Identification of High-Affinity Cyclopentane[b]thiophene -3-carboxamide Derivatives as Potential Anticancer Compounds: Insights from Docking and ADME

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Keywords: Thiophene, Anticancer, Kinesin Spindle Protein, Docking, ADME.

Abstract

The new three series of 98 compounds were designed having cyclopentane[b]thiophene-3-carboxamide derivatives of benzoic acid (series A), cyclopentane[b]thiophene-3-carboxylate derivatives of benzaldehyde (series B) and cyclopentane[b]thiophene-3-carboxylate derivatives of acetophenone (series C). Among all the designed derivatives, twelve compounds showed a better binding affinity with the x-ray crystallographic structure of KSP for anticancer (PDB Code: 2PG2) as compared with Raloxifene as standard drug. The compound 4 has the highest docking score with -7.037. The ADME properties were also described in the top 12 compounds. These 12 compounds can be further synthesized in the future against the anticancer cell line.

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Introduction

Compounds with heteroatoms play a vital role in medicinal chemistry as well as in organic chemistry due to their profound therapeutic value. One of such heterocyclic rings i.e., thiophene Fig. 1, a five membered ring having sulfur in place of oxygen in a furan ring with a molecular formula C_4H_4S [1]. The molecular mass of thiophene is 84.14 g/mol with a mass to volume ratio 1.051 g/ml. Thiophene melts at -38 °C [2]. Derivatives of thiophene possess different biological activities such as anticancer [3], analgesic and antimicrobial [4], anti-inflammatory [5][6], antioxidant [7], and anticonvulsant [8], etc.

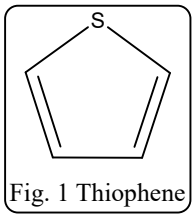


Fig. 1 Thiophene

Cancer is the second main leading cause of death in the world, after heart disease [9]. Globally, the most dominant form of cancer is lung cancer (about 11.6% of total cases), then breast cancer (11.6%), prostate cancer (7.1%), and colon cancer (6.1%) [10]. According to WHO, in 2018, 9.6 million deaths were estimated worldwide, or one in six deaths due to cancer [11]. In India, more than 1.1 million of 14 million new cancer cases were reported every year from all over the world [12]. Kinesin Spindle Protein (KSP) is also called Eg5. Microtubule-based motor protein, i.e., kinesin, belongs to the kinesin superfamily (KIF11) [13]. Kinesin is found in all proliferative tissue involved in human solid tumours, including breast, lung, ovarian, colon, and other cancers, as well as in leukaemia. Kinesin spindle protein inhibition causes cell death in proliferating tumour cells, but it has no effect in non-dividing cells; therefore, it may not induce peripheral neuropathy, which

is more prevalent with first-generation anti-mitotic medicines [14]. It controls the formation of the bipolar mitotic spindle. The spindle plays an essential role in mitosis and thus is a vital target for cancer therapy. This protein uses the energy produced by ATP hydrolysis to push microtubules, organelles, and vesicles. Inhibition of KSP prevents the formation of the bipolar spindle and leads to mitotic arrest and cell apoptosis [15].

Computer-Aided Drug Design

Drug discovery and development of a new medication is a lengthy, complicated, and expensive process. On average, drug discovery and development take 10-15 years and the US $500-800 million to establish a drug into the market, with synthesis and testing of lead analogs. Therefore, it is necessary to apply the computational method in drug discovery. Amid 5,000-10,000 screened compounds, around 250 compounds are selected for preclinical trials. Out of them, only five compounds enter into clinical trials, while the FDA approves only one [16]. There are two different approaches to CADD represented in Fig 2. (1) Structure-based drug design (SBDD) and (2) Ligand-based drug design (LBDD). SBDD depends on the detailed understanding of the third-dimensional structure of the protein that can be collected by X-ray diffraction, proton NMR spectroscopy, and comparative modeling [17]. Ligand-based techniques are developed using the known structures of ligands with proven therapeutic action. The main objectives of these approaches are to find out the bioactive molecules or to enhance the action of potential molecules. The QSAR method and pharmacophore modeling are the most prominent approaches for ligand-based drug discovery [18].

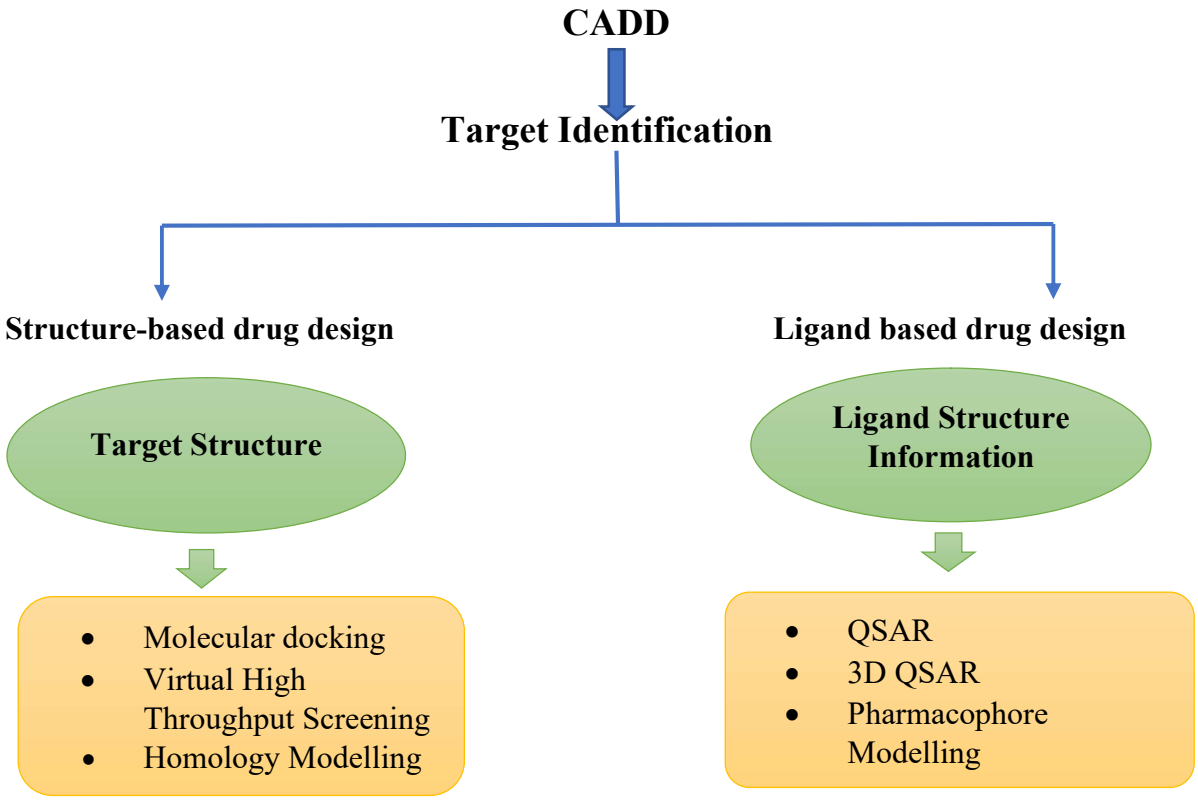


Fig. 2 Approaches to computer-aided drug design

Materials and methods

Ligand Preparation: The compounds were drawn by ChemDraw 20.1.1 and the energy was minimized using the MMF2 method with the help of Chem 3D software [19]. The compounds were optimized by LigPrep

(Schrodinger). In LigPrep, structures from 2D and 3D were converted, stereoisomers were made, counter ions were removed, the pH was ionized (7.0), defiant structures were removed, hydrogen atoms were added, and energy was minimized [20].

Protein Preparation: Several crystal structures are available in the protein data bank for KSP**;** for this work, we selected (PDB ID: 2PG2). 3D structure of receptor (KSP) (PDB ID: 2PG2) was retrieved from the web address (https://www.rcsb.org/) of the "protein data bank" for homo sapiens in PDB format. The protein was prepared using "protein preparation wizard" in Maestro 12.8 (Schrodinger suite). Protein preparation comprises the formation of hydrogen bonds, the assignment of bond orders, the creation of disulfide bridges, and the building of vacant cap terminals, loops, and branched chains, using Schrödinger Prime to model proteins residues [21]. The typical Optimized, Potential for Liquid Simulation (3 OPLS3e) field of force was used to decrease energy.

GRID generation: The receptor grid is a site on the receptor protein, which is more prone to binding with the ligand. The glide grid interface box in Schrödinger was used to create the receptors grid. The functional groups which are present at this site help to bind with the ligand [22].

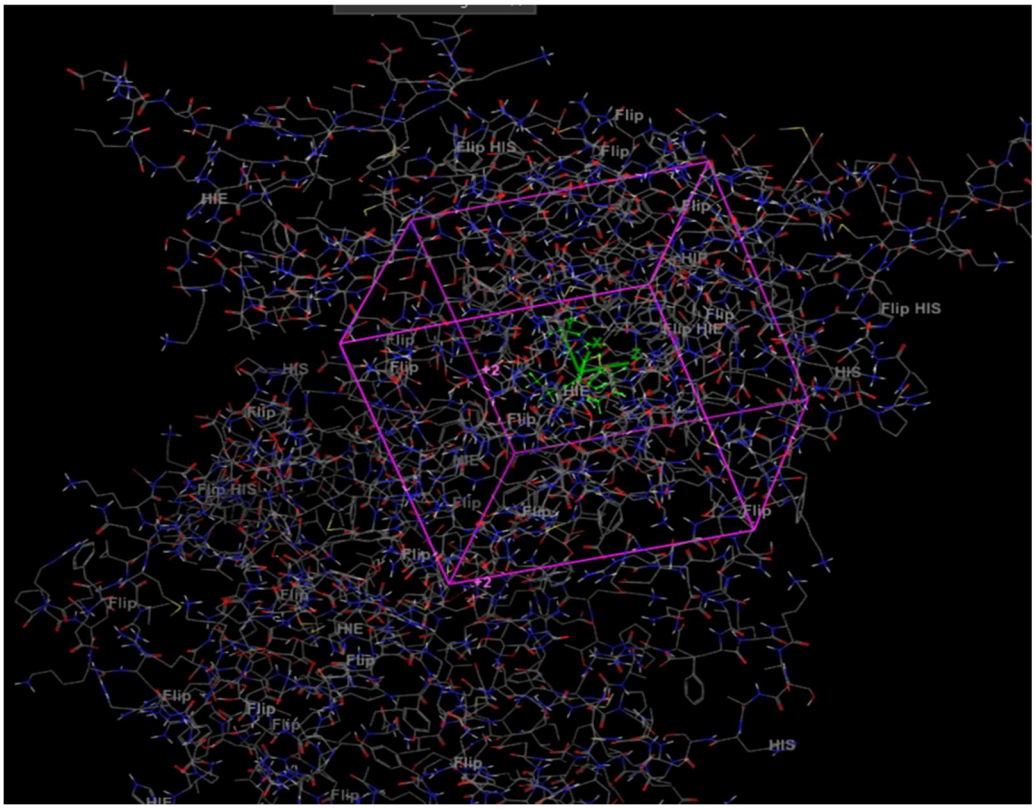


Fig 3 Grid generation

Molecular Docking method: The molecular docking studies were carried out utilizing an in-silico procedure. All compounds were subjected to molecular docking investigations using the molecular docking tool Glide v5.8 (Schrodinger suite 2021, LLC, New York) on the crystal structure of KSP with PDB ID: 2PG2. The X-ray crystal structure of KSP was retrieved from Protein Data Bank. The protein was prepared using "protein preparation wizard" in Maestro 12.8 (Schrodinger suite). The ligand was prepared using the LigPrep module to properly assign the types of atoms, the protonation states and the bond order. The glide grid interface box was used to create the receptors grid and docking was performed in

XP (extra precision) and a comparison was made on the basis of the docking score with the standard drug.

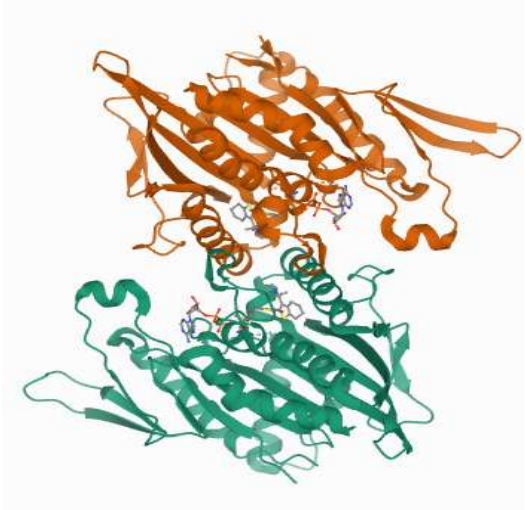


Fig. 4 2PG2 Crystal structure of KSP in complex with ADP and thiophene containing inhibitor 15

Ramachandran Plot

The Ramachandran plots have validated the protein structure. Based on how densely they are populated with data points obtained from the database of precisely designed protein structures, the various regions of the plot are clearly delineated. It displays the distribution of amino acids statistically. With the use of the Ramachandran plot, the protein molecule for the docking investigation was selected based on stability. According to Fig. 5, the majority of residues are found in the red area, also known as the allowed region. A modest number of residues are found in the forbidden regions of the all-anticancer protein. The identified anti-cancer protein molecules are structurally stable, as shown by the Ramachandran plot.

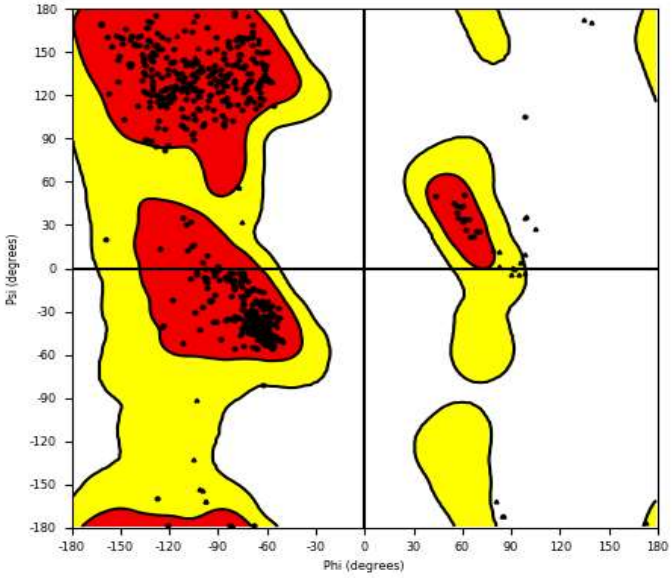


Fig. 5 Ramachandran plot of protein 2PG2

ADME Properties: The ADME characteristics of the drugs included in the study were determined. Several factors were calculated for appraising the substances from the SWISS-ADME database based on Lipinski's guidelines. The following are some of the parameters: H bond acceptor, H bond donor, Molecular weight, Log P, Number of rotatable bonds, and so on."

Results and Discussion

By using structure-based drug design, we have designed three series of 98 analogs and analyzed the influence of different groups in different positions on the inhibitor activity. Molecular docking studies were performed by using the Schrodinger suite Software. Table 1 contains the docking score of the designed compounds that shows the docking score more than the standard drug (Raloxifene) Fig. 6. Ninety-eight derivatives were docked in KSP. Twelve of the 98 derivatives exhibited superior binding affinity with the receptor, superposition, and docking scores. The docking study revealed that the docked molecules bind to the ligand binding site with moderate to good binding affinities. Furthermore, they fit into the active site pocket with moderate to good interactions with the amino acids, implying moderate to good biological activity as bioactive agents.

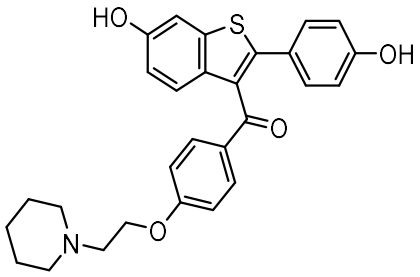
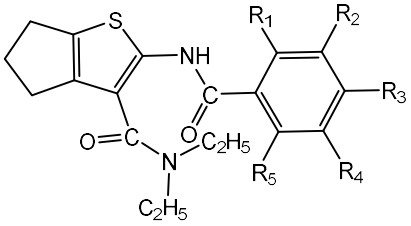


Fig. 6 Structure of Standard Drug (Raloxifene)

Table 1: Represents the docking score of designed ligands with the standard drug Raloxifene

Compound	Docking Score	Glide score	Glide energy
Compound 4	-7.037	-8.02	-47.775
Compound 11	-6.277	-6.765	-47.201
Compound 18	-6.492	-6.971	-48.891
Compound 20	-6.056	-6.977	-43.911
Compound 21	-6.216	-6.757	-47.158
Compound 25	-6.419	-6.927	-55.226
Compound 27	-6.676	-7.171	-40.353
Compound 35	-6.021	-7.126	-51.26
Compound 42	-6.298	-6.32	-42.971
Compound 58	-5.99	-6.015	-33.121
Compound 70	-6.096	-6.125	-32.4
Compound 88	-6.367	-6.389	-37.295
Raloxifene	-5.938	-5.941	-39.296

Designed ligands of Series A:



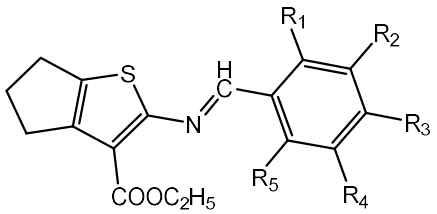
Benzoic acid Derivative

Table 2: Substitutions of benzoic acid derivatives

Compound	R_1	R_2	R_3	R_4	R_5
1	H	NH_2	H	H	H
2	H	H	NH_2	H	H
3	H	Cl	NH_2	Cl	H
4	**OH**	**H**	**H**	**NH_2**	**H**
5	H	OCH_3	H	H	H
6	H	H	OCH_3	H	H
7	OCH_3	H	H	H	H
8	H	H	H	H	H
9	H	H	C_6H_5	H	H
10	Br	H	H	H	H
11	**H**	**Br**	**H**	**H**	**H**
12	H	H	Br	H	H
13	Br	H	H	I	H
14	H	H	$C(CH_3)_3$	H	H
15	H	Cl	H	H	H
16	Cl	H	H	H	H
17	H	H	Cl	H	H
18	**Cl**	**H**	**NO_2**	**H**	**H**
19	Cl	H	H	NO_2	H
20	**H**	**NH_2**	**H**	**NH_2**	**H**
21	**Cl**	**H**	**Cl**	**H**	**H**
22	H	Cl	OH	Cl	H
23	OH	H	OH	H	H
24	H	OH	H	OH	H
25	**H**	**NO_2**	**H**	**NO_2**	**H**
26	NO_2	H	H	H	H
27	**H**	**H**	**OC_2H_5**	**H**	**H**
28	H	H	CHO	H	H
29	H	H	H	H	H
30	H	H	OH	H	H
31	H	OH	H	H	H
32	H	CH_3	OH	H	H
33	I	H	H	H	H
34	SH	H	H	H	H
35	**H**	**NO_2**	**H**	**H**	**H**
36	H	H	NO_2	H	H

37	H	OCH_3	NO_2	H	H
38	H	OCH_3	OH	OCH_3	H
39	I	I	H	I	H
40	H	OCH_3	OCH_3	H	H

Designed ligands of Series B:

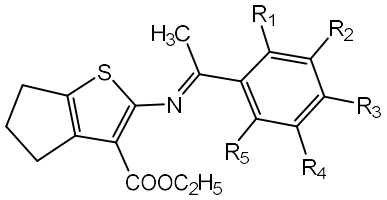


Benzaldehyde Derivative

Table 3: Substitutions of benzaldehyde derivatives

Compound	**R_1**	**R_2**	**R_3**	**R_4**	**R_5**
41	H	H	H	H	H
42	**H**	**H**	**$OCH_2C_6H_5$**	**H**	**H**
43	H	Br	H	H	H
44	H	H	Br	H	H
45	Cl	H	H	H	H
46	H	Cl	H	H	H
47	H	H	Cl	H	H
48	H	H	CN	H	H
49	Cl	H	Cl	H	H
50	H	Cl	Cl	H	H
51	F	H	H	H	F
52	H	OCH_3	OCH_3	H	H
53	H	OH	OH	H	H
54	H	H	$N(CH_3)_2$	H	H
55	H	H	OC_2H_5	H	H
56	H	H	F	H	H
57	OH	H	H	H	H
58	**H**	**OH**	**H**	**H**	**H**
59	H	H	OH	H	H
60	H	OH	OCH_3	H	H
61	H	H	OCH_3	H	H
62	CH_3	H	H	H	H
63	H	CH_3	H	H	H
64	H	H	CH_3	H	H
65	NO_2	H	H	H	H
66	H	NO_2	H	H	H
67	H	H	NO_2	H	H
68	H	H	C_5H_5N	H	H
79	H	OCH_3	OH	OCH_3	H
70	**H**	**OH**	**OH**	**OH**	**H**
71	H	OCH_3	OCH_3	OCH_3	H

Designed ligands of Series C:



Acetophenone Derivative

Table 4: Substitutions of acetophenone derivatives

Compound	R_1	R_2	R_3	R_4	R_5
72	H	H	H	H	H
73	H	H	NH_2	H	H
74	H	H	Cl	H	H
75	H	H	Br	H	H
76	Cl	H	Cl	H	H
77	Br	H	Cl	H	H
78	Br	H	OCH_3	H	H
79	H	Cl	Cl	H	H
80	H	Cl	H	H	H
81	OCH_3	H	OCH_3	H	H
82	OCH_3	H	H	OCH_3	H
83	H	OCH_3	OCH_3	H	H
84	OH	H	OH	H	H
85	OH	H	H	OH	H
86	OH	H	H	H	OH
87	OC_2H_5	H	H	H	H
88	**H**	**H**	**OC_2H_5**	**H**	**H**
89	H	H	F	H	H
90	OH	H	H	H	H
91	H	OH	H	H	H
92	H	H	OH	H	H
93	H	OCH_3	H	H	H
94	H	H	OCH_3	H	H
95	H	H	CH_3	H	H
96	H	NO_2	H	H	H
97	H	H	NO_2	H	H
98	$(Cl)_2$	H	Cl	H	H

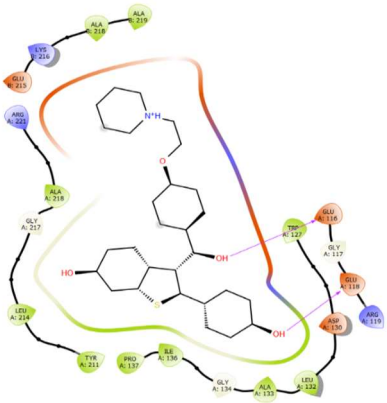
Ligand interaction diagram

Ligand interaction diagram represents the different interaction between the ligand and the receptor. There are 2 types of ligand interaction diagram (1) 2D ligand interaction diagram and (2) 3D ligand interaction diagram.

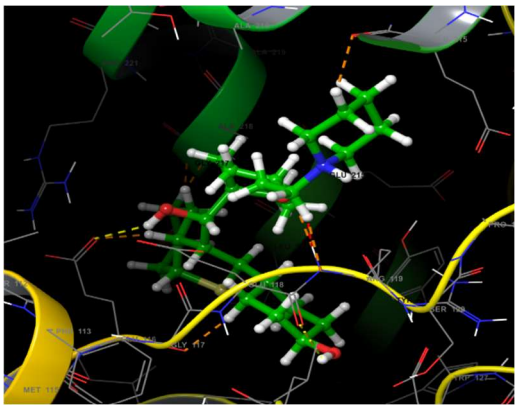
Ligand interaction diagram of reference compound

Raloxifene is a standard drug molecule that I was selected for my research work. Raloxifene interact with GLU 116, GLU 118 amino acid residue of receptor protein in order to produce biological activity.

Structure of Raloxifene



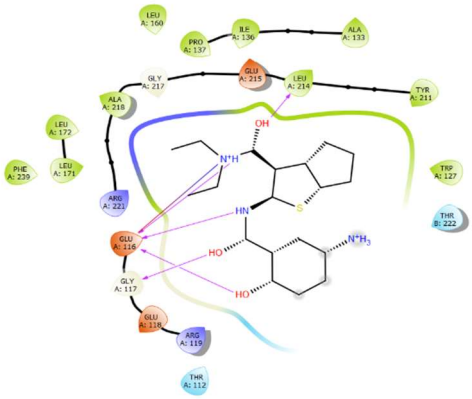
2D interaction diagram of Raloxifene



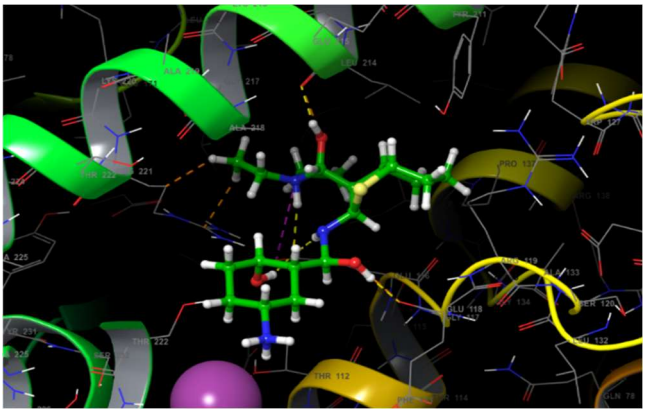
3D interaction diagram of Raloxifene

Ligand interaction diagram of compound 4

The compound 4 interacts with LEU 214, GLU 116, GLY 117 amino acids residue of receptor protein and form hydrogen bond between OH of second position and **GLU** 116 and this interaction is responsible for anticancer activity.



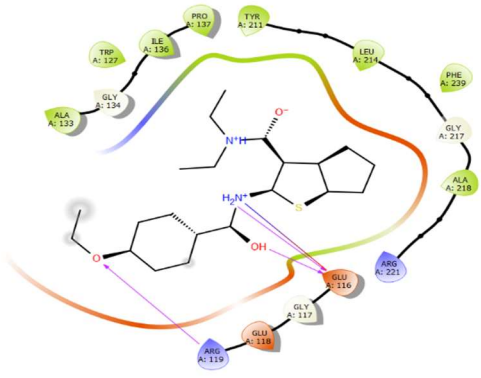
2 D ligand interaction diagram



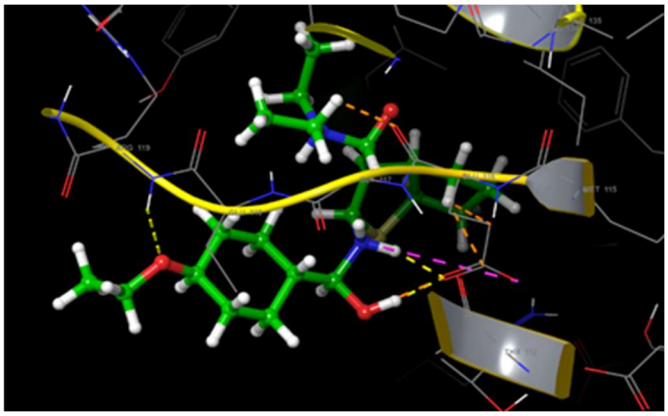
3 D ligand interaction diagram

Ligand interaction diagram of compound 27

The compound 27 interacts with GLU 116, ARG 119 amino acids residue of receptor protein**.**



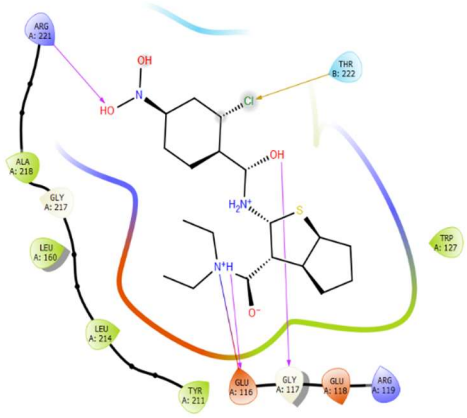
2D ligand interaction diagram



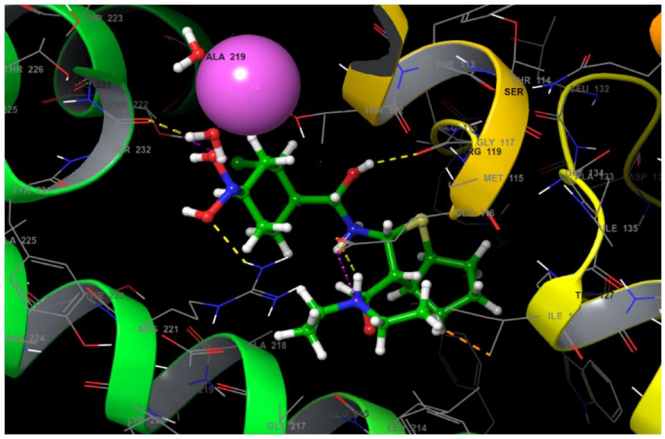
3D ligand interaction diagram

Ligand interaction diagram of compound 18

The compound 18 interacts with GLU 116, GLY 117, ARG 221, THR 222 amino acids residue of receptor protein



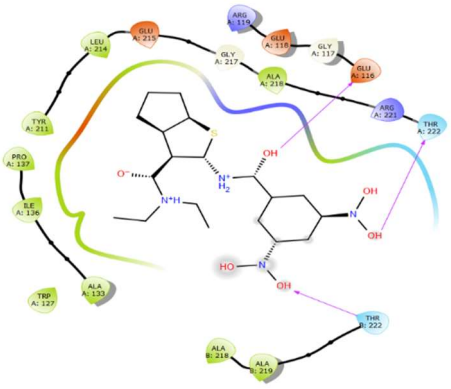
2D ligand interaction diagram



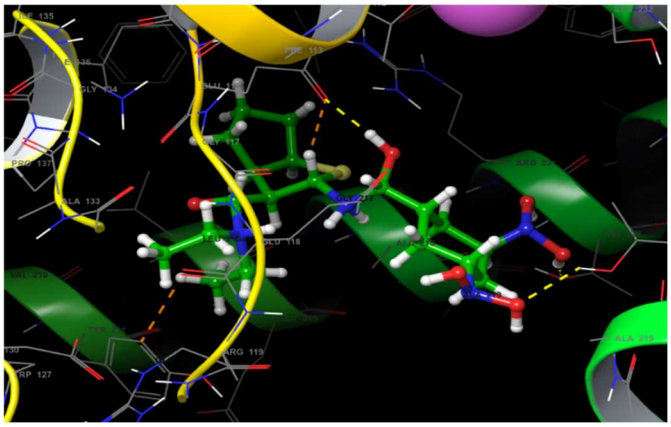
3D ligand interaction diagram

Ligand interaction diagram of compound 25

The compound 25 interacts with GLU 116, THR 222 amino acids residue of receptor protein



2D ligand interaction diagram

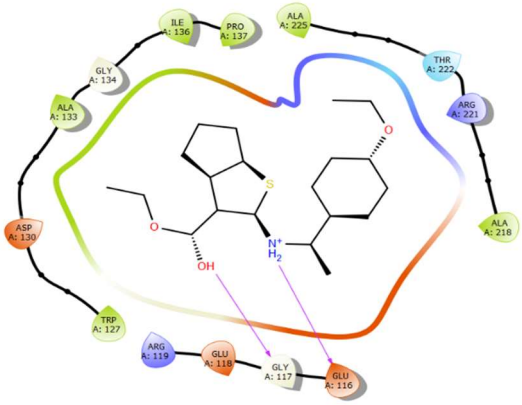


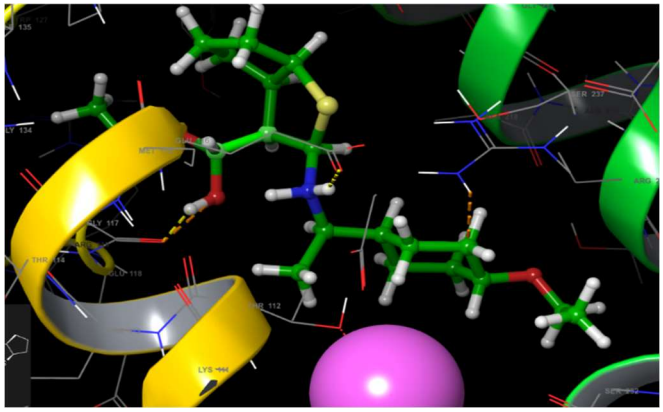
3D ligand interaction diagram

Ligand interaction diagram of compound 89

The compound 89 interacts with GLU 116, GLY117 amino acids residue of receptor protein

Structure of ligand 89





2D ligand interaction diagram **3D ligand interaction diagram of**

Table 5 Physicochemical, pharmacokinetics and drug-likeness

Comp. No.	Physicochemical properties				Pharmacokinetics			Drug likeness		Med. Chem.
	HBA	HBD	TPSA	Log Po/w	Log S	GIA	Log Kp	Lipinski	Vio	SA
4	3	3	123.9	2.92	-4.35	High	-5.94	Yes	0	3.47
11	2	1	77.65	4.38	-6.26	High	-5.4	Yes	0	3.44
18	4	1	123.47	3.71	-6.26	Low	-5.56	Yes	0	3.47
20	2	3	129.69	2.68	-5.11	High	-6.56	Yes	0	3.55
21	2	1	77.65	4.85	-6.85	High	-4.93	Yes	0	3.41
25	6	1	166.35	1.82	-4.43	Low	-6.96	Yes	0	4.7
27	3	1	86.88	4.1	-6.35	High	-5.43	Yes	0	3.59
35	4	1	120.53	2.59	-4.62	High	-6.57	Yes	0	4.58
42	3	1	75.8	4.53	-6.2	High	-4.28	Yes	0	3.73
58	4	1	87.13	3.73	-4.22	High	-5.53	Yes	0	3.49
70	6	3	127.59	2.93	-3.94	High	-6.23	Yes	0	3.57
88	4	0	76.13	4.69	-6.03	High	-5.14	Yes	0	3.76

HBA- Hydrogen Bond Acceptor, HBD- Hydrogen Bond Donor, TPSA- Topological Polar Surface Area, GIA- Gastrointestinal Absorption, SA- Synthetic Accessibility, Vio- Violence of Lipinski rule

Conclusion

In this study, three series of 98 derivatives of thiophene were designed by making different substitutions. The docking was performed on PDB ID: 2PG2 using Schrödinger software and it was concluded that benzoic acid derivatives show better binding affinity. Among all the designed derivatives, compounds 4, 11, 18, 20, 21, 25, 27, 35, 42, 58, 70 and 88 show the highest docking score than standard drug (Raloxifene) and which are mostly benzoic acid derivatives of thiophene. These derivatives bind to the protein and inhibit the KSP (Kinesin spindle protein), which prevents the formation of the bipolar spindle and leads to mitotic arrest and cell apoptosis. These twelve compounds can be further synthesized for in vitro experimental validation against anticancer.

References

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