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Piperazine appended 1,2,3 Triazole scaffolds: Drug design by Insilico Studies as validated target for EGFR & Her-2 as Potent Invitro Anti-Cancer and Anti-oxidant activities.

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ABSTRACT

Background: Cancer is major cause of death world wide, though many drugs are available to treat the challenging disease of cancer , mortality rate is high because of the irregular duplication of the cell. Therefore researchers are constantly working on that to produce new drug that can target any aspect of the cancer. Additionally, cell damage caused by free radicals also increases the risk of inflammation and other health issues.

Objectives: Triazoles have both the activity of reducing the abnormal multiplication as well as to reduce oxidative stress by trapping ROS can eliminate the harmful effects of reactive oxygen species, so new series of of 1, 2, 3 triazole appended piperazine compounds were synthesized to evaluate anti-cancer activity as well as anti-oxidant activity. The synthesized compounds were described by IR, NMR,Mass and assessed for invitro Anti-oxidant and Anticancer activity. Thus, the purpose of this work is to synthesize the novel piperazine dependent 1, 2, 3 triazole derivatives and to evaluate the pharmacological studies.

Procedure: Auto Dock Vina was used for in-silico planning of new analogues, and Swiss ADME software were used to assess 'Lipinski Rule of Five' and drug similarity properties. Anticancer action were tested by MTT assay method, and Anti-oxidant activity are determined by DPPH technique.

Results: Out of twenty five derivatives, Fifteen derivatives which obeyed the rule of five and having higher desired physio-chemical properties with highest docking score were synthesized. The synthesis has been carried out by two step process to determine their invitro antioxidant and anti-cancer activity. Among 15 compounds 7B and 25B displayed better anti-oxidant activity (IC 50 = 96.25 and IC 50 = 95.23 µg/ml) when compared with ascorbic standard (IC 50 = 8.4 µg/ml). The 19B and 25B (IC 50 = 52.6 and IC 50 = 54 µg/ml) in MCF cell line, and 15B and 19B (IC 50 = 213.51 and IC 50 = 163.8 µg/ml) in caco2 cell line shows better anti-cancer activity when compared with standard doxorubicin breast cancer (IC 50 = 1.14 µg/ml) and mitomycin C for colon cancer (IC 50 = 7.5 µg/ml).

KEW WORDS: 3PPO; Piperazine; 3QTK; Amines; Anti-oxidant; 4ZAU; Anticancer activity; ADME.

INTRODUCTION:

In the field of restorative research, 1, 2, and 3 triazoles are crucial importance in medicinal chemistry, since they might be used for the synthesis for numerous heterocyclic compounds with various activities, including antimicrobial.[1,2,3,4,5] Antioxidant [3],Anti-tuberculosis [6,7,8,9,10,11,12], Anti-fungal [13] , and Anticancer [14,15,16,17,] , activity. The nucleophilic expansion reaction between an amine, formaldehyde (or aldehyde), and a carbon corrosive produces mannich bases, which are beta-amino ketones. It has been used for the improvement of nitrogen-containing blends because the literature survey shown that Mannich bases are quite sensitive. Additionally, triazole is present in several common products, metabolic byproducts of organisms, primitive marine creatures, and so on. Further of their significance in industry, agri-business, and organic movement. We synthesize the 1,2,3-triazole subsidiaries that function with piperazine linked to many significant aromatic amines (Table 1) and to evaluate their invitro anticancer and anti-oxidant activity. Insilico configuration were done for twentyfive subordinates utilizing programming Auto Dock Vina , by utilizing pdb id: 3PP0, 3QTK, 4ZAU and compared with standard drug doxorubicin and mitomycin-C for Anti-cancer activity and Ascorbic acid for Anti-oxidant action. Out of twenty five derivatives, fifteen derivatives with the highest docking scores (Tables 2, 3, and 4) were synthesized (Table 5), and their structure were elucidated with FTIR, ^{1}H NMR, ^{13}C NMR, MASS, and elemental analysis. Anti-oxidant activity was observed through the use of DPPH method, among that Compounds 7B and 25B, exhibts better anti-oxidant activity (Table-6). Breast cancer MCF7 and colon cancer CaCO2 are two different cell lines used against in vitro anti-cancer activity. Compounds 19B and 25B, as well as 15B and 19B, showed anticancer activity against the cell line MCF-7 and CACO2 respectively, according to the study's findings (Tables 7, 8, and 9). Using Swiss ADME, we predicted drug similarity and ADME characteristics (Table-10).

1.EXPERIMENT SECTION:

Materials And Methods:

We bought all of the synthetic chemicals from Vasa Synthetic Substances in Malleshwaram, Bangalore. Using KBr pellets, ABB Bomem FTLA 2000-102 FTIR spectra were recorded in the 400–4000 cm-1 range. The NMR spectra of the ^{1}H and ^{13}C were recorded using Bruker Avance 300 (300 MHz) and Bruker 600 MHz. TMS is used as the internal benchmark, and the compound migrations are given in parts per million (ppm) at 300 and 75 MHz individually.

Synthesis of 1-((4-nitrophenyl) piperazin-1-yl) methyl)-1H -benzo(d)(1,2,3)-triazole

(Compound A):

Until the mixture was fully dissolved, para nitro benzoaldehyde (0.05mol), piperazine (0.01mol), and dissolved benzotriazole in ethanol (0.01Mol) were added. Reflux heating was used for eight hours to bring the reaction mixture to room temperature (27°C). The precipitate was further filtered and recrystallized with appropriate solvent (DMF and Ethanol).The reaction was confirmed by TLC using ethyl acetate and n-hexane ine the ratio of (3:7) . 90% yield, M.P. of 88ºC, light yellow colour.

Synthesis of substituted triazole and piperazine derivatives(Compound 1-25B):

The mixture of compound A (0.01Mol) and ethanol was mixed with formaldehyde (0.05mol), followed by the addition of the specific amines 1-25B (0.01Mol) until the mixture was completely dissolved. A reflux oven was set at room temperature for 8 hours at 27°C to heat the reaction mixture. The precipitate was isolated and recrystallized using suitable solvents, including dimethyl ether and ethanol and the reaction was confirmed by ethylacetate and n-hexane in the ratio (3:7).

2.BIOLOGICAL ACTIVITY:

2.1 Invitro Anti Oxidant- activity

Free radicals are automatically generated in our body through metabolism which contain one or more unpaired electrons causing the extraction of the electron to attain stability and leads to the damage of that molecule. Excess production of ROS leads to the free radicals and cause tissue damage and different disease. The development of many human diseases, including atherosclerosis, diabetes, hypertension, and AIDS, has been entangled with free radical oxidative pressure[18]. Antioxidant are group of compounds at low concentrations, function similarly to oxidiziable substrates, in that they effectively prevent or postpone oxidative reactions, although they are often oxidised themselves and also it plays the major role protecting our body from cell damage caused by ROS. Cell reinforcements might apply their impact on organic frameworks by various instruments including, metal particle chelation (subsequently killing possible free extremists), saving of cancer prevention agents (co-cell reinforcements)[19]. In order to evaluate a compound's vital properties, the DPPH seeking test is a simple synthetic experiment that requires little time and money and is expected to produce free radical rummaging activity. [20, 21].

Reagents:

(i) DPPH (EEC No.217-591-8,Sigma, USA), store at less than 0º C
(ii) Methanol, HPLC grade (Ranbaxy Chemicals)

Inhibitor (reference standard): Ascorbic aid (1mg/ml)
Preparation of working solutions.

DPPH: 1mg/ dissolved in 6 ml HPLC grade methanol.

Ascorbic aid: 1mg dissolved in 1ml methanol.

Procedure:

The DPPH test is conducted following the protocol laid forth by Rajakumar et al. To summarise, you will need 80 µl of DPPH solution, several concentrations of test solution, and enough HPLC grade methanol to make up 240 µl. The reference standard was evaluated at various concentrations: 0.9, 1.8, 3.7, 7.5, 15, µg/ml. In the test samples, different values between 31.25 and 500 µg/ml and 10-320 µg/ml were examined. A 15-minute incubation period is followed by mixing the reaction mixture at 25°C. At 517 nm, the semi-auto analyzer measures the absorbance. With the test sample removed, a control response is generated.

Statistical evaluation

Half maximum inhibitory concentration (IC50): this is the concentration of a substance required to half inhibit a biological process, such as that of an enzyme, cell, cell receptor, or bacterium. It is tested using a GraphPad prism.

Calculations:

Calculating percentage growth inhibition:

$$\%\ Inhibition = \frac{(OD\ of\ Control - OD\ of\ Sample)}{OD\ of\ Control} \times 100$$

2.2 Invitro Anticancer Activity:

Cell lines used in the study:

(1) MCF7 (human breast cancer cell line)

(2) CACO2 (human intestinal epithelial cell line)

Cells were sown in 96-well plates with a final volume of 100µl/well and a cell density of 10,000 cells/well. Once the cells had grown to 75% of their original size, they were introduced to different amounts of the special drug. The plates were put in the incubator for 24, 48, and 72 hours. Next, 100µl of MTT solution was added, at a concentration of 0.5 mg/ml. The plates were incubated for three to four hours at 37°C once more. Subsequently, 100 µl of DMSO was introduced into each well to dissolve the formazine crystals, and a thorough spinning of the mixture ensured their total dissolution. The percentage of viability was calculated using 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. SCHME:

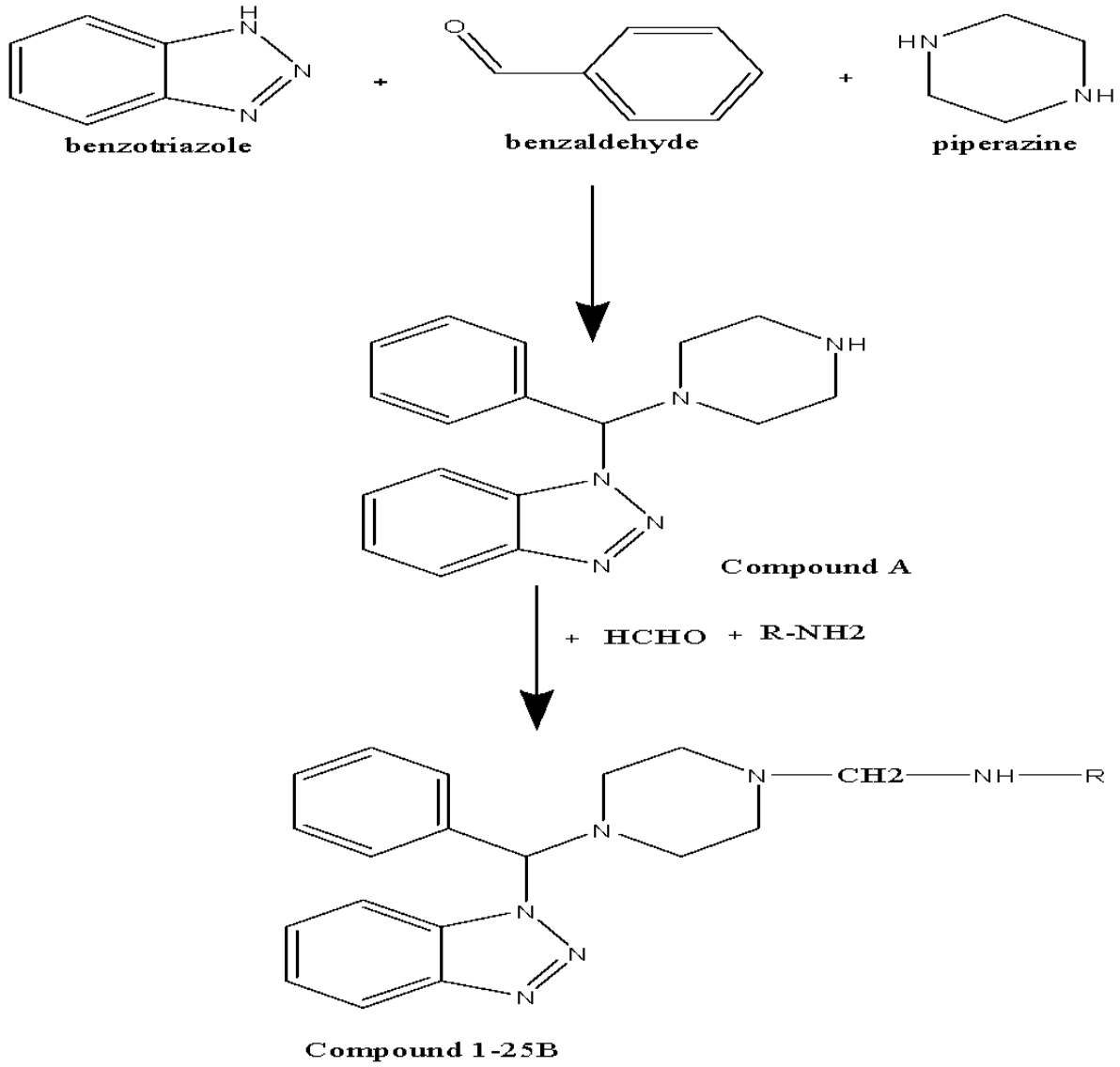
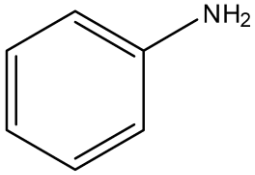
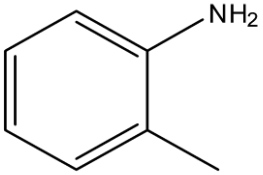
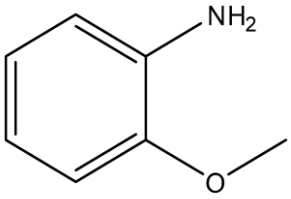
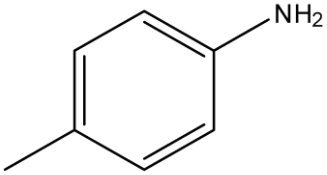
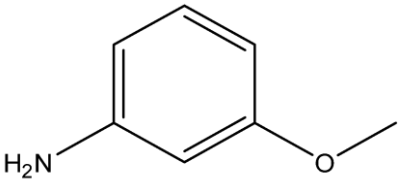
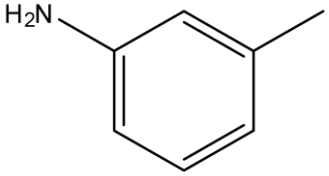
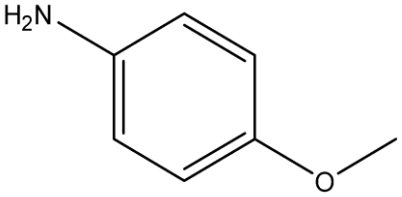
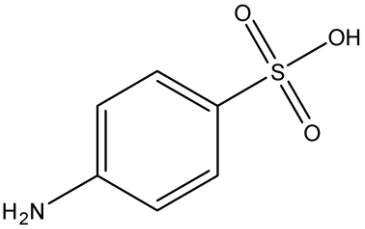
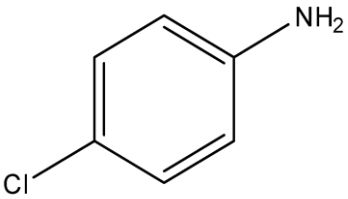
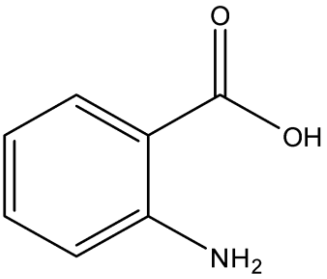
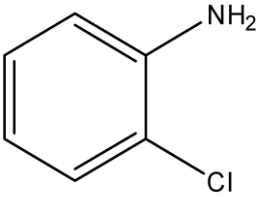
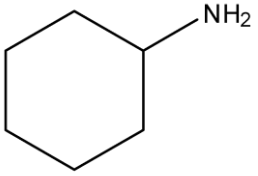
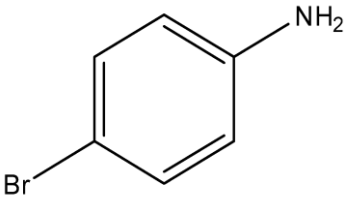
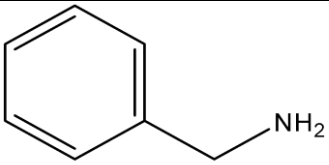
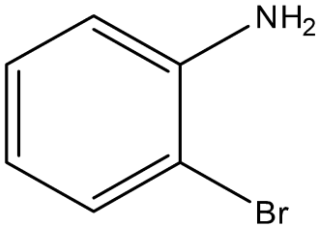
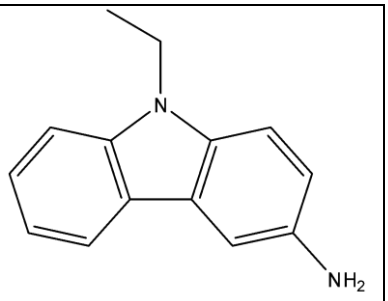
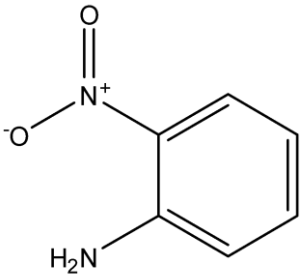
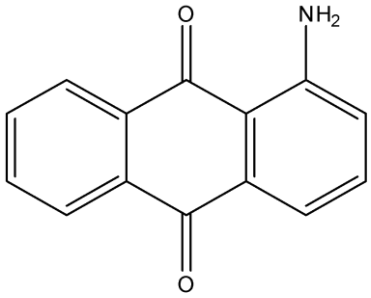
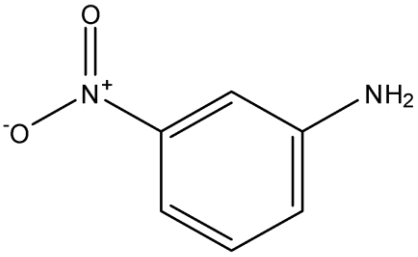
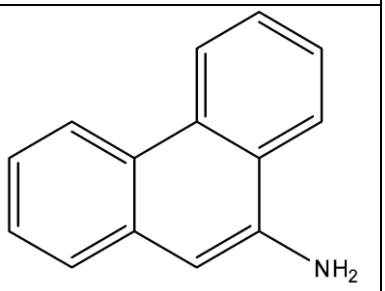
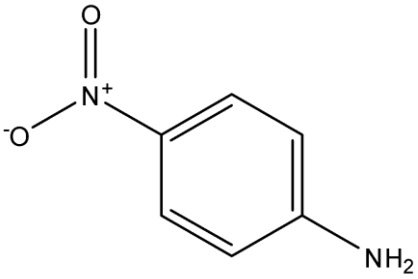
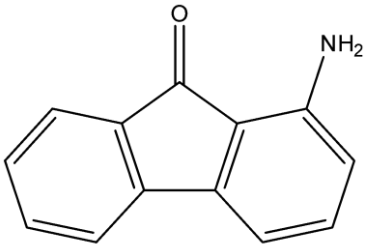
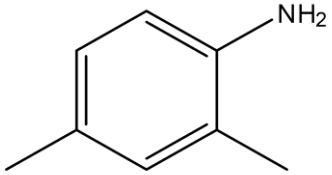
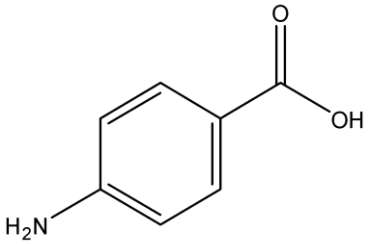
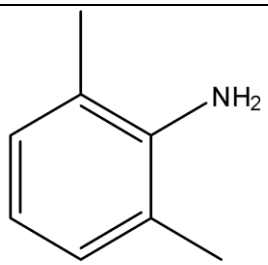


Table-01 List of aromatic primary amines used in the synthesis

Sl No	Compounds	R	Sl No	Compounds	R
1	1B	 ANILINE	14	14B	 ORTHO-TOLUIDINE

2	2B	 ORTHO ANISIDINE	15	15B	 PARA-TOLUIDINE
3	3B	 META ANISIDINE	16	16B	 META-TOLUIDINE
4	4B	 PARA ANISIDINE	17	17B	 SULPHANILIC ACID
5	5B	 4-CHLORO ANILINE	18	18B	 ANTHRANILIC ACID
6	6B	 2-CHLORO ANILINE	19	19B	 CYCLOHEXYLAMINE
7	7B	 4-bromo aniline	20	20B	 benzyl amine

8	8B	 2 bromo aniline	21	21B	 3 AMINO 9 ETHYL CARBAZOLE
9	9B	 2-NITRO ANILINE	22	22B	 1-aminoanthracene-9,10-dione
10	10B	 3-NITRO ANILINE	23	23B	 9-AMINO PHENANTHRENE
11	11B	 PARA-NITRO ANILINE	24	24B	 1-AMINO9-FLUORENONE
12	12B	 2,4,DIMETHYL ANILINE	25	25B	 4-AMINO BENZOIC ACID

13	13B	 2,6,DIMETHYL ANILINE			

4. MOLECULAR DOCKING [22,23,24,25,26.]

The optimised compounds were used to generate ligands, which were then saved using Spartan14 in the pdb file format before to the docking study. The protein bank was queried to get the three-dimensional structures of the VEFG, HER-2, and EGFR proteins (pdb ID: 3QTK, 3PPO, 4ZAU).In order to conduct the docking study, the enzyme was created using the Discovery Studio Visualizer. Throughout the preparation procedure, hydrogen was added to the crystal compound kept in the pbd file while water molecules, heteroatoms, and co-ligands were eliminated.

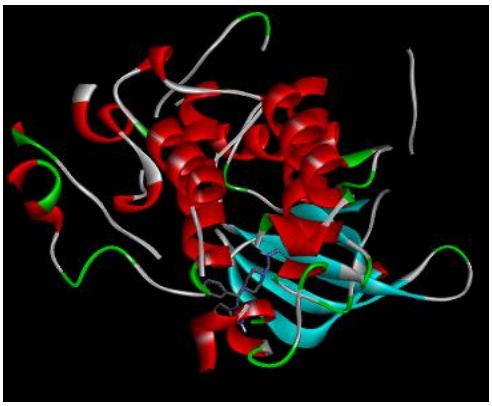
Using Autodock Vina in the Pyrex programme, the ligands were docked to the active site. If the docking procedure was successful, it was also feasible to reconstruct the complexes (ligand-receptor) using the Chimaera programme, enabling further research. The complexes' interactions were investigated using Discovery Studio Visualizer and pyMOL.

4.1 Table -02 Docking And Glide Score Of 3PPO (1-25B)

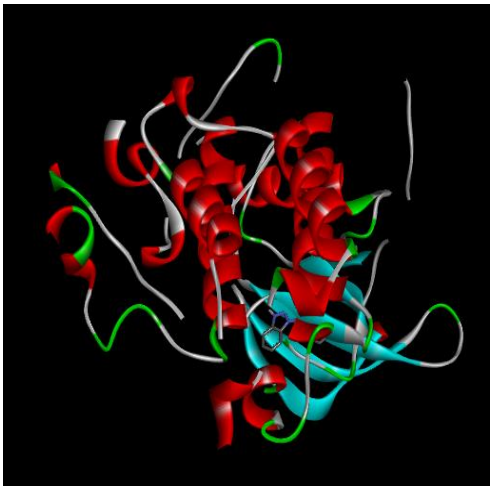
DOCKING AND GLIDE SCORE OF 3PPO-HER-2 (breast cancer)

Sl no	Compounds	3PPO	Interaction of amino acids
1.	Compound 1B	-7.7	Glu,Ser,Lys,Arg,Gly,Val,Ile,Ala
2.	Compound 2B	-6.7	Glu,Ser,Lys, , Leu, Phe
3.	Compound 3B	-5.9	Val,Ala, Leu, Asp, Cys
4.	Compound 4B	-7.9	Arg,Lys,Phe,Val,Trp
5.	Compound 5B	-8	Arg, Ala, Leu, Phe
6.	Compound 6B	-7.6	Val,Ala, Leu, Asp, Cys
7.	Compound 7B	-10.1	Arg, Ser,Lys, Phe, Ala, Thr
8.	Compound 8B	-6.2	Thr,Asp,Phe, Gly, Val
9.	Compound 9B	-5.5	Thr,Asp,Phe,Val
10.	Compound 10B	-8.4	Ala, Tyr, Val
11.	Compound 11B	-8.4	Gly, Val, Tyr, Ala,pro
12.	Compound 12B	-8.3	Val,Ala,Ser,Leu,Thr,Gly,

13.	Compound 13B	-9.8	Thr,Asp,Phe,Val,Lys,Leu,The,Arg
14.	Compound 14B	-7.9	Arg,Glu,Phe,Glu,Gly,Lys,Ser
15.	Compound 15B	-8.1	Gly,Leu,Met,Phe,Cys,Val,Thr
16.	Compound 16B	-5.5	Lys,Leu,The,Arg
17.	Compound 17B	-8.5	Asp,Tyr.Ala,Val,Arg,Ser
18.	Compound 18B	-6.1	Tyr.Ala,Val,Arg,Ser
19.	Compound 19B	-8.2	Phe,Arg,Gly,Ala,Leu,Cys
20.	Compound 20B	-10.7	Leu,Arg,Val,Asp,Cys,Ala
21.	Compound 21B	-5.1	Thr,Asp,Phe,Val
22.	Compound 22B	-5.6	Leu,Arg,Val,Asp
23.	Compound 23B	-5.2	Arg,Gly,Ala,Leu,Cys
24.	Compound 24B	-6.2	Val,Asp,Cys,Ala
25.	Compound 25B	-8.4	Tyr,Val, Thr,Asp,Phe



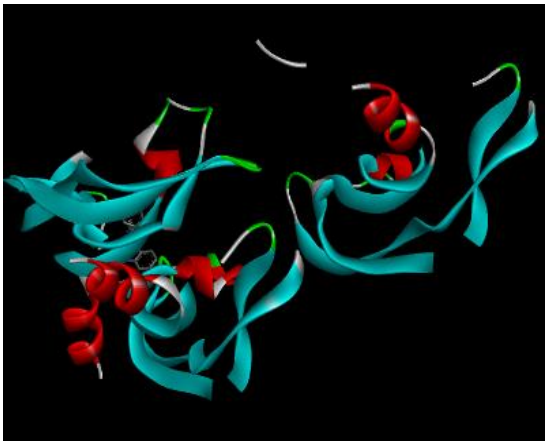
Compound 19B

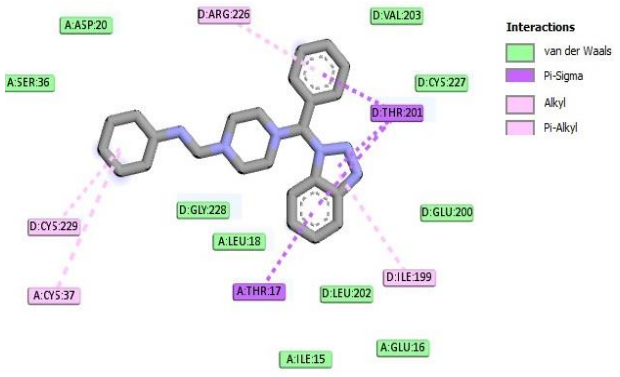


Compound 25B

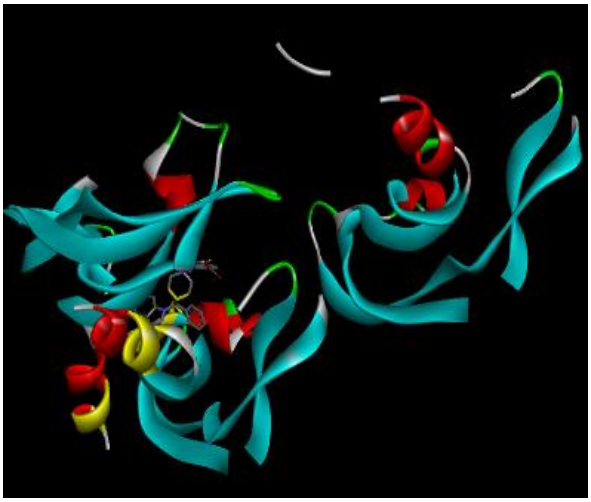
4.2 Table-03 Docking And Glide Score Of 3qtk(1-25B)-VEGF-Breast cancer

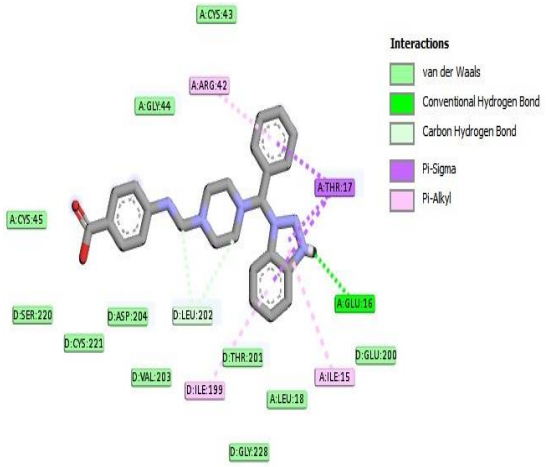
Sl no	Compounds	3QTK	Interaction of amino acids
1.	Compound 1B	-7	Cys, Phe, Asp, Cys
2.	Compound2B	-5.2	Phe, Ile, Lys, Lys, Asp
3.	Compound3B	-6.1	Glu, Cys, Leu, Cys
4.	Compound 4B	-7.5	Phe, Ile, Lys, Asp, Cys
5.	Compound 5B	-7.4	Phe,Ile, Asp, Cys,Glu
6.	Compound 6B	-7	Phe,Ile, Asp, Cys
7.	Compound 7B	-7.6	Glu, Cys, Leu, Lys
8.	Compound 8B	-5.2	Asp, Gly, Ser, Cys
9.	Compound 9B	-5.9	Ile, Lys, Asp, Cys
10.	Compound 10B	-8.1	Thr,Arg,Gly, Ser, Cys
11.	Compound 11B	-7.9	Cys, Ile, Asp, Thr,
12.	Compound 12B	-7.7	Cys, Ile, Asp, Glu, Phe
13.	Compound 13B	-7.6	Cys, Ile, Asp, Gly, Ser
14.	Compound 14B	-7.2	Gly,Cys,Asp,Ser,Ile
15.	Compound 15B	-7.5	Tyr,His,Glu,Cys
16.	Compound 16B	-6.0	Asp, Gly, Ser
17.	Compound 17B	-8.4	Thr,Arg,Gly,Cys,Asr,Asn,
18.	Compound 18B	-5.1	Thr,Arg,Gly,Cys
19.	Compound 19B	-6.9	Asp,Cys,Ser,Ile,
20.	Compound 20B	-7.1	Asp,Cys,Ser,Glu,Ile
21.	Compound 21B	-5.5	Cys,Ser,Glu,Ile, Ser,Ile,
22.	Compound 22B	-5.8	Gly,Cys,Asr,Asn
23.	Compound 23B	-5.9	Arg,Gly,Cys
24.	Compound 24B	-6.1	Arg,Gly, Ser, Cys
25.	Compound 25B	-8.1	Ser,Cys, Glu,Ile





Compound 19B





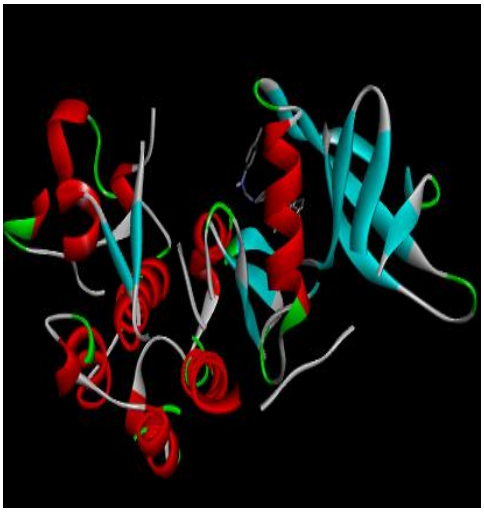
Compound 25B

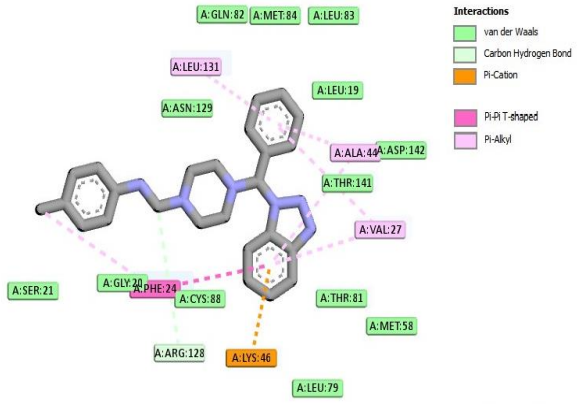
4.3 Table- 04 Docking And Glide Score Of 4ZAU (1-25B)

The way that the following substances bind to the 4ZAU active site

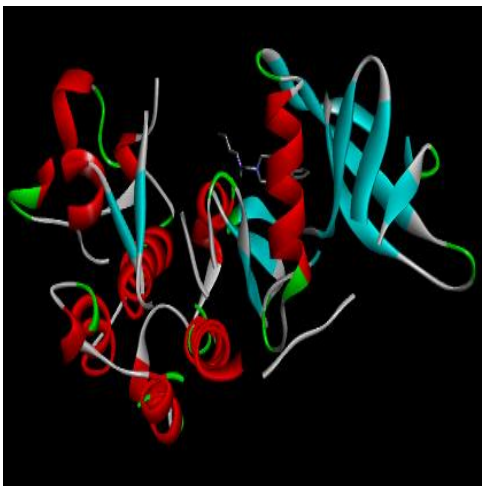
Sl no	Compounds	4ZAU	Interaction of amino acids
1.	Compound 1B	-9.2	Ala,Leu,Phe,Val,Arg
2.	Compound 2B	-6.2	Leu, Lys, Val, Phe
3.	Compound 3B	-5.5	Met,Lys,Ala,Leu,Val.
4.	Compound 4B	-9.3	Lys,Met,Leu,Phe,Val,Ala
5.	Compound 5B	-9.3	Lys,Met,Leu,Phe,Val,Ala
6.	Compound 6B	-9.9	Leu,Lys,Val,Phe, Ala
7.	Compound 7B	-9.4	Leu, Lys, Val, Phe, Met,Glu, Asp.
8.	Compound 8B	-6.4	Met,Lys,Ala,Leu,Val
9.	Compound 9B	-5.3	Asp,Met,Lys,Ala,Leu,Val
10.	Compound 10B	-9.2	Ser,Ala,Leu,Val,Lys
11.	Compound 11B	-9.5	Ser,Phe,Asp,Met,Lys,Ala,Leu,Val
12.	Compound 12B	-9.8	Leu,Lys,Val,Phe
13.	Compound 13B	-9.6	Asp,Lys,Val,Phe,Met,Leu,Ala
14.	Compound 14B	-9.3	Thr,Phe,Leu,Ala,Val,Lys,Phe
15.	Compound 15B	-9.2	Lys,Phe,Leu,Ala
16.	Compound 16B	-6.3	Met,Lys,Ala,Leu,Val
17.	Compound 17B	-9.4	Lys,Leu,Val,Phe,Ala
18.	Compound 18B	-5.5	Lys,Thr,Ala,Met
19.	Compound 19B	-9.6	Cys,Arg,Thr,Leu,Ala,Val,Lys
20.	Compound 20B	-9.9	Leu,Val,The,Lys,Thr,Ala,Met
21.	Compound 21B	-5.6	Asp,Met,Lys,Ala,Leu,Val
22.	Compound 22B	-5.4	Thr,Leu,Ala,Val,Lys
23.	Compound 23B	-5.6	Lys,Ala,Leu,Val

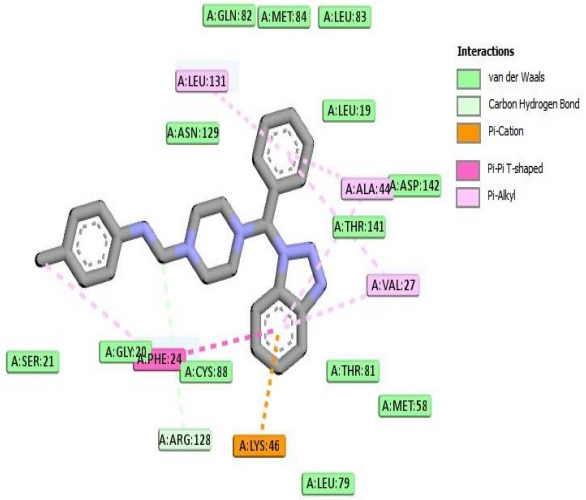
24.	Compound 24B	-5.7	Ala,Cys,Lys,Val
25.	Compound 25B	-9	Phe,Leu,Ala,Cys,Lys,Val





Compound 15B





Compound 19B

5. RESULT AND DISCUSSION: The structural details of the produced chemicals were clarified by using FTIR, ^{1}H NMR, ^{13}C NMR, and MASS. The spectra of the substances that were synthesised are listed below.

N-((4-((1*H* - benzo[*d*] [1,2,3]triazole-1-yl) (Phenyl)methyl) piperazin-1-yl) methyl)aniline (Compound 1B):

Yellow-white, M.P. 260ºC, Yield 35.88%, Mol Formula: C24H26N6, Mol Wt: 398.51, Elemental Analysis: C, 72.33; H, 6.58; N, 21.09. IR cm−1 (KBr): (CH) bending 736, (N=N) 1661, (NH) 3582, (CH2) 1488, cm-1. ^{1}HNMR (500 MHz, CDCl3): δ ppm (NH) 6.34(CH2) 2.71, (CH) 7. 75 ppm. ^{13}C NMR: (CH2) 49.9, (CH) 113.5, (C) 52.8, and (C) 126.2 ppm (125 MHz, DMSO d6, δ ppm). m/z%: 399.23 (M+1)+ 398.22 (Base peak).

N-((4-((1*H* – benzo [*d*] [1,2,3]triazole-1-yl) (phenyl)methyl) piperazin-1-yl)methyl)-4-methoxyaniline (Compound 4B):

Cream white, M.P .240ºC ,Yield 46.98%, Mol Formula: $C_{25}H_{25}N_7O_4$, Mol Wt: 428.23, Elemental Analysis C,70.07;H,6.59:N,19.61;O,3.73, IR cm^{-1} (KBr): (CH) bending 736, (N=N) 1661, (CH_2) 1488, (NH) 1582, (OCH3) 2840 , cm-1. ^{1}HNMR, (500 MHz, $CDCl_3$): δ ppm (NH) 6.34 (CH2) 2.71, (CH) 8.0 ppm. ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): (CH2) 74.6, (C) 52.8, (CH) 128.4, (C) 126.2 , *m/z*% : 428.23(Base peak) 429.44 $(M+1)^+$.

N-((4-((1*H*-benzo[*d*] [1,2,3]triazole-1-yl) (phenyl)methyl) piperazin-1-yl)methyl)-4-chloroaniline (Compound 5B):

Cream white, M.P..270ºC, Yield 41.94 percent, Mol Formula: C24H25ClN6, Mol Wt: 432.96, Elemental Analysis C,66.58;H,5.82:N,19.41;Cl,8.19, IR cm−1 (KBr): ^{1}HNMR, (500 MHz, CDCl3): δ ppm (NH) 6.34 (CH2) 2.71, (CH) 7.33 ppm, (CH) bending 736, (N=N) 1661, (NH) 1582, (Cl) 732 cm-1. ^{13}C NMR: (CH2) 74.6, (C) 52.8, (CH) 128.4, (C) 126.2, m/z percent: 432.18 (base peak), 433.12 (M+1)+.

N-((4-((1*H* - benzo[*d*] [1,2,3]triazole-1-yl) (Phenyl)methyl) piperazin-1-yl) methyl)-2-chloroaniline (Compound 6B):

White, M.P .274ºC ,Yield 55.94%, Mol Formula: $C_{24}H_{25}ClN_6$, Mol Wt: 432.96, Elemental Analysis C,66.58;H,5.82:N,19.41;Cl,8.19, IR cm^{-1} (KBr): (CH) bending 736, (N=N) 1661, (NH) 1582, 1660, (Cl) 782 cm-1. ^{1}HNMR, (500 MHz, $CDCl_3$): δ ppm (NH) 5.80 (CH2) 4.13, (CH) 7.33 (CH2) 2.71 ppm . ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm):, (CH2) 74.1 (C) 52.8 (CH) 128.4, (C) 126.2. *m/z* %: 432.18(Base peak) 433.12 $(M+1)^+$.

N-((4-((1*H* - benzo[*d*] [1,2,3]triazole-1-yl) (Phenyl)methyl) piperazin-1-yl) methyl)-4-bromoaniline (Compound 7B):

Brown, M.P.250ºC ,Yield 63.54%, Mol Formula: $C_{24}H_{25}BrN_6$, Mol Wt: 476.13, Elemental Analysis C,60.38;H,5.28:N,17.60;Br,16.74, IR cm^{-1} (KBr): (CH) bending at 736 cm-1, (N=N) at 1661 cm-1, (NH) at 1582 cm-1, and (Br) at 702 cm-1. ^{1}HNMR: δ ppm (NH) at 6.34 (CH2) at 4.13 (CH) at 7.33 (CH2) at 2.71 ppm. The ^{13}C NMR spectrum at 125 MHz in DMSO d6 with δ ppm shows the following structures: (CH2) 74.6, (C) 52.8, (CH) 128.4, (C) 110.0 m/z %:476.13 (base peak) 477.56 (M+1)+.

N-((4-((1*H* - benzo[*d*] [1,2,3]triazole-1-yl) (Phenyl)methyl) piperazin-1-yl) methyl)-3-nitroaniline (Compound 10B):

Mol Formula: C24H25N7O2, Mol Wt: 443.21, Elemental Analysis, Yellow colour, M.P..181ºC, Yield 63.78 percent (500 MHz, CDCl3): δ ppm (NH) 6.34 (CH2) 4.13 (CH) 7.33 (CH2) 2.71 ppm, (CH) bending 736, (NO2) 1660 (N=N) 1661, (NH) 1582. C,65.00;H,5.68:N,22.11;O,7.21, IR cm−1 (KBr): cm-1 ^{1}HNMR. The ^{13}C NMR base peak is 443.21, and the (CH2) 74.6 (C) 52.8 (CH) 128.4 (C) 110.0 m/z percent are both found.

N-((4-((1*H* - benzo[*d*] [1,2,3]triazole-1-yl) (Phenyl)methyl) piperazin-1-yl) methyl)-4-nitroaniline (Compound 11B):

Yellow colour, M.P .180ºC ,Yield 54.05%, Mol Formula: $C_{24}H_{25}N_7O_2$, Mol Wt: 443.21, Elemental Analysis C,65.00;H,5.68:N,22.11;O,7.21, IR cm^{-1} (KBr): (CH) bending 736, (NO_2) 1660 (N=N) 1661, (NH) 1582, cm-1 ^{1}HNMR, (500 MHz, $CDCl_3$): δ ppm (NH) 6.34 (CH2) 4.13, (CH) 7.33 ppm. ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm):(CH2) 74.6 (C) 52.8 (CH) 128.4, (C) 110.0 *m/z* %:443.21(Base peak).

N-((4-((1*H* - benzo[*d*] [1,2,3]triazole-1-yl) (Phenyl)methyl) piperazin-1-yl) methyl)-2,4-Dimethyl aniline (Compound 12B):

Creamy white, M.P .185ºC ,Yield 53%, Mol Formula: $C_{26}H_{30}N_6$, Mol Wt: 426.25, Elemental Analysis C,73.21;H,7.09:N,19.70; IR cm^{-1} (KBr): (CH) bending 736, (NO_2) 1660 (N=N) 1661, (NH) 1582, cm-1 ^{1}HNMR, (500 MHz, $CDCl_3$): δ ppm (NH) 5.80 (CH2) 4.13, (CH) 7.33 (CH3) 2.12 (CH2) 2.71 ppm. The ^{13}C NMR spectrum at 125 MHz in DMSO d6 with δ ppm shows the following: (CH2) 74.9, (C) 52.8, (CH) 128.4, (C) 110.0, and 17.9 m/z% of CH3: 426.25 (base peak)..

N-((4-((1*H* - benzo[*d*] [1,2,3]triazole-1-yl) (Phenyl)methyl) piperazin-1-yl) methyl)-2,6-Dimethyl aniline (Compound 13B):

White, M.P .189ºC,Yield 64.64%, Mol Formula: $C_{26}H_{30}N_6$, Mol Wt: 426.25, Elemental Analysis C,73.21;H,7.09:N,19.70; IR cm^{-1} (KBr): (CH) bending 736, (NO_2) 1660 (N=N) 1661, (NH) 1582, cm-1 ^{1}HNMR, (500 MHz, $CDCl_3$): δ ppm (NH) 5.34 (CH2) 4.13, (CH) 7.33 (CH3) 2.12, (CH2) 2.71 ppm. ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): (CH2) 75.2 (C) 52.8 (CH) 128.4 , (C) 110.0 , (CH3) 17.9. *m/z*%: 426.25 (Base peak) .

N-((4-((1*H* - benzo[*d*] 1,2,3]triazole-1-yl) (Phenyl)methyl) piperazin-1-yl) methyl)-2-Methyl aniline (Compound 14B):

White colour, M.P .180ºC ,Yield 58.78%, Mol Formula: $C_{25}H_{28}N_6$, Mol Wt: 412.24, Elemental Analysis C,72.79;H,6.84;N,20.37; IR cm^{-1} (KBr): (CH) bending 736, (NO_2) 1660 (N=N) 1661, (NH) 1582, cm-1 ^{1}HNMR, (500 MHz, $CDCl_3$): δ ppm (NH) 5.80 (CH2) 4.13, (CH) 7.33 (CH3) 2.01 , (CH2) 2.71 ppm. ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): (CH2) 75.2 (C) 52.8 , (CH) 128.4, (C) 110.0 (CH3) 17.6. *m/z*%: 412.54 (Base peak) .

N-((4-((1*H* - benzo[*d*] [1,2,3]triazole-1-yl) (Phenyl)methyl) piperazin-1-yl) methyl)-4-Methyl aniline (Compound 15B):

Yellowish white colour, M.P .190ºC ,Yield 51.2%, Mol Formula: $C_{25}H_{28}N_6$, Mol Wt: 412.24, Elemental Analysis C,72.79; H, 6.84; N,20.37; IR cm^{-1} (KBr): (CH) bending 736, (NO_2) 1660 (N=N) 1661, (NH) 1582, cm-1 ^{1}HNMR, (500 MHz, $CDCl_3$): δ ppm (NH) 6.34 (CH2) 4.13, (CH) 7.33 (CH3) 2.32, (CH2) 2.71 ppm. ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): (CH2) 75.2 (C) 52.8 (CH) 128.4 , (C) 110.0 (CH3) 17.6. *m/z*%: 412.54 (Base peak) .

N-(((4-((1*H* - benzo[*d*] [1,2,3]triazole-1-yl) (Phenyl)methyl) piperazin-1-yl) methyl) amino) benzenesulphonic acid (Compound 17B):

Creamy white, M.P .183ºC ,Yield 56%, Mol Formula: $C_{24}H_{26}N_6O_3S$, Mol Wt: 478.57, Elemental Analysis C,60.23;H,5.48;N,17.56;O,10.03;S,6.70; IR cm^{-1} (KBr): (CH) bending 736, (NO_2) 1660 (N=N) 1661, (NH) 1582, cm-1 ^{1}HNMR, (500 MHz, $CDCl_3$): δ ppm (NH) 6.34 (CH2) 4.13, (CH) 7.33 (CH3) 2.01, (CH2) 2.71 (OH) 8.5 ppm, ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): (CH2) 75.2 (C) 52.8 (CH) 128.4, (C) 110.0 (CH3) 17.6. *m/z*%: 478.57 (Base peak) .

N-((4-((1*H* - benzo [*d*] [1,2,3]triazole-1-yl) (Phenyl)methyl) piperazin-1-yl) methyl) cyclohexanamine (Compound 19B):

Creamy white, M.P .192ºC ,Yield 58.4%, Mol Formula: $C_{24}H_{32}N_6$, Mol Wt: 404.56, Elemental Analysis C,71.25; H,7.97;N,20.77; IR cm^{-1} (KBr): (CH) bending 736, 1660 (N=N) 1661, (NH) 1582, (C_6H_{12}) 1200 cm-1 ^{1}HNMR, (500 MHz, $CDCl_3$): δ ppm (CH2) 3.62, (CH) 7.33 (NH) 3.32 ppm, ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): (CH2) 70.3 (C) 52.8 (CH) 128.4 . *m/z* %: 478.57 (Base peak) .

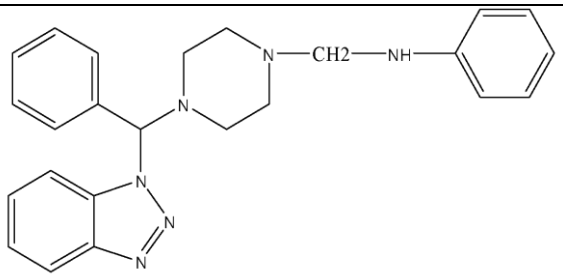
1-(4-((1*H* - benzo[*d*] [1,2,3]triazole-1-yl) (Phenyl)methyl) piperazin-1-yl)-*N*-Benzylmethylamine. (Compound 20B):

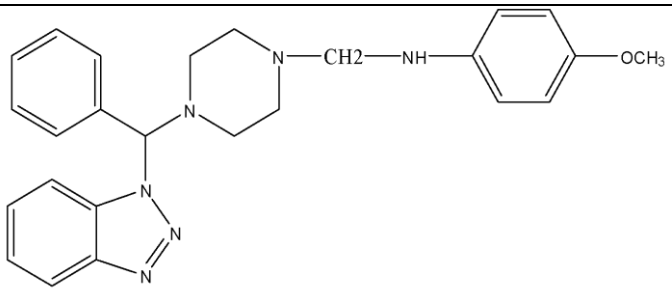
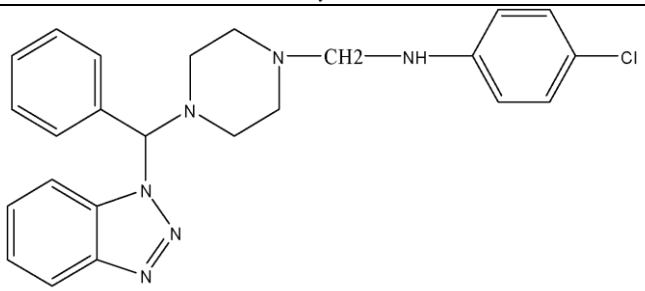
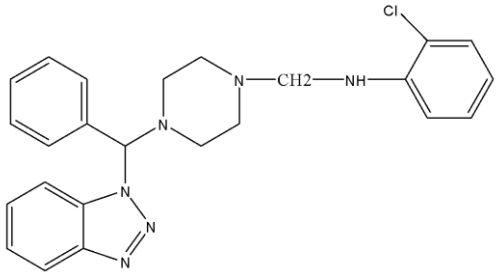
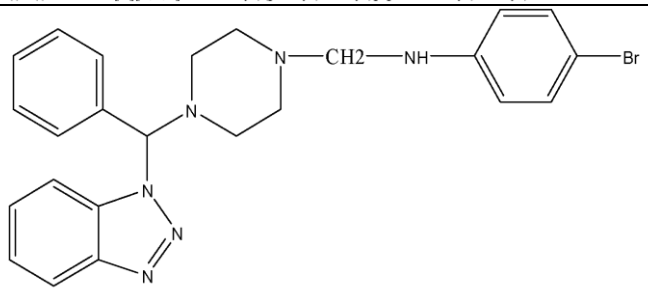
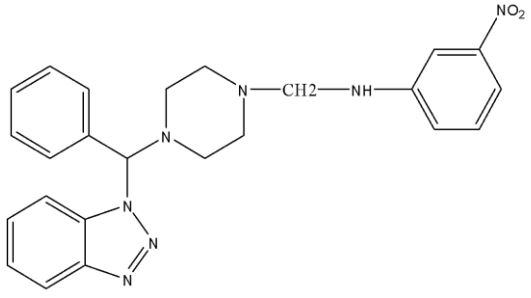
White, M.P .220ºC ,Yield 54.3%, Mol Formula: $C_{25}H_{28}N_6$, Mol Wt: 412.24, Elemental Analysis C,72.79;H,6.84;N,20.37; IR cm^{-1} (KBr): (CH) bending 736, 1660 (N=N) 1661, (NH) 1582, cm-1 ^{1}HNMR, (500 MHz, CDCl3): δ ppm (CH2) 3.62, (CH) 7.33 (NH) 4.16 ppm, ^{13}C NMR (125 MHz, DMSO d6), δ ppm): (CH2) 72.1 (C) 52.8 (CH) 128.4. m/z %: 412.24 (Base peak).

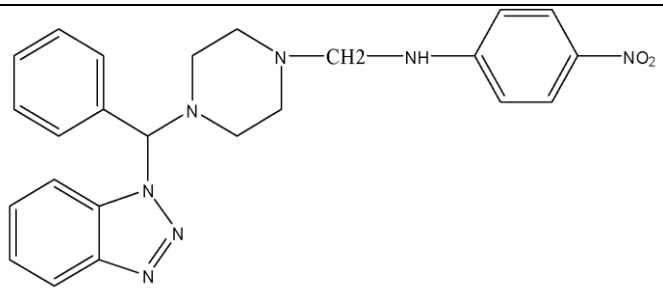
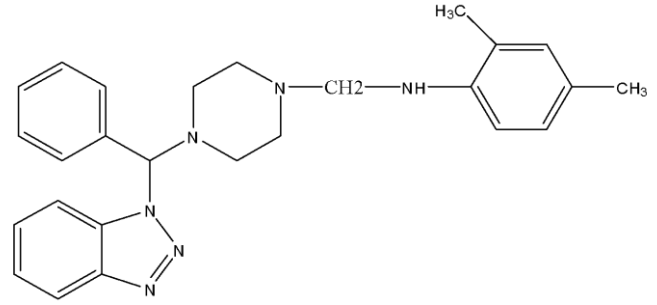
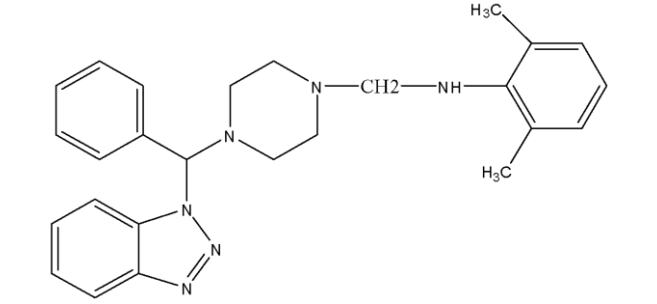
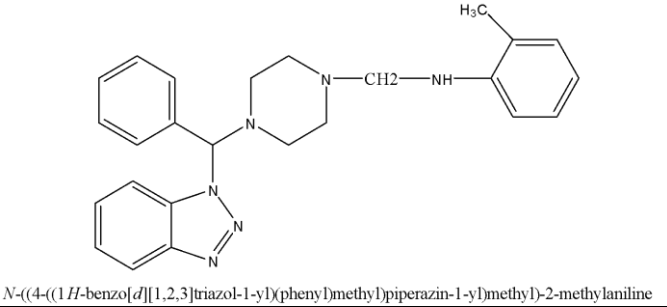
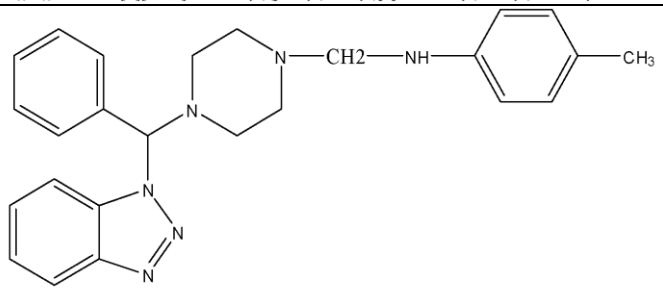
4-(((4-((1*H* - benzo[*d*] [1,2,3]triazole-1-yl) (Phenyl)methyl) piperazin-1-yl)methyl)amino)benzoic acid (Compound 25B):

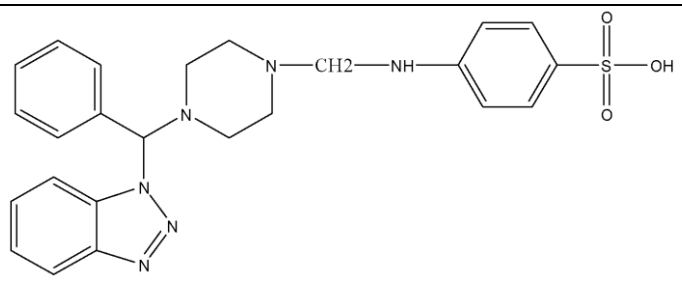
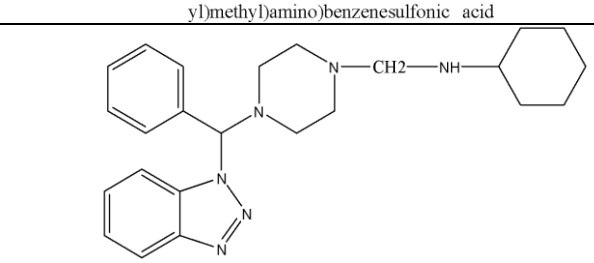
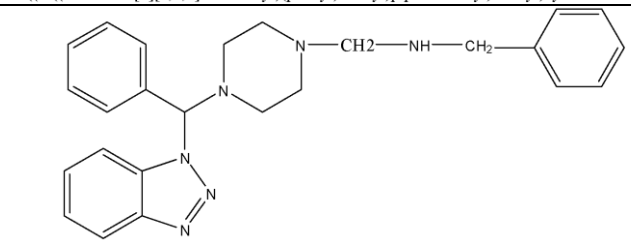
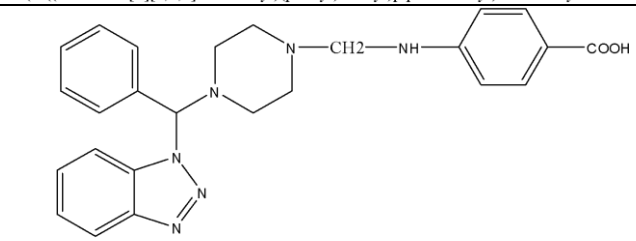
Brown, M.P.196ºC ,Yield 53.3%, Mol Formula: $C_{25}H_{26}N_6O_2$, Mol Wt: 442.21, Elemental Analysis C,67.86;H,5.92;N,18.99,O17.23 ; IR cm^{-1} (KBr): (CH) bending 736, 1660 (N=N) 1661, (NH) 1582, (COOH) 2800 cm-1 ^{1}HNMR, (500 MHz, $CDCl_3$): δ ppm (CH2) 4.13, (OH) 12.71 (CH) 7.33 (NH) 6.34 ppm, ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): (CH2) 74.6 (C) 52.8 (CH) 128.4 . *m/z*%: 442.21 (Base peak)" .

Table -05

Sl.No	Comp Code	Structure	Colour	M.P	Yield %
1.	1B	 *N*-((4-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)(phenyl)methyl)piperazin-1-yl)methyl)aniline	Yellowish white	260	35.88

2.	4B	 *N*-((4-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)(phenyl)methyl)piperazin-1-yl)methyl)-4-methoxyaniline	Cream white	240	46.98
3.	Compound 5B	 *N*-((4-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)(phenyl)methyl)piperazin-1-yl)methyl)-4-chloroaniline	Cream white	270	41.94
4.	Compound 6B	 *N*-((4-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)(phenyl)methyl)piperazin-1-yl)methyl)-2-chloroaniline	White	274	55.94
5.	Compound 7B	 *N*-((4-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)(phenyl)methyl)piperazin-1-yl)methyl)-4-bromoaniline	Brown	250	63.54
6.	Compound 10B	 *N*-((4-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)(phenyl)methyl)piperazin-1-yl)methyl)-3-nitroaniline	Yellow	181	63.78

7.	Compound 11B	 *N*-((4-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)(phenyl)methyl)piperazin-1-yl)methyl)-4-nitroaniline	Yellow	180	54.05
8.	Compound 12B	 *N*-((4-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)(phenyl)methyl)piperazin-1-yl)methyl)-2,4-dimethylaniline	Creamy white	185	53
9.	Compound 13B	 *N*-((4-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)(phenyl)methyl)piperazin-1-yl)methyl)-2,6-dimethylaniline	white	189	64.64
10	Compound 14B	 *N*-((4-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)(phenyl)methyl)piperazin-1-yl)methyl)-2-methylaniline	White	180	58.78
11	Compound 15B	 *N*-((4-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)(phenyl)methyl)piperazin-1-yl)methyl)-4-methylaniline	Yellowish white	190	51.2

12	Compound 17B	 4-(((4-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)(phenyl)methyl)piperazin-1-yl)methyl)amino)benzenesulfonic acid	Creamy white	183	56
13	Compound 19B	 *N*-((4-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)(phenyl)methyl)piperazin-1-yl)methyl)cyclohexanamine	Creamy white	192	58.4
14	Compound 20B	 1-(4-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)(phenyl)methyl)piperazin-1-yl)- *N*-benzylmethanamine	White	220	54.3
15	Compound 25B	 4-(((4-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)(phenyl)methyl)piperazin-1-yl)methyl)amino)benzoic acid	Brown	196	53

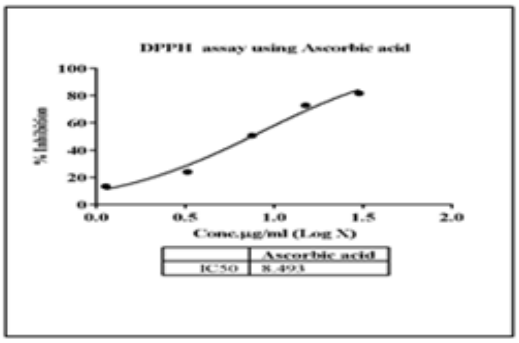
6.ANTI-OXIDANT ACTIVITY

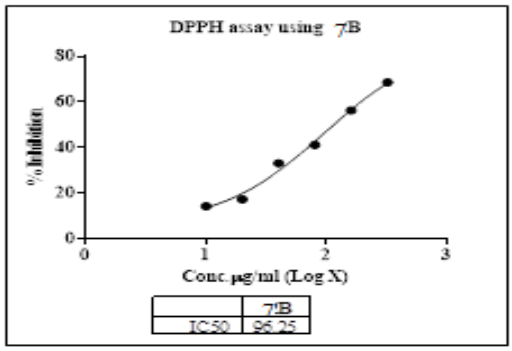
The DPPH technique has been used to study the antioxidant activity of compounds 1, 4, 5, 6, 7, 10, 11, 12, 13, 14, 15, 17, 19, 20, and 25 B. When compared to ascorbic acid (IC 50 = 8.4 µg/ml), 7B and 25B (IC 50 = 96.25 and 95.23 µg/ml) showed better among the 15 compounds in the anti-oxidant activity.Due to less inhibition unable to calculate IC 50 value for following compounds 1B,4B,5B,6B,12B,14B,15B,17B,19B and 20B.

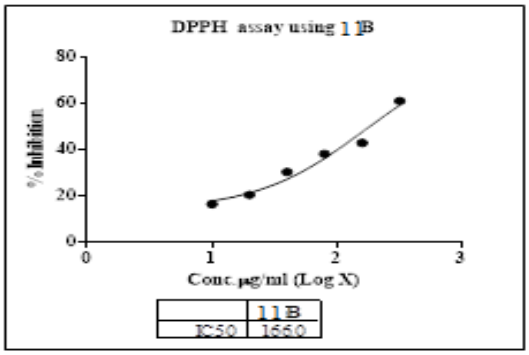
Results:

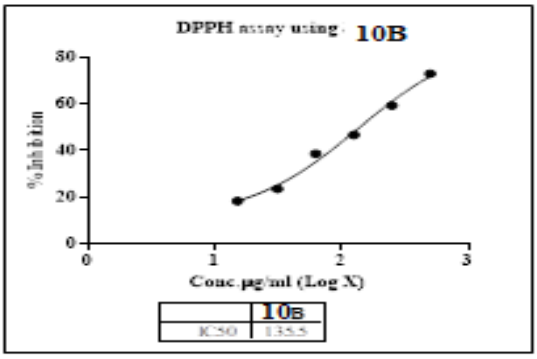
Compounds	**%Inhibition**	**IC 50**(µM)
Ascorbic acid	48.642	8.4
1B	26.26667	Due to less inhibition unable calculate IC 50
4B	23.46	Due to less inhibition unable calculate IC 50
5B	20.38	Due to less inhibition unable calculate IC 50
6B	12.96	Due to less inhibition unable calculate IC 50

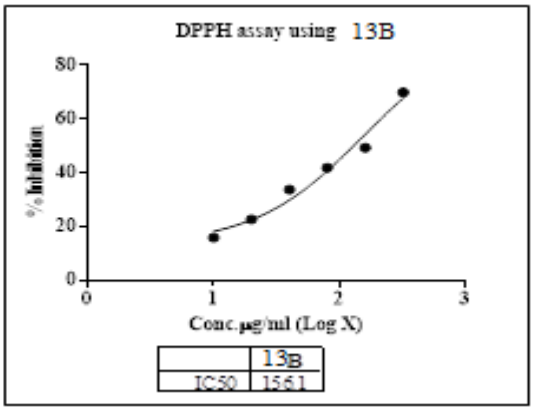
7B	38.54333	96.25
10B	43.25333	135.5
11B	34.78	166
12B	15.85	Due to less inhibition unable calculate IC 50
13B	38.85	156.1
14B	24.995	Due to less inhibition unable calculate IC 50
15B	24.995	Due to less inhibition unable calculate IC 50
17B	20.22	Due to less inhibition unable calculate IC 50
19B	18.23	Due to less inhibition unable calculate IC 50
20B	21.38	Due to less inhibition unable calculate IC 50
25B	37.53	95.23

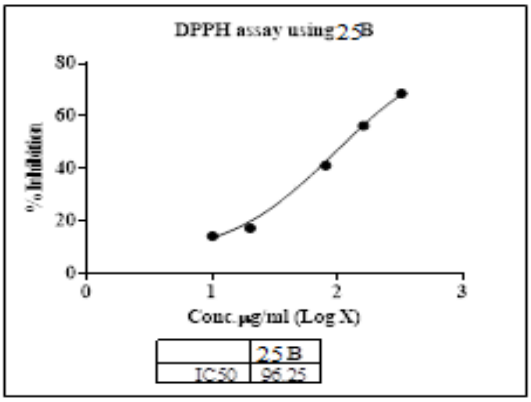










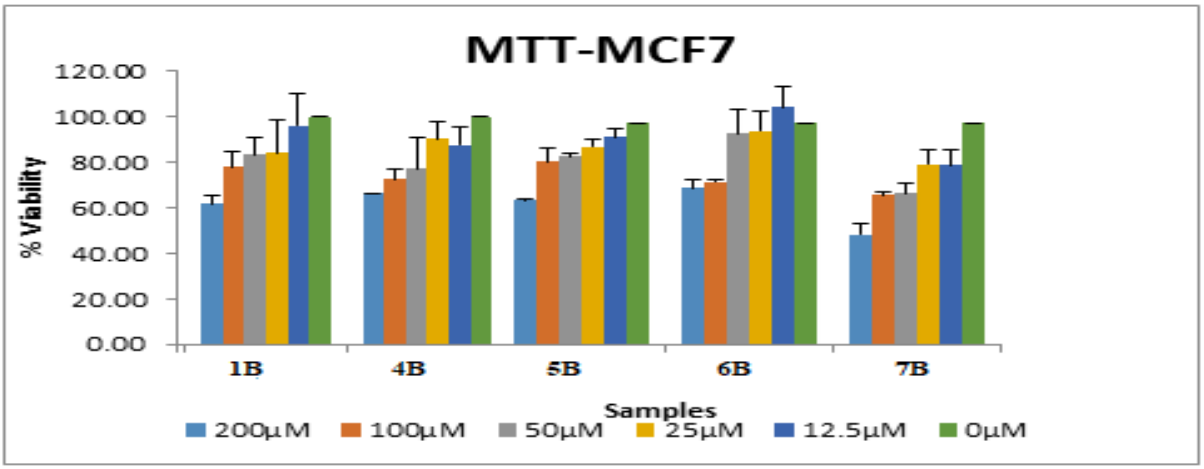


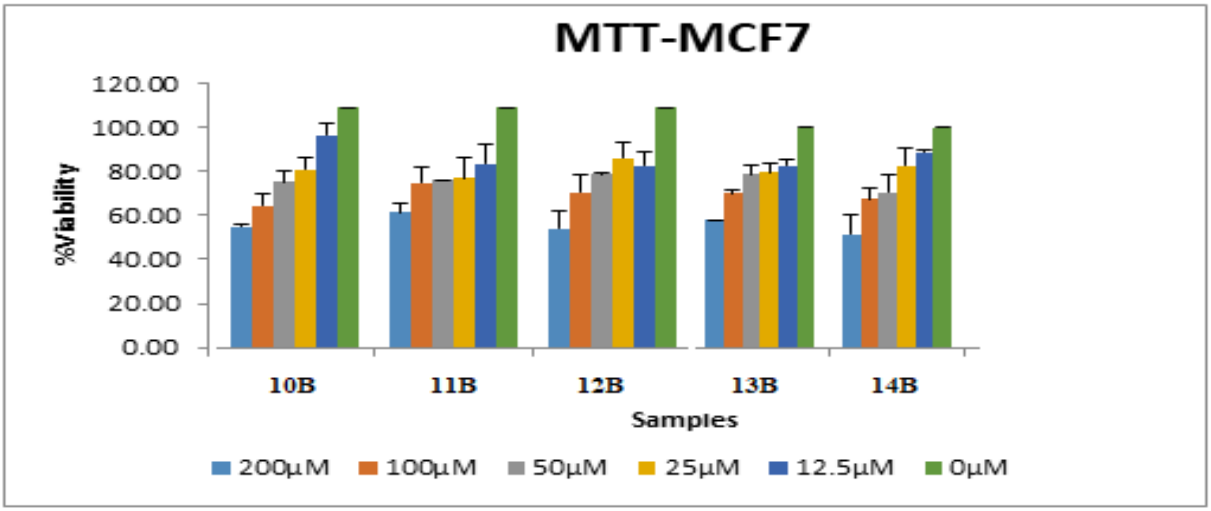
7. INVITRO ANTICANCER ACTIVITY

The potential anti-cancer properties of the following compounds have been investigated: 1B, 4B, 5B, 6B, 7B, 10B, 11B, 12B, 13B, 14B, 15B, 17B, 19B, 20B, and 25B. Among 15 compound's in in- vitro AntiCancer studies, compounds 19B and 25B in MCF cell line and 15B and 19B in caco2 cell line shows good cytotoxiccity activity.

7.1 Table-07 MCF7 CELL LINE:

%VIABILITY	200µM	100µM	50µM	25µM	12.5µM
control	100	100	100	100	100
1B	61.58	61.58	61.58	61.58	61.58
4B	66.22	66.22	66.22	66.22	66.22
5B	64.88	64.88	64.88	64.88	64.88
6B	70.46	70.46	70.46	70.46	70.46
7B	49.67	49.67	49.67	49.67	49.67
10B	50.38	50.38	50.38	50.38	50.38
11B	56.42	56.42	56.42	56.42	56.42
12B	49.49	49.49	49.49	49.49	49.49
13B	57.86	57.86	57.86	57.86	57.86
14B	51.45	51.45	51.45	51.45	51.45
15B	59.65	59.65	59.65	59.65	59.65
17B	55.26	55.26	55.26	55.26	55.26
19B	21.36	21.36	21.36	21.36	21.36
20B	20.52	20.52	20.52	20.52	20.52
25B	20.10	20.10	20.10	20.10	20.10


MTT-MCF7
% Viability
120.00
100.00
80.00
60.00
40.00
20.00
0.00
1B
4B
5B
6B
7B
Samples
200µM
100µM
50µM
25µM
12.5µM
0µM


MTT-MCF7
%Viability
120.00
100.00
80.00
60.00
40.00
20.00
0.00
10B
11B
12B
13B
14B
Samples
200µM
100µM
50µM
25µM
12.5µM
0µM

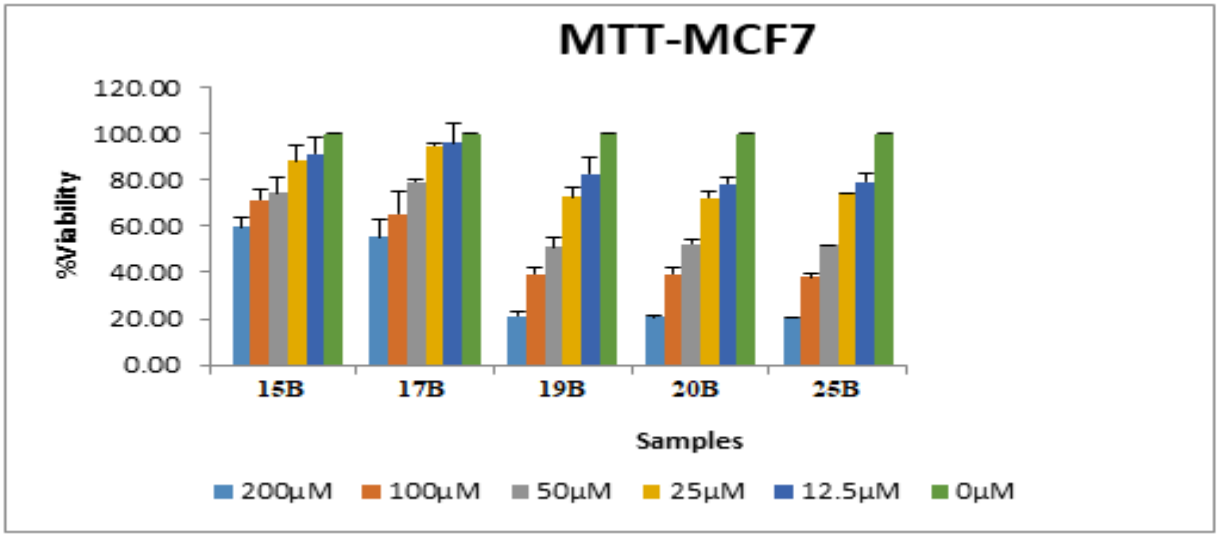
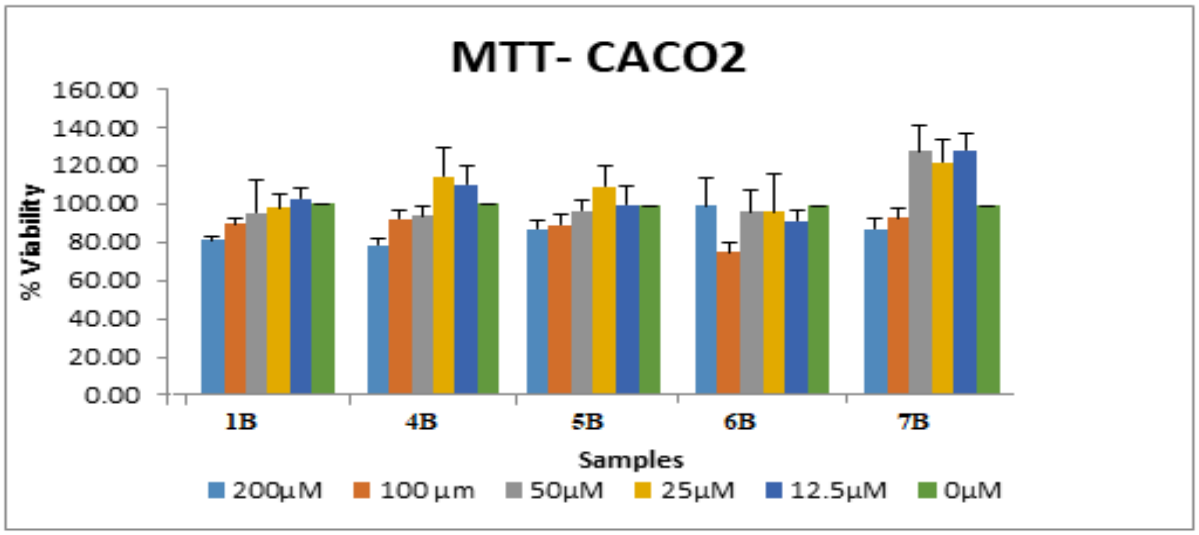
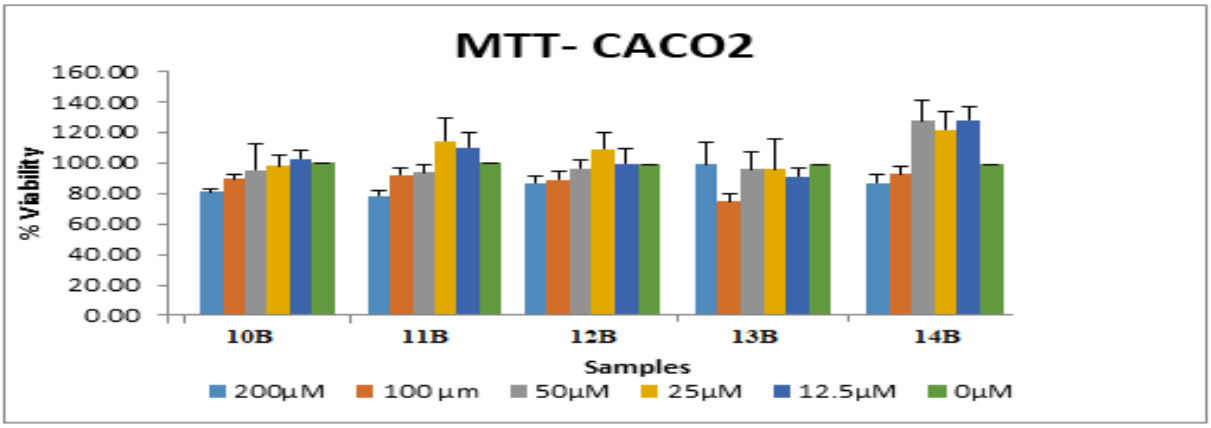
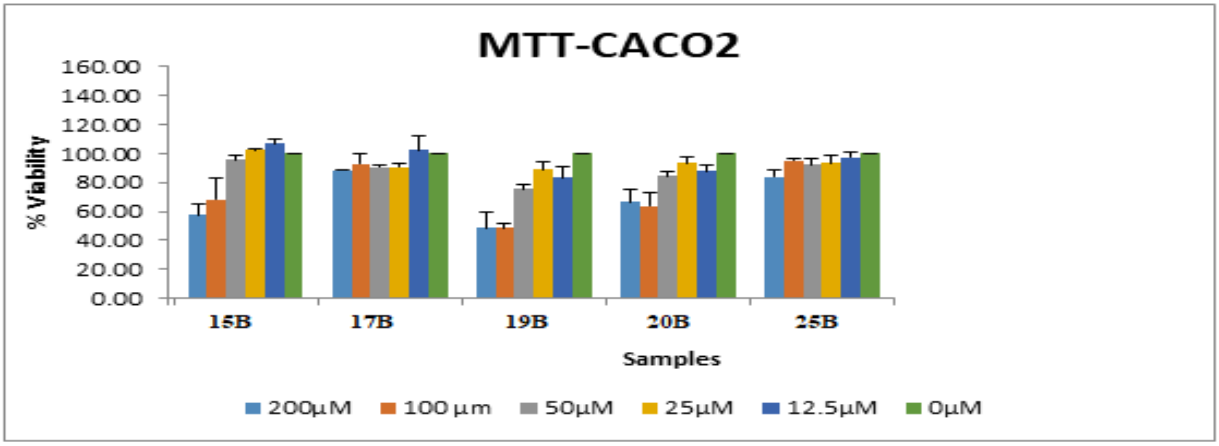

MTT-MCF7
%Viability
120.00
100.00
80.00
60.00
40.00
20.00
0.00
15B
17B
19B
20B
25B
Samples
200µM
100µM
50µM
25µM
12.5µM
0µM

Table-08 CACO2 Cell Lines

%VIABILITY	200µM	100µM	50µM	25µM	12.5µM
control	100	100	100	100	100
1B	81.16	81.16	81.16	81.16	81.16
4B	78.29	78.29	78.29	78.29	78.29
5B	87.72	87.72	87.72	87.72	87.72
6B	100.16	100.16	100.16	100.16	100.16
7B	87.68	87.68	87.68	87.68	87.68
10B	87.51	87.51	87.51	87.51	87.51
11B	94.26	94.26	94.26	94.26	94.26
12B	63.08	63.08	63.08	63.08	63.08
13B	67.10	67.10	67.10	67.10	67.10
14B	69.86	69.86	69.86	69.86	69.86
15B	57.76	57.76	57.76	57.76	57.76
17B	88.47	88.47	88.47	88.47	88.47
19B	48.42	48.42	48.42	48.42	48.42
20B	66.42	66.42	66.42	66.42	66.42
25B	83.81	83.81	83.81	83.81	83.81







IC50 Values (Table-9)

CELL LINE	MCF7(µM)	CACO2(µM)
COMPOUND CODE	IC50	IC50
1B	281.9	482.06
4B	300	350.6
5B	296.7	568.75
6B	289.3	229
7B	198.5	342.71
10B	197	483.3
11B	224.3	434
12B	196.3	224.9
13B	243.75	273.33
14B	219.5	287.26
15B	238.8	213.51
17B	204.3	746.5
19B	52.6	163.8
20B	55	312.7
25B	54	817.29

Pharmacokinetic parameters for in silico ADME prediction. (Table-10)

Sl.No	Compounds	MW	HBD	HBA	QPlog Po/W	QPlog BB	% Human Oral Absorption	Rule of Five
	Acceptable range	130.0 - 725.0	0 - 6	2 - 20	-2.0 – 1.2	-3.0 – 1.2	> 80% is high	Maximum is 4
1.	1B	398.514	1	6	**-2.736**	**0.358**	**94.894**	0
2.	4B	428.54	1	7	**-2.735**	**0.271**	**95.113**	0
3.	5B	432.959	1	6	**-2.735**	**-2.735**	**93.272**	0
4.	6B	432.959	1	6	**-2.735**	**0.514**	**90.875**	0
5.	7B	477.41	1	6	**-2.735**	**0.389**	**93.205**	0
6.	10B	443.511	1	8	**-2.735**	**-1.052**	**89.327**	0
7.	11B	443.511	1	8	**-2.735**	**-1.064**	**89.303**	0
8.	12B	426.568	1	6	**-2.736**	**0.413**	**95.446**	0
9.	13B	426.568	1	6	**-2.736**	**0.494**	**93.035**	0
10.	14B	412.541	1	6	**-2.735**	**0.527**	**92.334**	0
11.	15B	412.541	1	6	**-2.735**	**0.403**	**94.73**	0
12.	17B	478.578	1	8	**-2.735**	**-1.393**	**55.918**	0
13.	19B	404.562	1	6	**-2.738**	**0.322**	**92.445**	0
14.	20B	412.541	1	6	**-2.736**	**0.379**	**95.293**	0
15.	25B	515.665	1	7	**-2.735**	**0.439**	**93.965**	0

CONCLUSION:

As a result of the current study, The invitro anti-cancer activity shows that the compounds containing cyclohexylamine (19B) shows better cancer cell cytotoxicity in both breast and colon cancer. Moreover, The invitro anti-oxidant shows better inhibition of oxidative mechanism , when halogen and acid present in the para position of the compound. When comparing those two activities it is reveled that the compounds synthesized were more effective in anti-cancer agents than anti-oxidant agents. The results showed that cyclohexylamine imparted more on both breast and colon cancer activity. Thus the further substitutions with cyclohexylamine need to be studied potentially valuable new anti-cancer leads.

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CONFLICTS OF INTEREST:

The authors declare no conflict of interest.

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