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One-pot, four-component synthesis of novel cytotoxic agents 1-(5-aryl-1,3,4-oxadiazol-2-yl)-1-(1*H*-pyrrol-2-yl)methanamines

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ABSTRACT

A series of *N*-benzyl-1-(5-aryl-1,3,4-oxadiazol-2-yl)-1-(1*H*-pyrrol-2-yl)methanamines were synthesized via one-pot reaction of appropriate benzylamine, pyrrole-2-carbaldehyde, (*N*-isocyanimino)triphenylphosphorane, and a carboxylic acid. The anti-tumor potential of title compounds was tested against several cancer cell lines by using MTT assay. Some tested compounds including **5e**, **5p** and **5q** exhibited comparable or better cytotoxic activity against A549, HT29 or HT1080 cells in comparison to the reference drug doxorubicin. Also, the cytotoxic activity of compounds **5d** and **5n** against MCF-7 was better than that of doxorubicin. Compound **5n** with IC_{50} value of 4.3 μM was 4-fold more potent than doxorubicin. The structure–activity relationship study revealed that the introduction of halogen atoms on both 5-phenyl ring and *N*-benzyl part improved the cytotoxic activity against all tested cell lines.

1. Introduction

Currently cancer is a main cause of death worldwide. WHO proposed about 17 million cancer deaths per year with available cancer therapies by 2030 [1]. Despite many efforts to combat cancer, the success for treating the certain types of neoplasm has shown little progress due their metastasis and aggressiveness [2]. Among the existing cancer therapies, chemotherapy is the most significant treatment in cancer management [3]. Although several drugs have been developed for management of cancer, but most of them show no selectivity to the neoplastic cells, associated with reduced bioavailability, toxicity and drug-resistance [4–6]. Therefore, the development of novel anticancer agents remains a major challenge in the field of cancer chemotherapy.

The five-membered ring 1,3,4-oxadiazole is an important heterocycle which plays a significant role in organic synthesis [7] and medicinal chemistry, especially in the field of antimicrobial agents and chemotherapy. 1,3,4-Oxadiazole derivatives showed broad spectrum of bioactivities such as antiviral [8], antimicrobial [9,10], anti-tubercular [11], antifungal [12], and anticancer [13–17].

A series of 2,5-disubstituted-1,3,4-oxadiazoles have been reported by Ouyang et al. [18], and Tuma and co-workers [19] as tubulin polymerization inhibitors. *In vitro* studies indicated that compound **1** (Fig. 1) which interrupts mitotic division in breast carcinoma and squamous cell tumors were more effective than the taxane paclitaxel. Moreover, 1,3,4-oxadiazole derivatives possessing 1,4-benzodioxan moiety were described as potential anticancer agents [20]. Among the synthesized compounds, compound **2** exhibited good inhibitory activity against telomerase. Recently, Liu et al. reported the antiproliferative and EGFR inhibition properties

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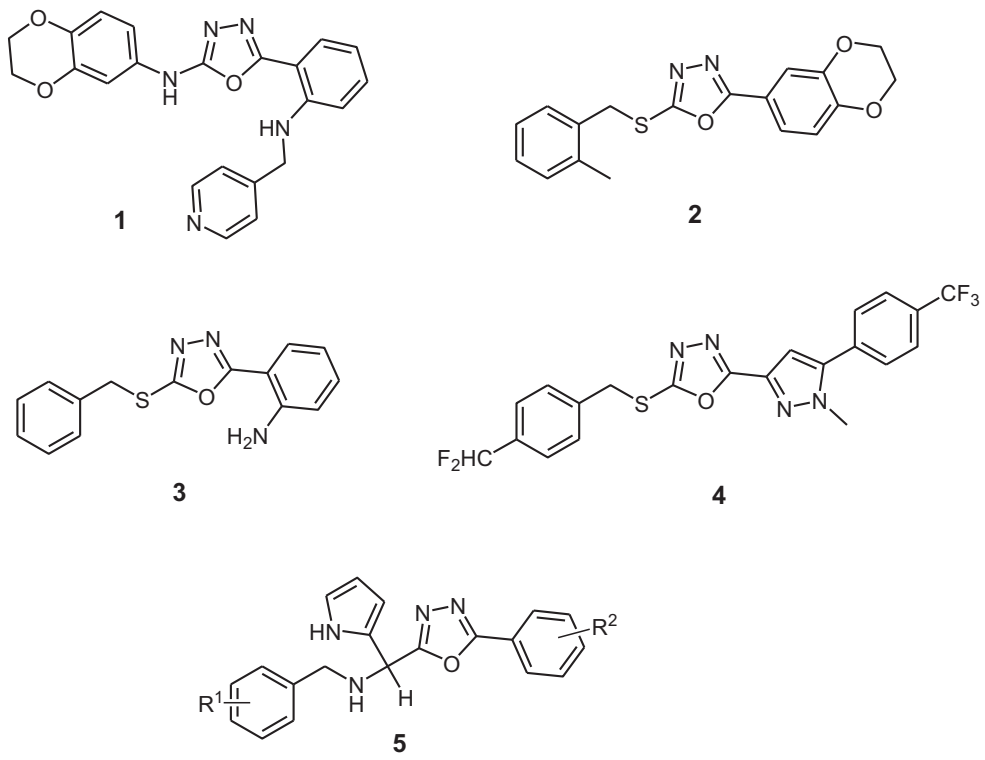


Fig. 1. Compounds **1**–**4** with 2,5-disubstituted 1,3,4-oxadiazole structure were reported as cytotoxic agents. Compounds **5** were designed as new potential cytotoxic agents.

of a series of 2-(benzylthio)-5-aryloxadiazole derivatives exemplified by compound **3** [21]. In a study by Puthiyapurayil et al., 1,3,4-oxadiazole **4** bearing *N*-methyl-4-(trifluoromethyl)phenyl pyrazole moiety was introduced as cytotoxic agent [22].

In continuation of our previous works in order to synthesize novel heterocyclic compounds [23], herein we synthesize a new series of 1,3,4-oxadiazoles **5** (Fig. 1) as cytotoxic agents.

2. Chemistry

The target compounds **5** were prepared by using one-pot, four-component reaction in CH_2Cl_2 (Scheme 1) [23]. The 1:1 imine intermediate generated by the condensation reaction of appropriate benzylamine **6** with pyrrole-2-carbaldehyde (**7**), was trapped by (*N*-isocyanimino)triphenylphosphorane (**8**) in the presence of benzoic acid derivatives **9** and led to the formation of 1,3,4-oxadiazole derivatives **5**. The reaction proceeds smoothly and cleanly under mild and neutral conditions and no side reactions were observed.

The structures of the products **5** were deduced from their IR, ^{1}H NMR, ^{13}C NMR spectra, mass spectrometry and elemental analysis. For example, The ^{1}H NMR spectrum of **5a** consists of a singlet for the NH proton (δ = 2.31 ppm), a singlet for the CH_2N group (δ = 3.85 ppm), a singlet for the CH proton (δ = 5.34 ppm), two multiplet for the protons of pyrrole (δ = 6.13–6.18 and 6.80–6.84 ppm), a singlet for the NH proton of pyrrole (δ = 9.02 ppm), and multiplet for the aromatic protons (δ = 7.27–8.03 ppm). The ^{1}H decoupled ^{13}C NMR spectrum of **5a** shows 16 distinct resonances, the assignment of these signals is given in the experimental section. The ^{1}H and ^{13}C NMR spectra of compounds **5b**–**q** are similar to those of compound **5a**, except for the aromatic parts, which exhibit characteristic signals with appropriate chemical shifts.

3. Pharmacology

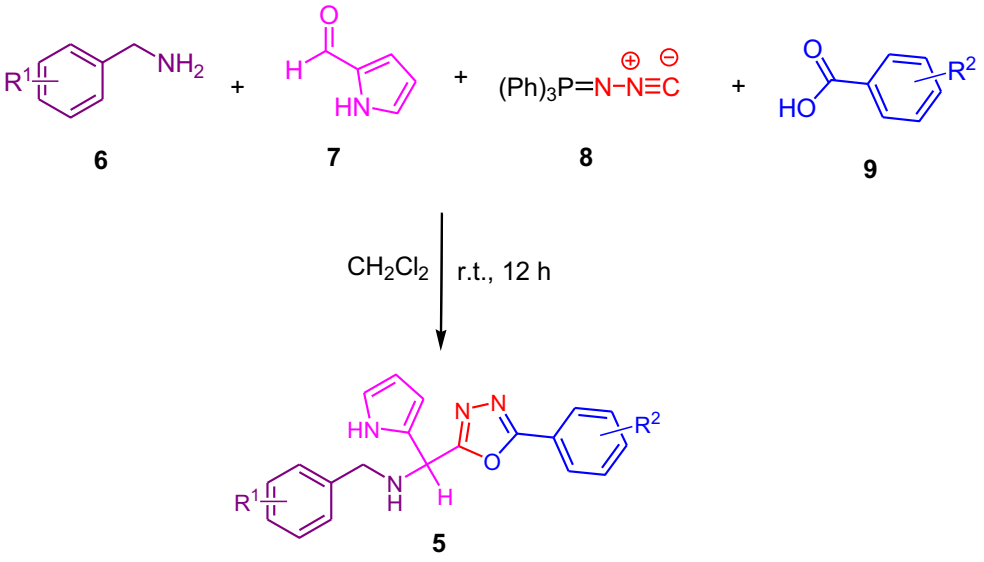
The *in vitro* effect of synthesized compounds **5a**–**q** on cancer cell viability was assessed using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay [24] against A549 (human alveolar basal epithelial adenocarcinoma), HT29 (human colorectal adenocarcinoma), HT1080 (human fibrosarcoma) and MCF-7 (human breast adenocarcinoma) cell lines. The percent of inhibition for each concentration of the compounds was calculated comparing to the control group. The IC_{50} of each compound was then determined using the achieved dose-percent of inhibition curve. Each experiment was repeated three times and the results were reported as mean ± SD (in μM).

4. Results and discussion

The cytotoxic activities of target compounds were summarized in Table 1 as IC_{50} values of tested compounds.

In general, some compounds showed good activity against tested cell lines. The IC_{50} values of compounds against A549 cell line demonstrated that compound **5p** with IC_{50} value of 13.3 μM showed the highest activity comparable to standard drug doxorubicin. Moreover, compounds **5d**, **5e** and **5q** exhibited good cytotoxic activity against A549 cells. Compound **5p** followed by **5e** and **5q** with IC_{50} values of 18–20 μM were the most active compounds against HT29 cells. Compounds **5d** and **5o** possessing IC_{50} values of 15.2 and 14.7 μM against HT1080 had comparable inhibitory activity in respect to doxorubicin. The most active compound against MCF-7 was **5n** (IC_{50} = 4.3 μM). It was 4-fold more potent than doxorubicin. Also, the cytotoxic activity of compound **5d** against MCF-7 cells was better than that of doxorubicin.

A survey on IC_{50} values of compounds **5a**–**f** against all tested cell lines revealed that compounds **5b**–**f** containing halogen



Scheme 1. One-pot four-component synthesis of 1,3,4-oxadiazoles **5**.

substituent on 5-phenyl ring were most potent than unsubstituted analog **5a**. Thus, the substitution of halogen on 5-phenyl ring significantly increased the cytotoxic activity against all tested cell lines. The observed results of 3- or 4-chloro derivatives (**5b** or **5c**) and 3,4-dichloro compound **5d** revealed that the introduction of second chlorine atom on phenyl ring was favorable for cytotoxic activity.

Although the regioisomers **5b** and **5c** showed same activity against HT1080 cells but in the cases of A549 and HT29 cells, 3-chloro derivative **5b** was more active than 4-chloro analog **5c**. In contrast, the cytotoxic activity of 4-chloro isomer **5c** against MCF-7 was higher than that of 3-chloro derivative **5b**. These findings indicated that the displacement of chlorine on 5-phenyl ring had different effect on activity depending on the type of tested cell line. By comparing the IC_{50} values of 4-chloro and 4-bromo derivatives (**5c** and **5f**, respectively), it was revealed that 4-chloro substitution was more favorable than 4-bromo group against all cell lines. In the case of methyl substituent on 5-phenyl ring, the 3-metyl analog **5h** displayed better activity against all cell lines respect to the 4-methyl isomer **5g**. The replacement of 4-methyl group with 4-methoxy substituent (**5i** vs. **5g**) improved the cytotoxic activity against all cancer cells with the exception of HT29.

The comparison of cytotoxic activities of compounds **5g**–**j** to those of unsubstituted compound **5a** demonstrated that the substitution of methyl or methoxy group on phenyl ring did not improve the activity. The introduction of 4-methoxy group on benzylamine moiety had positive or negative effect on cytotoxic activity, depending on the type of cell line (compound **5l** vs. **5a**). The substitution of 3-chlorophenyl in 4-methoxybenzylamine derivative **5l** resulted in more active compound **5m**. The cytotoxic activities of halo-substituted benzyl derivatives **5o**–**q** were better than those of corresponding non-halogenated analogs. Therefore, the insertion of halogen atom on benzyl group improved the cytotoxicity towards all tested cell lines. The observed IC_{50} values of 3-chlorophenyl derivatives (**5b**, **5m** and **5p**) demonstrated that the 3,4-dichlorobenzyl pendent group is more favorable. 4-Methoxybenzyl derivative **5l** showed better activity compared to the 2-methoxybenzyl isomer **5k**.

The IC_{50} values of tested compounds against different cell lines revealed that the relative susceptibilities of cell lines to each compound had a variable pattern. For example, while the MCF-7 is more susceptible to the compounds **5d**, and **5l**–**n**, but this cell line is more resistant towards **5f**–**k**.

5. Conclusion

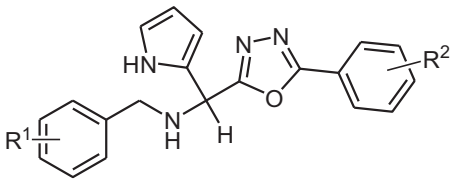
We have synthesized a series of *N*-benzyl-1-(5-aryl-1,3,4-oxadiazol-2-yl)-1-(1*H*-pyrrol-2-yl)methanamines via one-pot, four-component reaction. We believe that the reported method offers a mild, simple, and efficient route for the preparation of the 2,5-disubstituted 1,3,4-oxadiazole prototype **5**. The designed compounds showed good anti-proliferative activity against different cell lines. Some tested compounds including **5p**, **5e** and **5q** exhibited comparable or better cytotoxic activity against A549, HT29 or HT1080 cells in respect to the reference drug doxorubicin. Also, the cytotoxic activity of compounds **5d** and **5n** against MCF-7 were better than that of doxorubicin. Compound **5n** with IC_{50} value of 4.3 μM was 4-fold more potent than doxorubicin. The alteration of substituents on 5-phenyl ring and *N*-benzyl part revealed that the introduction of halogen atoms on both units improved the cytotoxic activity against all tested cell lines.

6. Experimental protocols

6.1. General chemistry

(*N*-Isocyanimino)triphenylphosphorane (**8**) was prepared based on reported procedure [25]. Other starting materials and solvents were obtained from Merck (Germany) and Fluka (Switzerland) and were used without further purification. Melting points were measured on an Electrothermal 9100 apparatus and are uncorrected. IR spectra were measured on a Jasco 6300 FTIR spectrometer. ^1H and ^{13}C NMR spectra ($CDCl_3$) were recorded on a BRUKER DRX-250 AVANCE spectrometer at 250.1 and 62.9 MHz, and a BRUKER AVANCE III spectrometer at 400.2 and 100.6 MHz, respectively. Elemental analyses were performed using a Heraeus CHN–O-Rapid analyzer. Mass spectra were recorded on a Finnigan MAT 8430 mass spectrometer operating at an ionization potential of 70 eV. Preparative layer chromatography (PLC) plates were prepared from Merck silica gel (F_{254}) powder.

Table 1
Cytotoxic activity (IC_{50}, μM)[a] of target compounds **5a**–**q** against different cancer cell lines in comparison with standard drug doxorubicin.



Compound	R^1	R^2	A549	HT29	HT1080	MCF-7
5a	H	H	187.0 ± 6.3	198.1 ± 13.5	231.0 ± 9.0	216.4 ± 13.6
5b	H	3-Cl	62.2 ± 3.1	48.3 ± 2.4	41.1 ± 2.4	56.7 ± 2.6
5c	H	4-Cl	73.8 ± 2.7	54.5 ± 2.0	41.0 ± 1.0	46.2 ± 2.2
5d	H	3,4-Cl_2	25.5 ± 0.7	41.7 ± 1.9	15.2 ± 0.5	13.9 ± 0.6
5e	H	2,4-Cl_2-5-F	27.5 ± 0.8	20 ± 0.7	25.6 ± 0.9	21.7 ± 1.0
5f	H	4-Br	87.2 ± 4.1	58.3 ± 5.0	70.6 ± 3.1	96.6 ± 4.1
5g	H	4-Me	744.5 ± 36.3	248.7 ± 12.0	909.3 ± 44.1	1118.2 ± 53
5h	H	3-Me	430.5 ± 11.3	201.5 ± 3.5	311.1 ± 15.2	480.3 ± 10.8
5i	H	4-OMe	326.5 ± 13.9	343.8 ± 16.7	169.5 ± 8.4	460.5 ± 13.9
5j	H	2,4-$(OMe)_2$	296.5 ± 8.8	392.4 ± 11.9	418.5 ± 12.9	471.0 ± 9.4
5k	2-OMe	H	362.5 ± 11.8	392.4 ± 9.8	278.5 ± 8.9	441.1 ± 10.1
5l	4-OMe	H	277.5 ± 8.8	285.6 ± 9.7	174.6 ± 7.9	141.6 ± 6.1
5m	4-OMe	3-Cl	77.5 ± 1.8	95.6 ± 2.7	84.6 ± 0.9	41.7 ± 3.0
5n	4-Me	4-Br	53.94 ± 0.5	49.5 ± 2.2	51.9 ± 2.2	4.3 ± 1.8
5o	4-F	4-OMe	52.8 ± 2.6	39.4 ± 1.8	14.7 ± 0.7	28.9 ± 1.3
5p	3,4-Cl_2	3-Cl	13.3 ± 0.8	18 ± 0.9	25.1 ± 1.8	19.0 ± 3.4
5q	3,4-Cl_2	4-Br	17.3 ± 0.8	20 ± 0.4	45.3 ± 2.9	31.0 ± 2.1
Doxorubicin			13.6 ± 0.5	16.0 ± 0.6	15.4 ± 0.6	16.9 ± 0.6

[a] Each experiment was repeated three times and the results were reported as mean ± SD.

6.2. General procedure for the preparation of compounds ***5a–q***

To a magnetically stirred solution of appropriate benzylamine **6** (1 mmol), pyrrole-2-carbaldehyde **7** (1 mmol) and (*N*-isocyanimino)triphenylphosphorane **8** (1 mmol) in CH_2Cl_2 (5 mL) was added dropwise a solution of benzoic acid derivatives **9** (1 mmol) in CH_2Cl_2 (5 mL) over 15 min. The mixture was stirred at room temperature for 12 h. Then, the solvent was removed under reduced pressure, and the viscous residue was purified by preparative thin layer chromatography (PTLC) (silica gel F_{254}), eluting with petroleum ether–ethyl acetate (4:1).

6.2.1. *N-Benzyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)-1-(1H-pyrrol-2-yl)methanamine **(5a)***

Brown powder; yield: 85%; m.p. 103–105 °C; IR (KBr): 3299, 2923, 2852, 1552, 1450, 705 cm^{-1}; ^1H NMR (250.0 MHz, $CDCl_3$): δ 2.31 (1H, s, NH amine), 3.85 (2H, s, CH_2), 5.34 (1H, s, CH), 6.13–6.18 (2H, m, 2CH pyrrole), 6.80–6.84 (1H, m, CH pyrrole), 7.27–8.03 (10H, m, CH Ar), 9.02 (1H, s, NH pyrrole). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 51.18, 51.77, 107.79, 108.59, 118.57, 126.98, 127.37, 128.31, 128.56, 129.00, 131.80, 123.70, 127.03, 138.93, 165.09, 166.12. Anal. Calcd for $C_{20}H_{18}N_4O$: C, 72.71; H, 5.49; N, 16.96; Found: C, 72.56; H, 5.67; N, 16.73.

6.2.2. *N-Benzyl-1-[5-(3-chlorophenyl)-1,3,4-oxadiazol-2-yl]-1-(1H-pyrrol-2-yl)methanamine **(5b)***

Brown powder; yield: 90%; m.p. 81–83 °C; IR (KBr): 3416, 2922, 2852, 1637, 1122, 613 cm^{-1}; ^1H NMR (250.0 MHz, $CDCl_3$): δ 2.20 (1H, s, NH amine), 3.87 (2H, s, CH_2), 5.36 (1H, s, CH), 6.19–6.21 (2H, m, 2CH pyrrole), 6.85 (1H, dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 4.4 Hz, CH pyrrole), 7.28–8.02 (9H, m, CH Ar), 8.97 (1H, s, NH pyrrole). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 51.24, 51.79, 107.90, 108.69, 118.69, 125.10, 126.98, 127.46, 128.34, 128.62, 130.41, 131.90, 125.30, 126.77, 135.20, 138.79, 164.00, 166.43. Anal. Calcd for $C_{20}H_{17}ClN_4O$: C, 65.83; H, 4.70; N, 15.36; Found: C, 65.54; H, 5.08; N, 15.14.

6.2.3. *N-Benzyl-1-[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]-1-(1H-pyrrol-2-yl)methanamine **(5c)***

Brown powder; yield: 83%; m.p. 129–131 °C; IR (KBr): 3416, 2922, 2852, 1608, 1484, 1094 cm^{-1}; ^1H NMR (250.0 MHz, $CDCl_3$): δ 2.21 (1H, s, NH amine), 3.87 (2H, s, CH_2), 5.35 (1H, s, CH), 6.19–6.21 (2H, m, 2CH pyrrole), 6.85 (1H, dd, $^4J_{HH}$ = 2.0 Hz, $^3J_{HH}$ = 4.0 Hz, CH pyrrole), 7.27–7.37 (5H, m, CH Ar), 7.49 (2H, d, $^3J_{HH}$ = 8.4 Hz, CH Ar), 7.97 (2H, d, $^3J_{HH}$ = 8.4 Hz, CH Ar), 8.98 (1H, s, NH pyrrole). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 51.22, 51.77, 107.87, 108.67, 118.66, 127.45, 128.27, 128.33, 128.61, 129.44, 122.16, 126.85, 138.16, 138.83, 164.33, 166.27. Anal. Calcd for $C_{20}H_{17}ClN_4O$: C, 65.84; H, 4.70; N, 15.36; Found: C, 66.71; H, 4.59; N, 15.49.

6.2.4. *N-Benzyl-1-[5-(3,4-dichlorophenyl)-1,3,4-oxadiazol-2-yl]-1-(1H-pyrrol-2-yl)methanamine **(5d)***

Brown powder; yield: 87%; m.p. 144–146 °C; IR (KBr): 3416, 2922, 2852, 1634, 1455, 729 cm^{-1}; ^1H NMR (400.2 MHz, $CDCl_3$): δ 2.49 (1H, s, NH amine), 3.87 and 3.95 (2H, ABq, $^2J_{HH}$ = 13.2 Hz, CH_2), 5.41 (1H, s, CH), 6.21 (2H, s, 2CH pyrrole), 6.88 (1H, s, CH pyrrole), 7.29–8.13 (8H, m, CH Ar), 9.13 (1H, s, NH pyrrole). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 51.25, 51.78, 107.92, 108.70, 118.74, 126.00, 127.47, 128.32, 128.62, 128.69, 131.25, 123.45, 126.45, 133.68, 136.37, 138.75, 163.33, 166.57. Anal. Calcd for $C_{20}H_{16}Cl_2N_4O$: C, 60.16; H, 4.04; N, 14.03; Found: C, 60.32; H, 4.16; N, 14.36.

6.2.5. *N-Benzyl-1-[5-(2,4-dichloro-5-fluorophenyl)-1,3,4-oxadiazol-2-yl]-1-(1H-pyrrol-2-yl)methanamine **(5e)***

Brown powder; yield: 86%; m.p. 107–109 °C; IR (KBr): 3417, 2922, 2851, 1634, 1457, 1085, 730 cm^{-1}; ^1H NMR (400.2 MHz, $CDCl_3$): δ 2.33 (1H, s, NH amine), 3.85 and 3.89 (2H, ABq, $^2J_{HH}$ = 13.6 Hz, CH_2), 5.39 (1H, s, CH), 6.18–6.21 (2H, m, 2CH pyrrole), 6.85 (1H, dd, $^4J_{HH}$ = 2.8 Hz, $^3J_{HH}$ = 5.0 Hz, CH pyrrole), 7.28–7.38 (3H, m, CH Ar), 7.64 (2H, d, $^3J_{HH}$ = 8.4 Hz, CH Ar), 7.80 (2H, d, $^3J_{HH}$ = 8.4 Hz, CH Ar), 9.01 (1H, s, NH pyrrole). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 51.26, 51.78, 107.96, 108.68, 118.76, 127.46, 128.33, 128.61,

133.01, 118.46 (d, $^2J_{CF}$ = 24.9 Hz, CH), 125.50, 126.56, 138.77, 140.96, 156.58 (d, $^1J_{CF}$ = 249.7 Hz, C), 161.93, 166.98. Anal. Calcd for $C_{20}H_{15}Cl_2FN_4O$: C, 57.57; H, 3.62; N, 13.43; Found: C, 57.33; H, 3.81; N, 13.64.

*6.2.6. N-Benzyl-1-[5-(4-bromophenyl)-1,3,4-oxadiazol-2-yl]-1-(1H-pyrrol-2-yl)methanamine **(5f)***

Brown powder; yield: 87%; m.p. 139–141 °C; IR (KBr): 3299, 2923, 2852, 1552, 1450, 705 cm^{-1}; ^1H NMR (250.1 MHz, $CDCl_3$): δ 2.41 (1H, s, NH amine), 3.83 (2H, s, CH_2), 5.32 (1H, s, CH), 6.18 (2H, s, 2CH pyrrole), 6.82–6.83 (1H, m, CH pyrrole), 7.27–7.32 (5H, m, CH Ar), 7.61 (2H, d, $^3J_{HH}$ = 8.5 Hz, CH Ar), 7.32 (2H, d, $^3J_{HH}$ = 8.5 Hz, CH Ar), 9.15 (1H, s, NH pyrrole). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 51.17, 51.74, 107.91, 108.57, 118.77, 127.42, 128.36, 128.59, 132.36, 122.51, 126.56, 126.81, 138.80, 164.39, 166.33. Anal. Calcd for $C_{20}H_{17}BrN_4O$: C, 58.69; H, 4.19; N, 13.69; Found: C, 59.37; H, 4.30; N, 13.51.

*6.2.7. N-Benzyl-1-[5-(4-methylphenyl)-1,3,4-oxadiazol-2-yl]-1-(1H-pyrrol-2-yl)methanamine **(5g)***

Brown powder; yield: 89%; m.p. 107–109 °C; IR (KBr): 3415, 2922, 2852, 1617, 1499, 1120, 728 cm^{-1}; ^1H NMR (250.1 MHz, $CDCl_3$): δ 2.20 (1H, s, NH amine), 2.45 (CH_3), 3.86 (2H, s, CH_2), 5.35 (1H, s, CH), 6.19–6.20 (2H, m, 2CH pyrrole), 6.85 (1H, dd, $^4J_{HH}$ = 2.0 Hz, $^3J_{HH}$ = 4.0 Hz, CH pyrrole), 7.26–7.36 (5H, m, CH Ar), 7.31(2H, d, $^3J_{HH}$ = 8.0 Hz, CH Ar), 7.92 (2H, d, $^3J_{HH}$ = 8.0 Hz, CH Ar), 9.04 (1H, s, NH pyrrole). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 21.66, 51.18, 51.76, 107.81, 108.58, 118.57, 126.97, 127.40, 128.36, 128.59, 129.73, 120.92, 127.07, 138.93, 142.42, 165.27, 165.83. Anal. Calcd for $C_{21}H_{20}N_4O$: C, 73.23; H, 5.85; N, 16.27; Found: C, 73.51; H, 5.56; N, 16.51.

*6.2.8. N-Benzyl-1-[5-(3-methylphenyl)-1,3,4-oxadiazol-2-yl]-1-(1H-pyrrol-2-yl)methanamine **(5h)***

Brown powder; yield: 89%; m.p. 99–101 °C; IR (KBr): 3445, 2922, 2852, 1635, 1619, 1551, 699 cm^{-1}; ^1H NMR (400.2 MHz, $CDCl_3$): δ 2.44 (3H, s, CH_3), 2.54 (1H, s, NH amine), 3.87 (2H, s, CH_2), 5.38 (1H, s, CH), 6.20–6.21 (2H, m, 2CH pyrrole), 6.86 (1H, dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 4.4 Hz, CH pyrrole), 7.27–7.86 (9H, m, CH Ar), 9.18 (1H, s, NH pyrrole). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 29.72, 51.19, 51.76, 107.79, 108.62, 118.55, 124.16, 127.41, 127.51, 128.34, 128.59, 128.94, 132.66, 123.55, 127.05, 138.90, 138.94, 165.30, 165.98. MS: *m/z* (%) = 258 (50), 185 (18), 139 (20), 106 (95), 91 (100), 71 (67), 57 (98). Anal. Calcd for $C_{21}H_{20}N_4O$: C, 73.23; H, 5.85; N, 16.27; Found: C, 73.50; H, 5.61; N, 16.48.

*6.2.9. N-Benzyl-1-[5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl]-1-(1H-pyrrol-2-yl)methanamine **(5i)***

Brown powder; yield: 88%; m.p. 96–98 °C; IR (KBr): 3415, 2923, 2851, 1616, 1172, 1026 cm^{-1}; ^1H NMR (250.0 MHz, $CDCl_3$): δ 2.34 (1H, s, NH amine), 3.88 (2H, s, CH_2), 3.90 (3H, s, OCH_3), 5.34 (1H, s, CH), 6.19–6.20 (2H, m, 2CH pyrrole), 6.84 (1H, dd, $^4J_{HH}$ = 2.0 Hz, $^3J_{HH}$ = 4.0 Hz, CH pyrrole), 7.01 (2H, d, $^3J_{HH}$ = 9.2 Hz, CH Ar), 7.27–7.37 (5H, m, CH Ar), 7.97(2H, d, $^3J_{HH}$ = 9.2 Hz, CH Ar), 9.02 (1H, s, NH pyrrole). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 51.18, 51.74, 55.48, 107.78, 108.58, 114.46, 118.54, 127.39, 128.35, 128.59, 128.79, 116.19, 127.12, 138.93, 162.41, 165.07, 165.57. Anal. Calcd for $C_{21}H_{20}N_4O_2$: C, 69.98; H, 5.59; N, 15.55; Found: C, 69.66; H, 5.82; N, 15.32.

*6.2.10. N-Benzyl-1-[5-(2,4-dimethoxyphenyl)-1,3,4-oxadiazol-2-yl]-1-(1H-pyrrol-2-yl)methanamine **(5j)***

Orange powder; yield: 86%; m.p. 98–100 °C; IR (KBr): 3441, 2922, 2852, 1613, 1455, 1119, 721 cm^{-1}; ^1H NMR (400.2 MHz, $CDCl_3$): δ 2.90 (1H, s, NH amine), 3.85 (2H, s, CH_2), 3.90 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 5.37 (1H, s, CH), 6.16–6.19 (2H, m, 2CH pyrrole), 6.81 (1H, s, CH pyrrole), 7.27–7.84 (8H, m, CH Ar), 9.39 (1H, s, NH pyrrole). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 51.09, 51.67, 55.57, 55.96, 98.98, 105.33, 107.72, 108.37, 118.81, 127.28, 128.37, 128.51, 131.63, 105.90, 127.33, 139.11, 159.37, 163.76, 165.24. MS: *m/z* (%) = 390 (1), 285 (94), 165 (78), 106 (87), 91 (100), 77 (69), 57 (35). Anal. Calcd for $C_{22}H_{22}N_4O_3$: C, 67.68; H, 5.68; N, 14.35; Found: C, 67.49; H, 5.51; N, 14.58.

*6.2.11. N-(2-Methoxybenzyl)-1-(5-phenyl-1,3,4-oxadiazol-2-yl)-1-(1H-pyrrol-2-yl)methanamine **(5k)***

Brown powder; yield: 82%; m.p. 106–108 °C; IR (KBr): 3439, 2926, 2836, 1443, 1161, 1085, 707 cm^{-1}; ^1H NMR (400.2 MHz, $CDCl_3$): δ 2.70 (1H, s, NH amine), 3.83 (3H, s, OCH_3), 3.83 and 3.93 (2H, ABq, $^2J_{HH}$ = 13.6 Hz, CH_2), 5.38 (1H, s, CH), 6.19–6.20 (2H, m, 2CH pyrrole), 6.85–8.04 (10H, m, CH Ar and CH pyrrole), 9.14 (1H, s, NH pyrrole). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 47.17, 51.80, 55.28, 107.69, 108.53, 110.34, 118.39, 120.50, 126.97, 128.82, 128.97, 130.34, 131.74, 123.79, 126.83, 127.22, 157.72, 165.00, 166.29. Anal. Calcd for $C_{21}H_{20}N_4O_2$: C, 69.98; H, 5.59; N, 15.55; Found: C, 70.22; H, 5.33; N, 15.30.

*6.2.12. N-(4-Methoxybenzyl)-1-(5-phenyl-1,3,4-oxadiazol-2-yl)-1-(1H-pyrrol-2-yl)methanamine **(5l)***

Brown powder; yield: 90%; m.p. 107–109 °C; IR (KBr): 3415, 2924, 2836, 1613, 1449, 1031,730 cm^{-1}; ^1H NMR (400.2 MHz, $CDCl_3$): δ = 2.97 (1H, s, NH amine), 3.77 (3H, s, OCH_3), 3.75 and 3.80 (2H, ABq, $^2J_{HH}$ = 14.0 Hz, CH_2), 5.36 (1H, s, CH), 6.17–6.18 (2H, m, 2CH pyrrole), 6.81–6.85 (1H, m, CH pyrrole), 7.24–8.03 (9H, m, CH Ar), 9.43(1H, s, NH pyrrole). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 50.63, 51.60, 55.27, 107.72, 108.61, 113.97, 118.50, 127.00, 129.03, 129.58, 131.83, 123.71, 127.08, 130.95, 158.94, 165.09, 166.13. Anal. Calcd for $C_{21}H_{20}N_4O_2$: C, 69.98; H, 5.59; N, 15.55; Found: C, 70.55; H, 5.23; N, 15.72.

*6.2.13. N-(4-Methoxybenzyl)-1-[5-(3-chlorophenyl)-1,3,4-oxadiazol-2-yl]-1-(1H-pyrrol-2-yl)methanamine **(5m)***

Brown powder; yield: 90%; m.p. 86–88 °C; IR (KBr): 3413, 2925, 2850, 1637, 1406, 1103, 773 cm^{-1}; ^1H NMR (400.2 MHz, $CDCl_3$): δ 2.42 (1H, s, NH amine), 3.79 (3H, s, OCH_3), 3.80 and 3.85 (2H, ABq, $^2J_{HH}$ = 13.2 Hz, CH_2), 5.38 (1H, s, CH), 6.19 (2H, s, 2CH pyrrole), 6.81–8.03 (9H, m, CH Ar and CH pyrrole), 9.08 (1H, s, NH pyrrole). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 50.65, 51.62, 55.26, 107.81, 108.63, 113.96, 118.63, 125.08, 126.95, 129.57, 130.39, 131.86, 125.30, 126.87, 130.86, 135.16, 158.94, 163.95, 166.50. Anal. Calcd for $C_{21}H_{19}ClN_4O_2$: C, 63.88; H, 4.85; N, 14.19; Found: C, 63.71; H, 4.60; N, 14.29.

*6.2.14. N-(4-Methylbenzyl)-1-[5-(4-bromophenyl)-1,3,4-oxadiazol-2-yl]-1-(1H-pyrrol-2-yl)methanamine **(5n)***

Yellow powder; yield: 86%; m.p. 136–138 °C; IR (KBr): 3431, 2921, 2852, 1603, 1480, 826 cm^{-1}; ^1H NMR (250.1 MHz, $CDCl_3$): δ 2.30 (3H, s, CH_3), 2.41 (1H, s, NH amine), 3.80 (2H, s, CH_2), 5.31(1H, s, CH), 6.13–6.17 (2H, m, 2CH pyrrole), 6.79–6.82 (1H, m, CH pyrrole), 7.11 (2H, d, $^3J_{HH}$ = 8.5 Hz, CH Ar), 7.20 (2H, d, $^3J_{HH}$ = 7.0 Hz, CH Ar), 7.61 (2H, d, $^3J_{HH}$ = 7.0 Hz, CH Ar), 7.81 (2H, d, $^3J_{HH}$ = 8.5 Hz, CH Ar), 9.08 (1H, s, NH pyrrole). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 21.06, 50.91, 51.67, 107.81, 108.56, 118.65, 128.27, 128.35, 129.24, 132.33, 122.54, 126.50, 126.88, 135.75, 137.05, 164.32, 166.36. Anal. Calcd for $C_{21}H_{19}BrN_4O$: C, 59.58; H, 4.52; N, 13.24; Found: C, 59.72; H, 4.27; N, 13.51.

*6.2.15. N-(4-Fluorobenzyl)-1-[5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl]-1-(1H-pyrrol-2-yl)methanamine **(5o)***

Orange powder; yield: 87%; m.p. 93–95 °C; IR (KBr): 3417, 1613, 1499, 1027, 836 cm^{-1}; ^1H NMR (250.1 MHz, $CDCl_3$): δ 2.30 (1H, s, NH amine), 3.79 (2H, s, CH_2), 3.86 (3H, s, OCH_3), 5.29 (1H, s, CH), 6.16–6.17 (2H, m, 2CH pyrrole), 6.79–6.82 (1H, m, CH pyrrole),

6.95–7.95 (8H, m, CH Ar), 9.23 (1H, s, NH pyrrole). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 50.37, 51.68, 55.44, 107.78, 108.53, 114.44, 118.58, 132.00, 115.31 (d, $^2J_{CF}$ = 21.3 Hz, CH), 129.90 (d, $^3J_{CF}$ = 8.1 Hz, CH), 116.13, 127.01, 134.71, 162.40, 162.00 (d, $^1J_{CF}$ = 251.6 Hz, C), 165.04, 165.46. Anal. Calcd for $C_{21}H_{19}FN_4O_2$: C, 66.66; H, 5.06; N, 14.81; Found: C, 66.49; H, 5.21; N, 14.63.

6.2.16. *N-(3,4-Dichlorobenzyl)-1-[5-(3-chlorophenyl)-1,3,4-oxadiazol-2-yl]-1-(1H-pyrrol-2-yl)methanamine* ***(5p)***

Brown powder; yield: 84%; m.p. 96–98 °C; IR (KBr): 3445, 2923, 2851, 1637, 1467, 1092, 794 cm^{-1}; ^{1}H NMR (400.2 MHz, $CDCl_3$): δ 2.37 (1H, s, NH amine), 3.82 (2H, ABq, $^2J_{HH}$ = 14.4 Hz, CH_2), 5.34 (1H, s, CH), 6.21(2H, s, CH pyrrole), 6.87 (1H, s, CH pyrrole), 7.16–7.99 (7H, m, CH Ar), 9.08 (1H, s, NH pyrrole). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 50.02, 51.87, 108.23, 108.75, 118.99, 125.07, 126.93, 127.58, 130.14, 130.45, 132.00, 126.28, 127.30, 131.35, 132.61, 135.23, 139.20, 164.05, 166.16. Anal. Calcd for $C_{20}H_{15}Cl_3N_4O$: C, 55.38; H, 3.49; N, 12.92; Found: C, 55.61; H, 3.58; N, 13.11.

6.2.17. *N-(3,4-Dichlorobenzyl)-1-[5-(4-bromophenyl)-1,3,4-oxadiazol-2-yl]-1-(1H-pyrrol-2-yl)methanamine* ***(5q)***

Brown powder; yield: 85%; m.p. 98–101 °C; IR (KBr): 3417, 2924, 2851, 1602, 1480, 1030, 792 cm^{-1}; ^{1}H NMR (400.2 MHz, $CDCl_3$): δ 2.19 (1H, s, NH amine), 3.82 and 3.86 (2H, ABq, $^2J_{HH}$ = 14 Hz, CH_2), 5.34 (1H, s, CH), 6.21 (2H, s, 2CH pyrrole), 6.87–6.88 (1H, m, CH pyrrole), 7.17–7.48 (3H, m, CH Ar), 7.66 (2H, d, $^3J_{HH}$ = 8.4 Hz, CH Ar), 7.89 (2H, d, $^3J_{HH}$ = 8.4 Hz, CH Ar), 9.03 (1H, s, NH pyrrole). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 50.00, 51.86, 108.21, 108.71, 118.99, 127.59, 128.36, 130.15, 130.45, 132.43, 122.39, 126.35, 126.71, 132.03, 132.13, 139.25, 164.33, 166.27. MS: *m*/*z* (%) = 390 (1), 285 (94), 165 (78), 106 (87), 91 (100), 77 (69), 57 (35). MS: *m*/*z* (%) = 478 (3), 303 (100), 183 (50), 159 (65), 140 (68), 94 (48), 57 (45). Anal. Calcd for $C_{20}H_{15}BrCl_2N_4O$: C, 50.24; H, 3.16; N, 11.72; Found: C, 50.41; H, 3.37; N, 11.51.

6.3. *Cell culture and cytotoxicity assay*

Four human cancer cell lines (A549, HT1080, HT29, and MCF-7) were purchased from the Iranian Biological Resources Center (IBRC, Tehran, Iran) and cultivated in DMEM media supplemented with fetal bovine serum (FBS, 10%), penicillin (100 units/mL) and streptomycin (100 μg/mL) at 37 °C in a CO_2 incubator (5% CO_2 and 95% relative humidity).

For the cytotoxicity assay, 15,000 cells in the exponential phase of growth were separately seeded in 96-well microplates and incubated for 24 h. Different concentrations of test compounds (1 μg/mL to 1000 μg/mL) were then prepared by dissolving each compound in DMEM media containing DMSO (0.5% v/v) and added into desired well and incubated for another 24 h. Negative control was provided by addition of same volume of DMEM medium to related well. Doxorubicin was used as positive control. Thereafter, the media were removed and 20 μL DMEM medium containing MTT (5 mg/mL) was added to each well followed by incubation for further 4 h and insertion of DMSO (100 μL) to dissolve formazan crystal. The optical density of each well was subsequently measured at 570 nm using Synergy 2 multi-mode microplate reader (BioTek, USA) [24].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.03.049.

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