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Design, Synthesis and Molecular Docking of Thiophene Derived Benzodiazepines as Anticancer Agents

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A new series of 1,4-benzodiazepines were synthesized based on the results of molecular docking. The designed compounds were docked in the groove of binding sites present in human estrogen receptor 2IOK and EGFR receptor 4WKQ. The physico-chemical properties, Lipinski's Rule of 5 and ADMET properties were calculated for the compounds by using the Qikprop application in Schrödinger software and on the basis of molecular docking fifteen compounds were designed. Because of poor yield in some cases, only ten compounds have been synthesized. All the newly synthesized compounds were established based on IR, ^{1}H NMR and mass spectral data. The new compounds were screened for *in vitro* anticancer activity. Among all the compounds, compound **BZ4** had the highest binding energies and showed the potential cytotoxicity when screened for anticancer activity by MTT assay.

Keywords: 1,4-Benzodiazepines, Chalcones, Molecular docking, Anticancer studies.

INTRODUCTION

Benzodiazepines are the bicyclic heterocyclic compounds with a seven-membered ring comprising a benzene nucleus with two atoms of nitrogen [1,2]. The diazepine ring is known for its antibacterial [3] and antidepressant properties [4]. The use of two pharmacophoric agents in a single molecule could be seen as a potential drug design approach for site-specificity [5]. Similarly, benzodiazepines exhibit the diversified activities such as anticonvulsants [6], antibacterial [7], anticancer [8], antitubercular [9], *etc.* and their synthesis in pharmaceutical and medicinal chemistry is very significant [1]. It also serves as an antifungal [11], antiproliferative [12] and the inhibitors of farnesyl-transferase [12]. Fused heterocyclic benzodiazepines are commonly used as antianxiety drugs, but they may be adversely affected by side effects such as neurological and psychomotor symptoms [13,14]. Some of these substances had antiproliferative effects on certain tumour cell lines. It highlights the mass potential anticancer agents [15]. Owing to their function on peripheral benzodiazepine (PBRs) receptors, some 1,4-benzodiazepine lipophilic derivatives are observed for their therapeutical CNS efficacy [16]. This can act as a specific selective and intracellular target for antineoplastic agents [17].

Some of the psychotropic drugs commonly used to treat insomnia and depression also have an impact on the cell proliferation. Surprisingly less work, however, has aimed themselves alone or in conjunction with proven cancer treatment. The most extensively studied benzodiazepine (BZ) receptor ligands have been demonstrated to have distinct, concentration-dependent effects on the progression and proliferation of both normal and malignant cells [18].

The most popular virtual screening technique is molecular docking and it plays a big role in the detection of original hits and optimization of hits in several investigations of similarity findings. This molecular docking method enables us to simulate the association of a small molecule with a protein on an atomic level, allowing us to differentiate the behaviour, both in the target proteins' binding sites and in elucidating basic biochemical processes [19]. The *in vitro* antitumor efficacy of these docked molecules was further investigated.

Nowadays, the prevalence of diseases such as cancer and tuberculosis is on the rise and the discovery of new molecules

is a challenging task for medicinal chemists. So, there is a need to find and design new compounds for the arising diseases.

Keeping in mind about these facts, this study aims to design, synthesize and screen some benzodiazepines derived from thiophenes for their anticancer efficacy. To investigate the intermolecular interaction between the designed ligand and the targeted enzyme, molecular docking was carried out on these molecules, which will assist in identifying the potential anticancer lead compounds.

EXPERIMENTAL

All the chemicals were procured from Sigma-Aldrich, India and used without further purification. The capillary technique was used to determine melting points, which are uncorrected. IR spectra (KBr pellets) were recorded by using Shimadzu Perkin-Elmer 8201 Pc IR Spectrometer, with frequencies given in cm^{-1}. ^1H NMR spectra were documented at 400 MHZ BRUKER ADVANCE II NMR SPECTROMETER by using $CDCl_3$ and DMSO as solvents. The mass spectra were obtained using the electron impact ionization technique on GC-MS Perkin-Elmer CLARUS680 Spectrometer.

Computational methodology: Schrödinger 2018-3 suite device Maestro (Ligprep, Glide XP docking, QikProp), which was developed on a 27-inch workstation by DELL Inc. and powered by an Intel Corei7-7700 CPU running at 3.60 GHz x8, 8GB of RAM, a 1000 GB hard drive and Linux -X664 as the operating system was used for computational analysis.

Molecular docking: For docking analysis, targets EGFR (PDB ID: 4WKQ) and human estrogen receptor (PDBID: 2IOK) [20] were selected and downloaded from PDB for lung and breast anticancer activity, respectively. The ligands and protein were prepared and minimized by the Ligprep and protein preparation wizard respectively. The molecular docking was then carried out between the grid-generated protein and the ligands, by the application glide (XP). The glide extra precision (XP) tool is used to validate the compatibility of acertain ligand molecule to a particular target's active site [21].

Drug likeness ADMET property: The characterization of physico-chemical properties is a common approach that has become quite popular in the area of pharmaceutical science. One significant task is to produce a product that blends biological activity with a sufficient physico-chemical profile, a pharmaceutical active feature. The compounds were evaluated for drug-like characteristics using the Lipinski Rule of Five and ADMET property prediction was carried out using the QikProp programme. QikProp, an algorithm of Schrödinger software, calculates the widest range of pharmaceutically related properties *viz*. QPlogBB, percentage human oral absorption, QPPCaco, Lipinski's rule RO5, SASA and Rule of Three.

In present work, the following steps were involved in the synthesis of 1,4-benzodiazepine derivatives.

Synthesis of novel thiophene derived chalcones: Aromatic ketone (0.005 M) and substituted benzaldehydes (0.005 M) dissolved in approximately 20-30 mL ethanol and stirred in the presence of 4-5 mL of freshly prepared 40% NaOH and maintained for 24 h of continuous stirring. After cooling to room temperature, the reaction mixture was placed onto crushed ice (100 mL) acidified with 5% HCl and stirred continously. The final product underwent purification, washing and recrystallization with absolute ethanol.

(*E*)-3-(4-Bromophenyl)-1-(5-chlorothiophen-2-yl)-prop-2-en-1-one (C1): Light yellow crystals (EtOH); m.p.: 261-263 ºC; yield: 81.23%; *m.f.* $C_{13}H_8OSBrCl$ (*m.w*. 327.62). IR (KBr, ν_{max}, cm^{-1}): 1648 (C=C *str.* in α, β unsaturated ketone), 1420 (C=C *str.*), 1224 (C-S-C), 680 (C-Br); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 7.2 (d, 1H, CH=CH), 7.3 (d, 1H, CH=CH), 7.27-7.87 (8H, m, Ar-H). Mass (*m/z*) (M +1): 327.60.

(*E*)-1-(5-Chlorothiophen-2-yl)-3-(4-fluorophenyl)-prop-2-en-1-one (C2): Pale yellow crystals (EtOH); m.p.: 202-204 ºC; yield: 83.22%; *m.f.* $C_{13}H_8OSClF$ (*m.w*. 266.72). IR (KBr, ν_{max}, cm^{-1}): 2556 (aromatic C–H), 1670 (C=O), 1600 (C=C *str.* in α,β unsaturated ketone), 1290 (C-S-C), 1156 (C-F); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 7.1 (d, 1H, CH=CH), 7.4 (d, 1H, CH=CH), 7.30-8.03 (8H, m, Ar-H). Mass (*m/z*) (M +1): 266.72.

(*E*)-3-(4-Chlorophenyl)-1-(5-chlorothiophen-2-yl)-prop-2-en-1-one (C3): Light green crystals (EtOH); m.p.: 231-233 ºC; yield: 85.93%; *m.f.* $C_{13}H_8OSCl_2$ (*m.w*. 283.17). IR (KBr, ν_{max}, cm^{-1}): 2506 (aromatic C–H), 1688 (C=O), 1602 (C=C *str.* in α,β unsaturated ketone), 1288 (C-S-C), 765 (C-Cl); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 7.0 (d, 1H, CH=CH), 7.2 (d, 1H, CH=CH), 7.25-8.13 (8H, m, Ar-H). Mass (*m/z*) (M +1): 283.17.

(*E*)-1-(5-Chlorothiophen-2-yl)-3-(4-hydroxyphenyl)-prop-2-en-1-one (C4): Off white crystals (EtOH); m.p.: 300-302 ºC; yield: 80.34%; *m.f.* $C_{13}H_9O_2SCl$ (*m.w*. 264.73). IR (KBr, ν_{max}, cm^{-1}): 3151 (C-OH), 2874 (aromatic C–H), 1665 (C=O), 1596 (C=C *str.* in α,β unsaturated ketone), 1428 (C=C *str.*), 1157 (C-S-C); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 6.9 (d, 1H, CH=CH), 7.1 (d, 1H, CH=CH), 9.79 (s, 1H, OH), 7.25-8.13 (8H, m, Ar-H). Mass (*m/z*) (M+1): 264.73.

(*E*)-1-(5-Chlorothiophen-2-yl)-3-(4-nitrophenyl)prop-2-en-1-one (C5): Light brown crystals (EtOH); m.p.: 285-287 ºC; yield: 83.74%; *m.f.* $C_{13}H_8NO_3Cl$ (*m.w*. 293.73). IR (KBr, ν_{max}, cm^{-1}): 2874 (aromatic C–H), 1665 (C=O), 1596 (C=C *str.* in α,β unsaturated ketone), 1428 (C=C *str.*), 1157 (C-S-C); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 6.7 (d, 1H, CH=CH), 7.3 (d, 1H, CH=CH), 7.2-8.11 (8H, m, Ar-H). Mass (*m/z*) (M+1): 293.73.

(*E*)-1-(5-Chlorothiophen-2-yl)-3-(4-(dimethylamino)-phenyl)prop-2-en-1-one (C6): Off white crystals (EtOH); m.p.: 518-520 ºC; yield: 82.33%; *m.f.* $C_{15}H_{14}NOSCl$ (*m.w*. 291.80). IR (KBr, ν_{max}, cm^{-1}): 2556 (aromatic C–H), 1555 (C=C *str.* in α,β unsaturated ketone), 1420 (C=C *str.*), 1157 (C-S-C); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 7.2 (d, 1H, CH=CH), 7.3 (d, 1H, CH=CH), 6.9-8.17 (8H, m, Ar-H). Mass (*m/z*) (M +1): 291.80.

(*E*)-1-(5-Chlorothiophen-2-yl)-3-(4-methoxyphenyl)-prop-2-en-1-one (C7): Pale yellow crystals (EtOH); m.p.: 234-236 ºC; yield: 78.84; *m.f.* $C_{15}H_{11}O_2SCl$ (*m.w*. 278.75). IR (KBr, ν_{max}, cm^{-1}): 2528 (arom. C–H), 1589 (C=C *str.* in α,β unsaturated ketone), 1410 (C=C *str.*), 1186 (C-S-C); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 7.0 (d, 1H, CH=CH), 7.1 (d, 1H, CH=CH), 7.3-8.01 (8H, m, Ar-H). Mass (*m/z*) (M +1): 278.75.

(*E*)-3-(Anthracen-2-yl)-1-(5-chlorothiophen-2-yl)prop-2-en-1-one (C10): Yellow crystals; m.p.: 369-371 ºC; yield: 86.87%; *m.f.* $C_{21}H_{13}OSCl$ (*m.w*. 348.85). IR (KBr, ν_{max}, cm^{-1}): 2585 (aromatic C–H), 1532 (C=C *str.* in α,β unsaturated ketone), 1456 (C=C *str.*), 1283 (C-S-C); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.1 (d, 1H, CH=CH), 7.4 (d, 1H, CH=CH), 6.7-7.76 (8H, m, Ar-H). Mass (*m/z*) (M +1): 348.85.

(*E*)-3-(2-Chlorophenyl)-1-(5-chlorothiophen-2-yl)-prop-2-en-1-one (C11): Light green crystals; m.p.: 231-233 ºC; yield: 69.30%; *m.f.* $C_{13}H_8OSCl_2$ (*m.w*. 283.17). IR (KBr, ν_{max}, cm^{-1}): 2506 (aromatic C–H), 1688 (C=O), 1602 (C=C *str.* in α,β unsaturated ketone), 1288 (C-S-C), 765 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.0 (d, 1H, CH=CH), 7.2 (d, 1H, CH=CH), 7.25-8.13 (8H, m, Ar-H). Mass (*m/z*) (M +1): 283.17.

(*E*)-1-(5-Chlorothiophen-2-yl)-3-(1*H*-imidazol-2-yl)-prop-2-en-1-one (C14): White crystals; m.p.: 305-307 ºC; yield: 75.23%; *m.f.* $C_{10}H_7N_2OSCl$ (*m.w*. 238.69). IR (KBr, ν_{max}, cm^{-1}): 2506 (aromatic C–H), 1688 (C=O), 1602 (C=C*str.* in α,β unsaturated ketone), 1288 (C-S-C), 765 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.2 (d, 1H, CH=CH), 7.5 (d, 1H, CH=CH), 7.2-8.05 (8H, m, Ar-H). Mass (*m/z*) (M +1): 238.69.

Synthesis of substituted 1,4-benzodiazepines (BZ 1-14): In presence of 100% ethanol and glacial acetic acid (30 mL), a mixture of chalcone (**C 1-14**) (0.005 mol) and *o*-phenylene-diamine (0.005 mol) were dissolved and the reaction mixture was then refluxed for around 10 h. When the reaction was performed, crushed ice was added to it. The product was filtered and rinsed with cold water (**Scheme-I**). Absolute ethanol was used to recrystallize the product.

4-(4-Bromophenyl)-2-(5-chlorothiophen-2-yl)-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepine (BZ 1): Light yellow crystals; m.p.: 261-263 ºC; yield: 81.23%; *m.f.* $C_{19}H_{14}N_2SBrCl$ (*m.w*. 237.62). IR (KBr, ν_{max}, cm^{-1}): 3468 (NH), 2921 (C-H), 679 (br), 1603 (C=N) 754 (C-S-C); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3, 3.3, 5.3 (d, 3H), 7.14 (s, 1H, NH) 6.80-8.06 (10H, m, Ar-H); Mass (*m/z*) (M +1) 418.06.

2-(5-Chlorothiophen-2-yl)-4-(4-fluorophenyl)-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepine (BZ 2): Yellow crystals; m.p.: 202-204 ºC; yield: 83.22%; *m.f.* $C_{19}H_{14}N_2SClF$ (*m.w*. 266.72). IR (KBr, ν_{max}, cm^{-1}): 3480 (NH), 3082 (C-H), 1128 (F), 1602 (C=N) 1291 (C-S-C); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 3.03.3, 4.0 (d, 3H), 7.2 (s, 1H, NH) 6.96-8.23 (10H, m, Ar-H). Mass (*m/z*) (M +1) 356.84.

4-(4-Chlorophenyl)-2-(5-chlorothiophen-2-yl)-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepine (BZ 3): Pale green crystals; m.p.: 231-233 ºC; yield: 85.93%; *m.f.* $C_{19}H_{14}N_2SCl_2$ (*m.w*. 283.17). IR (KBr, ν_{max}, cm^{-1}): 3468 (NH), 3027 (C-H), 758 (Cl), 1590 (C=N) 1090 (C-S-C); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.4, 4.5, 5.2 (d, 3H), 7.3 (s, 1H, NH), 7.22-8.19 (10H, m, Ar-H). Mass (*m/z*) (M +1) 373.26.

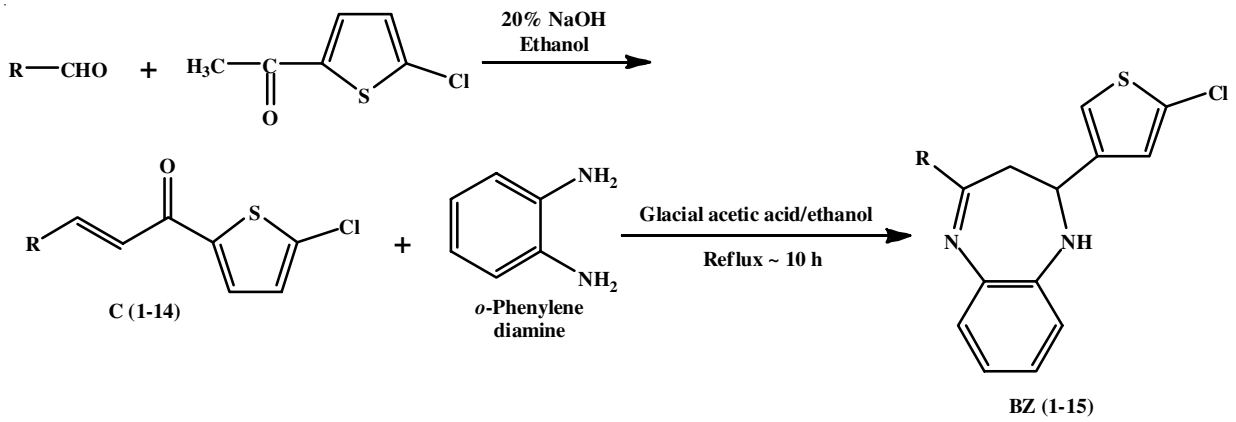
4-(2-(5-Chlorothiophen-2-yl)-2,3-dihydro-1*H*-benzo-[*b*][1,4]diazepin-4-yl)phenol (BZ 4): Off-white crystals; m.p.: 300-302 ºC; yield: 80.34%; *m.f.* $C_{19}H_{15}N_2OSCl$ (*m.w*. 264.73). IR (KBr, ν_{max}, cm^{-1}): 3623 (C-OH), 3385 (NH), 2919 (C-H), 1689 (C=N) 1241 (C-S-C); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 3.4, 3.9, 5.5 (d, 3H), 7.2 (s, 1H, NH), 6.67-8.02 (10H, m, Ar-H), 9.45 (s, 1H, OH); Mass (*m/z*) (M+1)354.406.

2-(5-Chlorothiophen-2-yl)-4-(4-nitrophenyl)-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepine (BZ 5): Light brown crystals; m.p.: 285-287 ºC; yield: 83.74%; *m.f.* $C_{19}H_{14}N_3O_2SCl$ (*m.w*. 293.73). IR (KBr, ν_{max}, cm^{-1}): 3685 (NH), 3109 (C-H), 1681 (C=N)1291 (C-S-C); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.3, 4.6, 5.1 (d, 3H), 7.1 (s, 1H, NH), 6.77-8.09 (10H, m, Ar-H). Mass (*m/z*) (M+1) 383.80.

4-(2-(5-Chlorothiophen-2-yl)-2,3-dihydro-1*H*-benzo-[*b*][1,4]diazepin-4-yl)-*N*,*N*-dimethylaniline (BZ 6): Light yellow crystals; m.p.: 256-258 ºC; yield: 82.33%; *m.f.* $C_{21}H_{20}N_3SCl$ (*m.w*. 291.80). IR (KBr, ν_{max}, cm^{-1}): 3458 (NH), 3128 (C-H), 2783 (N-CH_3), 1669 (C=N) 1235 (C-S-C); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 3.1, 3.6, 4.5 (d, 3H), 7.4 (s, 1H, NH), 7.10-8.08 (10H, m, Ar-H). Mass (*m/z*) (M+1)381.92.

2-(5-Chlorothiophen-2-yl)-4-(4-methoxyphenyl)-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepine (BZ 7): White crystals; m.p.: 234-236 ºC; yield: 78.84%; *m.f.* $C_{21}H_{20}N_2OSCl$ (*m.w*. 278.75). IR (KBr, ν_{max}, cm^{-1}): 3469 (NH), 3028 (C-H), 2837 (O-CH_3), 1679 (C=N) 1244 (C-S-C); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.5, 4.7, 5.2 (d, 3H), 7.16 (s, 1H, NH), 6.65-8.23 (10H, m, Ar-H). Mass (*m/z*) (M+1) 368.11.

4-(Anthracen-9-yl)-2-(5-chlorothiophen-2-yl)-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepine (BZ 10): Yellow crystals; m.p.: 369-371 ºC; yield: 86.87%; *m.f.* $C_{27}H_{19}N_2SCl$ (*m.w*. 348.85). IR (KBr, ν_{max}, cm^{-1}): 3369 (NH), 3151 (C-H), 1689 (C=N)1235 (C-S-C); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):



Scheme-I

3.1, 5.3, 5.7 (d, 3H), 7.1 (s, 1H, NH), 6.78 -8.14 (10H, m, Ar-H). Mass (*m/z*) (M+1) 438.97.

4-(2-Chlorophenyl)-2-(5-chlorothiophen-2-yl)-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepine (BZ 11): Light green crystals; m.p.: 231-233 ºC; yield: 69.30%; *m.f.* $C_{19}H_{14}N_2SCl_2$ (*m.w*. 283.17). IR (KBr, ν_{max}, cm^{-1}): 3460 (NH), 3027 (C-H), 758 (Cl), 1590 (C=N) 1090 (C-S-C); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.4, 4.5, 5.2 (d, 3H), 7.3 (s, 1H, NH), 7.22-8.19 (10H, m, Ar-H). Mass (*m/z*) (M +1) 373.26.

2-(5-Chlorothiophen-2-yl)-4-(1*H*-imidazol-2-yl)-2,3-dihydro-1*H*- benzo[*b*][1,4]diazepine (BZ 14): Off-white crystals; m.p.: 305-307 ºC; yield: 75.23%; *m.f.* $C_{16}H_{13}N_4SCl$ (*m.w*. 238.69). IR (KBr, ν_{max}, cm^{-1}): 3453 (NH), 3128 (C-H), 1665 (C=N) 1226 (C-S-C); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.4, 4.5, 5.2 (d, 3H), 7.3 (s, 1H, NH), 7.22-8.19 (10H, m, Ar-H). Mass (*m/z*) (M+1) 328.82.

Anticancer activity: MCF-7 cell lines were used for the anticancer activity. They were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS and 1% antibiotic-antimycotic solution added as supplements. Throughout the experiment, cells were kept at 37 ºC and 5% CO_2 in a humid environment. Methyl thiazolyl tetrazolium (MTT) test was used to determine the cytotoxicity of the synthesized compounds.

On a 96 well microtiter plates, the new cells were seeded at a density of 5000 cells per well. Following adherence, they were given treatments with the test substance at various doses, including 6.25, 12.5, 25, 50 and 100 µg/mL (solubilized in DMEM). MTT reagent was added to the wells and incubated at 37 ºC for 4 h. DMSO was used to solubilize the produced formazan crystals and a multimode microplate reader was used to measure the absorbance at 570 nm (FluoSTAR Omega, BMG Labtech). The test compound's cytotoxicity was estimated as a percentage of the untreated cell control. The standard drug used was tamoxifen [19].

RESULTS AND DISCUSSION

The aim of this study was to synthesize 2-(5-chlorothiophen-2-yl)-4-methyl-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepine derivatives using *o*-phenylenediamine and chalcone derivatives based on molecular docking results and assess their anticancer activities. The physico-chemical and ADMET properties were also screened for these compounds. Spectral data was used to confirm the structure of all the synthesized molecules.

All of the newly synthesized compounds were identified using spectral data. The IR spectra of compound **BZ4** revealed an absorption band at 2919 cm^{-1}, confirming the existence of an aromatic -CH group. Absorption bands at 3385 cm^{-1} for the NH group and 1689 cm^{-1} for the C=N group indicate the presence of the benzodiazepine ring. The absorption band at 1241 cm^{-1} for the C-S-C group indicates the presence of the thiophene ring. The other prominent peaks are at 3623 cm^{-1} indicating the presence of the C-OH group and 1439 cm^{-1} for the C=C group.

Further information concerning the structure of the molecule was gained by recording the mass spectra of the compound **BZ4** revealed a molecular ion peak at 354.4 *m/z* (M^+) peak that corresponded to the molecular formula $C_{19}H_{15}ClN_2OS$.

The ^{1}H NMR spectrum of compound **BZ4** revealed the existence of multiplet in the range δ 6.67-8.02 ppm, suggesting the presence of aromatic protons. At δ 9.45 ppm, a strong singlet peak was seen, indicating the existence of the OH group. The singlet peak at δ 7.2 ppm indicates the presence of NH proton and the peaks at δ 3.4, 3.9 and 5.5 (d) ppm indicates the presence of aliphatic protons of benzodiazepine.

Docking studies: Using Schrödinger software, *in silico* molecular docking investigations were performed on 15 designed analogues of thiophene-derived benzodiazepines. The compounds were docked in the groove of human estrogen receptor 2IOK and EGF receptor 4WKQ binding sites. The interactions of the compounds with receptors are listed in Tables 1 and 2 in terms of docking score.

The synthesized molecules bind to human estrogen receptor 2IOK with a binding free energy of between -9.71 and -8.954 kcal/mol. The active residues in 2IOK are Ile424, Met423, Val418, Gly521, Hid524, Leu 525, Met528, Met343, Leu346, Thr347, Ala350, Leu428, Leu391, Met388, Leu387, Leu384, Trp383, Phe404, Leu354 (Fig. 1).

TABLE-1
EXTRA PRECISION GLIDE DOCKING RESULTS WITH INTERACTING AMINO ACIDS IN THE ACTIVE SITE OF 2IOK

Compound	Glide XP docking score	Glide XP energy (kcal/mol)	Polar interaction with amino acids	HB	Pi-Pi stackings
BZ1	-9.710	-48.464	Hid524, Thr347	–	–
BZ2	-9.754	-44.324	Hid524, Thr347	–	Phe404
BZ3	-9.595	-46.961	Hid524, Thr347	–	–
BZ4	-9.954	-48.699	Hid524, Thr347	Glu353	Phe404
BZ5	-9.583	-50.134	Hid524, Thr347	–	–
BZ6	-9.568	-45.854	Hid524, Thr347	–	–
BZ7	-9.134	-41.739	Hid524, Thr347	–	Phe404
BZ8	-8.970	-38.646	Thr347	–	Phe404
BZ9	-8.707	-40.100	Thr347	–	Phe404
BZ10	-9.736	-31.345	Hid524, Thr347	–	Phe404
BZ11	-9.236	-41.176	Hid524, Thr347	–	–
BZ12	-8.893	-40.447	Thr347	–	Phe404
BZ13	-8.441	-41.176	Thr347	–	Phe404
BZ14	-8.302	-40.846	Thr347	Leu346	Phe404
BZ15	-8.954	-42.282	Hid524, Thr347	Leu346	Phe404
Tamoxifen (Std)	-10.435	-45.658	Hid524, Thr347	Leu346	–

TABLE-2
EXTRA PRECISION GLIDE DOCKING RESULTS WITH INTERACTING AMINO ACIDS IN THE ACTIVE SITE OF 4WKQ

Compound	Glide XP docking score	Glide XP energy (kcal/mol)	Polar interaction with amino acids	HB	Pi-Pi stackings
BZ1	-5.497	-42.188	Asn842, Thr790, Thr854	–	Nil
BZ2	-5.240	-40.495	Asn842, Thr790, Thr854, Gln791	–	–
BZ3	-5.143	-41.618	Asn842, Thr790, Thr854	–	–
BZ4	-6.309	-42.794	Asn842, Thr790, Thr854	Met793	–
BZ5	-4.291	-41.017	Asn842, Thr790, Thr854	–	–
BZ6	-4.761	-44.770	Asn842, Thr790, Thr854	Met793	–
BZ7	-5.886	-42.653	Asn842, Thr790, Thr854, Gln791	Met793	–
BZ8	-5.500	-40.350	Asn842, Thr790, Thr854	–	–
BZ9	-4.663	-43.491	Asn842, Thr790, Thr854, Gln791	Met793	–
BZ10	-3.337	-43.125	Asn842, Thr790, Thr854	–	–
BZ11	-5.100	-39.067	Asn842, Thr790, Thr854	–	–
BZ12	-4.545	-34.958	Thr790, Thr854, Gln791	Met793	–
BZ13	5.373	-40.996	Asn842, Thr790, Thr854, Gln791	–	–
BZ14	-4.804	-38.510	Asn842, Thr790, Thr854, Gln791	–	–
BZ15	-5.125	-39.942	Asn842, Thr790, Thr854, Gln791	–	–
Gefitinib (Std)	-8.639	-51.426	Thr854, Thr790, Gln791, Asn842	Met793, Csx797	–

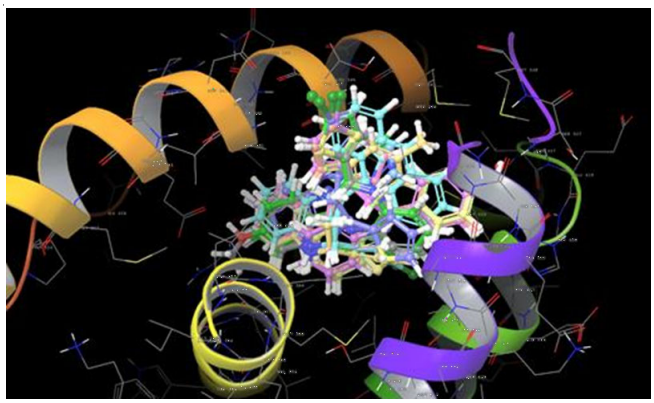


Fig. 1. All benzodiazepine derivatives (**BZ1-15**) at active site of 2IOK with surrounded amino acids

Of all the compounds docked with 2IOK, compound **BZ4** had the greatest binding energy of -9.954 and the highest affinity. The compounds **BZ2** and **BZ10** have docking scores of -9.754 kcal/mol and-9.736 kcal/mol, respectively and fit into the binding cleft of the 2IOK receptor.

Figs. 2-4 show the docking orientations of molecule **BZ4**, **BZ2** and **BZ10** with the 2IOK receptor.The hydrogen bond interactions are generated with Glu353 of 2IOK (Fig. 4). The Pi-Pi stacking formed with Phe404. The hydrophobic association between the ligand and the receptor is also a favourable interaction.

The binding free energy of the synthesized compounds with EGF receptor 4WKQ ranges from -5.497 to -5.125 kcal/mol. The active residues in 4WKQ were Val726, Thr854, Asp855,

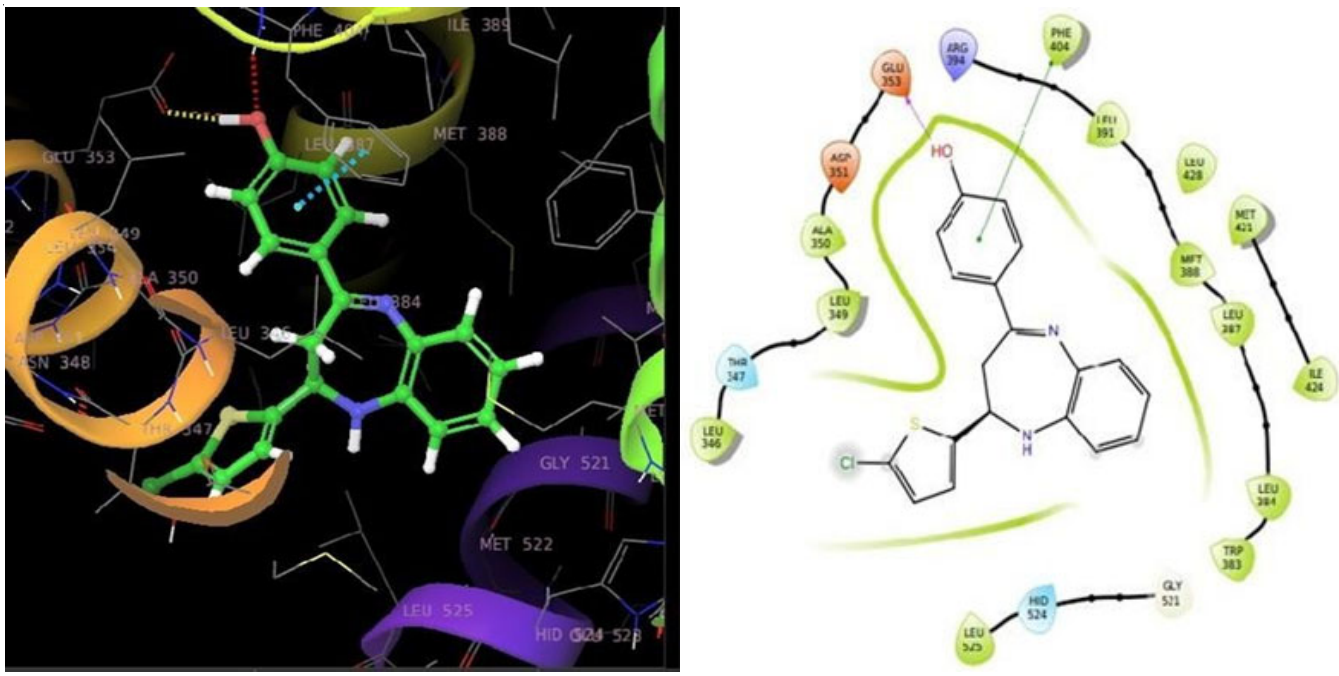


Fig. 2. 2D & 3D Interactions of compound **BZ4** with 2IOK

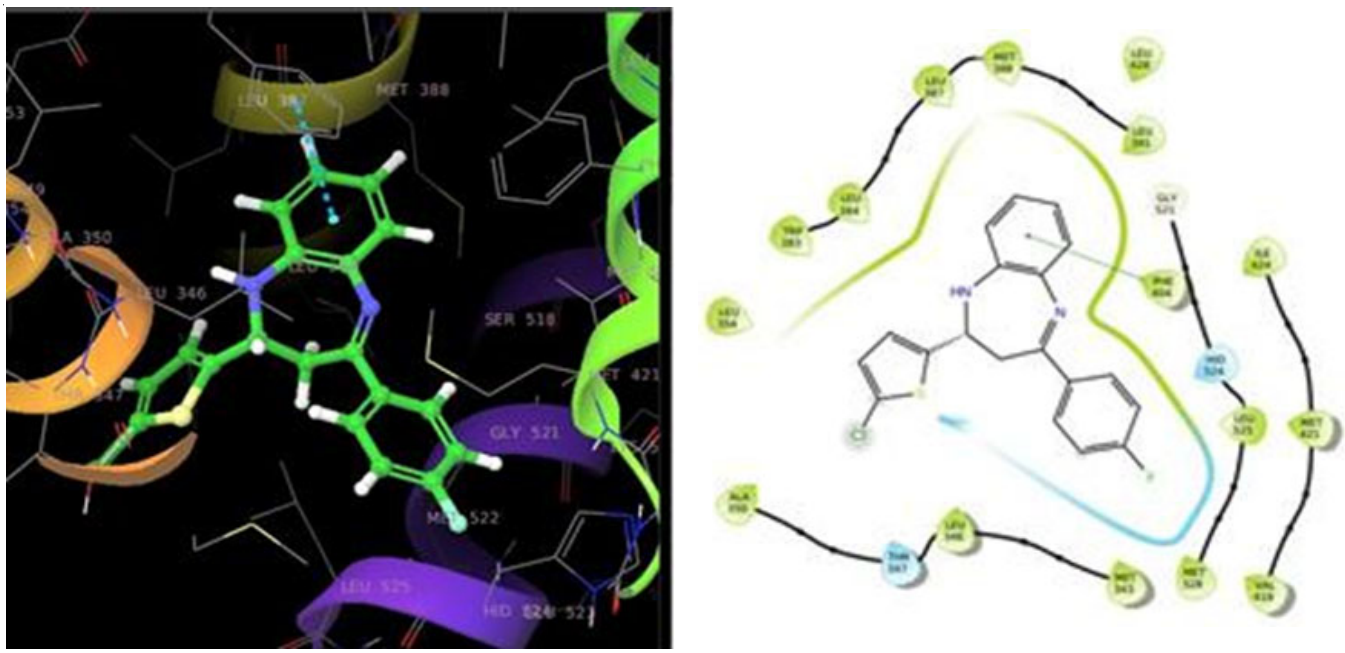


Fig. 3. 2D & 3D Interactions of compound **BZ2** with 2IOK

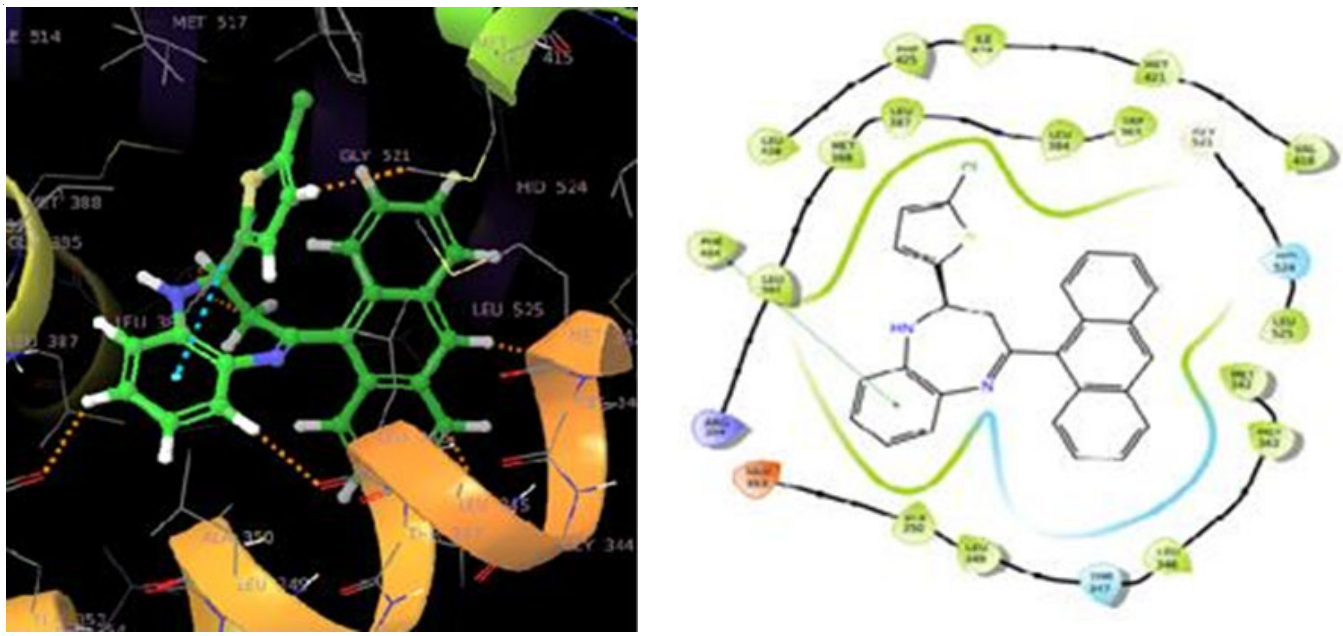


Fig. 4. 2D & 3D Interactions of compound **BZ10** with 2IOK

Met766, Leu777, Lys745, Ile744, Ala743, Leu788, Ile789, Thr790, Leu792, Met793, Pro794, Gly796, Csx797, Leu718, Leu844, Asn842, Arg842 (Fig. 5).

Among all compounds with 4WKQ, compound **BZ4** has the best binding energy of-6.309 and the highest affinity. With dock scores of -5.886 and -5.497, respectively, compounds **BZ7** and **BZ1** fit into the binding cleft of 4WKQ receptor. The hydrogen bond interactions are formed with Met793 (Fig. 6) of 4WKQ. The hydrophobic interaction between the ligand and the receptor is also a favourable one. The docking orientations of compounds **BZ4**, **BZ1** and **BZ7** with the 4WKQ receptor are represented in Figs. 6-8.

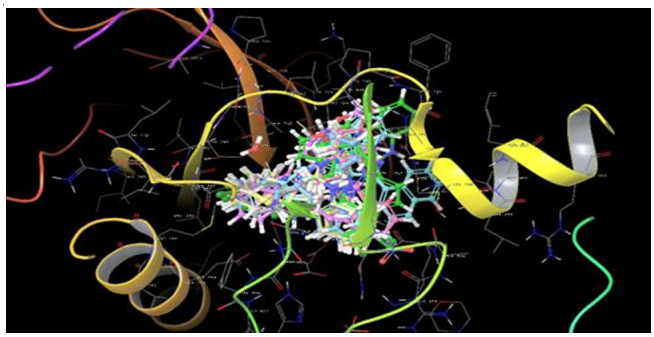


Fig. 5. All benzodiazepine derivatives (**BZ1-15**) at active site of 4WKQ with surrounded amino acids

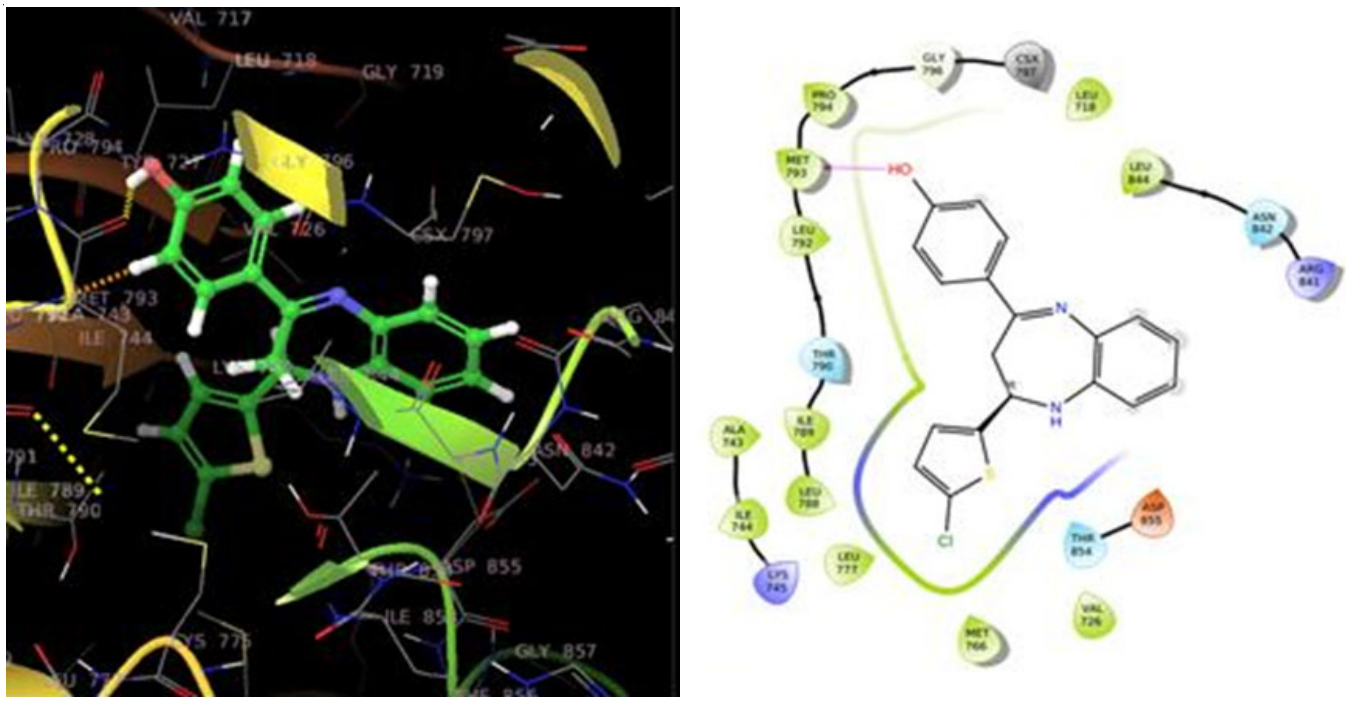


Fig. 6. 2D & 3D Interactions of compound **BZ4** with 4WKQ

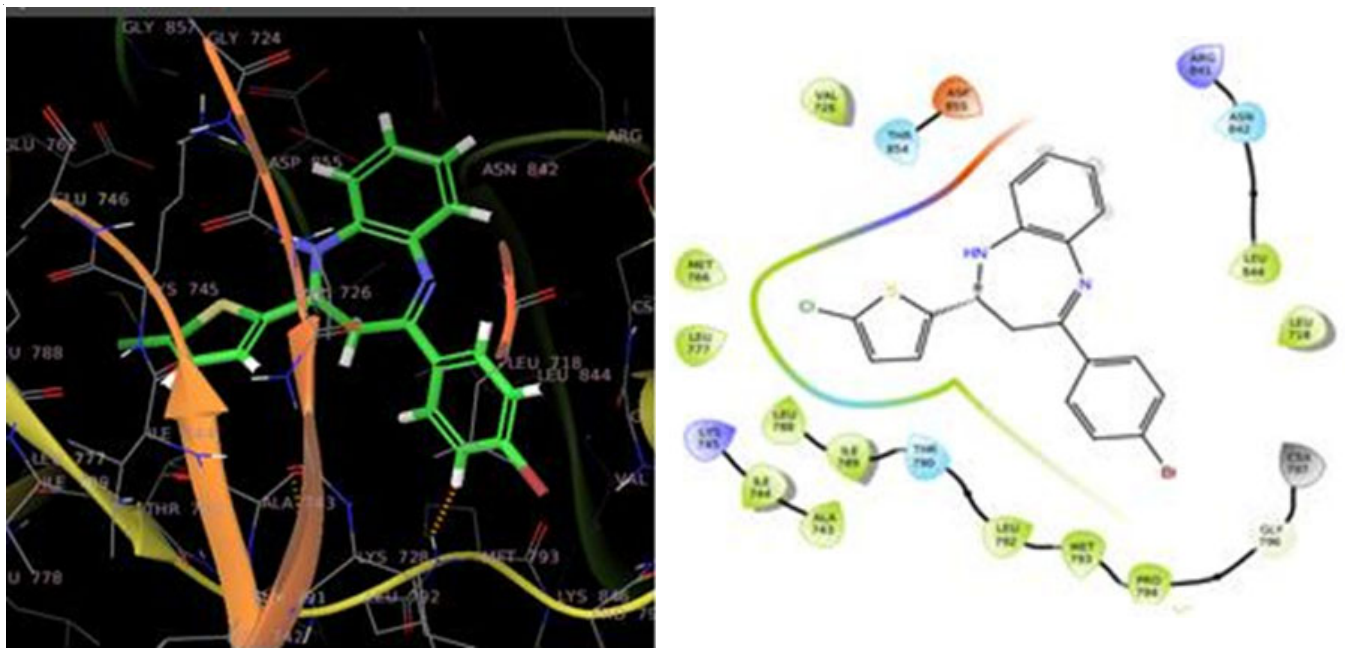


Fig. 7. 2D & 3D Interactions of compound **BZ1** with 4WKQ

All the benzodiazepine derivatives obeys the Lipinski's RO5 from the values obtained from the experimental methods. On comparing log P values with that of docking scores, we can conclude that there is no relationship between log P values and docking scores. The donor HB and acceptor HB values are within the normal range which shows that the constituents are obeying the Rule of Five (Table-3).

The ADMET investigations of the synthesized compounds contributed to the conclusion that all compounds exhibit high BBB penetration. The percentage human intestinal absorption and Caco2 cell permeability are within acceptable limits. All the compounds follow Lipinski's rules of five and Rule of three (Table-4).

Anticancer activity: Among the synthesized benzodiazepine derivatives, three compounds **BZ1**, **BZ2** and **BZ4** were selected based on docking scores and screened for anticancer activity by MTT assay. Test compound **BZ2** showed cytotoxicity on MCF-7 cells at 50 and 100 µg/mL and compound **BZ4** showed potential cytotoxicity at 12.5, 25, 50 and 100 µg/mL concentrations (Table-5), which contains hydroxyl group substitution at the *para* position.

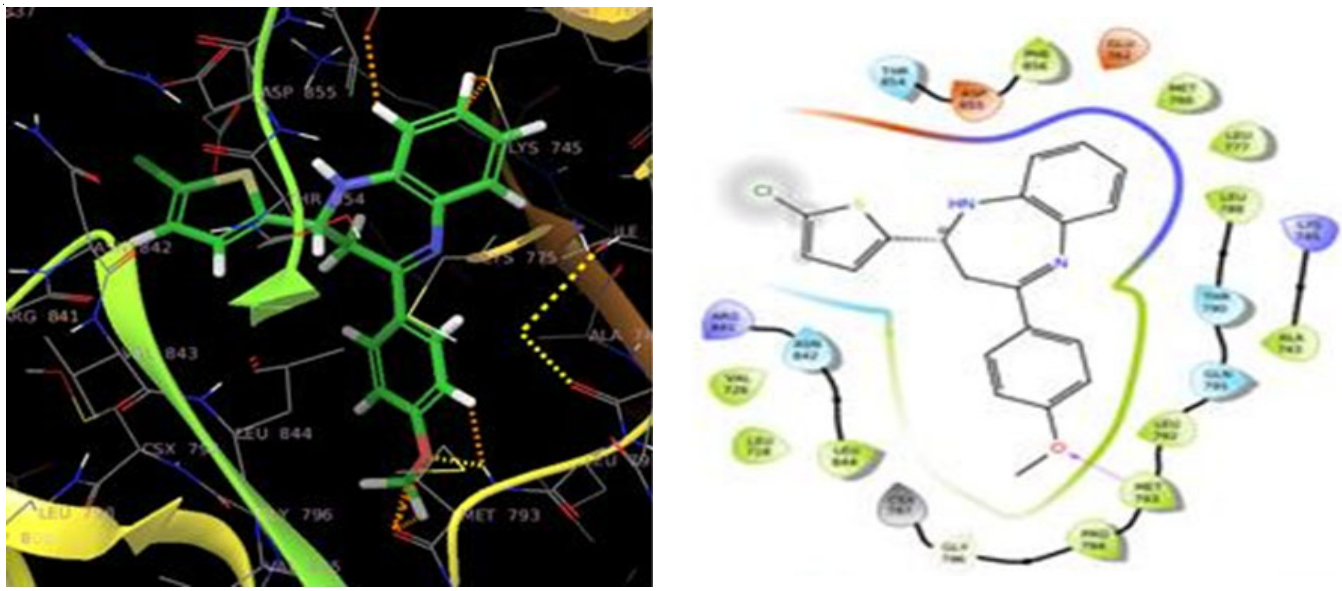


Fig. 8. 2D & 3D Interactions of compound **BZ7** with 4WKQ

TABLE-3
PHYSICO-CHEMICAL PROPERTIES OF COMPOUNDS **BZ 1-15**

Compound	m.w.	log P	Donor HB	Acceptor HB	PSA	Volume	Rotor
BZ1	417.750	5.73	1	2.00	26.632	1084.267	0
BZ2	356.844	5.05	1	2.00	26.619	1047.952	0
BZ3	373.299	5.45	1	2.00	26.629	1076.256	0
BZ4	354.853	4.51	2	2.75	49.198	1054.642	1
BZ5	383.851	5.72	1	3.00	71.555	1104.037	1
BZ6	381.930	5.18	1	4.00	27.630	335.460	3
BZ7	368.880	4.77	1	2.75	34.940	1105.636	1
BZ8	352.881	5.38	1	2.00	26.649	1091.745	0
BZ9	340.829	2.96	1	5.00	52.756	1001.249	0
BZ10	438.973	6.89	1	2.00	22.070	1295.699	0
BZ11	373.300	5.45	1	3.00	24.390	303.100	2
BZ12	328.815	3.51	1	2.50	36.981	953.838	0
BZ13	339.842	3.98	1	3.00	36.480	1011.366	0
BZ14	328.818	2.85	2	3.50	54.140	964.488	0
BZ15	344.876	4.88	1	2.00	27.337	1004.928	0
Tamoxifen (Std)	371.510	6.07	0	2.75	12.470	1308.708	9
Genfitinib (Std)	446.900	3.87	1	7.00	68.750	385.070	8

TABLE-4
ADMET PROPERTIES OF COMPOUNDS BZ 1-15

Compound	OPPCaco	QplogBB	% Human oral absorption	SASA	Rule of five	Rule of three
BZ1	6658.172	0.834	100	614.441	1	1
BZ2	6647.302	0.762	100	594.946	1	1
BZ3	6650.591	0.820	100	610.256	1	1
BZ4	2009.970	0.065	100	598.383	0	1
BZ5	883.752	-0.303	96.077	620.230	1	1
BZ6	1719.308	-0.003	100	600.532	0	2
BZ7	7350.245	0.629	100	618.348	1	1
BZ8	6630.249	0.642	100	618.021	1	1
BZ9	2122.162	0.179	100	564.876	0	1
BZ10	8549.487	0.747	100	708.869	1	1
BZ11	6058.422	0.624	100	571.040	1	1
BZ12	5827.769	0.619	100	539.362	0	0
BZ13	3706.803	0.425	100	553.320	0	1
BZ14	2992.173	0.333	100	553.585	0	0
BZ15	7321.961	0.795	100	574.388	1	1

TABLE-5
in vitro ANTICANCER ACTIVITY DATA BY MTT METHOD

Concentration (µg/mL)	Cytotoxicity (Mean ± SD)			
	BZ1	**BZ2**	**BZ4**	Tamoxifen
6.25	1.59 ± 0.49	1.99 ± 0.49	34.52 ± 7.15	72.38 ± 1.15
12.5	2.39 ± 1.69	18.10 ± 2.14	50.71 ± 0.95	85.58 ± 2.05
25	2.31 ± 2.06	44.49 ± 4.02	66.10 ± 2.03	85.72 ± 4.45
50	6.85 ± 1.53	53.82 ± 1.49	77.51 ± 1.26	88.51 ± 2.56
100	11.08 ± 0.96	65.47 ± 1.69	86.20 ± 1.36	89.4 ± 1.36

Conclusion

In present work, a novel series of 1,4-benzodiazepines have been docked (anticancer), synthesized and evaluated for anticancer activities and characterized by the spectral data. The docking interaction analysis of benzodiazepines has revealed that they can be considered promising lead molecules as anticancer agents. The selected 1,4-benzodiazepine derivatives were synthesized by the prescribed method resulting with good yield. *In vitro* anticancer study showed that compound **BZ4** has potent anticancer activity with hydroxyl group substitution at the *para* position, which already has the highest docking score when docked with human estrogen receptor 2IOK. So, it might be used as a lead molecule for future studies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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