Newer 1,2,3 Triazole Appended Piperazine: Molecular docking, ADME studies, Synthesis, Anti-Microbial andInvitro Anti-cancer studies.

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ABSTRACT

Drug discovery and development is a time-consuming, interdisciplinary and expensive process. Advances in computational procedures have empowered *in silico* routines, and specifically structure based drug design technique, to accelerate new target choice through the recognizable proof of hits for the improvement of lead compounds in the medication discovery process. Hence, the present work aims to identify the potent 1,2,3 triazole appended piperazine compounds for synthesis. In-silico design of novel analogues were carried out using Auto Dock Vina,Swiss ADME software was used to analyse 'Lipinski Rule of Five' and drug likeness properties. Ten derivatives which obeyed the rule of five and having desired physio-chemical properties and highest docking score were synthesized The synthesis has been carried out in two step process to determine their Anti-microbial and Invitro Anti-cancer activity.

KEW WORDS:Docking; 3PPO;Benzotriazole; Piperazine; Amines; Antibacterial; Anticancer activity; ADME.

INTRODUCTION:

1,2,3 triazole are of great importance in medicinal chemistry and can be used for the synthesis of numerous heterocyclic compounds with different biological activities such as, Anti-microbial[1,2,3,4] Anti hiv[5], Antibacterial [6,7,8] ,Anticancer [9,10,11,12] Anti-proliferative agents[13,14] Anti

tubercular activity [15,16] Anti-oxidant[17] Anthelmintic [18], Antipsychotic [19] ,Antimalarial [20]. Mannich bases is a beta-amino ketone [21,22,], which is formed by nucleophilic addition reaction of an amine, formaldehyde (or an aldehyde) and a carbon acid [23] . The literature survey had demonstrated that Mannich bases are very reactive so it has been utilized for development of Nitrogen containing mixes. Furthermore, triazole can be found in a variety of naturalgoods, metabolic products of fungus and primitive marine creatures etc. Because of their importance in industry, agriculture, andbiological activity.We synthesize a group of compounds containing 1,2,3 -Triazole derivatives in coordination with piperazine associated with various primary aromatic amines (Table1) and to evaluate their antibacterial potency and Invitro Anti-cancer activity. Insilico design were carried out for fifteen derivatives using software Auto Dock Vina , by using pdb id: 3QTK,3PP0,4ZAUand compared with standard drug ciprofloxacin and dauxorubicin . Ten derivatives which have highest docking score (Table2,Table3,Table4)were synthesized (Table-5) and their Structures were elucidated with FTIR ,^{1}H NMR,^{13}C NMR, MASS and elemental analysis. Antibacterialactivity was observed in the synthesized compounds by using disc diffusion method, among this compound 1A,2A,5A,12A shows significant anti-bacterial activity and compound 4A &13A shows appreciable anti-bacterial activity in *E.coli* and 2A & 5Ashows appreciable activity in *streptococcus*(Table6).Invitro Anti-cancer screening were conducted against a two different cancer cell line, breast cancer MCF7 , and colon cancer CaCO2. The results of this investigation showed that compounds 2A and 6A have anticancer activity against the cancer cell lines MCF-7 and CACO2 (Table7,Table8, Table9).ADME properties and drug-likeness prediction were carried out using Swiss ADME(Table-10).

1.EXPERIMENT SECTION:

Materials And Methods:

All the chemicals utilized in this study were obtained from vasa chemicals Malleshwaram,Bangalore. FTIR spectra were recorded on ABB Bomem FTLA 2000-102 FTIR instrument involving KBr pellets in the 400-4000 cm-1. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 300 (300 MHz) and Bruker 600MHz. The compound relocations are given in parts per million (ppm) involving TMS as interior norm at 300 and 75 MHz separately.

Synthesis of 1-((4-nitrophenyl) piperazin-1-yl)methyl)-1*H* -benzo(*d*)(1,2,3)-triazole(Compound A) [(24)]:

Benzotriazole (0.01Mol) was dissolved inethanol, and added to para nitro Benzaldehyde (0.05mol), and Piperazine (0.01mol) until the mixture completely dissolved. The reaction mixture was heated

to reflux for 8 hr at room temperature 27°C.The precipitate was filtered and recrystallized with suitable solvent (DMF & Ethanol).The reaction was confirmed by TLC using ethyl acetate& n-hexane (3:7).Light yellow colour, M.P .88ºC ,Yield 90%.

Synthesis of 1- (4-(1*H* - benzo(*d*) (1,2,3)-triazole-1-yl) (4-nitrophenyl)methyl) piperazin-1-yl) - *N* –benzylmethanamine (Compound 1A):

The mixture of compound A (0.01Mol) was dissolved in ethanol, and added to formaldehyde (0.05mol), and Benzyl amine (0.01Mol) until the mixture completely dissolved. The reaction mixture was heated to reflux for 8 hr at room temperature 27°C. The precipitate was filtered and recrystallized with suitable solvent (DMF & Ethanol).The reaction was confirmed byby TLC using ethyl acetate& n-hexane (3:7).

2.BIOLOGICAL ACTIVITY:

2.1Anti bacterial-activity[25,26,27]

The antibacterial activity of synthesized compounds was done by using disc diffusionmethodagainst that followingorganism as directed byEllen JBoron

E.coli-Gramnegative

Streptococcus - Gram positive

TestSample: 1A,2A,4A,5A,6A,11A,12A,13A,14A,15A

Preparationoftest and standard solutions:

The test sample 1A,2A,4A,5A,6A,11A,12A,13A,14A,15A were used in concentration of 100mg/ml, using dimethyl sulfoxide assolvent and ciprofloxacin in concentration, 50mg/ml usin DMSO as solvent.

PreparationofNutrient Agar:

Peptone	0.5%
Sodiumchloride	0.5%
Beefextract	0.5%
Agar	3.0%
Distilledwater	q.s
Phadjusted	7.2-7.4

Thenthe mediais distributedin 5mlquantityintocultureflask andsterilized byautoclaving.

DiscDiffusion Method:

To the sterile nutrient agar, suspension of *Escherichia coli*&*Streptococcus* was added at 45 degree Celsius andtransferred to sterile petri dish and allowed to solidify. Sterile discs of 5 mm in

diameter was madeusing Whatmann filter paper and sterilized. They were loaded with test compound and standard, Then they were placed on the surfaceofagarmedium. The plates were left standing for one hour at room temperature as a period of pre incubationdiffusiontominimizetheeffectofvariationintimebetweentheapplicationsofdifferentsolutions.Then the plates were incubated at 37 degree Celsius for 18 hours and observed for antibacterialactivity. The zone of inhibition was measured and tabulated.

2.2 Invitro Anticancer Activity:[28,29,30,31,32,33]

Cell lines used in the study:

(1) MCF7 (human breast cancer cell line)

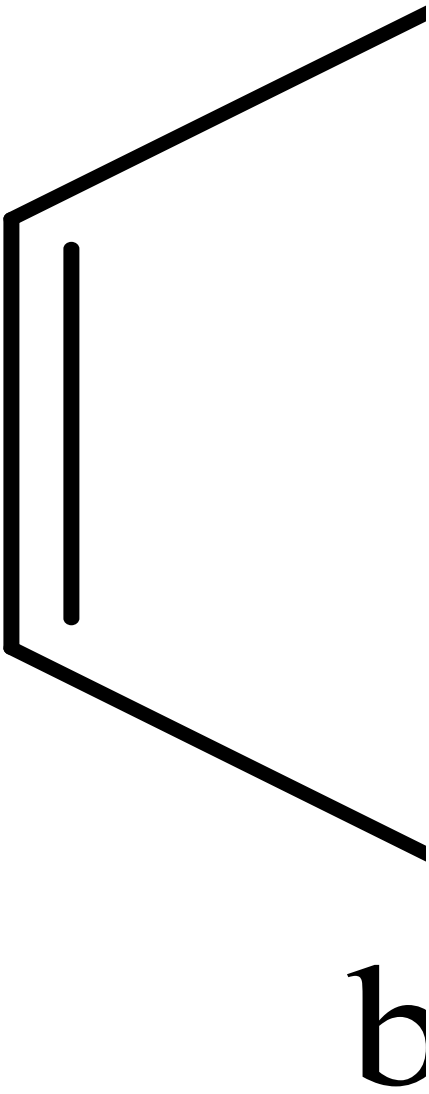
(2) CACO2 (human intestinal epithelial cell line)

Seeded cells in 96-well plates containing a final volume of 100µl/well with a cell density of 10,000 cells/well.Cells were treated with different concentrations of the given compound upon attainment of 75% cell confluency.The plates were kept in the incubator for 24, 48 and 72 hours. After that 100µl of 0.5mg/ml MTT solution was added.Again the plates were kept in the incubator for 3 to 4 hrs at 37°C. Then 100 µl of DMSO was added to each well to dissolve formazan crystals and mixed to ensure complete solubilization. The percentage viability was calculated by measuring the absorbance at 570 nm.

% viability = ((OD of sample-OD of Blank)/(OD of untreated-OD of blank))*100

Cell Culture Media: DMEM with 10% FBS (MCF7) and MEM with 20% FBS (CACO2)

3.SCHEME



Compounds 1-15A

Table-01 List of aromatic primary amines used in the synthesis

Sl No	Compounds	R	Sl No	Compounds	R
1	1A		7	7A	
2	2A		8	8A	
3	3A		9	9A	
4	4A		10	10A	
5	5A		11	11 A	
6	6A		12	12 A	

Sl No	Compounds	R1	Sl No	Compounds	R1
13	13 A		15	15 A	
14	14 A				

4.MOLECULAR DOCKING[34,35,36,37,38,39,40]

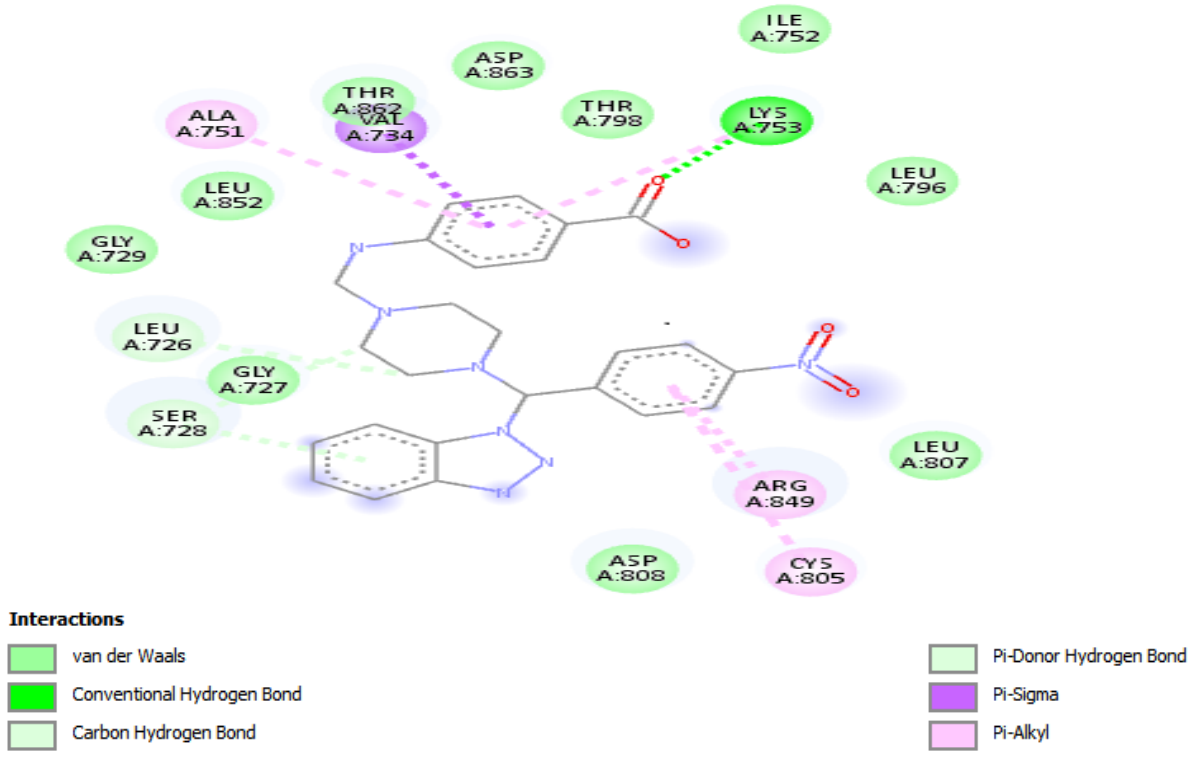
Before the docking analysis, ligands were prepared from the optimized Compounds and saved in pdb file format using spartan,14 .The 3D compound of VEGF, HER-2, EGFR protein were, downloaded from the protein bank (with pdb ID:3QTK,3PPO, 4ZAU).The enzyme was prepared with help of discovery studio visualizer for the docking analysis. In the course of the preparation, hydrogen was added, water molecule, heteroatoms and co-ligands were eliminated from the crystal Compound saved in pbd file.

The docking of the ligands to the active site was achieved with the help of pyrex software using Autodock vina. After successful docking protocol, reformation of the complexes (ligand-receptor) for further investigation was also achieved utilizing chimera software. Discovery studio visualizer and pyMOL were used to investigate the interactions of the complexes.

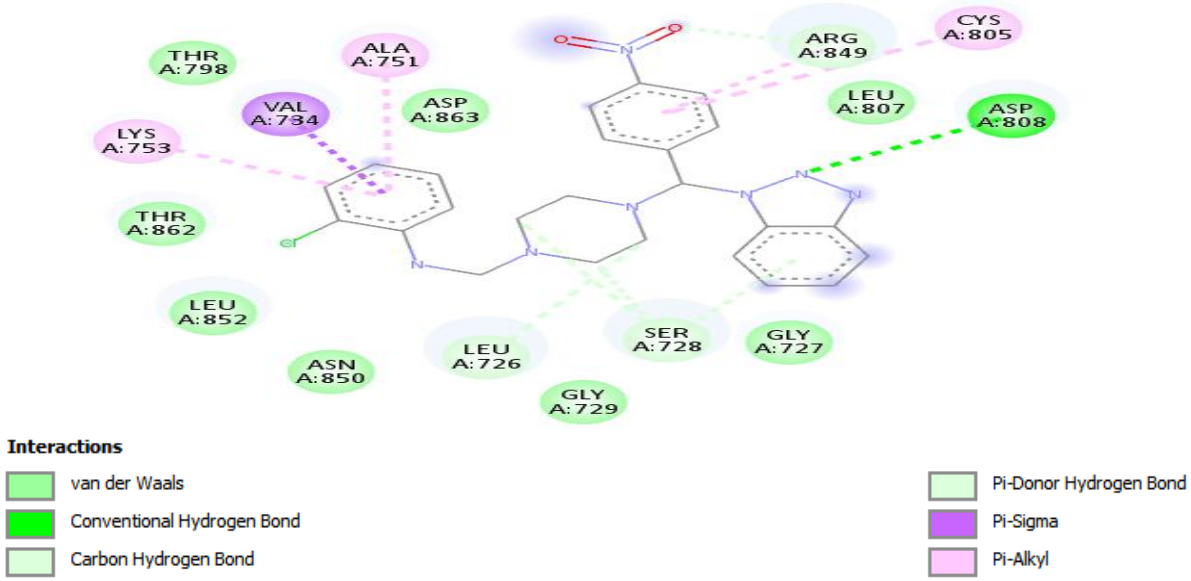
4.1 Table -02Docking And Glide Score Of 3PPO(1-15A)

Sl no	Compound Code	3PPO	Amino acids involved in ligand binding
1.	1A	-7.8	Ser,Arg,Val,Ala,Thr
2.	2A	-8.5	Ala,Val,Lys,Arg,Cys
3.	3A	-6.7	Ser,Arg,Val, Ala
4.	4A	-7.7	Gln,Tyr,Val,Val,Ala
5.	5A	-8.8	ASP,ASP ARG,Cys,Val,Lys,Leu,Ser
6.	6A	-8.4	Val,Lys,Ala,Arg,Cys,Asp
7.	7A	-6.8	Arg,Ser,Cys
8.	8A	-6.9	Leu,Ser,Arg
9.	9A	-5.2	Ala,Leu,Lys
10.	10A	-6.7	Val,Thr,Ala,Lys
11.	11A	-9.7	Arg,Ser,Cys,Arg,Leu,Val,Thr,Ala,Lys
12.	12A	-8.6	Lys,Val,Ala,Leu,Ser,Arg
13.	13A	-8	Leu,Val,Ala,Leu,Ser,Arg
14.	14A	-8.5	Val,Ala,Leu,Lys,Cys,Arg
15.	15A	-8.2	Val,Lys,Leu,Arg,Asp

COMPOUND 2A



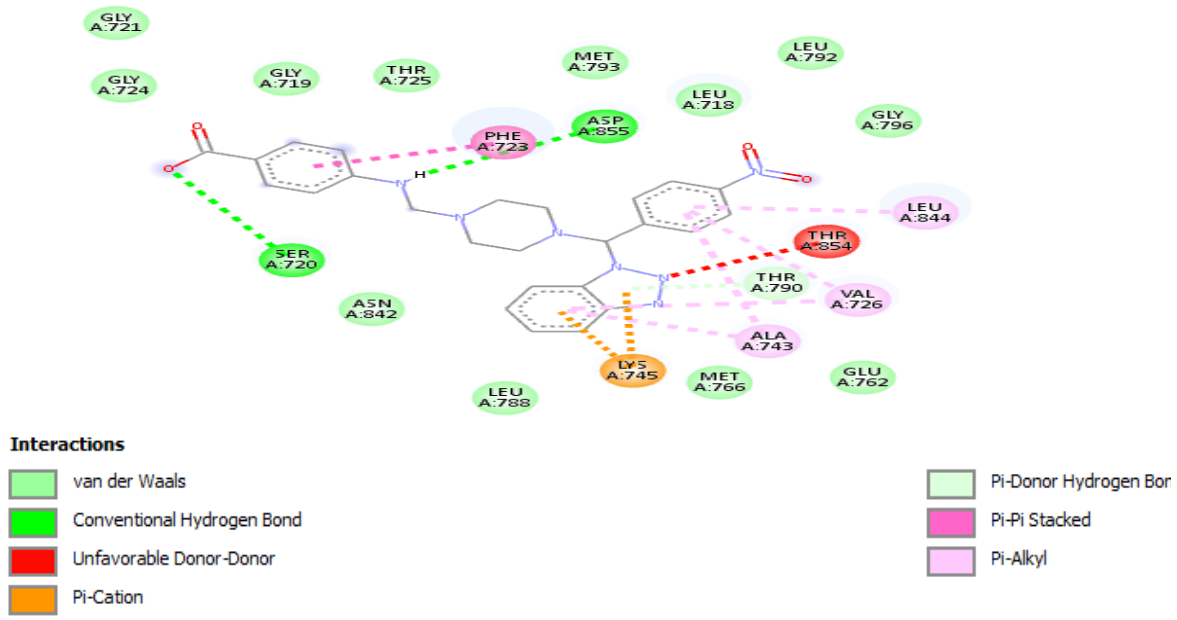
COMPOUND 6A



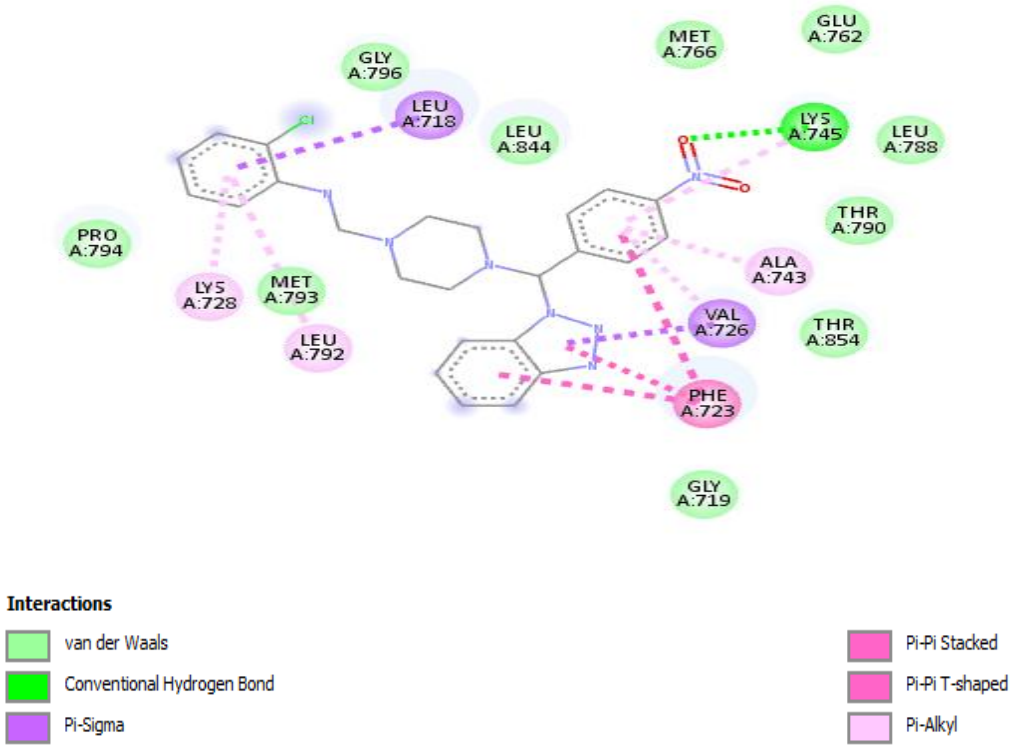
4.2 Table- 03Docking And Glide Score Of 4ZAU (1-15a)

Sl no	Compound Code	4ZAU	Amino acids involved in ligand binding
1.	1 A	-9.4	Phe,Lys,Ala,Thr,Val,Leu,Met,Leu
2.	2 A	-10.2	Ser,Phe,Asp,Thr,Lys,Ala,Val,Ala
3.	3A	-6.5	Asp,Thr,Lys,Ala
4.	4A	-8.7	Gly,Phe,Asp,Arg,Sas,Lys,Thr,Val
5.	5A	-9.9	Lys,Leu,Met,Lys,Val,Phe,Ala
6.	6A	-9.8	Leu,Val,Phe,Ala,Lys
7.	7A	-5.8	Sas,Lys,Thr,Val
8.	8A	-6.2	Lys,Leu,Met
9.	9A	-6.7	Val,Lys,Ala
10.	10A	-5.9	Met,Leu,Lys
11.	11A	-9.8	Met,Leu,Lys,Phe,Val,Lys,Ala
12.	12A	-10.1	Lys,Leu,Met,Phe,Val,Ala,Lys
13.	13A	-9.7	Leu,Gly,Pro,Phe,Val,Ala,Cys,Pro,Lys
14.	14A	-9.9	Leu,Lys,Phe,Val,Lys,Als,Val
15.	15A	-10	Phe,Met,Val,Ala,Lys,Leu

COMPOUND 2A



COMPOUND 6A

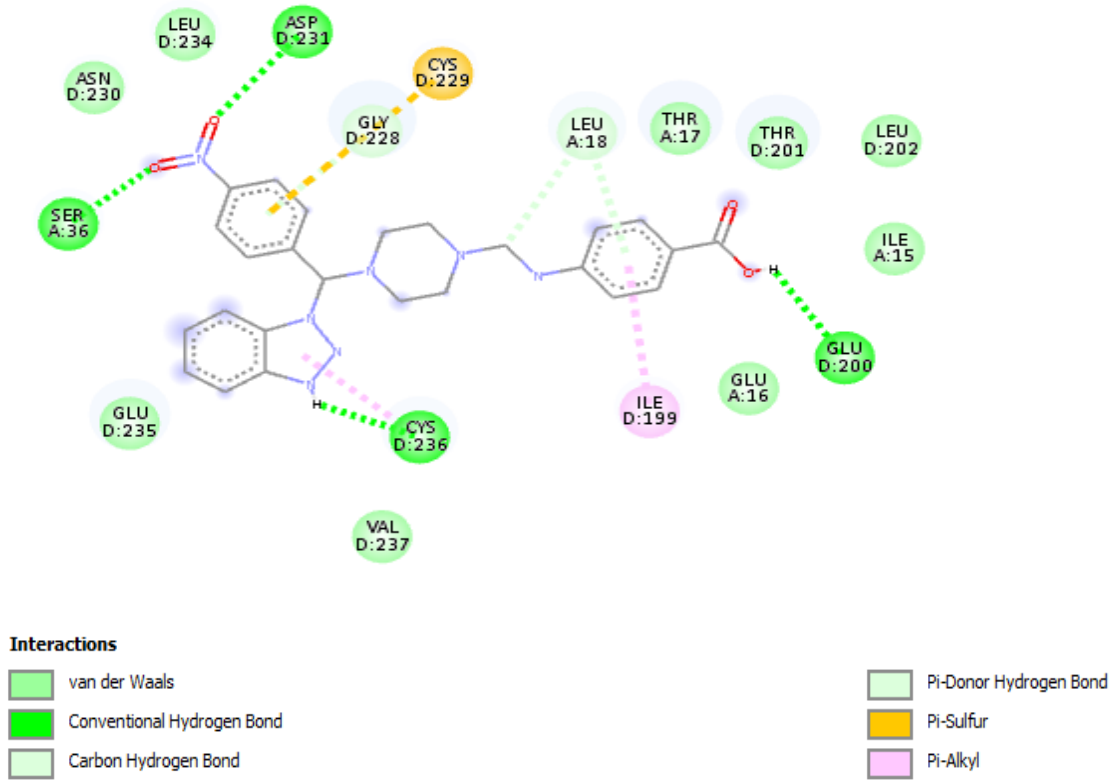


4.3 Table-04Docking And Glide Score Of 3qtk(1-15A)

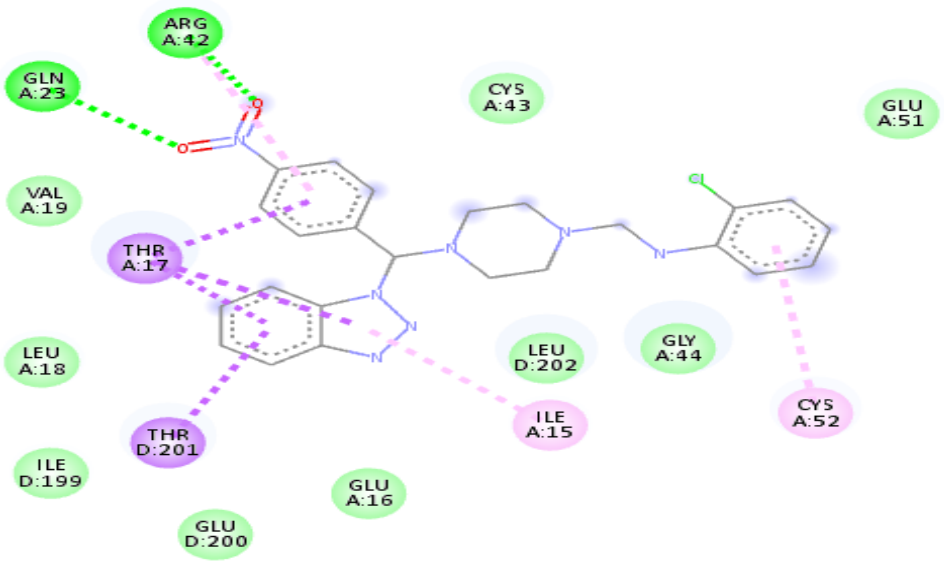
Sl no	Compound code	3QTK	Amino acids involved in ligand binding
1.	1A	-8.1	Arg,Gln,Thr,Ile,Gly,Cys,Ile
2.	2A	-8.4	Asp,Ser,Cys,Glu,Ile
3.	3A	-7.0	Arg,Gln,Thr
4.	4A	-8.2	Asp,Ser,Lys
5.	5A	-7.9	Gly,Thr,Lys,Ile,Arg
6.	6A	-8.1	Arg,Gly,Thr,Ile,Cys
7.	7A	-6.5	Thr,Glu,Ile,Cys
8.	8A	-6.8	Thr,Glu,Ile
9.	9A	-5.8	Gly,Asp,Cys,Ile
10.	10A	-6.1	Thr,Arg,Gln
11.	11A	-8.3	Arg,Thr,Glu,Ile,Cys
12.	12A	-8.2	Asp,Thr,Arg,Gln,
13.	13A	-8.1	Thr,Gln,Arg,Ile
14.	14A	-8.1	Thr,Ile,Gln,Arg,Cys

15.	15A	-8.3	Ser,Gly,Asp,Cys,Ile,Leu

COMPOUND 2A



COMPOUND 6A



5. RESULT AND DISCUSSION: The synthesized compounds were structurally elucidated using FTIR ,^{1}H NMR,^{13}C NMR and MASS. The spectral details of the synthesized compounds were given below.

1- (4-(1*H* - benzo(*d*) (1,2,3)-triazole-1-yl) (4-nitrophenyl)methyl) piperazin-1-yl) - *N* – benzylmethanamine (Compound 1A):

M.P .82ºC ,Yield 86%,Mol Formula: $C_{25}H_{27}N_7O_2$, Mol Wt: 457.22, Elemental Analysis C,65.63;H,5.95:N,21.43;O,6.99. IR (CH) bending 736, (NO) 1597 (N=N) 1661, (NH) 1582, (Aromatic) 1660, cm-1.^{1}HNMR(NH) 4.61(CH2) 2.71,(CH)8.0, ^{13}C NMR (CH2)52.8,(CH)119.6(C)140.2, *m/z* 457.22 (BASE PEAK) ION PEAK 458.23

4-(((4-(1*H* - benzo(*d*) (1,2,3)-triazole-1-yl) (4-nitrophenyl)methyl) piperazin-1-yl)methyl)amino)benzoic acid (Compound 2A):

Yellow colour, M.P .160ºC ,Yield 85%, Mol Formula: $C_{25}H_{25}N_7O_4$, Mol Wt: 487.52, Elemental Analysis C,61.59;H,5.17:N,20.11;O,13.13, IR (CH) bending 736, (NO_2) 1660 (N=N) 1661, (NH) 1582, (Aromatic) 1660,(COOH) 3100 cm-1 ^{1}HNMR, (NH) 6.34 (CH2) 2.71,(CH) 8.0 (OH) 12.71 ^{13}C NMR, (CH2) 49.9 (C) 146.2 (CH) 110.0 Benzene , *m/z* 487.20(BASE PEAK) 488.20, 489.20

***N*-((4-((1*H* - benzo(*d*) (1,2,3)triazole-1-yl) (4-nitrophenyl)methyl) piperazin-1-yl)methyl)040methoxyaniline(Compound 4A):**

Slight Yellow crystals, M.P .95ºC ,Yield 90%, Mol Formula: $C_{25}H_{27}N_7O_3$, Mol Wt: 473.54, Elemental Analysis C,63.41;H,5.75:N,20.71;O,10.14, IR (CH) bending 736, (NO) 1597 (N=N) 1661, (NH) 1582, (Aromatic) 1660,(OCH3) 2800 ^{1}HNMR, (NH) 6.34 (CH_2) 2.71,(CH) 8.0 (OH) 12.71 (CH_3) 3.81 ^{13}C NMR, (CH_2) 49.9 (C) 130.4,(C) In benzene 151.7 (CH) 110.0 Benzene , *m/z* 473.22(BASE PEAK) 474.22, 475.22

***N*-((4-((1*H* - benzo(*d*) (1,2,3)triazole-1-yl) (4-nitrophenyl)methyl) piperazin-1-yl)methyl)-4-chloroaniline(Compound 5A):**

Yellow crystals, M.P .86ºC ,Yield 89%, Mol Formula: $C_{24}H_{24}ClN_7O_2$, Mol Wt: 477.17, Elemental Analysis C,60.31;H,5.06:N,20.51; Cl 7.42, O,6.69, IR (CH) bending 736, (NO) 1597 (N=N) 1661, (NH) 1582, (Aromatic) 1660, (Cl) 800 cm^{-1}, ^{1}HNMR, (NH) 6.34 (CH_2) 2.71,(CH_2) methylene(CH) 4.13 (OH) 12.71 (CH_3) 3.81 ^{13}C NMR, (CH_2) 49.9 (CH_2) cyclohexane 52.6 (C) 130.4, (CH) 129.7 Benzene , *m/z* 477.17(BASE PEAK) 479.17, 478.17

***N*-((4-((1*H* - benzo(*d*) (1,2,3)triazole-1-yl) (4-nitrophenyl)methyl) piperazin-1-yl)methyl)-2-chloroaniline(Compound 6A):**

Yellowish brown crystals, M.P .65ºC ,Yield 82%, Mol Formula: $C_{24}H_{24}ClN_7O_2$, Mol Wt: 477.95, Elemental Analysis C,60.31;H,5.06: Cl 7.42, N,20.51;O,6.69, IR IR (CH) bending 736, (NO_2) 1650 (N=N) 1661, (NH) 1582, (Aromatic) 1660, (Cl) 800 cm^{-1} ^{1}HNMR, (NH) 5.80, (CH_2) 2.71,(CH_2) methylene 4.13 (CH) 6.11 ^{13}C NMR, (CH_2) 49.9 (CH_2) cyclohexane 52.8 (C) 130.7, (CH) 129.7 Benzene , *m/z* 477.17(BASE PEAK) 479.17, 478.17

***N*- (4-(1*H* - benzo(*d*) 1,2,3-triazole-1-yl) (4-nitrophenyl) piperazin-1-yl) methyl) 4-Nitroaniline(Compound 11A):**

Yellow crystals, M.P .85ºC ,Yield 83%, Molecular formula $C_{24}H_{24}N_8O_4$, IR (CH) bending 736, (CH_3) 1923, (NO) 1597 (N=N) 1661, (NH) 1582, cm-1. Elemental Analysis (C,59.01;H,4.95;N,22.94;0,13.10). ^{1}H NMR 6.602, (m, H), 6.581, (m, H),6.674, (m, H),6.810, (m, H),7.583, (m,H),7.938, (m,H),7.958(m,H), ^{13}C NMR, (CH_2) 49.9 (CH_2) cyclohexane 52.8 (C) 130.7, (CH) 129.7 Benzene *m/z* (161.09 Base peak 484.60(M^+) .

***N*- (4-(1*H* - benzo(*d*) 1,2,3-triazole-1-yl) (4-nitrophenyl) piperazin-1-yl) methyl) -2,4-dimethylaniline (Compound 12A):**

Brown colour, M.P .120ºC ,Yield 85%, Molecular formula $C_{26}H_{29}N_7O_2$ IR - bending CH-aromatic 811,(CH_3)1454, (NO) 1513, (N=N) 1598, (NH) 1627,cm^{-1},Elemental Analysis (C,66.22;H,6.20;N,20.79;0,6.79). ^{1}H NMR 6.991 (m,H),7.066(d,H),7.076(d,H),1.000, (s,3H) 2.292(s,3H),2.317(s,3H),2.493 (s,3H),2.497(s,3H), ^{13}C NMR, (CH_2) 49.9 (CH_2) cyclohexane 49.9 (C) 130.4, (CH) 119.6,(CH3) 17.9. Benzene. *m/z* 196 Base peak ,471.32**,** (M^+) 315.24 (M^++1).

***N*- (4-(1*H* - benzo(*d*) 1,2,3-triazole-1-yl) (4-nitrophenyl) piperazin-1-yl) methyl) -2,6-dimethylaniline(Compound 13A):**

Slighty Yellowish colour, M.P .158ºC ,Yield 87%, Molecular formula $C_{26}H_{29}N_7O_2$, IR (CH)-bending 737,1443,(NO) 1520 (N=N) 1603, (NH) 1644 cm^{-1}. Elemental Analysis

(C,66.22;H,6.20;N,20.79;0,6.79). ^{1}H NMR 2.067, (s,3H)2.500, (s,3H)2.486, (s,3H)6.937, (m,H),6.957, (m,H),6.976, (m,H),7.071, (m,H),7.089(m,H), ^{13}C NMR, (CH_2) 49.9 (CH_2) cyclohexane 52.8 (C) 130.4, (CH3) 17.9, (CH) 119.6 Benzene. *m/z* 215.03 Base peak 459.57(M^+ **)**

***N*- (4-(1*H* - benzo(*d*) (1,2,3) triazole-1-yl)(4-nitrophenyl)methyl) piperazin-1-yl)methyl) -2-methylaniline(Compound 14A):**

Yellow colour, M.P -84ºC ,Yield 85%, Mol Formula: $C_{25}H_{27}N_7O_2$, Mol Wt: 457.54, Elemental Analysis C,60.31;H,5.06: Cl 7.42, N,20.51;O,6.69, IR (CH)-bending 737,1443,(NO) 1520 (N=N) 1603, (NH) 1644, (CH_3)1400 cm^{-1}, ^{1}HNMR, (NH) 5.80, (CH_2) 2.71,(CH_2) methylene 4.13 (CH) 6.11 ^{13}C NMR, (CH_2) 49.9 (CH_2) cyclohexane 52.8 (C) 130.7, (CH) 129.7 Benzene , *m/z* (BASE PEAK) 479.17, 478.17

***N*- (4-(1*H* - benzo(*d*) (1,2,3)-triazole-1-yl) (4-nitrophenyl) piperazin-1-yl) methyl) -4-methylaniline(Compound 15A):**

Orange yellow colour, M.P .65ºC ,Yield 90%,Mol Formula: $C_{25}H_{27}N_7O_2$, Mol Wt: 457.22, Elemental Analysis C,65.63;H,5.95: Cl 7.42, N,21.43;O,6.99, IR (CH)-bending 737,1443,(NO) 1520 (N=N) 1603, (NH) 1644(CH_3)1350 cm^{-1}, ^{1}HNMR, (NH) 6.34, (CH_2) methylene 2.71 (CH)8.00 benztriazole(CH) 8.20 benzene ^{13}C NMR, (CH_2) 49.9 (CH_2) cyclohexane 52.8 (C) 130.7, (CH) 119.6 Benzene , ***m/z***(BASE PEAK) 457.22,458.23,459.23

Table-05 Physio chemical properties of synthesized compound

Sl.No	Structure	M.P	Yield%
1A		82	86

2A		160	85
4A		95	90
5A		86	89
6A		65	82

11A		85	83
12 A		120	85
13A		158	87
14A		84	85

15A		65	90

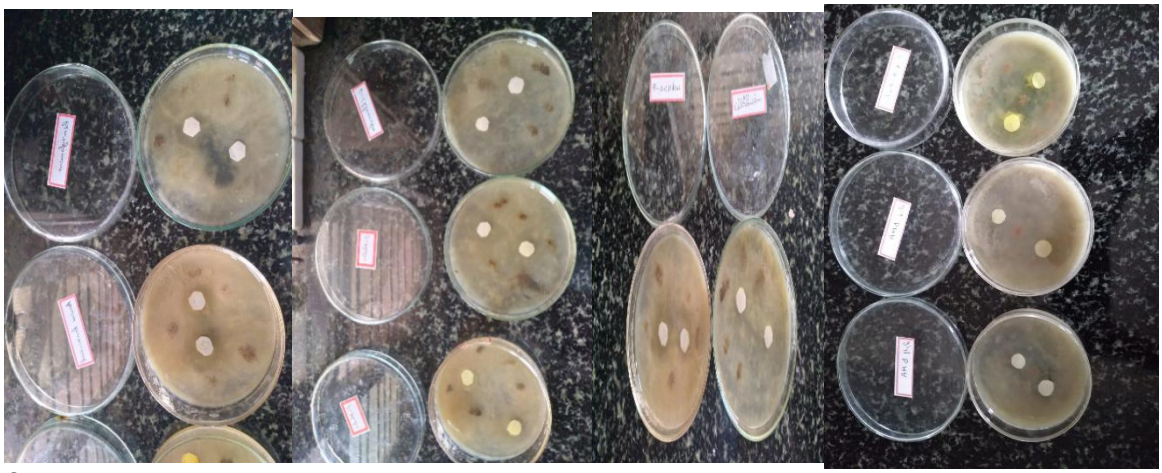
Anti-Bacterial activity and In vitroanticancer studies have been carried out for the following compounds 1A,2A,4A,5A, 6A,11A,12A,13A,14A,15A, in two different Bacteria (E.coli and Streptococcus) and two different cell line MCF7, and CaCO2 . Among the compounds tested, 1A,2A,5A,12A showed significant anti-bacterial activity in E.coli and compounds 2A and 5A also showed appreciable anti-bacterial activity in streptococcuswhencomparedtostandardCiprofloxacin 35 mm.

Compounds 2A,6A exhibited a significant activity against MCF7 cell line with the IC_{50} values of 86.26 and 94.03 µg/mL respectively. Compounds 2A,6A exhibited a significant activity against CaCO2 cell line with IC_{50} value of 108 and 131.3 µg/mL respectively.

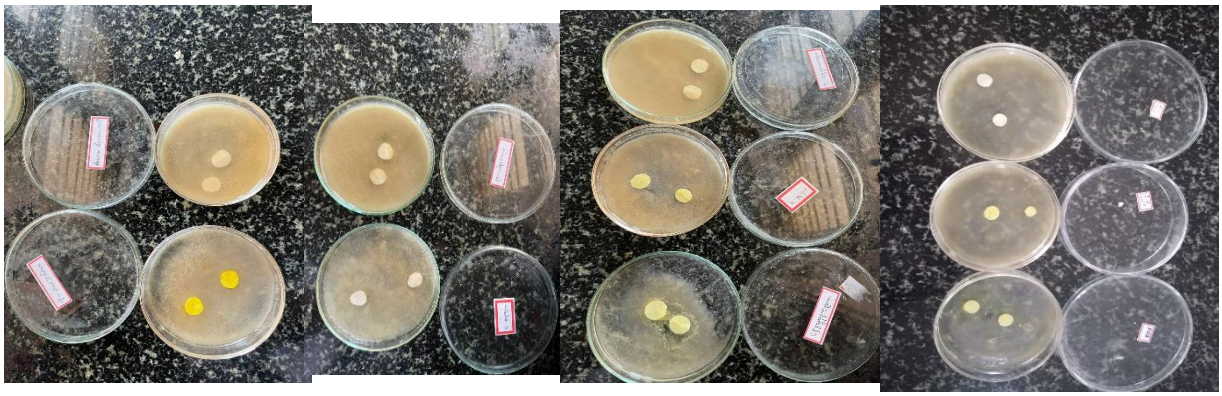
6.ANTIBACTERIALACTIVITY Table-06

Sl/N	**Compounds**	**Antibacterial activity Zone of Inhibition(mm)**	
		Escherichiacoli	*Streptococcus*
1	1A	25mm	15mm
2	2A	23mm	20mm
3	4A	17mm	25mm
4	5A	20mm	18mm
5	6A	15mm	14mm
6	11A	15mm	12mm
7	12A	20mm	10mm
8	13A	17mm	15mm
9	14A	10mm	19mm
10	15A	5mm	12mm
Std	Ciprofloxacin	35 mm.	

E.coli



Streptococcus



7. INVITRO ANTICANCER ACTIVITY

Table-07 MCF7 CELL LINE

%VIABILITY	200µM	100µM	5µM	25µM	12.5µM
Control	100	100	100	100	100
1A	23.9048	145.842	132.4328	104.2826	142.193
2A	20.97797	36.45095	86.21103	81.9797	93.01068
4A	31.77219	67.6065	83.14717	86.78286	88.83333
5A	30.34429	61.81188	67.18029	67.57703	87.95057
6A	19.59464	25.05297	63.14514	84.06038	90.22467
11A	18.78242	70.83273	78.18414	84.62788	95.81247
12A	54.76071	66.1438	80.16527	89.35987	99.74638
13A	20.64666	52.3609	81.17999	99.15667	88.85891
14A	22.34235	78.20146	83.84496	91.73872	105.4892
15A	30.50487	66.67344	75.48849	96.5173	92.50606

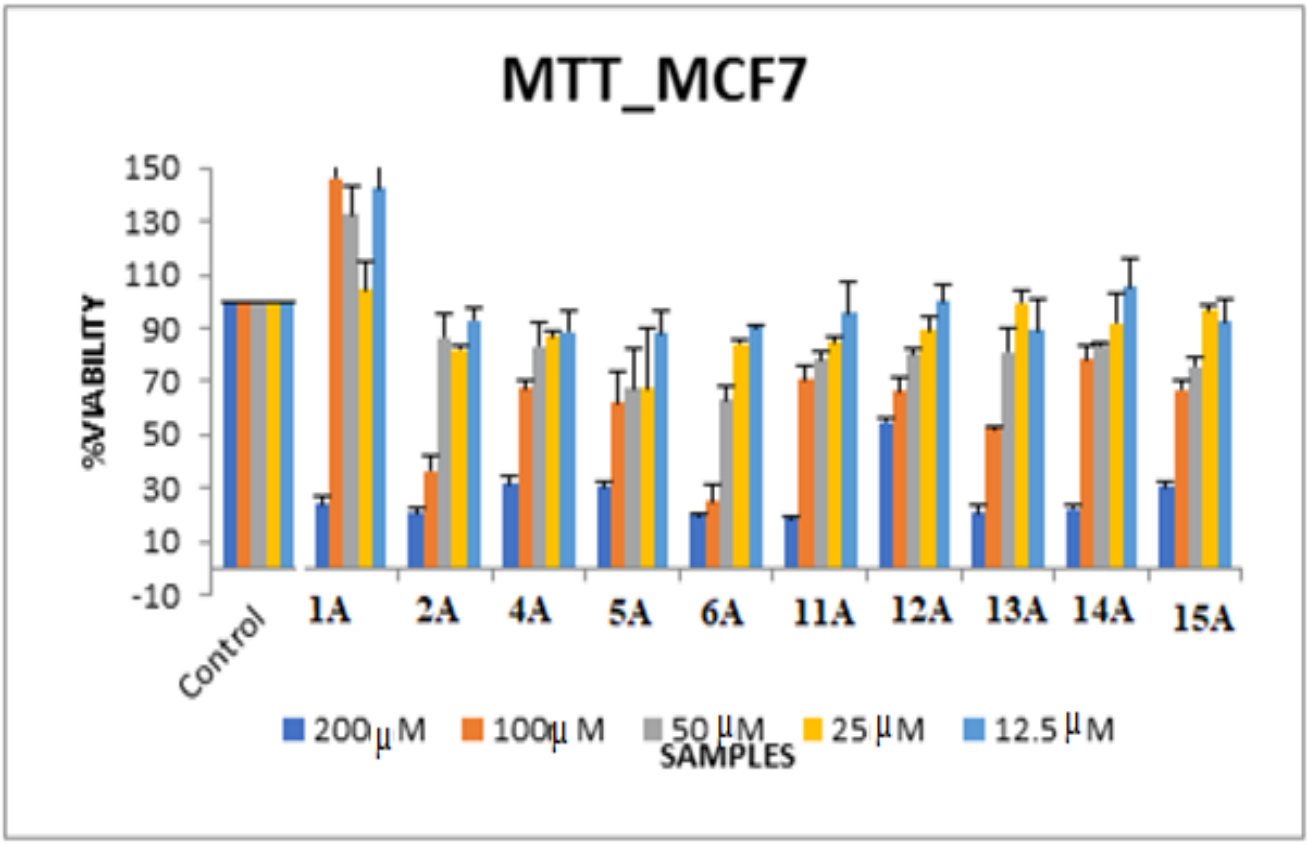
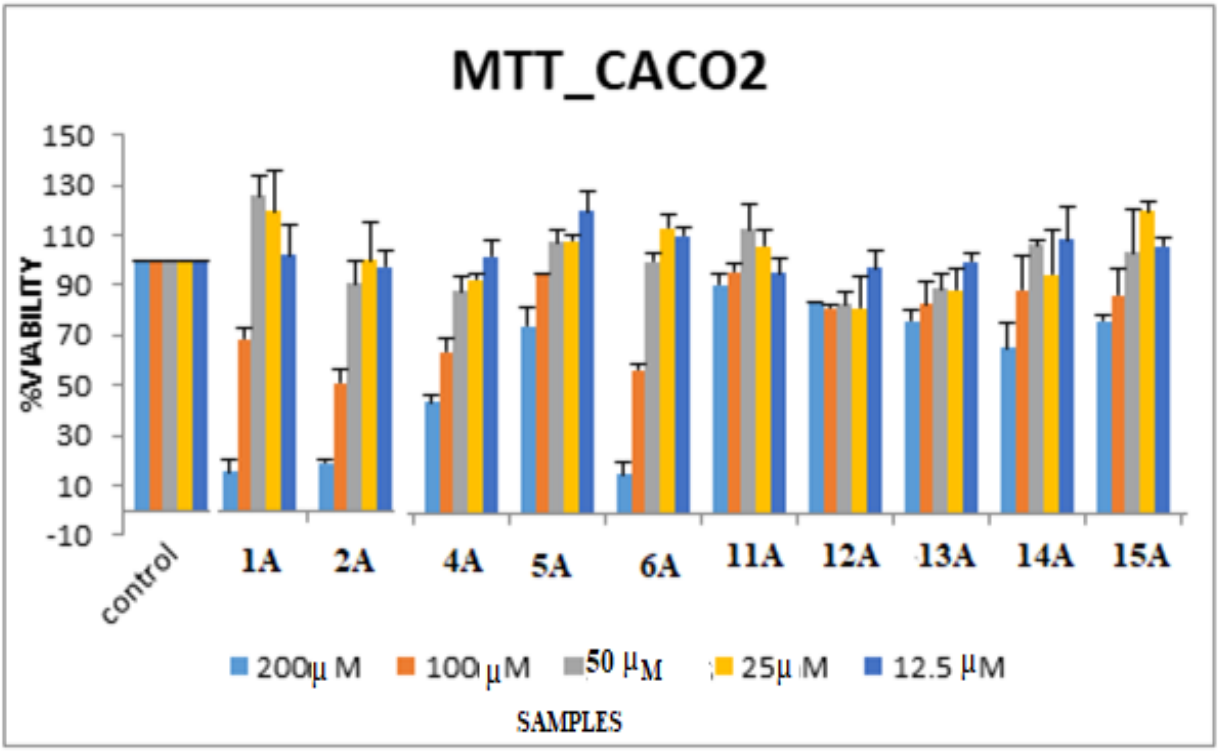


Table-08 CACO2 Cell Lines

%VIABILITY	200µM	100µM	50µM	25µM	12.5µM
control	100	100	100	100	100
1A	15.38221	68.45277	125.9489	119.3699	102.0307
2A	18.85965	50.83663	90.67227	100.0084	97.32367
4A	44.16988	64.49989	88.43283	93.13845	102.3748
5A	74.59459	95.36391	108.0198	108.6756	120.6442
6A	15.57018	57.05358	100.5801	113.7773	110.676
11A	91.22807	96.41764	113.5072	106.7587	95.94889
12A	84.02256	82.02907	83.32667	81.83205	97.88855
13A	76.56642	83.26323	89.94163	89.29973	100.2994
14A	66.00877	89.40368	107.0574	95.54996	109.4692
15A	76.72306	87.18145	104.2131	120.5605	106.829



The IC50 values for MCF7&CACO2Cell Line were displayed below. Table-09

CELL LINE	MCF7	CACO2
COMPOUND CODE	IC50	IC50
1A	191.57	142.62
2A	86.26	108
4A	147.37	170.5
5A	127.8	306.2
6A	94.03	131.3
11A	128.98	813.04
12A	204.09	400.59
13A	122.79	441.9
14A	142.26	280.3
15A	143.32	317.07

6. CONCLUSION:

As a result of the current study, The invitro anti-cancer activity shows that the compounds containing unsubstituted and disubstituted benzyl methyl amine in 4th position of piperazine ring system has no significant activity and mono substituted benzyl methyl amine in 4th position of piperazine ring system shows better cancer cell cytotoxicity. Among the screened compounds, the para acid substituted benzyl and ortho chloro benzyl substitution on the piperazine ring system have found to exhibit better in vitro anticancer activity. Moreover, the anti-bacterial activity of the same compounds has also been proved here, When comparing those two activities it is reveled that the compounds synthesized were more effective as anti-bacterial agents than anti-cancer agents. The results showed that the monosubstituted benzyl aminomethyl piperazine substitutions on N1 triazole imparted more on antibacterial activity. Thus the further substitutions on the N1 triazole systems need to be studied potentially valuable new anti-bacterial leads.

Table-10 ADMET[4]

Sl. No	Molecules	MW	HBD	HBA	GI Absorption	BBB Permeant	Log K_p (skin permeation)	TPSA	Rule of Five
Acceptable range	Acceptable range	130.0 - 725.0	0 - 6	2 - 20	HIGH-LOW	YES - NO	≤ 5	$< 140 Å^2$	Maximum is 4
1.	Compound 1A	457.53 g/mol	1	7	High	No	-6.48 cm/s	95.04 Å²	0
2.	Compound 2A	487.51 g/mol	2	8	High	No	-8.18 cm/s	132.34 Å²	1
3.	Compound 4A	471.55 g/mol	1	6	High	No	-5.91 cm/s	95.04 Å²	0
4.	Compound 5A	491.97 g/mol	1	6	High	No	-5.85 cm/s	95.04 Å²	0
5.	Compound 6A	477.95 g/mol	1	6	High	No	-5.72 cm/s	95.04 Å²	1
6.	Compound 11A	488.50 g/mol	1	8	Low	No	-6.35 cm/s	140.86 Å²	1
7.	Compound 12A	471.55 g/mol	1	6	High	No	-5.61 cm/s	95.04 Å²	0
8.	Compound 13A	471.55 g/mol	1	6	High	No	-5.61 cm/s	95.04 Å²	0
9.	Compound 14A	457.53 g/mol	1	6	High	No	-5.78 cm/s	95.04 Å²	0
10.	Compound 15A	457.53 g/mol	1	6	High	No	-5.78 cm/s	95.04 Å²	0

ACKNOWLEDGMENTS:

The authors are thankful to the management, Director, Principal, and faculties of R. R. College of Pharmacy, Chikkabanavaram, for rendering the facilities to complete my work successfully. Specially I would like to thank Dr. Vachala Pharmaceutical chemistry Dept who guided to complete my work.

CONFLICTS OF INTEREST:

The authors declare no conflict of interest

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