Research Article

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Synthesis, Cytotoxic Effect Assessment and Molecular Docking Studies of Disubstituted Thiadiazole Including Oxadiazole as Hybrid Component

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ABSTRACT

Cancer is the second leading cause of death in the world. Due to toxicity and resistance to common therapies, the attempt to develop new anticancer agents has become a major challenge. Oxadiazole and thiadiazole are of interest building blocks used in drug design. Hybrids of thiadiazole- oxadiazole have been synthesized with significant cytotoxic effects. Considering importance of mentioned scaffolds some of the thiadiazole-oxadiazole derivatives were synthesized by three steps in this study.

Firstly, thiol function of 2-amino-5-mercapto-1, 3, 4-thiadiazole was alkylated by benzyl chloride derivatives to give compounds (1a-c). The reaction of chloroacetyl chloride with amine group of compounds (1a–c) terminates to amide derivatives (2a-c). Definitive products were produced by treatment of corresponding amide derivatives with 5-(4-chlorophenyl)-1, 3, 4-oxadiazole-2-thiol. Synthesized compounds were evaluated by MTT assay against three cell lines. The final molecules were docked in the active sites of the epidermal growth factor receptor tyrosine kinase to assay the possible interactions. Final products showed range of cytotoxic activity of moderate to good against tested cell lines. Compound (3a) demonstrated a higher cytotoxic activity against MCF-7 (IC_{50}: 26 µM) and Lncap (IC_{50}: 37 µM) cell lines in comparison with other compounds. The highest docking score was -10.55 kcal/mol for compound 3a.

Keywords: Cytotoxic, Docking, Synthesis, Oxadiazol, Thiadiazole, MTT assay

1. Introduction

Cancer is a progressive proliferation of cells which belong to a part of the body. This life -threatening disease has become a global problem in the field of effective treatment without unwanted side effects and resistance. Furthermore, cancer is the most important disease that has been challenged in drug design [1-3]. Different classes of heterocyclic and fused heterocyclic derivatives have been finding out through experimental and theoretical screening as anticancer agents.1, 3, 4- Thiadiazole, an appropriate heterocyclic structure, occupy a prominent role among anticancer agents. Lipo solubility temper of 1, 3, 4-thiadiazole leads to good tissue permeability of this compound [4, 5]. The modifications in thiadiazole ring systems through various substitutions at different position in the ring can be modify biological effect [6, 7]. Several substituted thiadiazole derivatives have been reported with potential anticancer activity against various tumor cell lines [1]. The proposed anti-cancer mechanisms for thiadiazole include: potent Abl tyrosine kinase inhibitors [5], anti-tubulin agents [8]. Recent findings in the identification of epidermal growth factor receptor (EGFR) inhibitors may create hope for cancer treatment [9].Similarly, 1, 3, 4-oxadiazole derivatives are correlated with many types of biological virtues especially anticancer effects [8-11]. There are various reports citing oxadiazole as potential cytotoxic agent [4]. Some of the substituted 1, 3, 4-oxadiazole derivatives identified as anticancer agents due to inhibition of different growth factors such as tyrosine kinase [9]. Some of the thiadiazole derivatives incorporated with oxadiazole have been shown significant anti-cancer activity [4, 7, 12]. Amide and/or S-benzyl derivatives of thiadiazole have shown good cytotoxic effects in previous reports [1, 5, 8]. Some of the S-substituted 1, 3, 4-oxadiazole derivatives were identified as anticancer agents [9] (Figure1). Inspired by literature surveys, we decided to integrate s-benzyl thiadiazole derivatives with oxadiazole scaffold via amide linker. New derivatives of these two heterocyclic (3a-c) were designed by using pharmacophore hybridization strategy and evaluated against three cell lines by MTT experiment. Altintop et al explained probability of 1, 3, 4-thiadiazole as essential scaffold for tyrosine kinase inhibitors. They identified that the introduction of a hydrogen bonding substituent and a hydrophobic substituent into the thiadiazole scaffold would furnish promising inhibitors of Bcr-Abl tyrosine kinase [13]. Adamantine scaffold containing 1, 3, 4-thiadiazole derivatives were synthesized and evaluated against both wild EGFR and mutant EGFR by Wassel et al [14]. Due to the reported inhibitory effects of oxadiazole and thiadiazole derivatives on kinases (1, 11, 13, 15), especially tyrosine kinase [14, 16], molecular docking studies of final compounds on binding sites of the EGFR tyrosine kinase domain (PDB: 1M17) crystal structure were also performed and their binding energies were calculated.

2. Materials and Methods

2.1. Material

All chemical reagents were obtained from Merck Company [Germany]. Silica gel 60 F_{254} sheets [Merck, Germany] were applied for monitoring of reac-

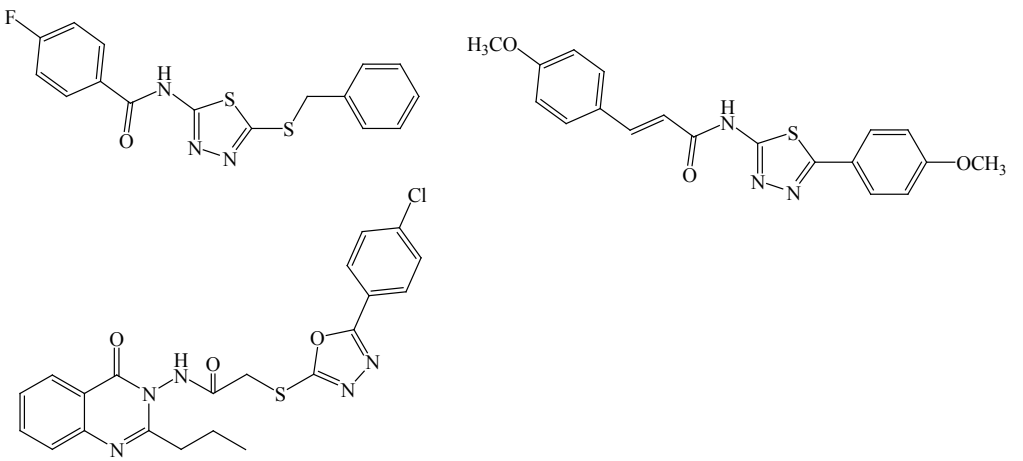

Figure 1. Structures of some of amide and/or S-benzyl of thiadiazole and oxadiazole derivatives with anticancer activity [1, 9].

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tions. Proton nuclear magnetic resonance (HNMR) [Bruker 400 MHz, Germany] was performed for ^{1}HNMR spectra acquisition. IR spectra were taken with a WQF-510 IR spectrophotometer [China]. Electrothermal9200 melting point instrument [England] was used for recording melting points and are uncorrected.

2.2. Methods

2.2.1. Chemistry

2.2.1.1. Procedure for the synthesis of 5-(benzyl thio)-1, 3, 4-thiadiazol-2-amine derivatives (1a-c)

Benzyl chloride derivatives (3mmol) were added to a solution of 2-amino-5-mercapto-1,3,4-thiadiazole (3mmol) and potassium hydroxide (3mmol) in (20 mL) absolute ethanol and refluxed for 2 h, after completion, the precipitated solid was filtered and solvent was evaporated to obtain yellow crystal products (**1a-c**)(Scheme 1)[1, 17].

2.2.1.2. Procedure for the synthesis of N-5-(benzyl thio)-1, 3, 4-thiadiazol-2-yl)-2-acetamide derivatives (2a-c)

To a solution of compound (**1a-c**) (1mmol) and triethylamine in anhydrous dichloromethane (DCM) (20mL) was slowly added chloroacetyl chloride (1.5 mmol). Stirring the mixture was performed at room temperature for 4 h. Obtained suspension was extracted with water and chloroform. Then chloroform solvent was removed to render orange powders (**2a-c**) (scheme1) [17].

2.2.1.3. Procedure for the preparation of N-5-(benzyl thio)-1, 3, 4-thiadiazol-2-yl)-2-(5 –(4-chlorophenyl)-1, 3, 4-oxadiazol-2-ylthio) acetamide derivatives (3a-c)

Compound 5-(4-chlorophenyl)-1, 3, 4-oxadiazole-2-thiol (2mmol) was dissolved in dry acetone (20mL). Anhydrous potassium carbonates (2mmol) [9, 18] and compound (**2a-c**) (2mmol) were added and refluxed for 3 h. The suspension was filtered off and the organic solution was concentrated, then recrystallized from ethanol to provide products (**3a-c**) (scheme1).

2.2.1.3.1. N-(5-(Benzylthio)-1,3,4-thiadiazol-2-yl)-2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-ylthio) acetamide (3a)

Yellow solid;yield:45 %;mp: 168°C > decomposed IR (KBr) (ν_{max}, cm^{-1}): 3360(NH), 3140 (C-H, Ar),1662 (C=O), 1089 (COC); ^{1}HNMR: (400 MHz; $CDCl_3$): δ 7.96 (2H, d, *J*=8Hz, H^A), 7.50(2H,d, *J*=8Hz, H^B),7.39-7.35 (3H, m, H-benzyl),7.34 (H, s, NH), 7.32-7.31 (2H, m, H-benzyl), 4.38(2H, s, CH_2), 4.15(2H, s, CH_2).

2.2.1.3.2. N-(5-(4-Chlorobenzylthio))-1, 3, 4-thiadiazol-2-yl)-2-(5-(4-chlorophenyl)-1, 3, 4-oxadiazol-2-ylthio) acetamide (3b)

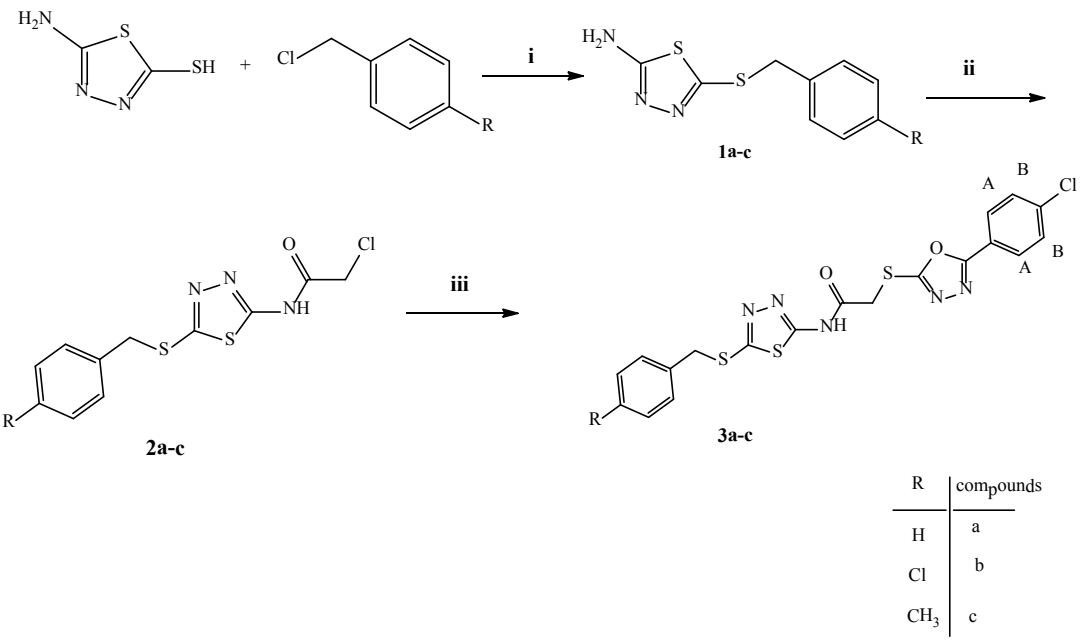
Pale yellow solid; yield: 30 %;mp: 176°C > decomposed. IR (KBr) (ν_{max}, cm^{-1}): 3422 (NH), 1696(C=O), 1086 (COC); ^{1}HNMR: (400 MHz; $CDCl_3$): δ7.95 (2H, d, *J*=8Hz, H^A),7.54 (1H, s, NH), 7.52 (2H, d, *J*=8Hz, H^B),7.35-7.31 (4H, m, H-benzyl), 4.44 (2H, s, CH_2), 4.2(2H, s, CH_2).

2.2.1.3.3. N-(5-(4-Methylbenzylthio))-1, 3, 4-thiadiazol-2-yl)-2-(5-(4-chlorophenyl)-1, 3, 4-oxadiazol-2-ylthio) acetamide (3c)

Pale yellow solid yield: 35 %; mp: 122°C > decomposed. IR (KBr) (ν_{max}, cm^{-1}):3222 (NH), 2911 (CH, Aliphatic), 1693(C=O), 1086 (COC); ^{1}HNMR: (400 MHz; $CDCl_3$): δ8.12(1H, s, NH), 7.94 (2H, d, *J*=8Hz, H^A),7.51 (2H, d, *J*=8Hz, H^B),7.23-7.19 (2H, m, H-benzyl),7.15-7.11 (2H, m, H-benzyl), 4.43 (2H, s, CH_2), 4.22 (2H, s, CH_2), 2.33 (3H, s, CH_3).

2.2.2. Cytotoxic Activity

MCF-7(breast cancer) and LnCAPcell lines were grown in RPMI 1640 completed with 5% v/v fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin and maintained at 37 °C in a humidified atmosphere (90%) containing 5% CO_2. Cell suspensions (180 µL)(5×10^4 cells/mL) were placed in 96-well plates and were kept in incubation condition for 24 h. The Stock solutions of **3a-c** (10 mM, 1 mL) were made in dimethyl sulfoxide (DMSO) and a serial dilution of 1000, 100 , 10 µM and 1 µM were prepared using the medium.After 24 h incubation, 20 µL of derivatives were added and incubation was re-established for 48 h. Paclitaxel was considered as positive controls. Three control wells of untreated cells were made in the absence



Scheme 1. Synthetic route to compounds **(3a-c). (i)** KOH**,** EtOH, 2h reflux; **(ii)**, $ClCOCH_2Cl$, DCM, TEA, room temperature; (iii) K_2CO_3, dry Acetone, 5-(4-chlorophenyl)-1, 3, 4-oxadizole-2-thiol, 3h reflux.

of tested sample. For the blank absorbance readings, medium alone ,were provided. To evaluate cell survival, treated cells were incubated with 20 µL of MTT solution for 4 h, afterwards, medium was thrown out,formazan crystals were dissolved by dimethyl sulfoxide (150µL). The absorbance was measured at 540 nm using an ELISA plate reader [11,18,19]. The experiments were accomplished in triplicate. Analysis of variance (ANOVA) and Tukey test was used to obtain the differences between groups with negative control.

Cell viability was calculated using the following equation:

$$Cellsurvival\ (\%) = \frac{\text{MA of treated wells} - \text{MA of blank}}{\text{MA of negative control} - \text{MA of blank}} \times 100$$

Where, MA is mean absorbance.

2.2.3. Insilico Studies

Tree-dimensional (3D) structures of the ligands were drawn in Hyperchem7.0 software then were optimized using the MM^+ molecular mechanical force field and 3D geometry optimization calculations. The ultimate conformations were calculated by AM1 as a semi-empirical method and molecular structures were optimized using the Polak Ribiere conjugate gradient algorithm. These optimized structures were used by AutoDock Tools for preparation PDBQT files as follows: Polar hydrogen atoms were added while non-polar hydrogen atoms were merged and then, Gasteiger partial atomic charges were assigned to the ligands. All rotatable bonds of ligands, defined by default of the program, were allowed to rotated during the automated docking process. The crystal structure of EGFR tyrosine kinase (PDB ID: 1M 17) with resolution 2.6 Å was obtained from the protein data bank (www.rcsb.org) [20]. PDBQT file of protein was prepared by AutoDock Tools as follows: water and ligand molecules were removed from the protein file. All missing hydrogens were added, and after determining the Kollman united atom charges, non-polar hydrogens were merged to their corresponding carbons [11, 21, 22]. The grid box dimensions were set in 60 × 60 × 60 Å points with a distance of 0.375 Å between the grid points. The grid box was centered with the coordinates x = 22.00Å, y = 0.204Å, z = 52.81 Å for EGFR tyrosine kinase (PDB ID: 1M 17). Docking was performed using the routine procedure and default parameters of molecular docking AutoDock 4.2 software. After 100 independent docking runs for each ligand, conformations were clustered according to the root mean square deviation tolerance of 2.0 Å

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and were ranked according to the binding free energy [21].

3. Results and Discussion

3.1. Synthesis and Characterization

Thiadiazole and oxadiazole derivatives, important nitrogenous pharmacophores, displayed various biological activities especially anticancer properties [2, 4, 8, 23, 24]. The biological results suggested that the substituents of phenyl ring attached to the 1, 3, 4-oxadiazole are important for adjusting anti proliferative properties against different tumor cell line [4]. In this work, oxadiazole moiety was incorporated to thiadiazole derivatives in order to obtain synergistic effect of these two active pharmacophores through a two-step procedure. The significant peaks corresponding to the major functional groups were obvious in the IR spectrum of final compounds. Amide N-H and carbonyl peaks were observed at 3422-3222 $cm^{-1}$and 1696-1662 cm^{-1}respectively. In the^{1}H NMR spectra of (**3a–c),** the aromatic ring protons attached to oxadiazole were observed in the range of 7.96-7.50 ppm. The multiplet peaks observed in the range of 7.39-7.19 ppm were attributed to the aromatic protons of s-benzyl. The characteristic singlet for methylene protons attached to carbonyl were observed around 4.44-4.38ppm and the characteristic singlet for methylene protons attached to s-thiadiazole were found around4.22-4.15 ppm.

3.2. Cytotoxic Activity

Final compounds were assayed for their cytotoxic effects on three human cell lines. Cytotoxic activity was represented in Table1 and Figures 2,3 and 4. Significant differences compared to the negative control on cell lines were observed for final compounds. All compounds (**3a-3c**) demonstrated moderate to good cytotoxic activity against the examined cell lines which can attributed to (4-chlorophenyl)-1, 3, 4-oxadiazol-2-ylthio constant moiety. The presence of chlorine group at the position 4 of phenyl ring attached to the 1, 3, 4-oxadiazole could improve activity. Compound **3a** without any electron withdrawing and donating moiety on the phenyl ring rendered significant cytotoxic effect against MCF-7 and Lncap, with IC_{50} value of 26 and 37µM respectively. In a study conducted by Ali abadi et al, amide and S-benzyl derivatives of thiadiazole were synthesized and evaluated against PC3 cell (prostate cancer), U87-C-531(gliobalstoma) and MDA-MB-231 (breast cancer). Compounds without any electron withdrawing and donating moiety on the phenyl ring, rendered the highest anticancer potency toward tested cell lines. Methoxy as an electron donating group caused an increase in the cytotoxic activity [1].

Structure activity relationship (SAR) study revealed that the presence of chlorine group (electron-withdrawing group) at the position 4 in the aryl moiety of the thiadiazole ring (**3b**) decreased the cytotoxic activity. The presence of electron-donating groups such as methyl at positions 4 in the aryl moiety of the thiadiazole ring (**3c**) improved cytotoxic activity comparison with chlorine in the same position. Since the final compounds differ only at positions 4 of benzyl attached to the thiadiazole ring, the effectiveness of the compound **3a** can be attributed to the substitution size. According to the observed IC_{50} values in this study cytotoxic effects compounds **3b** and **3c** against normal Huvec cell line is relatively similar and less than that 3a. However all three compound showed cytotoxic activity against normal Huvec cell line, which could be prevented by target therapy of these compounds.

3.3. Docking results

The docking results of compounds including the estimated free binding energy values (kcal/mol), and the interactions with key amino acid residues at the active site of enzymes are expressed in Table 2 and Figure5. The docking results revealed that the highest dock score and the lowest Ki (inhibition constant) belongs tocompound 3a which Met 769 has been detected for formation of hydrogen binds with nitrogen atoms of the 1,3,4-thiadiazole ring and amide band. This can relatively confirm the experimental results. The hydrogen bond formed between nitrogen atoms of the 1, 3, 4-thiadiazole ring and Met769 is one of the most important interactions. Compounds 3b and 3c showed similar results in docking study in terms of binding energy, inhibition constant and interference with similar amino acids. Glu 780 and Asp 776 are responsible for formation hydrogen binding with amide band in these two compounds. Farany et al were performed molecular docking studies of s-benzyl and amide derivatives of thiadiazole on Abl as well as Src tyrosine kinases .Their results showed that nitrogen atoms of the 1,3,4-thiadiazole ring has been formed two hydrogen bindings with

Table 1. The IC_{50} (μM) of final compounds against MCF-7, LnCAP and Huvec cell lines.

Tested Compounds	IC_{50}(μM)		
	MCF-7	**LnCAP**	**Huvec**
3a	26±3.2	37±2.5	14±2.3
3b	77±4.2	62±3.6	26±2.9
3c	49±4.3	46±1.3	26±2.9

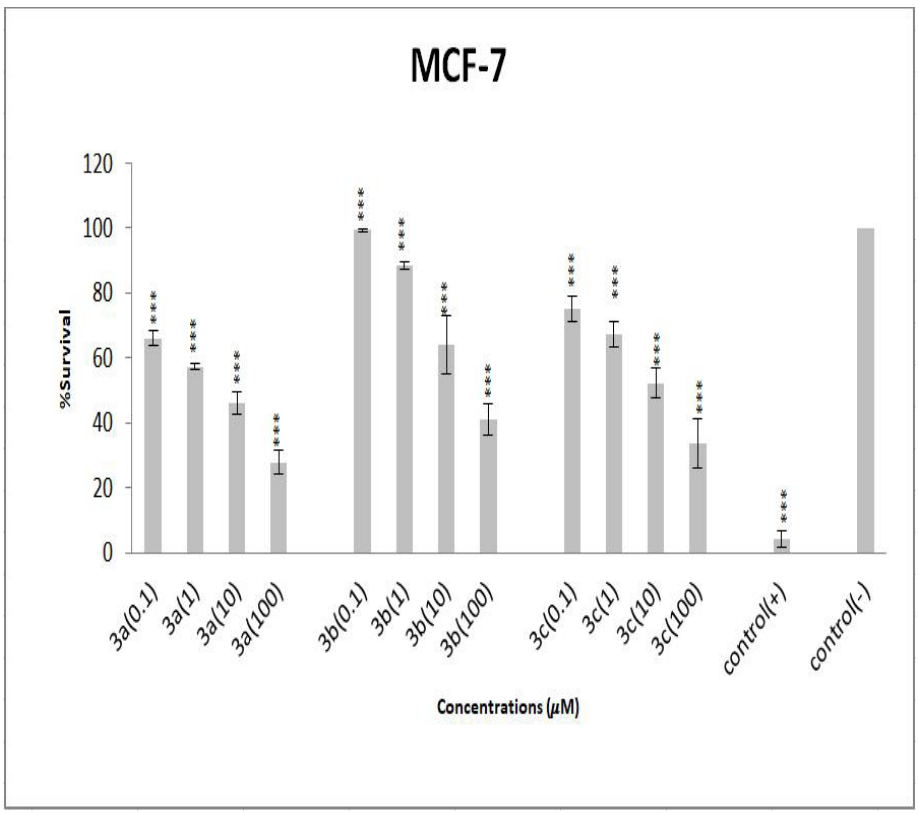

Figure 2. Cytotoxic results of compounds **(3a-c)** against MCF-7 cells. Data are expressed as mean ± SD, n = 3. *** $P < 0.001$ Show significant differences in comparison with negative control group.

Lys 295 and Ile 294 [25]. Rezaei Nasab et al, were assayed interactions between epidermal growth factor receptor tyrosine kinase and some of the inhibitors using combination of in-silico and in-vitro cytotoxicity methods. Their results showed that Met 769 had strong interaction with some of the derivatives. "Hing region key residue" Met769 of EGFR target is involved in the anticancer treatment strategies [21]. Stamos et al, assayed structure of the epidermal growth factor receptor kinase domain alone and in complex with erlotinib (4-anilinoquinazoline derivative). The N1 of the quinazoline in erlotinib structure accepts an H-bond from the Met769 amide nitrogen. The other quinazoline nitrogen atom (N3) is not within H-bonding distance of the Thr766 side chain (4.1 Å), but a water molecule bridges this gap [20].

4. Conclusion

Some of the oxadiazole -thiadiazole hybrid molecules were synthesized and evaluated for their cytotoxic effects against MCF-7 , Lncap and Huvec cell lines. Compound **3a** produced significant cytotoxic activity against cell lines in particular MCF-7 cell line. Compounds **3c** showed moderate cytotoxic activity against both cell lines in particular Lncap cell line. Based on the obtained results of docking and cytotoxic tests, compound **3a** seems to be a good

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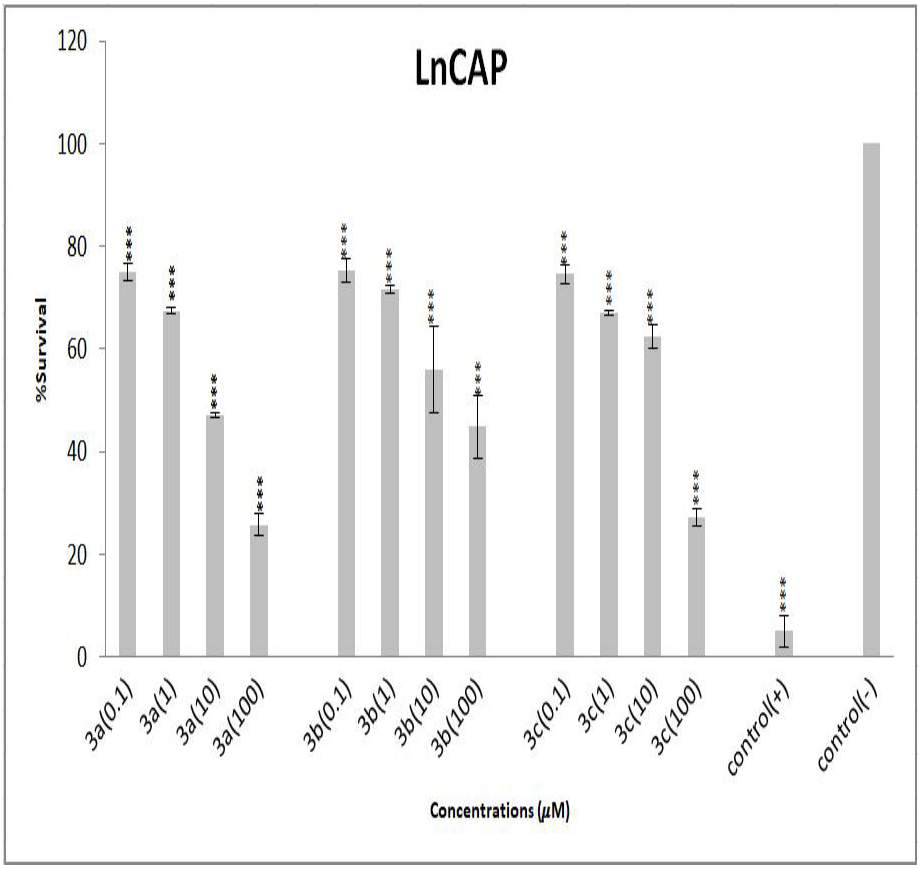

Figure 3. Cytotoxic results of compounds **(3a-c)** against Lncap cells. Data are expressed as mean ± SD, n = 3. *** $P < 0.001$ Show significant differences in comparison with negative control group.

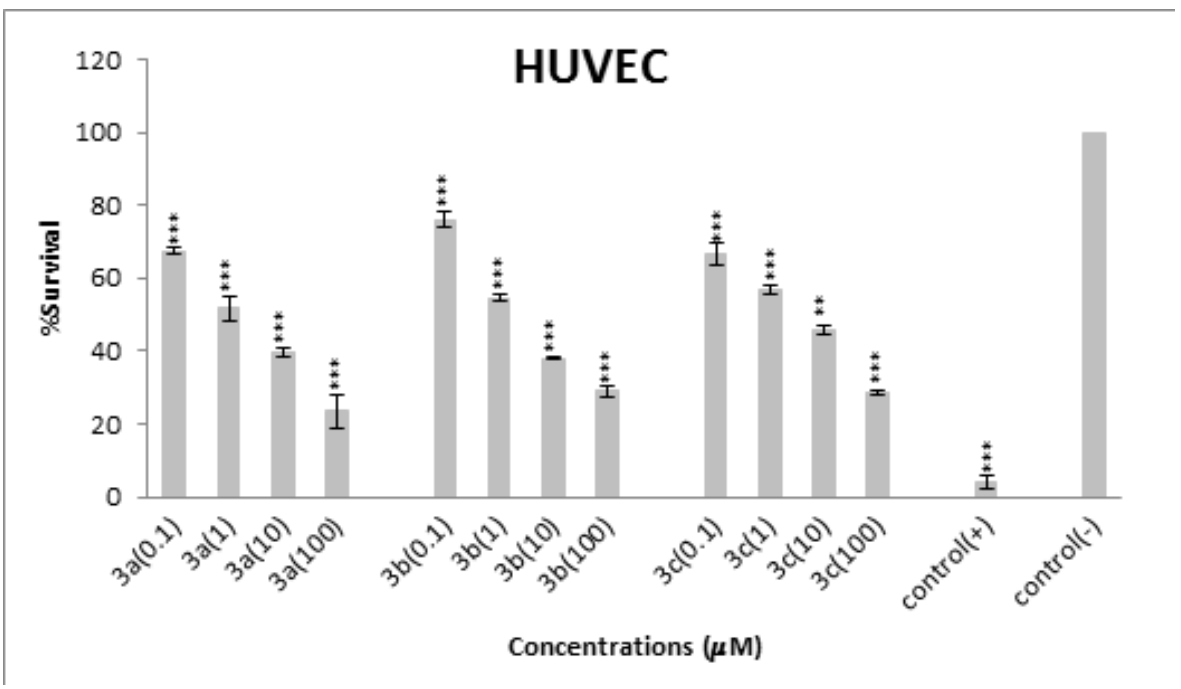

Figure 4. Cytotoxic results of compounds **(3a-c)** against Huveccells. Data are expressed as mean ± SD, n = 3. *** $P < 0.001$ Show significant differences in comparison with negative control group.

Table2. Energy-based interactions and hydrogen bonds for the final compounds

Tested Compounds	R	G bind(Kcal/mol)Δ	Hydrogen bond (Distance, Å)	Ki (µM)
3a	H	-10.55	Met 769 (1.95 Å) Cys 773	18.36 (nM)
3b	Cl	-6.03	Asp776 (2.019 Å) Glu 780 (2.13 Å)	38.19
3c	CH_3	-6.00	Cys 773 (1.96 Å) Glu 780 (2.13 Å) Asp 776	39.85

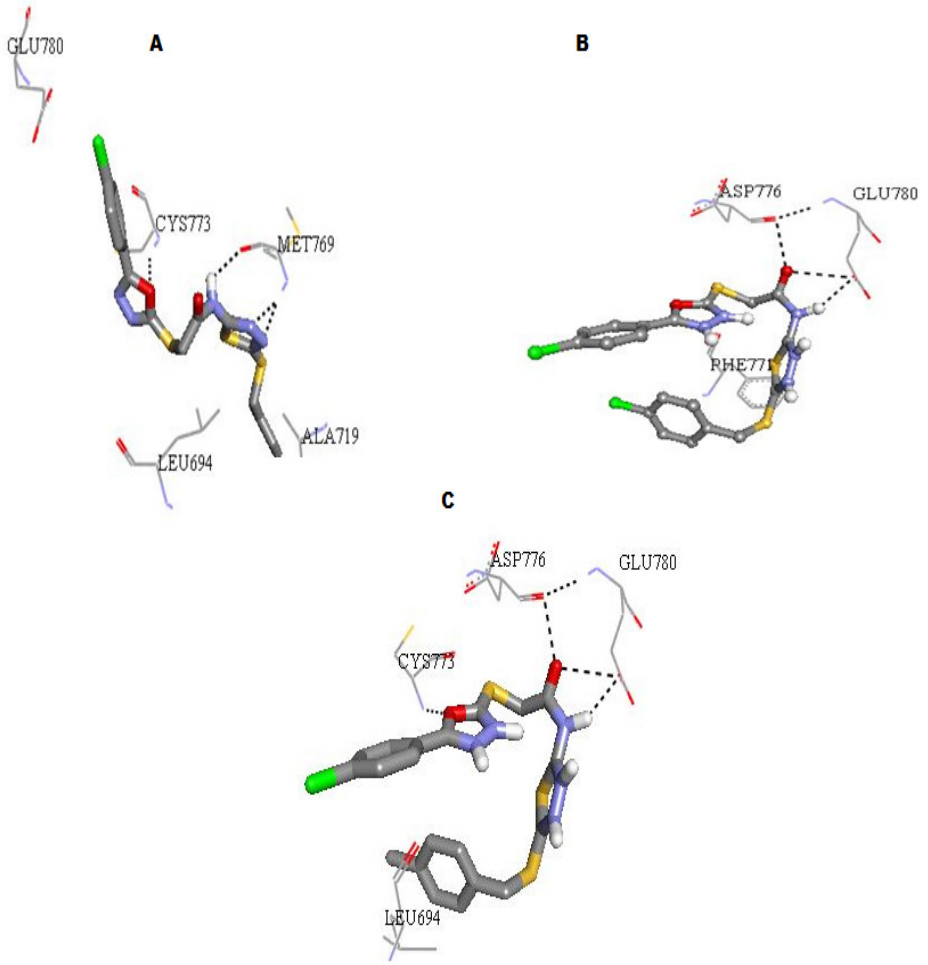

Figure 5. Docked conformation of compounds (A) 3a, (B) 3b, and (C) 3c in the binding site of epidermal growth factor receptor tyrosine kinase. Hydrogen bonds are shown by the black dashed line.

lead molecule. According to cytotoxic effect of this compound on the normal cell, target therapy should be considered.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Statement of Contribution of Researchers

Concept- F.H., E.J., S.Z., H.S.A.; Design- F.H., E.J.; Supervision- F.H., E.J., H.S.A.; Resources- E.J.; Ma-

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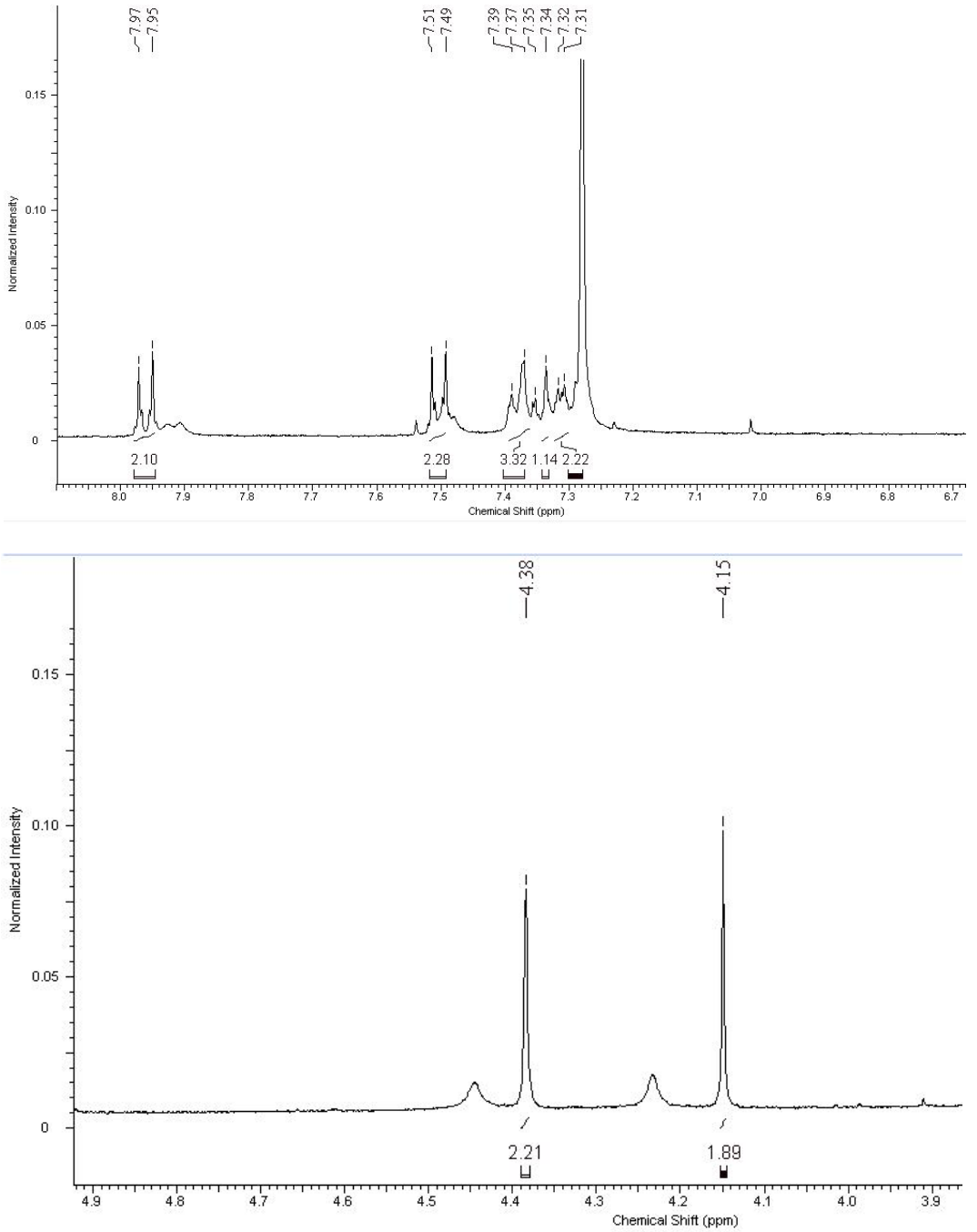

Figure 6. [1]HNMR spectra of compound 3a

terials- E.J.; Data Collection and/or Processing- F.H., E.J., S.Z., H.S.A.; Analysis and/or Interpretation- F.H., E.J., S.Z., H.S.A.; Literature Search- E.J., S.Z.; Writing- E.J., F.H.; Critical Reviews- E.J., F.H.

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