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Original Article

Synthesis, cytotoxic evaluation, and molecular docking studies of some new 1, 3, 4-oxadiazole-based compounds

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Abstract

Background and purpose: Oxadiazole-derived compounds have been shown to have a wide range of pharmacological activities. 2, 5-Disubstituted 1, 3, 4-oxadiazole derivatives have occupied a specific place in the design of anti-proliferative agents. In the present work a series of 2, 5-disubstituted 1, 3, 4-oxadiazoles derivatives containing amide group has been synthesized *via* a two-step reaction.

Experimental approach: A mixture of substituted carboxylic acid derivatives, semicarbazide, and phosphorus oxychloride in reflux condition yielded 2-amino-5-aryl-1, 3, 4-oxadiazole derivatives. Acylation of the amino group of the resultant oxadiazole with 6-chloronicotinoyl chloride in dry tetrahydrofuran/pyridine afforded the final products. The synthesized molecules were docked in the active sites of the epidermal growth factor receptor tyrosine kinase domain (PDB: 1M17) crystal structure to study the possible interactions with the active site. Cytotoxic activities of final products against HeLa and MCF-7 cells were also assessed by MTT assay.

Findings/Results: Compounds **IIb, IIc,** and **IIe** had a considerable cytotoxic activity with IC_{50} values of 19.9, 35, and 25.1 µM, respectively against HeLa cells. The highest docking score was -7.89 kcal/mol for compound **IIe**.

Conclusion and implications: Compound **IIe** exhibited remarkable cytotoxic activity against the two tested cell lines particularly HeLa cells which was in accordance with the *in silico* ΔG_{bind} result but further evaluations are necessary to prove these findings.

Keywords: Cytotoxicity; Molecular docking; Nicothionyl; Oxadiazole.

INTRODUCTION

Cancer treatment has been a major challenge due to the problems associated with available anticancer agents. Therefore, the development of novel anticancer agents is essential (1-3). The epidermal growth factor receptor (EGFR) is a cellular trans-membrane tyrosine kinase that is over-expressed in some of the human tumors (*e.g.* breast, ovarian, colon, renal, and prostate tumors). It is proposed that compounds which can inhibit tyrosine kinases activity may have an important role in cancer treatment (4). 1, 3, 4-Oxadiazole five-membered ring is one of the important scaffolds in medicinal chemistry researches. This heterocycle ring can engage in hydrogen bonding with receptor residues and improve pharmacological activities including anticancer, antimicrobial, anti-inflammatory, and anticonvulsant effects (5-11). Literature surveys have been shown that mono- and di-substituted 1,3,4-oxadiazole derivatives can be introduced as anticancer agents due to inhibition of different growth factors and enzymes such as tyrosine kinase (3,12), telomerase (13), tubulin polymerase (12,14) and thymidylate synthase (12).

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A series of 2, 5-disubstituted-1, 3, 4-oxadiazoles has been introduced by Ouyang *et al.* as tubulin polymerization inhibitors (14). 1, 3, 4-Oxadiazole derivatives possessing 1, 4-benzodioxan moiety have been reported as potential anticancer agents (2).

Structure-activity relationship studies have been shown that basic scaffold of 1, 3, 4-oxadiazoles is necessary for the broad spectrum cytotoxic activity towards various cell lines (3,8). It was also recognized that the substitution of various pharmacophores at position 5 of the oxadiazole may enhance anticancer properties (15,16). 2, 5 Di-substituted 1, 3, 4-oxadiazole derivatives with an amide group at position 2 were synthesized by bondock *et al.* Some of these compounds showed promising *in vitro* antitumor activity against several cell lines (3). In view of the above mentioned facts and as an attempt to obtain new anticancer agents, in the present research, a series of 2, 5-disubstituted 1, 3, 4-oxadiazol derivatives were synthesized **(IIa-d)**, along with some previously prepared oxadiazole derivatives **(IIe, f, and i)** (17), and evaluated for cytotoxic activity against MCF-7 and HeLa cell lines.

The final oxadiazole derivatives were docked into the binding sites of the EGFR tyrosine kinase domain (PDB: 1M17) crystal structure and their binding energies were calculated.

MATERIALS AND METHODS

Instrumentation

All starting materials, reagents, and solvents were purchased from commercial suppliers like Merck (Germany) and Aldrich (USA) companies. Merck silica gel 60 F_{254} plates (Germany) were applied for analytical thin-layer chromatography (TLC).

Proton nuclear magnetic resonance (^{1}HNMR) spectra were determined using a (Bruker 400 MHz, Germany) spectrometer, and chemical shifts are expressed as ppm with tetramethylsilane (TMS) as the internal standard. Infrared (IR, KBr discs) was measured with a WQF-510 FT-IR spectrophotometer (China). Melting points (mp) were determined using electrothermal 9200 melting point apparatus (England) and are uncorrected. All cell lines were purchased from the Pasteur Institute of Iran (Tehran, I.R. Iran).

General procedure for the synthesis of 2-amino-5-(substituted phenyl)-(1, 3, 4)-oxadiazole (Ia-d)

Benzoic acid derivatives (0.1 mol) and semicarbazide (0.2 mol) in phosphorous oxychloride (30 mL) were heated at 60 °C for 1 h and the temperature was raised to 95 °C and refluxed for 3 h. The mixture was then poured onto crushed ice and pH adjusted to 9-10 with 10 M NaOH and the obtained solid was filtered and recrystallized from methanol (Scheme1) (18,19).

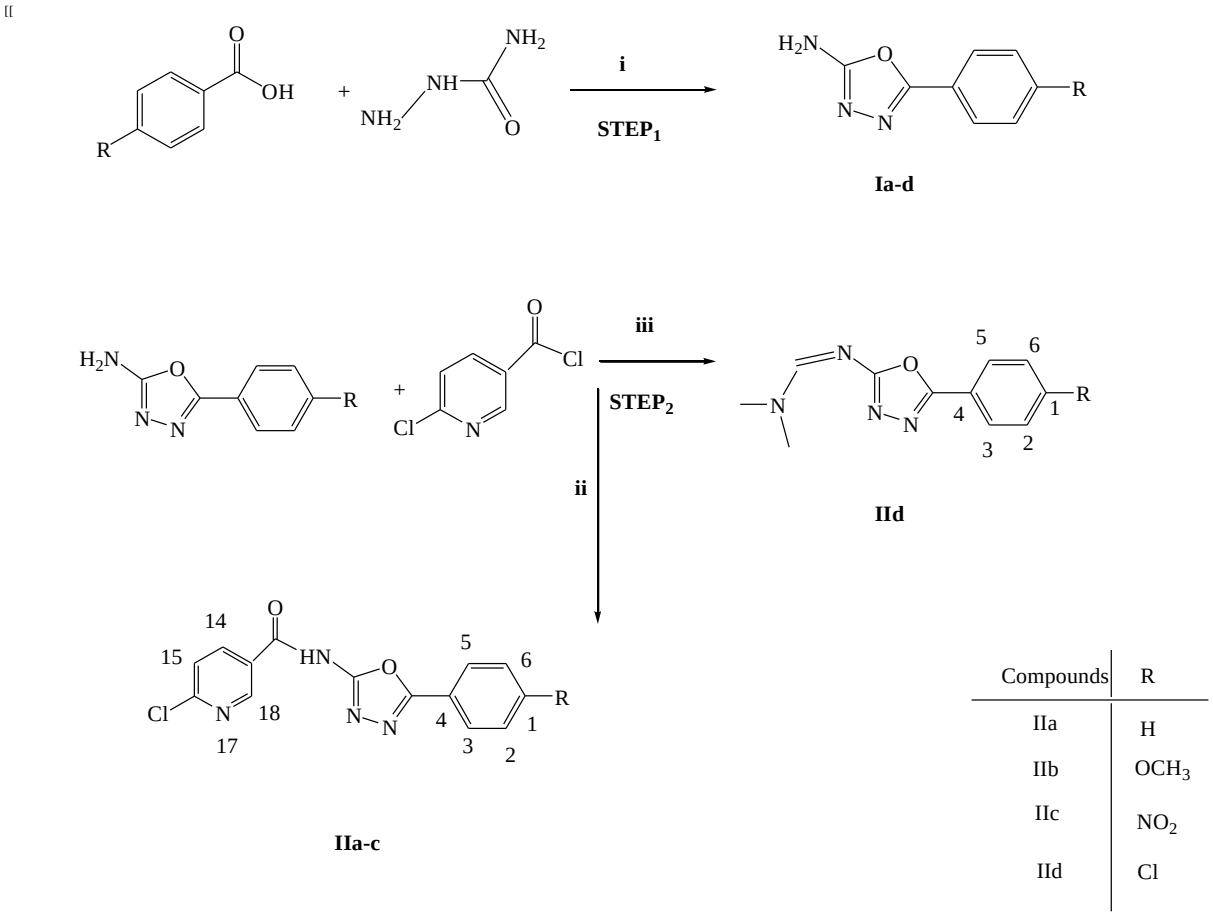
General procedure for the synthesis of compounds (IIa-c)

6-Chloronicotinoyl chloride (0.02 mol) in dry tetrahydrofuran (THF,10 mL) was added dropwise to a stirred solution of 2-amino-5-(substituted phenyl)-(1, 3, 4)-oxadiazole **(Ia-c)** (0.01 mol) in dry THF (15 mL) and dry pyridine (2 mL).

The reaction solution was stirred at room temperature for 2 h until a solid product was formed. The obtained solid was filtered, washed with water, dried and recrystallized from methanol to yield compounds (**IIa-c**) (Scheme1) (20).

Procedure for the synthesis of compound IId

6-Chloronicotinoyl chloride (0.02 mol) in dry dimethylformamide (DMF, 10 mL) was added dropwise into a solution of 5-(4-chlorophenyl)-1,3,4-oxadiazole -2-amine (**Id**, 0.01 mole) in dry DMF (10 mL) containing potassium carbonate (0.01 mmol). The reaction solution was stirred at room temperature for 2 h, and then was poured into ice-water; the obtained precipitate was collected and crystallized from methanol (Scheme1) (21).



Compounds	R
IIa	H
IIb	OCH_3
IIc	NO_2
IId	Cl

Scheme 1. Synthesis of the target compounds (**IIa-d**). (i) $POCl_3$, 3 h, reflux; (ii) dry tetrahydrofuran / pyridine, room temperature, 2 h; (iii) dimethylformamide , K_2CO_3, room temperature, 2 h.

Cytotoxic assay

Sample and culture media preparation

MCF-7 (breast cancer) and HeLa (cervical cancer) cells were obtained from the Pasture Institute of Iran (Tehran, I.R. Iran) and maintained at 37 °C in a humidified atmosphere (90%) containing 5% CO2. MCF-7 and HeLa cell lines were grown in RPMI 1640 completed with 5% v/v fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin. After 2-3 subcultures, 180 µL of the cell suspensions (5×10^4 cells/mL) were seeded in 96-well plates and incubated for 24 h (22). The stock solutions of the final compounds **IIa-d** and **IIe, f, and i** (10 mM, 1 mL) were prepared in the minimum volume of dimethyl sulfoxide (DMSO) and a serial dilution of 1000, 800, 400, 200, and 100 µM were prepared using the medium.

After 24 h incubation, 20 µL of different concentrations of the derivatives were added and the microplates were further incubated for 48 h. Paclitaxel was used as a standard anticancer drug for comparison. For the blank absorbance readings, three control well of medium alone were provided. To evaluate cell survival, treated cells were incubated with 20 µL of MTT solution (5 mg/mL in phosphate buffer solution) for 4 h, afterwards, the culture medium was aspirated and 150 µL DMSO was added to dissolve formazan crystals. The absorbance of each well was measured at 540 nm using an ELISA plate reader (22). The IC_{50} of each compound was determined using the achieved dose-percent of inhibition curve.

Cell viability was calculated using the following equation:

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

where, MA is mean absorbance

Molecular docking studies

The crystal structure of EGFR tyrosine kinase with its ligand (PDB ID: 1M 17) with resolution 2.6 Å was downloaded from the protein data bank (www.rcsb.org) (4,23).

Compounds **IIa-d** and **IIe, f, and i (**17) were subjected to molecular docking studies using autodock 4 software.

Structures of the ligands and the target protein were processed prior to the molecular docking studies (24). The protein was prepared for docking studies as follows: water and ligand molecules were removed from the protein file. All missing hydrogens were added, and after determining the Kolman united atom charges, non-polar hydrogens were merged to their corresponding carbons using Autodock tools.

The obtained model was then used for predicting the ligand-enzyme interaction at the active site.

For ligand preparation, three-dimensional (3D) structures of the ligands were drawn in HyperChem 7.0 software then the ligands 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 as inputs of the AutoDock tools. Then the partial charges of atoms were calculated using the Gasteiger-Marsili procedure implemented in the AutoDock tools package. Non-polar hydrogens of the compounds were merged and then rotatable bonds were defined. Prepared protein and ligand structures were saved in the PDBQT format for calculating energy grid maps. Grid box dimensions were 60 × 60 × 60 with a 0.375 Å grid points spacing.

The binding location of reference ligand was defined as a binding site for finding the best pose of all ligands (24-26).

The molecular docking technique was conducted using the Autodock 4.2 software package, with the implemented empirical free energy function and the Lamarckian genetic algorithm (24-26).

Statistical analysis

Each experiment was repeated three times and the results were reported as mean ± SD. One-way analysis of variance (ANOVA) followed by the Tukey post hoc test was used to determine the statistically significant differences between various groups.

RESULTS

Chemistry

*5-Phenyl-1, 3, 4-oxadiazol-2-amine (**Ia**)*

White solid; yield: 80%; mp: 240-241 °C (lit. 242-243 °C) (27), IR (KBr) (ν_{max}, cm^{-1}): 3320, 3136 (NH_2), 1650 (C=N), 1280 (COC); ^{1}HNMR: (400 MHz; DMSO-d_6): δ 7.86(2H, m, H-$C_{3,5}$), 7.58 (3H, m, H-$C_{1,2,6}$),7.32 (2H, s, NH_2).

*5-(4-Methoxyphenyl)-1, 3, 4-oxadiazol-2-amine (**Ib**)*

White solid; yield: 75%; mp: 251-252 °C (lit. 252-253 °C) (27), IR (KBr) (ν_{max}, cm^{-1}): 3338, 3154 (NH_2), 1656 (C=N), 1254 (COC); ^{1}HNMR: (400 MHz; DMSO-d_6): δ 7.79 (2H, d, *J* = 8 Hz, H-$C_{3,5}$), 7.2 (2H, s, NH_2), 7.14 (2H, d, *J* = 8 Hz, H-$C_{2,6}$), 3.88 (3H, s, OCH_3).

*5-(4-Nitrophenyl)-1, 3, 4-oxadiazol-2-amine (**Ic**)*

Yellow solid; yield: 80%; mp: 249-250 °C (lit. 251-252 °C) (27), IR (KBr) (ν_{max}, cm^{-1}): 3320, 3112 (NH_2), 1660 (C=N), 1529, 1346 (NO_2), 1047(COC); ^{1}HNMR: (400 MHz; $CDCl_3$): δ 8.38 (2H, d, *J* = 8 Hz, H-$C_{2,6}$), 8.31(2H, d, *J* = 8 Hz, H-$C_{3,5}$), 7.19 (2H, s, NH_2).

*5-(4-Chlorophenyl)-1, 3, 4-oxadiazol-2-amine (**Id**)*

White solid; yield: 78%; mp: 265-267 °C (lit. 260-264 °C) (27), IR (KBr) (ν_{max}, cm^{-1}): 3294, 3126 (NH_2), 1662 (C=N), 1289 (COC); ^{1}HNMR: (400 MHz; DMSO-d_6): δ 7.87 (2H, d, *J* = 8 Hz, H-$C_{3,5}$), 7.68 (2H, d, *J* = 8 Hz, H-$C_{2,6}$), 7.39 (2H, br, NH_2).

*6-Chloro-N -(5-phenyl-1,3,4-oxadiazol-2-yl)nicotinamide (**IIa**)*

White solid; yield: 50%; mp: 184-187 °C, IR (KBr) (ν_{max}, cm^{-1}): 3300 (NH), 3052 (C-H, Ar), 1679 (C=O); ^{1}HNMR: (400 MHz; DMSO-d_6): δ 8.89 (1H, s, H-C_{18}), 8.30(1H, d, *J* = 8 Hz, H-C_{14}),7.80 (2H, d, *J* = 8 Hz, H-$C_{3,5}$), 7.67(1H, d, *J* = 8 Hz, H-C_{15}),7.52-7.54 (3H, m, H-$C_{1,2,6}$), 7.26 (1H, s, NH).

*6-Chloro-N - (5-(4-methoxypheny) l-1, 3, 4-oxadiazol-2-yl) nicotinamide (**IIb**)*

White solid; yield: 48%; mp: 240-241 °C, IR (KBr) (ν_{max}, cm^{-1}): 3301 (NH), 1696 (C=O), 1255 (CO); ^{1}HNMR: (400 MHz; DMSO-d_6): δ 9.13 (1H, s, H-C_{18}), 8.51 (1H, d, *J* = 8 HZ, H-C_{14}), 8.02 (2H, d, *J* = 8 Hz, H-$C_{3,5}$), 7.85 (1H, d, *J* = 8 Hz, H-C_{15}), 7.28 (2H, d, *J* = 8 Hz, H-$C_{2,6}$), 3.97 (3H, s ,OCH_3).

*6-Chloro-N - (5-(4-nitropheny) l-1, 3, 4-oxadiazol-2-yl)nicotinamide (**IIc**)*

Light yellow solid; yield 40%; mp: 183-185 °C, IR (KBr) (ν_{max}, cm^{-1}): 3301 (NH), 1685 (C=O), 1582, 1369 (NO_2), 1295 (CO); ^{1}HNMR: (400 MHz; DMSO-d_6): 8.50 (2H, d, *J* = 8 Hz, H-$C_{2,6}$), 8.46 (2H, *J* = 8 Hz, H-$C_{3,5}$), 8.33(1H, s, H-C_{18}), 7.73 (1H, d, *J* = 8 Hz ,H-C_{14}), 7.50 (1H, d, *J* = 8 HZ, H-C_{15}).

*N'-(5-(4-Chloropheny) 1, 3, 4-oxadiazole-2-yl)-N, N-dimthylformamidine (**IId**)*

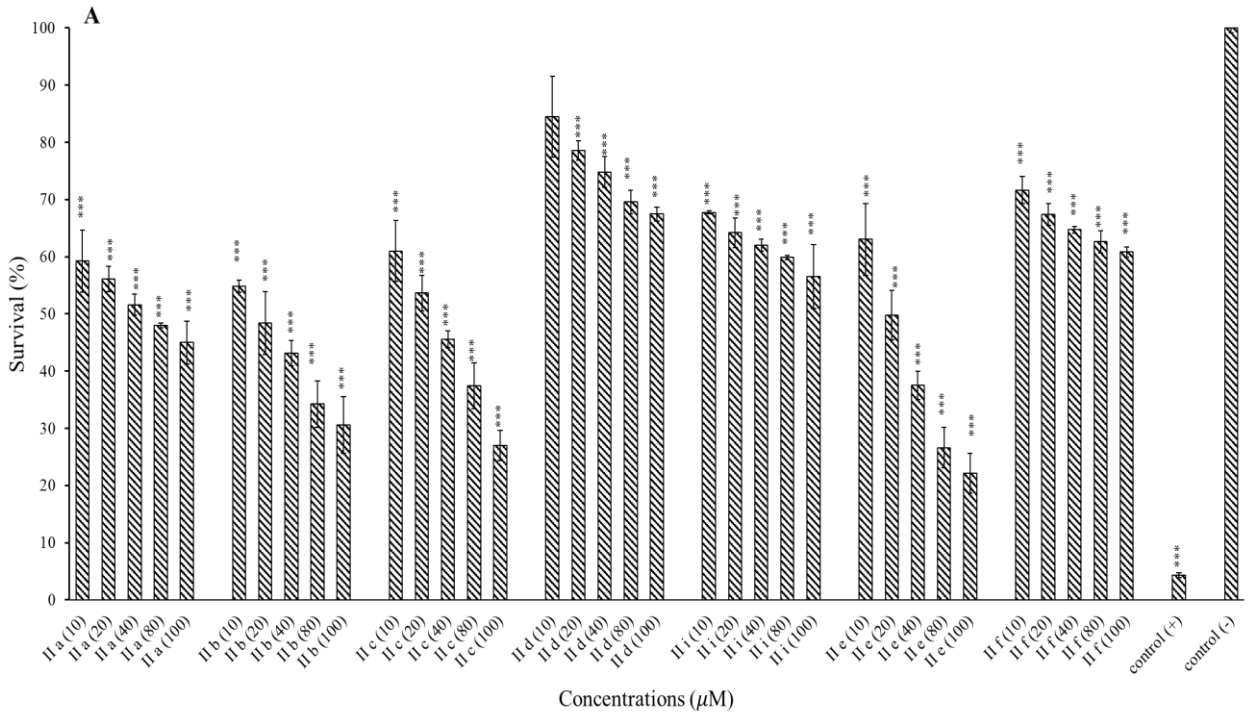
White solid; yield: 48%; mp: 163-166 °C, IR (KBr) (ν_{max}, cm^{-1}): 2930 (C-H), 3100 (C-H), 1636 (C=N), 1246 (CO); ^{1}HNMR: (400 MHz; DMSO-d_6): δ 8.53 (1H, s, CH), 7.92 (2H, d, *J* = 8 Hz, H-$C_{3,5}$), 7.62 (2H,d, *J* = 8 Hz, H-$C_{2,6}$), 3.18 (3H, s ,CH_3), 3.03 (3H, s ,CH_3).

Cytotoxic activity

The cytotoxicity of compounds was evaluated against HeLa and MCF-7 cell lines at 10, 20, 40, 80, and 100 µM using MTT assay. The results were summarized in Fig. 1A and B and Table 1. The results of the cytotoxic assay revealed that compounds **IIb**, **IIc**, and **IIe** with IC_{50} values of 19.4, 35, and 25.4 µM, respectively, showed the highest activity against HeLa cell line. Moreover, Compounds **IIe**, **IIc**, and **IIb** with IC_{50} values of 51.8, 54.2, and 78.7 µM, respectively were the most active compounds against MCF-7 cell line.

Docking

The docking results of compounds (Fig. 2) including the estimated free binding energy values of the docked positions, presented in kcal/mol, and the favorable interactions with key amino acid residues at the active site of enzymes are expressed in Table 2 and Figs 3.



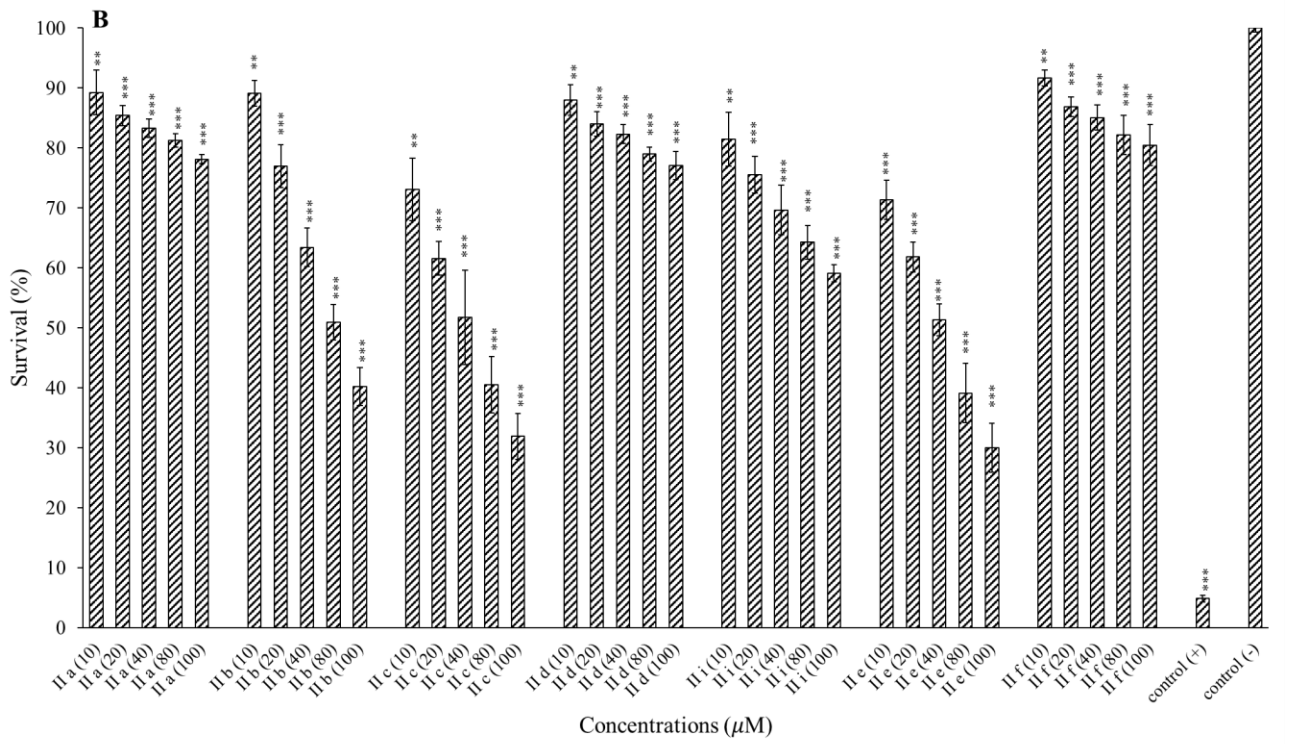


Fig. 1. Cytotoxic effect of synthetic compounds **IIa-d** and **IIe**, **IIf**, and **IIi** at 10, 20, 40, 80, and 100 μM on (A) HeLa and (B) MCF7 cells. Data are presented as mean ± SD, n = 3. $^{**}P < 0.01$ and $^{***}P < 0.001$ indicate significant differences in comparison with the negative control; paclitaxel at 200 μg/mL was used as a positive control

Table 1. The IC_{50} (μM) of tested compounds against MCF-7 and HeLa cell lines.

Cell lines	IC_{50} (μM)						
	IIa	**IIb**	**IIc**	**IId**	**IIe**	**IIf**	**IIi**
HeLa	63.6 ± 6.7	19.9 ± 7.6	35.0 ± 5.2	> 100	25.1 ± 8.1	> 100	> 100
MCF-7	> 100	78.7 ± 5.9	54.2 ± 1.8	> 100	51.8 ± 5.3	> 100	> 100

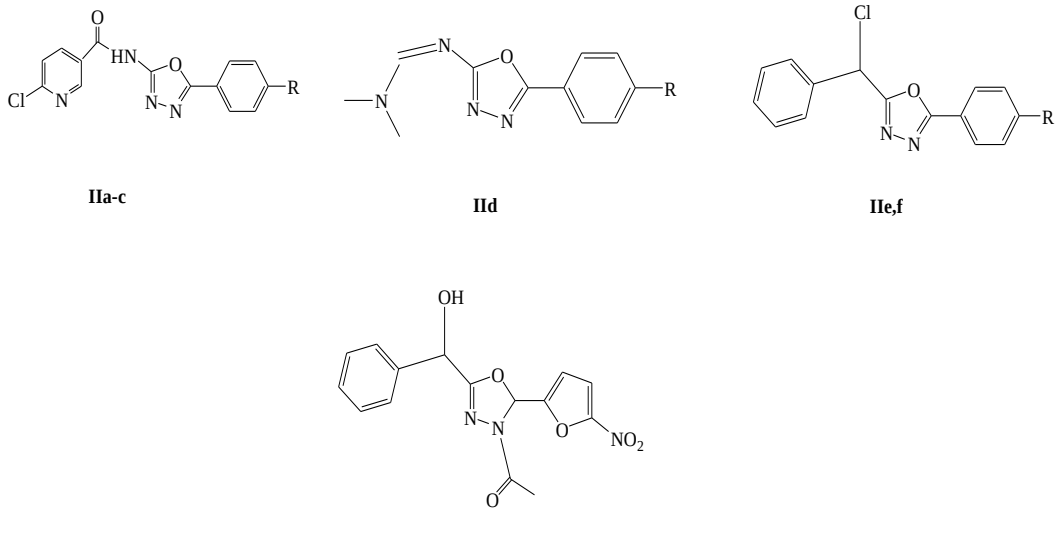


Fig. 2. Docked compounds into epidermal growth factor receptor tyrosine kinase.

Table 2. Energy-based interactions and hydrogen bonds for the oxadiazole derivatives docked into epidermal growth factor receptor tyrosine kinase.

compounds	R	ΔG_{bind} (Kcal/mol)[a]	Hydrogen bond
IIa	H	-7.57	Gln 767 (2.1 Å), Met769 (1.85 Å)
IIb	OCH_3	-7.19	Gln 767 (2.16 Å), Thr766 (2.16 Å), Asp 831(2.05 A°)
IIc	NO_2	-7.50	Lys721 (1.9 1 Å), Met 769 (2.018 Å),Thr830, Thr 766, Gln 767
IId	Cl	-6.26	-
IIe	Cl	-7.89	Gln 767 (2.1 Å), Met769 (2.03 Å), Thr766 (2.10 Å)
IIf	NO_2	-7.80	Gln 767 (2.14 Å)
IIi	-	-7.23	Asp831 (2.08 Å), Met769 (1.88 Å)

a, Values are calculated for the best pose of each structure.

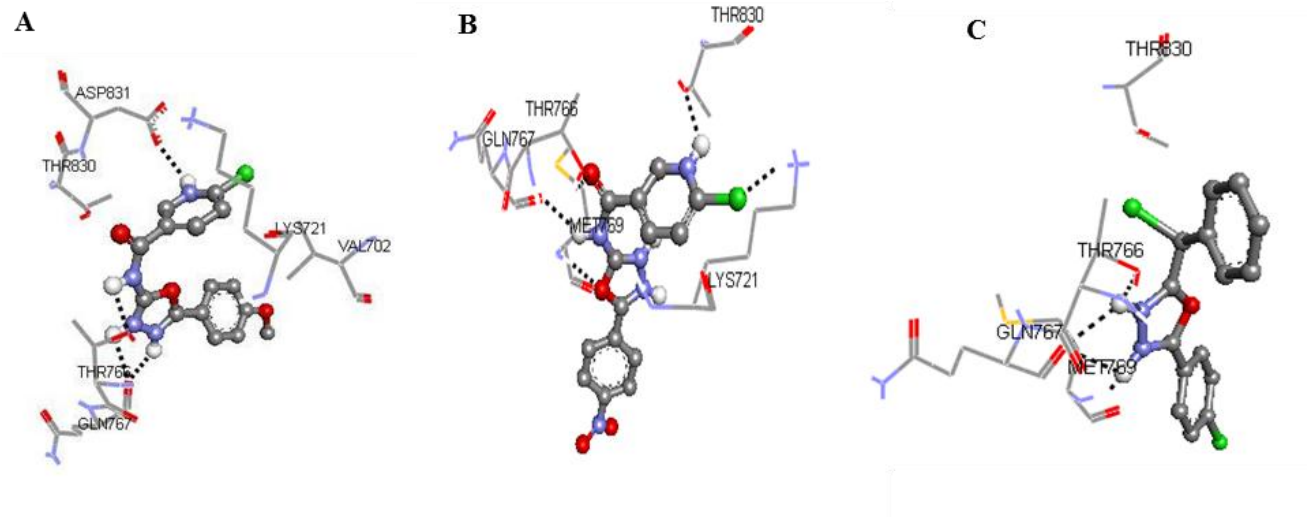


Fig. 3. Docked conformation of compounds (A) **IIb**, (B) **IIc**, and (C) **IIe** in the binding site of epidermal growth factor receptor tyrosine kinase. Hydrogen bonds are shown by the black dashed line.

DISCUSSION

A literature survey reveals that 1, 3, 4-oxadiazole is an interesting heterocyclic scaffold for the development of new anticancer agents (28-31). In the present study, some amide and imine derivatives of oxadiazole were synthesized in two steps, along with some previously prepared oxadiazole derivatives (17), and the cytotoxic effects were evaluated on two cell lines at different concentrations.

Interestingly, the obtained imine derivative could be explained by the participation of the solvent in reaction instead of 6-chloronicotinoyl chloride.

The results of cytotoxic evaluation revealed that the conversion of imine to amide derivatives increased the cytotoxic activity. Among amide analogues, compounds **IIb** and **IIc** with para positioning of the methoxy and nitro moiety, respectively, showed considerable cytotoxic activity compared to compound **IIa** against both cell lines**,** which indicated the introduction of methoxy or nitro groups to the phenyl ring, might improve the cytotoxic activity.

In the case of the previously prepared oxadiazole derivatives, compound **IIe** exhibited remarkable cytotoxic activity against both cell lines. It seems that the substitution of halogen on a 5-phenyl ring could significantly increase the cytotoxic activity against the tested cell lines.

The IC_{50} values of all tested compounds revealed that HeLa is more susceptible to compounds **IIb, IIc**, and **IIe**. The results also indicated that the cytotoxic activity of these compounds is concentration-dependent.

Literature surveys have been demonstrated that EGFR tyrosine kinase can be used as a target for anticancer drug design (4).

The docking results of the compounds revealed that the highest dock score was -7.89 kcal/mol for compound **IIe**. Three hydrogen

binding interactions have been detected for this ligand formed by Gln767, Met769, and Thr 766 amino acids at distances 2.1, 2.03, 2.1 Å, respectively. In the most compounds, nitrogen atoms of the 1, 3, 4-oxadiazole ring formed hydrogen bindings with Met 769. The rest of the tested compounds showed an appropriate dock score ranging from -6.26 to -7.80 kcal/mol. Among compounds containing amide group, dock score values were between -7.19 and -7.57 kcal/mol. The amide group on these analogues was oriented toward Met769 to make hydrogen bond interactions with this residue. This interaction was not observed for the derivative **IId** which was without an amide group, as its results of the cytotoxic test and docking study were not satisfactory.

CONCLUSION

In this study, synthesis, in silico, and cytotoxic evaluation of 2, 5-disubstituted 1, 3, 4-oxadiazole derivatives were reported. Compounds **IIb**, **IIc**, and **IIe** proved to be the most active derivatives in this study with special effectiveness against HeLa cell line. Compound **IIe** represented the best binding mode to the active site of EGFR tyrosine kinase. Based on the obtained results of docking and cytotoxic studies, compound **IIe** could be a suitable structure for inhibition of tyrosine kinase. But, further experimental tests such as *in vitro* inhibitory study against tyrosine kinases are essencial to confirm these findings.

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CONFLICT OF INTEREST STATEMENT

All authors declare no conflict of interest in this study.

AUTHORS' CONTRIBUTION

E.Jafari contributed in the conception and design of the work, conducting the study, analyzing the data, drafting, and revising the. F. Hassanzadeh contributed to the conception of the work, analyzing the data, revising the draft. M. Zarabi performed the experiments and analyzing the data. GH. Khodarahmi contributed to the conception of the work, conducting the study, revising the draft. G. Vaseghi analyzed the data. All authors agreed with all aspects of the work.

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