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Research Article

DESIGN AND SYNTHESIS OF DUAL HER-2 AND PI3 KINASE INHIBITORS FOR THE TREATMENT OF BREAST CANCER: A MULTIDIMENSIONAL APPROACH

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ABSTRACT

Breast cancer is the most commonly found cancer in women and the incidence is high in developing countries. Very few HER-2 tyrosine kinase inhibitors available in the market, but chronic use of some of these inhibitors may result in resistant to the treatment. The dual inhibition of HER-2 and PI3K tyrosine kinases can effectively treat breast cancers. In view of this, the present investigation is directed to the design of dual HER-2 and PI3K inhibitors employing a multidimensional drug design approach. In this approach, molecular docking studies of some existing HER-2 and PI3K inhibitors were carried out and physicochemical properties were also considered to identify common structural features for dual inhibition. From these results, new dual HER-2 and PI3K inhibitors were designed. Among some of the designed molecules new thienopyrimidines were synthesized and tested for anti-proliferative activity on breast cancer cell lines. Finally, six heterocyclic core moieties and important substituents were identified for HER-2 and PI3K selectivity. Among them thienopyrimidine was selected for the synthesis. Some of the synthesized compounds were found to show appreciable anti-proliferative activity in the *in vitro* cytotoxicity studies.

Keywords: Breast cancer, HER-2 and PI3K inhibitors, Structure based drug design, Molecular docking, Thienopyrimidine derivatives, Autodock.

1. INTRODUCTION

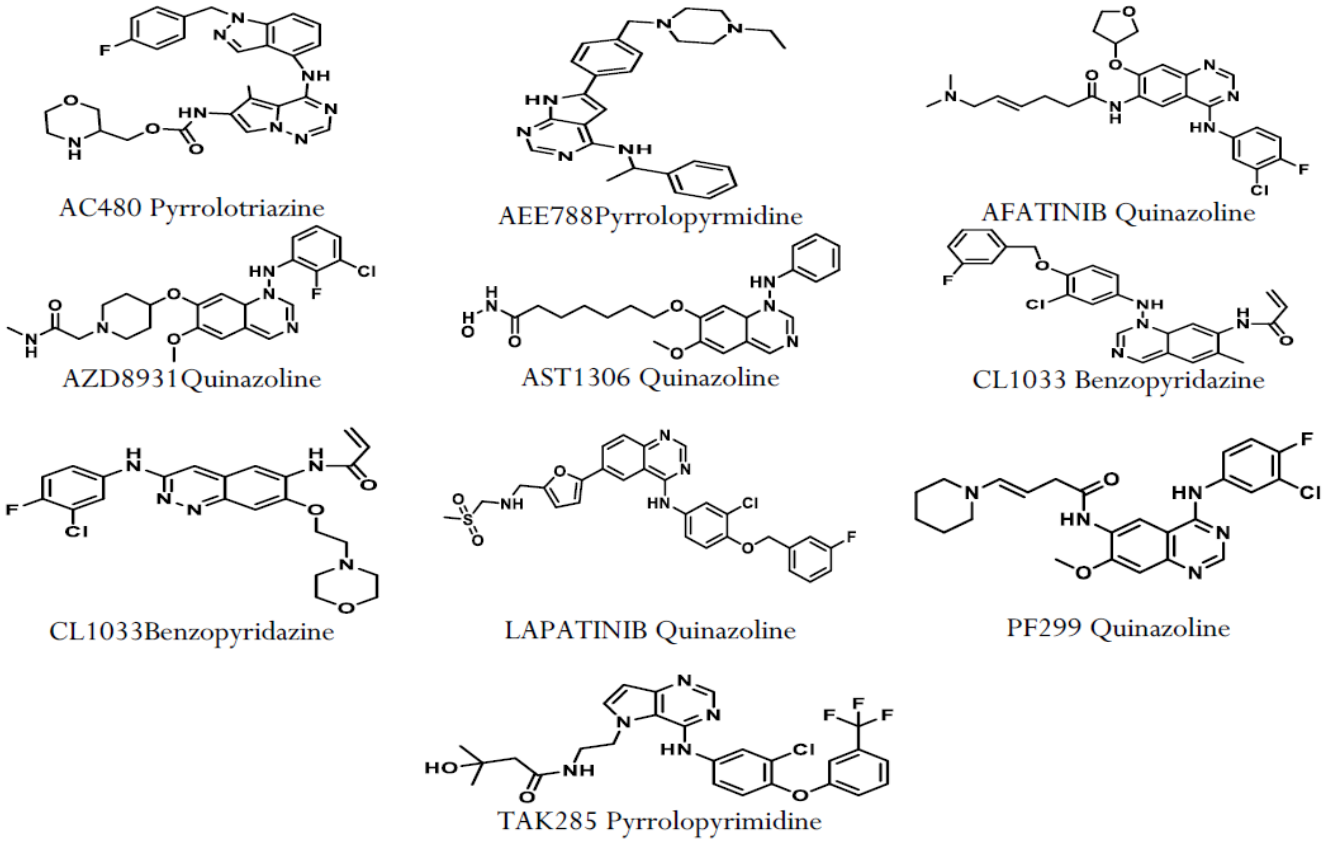
Breast cancer is the most common malignancy seen in women worldwide and still remains significance health challenge. Breast cancers have been classified into i) HER positive ii) ER negative/HER negative iii) Low proliferation ER+ve/HER-ve iv) High proliferation ER+ve/HER-ve. The HER-PI3k pathway is the most frequently deregulated and aberrantly amplified pathway in most of the breast cancers. According to the Countly *et al.*, [1] HER2 is over expressed in 25-30% of breast cancers and PIK3CA is the gene that encoded for p110 alpha subunit of PI3K and is mutated in 27% of breast cancers. Several anticancer agents that target these pathways have been approved by USFDA, Trastuzumab is a monoclonal antibody which is one of the first drugs of this class that acts by blocking HER-2/neu receptors on the cell surface. In addition, some Small molecule HER2 kinase inhibitors were also developed and approved. Lapatanib was one of the first drugs of this class [2, 3].

Although, patients with HER positive breast cancer respond to both mAb HER inhibitors and small molecule HER kinase inhibitors and increase the survival rate, but eventually acquire resistance to the HER therapy [4-6]. The underlying mechanism for this resistance is the aberrant activation of PI3K-Akt pathway either by loss of PTEN or activating mutations in PIK3CA [7-16]. Recent reports suggest that, the use of HER inhibitors alone [17-19] in breast cancer treatment may confer resistance, however, the combination of HER and PI3k inhibitors could prevent or delay the clinical resistance to HER therapy.

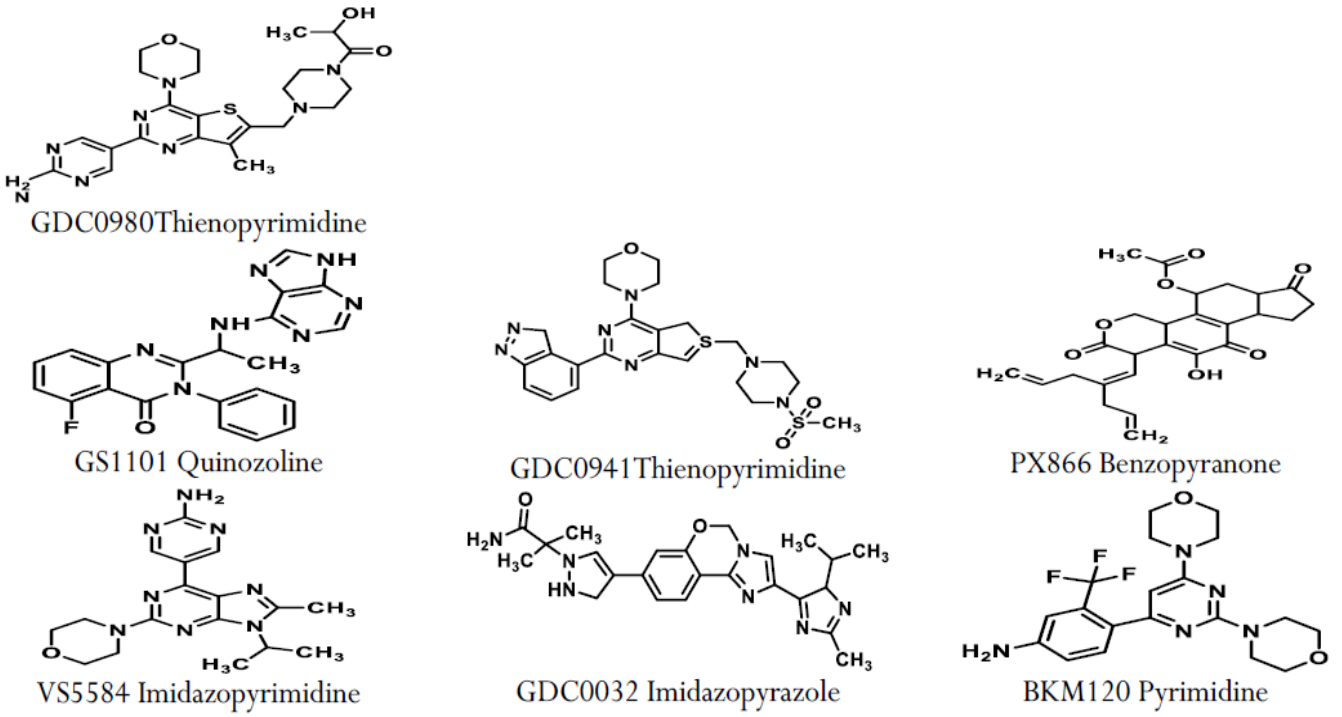
The rationale of the present study is that, as HER-PI3K pathway plays a crucial role in breast cancer development, progression and treatment, hence targeting both HER-2 and PI3 kinases is a unique strategy to design and develop novel agents to treat breast cancers efficiently. Hence, this investigation is aimed to design and develop some new molecules which act on both HER and PI3K pathway as multiple target inhibitors. Since no such inhibitors are reported till date, as dual HER2-PI3 Kinase inhibitors, these molecules could become potential drug candidates in the breast cancer treatment.

2. MATERIAL AND METHODS

The design of dual HER2 and PI3 kinase inhibitors was carried out by using a unique drug design strategy. In the first step, individual HER2 and PI3 kinase inhibitors reported in the literature were selected and retrieved their structures from the Zinc database contains 10 HER2 inhibitors and 9 PI3 kinase inhibitors possessing various heterocyclic ring systems.



Selected list of HER-2 inhibitors



Selected P13K inhibitors for the study

2.1. Molecular Property Calculation

The 2D structures downloaded from Zinc database were converted into 3D structures and energy minimized by using PRODRG server. The molecular properties such as molecular weight, rotatable bonds, LogP, topological surface area (TPSA), hydrogen bonds donors (HBD) and hydrogen bond acceptors (HBA) of the selected inhibitors were calculated by using Molinspiration tool (Table 1 & 2).

2.2. Molecular docking studies

Molecular docking studies on 3D structure of kinase domain enzyme HER-2 complexed with selective inhibitor SYR1270639 (PDB ID 3PP0) and PI3K gamma crystal structure complex with GDC0941 (PDB ID 3DBS) were obtained from RCSB protein bank and 3D structures were refined by removing water molecules and kollamann charges were added by using AUTODOCK 4.2.6 software to generate rigid protein PDBQTs and were used in further docking studies. The3D structures of all the ligands were converted into their PDBQTs. The active site for docking in the selected proteins were defined by their autogrid parameters with a grid box generated using grid points for the HER-2 (X=15.13, Y=16.347, Z=26.775) PI3K (X=22.563, Y=64.119, Z=20.121) with grid dimension 0.320Å leads to generate *gpf* file. Docking was carried out by setting up autodock parameters to their default values and the docking calculation were carried out using Lamarkian genetic algorithm in AUTODOCK 4.2.6.Graphic user interface Autodcok Tools (ADT) was used to perform all the docking steps and analyse the interactions after docking. The autogrid and autodock calculations were done using command line interface on the Ubuntu operating system.

2.3. Docking validation

Prior to the docking of inhibitors, the docking method discussed above was validated by redocking the existed molecules with co-crystallized inhibitor with appropriate target protein. The co-crystallized inhibitor SYR127063 was removed from the HER-2 and GEDC0941 from PI3K binding sites. The docking confirmation and co-crystallized confirmation of these inhibitors were superimposed to check the validity of the docking procedure.

2.4. Synthesis

The proposed thienopyridines and thienopyrimidines were synthesized by employing a systematic synthetic procedures outlined in Scheme 1 & 2. All the chemicals used were purchased from SD fine chemicals, Mumbai and are of synthetic grade. The progress of the reactions was monitored by using Silica Gel coated TLC plates visualised under UV light or in iodine chamber. The synthesized compounds were purified by recrystallization and purity was determined by measuring melting point in Kofler hot stage melting point apparatus and are uncorrected. The IR spectrums were recorded on Shimadzu FTIR spectrophotometer (1% KBR discs). ^{1}H NMR at 400MHz and ^{13}C NMR 100MHz was recorded on Bruker Avance II 400 MHz NMR spectrophotometer with TMS as internal standard mass spectrum was recorded using Agilent 1100.

2.4.1. Preparation of 2-amino-5-isopropyl-4-methylthiophene-3-carbonitrile (1)

Sulphur (0.01Mol) was dissolved in ethanol (20ml). To this solution, a mixture of 4-methyl pentan-2-one (0.01Mol) and malanonitrile (0.01Mol) was added and stirred at 60°C for 30 minutes. Then the morpholine (0.001Mol) was added drop wise and stirred at 60°C for 6 hours. The completion of the reaction was monitored by TLC. The reaction mixture was cooled to 0°C to get the crystalline product. The product was washed thoroughly with ethanol and recrystallized from ethanol.

%Yield =78%, M.P 180°C, Rf value 2.3, IR(KBr) cm^{-1}: 3,32O (NH_2 str) 2218 (str); ^{1}HNMR spectra (400MHZ, $CDCl_3$)δ 6.93 (s, 2H, NH_2), 2.98 (m, 1H, CH_3)2.28(s, 3H, CH_3), 1.20 (d,6H,CH_3), Mass (m/z) 181.10.

2.4.2. General procedure for the synthesis of 4,6-diamino-2-isopropyl-3-methylthieno[2,3-b]pyridine-5 carbonitrile (2)

To a solution of thiophene **(1)** (0.001Mol) in ethanol (20ml), malanonitrile (0.001Mol) and few drops of hydrochloric acid was added. The reaction mixture was stirred under reflux for 8 hours. The reaction mixture was cooled to room temperature and then poured into cold water (100ml). The solid product was filtered off, washed with water, dried and crystallized from absolute ethanol.

%Yield =74%,M.P 240°C, Rf value 2.5 cm, IR(KBr) cm^{-1}3, 450, 3,32O(brs 2NH), 2218(C-N str);^{1}HNMR spectra (400MHZ, $CDCl_3$)δ 6.89 (s, 2H, NH_2), 6.35 (s, 2H, NH_2),2.98(s, 1H, CH_3), 1.93 (s,3H, CH_3), 1.20 (d,6H,CH),Mass (m/z) 247.32.

2.4.3. Preparation of N,N'-(5-cyano-2-isopropyl-3-methylthieno[2,3-b]pyridine-4,6-diyl) acetamide (2a)

The thienopyridine **(2)** (0.001Mol) was dissolved in pyridine (15ml). To this solution, acetic anhydride (0.001Mol) was added and stirred for 24 hours at room temperature. The reaction mixture was neutralized and obtained precipitate was filtered off, washed with ethanol and crystallized from absolute ethanol.

%Yield=75%, M.P 230°C, Rf value 3.2 cm, Mass (m/z) 331.11; IR(KBr) cm^{-1} 3246 (NH), 2220 (CN), 1724 (C=O str); ^{1}HNMR spectra (400MHZ, $CDCl_3$) δ 10.61 (s, 1H,NH), 9.86 (s, 1H, NH), 3.12 (m,1H,CH), 2.04 (s, 3H,CH_3), 2.04 (s, 3H,CH_3), 1.20 (s, 3H, CH_3), 1.30 (s,6H,CH_3).

2.4.4. Preparation of N,N'-(5-cyano-2-isopropyl-3-methylthieno[2,3-b]pyridine-4,6-diyl)bis(4-methyl-benzenesulfonamide) (2b)

The thienopyridine **(2)** (0.001Mol) was dissolved in pyridine (15ml). To this solution, p-toluene sulfonylchloride (0.001Mol) was added and stirred for 24 hours at room temperature. The reaction mixture was neutralized and obtained precipitate was filtered off, washed with ethanol and crystallized from absolute ethanol.

%Yield=70%, M.P 225°C, Rf value 2.4 cm, IR(KBr) cm^{-1}3256 (NH str), 2280 (CN str), 1352 (SO_2 str); ^{1}HNMR spectra (400MHZ, $CDCl_3$)δ 11.38 (s, 1H, NH), 10.01(s, 1H, NH), 7.84(d, 2H, Ar-H), 7.68(d, 2H, Ar-H), 7.34(m, 4H, Ar-H), 2.98(m, 6H, CH_3), 2.43(s, 6H, CH_3), 1.93 (s,3H, CH_3), 1.20 (d,6H,CH_3), Mass(m/z)540.

2.4.5. Preparation of 2,2'-(5-cyano-2-isopropyl-3-methylthieno[2,3-b]pyridine-4,6-diyl)bis (azanediyl) diacetamide (2c)

The thienopyridine **(2)** (0.001Mol) was dissolved in pyridine (15ml). To this solution, ethyl chloroacetate (0.001Mol) was added and refluxed for 24 hours. The reaction mixture was poured in cold water and neutralized. The obtained precipitate was filtered off, washed with ethanol and crystallized from absolute ethanol. TLC solvent system:n-hexane: Ethyl acetate-70: 30

%Yield=77%, M.P 210°C, , Rf value 2.1cm, IR(KBr) cm^{-1}332O(NH str), 2218 (CN str), 1705 (C=O str); ^{1}HNMR spectra (400MHZ, $CDCl_3$), δ 9.10 (s, 1H, NH), 5.03 (s, 1H, NH), 4.16(m, 4H, CH_2), 4.02(s, 4H, CH_2), 2.98 (m, 1H, CH_3),1.93 (s,3H, CH_3), 1.22 (d,6H,CH), 1.20 (d,6H,CH_3), Mass (m/z) 419.17.

2.4.6. Preparation of 6-isopropyl-2,5-dimethylthieno [2,3-d]pyrimidine-4-amine (3)

To a solution of thiophene **(1)** (0.001Mol) in methanol (20ml), acetonitrile (0.001Mol) and sodium methoxide (0.001Mol) was added. The reaction mixture was refluxed for 8 hours, excess amount of solvent was removed under vaccum. The obtained product was poured in cold water (100ml), solid product was filtered off, washed with ethanol and crystallized from absolute ethanol.

%Yield=76%, M.P 220°C, Rf value 2.6 cm, IR(KBr) cm^{-1}3400(brs NH_2), 2920(CH_3 str); ^{1}HNMR spectra (400MHZ, $CDCl_3$)δ 6.89(s, 2H, NH), 6.35(s, 2H, NH), 2.98(s, 1H, CH_3), 1.93 (s,3H, CH_3), 1.20 (d,6H,CH),Mass (m/z) 222.10.

2.4.7. Preparation of N-(6-isopropyl-2,5-dimethyl-thieno [2,3-d]pyrimidin-4-yl) acetamide (3a)

The thienopyrimidine **(3)** (0.001Mol) was dissolved in pyridine (15ml). To this solution, acetic anhydride (0.001Mol) was added and stirred for 24 hours at room temperature. The reaction mixture was neutralized and obtained precipitate was filtered off, washed with ethanol and crystallized from absolute ethanol.

Yield 71%, M.P 215°C, Rf value 2.4 cm; IR(KBr) cm^{-1}342O (NH str), 1715(C=O str), 2910(CH_3 str); ^{1}HNMR spectra (400MHZ, $CDCl_3$)δ 10.61 (s, 1H, NH), 3.12 (m,1H, CH), 2.4 (s, 3H, CH_3), 2.6 (s, 3H, CH_3),1.30 (S, 3H, CH_3), 1.20(S, 6H, CH_3), Mass (m/z) 264.35.

2.4.8. Preparation of N-(6-isopropyl-2, 5-dimethylthieno [2, 3-d]pyrimidin-4-yl)-4-methyl-benzene sulfonamide(3b)

The thienopyrimidine **(3)** (0.001Mol) was dissolved in pyridine (15ml). To this solution p toluene sulfonyl chloride (0.001Mol) was added and stirred for 24 hours at room temperature. The reaction mixture was neutralized and obtained precipitate was filtered off, washed with ethanol and crystallized from absolute ethanol.

%Yield 72%; M.P 235°C, Rf value 1.8 cm, IR(KBr) cm^{-1}, 321O(NH str), 2900(CH_3 str), 1345 (SO_2 str); ^{1}HNMR spectra (400MHZ, $CDCl_3$)δ, 10.01 (s,1H, NH), 7.32(m, 4H, Ar-H), 3.12(s, 1H, CH), 2.6 (s, 3H, CH_3),2.4 (s, 3H, CH3), 1.20 (s, 6H, CH_3), 1.30 (s, 3H, CH3), Mass (m/z) 376.

2.4.9. *Preparation of ethyl 2-(6-isopropyl-2,5-dimethylthieno[2,3-d]pyrimidin-4-ylamino) acetate (3c)*

The thienopyrimidine **(3)** (0.001Mol) was dissolved in pyridine (15ml). To this solution, ethyl chloroacetate (0.001Mol) and ethanol was added and refluxed for 24 hours. The reaction mixture was poured in cold water and neutralized. The obtained precipitate was filtered off, washed with ethanol and crystallized from absolute ethanol. %Yield=74%,M.P 225°C, Rf value 2.2 cm, IR (KBr) cm^{-1}, 3320 (NH str), 2923(CH_3 str), 1705 (C=O str); ^{1}HNMR spectra (400MHZ, $CDCl_3$)δ, 7.39(s, 1H, NH),4.16(m, 2H, CH_2),4.02 (s, 2H, CH_2), 2.98 (s, 1H, CH), 2.40 (s, 3H, CH_3), 1.93, (s, 3H, CH_3), 1.22 (d,3H,CH_3), 1.20 (d,6H,CH_3), Mass (m/z) 308.25.

3. RESULTS AND DISCUSSION

3.1. Molecular properties calculation

The molecular properties such as molecular weight, no of rotatable bonds, LogP values, topological surface area (TPSA), hydrogen bond donors(HBD), hydrogen bond acceptors (HBA) of the selected inhibitors were calculated by using MOLINSPIRATION; one of the online tool.

The idea behind the consideration of molecular properties in this study is, firstly to examine or to identify the relationship between the molecular properties of selected inhibitors molecules and second is to assess any possible relationship between the molecular properties HER-2 kinase inhibitors and PI3K inhibitors. These findings could be helpful in the designing of novel agents in this study.

Based on the calculated properties, some limits have been set to design the novel inhibitors to achieve dual inhibition. The molecular weight is between 350 and 590, the number of rotatable bonds kept between 4 and 7 for the flexibility in the active site of both enzymes. The logP values were kept between 0.9 and 5.38, the hydrogen bond donors and hydrogen bond acceptors were kept 1-5 and 6-8 respectively, the TPSA value was kept between 57 and 122. Moreover, it is known that some of the properties may vary with the molecular structure and it may or may not influence the binding affinity/pattern of the molecule within the targets and the activity as well.

Hence, further molecular docking studies were carried out with the selected inhibitors on the appropriate target.

Table 1: Molecular properties of the PI3K inhibitors

Drug	Mol. Wt	Rot. Bonds	LogP	TPSA	HBD	HBA
AC480	361.207	4	-2.68	211	5	13
AEE788	440.595	7	3.94	57	1	6
AFATINIB	438.507	5	1.74	122	7	8
AZD8931	473.936	7	3.32	89	2	8
CI1033	485.947	9	4.35	89	2	8
CUDC	434.496	11	3.86	106	3	8
LAPATINIB	582.077	11	6.16	110	3	8
PF 299	470.956	7	5.02	81	3	7
TAK285	547.965	10	5.52	101	3	8
AST1306	448.11	7	5.81	76	2	6

Table 2: Molecular properties of theHER-2 inhibitors

Drug	Mol. Wt	Rot. Bons	Log P	TPSA	HBD	HBA
GS1101	415.432	5	3.68	101	2	8
BKM120	410.4	4	2.36	90	2	8
GDC0941	514.657	5	2.44	109	2	10
IP1145	416.872	4	4.56	88	2	7
GDC0980	499.621	5	0.87	135	4	11
GDC0032	461.55	5	2.01	120	3	10
BYL719	469.548	3	5.77	77	0	6
VS5584	354.418	3	1.22	108	2	9
PX866	524.59	9	2.59	122	0	9

3.2. Molecular docking studies

Molecular docking [20-21] was employed to study the binding patterns of the selected inhibitors with the appropriate target proteins (HER-2 and PI3K). In order to validate the docking methodology followed in the docking studies, the co-crystallized inhibitors bound to the each target protein were removed. Further, the inhibitors were re-docked into the respective binding site of the proteins. The docked (low energy) and co-crystallized conformations were further superimposed to check their conformational relevance. The current docking procedure followed, re-produced the conformation almost equal to the co-crystallized conformation of the inhibitors used SYR127063 and GDC0941 in the active site of HER-2 and PI3 kinase respectively.

The results suggested that the current docking methodology is valid and it could be used for docking of selected inhibitors.

Hence this validated docking procedure was used to carry out the molecular docking studies of selected inhibitors with their appropriate targets. After completion of the docking, the docking results were extracted from the appropriate *.dlg* file.

When re-docked the co-crystallized inhibitors with appropriate proteins, the obtained docking energy of the best conformation was -10.74kcal/mol for HER-2-SYR12703 and -10.88 kcal/mol for PI3K-GDC0941. The main residues involved were identified within the active site of HER-2-SYR12703 are LEU 852, MET 801, THR 862, PHE 864, GLY 799, THR 798, LEU 726, LYS 753, ALA 751, LEU 796 and ASP 863. The main residues are involved in the active site of PI3K-GDC0941 are THR 887, ASP 950, MET 953, ILE 963, LYS 833, VAL 882, ILE 881 and GLU 880. Later, all the selected HER-2 and PI3K inhibitors were docked with appropriate target protein following the same procedure. The binding affinity and interaction of the each of the inhibitor was studied considering the least energy conformation of the inhibitor.

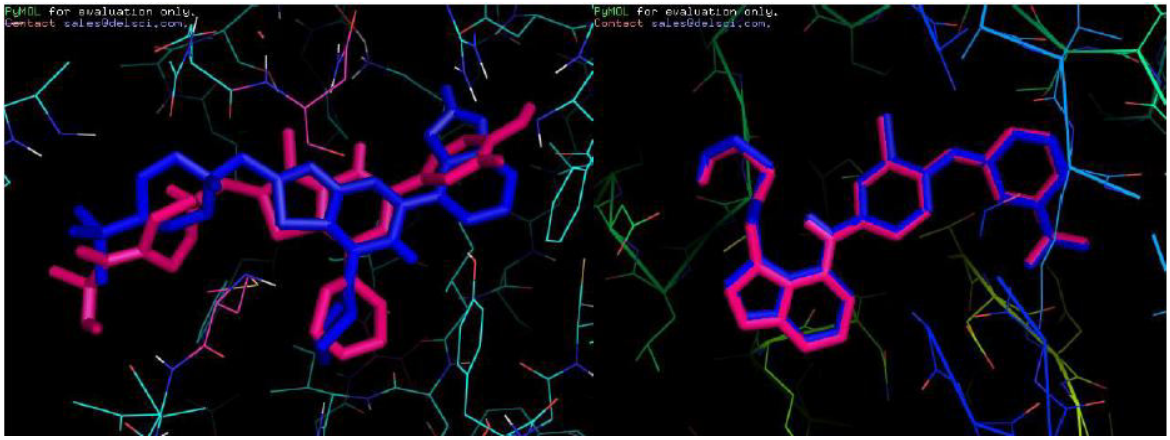


Fig. 1: The superimposition of SYR127063 and suprimposition of GDC0941

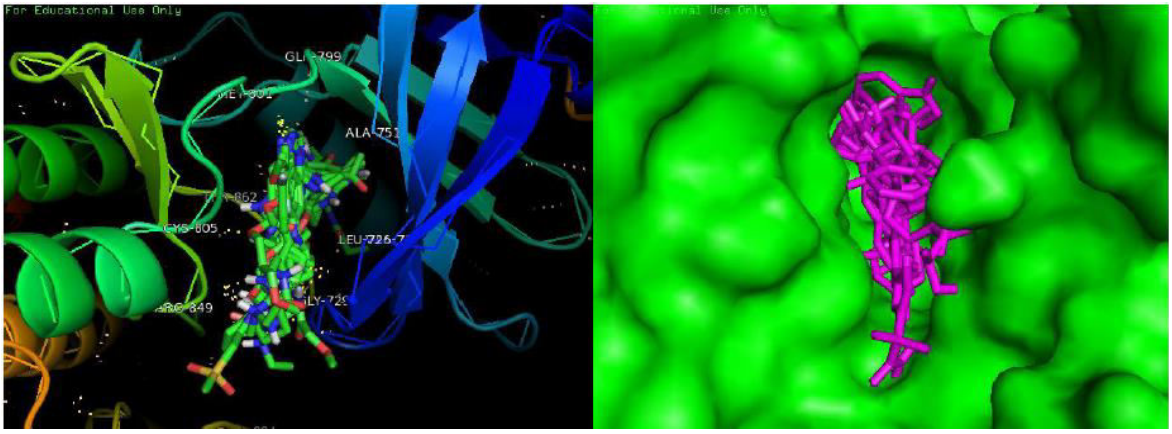


Fig. 2: Superimposition of all the HER-2 inhibitors in the active site of the HER-2 Kinase (3PP0) and superimposition of all the PI3K inhibitors in the active site of the PI3 Kinase (3DBS)

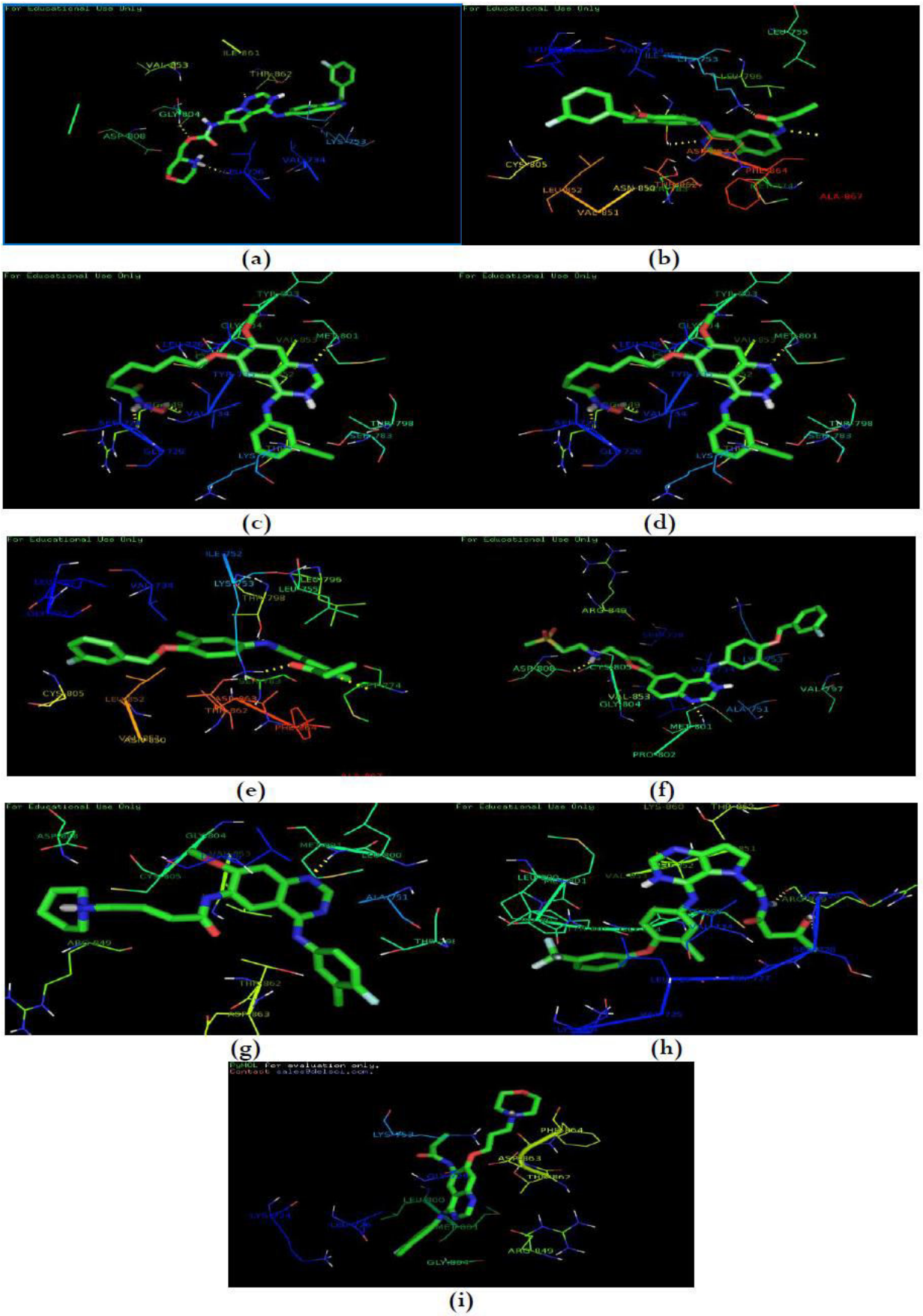


Fig. 3: Interaction of a) AC480, b) AEE788, c) AZD8931, d) CUDC101, e) AST1306, f) LAPATINIB, g) PF299, h) TAK285 and i) CL1033 with HER-2 (PDB ID 3PP0)

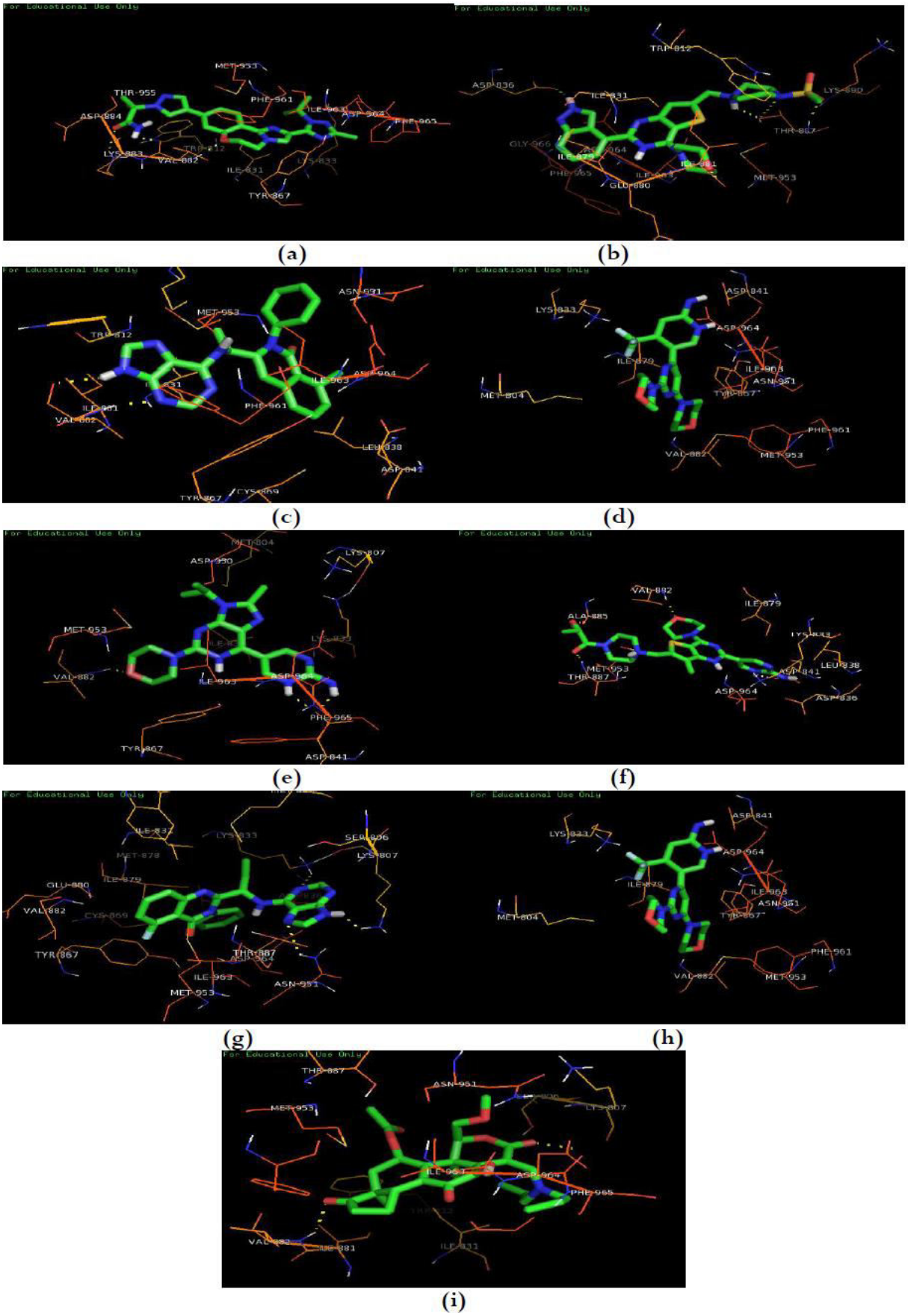


Fig. 4: Interaction of a) GDC0032, b) GDC0941, c) IPI145 d) BEZ235, e) GDC0980, f) VS5584, g) GS1101, h) BKM120 and i) PX866 with PI3K (PDB ID 3DBS).

Table 3: Selected HER-2 kinase inhibitors their molecular docking results

Entry	HER-2 inhibitors	Binding energies	Interacting amino acids within the active site
1	AC480	-12.64	Met801, Lys753, Ala751, Asp863
2	LAPATINIB	-12.31	Met801, Lys753, Val797, Asp863
3	AST1306	-11.45	Mt801, Lys753, Asp863
4	AEE788	-10.33	Met801, Lys753,Val734, Asp863
5	AZD8931	-9.64	Asp863, Met801, Lys753, Leu726
6	CI103	-9.32	Met801, Lys753, Tyr735, Asp863
7	PF299	-9.17	Met801, Lys753, Ala751, Asp863
8	TAK285	-7.75	Met801, Lys753, Ala751, Asp863

Table 4: Selected PI3K inhibitors their molecular docking results

Entry	PI3k inhibitors	Binding Energies	Interacting amino acids within the active site
1	GDC0032	-11.46	Val882, Asp867, Ala805, Lys802
2	GDC0941	-11.06	Val882, Asp867, Lys802
3	IPI145	-10.34	Val882,Asp841,Tyr867
4	BEZ235	-10.21	Val882,Tyr867, Ile963, Asp841
5	GDC0980	-9.57	Asp841,Val882, Ala805
6	VS5584	-9.26	Val882,Tyr867,Asp841
7	GS1101	-8.57	Val882, Asp841, Asp964, Lys802
8	BKM120	-8.48	Val882, Tyr867, Ile963, Asp841

3.3. Design and synthesis of dual HER-2 and PI3K analogues

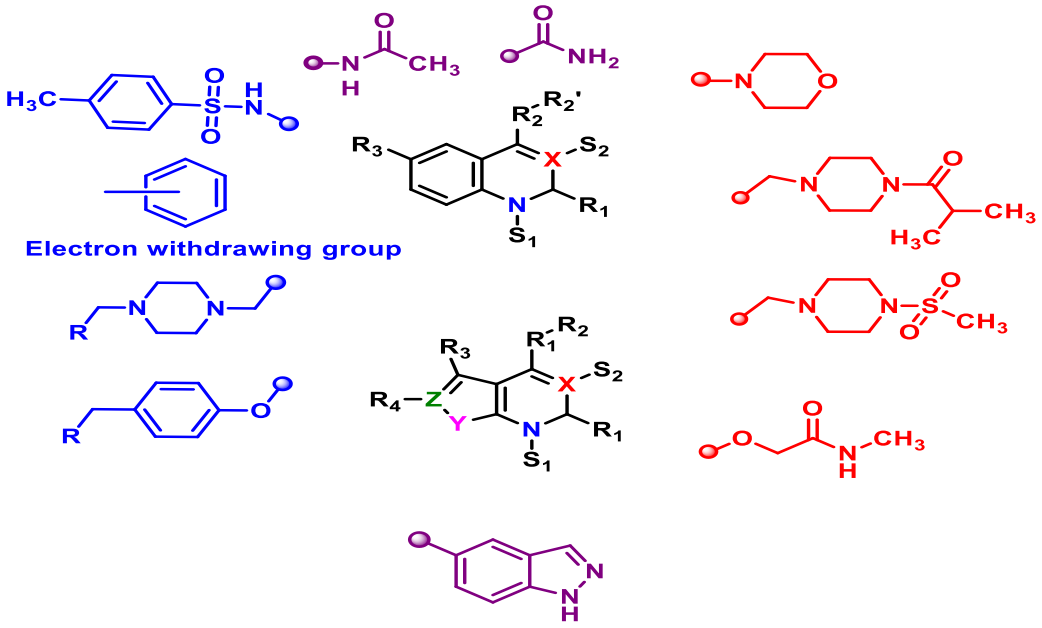
In the current investigation, the design of dual HER-2 and PI3K inhibitors was carried out by employing multiple designing strategies. The proposed inhibitors for HER-2 and PI3K kinases were designed based on the results of molecular property calculations and molecular docking studies.

Based on the study conducted on the HER-2 and PI3K inhibitors, to achieve the dual inhibition heterocyclic core systems such as quinazoline, quinolone, pyrrolo-pyrimidine, thienopyrimidine and pyrrazolopyrimidines were taken as the important scaffolds. The reason behind the selection of core moieties is that, these structures have affinity to the ATP binding site of both of the enzymes. Onto the core structure at R^1, R^2, $R^{2'}$, R^3 and R^4 positions various important substituents and side chains were incorporated in order to make favorable interactions with the active site of both enzymes. Position S1 can be substituted but however, S2 position is remain unsubstituted for HER-2 selectivity. To facilitate hydrogen bond formation with the important residues of the both enzymes, 4-amino substitution would be useful.

According to the docking results, the ring nitrogen plays an important role in hydrogen bond formation within the hinge region of the HER-2. For the PI3K inhibition, at least one C-O-C oxygen is required to form an additional hydrogen bond which can be incorporated in the form of acyclic side chain or within a ring system such as morpholine. However, the carbonyl of amine also would be helpful in this context. A methyl substituted piperizenyl side chain is important for dual inhibition at positions either R^3 or R^4 attached by means of ether bridge. The piperezenyl ring could be replaced with either morpholine or piperidine rings. Other substituents like aromatic groups substituted with one or more electron withdrawing groups can be incorporated at various positions on the core systems without disturbing the other important molecular parameters, the monocyclic ring can also substituted with bicyclic ring systems such as benzopyrazole and imidazopyrimidines.

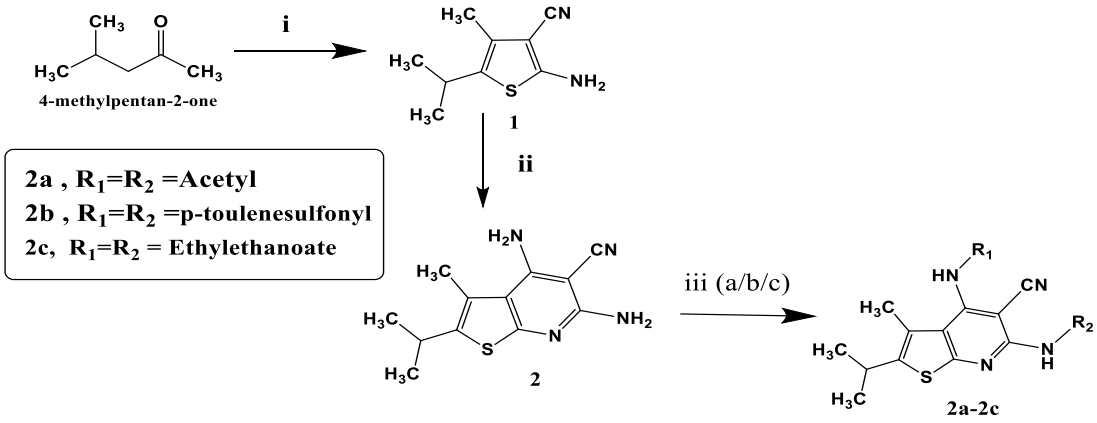
Based on the above findings, dual inhibitors were designed by setting some molecular property limitations. From the designed molecules thienopyridine and thienopyrimidine class of inhibitors were selected for the synthesis.

Based on the results obtained some novel inhibitor could be designed by the combination of any heterocyclic ring and other important molecular fragments as mentioned earlier. For the synthesis, thienopyridine and thienopyrimidine systems were selected and proposed inhibitor molecules were designed by incorporating important side chains. The proposed compounds were synthesized by employing a systematic synthetic procedures outlined in Scheme 1 & 2.



X=N; Quinoline: X=CH;Thienopyrimidine: X=N, Y=S, Z=CH; Pyrrolopyrimidine (Id): X=N, Y=N, Z=CH; Pyrrolopyrimidine (Ic): X=N, Y=N, Z=N

Fig. 5: Designed Molecules Quinazolines



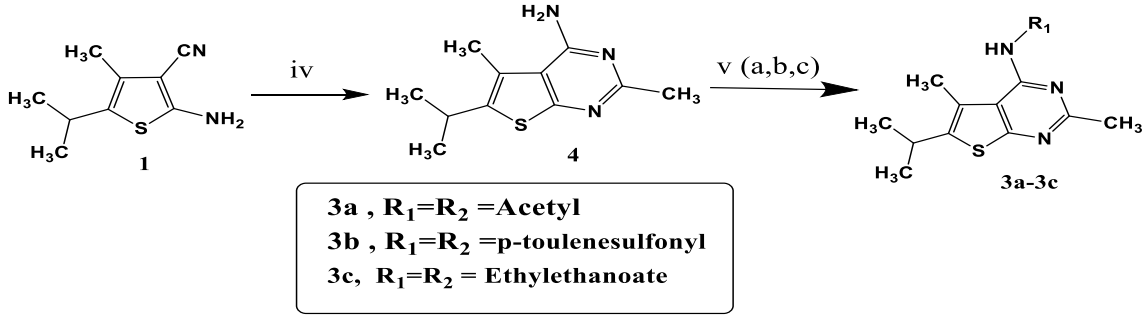
i = Malanonitrile, Sulphur, Morpholine in Ethanol, Stirring at 55-60^{0}C, 7h
ii = Malanonitrile, Ethanaol, HCl, Reflux, 8h
iii a= Acetic Anhydride, Pyridine, Stirring, RT, 24hr
iii b = p-toulenesulfonylchloride, Pyridine, Stirring, RT, 24hr
iii c = Ethyl chloroacetate, Etanol, Pyridine, reflux, 8hr

Scheme 1

The proposed thienopyridine class inhibitors were designed. In the scheme 1, 2-amino-5-isopropyl-4-methylthiophene-3-carbonitrile **(1)** was prepared by the cyclisation of 4-methyl pentan-2-one and sulphur with malanonitrile. The purity of the compound was confirmed by a single spot in TLC and melting point and the structure was confirmed by FTIR. The IR spectrum showed absorption peaks at 3,320 cm^{-1} and 2218 cm^{-1} due to NH stretch of amine and C-N stretch of carbonitrile.Compound 4, 6-diamino-2-isopropyl-3-methylthieno [2,3-b]pyridine-5-carbonitrile **(2)** was synthesized by condensation of compound **(1)** with malanonitrile in hydrochloric acid under reflux for 8 hr. The purity of resulting compound was confirmed by a single spot in TLC and sharp melting point. The structure was confirmed by FTIR. The IR spectrum of compound **(2)** showed an absorption peak at 3,450 cm^{-1}and 2218 cm^{-1}due to NH stretch of amine and C-N stretch of carbonitrile. The compound **(2a & b)** was synthesized by condensation of compound **(2)** with acetic anhydride and *p*-toluene sulfonylchloride in pyridine stirred for 24 hr at room temperature. The

purity of the compound was confirmed by a single spot in TLC and sharp melting point. The structure was confirmed by FTIR, mass and ^{1}HNMR spectral data. The IR spectrum of compounds showed absorption peak at 1724 cm^{-1} due to C=O stretch of amide, and a peak of 2280 cm^{-1} due to C-N stretch ofcarbonitrile. The ^{1}HNMR of compound **(2a)** showed a singlet at δ 10.61 correspondents to one proton of NH and the proton of CH appeared at δ 3.12. The two methyl protons appeared as two separate singlets at δ 1.93 and 2.04. The three protons of CH_3 appeared as singlet around δ 1.20. The compound **(2c)** was synthesized by condensation of compound **(2)** with ethyl chloroacetate in pyridine refluxing for 24 hr. The purity of the compound was confirmed by a single spot in TLC and sharp melting point. The structure was confirmed by FTIR. The IR spectrum of compound showed absorption peak at 3,320 cm^{-1} and 1705 due to C-N str of pyridine and C=O str of ester.

In the scheme 2, 6-isopropyl-2,5-dimethylthieno [2,3-d] pyrimidine-4-amine **(4)** was synthesized by condensation of acetonitrile with compound (1) in methanol refluxing for 7 hr. The purity of the compound was confirmed by a single spot in TLC and mp and the structure was confirmed by FTIR. The IR spectrum showed absorption peaks at 3400 cm^{-1}and 2920 cm^{-1} due to N-H strectch of amine and C-H stretch of CH_3. The compounds **(3a &b)** was synthesized by condensation of compound (3) with acetic anhydride and *p*-toluene sulfonylchloride in pyridine stirred for 24 hr at room temperature. The purity of the compound was confirmed by a single spot in TLC and sharp melting point. The structure was confirmed by FTIR, mass and ^{1}HNMR spectral data.



iv = CH_3ONa, CH_3OH, Reflux, 7h
v a = Acetic Anhydride, Pyridine, Stirring, RT, 24hr
v b = p-toulenesulfonylchloride, Pyridine, Stirring, RT, 24hr
v c = Ethyl chloroacetate, Etanol, Pyridine, reflux, 8hr

Scheme 2

The ^{1}HNMR of compound **(3a)** showed a singlet at δ 10.61 correspondents to one proton of NH and the proton of CH appeared as multiplet at δ 3.12. The two methyl protons appeared as two separate singlets at δ 1.93 and 2.04. The three protons of CH_3 appeared as singlet around δ 1.20. The compound **(3b)** showed a singlet at δ 10.1 correspondents to one proton of NH and aromatic protons appeared at 7.39 which account for 4 protons. The proton of CH appeared at δ 3.12 and the two methyl protons appeared as singlet around δ 1.93.The IR spectrum of compounds showed absorption peak at 1715 cm^{-1} due to C=O stretch of amide, and a peak of 2910 cm^{-1} due to C-H stretch of CH_3.

The compound **(3c)** was synthesized by condensation of compound **(3)** with ethyl chloroacetate in pyridine refluxing for 24 hr. The purity of the compound was confirmed by a single spot in TLC and sharp melting point. The structure was confirmed by FTIR. The IR spectrum of compound showed absorption peak at 1705 cm^{-1} and 2923cm^{-1} due to C=O stretch of ester and C-H stretch of CH_3. All the compounds showed molecular ion peak corresponding to their molecular weights conformed their structures. All the compounds were confirmed on the basis of physical and spectral data.

3.4. Antiproliferative activity

MTT Assay was employed to screen the *in vitro* antiproliferative activity on breast cancer cell lines. The IC_{50} values for standard Dabrafenib and test compounds were determined from the absorbance of the test and standard drug. The detailed procedure followed for this study was mentoned in our previous article [22]. The results showed that among the two series of compounds synthesized 4-amino Thienopyrimidine derivatives (3a-3c) were found to possess excellent anticancer potency

than the 2,4-diamino thienonpyridines. However, compound 3b with paratoulene sulphonyl substitution exhibited excellent activity. Compound 2b also showed similar potency on both the cell lines with IC_{50} value 1.2µM

Table 5: *In vitro* Anti proliferative activity of synthesized compounds (IC_{50} µM)

Entry	Compound	MCF 7	MCF 12
1	2a	50±0.1	64±0.01
2	2b	>100	1.2±0.001
3	2c	74±0	75±0.03
4	3a	1.5±0.01	1.5±0.01
5	3b	1.2±0.02	1.2±0.03
6	3c	1.6±0.02	18±0.01
Standard	dabrafenib	0.001	0.001

4. CONCLUSION

In conclusion, the present study highlights the treatment of breast cancer by avoiding the resistance in the design of novel dual HER-2 and PI3K inhibitors by the multidimensional approach. Some new molecular frame works were designed for dual inhibition using computational and medicinal chemistry tools. From the designed compounds, two series of analogues were synthesized and tested for anticancer activity. The results showed that among the two series of compounds synthesized, 4-amino Thienopyrimidine derivatives (3a-3c) were found to possess excellent anticancer potency than the 2,4-diamino thienonpyridines. These synthesized compounds 2a, 2b, 2c, 3a, 3b and3c were tested against human breast cancer cell lines and showed better antiproliferative activity by comparing with standard drug debrafenib whose IC_{50} value was 0.001µM.

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Conflict of interest

None declared

Source of Funding

None declared

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