflammatory agent. B_6 and B_7 also showed good interactions with DNA gyrase enzyme. The influence of substitution on phenyl ring on binding interactions and ΔG was found similar even with COX-2 enzyme; all substituted phenyl derivatives showed improved affinity towards the target active site when compared to the unsubstituted phenyl ring (B_1; ΔG = -7.55 kcal/mol). It was noted that 2-hydroxy-3,5-dinitro derivative (B_8) displayed better affinity with ΔG of -8.75 kcal/mol and all other derivatives showed ΔG in the range -7.45 to -8.75 kcal/mol.

Binding modes of highly interactive stearyl, oleayl substituted derivatives (B_6 and B_7) and celecoxib are shown in Figs. 3 and 4, respectively. The designed compounds were found to interact with the active site through hydrogen bonds with ASN 19, ARG 139, THR 198, GLU 222, GLU 275, GLU 276, ASP



(a) (b)

Fig. 3. Binding interactions of B_6 and B_7 with COX-2 representation in ribbon mode

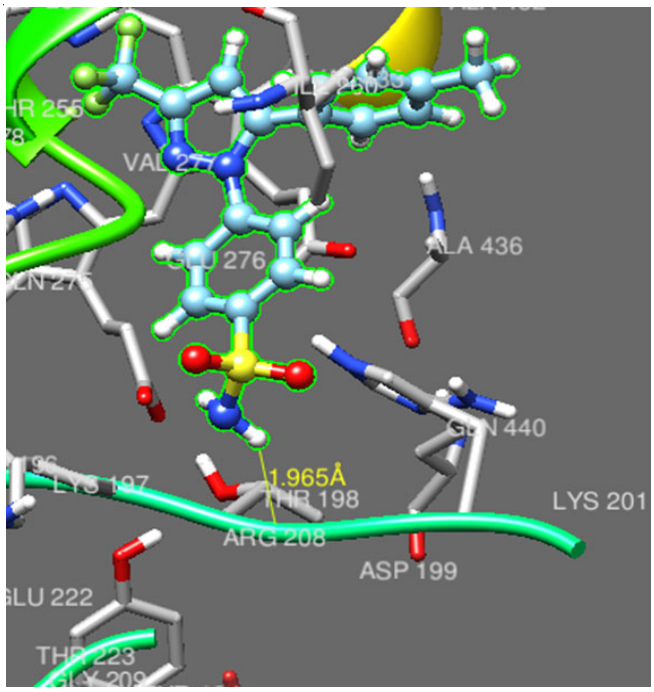


Fig. 4. Interactive ribbon representation of celecoxib with COX-2 interacting with THR 198, ASP 215

333, GLN 440, PHE 566 and HEME 605. Oleayl derivative (B_7) interacted with GLN 440 and HEME 605 and it was observed hydrogens attached to the free amino group substituted at 4th position of 1,2,4-triazole ring formed hydrogen bonds with oxygen of THR 198, hydrogens attached to the free thiol group substituted at 3rd position of 1,2,4-triazole ring formed hydrogen bonds with oxygen of HEME 605 and nitrogen at 2nd position of 1,2,4-triazole ring formed hydrogen bonds with hydrogen of GLN 275 with bond lengths of 2.107, 2.075 and 2.360 Å, respectively. Similarly the stearyl derivative (B_6) also participated in hydrogen bond by the hydrogens attached to the free amino group substituted at 4th position of 1,2,4-triazole ring and formed hydrogen bonds with oxygen of ASP 215. The hydrogens attached to the free thiol group substituted at 3rd position of 1,2,4-triazole ring formed hydrogen bonds with oxygen of GLY 221 within the distance 1.921 Å and 2.140 Å, respectively. Binding modes of stearyl, oleayl substituted derivatives and celecoxib as shown in Figs. 5 and 6, respectively.

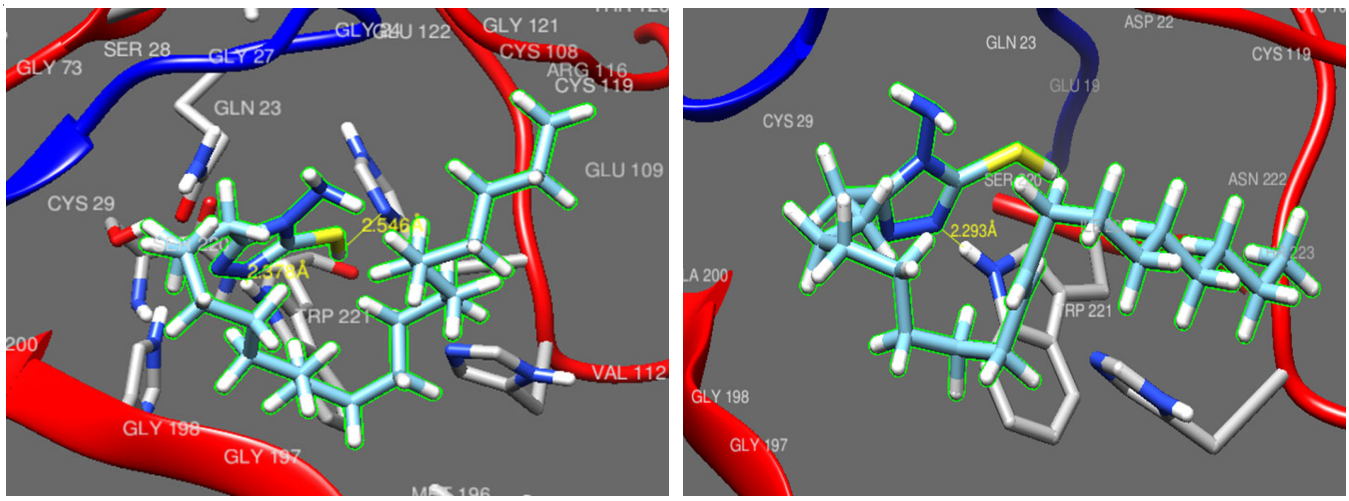
Molecular docking studies of designed ligands with cathepsin B (PBD ID: 1SP4): Cathepsin, a cysteine protease enzyme that belongs to papain super family. It is highly implicated in tumerigenesis and also acts as prognostic marker for several cancer types. Cysteine cathepsins have crucial role in metastasis, as bone is the most common site of distant metastasis in breast cancer patients, identifying cysteine cathepsins role in bone metastasis is a difficult task. Thus it was suggested that cathepsin B is an efficient therapeutic target for the management of breast cancer patients with metastatic disease. Selective cathepsin B inhibition is a significant way to reduce metastasis and also produces therapeutic potential [31]. Design of cathepsin B inhibitors is a challenging and difficult task, because of central and crucial role played by it in tumour progression and invasion [32].

Molecular docking studies were performed by retrieving the crystal structures of ligand and NS134 from Protein Data Bank (RCSB) (http://www.rcsb.org/pdb). Molecular docking studies showed that among all 18 designed compounds, higher binding affinity was observed with stearyl and oleayl derivatives (B_6 and B_7) with the scores -9.11 and -9.39 kcal/mol, respectively and that is comparable to NS-134 (-10.35 kcal/mol), a good anticancer agent (Table-3). Remaining compounds were moderately interactive with the binding scores in the range -7.23 to -8.10 kcal/mol. The designed compounds were found to form hydrogen bond interactions with GLU 122, HSE 110, HSD 111, CYS 119, HSD 145 and TRP 221 in the active site. The following interactions were observed for stearyl and oleayl derivative (B_6 and B_7):

i) Hydrogens attached to the free amino group substituted at 4th position of 1,2,4-triazole ring formed hydrogen bonds with oxygen of GLU 122 (bond length: 2.155 and 2.336 Å).

ii) Hydrogens attached to the free thiol group substituted at 3rd position of 1,2,4-triazole ring formed hydrogen bonds with nitrogen of HSE 110 (bond length: 2.132 and 2.546 Å).

iii) Nitrogen at 2nd position of 1,2,4-triazole ring formed hydrogen bonds with hydrogen of TRP 221(bond length: 2.293 and 2.378 Å) (Figs. 5 and 6).



(a) (b)

Fig. 5. Binding interactions of B_6 and B_7 with cathepsin B representation in ribbon mode

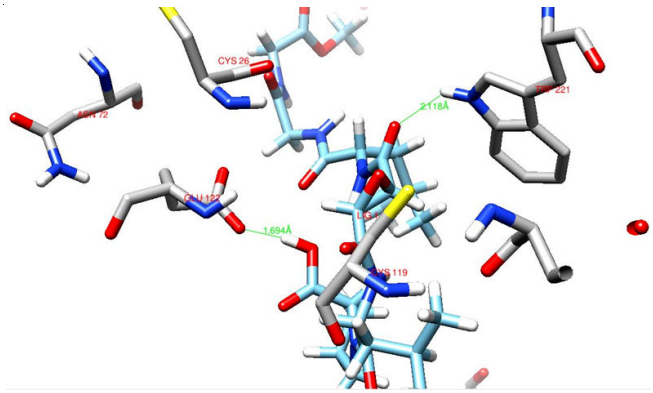


Fig. 6. Interactive representation of NS 134 with cathepsin B (PDB ID: 1SP4) with TRP 221 and GLU 122

In summary, designed compounds showed relatively good affinity for cathepsin B and DNA gyrase enzymes when compared to COX-2. The compounds were able to attain binding poses so as to interact with key residues of the selected targets. The most significant interactions that contributed to protein-ligand binding free energy include participation of free thiol and amino groups positioned at 3rd and 4th positions of 1,2,4-triazole ring. Substitution at 5th position significantly improved the affinity of the compounds for the active sites. Presence of phenyl ring substituted with electron withdrawing groups seems to be more favourable in this aspect. Styryl and pyridyl rings also enhanced the binding abilities of the molecules. Moreover, compounds B_6 and B_7 exhibited higher selectivity (comparable to the standard drugs) towards all the target enzymes as the non polar chains were well accommodated in the binding pockets. On the basis of the results produced from the *in silico* studies and docking, active compounds will be synthesized and evaluated for their therapeutic efficacy in the treatment of bacterial infections, inflammation and cancer.

Conclusion

Good binding affinities were observed by the title compounds towards the targets (DNA gyrase sub unit B, COX-2 and cathepsin B). The key scaffold, 4-amino-4*H*-1,2,4-triazole-3-thiol ring formed several hydrogen bonds with crucial amino acids of the selected targets. Interesting results were obtained when 5th position is substituted and it is evident that stearyl and oleayl group substitution (B_6 and B_7) is promising. This study also implies that the designed compounds have favourable pharmacokinetic properties and their cellular uptake is not affected by P-glycoprotein.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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