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**Der Pharma Chemica, 2015, 7(8):130-136**
*(http://derpharmachemica.com/archive.html)*

**ISSN 0975-413X**
**CODEN (USA): PCHHAX**

# Synthesis, docking study and anticancer activity of benzoic acid Substituted derivatives of quinazolinones

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**ABSTRACT**

*Quinazolinone derivatives have played a vital part in the development of heterocyclic compounds. During the last 2 decades, the study of the biological activities of quinazolinone derivatives has been the aim of many researchers. Based on these findings, a series of benzoic acid substituted quinazolinone were synthesized by refluxing $CS_2$ and 2-aminobenzoicacid in acetic acid. Compounds were characterized by IR, $^1H$ NMR, and $^{13}C$ NMR. All the compounds were tested for their anticancer activity against MCF-7 breast cancer cell line with MTT assay. Most of the compounds showed moderate to good anti-breast cancer activity. Docking studies of the synthesized compounds was done with the help of iGEMDOCKv2.1 software using GRIP batch docking method to study their observed activity. Docking study was done and the compounds QZ 5 were found to fit well with the target protein BARC-1.*

**Keywords**: Anticancer, MCF-7 cell line, docking, protein BARC-1

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## INTRODUCTION

Cancer is the uncontrolled growth and spread of cells. It can affect any part of the body. The growth of often invade surrounding tissue and metastasize to distant sites. Cancer can be caused 90-95% by factors such as tobacco, obesity, infections, radiation and 5-10% due to heredity [1]. Thus, invention of newer anti-cancer agents has now become a key aim worldwide. The approach to practice medicinal chemistry has developed from an empirical one involving organic synthesis of new compound, based largely on modification of structures of known activity. According to Manfred Wolf, present development of medicinal chemistry has resistance, stating that "underlying the new age in foundation that includes explosive development of molecular biology since 1960, the advances in physical chemistry and physical organic chemistry has made possible by high speed computers and new powerful analytical methods. Numerous heterocyclic compounds, cyclic anhydrates, cyclic imides, cyclic acetals of dihydroxy alcohols, the solvents, dioxanes and tetrahydrofuran, in all of these, the chemistry is essentially that of their open-chain analogues. Heterocyclic intermediates are being used more and more in synthesis as protecting groups, readily generated, and readily removed.

Quinazolines are classes of fused heterocycles that are of considerable interest because of the diverse range of their biological properties, for example, anticancer, diuretic, anti-inflammatory, anticonvulsant and antihypertensive activities. Quinazolinones will be classified into the following five categories, based on the substitution patterns of the ring system. Out of the three quinazolinone structures, 4(3H)-quinazolinones are most prevalent, either as intermediates or as natural products in many proposed biosynthetic pathways. This is partly due to the structure being derived from the anthranilates (anthranilic acid or various esters, isatoicanhydride, anthranilamide and anthranilonitrile) while the 2(1H)-quinazolinone is predominantly a product of anthranilonitrile or benzamides with nitriles [2].

According to literature survey, quinazolinone containing moiety were reported to possess antimicrobial [3], analgesic [4], anti-inflammatory [5], anti-cancer [6], anti-tubercular [7], anthelmintic [8] and diuretic activities [9]. In addition quinazolinone have been reported to have broad biological activities like analgesic [10], anti-inflammatory [11], anti-cancer [12], anti-HIV [13], anti-Parkinson [14], anti-bacterial [15], anti-fungal and anti-tubercular agents. The synergism of both the heterocyclic moieties in a single entity may result in the formation of some worthwhile molecules with promising biological activities

## MATERIALS AND METHODS

### Reagents

All the chemicals and solvents used were of AR-grade obtained from Sigma- Aldrich, Sisco Research Laboratories, Qualingens, Hi-media, nice chemicals, Spectrochem and were used without further purification.

### Equipment

All melting points were taken in open capillaries and are uncorrected. Elemental analysis was performed on a Perkin-Elmer analyzer. IR spectral were recorded in KBr on Shimadzu spectrometer, $^{1}$H-NMR and $^{13}$C-NMR in DMSO-d6 on a Bruker AC-400 spectrometer using TMS as an internal standard. The microorganisms were obtained from National Chemical Laboratory, Pune.

### TLC analysis

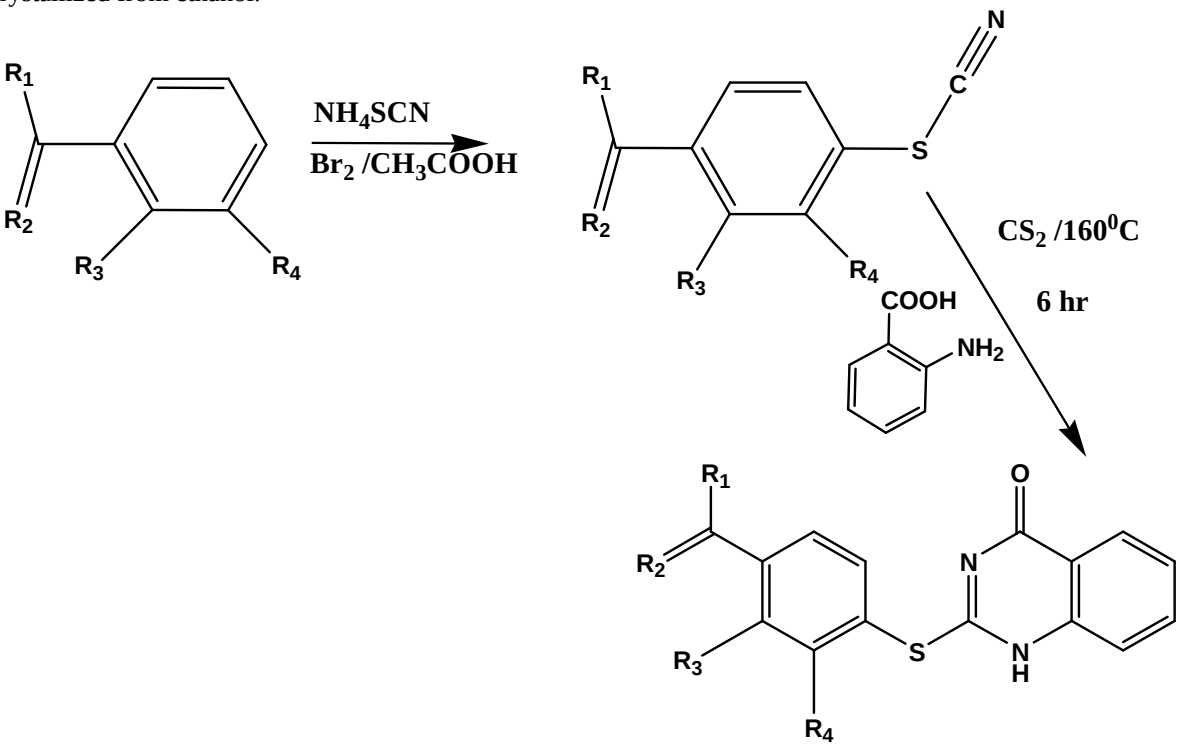
Thin-layer chromatography (TLC) was performed on pre-coated aluminium plates (silica gel 60F254, Merck). Plates were visualized by UV light and iodine vapour.

### General procedure for the synthesis of thiocyanate (TC1-TC5)

The substituted/unsubstituted benzoic acid (0.5 mol) was dissolved in acetic acid (125 ml) and the solution was added to the solution of ammonium thiocyanate (1.05mol, 80 g) in glacial acetic acid (250 ml). This solution was cooled to 10-20º C. To this well stirred solution, a solution of bromine (0.5 mol, 25.7 ml) in acetic acid (250 ml) was added drop wise for thirty minutes and the temperature was maintained below 20ºC. After the addition of bromine, it was kept at room temperature for ten minutes and then it was diluted with an equal amount of water. The solid material was filtered, washed, dried and recrystallized from ethanol.

### General procedure for the synthesis of Quinazolinones (Compound QZ 1-QZ 5)

A mixture of thiocyanate TC1-TC 5 (0.01 mol), 2-amino benzoicacid (0.01mol, 1.08g) and carbon disulphide (0.1 mol, 8 ml) was heated in an oil bath at 160$^{o}$C for 6 hours. The resultant quinazolinones was cooled and recrystallized from ethanol.



| | R1 | R2 | R3 | R4 |
|---|---|---|---|---|
| **TC1, QZ 1,** | **OH** | **O** | **H** | **H** |
| **TC2, QZ 2,** | **OH** | **O** | **$Cl_2$** | **H** |
| **TC3, QZ 3,** | **OH** | **O** | **Br** | **H** |
| **TC4, QZ 4,** | **OH** | **O** | **H** | **$NO_2$** |
| **TC5, QZ 5,** | **OH** | **O** | **$OCH_3$** | **H** |

## RESULTS AND DISCUSSION

**Table 1 : Analytical data of thiocyanate (TC1-TC5)**

| Thiocyanates | Yld (%) | M. Pt (° C) | Molecular Formula | Elemental Analysis (%) Reported (Calculated) | | | | | | | M.wt |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | C | H | N | O | Cl | Br | S | |
| TC 1 | 76 | 205-206 | $C_8H_5SN_3O_2$ | 53.62 (53.60) | 2.81 (2.88) | 7.82 (7.88) | 17.86 (17.87) | - | - | 17.89 (17.86) | 179 |
| TC 2 | 62 | 247-248 | $C_8H_4$ Cl S N $O_2$ | 44.98 (45.07) | 1.89 (1.94) | 06.56 (06.60) | 14.98 (15.05) | 16.59 (16.60) | | 15.01 (15.04) | 213 |
| TC 3 | 97 | 277-278 | $C_8H_4$S Br N $O_2$ | 37.23 (37.27) | 1.56 (1.56) | 5.43 (5.48) | 12.40 (12.45) | - | 30.96 (30.99) | 12.42 (12.46) | 258 |
| TC 4 | 75 | 248-249 | $C_8H_4N_2SO_4$ | 42.86 (42.90) | 1.80 (1.84) | 12.50 (12.56) | 28.55 (28.59) | - | - | 14.30 (14.34) | 224 |
| TC 5 | 80 | 251-252 | $C_9H_7SNO_3S$ | 51.67 (51.70) | 3.37 (3.41) | 6.69 (6.73) | 22.94 (22.99) | - | - | 15.33 (15.38) | 209 |

**IR data for the thiocyanate (TC 1-TC 5)**

*TC-1 (4-thiocyanatobenzoicacid) - $^{v}$C≡N: 2220cm-1*
*TC-2 (2-chloro-4-thiocyanatobenzoicacid) - $^{v}$C≡N: 2231 cm-1*
*TC-3 (2-bromo-4-thiocyanatobenzoicacid) - $^{v}$C≡N: 2221cm-1*
*TC-4 (3-nitro-4-thiocyanatobenzoicacid) - $^{v}$C≡N: 2210 cm-1*
*TC-5 (2-methoxy-4-thiocyanatobenzoicacid) - $^{v}$C≡N: 2192cm-1*

**Table 2: Analytical data of quinazolinones (QZ 1-QZ 5)**

| Quinazolinone | Yld (%) | M. Pt (° C) | Molecular Formula | Elemental Analysis (%) Reported (Calculated) | | | | | | | M.wt |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | C | H | N | O | Cl | Br | S | |
| QZ 1 | 73 | 505-507 | $C_{15}H_{10}SN_2O_3$ | 60.39 (60.42) | 3.38 (3.37) | 9.39 (9.41) | 16.09 (16.11) | - | - | 10.75 (10.77) | 298 |
| QZ 2 | 69 | 547-549 | $C_{15}H_9ClSN_2O_3$ | 54.14 (54.16) | 2.73 (2.76) | 8.42 (8.45) | 14.42 (14.46) | 10.65 (10.62) | - | 9.64 (9.65) | 332 |
| QZ 3 | 77 | 577-579 | $C_{15}H_9BrSN_2O_3$ | 47.76 (47.78) | 2.40 (2.42) | 7.43 (7.45) | 12.72 (12.74) | - | 21.18 (21.20) | 8.50 (8.53) | 377 |
| QZ 4 | 71 | 490-491 | $C_{15}H_9SN_3O_5$ | 52.48 (52.50) | 2.64 (2.66) | 12.24 (12.26) | 23.30 (23.32) | - | - | 9.34 (9.36) | 343 |
| QZ 5 | 80 | 551-552 | $C_{15}H_{12}SN_2O_4$ | 58.53 (58.55) | 3.68 (3.67) | 8.53 (8.55) | 19.49 (19.51) | - | - | 9.77 (9.79) | 328 |

**IR data for the quinazolinones (QZ 1 -QZ 5)**

**Compound QZ 1:** *4-(4-oxo-1, 4-dihydro-quinazolin-2-ylsulfanyl) benzoic acid*
IR KBr (cm ¯1): 1677(C=Nstr), 3379 (NHstr), 2924.39 (OH str), $^{1}$H-NMR: δ 7.79 (Ar-H, multiplet), δ 10.7 (Ar-OH, singlet).$^{13}$C-NMR: δ 161 (OH), δ 151(C=N).

**Compound QZ 2**: *4-(4-oxo-1, 4-dihydro-quinazolin-2-ylsulfanyl)-2-chlorobenzoic acid*
IR KBr (cm ¯1): 1670(C=Nstr), 3373 (NHstr), 2922 (OH str), 750(C-Cl str) 1H-NMR: δ 7.8 (Ar-H, multiplet), δ 11.2 (Ar-OH, singlet).$^{13}$C-NMR: δ 174 (OH), δ 152(C=N).

**Compound QZ 3*:*** *4-(4-oxo-1, 4-dihydro-quinazolin-2-ylsulfanyl)-2-bromobenzoic acid*
IR KBr (cm ¯1): 1674(C=Nstr), 3472 (NHstr), 2924.56 (OH str), 529(C-Br str) 1H-NMR: δ 7.8 (Ar-H, multiplet), δ 10.9 (Ar-OH, singlet).$^{13}$C-NMR: δ 168 (OH), δ 147(C=N).

**Compound QZ 4**: *4-(4-oxo-1, 4-dihydro-quinazolin-2-ylsulfanyl)-3-nitrobenzoic acid*
IR KBr (cm ¯1): 1663(C=Nstr), 3325 (NHstr), 2921 (OH str), 1424(C-$NO_2$ str) 1H-NMR: δ 7.6 (Ar-H, multiplet), δ 11.1 (Ar-OH, singlet).$^{13}$C-NMR: δ 173 (OH), δ 149(C=N).

**Compound QZ 5**: *4-(4-oxo-1, 4-dihydro-quinazolin-2-ylsulfanyl)-2-methoxybenzoic acid*
IR KBr (cm ¯1): 1673(C=Nstr), 3373 (NHstr), 2924.73 (OH str), 2857(C-$OCH_3$ str) 1H-NMR: δ 7.6 (Ar-H, multiplet), δ 10.8 (Ar-OH, singlet).$^{13}$C-NMR: δ 172 (OH), δ 153(C=N).

**Biological evaluation**

**Anticancer activity**

Growth of breast cancer cells was quantitated by the ability of living cells to reduce the yellow MTT to purple formazan products. The amount of formazan product formed is directly proportional to the number of living cells. Synthesized compounds were prepared as 4.0 mM top stock solutions, dissolved in DMSO. MCF-7 human breast cancer cells (human breast adenocarcinoma cell line originally obtained in 1973 from Michigan Cancer Foundation) were cultivated at 37 °C in an atmosphere of 5% CO2 in Dubecco's modified Eagle's minimal medium (DMEM) supplemented with 3.0 mML glutamine with 10% fetal bovine serum were routinely sub cultured twice weekly to maintain in continuous logarithmic growth. Cells were trypsinized for the passage into the well plate and plated at 10,000 cells/well in 100 $\mu$L of medium in 96-well plates. Cells were allowed to adhere to the surface of well plates. After 24 h, medium was removed and 100 $\mu$L of drug solutions (prepared at 10, 50, 100 and 250 $\mu$M concentrations) were added into the wells. 100 $\mu$L of fresh medium without cells was added as control. 4 wells were used for each concentration of drug solution, while 4 wells were reserved for cell culture control, which contained the corresponding amount of DMSO. The total drug exposure was 48 h. After 48 h, contents of the well were removed and 20 $\mu$L of MTT solution (5 mg in 1 mL of phosphate buffer saline) was added to each well. Incubation at 37 °C for 4 h allowed reduction of MTT by mitochondrial dehydrogenase to an insoluble formazan product. Well contents were removed and the formazan product was solubilized by addition of 100 $\mu$L DMSO [17]. The purple colour was produced. Absorbance of each well was read on Tenac 200 plate reader at 570 nm. From the absorbance, the % inhibition was calculated as % growth inhibition = Ac – At / Ac X 100. Where *A*c is the mean absorbance of control and *A*t is the mean absorbance of test (Table 3).

**Table 3 % Growth inhibition of MCF-7 cells**

| Compound | % Growth inhibition | | | | |
|---|---|---|---|---|---|
| | 0.25 µM | 2.5 µM | 25 µM | 50 µM | 100 µM |
| QZ 1 | 0.2958 | 2.4785 | 16.4543 | 27.4545 | 47.5861 |
| QZ 2 | 0.6754 | 2.9586 | 19.7865 | 25.5668 | 39.6875 |
| QZ 3 | 0.3867 | 2.5645 | 18.4443 | 31.4775 | 40.1123 |
| QZ 4 | 0.1221 | 1.5878 | 20.1257 | 26.8965 | 43.4568 |
| QZ 5 | 0.4958 | 1.7589 | 17.8592 | 30.1235 | 58.5239 |

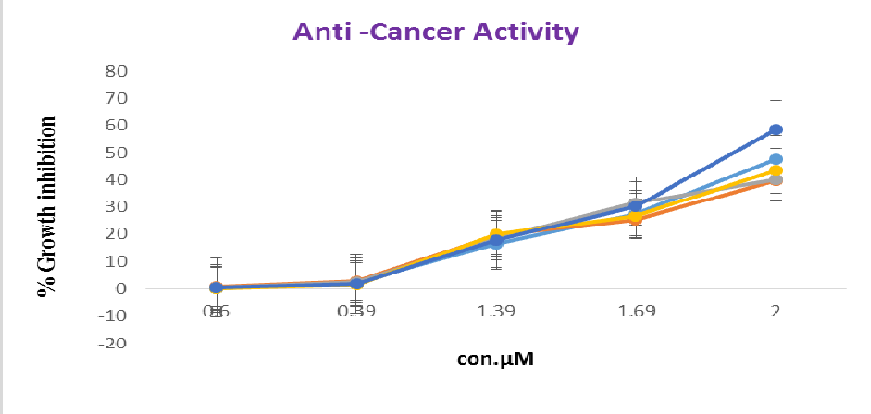



**Fig.1 % Growth of inhibition of synthesized quinazolinones**

**Anti-microbial Activity**

The anti-microbial activity for the sample was carried out by Disc Diffusion Technique [17]. The test microorganisms (Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Aspergillus Niger) maintained by periodical subculturing on nutrient agar and sabouraud dextrose agar medium for bacteria and fungi respectively. The test microorganisms were obtained from National Chemical Laboratory NCL), Pune and maintained by periodical sub culturing on nutrient agar and sabouraud dextrose agar medium for bacteria and fungi respectively. The effects produced by the sample were compared with the effect produced by the positive control (Reference standard ciprofloxacin 5 µg/disc for bacteria; Nystatin 100 units/disc for fungi).

**Table 4 Anti-microbial activity of the synthesized compounds**

| S.No | Name of the Microorganisms | Zone of inhibition in mm | | | | | |
|---|---|---|---|---|---|---|---|
| | | OX 1 | OX 2 | OX 3 | OX 4 | OX 5 | Std |
| 1. | *Staphylococcus aureus* (NCIM 2079) | 20 | 15 | 20 | 20 | 30 | 35 |
| 2. | *Bacillus Subtilis* ( NCIM 2063) | 20 | 16 | 21 | 23 | 26 | 40 |
| 3. | *Klebsiella aerogenes* (NCIM 2098) | 26 | 18 | 20 | 25 | 24 | 30 |
| 4. | *Pseudomonas aeruginosa* (NCIM 2036) | 20 | 18 | 21 | 25 | 25 | 40 |
| 5. | *Aspergillus niger* ( NCIM 20105) | 22 | 20 | 30 | 26 | 30 | 35 |
| 6. | *Candida albicans* (NCIM 3102) | 20 | 22 | 20 | 23 | 28 | 32 |

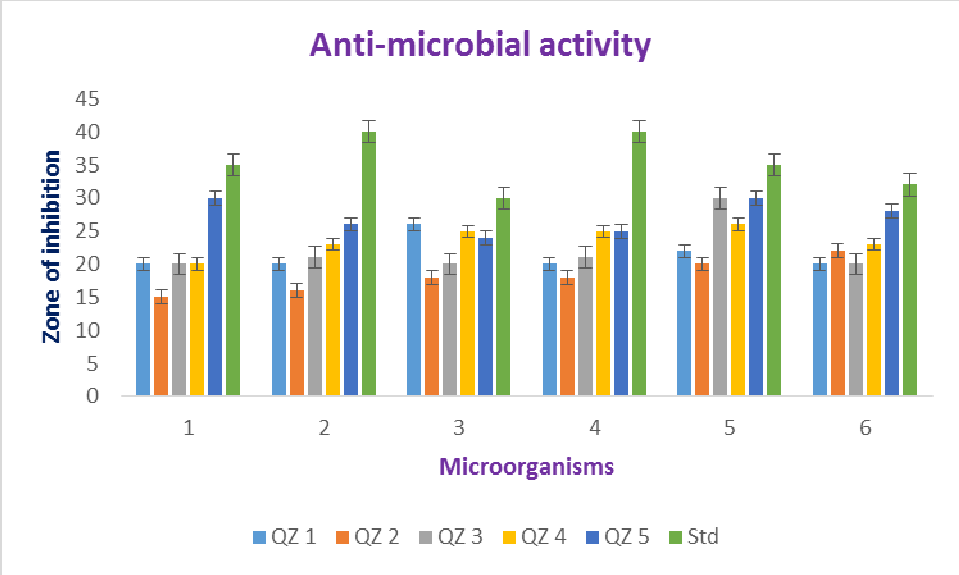



**Fig.2 Zone of inhibition of synthesized quinazolinones against anti-microbial activity**

**Docking studies**

Over activation of receptor tyrosine kinase (RTK) signalling pathways is strongly associated with carcinogenesis. So it is becoming increasingly clear that impaired deactivation of RTKs may be a mechanism in cancer. On this basis, we selected RTK as a biological target for docking study of synthesized compounds. The crystal structure of EGFR kinase domain (PDB ID: 2a91) in complex with an irreversible inhibitors was obtained from the protein data bank [18]. The crude PDB structure of receptor was then refined by completing the incomplete residues. The crystallized ligand lying within the receptor was modified by assigning missing bond order and hybridization states. The side chain hydrogen was then added to the crystal structure and their positions were optimized up to the rms gradient 1 by aggregating the other part of the receptor.

**Target Protein Structure**

The structure of the target protein was downloaded from PDB

Target PDB: THE CRYSTAL STRUCTURE OF WILD TYPE DIPHTHERIA TOXIN
PDB ID: 1FOL. Structures of the compounds. The structures of the different compounds were drawn using ChemSketch software and the files were processed and saved as MOL files.

The PDB structure with the ID 1FOL was loaded in to the iGEMDOCK software. The binding site for the target was prepared with the radius of 4 A0. The different ligands were drawn, prepared and uploaded into the software. The following parameters were set. Population size: 100, Generations: 50, Number of solutions: 2. the output path was set. 'Start docking' option was clicked and when docking was complete post analysis of the docked ligands was done. The predicted poses and the energy list of these poses will be outputted into the "best Pose" and "fitness.txt" of the output location, respectively. The predicted poses and scores of ligands are saved in the user defined output path. Fitness is the total energy of a predicted pose in the binding site. The empirical scoring function of

iGEMDOCK is estimated as: Fitness = vdW + Hbond + Elec. Here, the vdW term is van der Waal energy. Hbond and Elect terms are hydrogen bonding energy and electro statistic energy, respectively.

The interaction residues and energy values of the synthesis compounds with the target.

**Table 5: Energy values of the synthesized compounds**

| S.No | Compound | Energy | VdW | HBond |
|---|---|---|---|---|
| 1 | QZ1 | -82.937 | -80.465 | -2.472 |
| 2 | QZ2 | -80.0797 | -79.0677 | -1.012 |
| 3 | QZ3 | - 102.861 | -96.7069 | -6.1534 |
| 4 | QZ4 | -43.9235 | -44.8536 | -0.9301 |
| 5 | QZ5 | -109.36 | -102.38 | -7.4302 |

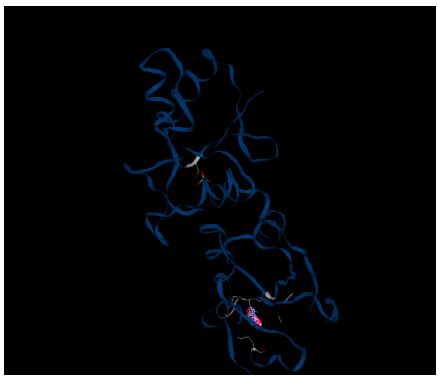


**Fig.3 Interaction of QZ-1 with *BRCA1***

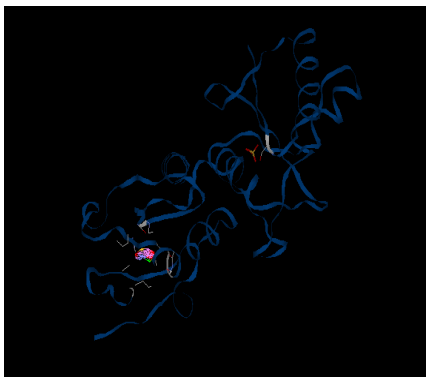


**Fig.4 Interaction of QZ-2 with *BRCA1***

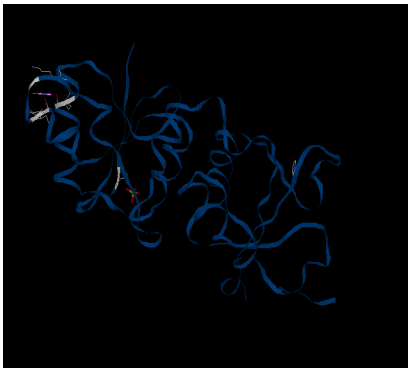


**Fig.5 Interaction of QZ-3 with *BRCA1***

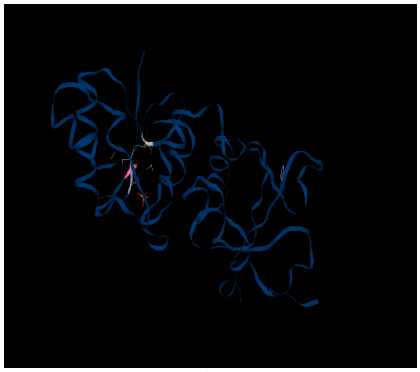


**Fig.6 Interaction of QZ-4 with *BRCA1***

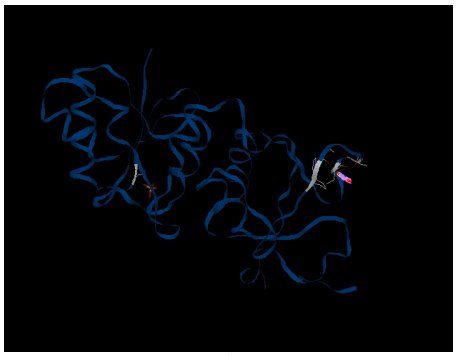


**Fig.7 Interaction of QZ-2 with *BRCA1***

## DISCUSSION

All the synthesized compounds were screened for cytotoxicity on MCF-7 cell lines by MTT method. Cytotoxicity was checked at 24 hours and 48 hours duration. It was found that the activity of the compounds was increased after 48 hours as compared to 24 hours. Among the tested compounds QZ 3 and QZ 4 showed potent activity and their % growth inhibition was 45.568 and 42.236 at 100 µM/ml. Compounds QZ 3 and QZ 4 were showed IC50 50 µM/ml and 32.466 µM/ml. Docking studies was carried out by taking tyrosine kinase domain as a target for anticancer

activity, the compound QZ 5 was found to have highest negative dock score (-109.36). It means that it can fit well in the receptor cavity forming energetically most stable drug receptor complex. QZ 5 Compound shows more activity than the other compounds against *Staphylococcus aureus, Bacillus Subtilis, Klebsiella aerogenes, Pseudomonas aeruginosa, Aspergillus niger, Candida albicans.*

## CONCLUSION

As the concentration of compound being tested increased, the *in- vitro* anticancer activity also increased. The docking score of the synthesized compounds could not be correlated with the *invitro* anticancer activity and conclusion could not be drawn on their exact mechanism of action. So further molecular modification is required in order to arrive at more accurate structure activity relationship with their anticancer activity on breast cancer cell lines or different crystal structure of tyrosine kinase domain could be selected from PDB to study their mechanism of action. The Anti-microbial activity is studied and the compound QZ5 shows maximum activity

**Acknowledgement**
The authors thank the Principal, Management and PG & Research Department of Chemistry, of Bishop Heber College, for the facilities provided to carry out this work.

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