

# Synthesis and cytotoxic activity evaluation of some new 1, 3, 4-oxadiazole, 1, 3, 4-thiadiazole and 1, 2, 4-triazole derivatives attached to phthalimide

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## Abstract

**Background and purpose:** In the last few decades, nitrogen-rich heterocyclic compounds such as 1, 3, 4-thiadiazoles, 1, 2, 4-triazoles and 1, 3, 4-oxadiazoles have received considerable attention because of their notable biological properties, especially cytotoxic effects. The small molecules of mentioned azole derivatives revealed very intensive antitumor activity. In addition, phthalimide-thiadiazole and naphthalimide-triazole hybrid derivatives have shown remarkable cytotoxic effects. According to these observations, some of the hybrid derivatives containing the phthalimide-five-membered azoles were prepared in three steps in this research.

**Experimental approach:** The thiol group of azoles was treated with ethyl chloroacetate which was followed by a reaction with hydrazine hydrate to provide acid hydrazide derivatives. Subsequently, the corresponding acid hydrazides were utilized to prepare the final derivatives through the reaction with phthalic anhydride. Cytotoxic activity of final compounds was evaluated against MCF-7 and HeLa cell lines using MTT assay.

**Findings/Results:** Compound **3d** containing two phthalimide moieties in its structure showed a significant improvement in cytotoxic activity with an  $IC_{50}$  value of 29  $\mu$ M against HeLa cell line. Compounds **3a-3c** showed less cytotoxic effects against both cell lines.

**Conclusion and implications:** The combination of the thiadiazole nucleus with two phthalimide structures increased the cytotoxic activity against the HeLa cell line. This increase in cytotoxic activity is probably due to its being more lipophilic characteristic and interaction of this derivative with the biological targets of two directions.

**Keywords:** Cytotoxic activity; Phthalimide; 1, 3, 4-Oxadiazole; 1, 3, 4-Thiadiazole; 1, 2, 4-Triazole.

## INTRODUCTION

Cancer treatment is still a major public health concern and a situation demanding serious investigation for more safe and effective chemotherapeutics, especially in view of metastasis and drug resistance development. Therefore, the development of novel anticancer agents has remained a major challenge in the field of cancer chemotherapy (1,2).

Five-membered heterocyclics are used as a pivotal scaffold of many compounds in medicinal chemistry due to their similarity with biologically active compounds within our

body and provide a wide range of biological activities such as anticonvulsant, anticancer, antimicrobial, and anti-inflammatory effects (2,3). In this family, azoles, including 1, 3, 4-oxadiazoles, 1, 3, 4-thiadiazoles and 1, 2, 4-triazoles have been involved as “privileged” construction in plentiful therapeutic areas particularly anticancer chemotherapy (2-9). Literature survey indicated that a minor alteration in the structure of 1,3,4-oxadiazoles, 1,2,4-triazoles and 1,3,4-thiadiazines can lead to changes in their biological activities (10).

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During recent years, some small molecules containing the aforementioned azoles were synthesized as potential anticancer agents (7,9,11-13). Structure-activity relationship studies have indicated that a basic scaffold of 1, 3, 4-oxadiazoles is necessary for the broad spectrum cytotoxic activity towards diverse cell lines (14). 1,3,4-Oxadiazole is bioisosteres of amides and esters, which can participate in hydrogen bonding interactions with the receptors (2,6). Mono and di-substituted 1, 3, 4-oxadiazoles have indicated remarkable antitumor activities (2). The anticancer mechanisms for oxadiazoles are related to the inhibition of different growth factors, enzymes, and kinases (2,14). Zhang *et al.* synthesized new 1,3,4-oxadiazole derivatives containing pyridine and acyl hydrazone moieties as potential telomerase inhibitors (15). Some of the compounds bearing 1, 2,4-triazole moiety are approved as anticancer drugs (7,16). Mercapto substituted 1,2,4-triazole have also shown antitumor properties (16).

The sulfur atom of thiadiazole (another important five-membered azole) can improve liposolubility of 1, 3, 4-thiadiazole which in turn leads to good tissue permeability (12). Some 1,3,4- thiadiazole derivatives have already been synthesized as kinase inhibitors with potential anticancer activity (17). Many differently substituted 1, 3, 4-thiadiazole and 1, 2, 4-triazole derivatives, in particular, 2-alkythio derivatives have shown anticancer activities (7,16,18,19).

On the other hand, phthalimides, have exhibited remarkable pharmacological effects, including anti-inflammatory, antimycobacterial, analgesic, anticonvulsant, and anticancer activities (20-23). Some derivatives of thiadiazole and triazole containing phthalimide pharmacophore were reported as anticancer agents (24-26). The synthesis of some derivatives of naphthalimide substituted triazole with a high affinity towards DNA has been reported. The enhanced cytotoxicity has been attributed to the presence of the triazole nucleus and planarity of naphthalimide (24). Kushwaha *et al.* prepared some of the different heterocycle substituted phthalimide derivatives including

1,3,4-thiadiazine, pyrazole, thiazole, and thiazoline with potent cytotoxic activities (25). In light of the therapeutic importance of these azoles and phthalimide, a series of novel hybrid molecules containing 1,3,4-oxadiazole, 1,3,4-thiadiazole, or 1,2,4-triazole bearing phthalimide moiety were synthesized and evaluated against two cancer cell lines. The combination of different heterocyclic with phthalimide may show a synergistic effect. The introduction of different azoles in the structure of the compound may increase the interaction of these molecules with the biological targets translating to enhanced therapeutic effect.

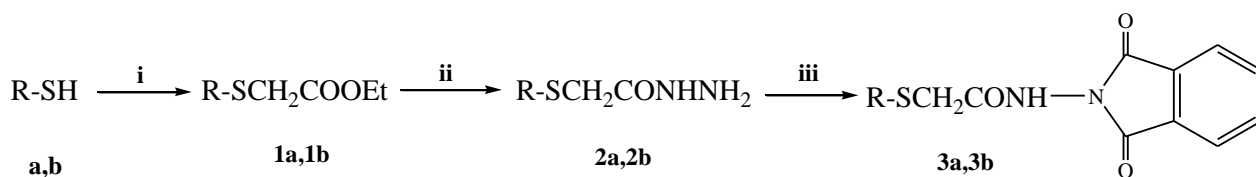
## MATERIALS AND METHODS

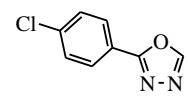

### Instrumentation

Reagents and starting materials were bought from commercial suppliers like Merck (Germany) and Aldrich (USA) companies. Thin-layer chromatography (TLC) was conducted on aluminum-based silica gel 60 F<sub>254</sub> plates (Germany). Proton nuclear magnetic resonance (HNMR) spectrometer (Bruker 400 MHz, Germany) was used for recording chemical shifts expressed as (ppm) with tetramethylsilane (TMS) as internal standard. Infrared (IR, KBr discs) was obtained with a WQF-510 Fourier-transform (FT)-IR spectrophotometer (China). Melting points was obtained using electrothermal 9200 melting point apparatus (England) and are uncorrected. Mass spectra were performed on Agilent Technologies 5975C mass spectrometer (USA).

### Synthesis of ethyl 2-(5-(4-chlorophenyl)-1, 3, 4-oxadiazol-2-ylthio) acetate (1a)

Compound 5-(4-chlorophenyl)-1, 3, 4-oxadiazole-2-thiol (0.02 mol) was dissolved in dry acetone (20 mL). Anhydrous potassium carbonates (0.02 mol) and ethyl chloroacetate (0.02 mol) were added to the above solution and the mixture was stirred for 2 h at room temperature. The reaction mixture was filtered off and the organic solution was concentrated *in vacuo* to give a crude product which was crystallized from methanol. (15,19) (Scheme 1).



Compounds	R
	a
	b

**Scheme 1.** Synthetic route for the preparation of compounds(3a and 3b). (i),  $\text{ClCH}_2\text{COOEt}$ . Acetone/ $\text{K}_2\text{CO}_3$ ; (ii),  $\text{NH}_2\text{NH}_2$ ; (iii) phthalic anhydride, glacial acetic acid, reflux.

#### Synthesis of ethyl 2-(4-methyl-4H-1, 3, 4-triazol-3-ylthio) acetate (1b)

Compound 4-methyl-4H-1, 2, 4-triazole-3-thiol (0.02 mol) was dissolved in dry acetone (10 mL). The mixture was basified by anhydrous potassium carbonates (0.02 mol) then, ethyl chloroacetate (0.02 mol) was added and the reaction contents were reflux for 24 h and monitored by TLC. The precipitates were collected through filtration and the organic solution was concentrated *in vacuo* to give a crude product which crystallized by chloroform (5,27,19) (Scheme 1).

#### Synthesis of 2-(5-(4-chlorophenyl)-1, 3, 4-oxadiazole-2-ylthio) acetohydrazide (2a)

The ester **1a** (0.01 mol) was dissolved in methanol (15 mL) and (0.1 mol) 80% hydrazine hydrate was added followed by stirring for 3-4 h at room temperature. TLC was processed to monitor the reaction completion. The product was acquired by the addition of excess cold water and separated by filtration, washed by n-hexane, and dried (28,29) (Scheme 1).

#### Synthesis of 2-(4-methyl-4H-1, 2, 4-triazol-3-ylthio) acetate (2b)

A mixture of the ester **1b** (0.01 mol) and hydrazine hydrate (0.02 mol) in ethanol (20

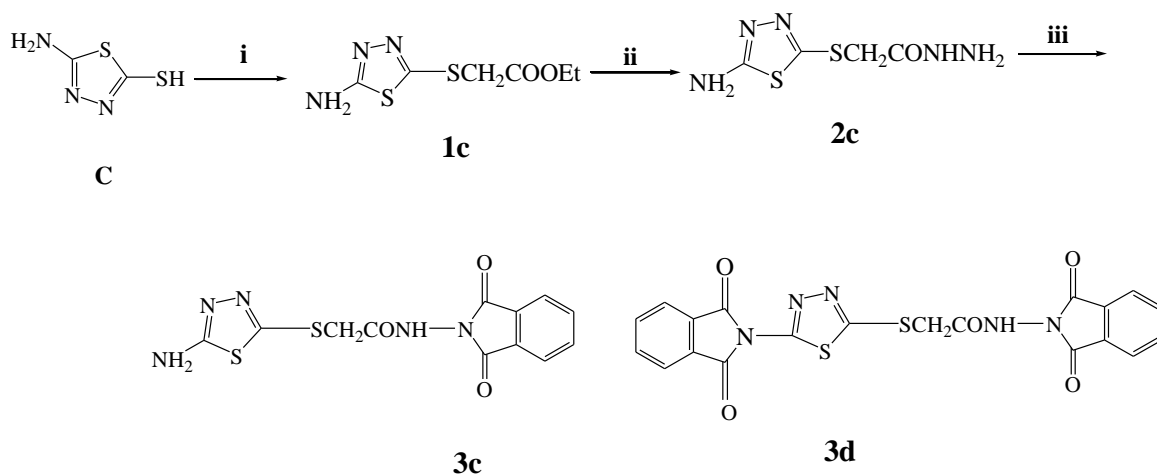
mL) was stirred at room temperature for 10 h and then filtered off (30)(Scheme 1).

#### Synthesis of (5-(4-chlorophenyl)-1, 3, 4-oxadiazole-2-ylthio)-N-(1, 3-dioxoisindolin-2-yl) acetamide and N-(1, 3-dioxoisindolin-2-yl)-2-(4-methyl-4h-1, 2, 4-triazol-3-ylthio) acetamide (3a,3b)

A mixture of the acid hydrazide (**2a** or **2b**; 0.01 mol), phthalic anhydride (0.02 mol) in glacial acetic acid (20 mL) was heated under reflux for 4-5 h (31). The precipitate was filtered and **3a** recrystallized from methanol and **3b** recrystallized from methanol, then petroleum ether/chloroform (2/1) (Scheme 1).

#### Synthesis of ethyl 2-(5-amino-1, 3, 4-thiadiazole-2-yl-thio) acetate (1c)

2-Amino-5-mercapto-1,3,4-thiadiazole (0.02 mol) was dissolved in absolute ethanol (20 mL) and treated with potassium hydroxide (0.02 mol). The mixture was stirred at room temperature for 15 min. Then, ethylchloroacetate (0.02 mol) was added and the mixture was refluxed for 2 h. After completion of the reaction, the solvent was evaporated under reduced pressure. Extraction was carried out with chloroform and water. Chloroform was evaporated and the remaining solid was recrystallized from ethanol and collected as white crystal (32,33) (Scheme 2).



**Scheme 2.** General procedure applied for the synthesis of the target compounds (**3c** and **3d**). (i),  $\text{ClCH}_2\text{COOEt}$ ,  $\text{KOH}$ , ethanol; (ii),  $\text{NH}_2\text{NH}_2$ ; (iii), phthalic anhydride, glacial acetic acid, reflux.

### Synthesis of 2-(5-amino-1, 3, 4-thiadiazole-2-ylthio) acetohydrazide (2c)

A mixture of compound **1c** (0.01 mol) and hydrazine hydrate (0.02 mol) in ethanol (20 mL) was refluxed for 3 h. The obtained precipitate was filtered, washed with cold water, dried and recrystallized from ethanol to get compound **2c** (22) (Scheme 2).

### Synthesis of 2-(5-amino-1,3,4-thiadiazole-2-ylthio)-N-(1,3-dioxisoindolin-2-yl)acetamide (3c)

A mixture of acid hydrazide (**2c**, 0.01 mol), phthalic anhydride (0.01 mol) in glacial acetic acid (20 mL) was heated under reflux for 4-5 h (31). The precipitate was filtered and recrystallized from ethanol then, petroleum ether/chloroform (2/1) (Scheme 2).

### Synthesis of N-(1, 3-dioxisoindolin-2-yl)-2-(5-(1, 3-dioxindolin-2-yl)-1,3,4-thiadiazol-2-ylthio)acetamide (3d)

A mixture of the acid hydrazide (**2c**, 0.01 mol), phthalic anhydride (0.02 mol) in glacial acetic acid (20 mL) was heated under reflux for 4-5 h. The obtained precipitate was filtered and crystallized from ethanol then, petroleum ether/chloroform (2/1) (31) (Scheme 2).

### Cytotoxicity assay

The cancerous cell lines; MCF-7 (breast cancer) and HeLa (cervical cancer), were received from Pasture Institute (Tehran, I.R. Iran) and were grown in Roswell Park

Memorial Institute (RPMI) 1640 which was supplemented with 100 units/mL penicillin, 100  $\mu\text{g/mL}$  streptomycin, and 5% v/v fetal bovine serum (FBS). After 2-3 subcultures, cells were cultured in a 96-well plate at a concentration of ( $5 \times 10^4$  cells/mL) and incubated for 24 h. Then, the cells were treated with various concentrations of the synthesized compounds. Paclitaxel (180  $\mu\text{g/mL}$ ) and dimethyl sulfoxide (DMSO, 1%) were regarded as the positive and negative controls, respectively.

Incubation was performed in a humidified atmosphere condition including 5%  $\text{CO}_2$  and 37  $^\circ\text{C}$  for 48 h. After 48 h exposure period, 20  $\mu\text{L}$  of MTT dye (5 mg/mL) was added to each well and incubated for another 3 h at the same condition. DMSO (150  $\mu\text{L}$  per well) was used to dissolve obtained formazan crystals and absorbance was recorded at 570 nm using an ELISA plate reader (34,35). 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:

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

where, MA is mean absorbance.

### Statistical analysis

The data are expressed as mean  $\pm$  SD. The experiments were accomplished in triplicate.

Analysis of variance (ANOVA) and Tukey post-hoc test was used to obtain the differences between the groups with negative control. A *P* value less than 0.05 was considered statistically different.

## RESULTS

### Chemistry

*Ethyl 2-(5-(4-Chlorophenyl)-1, 3, 4-oxadiazol-2-ylthio) acetate (1a)*

Yield: 80%, white solid, m.p. 83-85 °C (lit: 85-86 °C (36)); IR (KBr,  $\nu_{\max}$  cm<sup>-1</sup>): 2938 (C-H), 1738 (C=O), 1119 (C-O); <sup>1</sup>HNMR: (400 MHz; CDCl<sub>3</sub>):  $\delta$  7.88 (2H, d, *J* = 8 Hz, H-Ar), 7.40 (2H, d, *J* = 8 Hz, H-Ar), 4.19 (2H, q, *J* = 4 Hz, OCH<sub>2</sub>), 4.04 (2H, s, CH<sub>2</sub>), 1.23 (3H, t, *J* = 4 Hz, CH<sub>3</sub>).

*2-(5-(4-Chlorophenyl)-1, 3, 4-oxadiazole-2-ylthio)acetohydrazide (2a)*

Yield: 75%, white solid, m.p. 169-171 °C (lit: 178-180 °C (37)); IR (KBr,  $\nu_{\max}$  cm<sup>-1</sup>): 3299 (NH), 3300 (NH), 1685 (C=O).

*(5-(4-Chlorophenyl)-1, 3, 4-oxadiazole-2-ylthio)-N-(1, 3-dioxoisindolin-2-yl) acetamide (3a)*

Yield: 65%, white solid, m.p. 132-135 °C; IR (KBr,  $\nu_{\max}$  cm<sup>-1</sup>): 3295 (NH), 3050 (C-H, Ar), 2956 (C-H), 1736 (C=O); <sup>1</sup>HNMR: (400 MHz; DMSO-d<sub>6</sub>):  $\delta$  11.15 (1H, s, NHCO), 8.13-8.06 (4H, m, H-phthalic), 7.80 (2H, d, *J* = 8 Hz, H-Ar), 7.54 (2H, d, *J* = 8 Hz, H-Ar), 4.52 (2H, s, CH<sub>2</sub>), MS (m/z): 414 (M<sup>+</sup>); calculated 414.82 g/mol.

*N-(1, 3-dioxoisindolin-2-yl)-2-(4-methyl-4h-1, 2, 4-triazole-3-ylthio) acetamide (3b)*

Yield: 54%, white solid, m.p. 225 °C (decomposed); IR (KBr,  $\nu_{\max}$  cm<sup>-1</sup>): 3295 (NH), 1737, 1699 (C=O); <sup>1</sup>HNMR: (400 MHz; DMSO-d<sub>6</sub>): 11.04 (1H, s, NHCO), 8.60 (1H, s, H-triazole), 7.97-7.92 (4H, m, H-phthalic), 4.06 (2H, s, CH<sub>2</sub>), 3.61 (3H, s, CH<sub>3</sub>); MS (m/z): 317 (M<sup>+</sup>); calculated M.W. 317.2 g/mol.

*Ethyl 2-(5-amino-1, 3, 4-thiadiazole-2-yl-thio) acetate (1c)*

Yield: 57%, white solid, m.p. 82-83 °C (lit: 83-84 °C (38)); IR (KBr,  $\nu_{\max}$  cm<sup>-1</sup>): 3396

(NH<sub>2</sub>), 3280 (NH<sub>2</sub>), 2923 (C-H), 1735 (C=O), 1300 (C-O) cm<sup>-1</sup>.

*2-(5-Amino-1, 3, 4-thiadiazole-2-ylthio) acetohydrazide (2c)*

Yield: 53.78%, white solid, m.p. 150-151 °C (lit: 150-151 °C (39)); IR (KBr,  $\nu_{\max}$  cm<sup>-1</sup>): 3300, 3274, 3029 (NH<sub>2</sub>, NH), 1685 (C=N), 1648 (C=O) cm<sup>-1</sup>; <sup>1</sup>HNMR: (400 MHz; DMSO-d<sub>6</sub>): 9.28 (1H, s, NHCO), 7.32 (2H, s, NH<sub>2</sub>), 4.31 (2H, s, CH<sub>2</sub>), 3.71 (2H, s, NH<sub>2</sub>). MS (m/z): 205 (M<sup>+</sup>); calculated M.W. 205 g/mol.

*2-(5-Amino-1, 3, 4-thiadiazole-2-ylthio)-N-(1, 3-dioxoisindolin-2-yl) acetamide (3c)*

Yield: 30%, white solid, m.p. 265 °C (decomposed); IR (KBr,  $\nu_{\max}$  cm<sup>-1</sup>): 3300, 3274 (NH<sub>2</sub>, NH), 1745 (C=O), 8.15-8.13 (2H, m, H-phthalic), 8.09-8.06 (2H, m, H-phthalic), 7.71 (2H, s, NH<sub>2</sub>), 3.41 (2H, s, CH<sub>2</sub>); MS (m/z): 335 (M<sup>+</sup>), 292; calculated M.W. 335.3 g/mol.

*N-(1, 3-Dioxoisindolin-2-yl)-2-(5-(1, 3-dioxoisindolin-2-yl)-1, 3, 4-thiadiazol-2-ylthio) acetamide (3d)*

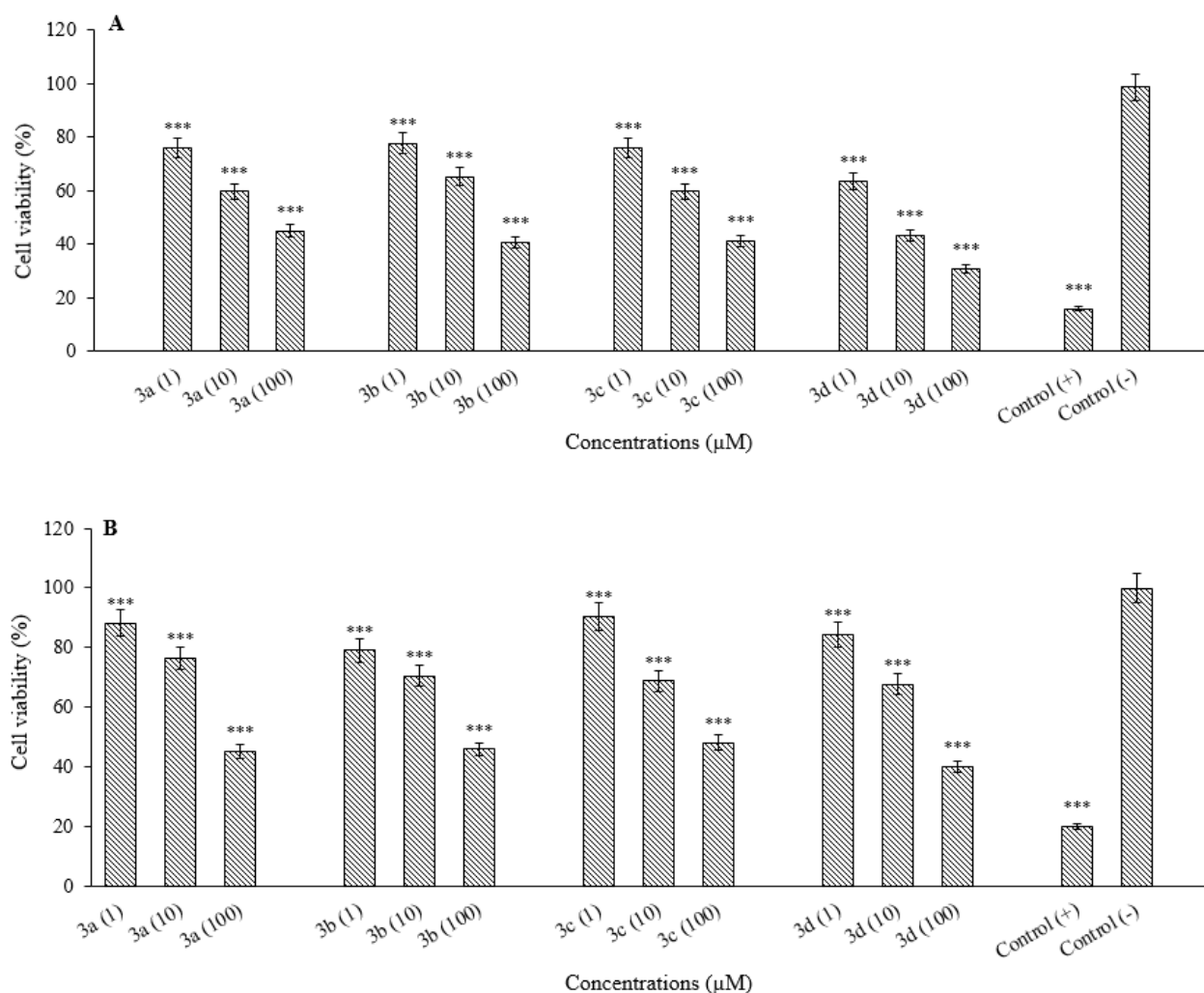
Yield: 50 %, white solid, m.p. 267 °C (decomposed); IR (KBr,  $\nu_{\max}$  cm<sup>-1</sup>): 3400 (NH), 3110 (C-H, Ar), 1785, 1749 (C=O); <sup>1</sup>HNMR: (400 MHz, DMSO-d<sub>6</sub>): 11.16 (1H, s, NHCO), 8.07-8.05 (2H, m, H-phthalic), 7.98-7.95 (6H, m, H-phthalic), 4.42 (2H, s, CH<sub>2</sub>); MS (m/z): 465 (M<sup>+</sup>), calculated M.W. 465 g/mol.

### Cytotoxic assay

Final compounds were assessed for their cytotoxic effects on two human cancer cell lines, MCF-7 and HeLa. Cytotoxic activity results were presented in Table 1 and Fig. 1. Significant differences (*P* ≤ 0.001) in viability compared to the negative control were observed on both cell lines for final compounds. None of the compounds were found as effective as paclitaxel.

**Table 1.** The IC<sub>50</sub> of the final compounds against MCF-7 and HeLa cell lines.

Compounds	IC <sub>50</sub> (μM) MCF-7	IC <sub>50</sub> (μM) HeLa
<b>3a</b>	87 ± 3.5	77 ± 3.3
<b>3b</b>	86 ± 3.9	71 ± 3.6
<b>3c</b>	92 ± 3.3	67 ± 4.1
<b>3d</b>	73 ± 3	29 ± 2.9
Paclitaxel	5.25-11.03 (μg/mL) 6.1 (μM)	7.76 (μg/mL) 9.08 (μM)



**Fig. 1.** Cytotoxic effect of compounds **3a-3c** on (A) HeLa and (B) MCF-7 following exposure different concentrations (1, 10, and 100  $\mu\text{M}$ ). Data are presented as mean  $\pm$  SD,  $n = 3$ . Paclitaxel was used as the positive control. \*\*\* $P \leq 0.001$  Indicate significant differences compared with the negative control group.

## DISCUSSION

In the light of reported cytotoxic activity of azoles (7,9,40), cyclic imides, and conjugated azole-imide (24,25), and in continuing efforts to develop potent cytotoxic agents, some phthalimide-based azoles including S- $\text{CH}_2\text{CONH}$ -linker were designed and prepared after exploring molecular hybridization approaches in a three-step procedure as depicted in Schemes 1 and 2. Molecular hybridization is one of the useful approaches in the design of new therapeutic compounds. One important consideration in hybrid compound design is the choice of a linker, as the nature of the linker and the distance between the pharmacophores can play an essential role (41). The choice of these structures was in accordance with the fact that

the conjugated azole-phthalimide and 2-alkythio derivatives of azoles (18) showed anticancer effects in the previous studies (24,25).

The thiol group of azoles was alkylated using ethyl chloroacetate. 2-Alkythio derivatives of azoles are important compounds used as key intermediates in organic and inorganic synthesis (36). Corresponding 2-alkythio derivatives of azoles were treated with hydrazine hydrate to provide acid hydrazide derivatives, which were followed by a reaction with phthalic anhydride to obtain final products. The formation of final compounds was confirmed by recording their IR,  $^1\text{H}$ NMR, and mass spectra. IR analysis of final compounds showed the peaks at 3295, 3300, 3274, and 3400  $\text{cm}^{-1}$ , which were due to the NH groups. The absorption bands at 1699,

1736, 1737, 1745, 1749, and 1785  $\text{cm}^{-1}$  were due to the C=O groups. The  $^1\text{HNMR}$  spectra of compounds in  $\text{DMSO-d}_6$  solvent showed a singlet peak around 11 ppm, which was attributed to the NH proton of amide groups. All protons belonging to the phthalic ring appeared at ranges of  $\delta$  8.15-7.92 ppm as multiple signals. The protons of the methylene linker appear as a singlet at ranges of 4.52-3.41 ppm. In compound **3a**, two doublet peaks at regions 7.80 and 7.54 ppm were assigned to protons of the Cl-phenyl ring attached to the oxadiazole ring.

In compound **3b**, the characteristic peak of the triazole was observed as a singlet at 8.60 ppm and one singlet peak corresponding to the three protons of methyl substitution appeared at 3.61.

All the final products were subjected for evaluation of cytotoxic effects against MCF-7 and HeLa cell lines using MTT-colorimetric assay. Cytotoxic results indicated that oxadiazole and triazole derivatives containing phthalimide moiety exhibited relatively similar cytotoxic activity against both cell lines.

The  $\text{IC}_{50}$  results rendered the thiadiazole derivatives **3c** and **3d** to be more effective against the HeLa cell line as compared to the other azoles in this study. The structure of intermediate **2c** allows realizing change in two directions of the thiadiazole ring. Thiadiazole derivative **3d** containing two phthalimide moieties in structure with  $\text{IC}_{50}$  value of 29  $\mu\text{M}$  and 73  $\mu\text{M}$  showed the highest activity against HeLa and MCF-7, respectively.

The presence of the second phthalimide in the structure of compound **3d** caused more lipophilic characteristics compared to the **3c** derivative which may be a logical reason for the improvement of its cytotoxic effect. In addition, it may enhance the interaction of this derivative with the biological targets.

$\text{IC}_{50}$  results determined that in these series of azole derivatives the type of heterocyclic ring plays an important role in developing the cytotoxic properties as seen in thiadiazole derivatives compared to oxadiazole and triazole.

According to the results, it can be inferred that the cytotoxic effects of the tested compounds were concentration-dependent and the compounds with higher concentrations had more cytotoxic effects. Tested compounds

exhibited significant differences in cell viability compared to the negative control on both cell lines which is presented in Fig. 1.

## CONCLUSION

In summary, the novel derivatives of five-membered azoles bearing phthalimide moiety were synthesized in three steps and their *in vitro* cytotoxic activities were assayed against MCF-7 and HeLa cell lines. Results of  $\text{IC}_{50}$  demonstrated that oxadiazole and triazole-phthalimide derivatives had the lowest cytotoxic against both cell lines and compound **3d** with two phthalimide moieties in structure exhibited much better cytotoxic activity against the HeLa cell line. It can be concluded that the HeLa cell line is more susceptible than MCF-7 to compound **3d**.

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## Conflict of interest statement

The authors declared no conflict of interest in this study.

## Authors' contribution

E. Jafari contributed to the conception and design of the work, conducting the study, analyzing the data, drafting and revising the manuscript. F. Hassanzadeh contributed to the conception of the work, analyzing the data, revising the manuscript. F. Shojaei performed the experiments and analyzing the data. H. Sadeghi-Aliabadi contributed to the conception of the work, conducting the study, revising the manuscript. All authors agreed with all aspects of the work.

## REFERENCES

1. Ramazani A, Khoobi M, Torkaman A, Nasrabadi FZ, Forootanfar 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.
2. 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.
3. Singh AK, Sahu VK, Yadav D. Biological activities of 2,5-disubstituted-1,3,4-oxadiazoles. *Int J Pharm Sci Res.* 2011;2(6):135-147.
  4. 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.
  5. Hassanzadeh F, Sadeghi-Aliabadi H, Jafari E, Sharifzadeh A, Dana N. Synthesis and cytotoxic evaluation 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.
  6. 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 (3*H*)-thione quinolone derivatives as novel anticancer agent. *Eur J Med Chem.* 2013; 60:23-28.  
DOI: 10.1016/j.ejmech.2012.11.039.
  7. Shi YJ, Song XJ, Li X, Ye TH, Xiong Y, Yu LT. Synthesis and biological evaluation of 1,2,4-triazole and 1,3,4-thiadiazole derivatives as potential cytotoxic agents. *Chem Pharm Bull (Tokyo).* 2013;61(11):1099-1104.  
DOI: 10.1248/cpb.c13-00242.
  8. Yang SJ, Lee SH, Kwak HJ, Gong YD. Regioselective synthesis of 2-amino-substituted 1,3,4-oxadiazole and 1,3,4-thiadiazole derivatives via reagent-based cyclization of thiosemicarbazide intermediate. *J Org Chem.* 2013;78(2):438-444.  
DOI: 10.1021/jo302324r.
  9. Waghmare S, Piste P. Pharmacological activities of triazole, oxadiazole and thiadiazole. *Int J Pharm Bioorg Sci.* 2013;4(3):310-332.
  10. Ahmad A, Varshney H, Rauf A, Sherwani A, Owais M. Synthesis and anticancer activity of long chain substituted 1, 3, 4-oxadiazol-2-thione, 1, 2, 4-triazol-3-thione and 1, 2, 4-triazolo [3, 4-b]-1, 3, 4-thiadiazine derivatives. *Arabian J Chem.* 2017;10:S3347-S3357.  
DOI: org/10.1016/j.arabjc.2014.01.015.
  11. El-Hamouly WS, Amin KM, El-Assaly SA, El-Meguid EAA. Synthesis and antitumor activity of some new 1,3,4-oxadiazole, pyrazole and pyrazolo [3,4-d] pyrimidine derivatives attached to 4-benzothiazol-2-yl phenyl moiety. *Der Pharma Chem.* 2011;3(6):282-292.
  12. Zhang K, Wang P, Xuan LN, Fu XY, Jing F, Li S, *et al.* Synthesis and antitumor activities of novel hybrid molecules containing 1,3,4-oxadiazole and 1,3,4-thiadiazole bearing Schiff base moiety. *Bioorg Med Chem Lett.* 2014;24(22):5154-5156.  
DOI: 10.1016/j.bmcl.2014.09.086.
  13. Bhinder CK, Kaur A. Review: 1,3,4-oxadiazole as anticancer agent. *Int J Pharm Res Sci.* 2014;3(4):457-479
  14. 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
  15. Zhang F, Wang XL, Shi J, Wang SF, Yin Y, Yang YS, *et al.* Synthesis, molecular modeling and biological evaluation of *N*-benzylidene-2-((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)thio) acetohydrazide derivatives as potential anticancer agents. *Bioorg Med Chem.* 2014;22(1):468-477.  
DOI: 10.1016/j.bmc.2013.11.004.
  16. Mavrova AT, Wesselinova D, Tsenov YA, Denkova P. Synthesis, cytotoxicity and effects of some 1,2,4-triazole and 1,3,4-thiadiazole derivatives on immunocompetent cells. *Eur J Med Chem.* 2009;44(1):63-69.  
DOI: 10.1016/j.ejmech.2008.03.006.
  17. Radi M, Crespan E, Botta G, Falchi F, Maga G, Manetti F, *et al.* Discovery and SAR of 1,3,4-thiadiazole derivatives as potent Abl tyrosine kinase inhibitors and cytodifferentiating agents. *Bioorg Med Chem Lett.* 2008;18(3):1207-1211.  
DOI: 10.1016/j.bmcl.2007.11.112.
  18. Aliabadi A, Eghbalian E, Kiani A. Synthesis and evaluation of the cytotoxicity of a series of 1, 3, 4-thiadiazole based compounds as anticancer agents. *Iran J Basic Med Sci.* 2013;16(11):1133-1138.  
PMID: 24494064.
  19. Hassanzadeh F, Sadeghi-Aliabadi 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.
  20. Jafari E, Taghi jarah-Najafabadi N, Jahanian-Najafabadi A, Poorirani S, Hassanzadeh F, Sadeghian-Rizi S. Synthesis and evaluation of antimicrobial activity of cyclic imides derived from phthalic and succinic anhydrides. *Res Pharm Sci.* 2017;12(6):526-534.  
DOI: 10.4103/1735-5362.217433.
  21. Hassanzadeh F, Jafari E. Cyclic imide derivatives: as promising scaffold for the synthesis of antimicrobial agents. *J Res Med Sci.* 2018;23:53-59.  
DOI: 10.4103/jrms.JRMS\_539\_17.
  22. Al-Azzawi AM, Hamd AS. Synthesis, characterization and antimicrobial activity evaluation of new cyclic imides containing 1,3,4-thiadiazole and 1,3,4-oxadiazole moieties. *Int J Res Pharm Chem.* 2013;3:890-897.
  23. Sondhi SM, Rani R, Roy P, Agrawal SK, Saxena AK. Microwave-assisted synthesis of *N*-substituted cyclic imides and their evaluation for anticancer and anti-inflammatory activities. *Bioorg Med Chem Lett.* 2009;19(5):1534-1538.  
DOI: 10.1016/j.bmcl.2008.07.048.
  24. Shankaraiah N, Kumar NP, Tokala R, Gayatri BS, Talla V, Santos LS. Synthesis of new 1,2,3-triazolo-naphthalimide/phthalimide conjugates via 'Click' reaction: DNA intercalation and cytotoxic studies. *J Braz Chem Soc.* 2019;30(3):454-461.  
DOI: 10.21577/0103-5053.20180111.



25. Kushwaha N, Kaushik D. Recent advances and future prospects of phthalimide derivatives. *J App Pharm Sci.* 2016;6(3): 159-171. DOI: 10.7324/JAPS.2016.60330.
26. Rezaei Z, Moghimi S, Javaheri R, Asadi M, Mahdavi M, Shabani S, et al. Synthesis and biological evaluation of 1,3,4-thiadiazole linked phthalimide derivatives as anticancer agents. *Lett Drug Des Discov.* 2017;14(10): 1138-1144. DOI: 10.2174/1570180814666170127164759.
27. Cavusoglu BK, Yurttaş L, Cantırk Z. The synthesis, antifungal and apoptotic effects of triazole-oxadiazoles against *Candida species*. *Eur J Med Chem.* 2018;144:255-261. DOI: 10.1016/j.ejmech.2017.12.020.
28. Rehman AU, Rasool S, Abbasi MA, Siddiqui SZ, Sattar A, Khan KM, et al. Synthesis, characterization and biological activity of some new *S*-substituted derivatives of 5-(2-chlorophenyl)-1,3,4-oxadiazol-2-thiol. *J Chem Soc Pak.* 2015;37(4):776-786.
29. Rasool S, Rehman AU, Abbasi MA, Siddiqui SZ, Shah SAA, Ahmad I, et al. Antibacterial and enzyme inhibition screening of some new acetamide and azomethine derivatives. *J Chil Chem Soc.* 2015;60(4):2704-2710. DOI: 10.4067/S0717-97072015000400014.
30. Popiolek Ł, Kosikowska U, Mazur L, Dobosz M, Malm A. Synthesis and antimicrobial evaluation of some novel 1,2,4-triazole and 1,3,4-thiadiazole derivatives. *Med Chem Res.* 2013;22(7): 3134-3147. DOI: 10.1007/s00044-012-0302-9.
31. Kadi AA, El-Azab AS, Alafeefy AM, Abdel-Hamide SG. Synthesis and biological screening of some new substituted 2-Mercapto-4-(3*H*)-quinazolinone analogs as anticonvulsant agents. *Az J Pharm Sci.* 2006;34:135-155.
32. Alwan SM. Synthesis and preliminary antimicrobial activities of new arylideneamino-1,3,4-thiadiazole-(thio/dithio)-acetamido cephalosporanic acids. *Molecules.* 2012;17(1):1025-1038. DOI: 10.3390/molecules17011025.
33. Baghel US, Kaur H, Chawla A, Dhawan RK. Synthesis and antimicrobial evaluation of thiadiazole derivatives. *Der Pharma Chem.* 2014;6(2):66-69.
34. Hassanzadeh F, Jafari E, Hakimelahi GH, Rahmani Khajouei M, Jalali M, Khodarahmi GA. Antibacterial, antifungal and cytotoxic evaluation of some new quinazolinone derivatives. *Res Pharm Sci.* 2012;7(2):87-94.
35. Khodarahmi GA, Rahmani Khajouei M, Hakimelahi GH, Abedi D, Jafari E, Hassanzadeh F. Antibacterial, antifungal and cytotoxic evaluation of some new 2,3-disubstituted 4(3*H*)-quinazolinone derivatives. *Res Pharm Sci.* 2012;7(3):151-158.
36. Soleiman-Beigi M, Alikarami M, Hosseinzadeh T. One-pot synthesis of 2-alkylthio-1,3,4-oxadiazole and bis-(1,3,4-oxadiazole-2-yl)thio alkyl derivatives from acid hydrazides and CS<sub>2</sub>. *Asian J Chem.* 2013;25(9):4939-4942. DOI: 10.14233/ajchem.2013.14148.
37. Rasool S, Rehman A, Abbasi MA, Gul S, Akhtar MN, Ahmad I, et al. Synthesis of *N*-Substituted-2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide derivatives as suitable antibacterial agents. *Trop J Pharm Res.* 2015;14(6):1081-1088. DOI: 10.4314/tjpr.v14i6.21.
38. Rad O, Tibor R, Duma M, Vlase L, Pîrnău A, Tîpceriuc B, et al. Synthesis and antimicrobial activity of some 5-amino-2-mercapto-1,3,4-thiadiazole derivatives thioethers and Schiff bases. *Studia Ubb Chemia.* 2016;61:17-32.
39. Hassanzadeh F, Jafari E, Saeedi M, Saberi S. Synthesis and evaluation of thiadiazole-based antileishmanial agents. *J Rep Pharm Sci.* 2020;9(2):189-195. DOI: 10.4103/jrptps.JRPTPS\_3\_20.
40. Hassanzadeh F, Jafari E, Zarabi M, Khodarahmi G, Vaseghi G. Synthesis, cytotoxic evaluation, and molecular docking studies of some new 1,3,4-oxadiazole-based compounds. *Res Pharm Sci.* 2020;15(5):454-462. DOI: 10.4103/1735-5362.297848.
41. Sunil RJ, Pal S, Jayashree A. Molecular hybridization-an emanating tool in drug design. *Med Chem.* 2019;9(6):93-95.