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

Farshid Hassanzadeh¹, Elham Jafari¹*, Mohammadreza Zarabi¹, Ghadamali Khodarahmi¹, and Golnaz Vaseghi²

¹Department of Medicinal Chemistry and Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.
²Applied Physiology Research Center and Isfahan Cardiovascular Research Center, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.

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 Ile had a considerable cytotoxic activity with IC₅₀ values of 19.9, 35, and 25.1 µM, respectively against HeLa cells. The highest docking score was -7.89 kcal/mol for compound Ile.

**Conclusion and implications:** Compound Ile 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).
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 Disubstituted 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 F254 plates (Germany) were applied for analytical thin-layer chromatography (TLC).

Proton nuclear magnetic resonance (1H NMR) 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 IIId

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).
Scheme 1. Synthesis of the target compounds (IIa-d). (i) POCl₃, 3 h, reflux; (ii) dry tetrahydrofuran / pyridine, room temperature, 2 h; (iii) dimethylformamide, K₂CO₃, room temperature, 2 h.

### Cytotoxic assay

**Sample and culture media preparation**

MCF-7 (breast cancer) and HeLa (cervical cancer) cells were obtained from the Pasteur 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 × 10⁴ 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 IC₅₀ of each compound was determined using the achieved dose-percent of inhibition curve.

Cell viability was calculated using the following equation:

\[
\text{Cell survival} \% = \frac{\text{MA of treated wells} - \text{MA of blank}}{\text{MA of negative control} - \text{MA of blank}} \times 100
\]

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) (νmax, cm⁻¹): 3320, 3136 (NH₂), 1650 (C=N), 1280 (COC); 1HNMR: (400 MHz; DMSO-d₆): δ 7.86(2H, m, H-C₃,5), 7.58 (3H, m, H-C₁,2,6), 7.32 (2H, s, NH₂).

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) (νmax, cm⁻¹): 3338, 3154 (NH₂), 1656 (C=N), 1254 (COC); 1HNMR: (400 MHz; DMSO-d₆): δ 7.79 (2H, d, J = 8 Hz, H-C₃,5), 7.2 (2H, s, NH₂), 7.14 (2H, d, J = 8 Hz, H-C₂,6), 3.88 (3H, s, OCH₃).

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) (νmax, cm⁻¹): 3320, 3112 (NH₂), 1660 (C=N), 1529, 1346 (NO₂), 1047(COC); 1HNMR: (400 MHz; CDCl₃): δ 8.38 (2H, d, J = 8 Hz, H-C₂,6), 8.31(2H, d, J = 8 Hz, H-C₃,5), 7.19 (2H, s, NH₂).

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) (νmax, cm⁻¹): 3320, 3112 (NH₂), 1660 (C=N), 1529, 1346 (NO₂), 1047(COC); 1HNMR: (400 MHz; DMSO-d₆): δ 8.38 (2H, d, J = 8 Hz, H-C₂,6), 8.31(2H, d, J = 8 Hz, H-C₃,5), 7.19 (2H, s, NH₂).

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

White solid; yield: 50%; mp: 184-187 °C, IR (KBr) (νmax, cm⁻¹): 3300 (NH), 3052 (C-H, Ar), 1679 (C=O); 1HNMR: (400 MHz; DMSO-d₆): δ 8.89 (1H, s, H-C₁₈), 8.30(1H, d, J = 8 Hz, H-C₁₄) , 7.80 (2H, d, J = 8 Hz, H-C₃,5), 7.67(1H, d, J = 8 Hz, H-C₁₅), 7.52-7.54 (3H, m, H-C₁,2,6), 7.26 (1H, s, NH).
6-Chloro-N - (5-(4-methoxyphenyl)-1, 3, 4-oxadiazol-2-yl) nicotinamide (IIb)

White solid; yield: 48%; mp: 240-241 °C, IR (KBr) (νmax, cm⁻¹): 3301 (NH), 1696 (C=O), 1255 (CO); ¹HNMR: (400 MHz; DMSO-d₆): δ 9.13 (1H, s, H-C₁₈), 8.51 (1H, d, J = 8 Hz, H-C₁₄), 8.02 (2H, d, J = 8 Hz, H-C₃₅), 7.85 (1H, d, J = 8 Hz, H-C₁₅), 7.28 (2H, d, J = 8 Hz, H-C₂₆), 3.97 (3H, s ,OCH₃).

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

Light yellow solid; yield 40%; mp: 183-185 °C, IR (KBr) (νmax, cm⁻¹): 3301 (NH), 1685 (C=O), 1582, 1369 (NO₂), 1295 (CO); ¹HNMR: (400 MHz; DMSO-d₆): 8.50 (2H, d, J = 8 Hz, H-C₂₆), 8.46 (2H, d, J = 8 Hz, H-C₃₅), 8.33 (1H, s, H-C₁₈), 7.73 (1H, d, J = 8 Hz, H-C₁₄), 7.50 (1H, d, J = 8 Hz, H-C₁₅).

N’-(5-(4-Chlorophenyl)-1, 3, 4-oxadiazole-2-yl)-N, N-dimethylformamidine (IIId)

White solid; yield: 48%; mp: 163-166 °C, IR (KBr) (νmax, cm⁻¹): 2930 (C-H), 3100 (C-H), 1636 (C=N), 1246 (CO); ¹HNMR: (400 MHz; DMSO-d₆): δ 8.53 (1H, s, CH), 7.92 (2H, d, J = 8 Hz, H-C₃₅), 7.62 (2H,d, J = 8 Hz, H-C₂₆), 3.18 (3H, s ,CH₃), 3.03 (3H, s ,CH₃).

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 IC₅₀ 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 IC₅₀ 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.
Synthesis and cytotoxicity of some oxadiazole-based compounds

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 | III | III |
| HeLa       | 63.6 ± 6.7 | 19.9 ± 7.6 | 35.0 ± 5.2 | > 100 | 25.1 ± 8.1 | > 100 |
| MCF-7      | > 100 | 78.7 ± 5.9 | 54.2 ± 1.8 | > 100 | 51.8 ± 5.3 | > 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      | ΔGbind (Kcal/mol)* | Hydrogen bond                                                                 |
|-----------|--------|--------------------|-------------------------------------------------------------------------------|
| IIa       | H      | -7.57              | Gln 767 (2.1 Å), Met769 (1.85 Å)                                             |
| IIb       | OCH₃   | -7.19              | Gln 767 (2.16 Å), Thr766 (2.16 Å), Asp 831 (2.05 Å)                           |
| IIc       | NO₂    | -7.50              | Lys721 (1.9 Å), Met 769 (2.018 Å), Thr830, Thr 766, Gln 767                 |
| IIId      | Cl     | -6.26              | -                                                                             |
| IIe       | Cl     | -7.89              | Gln 767 (2.1 Å), Met769 (2.03 Å), Thr766 (2.10 Å)                            |
| IIIf      | NO₂    | -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 IC₅₀ 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 IIId 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.

ACKNOWLEDGEMENTS

This work was financially supported, through the Grant No. 295081, by the Vice-Chancellor of Research of Isfahan University of Medical Sciences, Isfahan, I.R. Iran

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. 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. Vasegh analyzed the data. All authors agreed with all aspects of the work.

REFERENCES

1. Kumar A, Gupta A, Kashaw V, Shukla G, Mishra V, Kashaw SK. Syntheses and anticancer evaluation of some novel 3-[5-(4-substituted) phenyl-1,3,4-oxadiazole-2-yl]-2-phenylquinazoline-4(3H)-ones. Int J Pharm Pharm Sci. 2012;4(1):502-506.
2. Ramazani A, Khoobi M, Torkaman A, Nasrabad FZ, Foroootanfar H, Shakibaie M, et al. One-pot, four-component synthesis of novel cytotoxic agents 1-(5-aryl- 1,3,4- oxadiazol-2-yl)-1-(1H-pyrrol-2-yl) methanamines. Eur J Med Chem. 2014;78:151-156. DOI: 10.1016/j.ejmech.2014.03.049.
3. Bondock S, Adel S, Etman HA, Badria FA. Synthesis and antitumor evaluation of some new 1,3,4-oxadiazole-based heterocycles. Eur J Med Chem. 2012;48:192-199. DOI: 10.1016/j.ejmech.2011.12.013.
4. Ahsan MJ, Rathod VPS, Singh M, Sharma R, Jadav SS, Yasmin S, et al. Synthesis, anticancer and molecular docking studies of 2-(4-chlorophenyl)-5-aryl-1,3,4-oxadiazole analogues. Med Chem. 2013;3(4):294-297.
5. Singh R, Chouhan A. Various approaches for synthesis of 1,3,4-oxadiazole derivatives and their pharmacological activity. World J Pharm Pharm Sci. 2014;3(10):1474-1505.
6. Pangal A, Shaikh JA. Various pharmacological aspects of 2,5-disubstituted 1,3,4-oxadiazole derivatives: a review. Res J Chem Sci. 2013;3(12):79-89.
7. Sharma S, Sharma PK, Kumar N, Dudhe R. A review: oxadiazole their chemistry and pharmacological potentials. Der Pharma Chemica. 2010;2(4):253-263.
8. Nagara J, Chaluvaraju KC, Nirajan MS, Kiran S. 1,3,4-Oxadiazole: a potent drug candidate with various pharmacological activities. Int J Pharm Pharm Sci. 2011;3(3):9-16.
9. Somani RR, Chipulkar S, Vetale SP, Makhija DT, Shirodkar PY. Evaluation of apoptosis inducing activity of an oxadiazole based potential anticancer compound. Int J PharmTech Res. 2013;5(3):1233-1240.
10. Bhinder CK, Kaur A, Kaur A. Review: 1,3,4-oxadiazole as anticancer agent. Int J Pharm Res Bio Sci. 2014;3(4):457-479.
11. Sharma R, Kumar N, Yadav R. Chemistry and pharmacological importance of 1,3,4-oxadiazoles derivatives. J Chem. 2015;4(2):1-27.
12. Bajaj S, Asati V, Singh J, Roy PP. 1,3,4-Oxadiazoles: an emerging scaffold to target growth factors,
enzymes and kinases as anticancer agents. Eur J Med Chem. 2015;97:124-141. DOI: 10.1016/j.ejmech.2015.04.051.

13. Zachariah SM, Ramkumar M, George N, Ashif MS. A review on oxadiazole. Res J Pharm Bio Chem Sci. 2015;6(2):205-219.

14. Ouyang X, Piatnitski EL, Pattaropong V, Chen X, He HY, Kiselyov AS, et al. Oxadiazole derivatives as a novel class of antimitic agents: synthesis, inhibition of tubulin polymerization and activity in tumor cell lines. Bio Org Med Chem Lett. 2006;16:1191-1196. DOI: 10.1016/j.bmcl.2005.11.094.

15. Adimule V, Medapa S, Jagadeesha AH, Kumar LS, Rao PK. Synthesis, characterization and cytotoxic evaluation of novel derivatives of 1,3,4-oxadiazole containing 5-phenyl thiophene moiety. J Pharm Bio Sci. 2014;9(5):42-48.

16. Biju CR, Ilango K. Docking investigation, synthesis and cytotoxic studies of substituted oxadiazole derivatives. Int J Pharm Pharm Sci. 2012;4(3):262-265.

17. Jafari E, Mohammadi T, Jahanian-Najafabadi A, Hassanzadeh F. Synthesis and antimicrobial evaluation of some 2,5 disubstituted 1,3,4-oxadiazole derivatives. Res Pharm Sci. 2017;12(4):330-336. DOI: 10.4103/1735-5362.212051.

18. Deshmukh R, Jha AK, Thakur AS, Dewangan D. Synthesis and antibacterial activity of some 1,3,4-oxadiazole derivatives and their thiourea analogues. Int J Pharm Bio Sci. 2011;2(1):215-219.

19. Kumar CTK, Keshavayya J, Rajesh TN, Peethambar SK, Shoukat Ali AR. Synthesis, characterization, and biological activity of 5-phenyl-1,3,4-thiadiazole-2-amino incorporated azo dye derivatives. Org Chem Int. 2013;2013:1-7. DOI: 10.1155/2013/370626.

20. Cressier D, Prouillac C, Hernandez P, Amourette C, Diserbo M, Lion C, et al. Synthesis, antioxidant properties and radioprotective effects of new benzothiazoles and thiadiazoles. Bioorg Med Chem. 2009;17(14):5275-5284. DOI: 10.1016/j.bmc.2009.05.039.

21. Taher AT, Georgey HH, El-Subbagh HI. Novel 1,3,4-heterodiazole analogues: synthesis and in-vitro antitumor activity. Eur J Med Chem. 2012;47(1):445-451. DOI: 10.1016/j.ejmech.2011.11.013.

22. Hassanzadeh F, Sadeghi-Allabadi H, Nikooei S, Jafari E, Vaseghi G. Synthesis and cytotoxic evaluation of some derivatives of triazole-quinazolinone hybrids. Res Pharm Sci. 2019;14(2):130-137. DOI: 10.4103/1735-5362.253360

23. Qin X, Li Z, Yang L, Liu P, Hu L, Zeng C, et al. Discovery of new [1,4]dioxino[2,3-f]quinazoline-based inhibitors of EGFR including the T790M/L858R mutant. Bioorg Med Chem. 2016;24(13):2871-2881. DOI: 10.1016/j.bmc.2016.01.003.

24. Rezaee Nasab R, Mansourian M, Hassanzadeh F. Synthesis, antimicrobial evaluation and docking studies of some novel quinazolinone Schiff base derivatives. Res Pharm Sci. 2018;13(3):213-221. DOI: 10.4103/1735-5362.228942.

25. Sirous H, Zabihollahi R, Aghasadeghi MR, Sadat SM, Saghaie L, Fasshi A. Docking studies of some 5-hydroxypyridine-4-one derivatives: evaluation of integrase and ribonuclease H domain of reverse transcriptase as possible targets for anti-HIV-1 activity. Med Chem Res. 2015;24:1215-2122. DOI: 10.1007/s00044-014-1289-1.

26. Ranjbar S, Edraki N, Khoshneviszadeh M, Foroumadi A, Miri R, et al. Design, synthesis, cytotoxicity evaluation and docking studies of 1,2,4-triazine derivatives bearing different arylidene-hydrazinyl moieties as potential mTOR inhibitors. Res Pharm Sci. 2018;13(1):1-11. DOI: 10.4103/1735-5362.220962.

27. Niú P, Kang J, Tian X, Song L, Liu H, Wu J, et al. Synthesis of 2-amino-1,3,4-oxadiazoles and 2-amino-1,3,4-thiadiazoles via sequential condensation and I2-mediated oxidative C-O/C-S bond formation. J Org Chem. 2015;80(2):1018-1024. DOI: 10.1021/jo502518c.

28. Tu G, Yan Y, Chen X, Lv Q, Wang J, Li S. Synthesis and antiproliferative assay of 1,3,4-oxadiazole and 1,2,4-triazole derivatives in cancer cells. Drug Discov Ther. 2013;7(2):58-65.

29. Oliveira CS, Lira BF, Barbosa-Filho JM, Lorenzo JGF, Athayde-Filho PF. Synthetic approaches and pharmacological activity of 1,3,4-oxadiazoles: a review of the literature from 2000-2012. Molecules. 2012;17(9):10192-10231. DOI: 10.3390/molecules170910192.

30. Sun J, Zhu H, Yang ZM, Zhu HL. Synthesis, molecular modeling and biological evaluation of 2-aminomethyl-5-(quinolin-2-yl)-1,3,4-oxadiazole-2(3H)-thione quinolone derivatives as novel anticancer agent. Eur J Med Chem. 2013;60:23-28. DOI: 10.1016/j.ejmech.2012.11.039.

31. Hassanzadeh F, Sadeghi-Allabadi H, Jafari E, Sharifzadeh A, Dana N. Synthesis and cytotoxic valuation of some quinazolinone-5-(4-chlorophenyl) 1,3,4-oxadiazole conjugates. Res Pharm Sci. 2019;14(5):408-413. DOI: 10.4103/1735-5362.268201.