Section A-Research Paper
ISSN 2063-5346

DOCKING, SYNTHESIS AND EVALUATION OF ANTICANCER ACTIVITY OF NOVEL PYRIMIDINE DERIVATIVES

Vidya K. Magar[1, 2*], Lalit Sonawane [1], Shailesh **Patwekar[1]**

1. School of Pharmacy, Swami Ramanand Teerth Marathwada University, Vishnupuri, Nanded, Maharashtra, India-431606.
2. BGPS's, Srinath College of Pharmacy, P-119, Bajaj Nagar, MIDC Waluj, Aurangabad, Maharashtra, India - 431133

Address for Correspondence:

Mrs. Vidya K. Magar, ***(Asst. Professor)***

Dept. of Pharmaceutical Chemistry,

BGPS's, Srinath College of Pharmacy,

Aurangabad, Maharashtra, India - 431133

E-Mail: vidyamagar087@gmail.com

Mobile No.: +918237509537.

DOI: 10.48047/ecb/2023.12.si4.1614

ABSTRACT

In the present work we have designed and synthesized novel pyrimidine derivatives by condensing Acetyl benzimidazole with various aromatic and heteroaromatic aldehyde and cyclized into pyrimidine derivatives by reacting with Guanidine HCl. These synthesized pyrimidine derivatives screened for anticancer activity. Among the 12 Pyrimidine derivatives, four compounds shown IC_{50} values in the range 15.45-40.08 µg/ml range and found more potent than standard anticancer drug 5-Flurouracil which shown IC_{50} value 41.56 µg/ml on the MCF-7 cell line. Among 12 pyrimidine derivatives, IC 23 $^{rd.}$ pyrimidine derivative shown IC_{50} Value 15.45 µg/ml on the MCF-7 cell line which demonstrate promising anticancer activity. Molecular docking study also performed on telomerase active pocket to find out favourable interactions of pyrimidine derivatives, result shown that all compounds have good binding interaction with receptor.

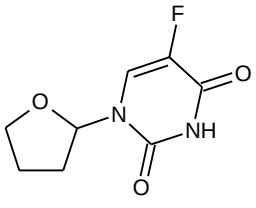
Keywords: Anticancer drug, Pyrimidine derivative, Telomerase enzyme

Section A-Research Paper
ISSN 2063-5346

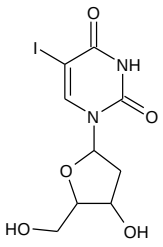
INTRODUCTION

Cancer is disorder in which there is abnormal cell growth that arise from genetic or epigenetic modification in somatic cells which has abnormal cell growth and may be spread to other body parts.[1]. Only 5-10% of all cancer cases are due to genetic defects and remaining 90-95% cause is environment and life style. In lifestyle factor mainly include cigarette smoking, diet, alcohol, stress and physical inactivity[2]. Cancer is a major cause of death in world, there are nearly 10 million deaths in 2020.[3] and current medicinal therapy suffer from many drawbacks and so there is need for development of new and efficient drug therapy which can overcome draw backs of conventional therapy. Pyrimidine is six membered heterocycle rings containing two nitrogen at 1 and 3 position, it is widely observed in nature and are building blocks of nucleic acid like DNA and RNA in form of cytosine, thymine, and uracil. Pyrimidine possesses various biological activities such as antiallergic antibacterial and antifungal activity, antihypertensive, cardiotonic, antiasthmatics, bronchodilator or antitumor activity.[4] In biological activity consideration, fused heteroaromatic system are much greater than the monocyclic compound. Fused pyrimidine is versatile nucleus because of its broad biological potential[5]. Benzimidazole nucleus contains benzene ring fused with imidazole ring it has similarity with Purine and therefore they can show interaction with biomolecules and possesses wide range of activities like anti-bacterial, anti-cancer, anti-fungal anti-histaminic.[6-7]

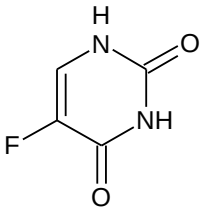
Various potent drugs containing Pyrimidine nucleus are available in market for example Tegafur, Fluorouracil, Methotrexate (As the anticancer agents) Broxuridine, Idoxuridine (As the antiviral agents) Trapidil, Dipyridamole (as the vasodilators)[8]



Tegafur



Idoxuridine



Flurouracil

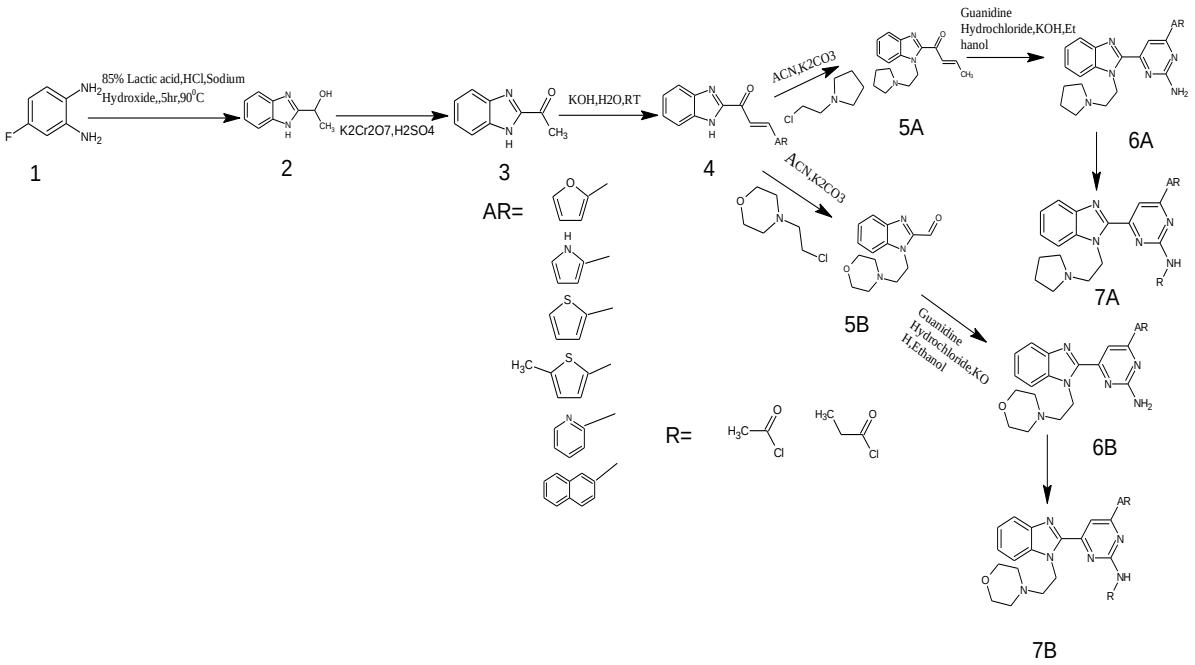
From literature survey it is found that Benzimidazole nucleus condensed with pyrimidine shown potent anticancer activity.[9] So, we have made conjugate of benzimidazole and

pyrimidine nucleus to get adductive efficacy and to reduce side effects of conventional anticancer agents.

MATERIALS AND METHODS

In present work we have synthesized 12 novel pyrimidine derivatives by condensing acetyl benzimidazole with various aromatic and antiaromatic aldehyde in presence of sodium hydroxide and cyclized by reacting with guanidine HCl. These all-pyrimidine derivatives then screened for anticancer activities and molecular docking study also performed on telomerase enzyme.

Scheme for Synthesis:



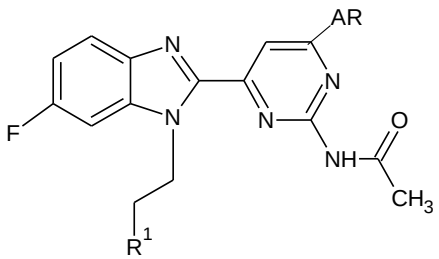
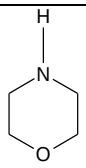
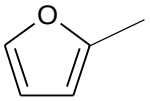
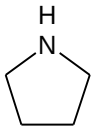
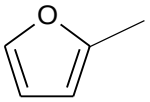
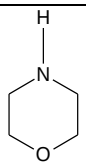
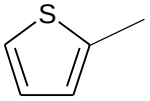
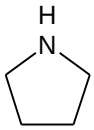
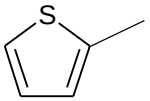
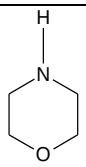
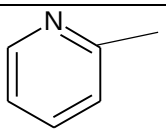
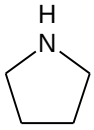
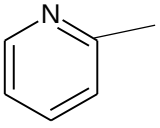
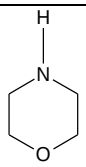
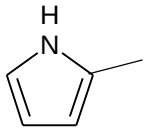
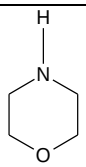
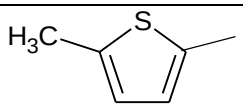
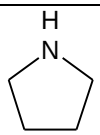
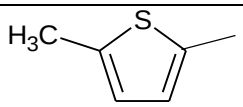
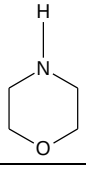
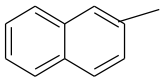
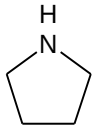
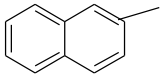
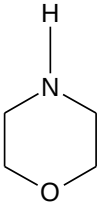
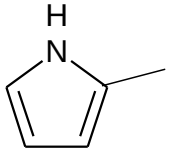


Table 1: Novel Pyrimidine Derivatives

Sr. No	Compound Code	R^1	R^2
1.	**IC-13**		
2.	**IC-14**		
3.	**IC-15**		
4.	**IC-16**		
5.	**IC-17**		
6.	**IC-18**		
7.	**IC-19**		
8.	**IC-21**		
9.	**IC-22**		
10.	**IC-23**		

11.	**IC-24**		
12.	**IC-25**		

SYNTHESIS

Synthesis of 1-(6-Fluoro-1*H*-benzo[*d*]imidazol-2-yl)ethanol

4-Fluro o-Phenylenediamine (5.05g,40 mmol) is transferred to the round bottom flask and added lactic acid (3.96g,44 mmol) then to above stirred solution was added hydrochloric acid (4.0 N, 25 mL) then this mixture is refluxed for 16 hours, reaction is monitored with TLC. After completion of the reaction, reaction mixture is neutralized with sodium hydroxide solution and recrystallize to obtain (6.49 g) in 90% yield as a greenish-brown solid.

Synthesis of 1-(6-Fluoro-1*H*-benzo[*d*]imidazol-2-yl)ethanone

1-(6-Fluoro-1*H*-benzo[*d*]imidazol-2-yl) ethanol (8.1 g, 50 mmol) was transferred in conical flask then added H2SO4 (5%; 40 ml), mixture stirred for five minutes and to this solution added dropwise with stirring a solution of Potassium Dichromate (19.8 g, 150 mmol) in aq. H2S04 (25%, v/v; 80 ml) at RT over a period of 20 min. Stirring continued at RT for 2 h. The orange solid was separated and washed with water, then suspended in water (50 ml) and neutralized with aq. NH_3 to a pH of 6.0-10.5. The separated solid was washed with water, dried (5.76 g, 72%) and crystallized from boiling ethyl acetate to obtain pure off-white solid.

Synthesis of Benzimidazole-Chalcone Derivatives:

1-(6-Fluoro-1*H*-benzo[*d*]imidazol-2-yl) ethanone (0.02 mol) were dissolved in ethanol (20 ml) and added sodium hydroxide (10%, 8ml) and Heteroaromatic or aromatic aldehyde (0.02 mol) to it. reaction mixture was stirred at room temperature for 5 hours to obtain product.

Synthesis of N-Pyrrolidine or N-Morpholine Benzimidazolyl-Chalcone Derivatives:

Benzimidazole-chalcone (1.0 equiv) and acetonitrile (10 ml) were transferred in round bottom flask then potassium carbonate (5.0 equiv) was added and it stirred for 10 minutes and

2-Chloroethyl morpholine hydrochloride (1.1 equiv.) or 2-Chloroethyl Pyrrolidine hydrochloride (1.1 equiv) transferred to the reaction mixture and refluxed for 6 after completion of reaction stick mass was partitioned between water and ethyl acetate. The organic layer was then separated and concentrated under chromatography to obtain the desired compound.

Benzimidazole Pyrimidine Conjugate:

To a previously stirred solution of N-Pyrrolidine or N-Morpholine Benzimidazolyl-Chalcone (1.0 equiv.) and guanidine hydrochloride (1.5 equiv.) in ethanol (10 mL) was added a solution of sodium hydroxide (2.0 equiv.) in water (1 mL) at room temperature. The resulting reaction mixture was heated to reflux for 3 h. After 3 h, the reaction mixture was cooled down to room temperature and poured into ice-cold water to obtain the brown precipitates. These precipitates are filtered and washed with water. The resulting solid was dried under reduced pressure to obtain the desired compound.

Synthesized N-Acetyl or Propionyl Benzimidazole Pyrimidine derivatives:

Acetyl chloride (1 equi.) was added to stirring solution of Benzimidazole Pyrimidine (1 equi) and potassium carbonate in dry acetone (25 mL). The reaction mixture was subsequently stirred at room temperature for 5 h and quenched with water (5 mL) leading to a precipitation of the product. The reaction mass was filtered to obtain the crude product which was purified by column chromatography and offered the final compound.

FINAL COMPOUND ANALYSIS

1. **N-(4-(6-Fluoro-1-(2-morpholinoethyl)-1H-benzo[d]imidazol-6-yl)-10-(furan-6-yl) pyrimidin-6-yl) acetamide:**

Reaction mixture of 4-(6-Fluoro-1-(2-morpholinoethyl)-1*H*-benzo[*d*]imidazol-2-yl)-6-(furan-2-yl) pyrimidin-2-amine (1.22 mmol) and potassium carbonate (3.66 mmol) was prepared in dry acetone (25 mL). Acetyl chloride (1.22 mmol) was added to above solution with continuous stirring. The reaction mixture was subsequently stirred at room temperature for 5 h and quenched with water (5 mL) leading to a precipitation of the product. The reaction mass was filtered to obtain the crude product which was purified by column chromatography and offered the final compound IC-13 (0.21 g, 38 % yield) as a white solid. It was confirmed by IR, NMR and Mass spectroscopy.

IR:3260,2812,1682,1587,1537,1468,1407,1303,1251,1110,1018$^{(-cm)}$

NMR:2.2(s,7H),3.1(s,4H),3.3(s,2H),5.2(t,2H),6.8(m,1H),7.2(t,1H),7.5(d,1H),7.7(d,1H),7.8(q,1H),8.0(s,1H),8.2(s,1H),10.9(s,1H)

Mass: 451.40

2. N-(4-(6-Fluoro-1-(2-(pyrrolidin-1-yl)ethyl)-1H-benzo[d]imidazol-6-yl)-10-(furan-6-yl)pyrimidin-6-yl)acetamide

Acetyl chloride (1.27 mmol) was added in the solution of 4-(6-Fluoro-1-(2-(pyrrolidin-1-yl)ethyl)-1*H*-benzo[*d*]imidazol-2-yl)-6-(furan-2-yl)pyrimidin-2-amine(1.27 mmol) and potassium carbonate (3.81 mmol) in dry acetone (25 mL) with continuous stirring, The reaction mixture was subsequently stirred at room temperature for 5 h and quenched with water (5 mL) leading to a precipitation of the product. The reaction mass was filtered to obtain the crude product which was purified by column chromatography and offered the final compound IC-14 (0.22 g, 37% yield) as a cream-white solid. It was confirmed by IR, NMR and Mass spectroscopy.

IR:2995,1678,1604,1537,1483,1444,1410,1373,1297,1221,1173,1010

NMR:1.4(s,4H),2.2(s,3H),2.3(s,4H),2.7(t,2H),5.1(t,2H),6.7(1H),7.1(t,1H),7.4(m,1H),7.6(m,1H),7.7(m,1H),8.0(s,1H),8.1(s,1H),10.8(s,1H)

Mass:435

3. N-(4-(6-Fluoro-1-(2-morpholinoethyl)-1H-benzo[d]imidazol-6-yl)-10-(thiophen-6-yl)pyrimidin-6-yl)acetamide

Acetyl chloride (1.18 mmol) was added to the reaction mixture containing 4-(6-Fluoro-1-(2-morpholinoethyl)-1*H*-benzo[*d*]imidazol-2-yl)-6-(thiophen-2-yl)pyrimidin-2-amine(1.18 mmol) and potassium carbonate (03.54 mmol) in dry acetone (25 mL). The reaction mixture was subsequently stirred at room temperature for 5 h and quenched with water (5 mL) leading to a precipitation of the product. The reaction mass was filtered to obtain the crude product which was purified by column chromatography and offered the final compound IC-15 (0.19 g, 35 % yield) as a white solid

IR:3350,3061,2950,2820,1715,1588,1539,1496,1467,1433,1398,1333,1281,1247,1188,1108,1063$^{(-cm)}$

NMR:2.2(d,7H),3.1(s,4H),3.3(s,2H),5.2(s,2H),7.1(t,1H),7,3(t,1H),7.6(d,1H),7.8(q,1H),7.9(d, 1H),8.2(d,1H),8.3(s,1H),10.8(s,1H)

Mass:467.40

4. N-(4-(6-Fluoro-1-(2-(pyrrolidin-1-yl)ethyl)-1H-benzo[d]imidazol-6-yl)-10-(thiophen-6-yl)pyrimidin-6-yl)acetamide

Acetyl chloride (0.096 g, 1.22 mmol) was added to stirring solution containing 4-(6-Fluoro-1-(2-(pyrrolidin-1-yl)ethyl)-1*H*-benzo[*d*]imidazol-2-yl)-6-(thiophen-2-yl)pyrimidin-2-amine (1.22 mmol) and potassium carbonate (3.66 mmol) in dry acetone (25 mL). The reaction mixture was subsequently stirred at room temperature for 5 h and quenched with water (5 mL) leading to a precipitation of the product. The reaction mass was filtered to obtain the crude product which was purified by column chromatography and offered the final compound IC-16 (0.19 g, 35 % yield) as a white solid:

IR:3063,2383,2311,1717,1671,1582,1538,1432,1387,1294,1174$^{(-cm)}$

NMR:1.5(s,4H),1.9(s,2H),2.2(s,3H),2.5(s,2H),3.3(s,2H),5.1(s,2H),7.2(t,1H),7.27(t,1H),7.7(d, 1H),7.8(q,1H),7.9(d,1H),8.2(d,1H),8.3(s,1H),10.8(s,1H)

Mass:451.400

5. N-(4-(6-Fluoro-1-(2-morpholinoethyl)-1H-benzo[d]imidazol-6-yl)-10-(pyridin-6-yl)pyrimidin-6-yl)acetamide

Acetyl chloride (1.19 mmol) was added to the reaction mixture containing 4-(6-Fluoro-1-(2-(2-morpholinoethyl)-1*H*-benzo[*d*]imidazol-2-yl)-6-(pyridin-2-yl)pyrimidin-2-amine(1.19 mmol) and potassium carbonate (3.57 mmol) in dry acetone (25 mL). The reaction mixture was subsequently stirred at room temperature for 5 h and quenched with water (5 mL) leading to a precipitation of the product. The reaction mass was filtered to obtain the crude product which was purified by column chromatography and offered the final compound IC-17 (0.15 g, 28 % yield) as a white solid:

IR:3034,2882,2820,1683,1627,1585,1543,1476,1419,1369,1328,1286,1174,1120$^{(-cm)}$

NMR:2.2(d,7H),2.5(m,2H),3.1(s,4H)5.2(s,2H),7.2(t,1H),7.6(q,2H),7.8(q,1H),8.1(t,1H),8.4(d, 1H),8.8(d,1H),8.8(s,1H),10.9(s,1H)

Mass: 462.450

6. N-(4-(6-Fluoro-1-(2-(pyrrolidin-1-yl)ethyl)-1H-benzo[d]imidazol-6-yl)-10-(pyridin-6-yl)pyrimidin-6-yl)acetamide

Acetyl chloride (0.097 g, 1.24 mmol) was added to reaction mixture containing 4-(6-Fluoro-1-(2-(pyrrolidin-1-yl)ethyl)-1*H*-benzo[*d*]imidazol-2-yl)-6-(pyridin-2-yl)pyrimidin-2-amine (1.24 mmol) and potassium carbonate (3.72 mmol) in dry acetone (25 mL). The reaction mixture was subsequently stirred at room temperature for 5 h and quenched with water (5 mL) leading to a precipitation of the product. The reaction mass was filtered to obtain the crude product which was purified by column chromatography and offered the final compound IC-18 (0.14 g, 24 % yield) as a white solid:

IR: 3033,2958,2885,1677,1583,1541,1472,1417,1367,1288,1168,1010,877$^{(-cm)}$
NMR:1.4(S,4H),2.5(s,6H),2.7(t,2H),5.2(t,2H),7.2(t,1H),7.6(m,2H),7.8(m,1H),,8.1(t,1H),8.4(d,1H),8.8(d,2H),10.9(s,1H)
Mass:446.450

7. N-(4-(6-Fluoro-1-(2-morpholinoethyl)-1H-benzo[d]imidazol-6-yl)-10-(1H-pyrrol-6-yl)pyrimidin-6-yl)acetamide

Acetyl chloride (1.23 mmol) was added to the solution of 4-(6-Fluoro-1-(2-morpholinoethyl)-1*H*-benzo[*d*]imidazol-2-yl)-6-(1*H*-pyrrol-2-yl) pyrimidin-2-amine (1.23 mmol) and potassium carbonate (3.69 mmol) in dry acetone (25 mL). The reaction mixture was subsequently stirred at room temperature for 5 h and quenched with water (5 mL) leading to a precipitation of the product. The reaction mass was filtered to obtain the crude product which was purified by column chromatography and offered the final compound IC-19 (0.12 g, 22 % yield) as a light brown solid:
IR:3735,3034,2881,2822,2311,1710,1585,1537,1417,1477,1329,1293,1176,1107$^{(cm-)}$
NMR:2.2(m,4H),2.3(s,2H),3(d,1H),3.1(s,3H),3.5(d,1H),3.7(d,1H),3.8(d,1H),5.3(2H),6.2(s,1H),7.1(s,2H),7.2(1H),7.7(m,1H),7.8(m,1),8.2(m,1H),10.59(s,1H).
Mass: 450.4

8. *N*-(4-(6-Fluoro-1-(2-morpholinoethyl)-1*H*-benzo[*d*]imidazol-2-yl)-6-(5 methylthiophen-2-yl)pyrimidin-2-yl)acetamide

Acetyl chloride (1.14 mmol) was added to stirring solution of 4-(6-Fluoro-1-(2-morpholinoethyl)-1*H*-benzo[*d*]imidazol-2-yl)-6-(5-methylthiophen-2-yl) pyrimidin-2-amine (1.14 mmol) and potassium carbonate (3.42 mmol) in dry acetone (25 mL). The reaction mixture was subsequently stirred at room temperature for 5 h and quenched with water (5 mL) leading to a precipitation of the product. The reaction mass was filtered to obtain the crude product which was purified by column chromatography and offered the final compound IC-21 (0.18 g, 34 % yield) as a yellow solid:

IR:3272,2882,1795,1717,1685,1585,1536,1466,1404,1383,1330,1263,1175,1122,1086(cm^{-})
NMR:2.2(s,4H),2.5(d,3H),3.1(s,3H),3.5(s,3H),5.2(s,2H),6.9(s,1H),7.2(s,1H),7.8(s,2H),8.0(d, 1H),8.2(s,1H),10.4(s,1H)
Mass:481.45

9. N-(4-(6-Fluoro-1-(2-(pyrrolidin-1-yl)ethyl)-1H-benzo[d]imidazol-2-yl)-6-(5-methylthiophen-2-yl)pyrimidin-2-yl)acetamide

Acetyl chloride (1.18 mmol) was added to stirring solution of 4-(6-Fluoro-1-(2-(pyrrolidin-1-yl) ethyl)-1*H*-benzo[*d*]imidazol-2-yl)-6-(5-methylthiophen-2-yl) pyrimidin-2-amine
(1.18 mmol) and potassium carbonate (3.54 mmol) in dry acetone (25 mL). The reaction mixture was subsequently stirred at room temperature for 5 h and quenched with water (5 mL) leading to a precipitation of the product. The reaction mass was filtered to obtain the crude product which was purified by column chromatography and offered the final compound IC-22 (0.19 g, 36 % yield) as a yellow solid:
IR:2913,2842,2384,2311,1723,1670,1584,1536,1467,1413,1384,1296,1215,1167,1091,1047, 1008(cm^{-})
NMR:1.5(s,3H),1.9(s,2H),2.2(s,3H),2.5(m,3H),2.6(s,3H),3(s,2H),5.1(t,2H),6.9(s,1H),7.2(t,1 H),7.7(t,1H),7.8(t,1H),8(s,1H),8.2(s,1H),10.8(s,1H)
Mass:465.4

10. N-(4-(6-Fluoro-1-(2-morpholinoethyl)-1H-benzo[d]imidazol-2-yl)-6-(naphthalen-2-yl)pyrimidin-2-yl)acetamide

Acetyl chloride (1.07 mmol) was added to the reaction mixture containing 4-(6-Fluoro-1-(2-morpholinoethyl)-1*H*-benzo[*d*]imidazol-2-yl)-6-(naphthalen-2-yl)pyrimidin-2-amine (1.07 mmol) and potassium carbonate (3.21 mmol) in dry acetone (25 mL). The reaction mixture was subsequently stirred at room temperature for 5 h and quenched with water (5 mL) leading to a precipitation of the product. The reaction mass was filtered to obtain the crude product which was purified by column chromatography and offered the final compound IC-23 (0.21 g, 38 % yield) as a white solid:

IR: 3278,2961,2815,1716,1677,1586,1533,1468,1404,1383,1329,1268,1179,1123

NMR:2.2(d,7H),2.5(m,2H),3.1(s,4H),5.2(s,2H),7.2(t,1H),7.7(m,3H),7.8(m,1H),8.0(m,1H),8.1(m,2H),8.3(d,1H),8.6(s,1H),8.9(s,1H),10.9(s,1H)

Mass:511.450

11. **N-(4-(6-Fluoro-1-(2-(pyrrolidin-1-yl)ethyl)-1H-benzo[d]imidazol-2-yl)-6-(naphthalen-2-yl)pyrimidin-2-yl)acetamide**

Acetyl chloride (0.086 g, 1.10 mmol) was added to the mixture of 4-(6-Fluoro-1-(2-(pyrrolidin-1-yl)ethyl)-1*H*-benzo[*d*]imidazol-2-yl)-6-(naphthalen-2-yl)pyrimidin-2-amine(0.50 g, 1.10 mmol) and potassium carbonate (0.46 gm, 3.30 mmol) in dry acetone (25 mL). The reaction mixture was subsequently stirred at room temperature for 5 h and quenched with water (5 mL) leading to a precipitation of the product. The reaction mass was filtered to obtain the crude product which was purified by column chromatography and offered the final compound IC-24 (0.19 g, 34 % yield) as a light solid:

IR: 3278.2959,2815,2815,1684,1590,1534,1468,1414,1371,1330,1249,1174,1124

NMR:1.4(s,4H),2.2(s,7H),2.7(t,2H),5.2(t,2H),7.2(t,1H),7.6(m,3H),7.8(q,1H),8(d,1H),8.1(d,2H),8.3(d,1H),8.5(s,1H),8.9(s,1H),10.9(s,1H)

Mass: 495

12. N-(4-(1-(2-Morpholinoethyl)-1H-benzo[d]imidazol-6-yl)-10-(1H-pyrrol-6-yl) pyrimidin-6-yl)propionamide

Propionyl chloride (1.28 mmol) was added to stirring solution of 4-(1-(2-Morpholinoethyl)-1*H*-benzo[*d*]imidazol-2-yl)-6-(1*H*-pyrrol-2-yl) pyrimidin-2-amine (1.28 mmol) and potassium carbonate (3.84 mmol) in dry acetone (25 mL). The reaction mixture was

subsequently stirred at room temperature for 5 h and quenched with water (5 mL) leading to a precipitation of the product. The reaction mass was filtered to obtain the crude product which was purified by column chromatography and offered the final compound IC-25 (0.17 g, 29 % yield) as a brown solid:

IR:3735,3278,2961,2816,2311,1680,1587,1536,1467,1409,1307,1176,1112.1020

NMR:2.3(m,2H),2.6(m,3H),3(d,1H)3.1(s,2H),3.5(t,1H),3.7(d,1H),3.8(s,1H),4,2(t,1H),5.2(s,1H),5.4(s,1H),6.2(s,1H),7.1(s,2H),7.4(m,3H),7.7(m,2H),7.9(d,1H),8.2(d,1H),10.5(s,2H)

Mass: 446

DOCKING STUDY

Telomer is functional unit which is present at the end of eukaryotic chromosomes, it is required to maintain the integrity and stability of genome. Telomerase is RNA-dependent DNA polymerase that is essential for synthesis of telomers. Telomerase is general in most cancer cells and is critical for cancer cell development. [13] Telomeres and telomerase are important target for anticancer therapy. Telomerase is involved in most cancer cells, Telomerase is highly expressed in human tumors and various tumour-derived cell lines, about 85–90%[14] Similarly, Telomerase is predominantly expressed in breast cancer patients (86%) [15]

Molecular docking studies were carried out on Schrodinger suite 2012 was used in the study

Protein Crystal structure

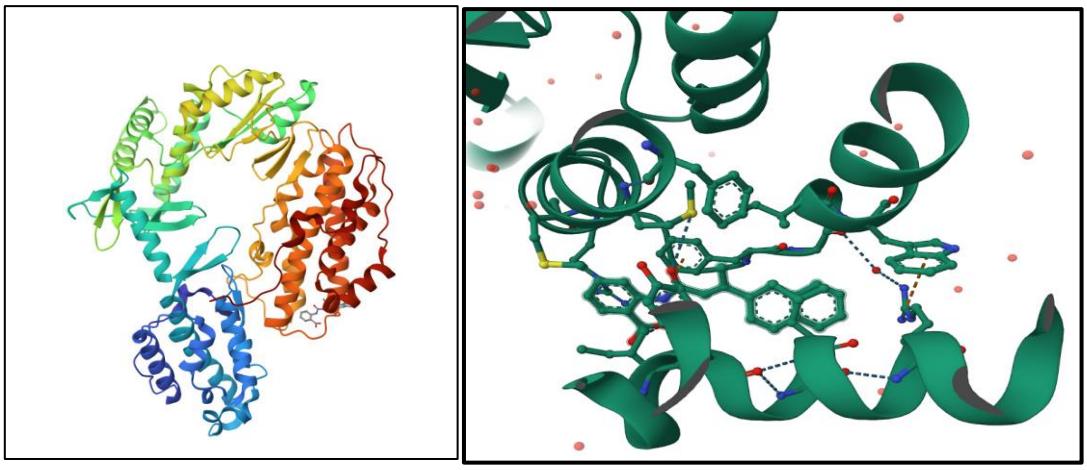


Fig. 1: 5CQG Structure of Tribolium telomerase in complex with the highly specific inhibitor BIBR1532

RESULTS AND DISCUSSIONS

Table No: 2 Docking score of Pyrimidine Derivatives

Sr. No	Ligand ID	Docking score
1.	IC 13	-10.257
2.	IC 14	-9.275
3.	IC 15	-8.448
4.	IC 16	-8.39
5.	IC 17	-7.767
6.	IC 18	-8.729
7.	IC 19	-9.444
8.	IC 21	-8.89
9.	IC 22	-9.393
10.	IC 23	-9.97
11.	IC 24	-8.975
12.	IC 25	-8.623
	Native ligand	**-9.148**

5CQG

12 Novel pyrimidine derivatives ligands were docked against protein Structure of Tribolium telomerase in complex with the highly specific inhibitor BIBR1532, (PDB ID: 5CQG). Out of the tested compounds, docking score of native ligands was found to be **-9.148**. It was clearly indicated that the binding ability of the most active compound 13 was found to be more when compared to the native ligand. Molecular Docking Scores and Residual Amino Acid Interactions of top three Compounds against Binding Domain is given in below **Table 3.**

Sr. No.	Ligand id	Docking score	Interacting amino acid with type of interaction
1	13	-10.257	ARG 486 (H-Bond), ILE 550(H-Bond)
2	23	-9.993	PHE 494 (H-Bond), PHE 494(Pi-Pi stacking), TYR 551(Pi-Pi stacking)
3	19	-9.444	PHE 494(H-Bond) PHE 494 (Pi-Pi stacking), TYR 551(Pi-

			Pi stacking)

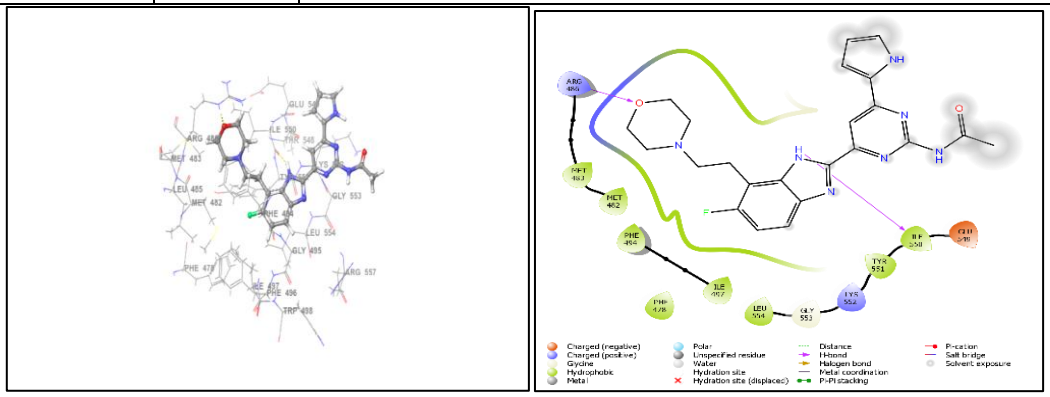


Fig.2: 3D and 2D interaction diagram for compound 19 bound to active site of 5CQG

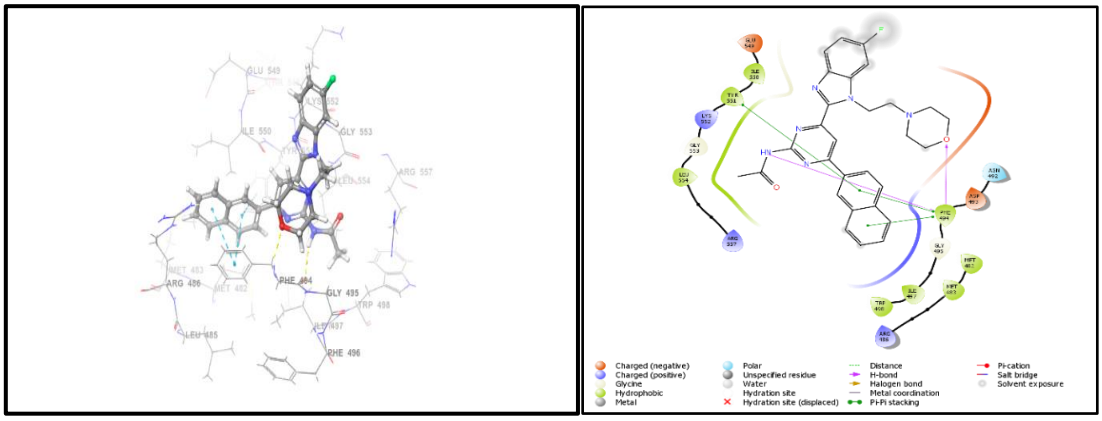


Fig. 3: 3D and 2D interaction diagram for compound 23 bound to active site of 5CQG

In another method the selected 12 molecules were docked into the crystal structure of the Structure of Tribolium telomerase in complex with the highly specific inhibitor BIBR1532 (PDB ID: 5CQG) which is a key enzyme in anti-cancer drug development. It was observed that these molecules when docked into the binding pocket of the selected protein, compound 13 exhibited greatest binding affinity followed by compound 23,19. Compound 13,23,19 were found to have better docking score when compared to co-crystallized ligand.

ANTICANCER ACTIVITY

All the novel Pyrimidine derivatives were evaluated against human breast adenocarcinoma (MCF-7), cell lines.it is compared with the 5-Fluro Uracil (a standard antitumor drug) and cytotoxicity were screened under identical conditions. MTT (3-(4,5-dimethylthiazol-6-yl)-6,5-diphenyl tetrazolium bromide) assays were performed and used to calculate the IC_{50} value. The IC_{50} value (the dose required to causes a 50% reduction in the survival value)

found via MTT assay is considered to evaluate the potential anticancer activity of compounds. The IC_{50} values of synthesized pyrimidine derivatives are summarized in **Table 4**.

Among the 12 benzimidazole derivatives, five compounds shown IC_{50} values in the range 15.45-28.19 µg/ml range and found more potent than 5-Flurouracil which shown IC_{50} value 41.56 µg/ml and few compounds in the 38.43- 42.48 µg/ml range on the MCF-7 cell line. Among 12 pyrimidine derivatives, IC-23rd pyrimidine derivative shown IC_{50} Value 15.45 µg/ml on the MCF-7 cell line which demonstrated promising anticancer activity.

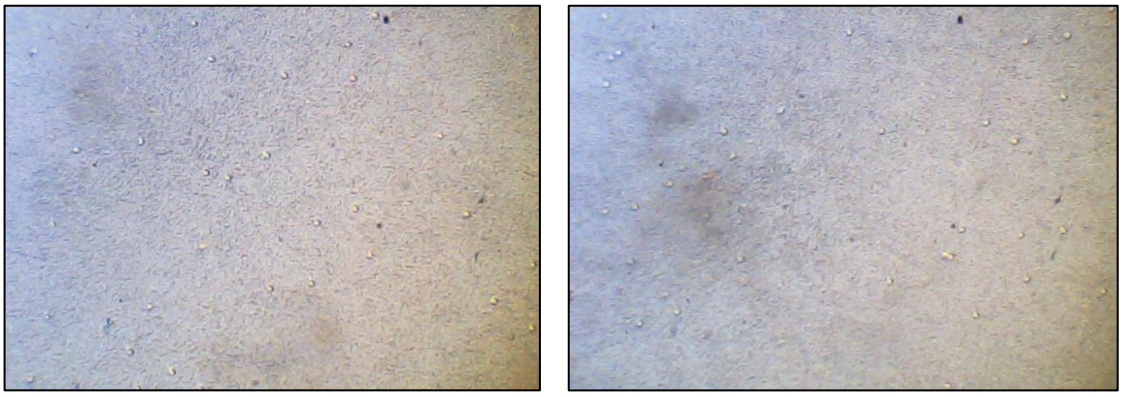


Fig.5: Cytotoxic effect of IC 23rd and IC 17th pyrimidine Derivatives

Table 4: Effects of compound against MCF-7 (Breast Cancer cell line) by MTT assay

Sr. No	Sample ID	IC_{50} (µg/ml))
1.	IC 13	44.13
2.	IC 14	45.65
3.	IC 15	61.06
4.	IC 16	46.26
5.	IC 17	24.15
6.	IC 18	24.15
7.	IC 19	41.52
8.	IC 21	40.08
9.	IC 22	116.60
10.	IC 23	15.45
11.	IC 24	50.28
12.	IC 25	49.74
13.	Std. 5 FU	41.56

CONCLUSION

Novel pyrimidine derivatives condensed with Benzimidazole Nucleus were synthesized, characterized, and evaluated for their in vitro anticancer activity against MCF-7 cell line.

Compounds IC 23 shown highest Anticancer activity against MCF-7 cell lines compared to 5-Flurouracil. The incorporation of N-Morpholine and Naphthalene in pyrimidine and Benzimidazole conjugate was found to significantly enhance the anticancer activity where compound IC-17 and IC-18 also found more cytotoxic compared to the standard drug 5-Fluro uracil. Further, molecular docking study demonstrated that Pyrimidine derivative IC 23 showed best docked score with anticancer potency. It is suggested from results that Pyrimidine-Benzimidazole conjugate might be suitable candidates for further chemical modification in order to obtain more potent and selective anticancer agents.

ACKNOWLEDMENTS:

I wish to thank the Director and Teachers of School of Pharmacy, S.R.T.M. University, Nanded for continues help and encouraging discussions.

I am thankful to the Management and Principal (Dr. Santosh Shelke) of BGPS's, Srinath College of Pharmacy, Aurangabad for providing facilities for my research work.

REFERENCES

1. Saini A, Kumar M, Bhatt S, Saini V, Malik A. Cancer causes and treatments. International Journal of Pharmaceutical Sciences and Research. 2020; 11 (7):3121-74.
2. Anand P, Kunnumakara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB. Cancer is a preventable disease that requires major lifestyle changes. Pharmaceutical research. 2008 Sep; 25:2097-116.
3. World Health Organization, 3 February 2022.
4. Bhat AR. Biological activity of pyrimidine derivativies: a review. Organic & Medicinal Chemistry International Journal. 2017; 2(2):23-10.
5. Prabhakar V, Babu KS, Ravindranath LK, Latha J. Design, synthesis, characterization and biological activity of novel thieno [2, 3-d] pyrimidine derivatives. Indian J. Adv. Chem. Sci. 2017; 5(1):30-82.
6. Foye WO. Foye's principles of medicinal chemistry. Lippincott williams & wilkins; 2008.
7. Rang H. HP Rang, M. Maureen Dale. Rang & Dale's Pharmacology. 7th ed. Edinburgh: Elsevier Churchill Livingstone. 2012:113.

8. Selvam TP, James CR, Dniandev PV, Valzita SK. A mini review of pyrimidine and fused pyrimidine marketed drugs. Research in Pharmacy. 2015 Oct 29; 2(4).

9. Hsieh CY, Ko PW, Chang YJ, Kapoor M, Liang YC, Chu HL, Lin HH, Horng JC, Hsu MH. Design and synthesis of benzimidazole-chalcone derivatives as potential anticancer agents. Molecules. 2019 Sep 6; 24(18):3259.

10. Abdel-Mohsen HT, Ragab FA, Ramla MM, El Diwani HI. Novel benzimidazole–pyrimidine conjugates as potent antitumor agents. European journal of medicinal chemistry. 2010 Jun 1; 45(6):2336-84.

11. Anand K, Wakode S. Synthesis, characterization and biological evaluation of benzimidazole derivatives. International journal of pharmaceutical sciences and research. 2018 Feb 1; 9(2):617-64.

12. Kumar Ganesan ID and Baojun Xu,Telomerase Inhibitors from Natural Products and Their Anticancer Potential, Int. J. Mol. Sci. 2018, 19, 13; doi:10.3390/ijms19010013.

13. Cong YS, Wright WE, Shay JW. Human telomerase and its regulation. Microbiol Mol Biol Rev. 2002 Sep; 66(3):407-65, table of contents. doi: 10.1128/MMBR.66.3.407-825.2002.

14. Oztas, E.; Kara, H.; Kara, Z.P.; Aydogan, M.U.; Uras, C.; Ozhan, G. Association between human telomerase reverse transcriptase gene variations and risk of developing breast cancer. Genet. Test. Mol. Biomarkers 2016, 20, 459–464.

15. Hiyama, E.; Hiyama, K. Telomere and telomerase in stem cells. Br. J. Cancer 2007, 96, 1020–1024.

16. Salib SB. Synthesis and antitumor activity of novel thienopyrimidine derivatives containing thiosemicarbazide moiety. Open Access Library Journal. 2016; 3(07):1.

17. Tylińska B, Wiatrak B, Czyżnikowska Ż, Cieśla-Niechwiadowicz A, Gębarowska E, Janicka-Kłos A. Novel pyrimidine derivatives as potential anticancer agents: Synthesis, biological evaluation and molecular docking study. International Journal of Molecular Sciences. 2021 Apr 7; 22(8):3825.

18. Mahapatra A, Prasad T, Sharma T. Pyrimidine: A review on anticancer activity with key emphasis on SAR. Future Journal of Pharmaceutical Sciences. 2021 Jun 19; 7(1):123.

19. Sridhar S, Prasad YR, Dinda SC. Synthesis and anticancer activity of some novel pyrimidine derivatives. International Journal of Pharmaceutical Sciences and Research. 2011 Oct 1; 2(10):2562.

20. Zhou W, Zhang W, Peng Y, Jiang ZH, Zhang L, Du Z. Design, synthesis and anti-tumor activity of novel benzimidazole-chalcone hybrids as non-intercalative topoisomerase II catalytic inhibitors. Molecules. 2020 Jul 12; 25 (14):3180.

21. Elbouhi M, Badaoui H, Ouabane M, Alaoui MA, Koubi Y, Mokhlis Y, ElKamel K, Lakhlifi T. Anti-Tumor Activity of Novel Benzimidazole-Chalcone Hybrids as non-Intercalative Topoisomerase II Catalytic Inhibitors: 2D-QSAR study. RHAZES: Green and Applied Chemistry. 2022 Mar 4; 14:62-75.