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In silico molecular docking studies and MM/GBSA analysis of coumarin-carbonodithioate hybrid derivatives divulge the anticancer potential against breast cancer

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Abstract

Background: There are many biomarkers associated with breast cancer. Higher expression of PIK3CA (Phosphoinositide 3-kinase Cα), in its upregulated form, is associated with Hr^{+} and $Her2^{-}$ breast cancer; therefore, many drugs were synthesized against this protein to treat breast cancer patients. FDA recently approved that the drug alpelisib also inhibits PI3KCα (PDB ID-5DXT) in BC patients with Hr^{+} and $Her2^{-}$. In present study, we have exploited fourteen coumarin-carbonodithioate derivatives and alpelisib against this protein along with eighteen others which are responsible for causing BC through computational analysis. We have used Schrödinger Maestro 11.2 version for our in silico docking study, and to calculate relative binding energies of ligands, we used prime MM-GBSA module.

Result: Docking study revealed that among all fourteen compounds, 2f, 2a, 2d, and 2e showed the highest *G* score than the alpelisib and coumarin against PI3KCα with − 9.3, − 9.0, − 9.0 and − 9.1 kcal/mol respectively, along with individual *G* score of alpelisib (− 8.9) and coumarin (− 7.9). Prime MM-GBSA analysis gave the relative binding energies of alpelisib, 2f, and 2e with − 19.94864535, − 18.63076296 and − 13.07341286 kcal/mol sequentially.

Conclusion: This study provides an insight into the coumarin-carbonodithioate derivatives that could act as inhibitors of PI3KCα like alpelisib. Further prime MM-GBSA study revealed ligand binding energies and ligands strain energies.

Keywords: Coumarin-carbonodithioate, MM-GBSA, *G* score, Alpelisib, Binding energy, PIK3Cα, Breast cancer, 5DXT

1 Background

Breast cancer (BC) is a very common cancer worldwide in females and has overtaken cervical cancer in Indian women [17]. Many different pathways mainly mediate the BC aetiology [43]. There are many factors like lifestyle, family history, hormonal, genetic variation affecting BC, having said that, the one specific factor causing BC was out of reach [2]. The paramount challenges for researchers of BC are to understand the molecular changes in the genes associated with cell cycle progression, whose mutation or overexpression leads to BC [25]. Numerous biomarkers were presently used for the diagnosis of BC [5]. There are several known overexpressed genes associated with BC.

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Table 1 Different proteins upregulated in BC and their respective PDB IDs

Protein	PDB ID
MAP25K	2O2V
FGFR2	1GJO
CTLA-4	3OSK
MRE11	3T2f
BARD1 BRCT	2NTE
PALB2	2 W18
P53R2	3HF1
CDK5/P25	3IG7
CDK4	2 W96
AKT1	4EKL
HBRD4	4ZW1
MDM2	1RV1
PI3Kα	5DXT
BRCA2	2fYJ
BRCA1	4IGK
P7056K1	3A60
CDK8	3RGF
RAD51	1SZP

Table 2 Schrödinger Maestro Docking score (kcal/mol) of compounds against selected up regulated proteins in BC

PDB ID	2a	2b	2c	2d	2e	2f
2O2V	− 3.5	− 3.2	− 4.2	− 2.7	− 3.9	− 3.6
1GJO	− 4.5	− 4.9	− 4.5	− 4.2	− 4.8	− 3.8
3OSK	− 3.8	− 3.6	− 3.4	− 3.2	− 6.0	− 3.0
3T2f	− 4.2	− 4.3	− 4.7	− 4.1	− 4.3	− 4.2
2NTE	− 5.2	− 4.7	− 5.2	− 5.3	− 5.1	− 3.6
2 W18	− 4.5	− 5.1	− 4.9	− 6.2	− 5.0	− 3.8
3HF1	− 5.5	− 5.4	− 5.6	− 5.4	− 5.3	− 5.2
2 W96	− 3.8	− 4.5	− 4.5	− 4.5	− 4.0	− 4.8
4EKL	− 5.6	− 5.5	− 5.7	− 5.8	− 5.5	− 4.3
4ZW1	− 7.1	− 8.2	− 7.2	− 7.4	− 7.5	− 6.4
1RV1	− 4.4	− 4.8	− 3.7	− 4.3	− 4.3	− 4.5
2fYJ	− 4.3	− 3.9	− 4.2	− 4.1	− 3.6	− 3.1
4IGK	− 4.6	− 4.3	− 4.7	− 4.1	− 4.4	− 4.5
3A60	− 6.5	− 6.6	− 6.9	− 6.7	− 6.8	− 6.8
1SZP	− 3.1	− 3.3	− 3.2	− 3.5	− 3.2	− 3.5
5DXT	**− 9.0**	**− 8.8**	− 8.4	**− 9.0**	**− 9.1**	**− 9.3**
3IG7	− 7.3	− 7.7	**− 8.9**	− 7.2	− 8.2	− 7.8
3RGF	− 6.2	− 8.0	− 6.9	− 7.4	− 7.5	− 6.8

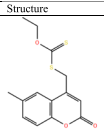
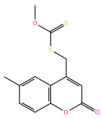
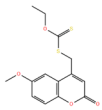
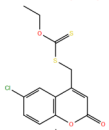
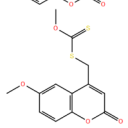
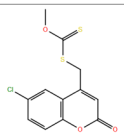
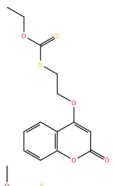
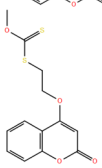
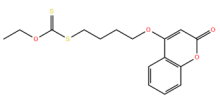
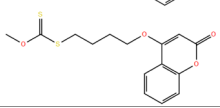
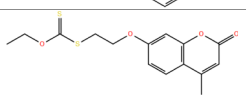
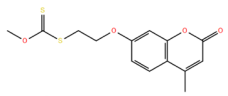
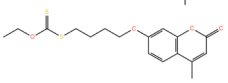
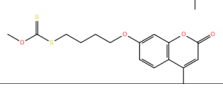
Phosphoinositide 3-kinase is classified under the lipid kinase family mainly involved to regulate some biologically important functions like cell proliferation, differentiation, migration, etc. [40]. There are three classes of PI3K in which class I (PI3Kα, ß, γ, and δ) express drastically the overexpression of PI3Kα which is seen in BC patients [31, 34]. In recent years, inhibitors of PI3Kα were tested clinically in patients with advanced BC malignancies which unveiled differential effectiveness [16]. Almost more than 70% of BCs are hormone receptor (HR^+) and ($HER2^-$) human epidermal growth factor receptor 2 [14, 38]. The higher mutation rate in PI3KCα is seen in most of the HR^+ and $HER2^-$ and has a high survival rate among BC patients with PIK3CA-mutated patients. Lately, alpelisib had received Food and Drug Administration approval for treating postmenopausal women and men with metastatic BC whose tumours were having a mutation with the PIK3CA gene [41].

Cyclin-dependent kinase 5 (CDK-5) is a Ser/Thr kinase, a regulator protein in the cell cycle that phosphorylates and activates the ATM pathway in a double standard DNA repair pathway [36]. The microarray expression study showed significant observation of the upregulation of CDK-5 in BC, colon cancer, lung, bladder, ovarian cancer, etc. In contrast, oesophageal and blood cancer exhibited downregulation of CDK5 expression [21]. The MCF7 cell lines compared to normal cell lines were correlated with the association of CDK-5 overexpression [42].

Several studies reported that compounds like purine, pyramidine [3], and coumarin derivatives exhibit anticancer activity [13]. The plant-derived natural product coumarin derivatives play a vital role in medicinal chemistry with a wide range of pharmacological activities including anticancer effects. However, the molecular mechanism of coumarin compounds exerting their anticancer effects remained unknown [44]. Alkyldithiocarbonate ligands and also with few metal complexes have also appreciable attention for their interactions and broad range of applications. Dithiocarbamate and dithiocarbonate sulphur containing ligands have been used extensively in agriculture as well as in medicinal chemistry [4].

Hence, the coumarin-carbonodithioate derivative compounds (A to F) have been utilized as the secondary data from the earlier reported study [19]. Remaining (E to N) compounds identified for their antifungal and antibacterial activity in previously reported synthesis and molecular docking studies of potent coumarin–carbonodithioate hybrids [27]. Our study analysis brought about an understanding of the anticancer potential of these compounds through in silico studies and comparing both sets of ligands with standard coumarin and alpelisib. Therefore, present investigation obtained

Table 3 Coumarin-carbonodithioate derivatives with their chemical name and structure respectively

Ligand Chemical Name	Structure
O-ethyl S-(6-methyl-2-oxo-2e-chromen-4-yl)methylcarbonodithioate	
O-methyl S-(6-methyl-2-oxo-2e-chromen-4-yl)methylcarbonodithioate	
O-ethyl S-(6-methoxy-2-oxo-2e-chromen-4-yl)methylcarbonodithioate	
S-(6-chloro-2-oxo-2e-chromen-4-yl)methyl O-ethyl carbonodithioate	
S-(6-methoxy-2-oxo-2e-chromen-4-yl)methylOmethylcarbonodithioate	
S-(6-chloro-2-oxo-2e-chromen-4-yl)methyl O-methyl carbonodithioate	
S-2-(2-oxo-2e-chromen-4-yloxy)ethyl O-ethyl carbonodithioate	
S-2-(2-oxo-2e-chromen-4-yloxy)ethyl O-methyl carbonodithioate	
S-4-(2-oxo-2e-chromen-4-yloxy)butyl O-ethyl carbonodithioate	
S-4-(2-oxo-2e-chromen-4-yloxy)butyl O-methyl carbonodithioate	
S-2-(4-methyl-2-oxo-2e-chromen-7-yloxy)ethyl O-ethyl carbonodithioate	
S-2-(4-methyl-2-oxo-2e-chromen-7-yloxy)ethyl O-methyl carbonodithioate	
S-4-(4-methyl-2-oxo-2e-chromen-7-yloxy)butyl O-ethyl carbonodithioate	
S-4-(4-methyl-2-oxo-2e-chromen-7-yloxy)butyl O-methyl carbonodithioate	

14 different coumarin-carbonodithioate derivatives from the literature survey [16]. Docking analysis of 14 derivatives was carried out against 18 BC upregulated proteins. Furthermore, the selection of PI3KCα (PDB ID-5DXT) protein out of 18 proteins leads to a docking investigation of all 14 ligands. This protein was also docked with coumarin and with its known inhibitor to compare the dock scores with our compounds.

In silico molecular docking studies lead to innovation in synthesizing novel drugs as this study requires less time and can be performed with a large number of ligands and easy to compare between the ligand scores [7, 29]. The in silico studies also bring about the understanding of the solvation effect, molecular dynamic simulation, and molecular electrostatic potential studies with the interpretation of behaviour of the compounds in different solutions [1, 28]. In silico computational screening plays a vital role in the discovery of drugs that have success stories [33, 37].

Objectives of the work are, to study the effect of coumarin-carbonodithioate derivatives on breast cancer overexpressed proteins through the computational molecular docking method. To calculate the ligand binding energies and ligand strain energies for a given set of ligands 2a, 2f, 2c, 2d, 2 h, and 2i against a single receptor PI3KCα. And also to predict the relative binding free energies and energy properties of individual ligand, receptor, and complex structures that contribute to total binding energies using the prime MM-GBSA method.

2 Methods

2.1 Selection of proteins for docking

Literature study shows that the following proteins are in close association with BC, like FGFR2 [20], CTLA4 [10], MREII [18], BRAD1 [15], PALB2 [22], p53R2 [23], CDK 8 [6], CDK 4/6 [32], AKT1 [35], BRD4 [42], MDM2 [12], BRCA 1 and 2 [9], and RAD51 [24]. The 3D structure proteins and their respective protein data bank (PDB) Ids are given in Table 1. All the mentioned proteins were retrieved from PDB and subjected to the protein pre-process module of Schrödinger Maestro 11.2 version.

2.2 Preparation of proteins for docking

All 18 proteins used for docking were prepared using protein preparation wizard [26]. The preparation involved the assignment of the hydrogen bonds, bond orders, addition of hydrogens, optimization, minimization of the proteins, and deletion of waters beyond 5 from the het group. Determination of highly potential binding sites of ligands on proteins was carried out using SiteMap tool analysis [11]. Using Glide application protein receptor grid was generated (assignment of ligand binding site for docking). Additionally, docking of all ligands

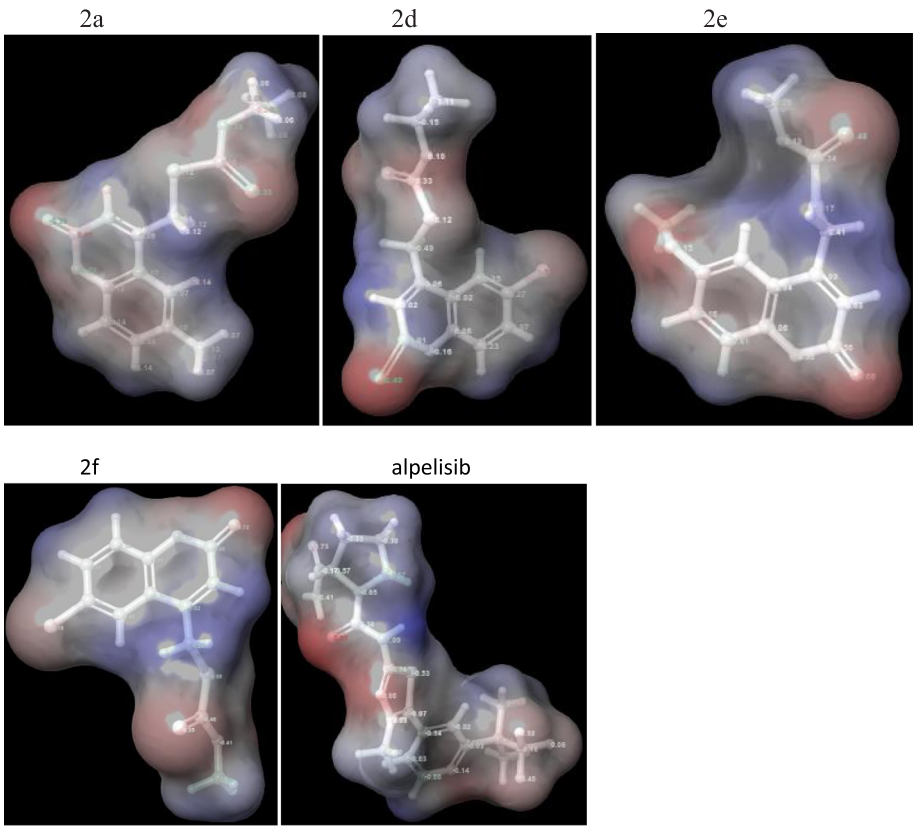


Fig. 1 Electrostatic potential value plotted on the surface of 2a, 2d, 2e, 2f, and alpelisib respectively using electro-static potential fitting charge (ESP) atomic charges by OPLS2005 force field (electropositive charge, blue; electronegative charge, red; neutral, white)

were carried out using glide's ligand docking module. Extra-precision (XP-visualizer module) was used for the visualization of glide score (*G* score). Docking results of 2a to 2f with all 18 proteins are briefly given in Table 2.

2.3 Preparation of ligands

The 3D structures of all 14 ligands are prepared using Schrödinger Maestro software. Minimization of all ligands was carried out using the OPLS-2005 force field module [8]. All ligand structures and their respective chemical names are stated in Table 3. The electrostatic potential values plotted on the surface of ligands using electro-static potential fitting charge (ESP) atomic charges by OPLS2005 force field are given in Fig. 1.

2.4 Prime MM-GBSA

The prime MMGBSA method (Prime Version 4.8) exhibited the relative binding-free energy (ΔG bind) of each ligand molecule, and results are given in Table 4.

Formula expanded is given below:

$\Delta G(\text{bind}) = \Delta G(\text{solv}) + \Delta E(\text{MM}) + \Delta G(\text{SA})$

where:

- Δ*G*solv is the difference in GBSA solvation energy of the PIK3CA-inhibitor complex and the sum of the solvation energies for unliganded PIK3CA and inhibitor.
- Δ*E*MM is a difference in the minimized energies between PIK3CA-inhibitor complex and the sum of

Table 4 The relative binding-free energies (kcal/mol) obtained by Prime MM–GBSA, where MMGBSA dG Bind = Complex − Receptor − Ligand and MMGBSA dG Bind(NS) = Complex − Receptor(from optimized complex) − Ligand(from optimized complex) = MMGBSA dG Bind − Receptor Strain − Ligand Strain. NS in the table is no strain; it is the binding energy without considering for the receptor and ligand conformational changes needed for the formation of complex

Compound	MMGBSA-dG-binding energy	MMGBSA-dG-bind in Coulomb	MMGBSA-dG-bind(NS)	MMGBSA-dG bind(NS)-Coulomb
Alpelisib	− 19.9486	− 16.626	− 19.1304	− 6.18978
2f	− 18.6308	− 7.17531	− 17.3177	− 7.22135
2e	− 13.0734	− 0.6329	− 21.3911	− 0.3561

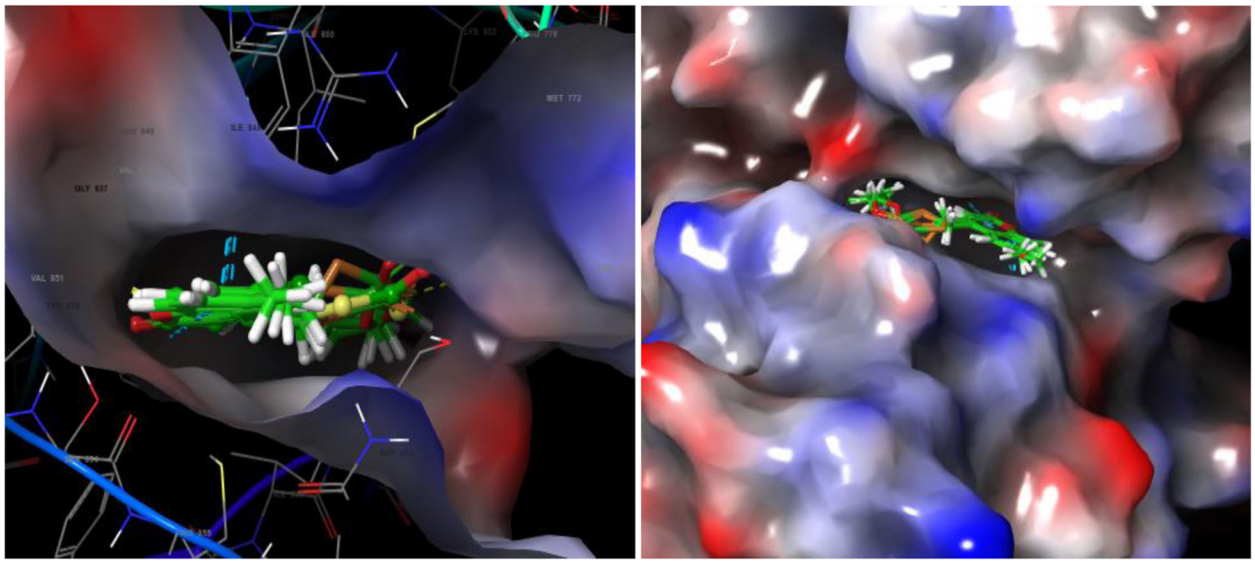


Fig. 2 Both the figures depicts interaction of all compounds with PIK3Cα (PDB ID-5DXT) protein active site. Electrostatic potential value plotted surface of PIK3Cα with the conformations of ligand compounds and crystal structure of proteins. The carbon atoms PIK3Cα are coloured by magenta, cyan, and green, respectively (electropositive charge, blue; electronegative charge, red; and neutral, white)

Table 5 Schrödinger Maestro Docking score (kcal/mol) of compounds against PIK3CA (PDB ID: 5DXT)

Name of compound	*G* score	Dock score	Lipophilic score	H-bond score
Alpelisib	− **8.9**	− 8.9	− 4.9	− 1.3
Coumarin	− 7.9	− 7.9	− 3.5	− 0.7
2a	− **9.0**	− 9.0	− 4.8	− 0.6
2b	− 8.8	− 8.8	− 4.9	− 0.6
2c	− 8.4	− 8.4	− 4.7	− 0.7
2d	− **9.0**	− 9.0	− 4.7	− 0.6
2e	− **9.1**	− 9.1	− 4.8	− 0.6
2f	− **9.3**	− 9.3	− 4.7	− 1.2
2 g	− 8.3	− 8.3	− 4.8	− 0.7
2 h	− 8.1	− 8.1	− 4.7	− 0.9
2i	− 6.6	− 6.6	− 4.5	− 0.8
2j	− 7.0	− 7.0	− 4.5	− 0.8
2 k	− 7.9	− 7.3	− 4.9	− 1.1
2 l	− 8.1	− 8.1	− 4.7	− 1.1
2 m	− 7.3	− 7.3	− 4.9	− 1.1
2n	− 8.5	− 8.5	− 5.5	− 0.7

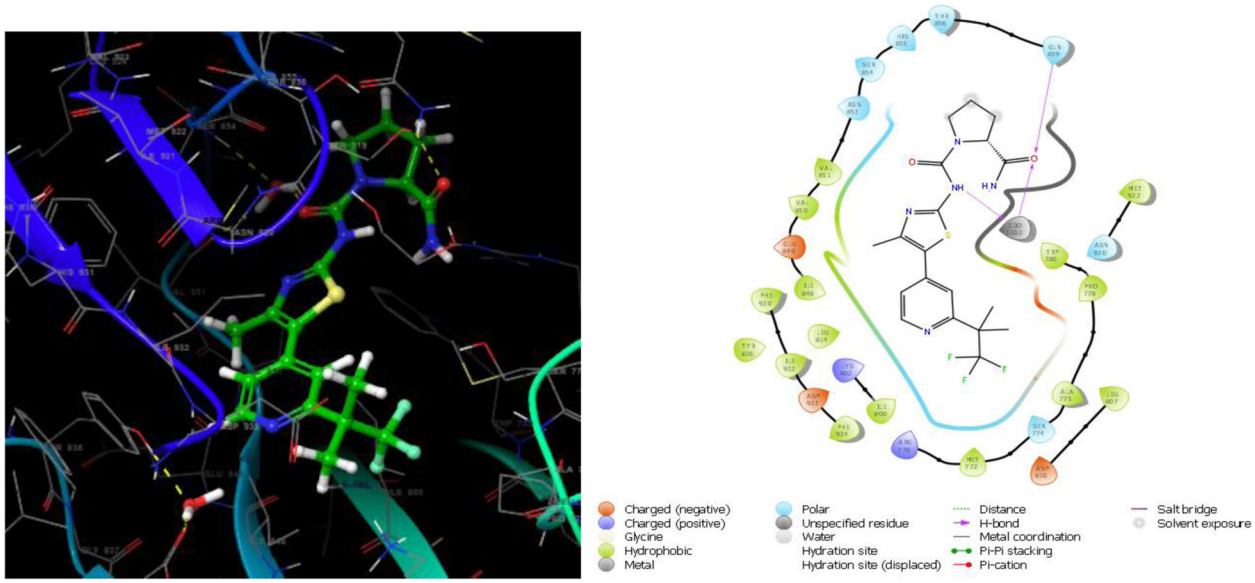


Fig. 3 Interaction of Alpelisib with PIK3Cα, both 3D and 2D diagrams, is given below with yellow dotted line represents H-bond

the energies of the unliganded PIK3CA and inhibitor.

- ΔGSA is a difference in surface area energies of the complex and the sum of the surface area energies for the unliganded PIK3CA and inhibitor.

Prime MM-GBSA calculates the energy of optimized free receptors, free ligand, and a complex of the ligand with a receptor. It also calculates the ligand strain energy by placing ligand in a solution which was autogenertated by VSGB 2.0 suit. The prime energy visualizer presented the visualization of energy.

3 Results

Various molecular changes in different types of genes cause BC. There are many identified biomarkers for BC. In our study, we used 18 upregulated proteins in BC against 14 coumarin-carbonodithioate derivatives. The accomplishment of all 18 proteins receptor grid generation using SiteMap module predicted the top three binding sites for ligands on proteins surfaces. Our present work considered the greatest *G* score exhibiting receptors of the respective protein. Among all proteins, 5DXT has shown the highest *G* score from 2a, 2b, 2d, 2e, and 2f compounds. As a screening result, we identified greater binding energy for PIK3Cα protein. Evidently, PIK3Cα gene overexpression leads to many different cancer types, mainly breast cancer in most of the cases around the globe. Hence, PIK3Cα is an attractive target for numerous therapeutic approaches. The binding of all compounds to the active site of protein is given in Fig. 2. Alpelisib is a known inhibitor of PIK3Cα which was recently approved as a drug for BC patients mainly with HR^+ and $HER2^-$ by FDA. Therefore, in this study, we used 14 coumarin-carbonodithioate derivatives for docking study along with alpelisib and coumarin, and the *G* score values are given in Table 5.

Alpelisib has a binding affinity *G* score of − 8.9 kcal/mol with three hydrogen bonds (Fig. 3), whereas coumarin with − 7.9 kcal/mol with one hydrogen bond against 5DXT protein given in Fig. 4. The best-docked compound is 2f with − 9.3 kcal/mol. Out of 14 compounds, A, D, H, and I had greater *G* score than the standard Alpelisib and coumarin. As depicted in Fig. 5, compound 2f interacts with 5DXT more tightly than other compounds with H-bond of length 2.14 Å with 851st Valine residue and Pi-Pi stacking between Trp780 and Tyr 836th residue. Similarly, in Fig. 6, compounds 2d, 2e, and 2a form H-bond of length 2.18 Å, 2.16 Å, and 2.15 Å respectively with Val 851st residue. 2e and 2d form two pi-pi stacking with Tyr836 and Trp 280th residue, whereas 2a forms only one pi-pi stacking with Trp 780th residue. Prime MM-GBSA analysis revealed the binding energy ΔG of alpelisib to 5DXT as − 19.94 kcal/mol compared with best-docked compound 2f with − 18.63 kcal/mol. Prime energy calculation analysis describes the relative binding energies of each molecule.

4 Discussion

Coumarin and their synthetic analogous contain antitumor, anti-HIV, antibacterial, and anti-inflammatory properties [30]. Scopoletin is a known coumarin that

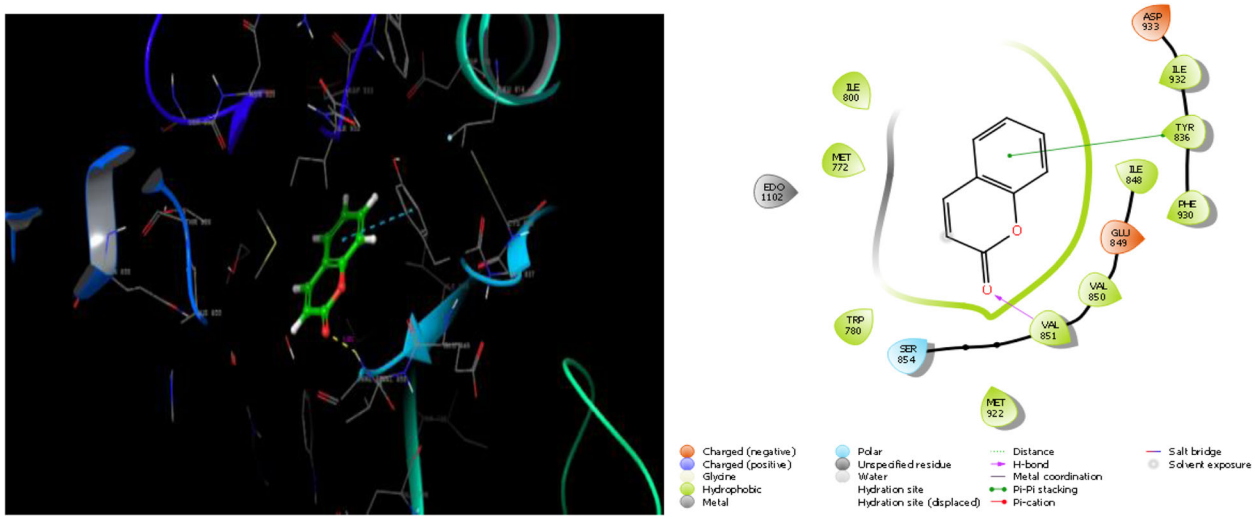


Fig. 4 Interaction of coumarin with PIK3Cα, both 3D and 2D diagrams, is given below with yellow dotted line in 3D figure represents H-bond

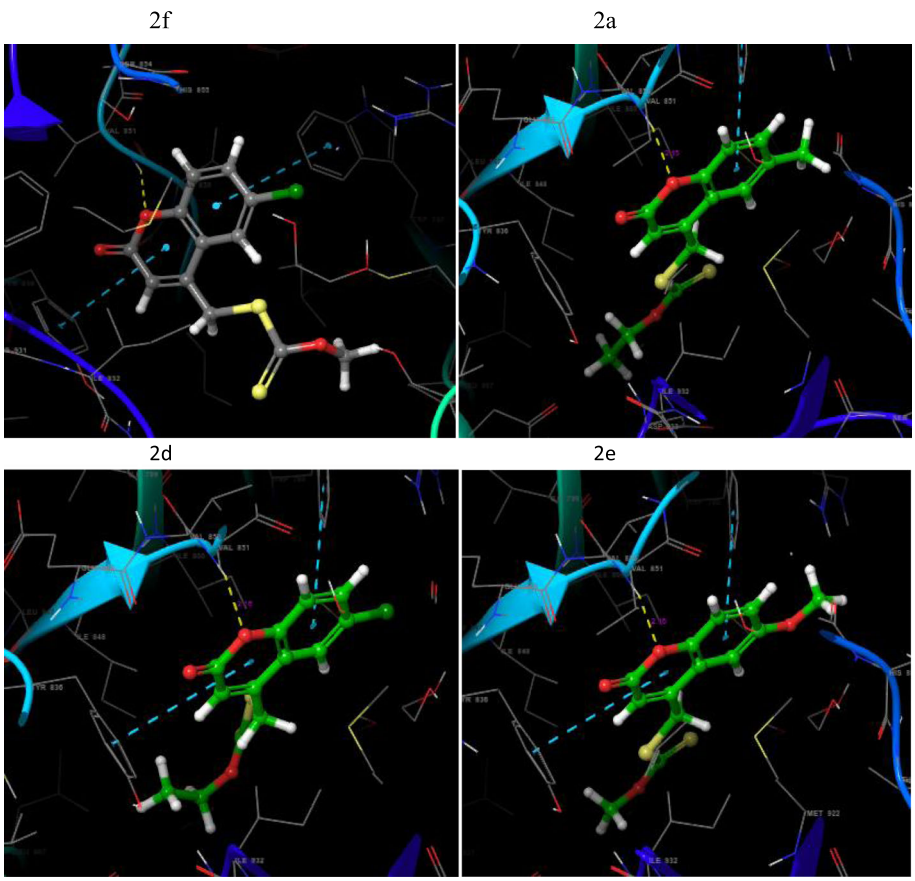


Fig. 5 Interaction of 2f, 2a, 2d, and 2e respectively with PIK3Cα 3D diagrams is given below with yellow dotted line represents hydrogen bond sky blue dotted lines represents pi-pi stacking interaction

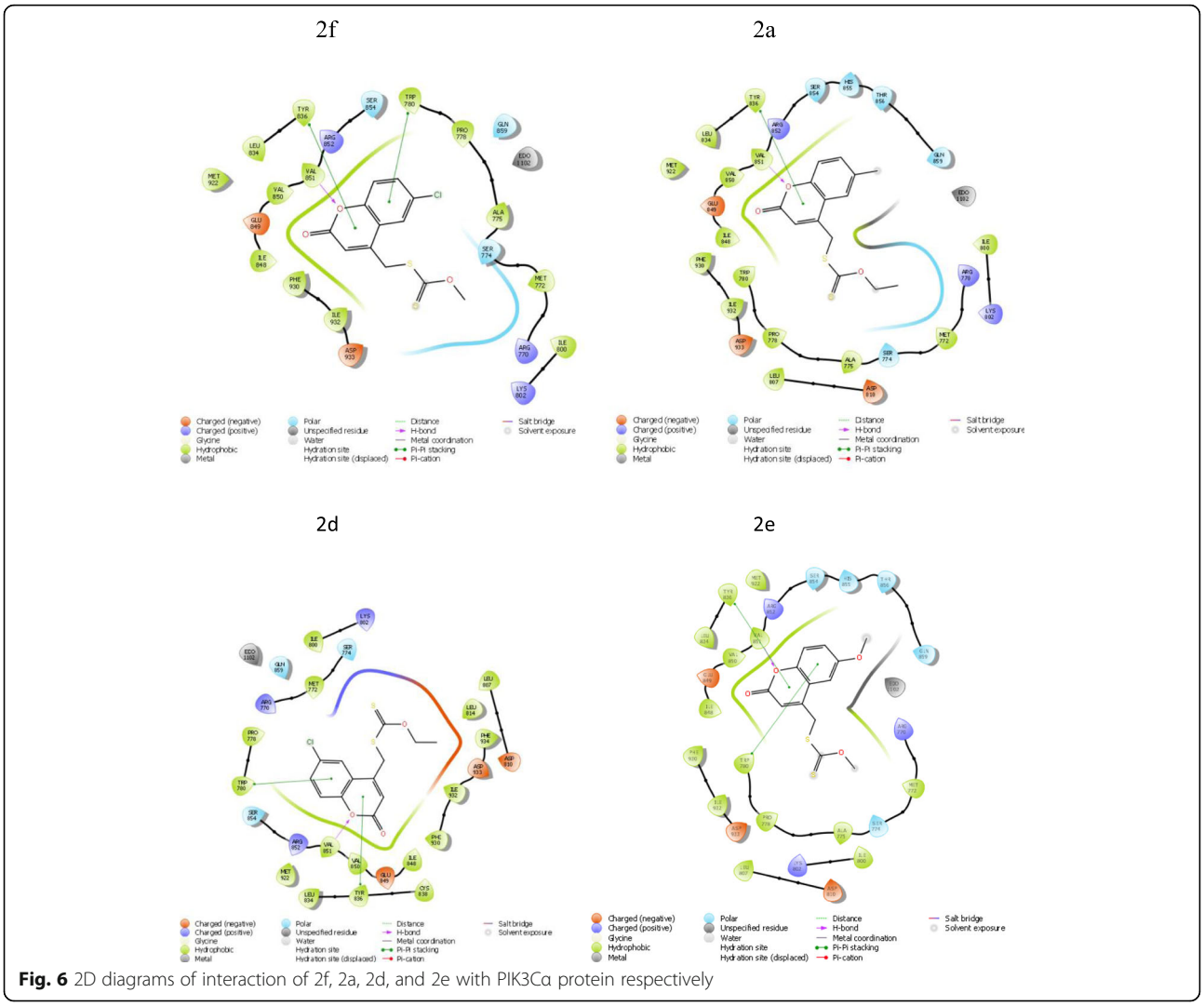


Fig. 6 2D diagrams of interaction of 2f, 2a, 2d, and 2e with PIK3Cα protein respectively

acts as an antiangiogenic compound [39]. Thus, our assay comprised of coumarin derivatives to understand their ability as anticancer agents through in silico study. Some of these derivatives had a greater *G* score than the standard drug used against PI3KCα protein alpelisib. Among 2a to 2f, five of them have shown greater *G* score for 5DXT one among 18 proteins (Table 2). We have analysed, most of the derivatives showed the H-bonding interaction between compounds and valine (851) amino acid residues of the protein, although PI3KCα inhibitors were involved in treating specific hormone receptor-mediated BC and not involved in all subtypes of BC. Henceforth, the present study is applicable for HR^+ and $HER2^-$ breast cancer.

5 Conclusion

The molecular docking studies revealed the binding energy in kcal/mol of ligands against 18 proteins in which most of the compounds have a greater *G* score for PIK3Cα. Among 14 coumarin-carbonodithioate compounds, 2a, 2d, 2e, and 2f exhibited greater *G* score than the known inhibitor of PIK3Cα protein called alpelisib. Prime MM-GBSA analysis improves the binding energy calculations than the molecular docking energies. Thereupon, MM-GBSA analysis reveals the stronger binding of the ligands to the receptors. Our study unveiled that alpelisib and compound 2f exhibit stronger binding to PIK3Cα, compared to other ligands. Therefore, indisputably 2a, 2d, 2e, and 2f molecules could serve as a lead compounds for designing the inhibitors for PIK3Cα.

6 Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s43088-020-00059-7.

Additional file 1. Prime MM-GBSA results.

Abbreviations
PIK3CA: Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform; Hr: Hormone receptor; Her2: Human epidermal growth factor receptor 2; FDA: Food and Drug Administration; PDB: Protein data bank; MM-GBSA: Molecular Mechanics/Generalized Born Surface Area; BC: Breast cancer; CDK 5: Cyclin dependent kinase 5; FGFR2: Fibroblast growth factor receptor 2; CTLA4: Cytotoxic T-lymphocyte-associated protein 4; PALB2: Partner and localizer of BRCA2; p53R2: p53-inducible ribonucleotide reductase; CDK 8: Cyclin-dependent kinase 8; CDK 4/6: Cyclin dependent kinase 4/6; BRD4: Bromodomain-containing protein 4; MDM2: Mouse double minute 2

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Authors' contributions
SVP and CMK designed the research work; SAA and SSK conducted the review and editing part. SVP conducted the research work and prepared the manuscript. Finally, all authors have read and approved the final research manuscript for publication.

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Availability of data and materials
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Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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