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Synthesis and evaluation of some new 1,3,4-oxadiazoles bearing thiophene, thiazole, coumarin, pyridine and pyridazine derivatives as antiviral agents

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In an attempt to produce heterocyclic compounds based on 1,3,4-oxadiazole derivatives with potential antiviral activity, synthesis of compound **1** [2-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)acetonitrile] was performed through the reaction of cyanoacetic acid hydrazide with carbon disulfide in alcoholic potassium hydroxide. Compound **1** has an activating methylene group, so it was directed toward some specific reactions. Thus, aryldiazonium chlorides reacted with compound **1** affording hydrazono derivatives **2a-c**. Also, aromatic aldehydes reacted with compound **1** to produce compounds **3a**,**b**. Furthermore, cyclic ketones were subjected to the synthesis of fused thiophene derivatives **4a**,**b** *via* reaction with compound **1** in the presence of elemental sulfur. In addition, 1,3,4-oxadiazole derivative **1,** when reacted with isothiocyanates, salicylaldehyde or 1,3-dicarbonyl compounds, formed thiazole derivatives **5a**,**b**, coumarin derivative **6** and alkenyl derivatives **7a**,**b** resp. Compound **7b** underwent cyclization to afford pyridine derivative **8**. Arylhydrazono derivatives **9a**,**b** were produced through the reaction of compound **7a** with aryldiazonium chlorides. Products **9a**,**b** underwent cyclization to produce pyridazine derivatives **10a**,**b**. Finally, 1,3,4-oxadiazole derivative **1** was directed toward reaction with hydrazine derivatives, bromoacetophenone and ethylchloroacetate affording compounds **11a**,**b**, **12** and **13**, resp. Fused thiophene derivatives **14a**,**b** were produced *via* reaction of compounds **4a**,**b** with a mixture of malononitrile and ethylorthoformate. Antiviral activity of the synthesized products showed that 5-(4-amino-3-ethyl-2-thioxo-2,3-dihydrothiazol-5-yl)-1,3,4-oxadiazole-2(3*H*)-thione (**5a**) and 5-(4-amino-3-phenyl-2-thioxo-2,3-dihydrothiazol-5-yl)-1,3,4-oxadiazole-2(3*H*)-thione (**5b**) acted as the most active agents against *Feline herpes virus, Feline corona virus*, *Herpes simplex virus-1* and *Herpes simplex virus-2*, whereas compound 2-(5-(2-phenylhydrazono)-4,5-dihydro-1,3,4-oxadiazol-2-yl)acetonitrile (**11b**) was the most effective against *Vaccinia virus*, *Herpes simplex virus* (TK-KOS-ACVr), *Coxsackie virus B4* and *Vesicular stomatitis virus*.

Keywords: 1,3,4-oxadiazole derivatives, thiophene, coumarin, thiazole, pyridine, pyridazine, antiviral activity

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1,3,4-Oxadiazole heterocyclic ring is one of the most important heterocyclic moieties because of its versatile biological actions. In particular, heterocyclic compounds bearing such a ring are known to exert potential antitubercular activity (1), antiinflammatory and analgesic activities (2), antiviral activity (3), anticancer activity and antimicrobial activity (4, 5). Also, 1,3,4-oxadiazole bearing fused thiophene derivatives have antioxidant activity (6). In addition, 1,3,4-oxadiazole containing thiazole moiety shows antimicrobial and cytotoxic activities (7). It was found that oxadiazole bearing chromene derivatives have potential antibacterial and antifungal properties (8). Furthermore, pyridazine derivatives show interesting antifungal activity (9). Also, phenyl-bearing oxadiazole exerts versatile biological properties, such as antimicrobial and cytotoxic activity (10, 11) and anti-inflammatory activity (12). On the other hand, 5-substituted-2-mercapto-1,3,4-oxadiazoles show analgesic, antitubercular and anticonvulsant properties (13).

EXPERIMENTAL

Melting points of all the synthesized compounds were determined in open capillaries and were uncorrected. Elemental analyses were performed on a Yanaco CHNS Corder elemental analyzer (Japan). Structures of the synthesized compounds were established using IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry techniques (Tables I and II). IR spectra were measured using KBr discs on a Pye Unicam SP-1000 spectrophotometer (Pye Unicam, UK). ^{1}H NMR and ^{13}C NMR spectral analyses were performed on a Varian EM 390-200 MHz instrument (Varian, Germany) in CD_3SOCD_3 as solvent and TMS as internal standard. Chemical shifts were expressed as δ ppm. Mass spectra were recorded on Kratos (75 eV) MS equipment (Germany).

Syntheses

*2-(5-Thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)acetonitrile (**1**).* – A mixture of potassium hydroxide aqueous solution in ethanol (2.5 g in 50 mL) and carbon disulfide (9.0 mL, 0.15 mol) was added to cyanoacetic acid hydrazide (4.95 g, 0.05 mol). The reaction mixture was heated under reflux until the hydrogen sulfide odor disappeared (within 10 h). The mixture was then poured onto ice-water containing a few drops of 2 mol L^{-1} HCl. The formed solid product was filtered out, washed with water, dried and recrystallized from ethanol to afford the desired product.

N′*-phenyl-5-thioxo-4,5-dihydro-1,3,4-oxadiazole-2-carbohydrazonoyl cyanide (**2a**),* N′*-(4-chlorophenyl)-5-thioxo-4,5-dihydro-1,3,4-oxadiazole-2-carbohydrazonoyl cyanide (**2b**) and* N′*-(4-methoxyphenyl)-5-thioxo-4,5-dihydro1,3,4-oxadiazole-2-carbohydrazonoyl cyanide (**2c**).* – Either benzenediazonium chloride, 4-chlorobenzenediazonium chloride or 4-methoxybenzenediazonium chloride (0.003 mol) [prepared by adding an aqueous sodium nitrite solution (0.207 g, 0.003 mol) to a cold solution of either aniline, 4-chloroaniline or 4-methoxyaniline (0.003 mol) in the appropriate amount of glacial acetic acid at 0–5 °C, under continuous stirring] was added to a cold solution (0–5 °C) of 1,3,4-oxadiazole derivative **1** (0.423 g, 0.003 mol) in ethanol (50 mL) containing sodium acetate (0.246 g, 0.003 mol), under continuous stirring. The reaction mixture was stirred at room temperature for additional 3 h and the solid product so formed, in each case, was collected by filtration and recrystallized from ethanol.

*3-Phenyl-2-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)acrylonitrile (**3a**) and 3-(4-chlorophenyl)-2-(5-thioxo-4,5-dihydro1,3,4-oxadiazol-2-yl)acrylonitrile (**3b**).* – Either benzaldehyde (0.318 g, 0.003 mol) or 4-chlorobenzaldehyde (0.421 g, 0.003 mol) was added to a solution of compound **1** (0.423 g, 0.003 mol) in ethanol (50 mL) containing piperidine (0.5 mL). The reaction mixture was heated under reflux for 4 h and then poured onto ice/water containing a few drops of HCl. Suction filtration was used to collect the precipitate.

*5-(3-Amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophen-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione (**4a**) and 5-(3-amino-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione (**4b**).* – Either cyclopentanone (0.252 g, 0.003 mol) or cyclohexanone (0.294 g, 0.003 mol) together with elemental sulfur (0.096 g, 0.003 mol) were added to a solution of 1,3,4-oxadiazole derivative **1** (0.423 g, 0.003 mol) in ethanol (40 mL) containing triethylamine (0.5 mL). The reaction mixture was heated under reflux for 3 h, cooled and poured onto an ice/water mixture containing a few drops of HCl. The solid product that formed in each case was collected by filtration and recrystallized from 1,4-dioxane.

*5-(4-Amino-3-ethyl-2-thioxo-2,3-dihydrothiazol-5-yl)-1,3,4-oxadiazole-2(3*H*)-thione (**5a**) and 5-(4-amino-3-phenyl-2-thioxo-2,3-dihydrothiazol-5-yl)-1,3,4-oxadiazole-2(3*H*)-thione (**5b**).* – Either ethylisothiocyanate (0.261 g, 0.003 mol) or phenylisothiocyanate (0.406 g, 0.003 mol) was added to a solution of 1,3,4-oxadiazole derivative **1** (0.423 g, 0.003 mol) in ethanol (50 mL) containing 0.5 mL of triethylamine. The reaction mixture was refluxed for 3 h, cooled and poured onto an ice/water mixture containing a few drops of HCl. The formed precipitate in each case was filtered off and recrystallized from 1,4-dioxane.

*3-(5-Thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one (**6**).* – Salicylaldehyde (0.366 g, 0.003 mol) was added to a solution of 1,3,4-oxadiazole derivative **1** (0.423 g, 0.003 mol) in 1,4-dioxane (40 mL) containing piperidine (0.5 mL). The reaction mixture was heated under reflux for 4 h and then evaporated under vacuum. The solid product was triturated with ethanol and the formed solid product was collected by filtration and recrystallized from 1,4-dioxane.

*3-Methyl-5-oxo-2-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)hex-2-enenitrile (**7a**) and 4-cyano-3-methyl-*N*-phenyl-4-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)but-3-enamid (**7b**).* – A mixture of 1,3,4-oxadiazole derivative **1** (0.423 g, 0.003 mol) and either acetyl acetone (0.3 g, 0.003 mol) or acetoacetanilide (0.531 g, 0.003 mol) containing a catalytic amount of piperidine (0.5 mL) was fused at 140 °C for 30 min. It was then left to cool and the formed solid product in each case was collected by filtration and dried. It was then recrystallized from ethanol.

*6-Amino-4-methyl-1-phenyl-5-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)pyridin-2(1*H*)-one (**8**).* – Compound (**7b**, 0.3 g**,** 0.001 mol.) was dissolved in 50 mL of ethanol and 0.5 mL of triethylamine was added. The solution was then subjected to heating under reflux for 3 h aiming at formation of a cyclized product. The solution was cooled, poured onto ice water, a few drops of HCl were added to enhance precipitate formation and the latter product was then collected by filtration and recrystallized from ethanol.

*3-Methyl-5-oxo-4-(2-phenylhydrazono)-2-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)hex-2-enenitrile (**9a**) and 4-(2-(4-chlorophenyl)hydrazono)-3-methyl-5-oxo-2-(5-thioxo-4,5-dihydro*

*-1,3,4-oxadiazol-2-yl)hex-2-enenitrile (**9b**).* – Either benzenediazonium chloride or 4-chlorobenzenediazonium chloride (0.002 mol) [prepared by adding an aqueous sodium nitrite solution (0.138 g, 0.002 mol) to a cold solution of either aniline or 4-chloroaniline (0.002 mol) in an appropriate amount of conc. HCl at 0–5 °C, under continuous stirring] was added to a cold solution (0–5 °C) of compound **7a** (0.446 g, 0.002 mol) in ethanol (50 mL), containing sodium hydroxide (0.08 g, 0.002 mol), under continuous stirring. The reaction mixture was stirred at room temperature for additional 4 h and the formed solid product in each case was collected and recrystallized from 1,4-dioxane.

*1-(6-Imino-4-methyl-1-phenyl-5-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)-1,6-dihydropyridazin-3-yl) ethanone (**10a**) and 1-(1-(4-chlorophenyl)-6-imino-4-methyl-5-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)-1,6-dihydropyridazin-3-yl)ethanone (**10b**).* – A solution of each compound **9a** (0.654 g, 0.002 mol) or **9b** (0.724 g, 0.002 mol) in 50 mL ethanol (containing catalytic amount of trimethylamine, 0.5 mL) was heated under reflux for 4 h and poured onto an ice/water mixture containing a few drops of hydrochloric acid. The solid product so formed was collected by filtration and recrystallized from ethanol.

*2-(5-Hydrazono-4,5-dihydro-1,3,4-oxadiazol-2-yl)acetonitrile (**11a**) and 2-(5-(2-phenylhydrazono)-4,5-dihydro-1,3,4-oxadiazol-2-yl)acetonitrile (**11b**).* – Either hydrazine hydrate (0.15 g, 0.003 mol) or phenyl hydrazine (0.324 g, 0.003 mol) was added to a solution of compound **1** (0.423 g, 0.003 mol) in ethanol (50 mL). The reaction mixture was stirred at room temperature for 24 h and the formed solid product, in each case, was collected by filtration and recrystallized from 1,4-dioxane.

*2-(5-(2-Oxo-2-phenylethylthio)-1,3,4-oxadiazol-2-yl)acetonitrile (**12**).* – Phenacylbromide (0.6 g, 0.003 mol) was added to the dry solid of compound **1** (0.423 g, 0.003 mol) in ethanol (50 mL). The reaction mixture was heated under reflux for 3 h and then left to cool. The remaining product was triturated with diethyl ether and the formed solid product was collected by filtration. It was recrystallized from 1,4-dioxane.

*Ethyl 2-(5-(cyanomethyl)-1,3,4-oxadiazol-2-ylthio)acetate (**13**).* – A suspension of compound **1** (0.423 g, 0.003 mol) in ethanol (50 mL) was added to ethyl chloroacetate (0.37 g, 0.003 mol). The reaction mixture was heated under reflux for 5 h, then left to cool and the remaining product was triturated with diethyl ether. The solid product so formed was collected by filtration and recrystallized from ethanol.

*2-((2-(5-Thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)-5,6-dihydro-4*H*-cyclopenta[*b*]-thiophen-3-yl-imino)methyl)malononitrile (**14a**) and 2-((2-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)-4,5,6,7-tetrahydrobenzo [*b*] thiophen-3-ylimino)methyl)malononitrile (**14b**).* – To a mixture of either compound **4a** (0.478 g, 0.002 mol) or **4b** (0.507 g, 0.002 mol) and triethyl orthoformate (0.296 g, 0.002) and malononitrile (0.132 g, 0.002 mol), a catalytic amount of piperidine was added. The reaction mixture was heated in an oil bath at 120 °C for 3 h. The mixture was then boiled in ethanol for a few minutes, poured onto an acidified ice/water mixture and the formed solid product, in each case, was collected by filtration and recrystallized from 1,4-dioxane.

Synthetic pathways are presented in Schemes 1–3. Structures of all the synthesized compounds were confirmed based on analytical and spectral data; physicochemical and spectral data of the synthesized compounds are given in Tables I and II.

Biological evaluations

Antiviral activity tests: material and methods. – Antiviral activities of the synthesized compounds were evaluated following the procedures described before (14). We conducted

Table I. Physicochemical data for the synthesized compounds

Compd.	Mol. formula (M_r)	M. p. (°C)	Yield (%)	Calcd./found (%)			
				C	H	N	S
1	$C_4H_3N_3OS$ (141.15)	143–145	61	34.04 34.33	2.14 2.29	29.77 29.55	22.72 22.48
2a	$C_{10}H_7N_5OS$ (245.26)	168–171	55	48.97 49.22	2.88 2.61	28.55 28.34	13.07 13.29
2b	$C_{10}H_6ClN_5OS$ (279.71)	181–183	47	42.94 42.77	2.16 2.33	25.04 25.30	11.46 11.72
2c	$C_{10}H_9N_5O_2S$ (275.29)	158–160	56	47.99 47.71	3.30 3.56	25.44 25.70	11.56 11.43
3a	$C_{11}H_7N_3OS$ (229.26)	86–88	61	57.63 57.37	3.08 2.84	18.33 18.59	13.99 13.73
3b	$C_{11}H_6ClN_3OS$ (263.70)	102–103	53	50.10 50.26	2.29 2.53	15.93 15.66	12.16 12.44
4a	$C_9H_9N_3OS$ (239.32)	242–244	71	45.17 45.37	3.79 3.93	17.56 17.29	26.80 26.55
4b	$C_{10}H_{11}N_3OS$ (253.34)	207–209	66	47.41 47.64	4.38 4.65	16.59 16.81	25.31 25.07
5a	$C_7H_8N_4OS_3$ (260.36)	173–175	70	32.29 32.05	3.10 3.37	21.52 21.28	36.95 36.71
5b	$C_{14}H_8N_4OS_3$ (308.40)	188–191	64	42.84 42.66	2.61 2.83	18.17 18.29	31.19 31.43
6	$C_{11}H_6N_2O_3S$ (246.24)	198–200	58	53.65 53.37	2.46 2.71	11.38 11.55	13.02 13.28
7a	$C_9H_9N_3O_2S$ (223.25)	210–212	77	48.42 48.22	4.06 4.34	18.82 18.58	14.36 14.09
7b	$C_{14}H_{12}N_4O_2S$ (300.34)	238–240	55	55.99 55.73	4.03 4.27	18.65 18.41	10.68 10.89
8	$C_{14}H_{12}N_4O_2S$ (300.34)	93–95	45	55.99 56.24	4.03 3.83	18.65 18.92	10.68 10.95
9a	$C_{15}H_{13}N_5O_2S$ (327.36)	120–122	65	55.03 54.76	4.00 3.75	21.39 21.14	9.79 9.98
9b	$C_{15}H_{12}ClN_5O_2S$ (361.81)	138–140	61	49.79 49.53	3.34 3.61	19.36 19.09	8.86 8.58

10a	$C_{15}H_{13}N_5O_2S$ (327.36)	190–192	58	55.03 55.31	4.00 4.27	21.39 21.11	9.79 10.04
10b	$C_{15}H_{12}ClN_5O_2S$ (361.81)	155–157	63	49.79 50.03	3.34 3.07	19.36 19.12	8.86 8.61
11a	$C_4H_5N_5O$ (139.12)	110–112	67	34.53 34.76	3.62 3.37	50.34 50.11	–
11b	$C_{10}H_9N_5O$ (215.21)	129–131	60	55.81 55.94	4.22 3.99	32.54 32.26	–
12	$C_{12}H_9N_3O_2S$ (259.29)	219–221	71	55.59 55.32	3.50 3.77	16.21 16.45	12.37 12.64
13	$C_8H_9N_3O_3S$ (227.24)	98–101	58	42.28 42.55	3.99 3.72	18.49 18.75	14.11 14.34
14a	$C_{13}H_9N_5OS_2$ (315.37)	160–162	75	49.51 49.80	2.88 3.16	22.21 22.41	20.33 20.59
14b	$C_{14}H_{11}N_5OS_2$ (329.40)	171–173	73	51.05 51.33	3.37 3.09	21.26 21.53	19.47 19.18

antiviral and cytotoxic investigations for all synthesized compounds against 8 viruses in 3 different cell lines. They included *Herpes simplex virus-1* (KOS), thymidine kinase-deficient HSV-1 KOS strain resistant to acyclovir (ACVr) (TK-KOS-ACVr), *Herpes simplex virus-2* (G), *Vaccinia virus* in HEL cell cultures; *Vesicular stomatitis virus* and *Coxsackie virus B4* in HeLa cell cultures, and *Feline herpes virus* (FHV) and *Feline corona virus* (FCoV) in CRFK cell cultures. The HEL, HeLa and CRFK cell lines (Novartis Institutes for Biomedical Research, Basel, Switzerland) were monitored for mycoplasma contamination and were found to be mycoplasma free.

Inhibition of virus-induced cytopathogenicity in vitro. – Confluent cell cultures in microtiter trays were inoculated with 100 $CCID_{50}$ (1 $CCID_{50}$ corresponding to the virus stock dilution proven for infection of 50 % of cell cultures). After 1 h of virus adsorption to the cells, the residual virus was removed and replaced by cell culture medium (Eagle's Minimum Essential Medium) containing 3 % fetal calf serum and various concentrations of test compounds. Viral cytopathogenicity was recorded as soon as it reached completion in the untreated virus-infected cell cultures, that is, in 1 to 2 days for *Vesicular stomatitis virus*, in 2 days for *Coxsackie virus*, in 2 to 3 days for *Vaccinia virus* and *Herpes simplex virus* types 1 and 2. Antiviral activity of the compounds is expressed as the concentration required to inhibit viral cytopathogenicity by 50 % (IC_{50}).

Cytotoxicity. – Cytotoxic activity was measured as an alteration of normal cell morphology. Evaluation of cell morphology concerning confluent cell cultures that were not infected but were treated with various concentrations of test compounds were incubated in parallel with the virus-infected cell cultures and examined microscopically at the same time as viral cytopathogenicity was recorded in the virus-infected cell cultures. Disruption of the cell monolayer, *e.g.*, rounding up or detachment of cells, was considered as evidence for cytotoxicity. Cytotoxicity of both synthesized compounds and reference stan-

Table II. Spectral data for the synthesized compounds

Compd.	MS (*m*/*z*)	^{13}C NMR (δ, ppm)	^{1}H NMR (δ, ppm)	IR (ν, cm^{-1})
1	141	45.7 (CH_2), 141.6 (CN), 156.2 (C=N), 161.9 (C=S)	3.23-3.37 (s, 2H, CH_2), 11.72 (s, 1H, D_2O-exchangeable, NH)	3327-3288 (NH), 2889 (CH_2), 2228 (CN), 1598 (C=S), 1564 (C=N)
2a	245	123.2 (CN), 125.5, 127.7, 130.8, 134.2, 137.6, 141.8 (C_6H_5), 153.6, 159.4 (2C=N), 164.2 (C=S)	6.85-7.22 (m, 5H, benzene ring), 8.76, 10.82 (2s, 2H, D_2O-exchangeable, 2NH)	3368-3320 (2NH), 3048 (CH aromatic), 2225 (CN), 1567 (C=S), 1559 (C=N)
2b	279	121.9 (CN), 127.6, 129.5, 132.7, 134.9, 136.5, 140.1 (C_6H_4), 151.9, 158.7 (2C=N), 162.5 (C=S)	7.12-7.34 (dd, 4H, C_6H_4), 9.34, 10.94 (2s, 2H, D_2O-exchangeable, 2NH)	3343-3284 (2NH), 3053 (CH aromatic), 2224 (CN), 1558 (C=S), 1548 (C=N)
2c	275	36.2 (CH_3), 125.7 (CN), 127.3, 129.7, 131.8, 133.8, 135.7, 141.2 (C_6H_4), 153.3, 157.3 (2C=N), 168.2 (C=S)	2.83 (s, 3H, CH_3), 6.84-7.12 (dd, 4H, C_6H_4), 9.41, 11.33 (2s, 2H, D_2O-exchangeable, 2NH)	3362-3331 (2NH), 3051 (CH aromatic), 2976 (CH_3), 2228 (CN), 1565 (C=S), 1552 (C=N)
3a	229	67.3, 80.2 (C=CH), 121.7 (CN), 123.5, 126.4, 130.4, 133.6, 135.5, 140.2 (C_6H_5), 148.7 (C=N), 153.1 (C=S)	5.38 (s, 1H, CH), 7.12-7.37 (m, 5H, benzene ring), 9.55 (s, 1H, D_2O-exchangeable, NH)	3344 (NH), 3056 (CH aromatic), 2224 (CN), 1553 (C=S), 1546 (C=C)
3b	263	73.5, 84.4 (C=CH), 122.7 (CN), 125.3, 127.1, 131.2, 133.9, 136.7, 139.5 (C_6H_4), 146.8 (C=N), 150.6 (C=S)	5.59 (s, 1H, CH), 7.23-7.48 (dd, 4H, benzene ring), 10.15 (s, 1H, D_2O-exchangeable, NH)	3318 (NH), 3053 (CH aromatic), 2226 (CN), 1560 (C=S), 1548 (C=C)
4a	239	21.7, 26.2, 28.9 (3CH_2), 130.7, 133.4, 138.7, 141.5, 145.6, 155.3 (thiophene C, 1,3,4-oxadiazole C)	1.87-2.05 (m, 6H, 3CH_2), 4.42 (s, 2H, D_2O-exchangeable, NH_2), 10.65 (s, 1H, D_2O-exchangeable, NH)	3423-3344 (NH_2, NH), 2887 (CH_2), 1660 (C=S), 1647 (C=C)
4b	253	24.6, 26.8, 29.2, 30.8 (4CH_2), 129.6, 132.8, 136.6, 140.7, 143.8, 152.3 (thiophene C, 1,3,4-oxadiazole C)	1.87-2.21 (m, 8H, 4CH_2), 4.63 (s, 2H, D_2O-exchangeable, NH_2), 10.23 (s, 1H, D_2O-exchangeable, NH)	3383-3321 (NH_2, NH), 2889 (CH_2), 1663 (C=S), 1652 (C=C)
5a	260	16.5 (CH_3), 37.8 (CH_2), 135.2, 139.1, 145.7, 158.4, 167.5 (1,3,4-oxadiazole C, thiazole C)	1.45 (t, 3H, J = 6.46 Hz, CH_3), 4.12 (q, 2H, J = 6.46 Hz, CH_2), 4.88 (s, 2H, D_2O-exchangeable, NH_2), 10.28 (s, 1H, D_2O-exchangeable, NH)	3356-3238 (NH_2, NH), 2960 (CH_3), 2876 (CH_2), 1668, 1660 (2C=S), 1654 (C=C)

Compd.	MS (*m*/*z*)	^{13}C NMR (δ, ppm)	^{1}H NMR (δ, ppm)	IR (ν, cm^{-1})
5b	308	127.5, 129.8, 133.4, 135.7, 136.3, 139.1, 145.7, 158.4, 167.5 (benzene C, 1,3,4-oxadiazole C, thiazole C)	5.12 (s, 2H, D_2O-exchangeable, NH_2), 7.11-7.34 (m, 5H, benzene ring), 9.45 (s, 1H, D_2O-exchangeable, NH)	3383-3265 (NH_2, NH), 3050 (CH aromatic), 1657, 1652 (2C=S), 1644 (C=C)
6	246	126.3, 129.9, 133.6, 138.2, 141.7, 145.3, 148.5, 150.3, 154.2 (1,3,4-oxadiazole C, coumarin C), 172.6 (C=O)	6.45 (s, 1H, coumarin H-4), 7.34-7.56 (m, 4H, C_6H_4), 10.27 (s, 1H, D_2O-exchangeable, NH)	3328-3298 (NH), 3056 (CH-aromatic), 1827 (C=O), 1663 (C=S), 1653 (C=N), 1645 (C=C)
7a	223	24.7, 28.3 ($2CH_3$), 39.2 (CH_2), 69.3, 77.2 (C=C), 128.6 (CN), 149.3, 159.7 (1,3,4-oxadiazole C), 178.4 (C=O)	2.23, 2.38 (2s, 6H, $2CH_3$), 4.18 (s, 2H, CH_2), 11.13 (s, 1H, D_2O-exchangeable, NH)	3331 (NH), 2987 (CH_3), 2227 (CN), 1705 (C=O), 1559 (C=S), 1542 (C=C)
7b	300	22.3, (CH_3), 47,1 (CH_2), 63.4, 72.8 (C=C), 121.8 (CN), 124.3, 126.3, 128.4, 129.7, 130.7, 132.5, 144.8, 154.4 (C_6H_5, 1,3,4- oxadiazole C), 174.3 (C=O)	2.31 (s, 3H, CH_3), 4.34 (s, 2H, CH_2), 7.12-7.32 (m, 5H, C_6H_5), 8.23, 10.73 (2s, 2H, D_2O-exchangeable, 2NH)	3339-3327 (NH), 3058 (CH-aromatic), 2976 (CH_3), 2224 (CN), 1675 (C=O), 1568 (C=S), 1554 (C=C)
8	300	25.1, (CH_3), 125.7, 128.2, 129.9, 131.6, 133.6, 137.4, 138.5, 142.4, 145.9, 147.4, 148.9, (C_6H_5, pyridine C, 1,3,4- oxadiazole C), 168.5 (C=O)	1.95 (s, 3H, CH_3), 5.23 (s, 2H, D_2O-exchangeable, NH_2), 6.92- 7.41 (m, 5H, C_6H_5, 1H, pyridine ring), 9.82 (s, 1H, D_2O-exchangeable, NH)	3388, 3316, 3224 (NH_2, NH), 3053 (CH-aromatic), 2965 (CH_3), 1662 (C=O), 1551 (C=S), 1543 (C=C)
9a	327	21.9, 27.4 ($2CH_3$), 58.3, 61.2, 73.6 (C=C, C=N), 125.3 (CN), 128.7, 130.4, 132.5, 134.9, 137.5, 139.2, 147.3, 155.8 (1,3,4- oxadiazole C, C_6H_5), 173.1 (C=O)	1.29, 1.62 (2s, 6H, $2CH_3$), 7.54-8.81 (m, 5H, C_6H_5), 11.74, 12.28 (2s, 2H, D_2O-exchangeable, 2NH)	3357, 3319 (2NH), 3048 (CH-aromatic), 2977 (CH_3), 2225 (CN), 1734 (C=O), 1553 (C=S), 1540 (C=C)
9b	361	23.7, 35.2 ($2CH_3$), 56.7, 60.3, 71.4 (C=C, C=N), 122.4 (CN), 127.5, 139.6, 133.1, 135.8, 139.6, 143.1, 146.5, 152.7 (1,3,4-oxadiazole C, C_6H_4), 179.3 (C=O)	2.05, 2.34 (2s, 6H, $2CH_3$), 7.43-7.58 (dd, 4H, C_6H_4), 8.78, 9.94 (2s, 2H, D_2O-exchangeable, 2NH)	3195, 3131 (2NH), 3059 (CH-aromatic), 2972 (CH_3), 2233 (CN), 1743 (C=O), 1603 (C=S), 1547 (C=C)
10a	327	19.0, 23.9 ($2CH_3$), 109.9, 121.4, 123.9, 129.2, 133.5, 138.0, 144.2, 145.3, 147.3, 150.4, 156.0, (1,3,4- oxadiazole C, pyridazine C, C_6H_5), 160.2 (C=O)	1.88, 2.34 (2s, 6H, $2CH_3$), 7.17-7.35 (m, 5H, C_6H_5), 9.23, 9.86 (2s, 2H, D_2O-exchangeable, 2NH)	3266, 3211 (2NH), 3053 (CH-aromatic), 2983 (CH_3), 1764 (C=O), 1559 (C=S), 1542 (C=C)

Compd.	MS (*m*/*z*)	^{13}C NMR (δ, ppm)	^{1}H NMR (δ, ppm)	IR (ν, cm^{-1})
10b	361	22.4, 32.6 (2CH_3), 124.3, 126.7, 129.5, 133.2, 135.5, 138.2, 140.7, 142.1, 143.7, 145.9 (1,3,4-oxadiazole C, pyridazine C, C_6H_4), 176.3 (C=O)	1.95, 2.26 (2s, 6H, 2CH_3), 7.34-7.56 (dd, 4H, C_6H_4), 8.65, 9.79 (2s, 2H, D_2O-exchangeable, 2NH)	3297, 3209 (2NH), 3054 (CH-aromatic), 2986 (CH_3), 1758 (C=O), 1564 (C=S), 1552 (C=C)
11a	139	52.6 (CH_2), 127.4, 134.6 (1,3,4-oxadiazole C), 141.6 (CN)	3.39-3.44 (s, 2H, CH_2), 5.17 (s, 2H, D_2O-exchangeable, NH_2), 9.55 (s, 1H, D_2O-exchangeable, NH)	3328-3166 (NH_2, NH), 2877 (CH_2), 2227 (CN), 1563 (C=N)
11b	215	46.9 (CH_2), 124.4, 126.3, 131.5, 133.2, 136.3, 137.9, 139.4, 142.3 (1,3,4-oxadiazole C, C_6H_5), 144.8 (CN)	3.17-3.29 (s, 2H, CH_2), 7.16-7.44 (m, 5H, C_6H_5), 9.38, 9.57 (2s, 2H, D_2O-exchangeable, 2NH)	3363-3214 (2NH), 3052 (CH-aromatic), 2869 (CH_2), 2225 (CN), 1553 (C=C)
12	259	43.2, 48.7 (2CH_2), 125.1, 127.2, 132.6, 136.1, 139.2, 141.7, 143.4, 145.2 (1,3,4-oxadiazole C, C_6H_5), 149.3 (CN), 175.4 (C=O)	3.08, 3.23 (2s, 4H, 2CH_2), 7.41-7.65 (m, 5H, C_6H_5)	3053 (CH-aromatic), 2858 (CH_2), 2228 (CN), 1739 (C=O), 1555 (C=C)
13	227	20.4 (CH_3), 46.4, 49.8, 55.7 (3CH_2), 129.5, 133.7 (1,3,4-oxadiazole C), 151.2 (CN), 179.3 (C=O)	2.05 (t, 3H, CH_3), 3.11, 3.69, 4.31 (2s, q, 6H, 3CH_2)	2955 (CH_3), 2852 (CH_2), 2224 (CN), 1797 (C=O)
14a	315	26.7, 29.5, 32.2 (3CH_2), 55.6, 63.4 (2CH), 127.6, 129.4, 135.2, 140.4, 143.2, 151.1 (thiophene C, 1,3,4-oxadiazole C), 153.7, 158.3 (2CN)	1.98-2.17 (m, 6H, 3CH_2), 4.57, 5.68 (2d, 2H, CH, CH=), 10.86 (s, 1H, D_2O-exchangeable, NH)	3344 (NH), 2861 (CH_2), 2227, 2223 (2CN), 1665 (C=S), 1653 (C=C)
14b	329	25.3, 28.2, 32.7, 37.3 (4CH_2), 53.9, 61.2 (2CH), 123.9, 128.5, 134.4, 138.7, 144.2, 147.2 (thiophene C, 1,3,4-oxadiazole C), 155.7, 159.8 (2CN)	1.83-2.19 (m, 8H, 4CH_2), 4.43, 5.73 (2d, 2H, CH, CH=), 9.45 (s, 1H, D_2O-exchangeable, NH)	3265 (NH), 2874 (CH_2), 2228, 2225 (2CN), 1663 (C=S), 1651 (C=C)

dard drugs was determined as the minimum concentration required to cause a microscopically detectable alteration of normal cell morphology.

Fifty percent of effective concentration or concentration producing 50 % inhibition of virus-induced cytopathic effect (CC_{50}) was determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

DMSO was used as solvent for reference drugs and tested compounds while phosphate buffer saline was used as diluent.

RESULTS AND DISCUSSION

Chemistry

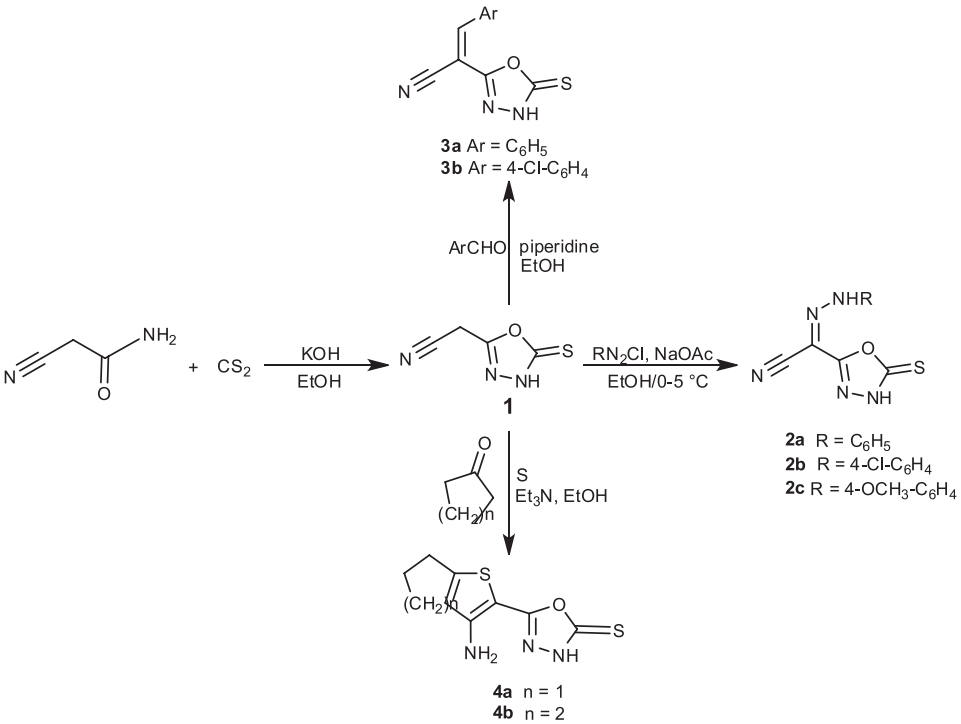
In the framework of our research group (15, 16), we here introduce some novel heterocyclic compounds based on the 1,3,4-oxadiazole ring with potential antiviral and cytotoxic activities. In this article, we report the synthesis of thiophene-, thiazole-, coumarin-, pyridine- and pyridazine-bearing 1,3,4-oxadiazoles.

Cyanoacetic acid hydrazide reacted with carbon disulphide in ethanolic KOH to produce 1,3,4-oxadiazole derivative **1**. The structure of compound **1** was in agreement with ^{1}H NMR, which showed a singlet at δ 3.23-3.37 ppm due to the presence of the methylene group and a singlet at δ 11.72 ppm due to the existence of NH. Mass spectral fragmentation was consistent with the structure of compound **1**. Compound **1** underwent a series of chemical reactions, thus aryldiazonium chlorides reacted with 1,3,4-oxadiazole derivative **1** to produce arylhydrazone derivatives **2a**-**c**. Structures of the latter compounds were verified by elemental analysis and spectral data: in compound **2a,** ^{1}H NMR spectrum indicated the presence of a multiplet at δ 6.85-7.22 ppm which could be assigned to the 5H of the benzene ring and two singlets at δ 8.76 and 10.82 ppm corresponding to two NH groups. In the reaction of compound **1** with arylaldehydes, compounds **3a**,**b** were obtained. Also, compound **1** reacted with either cyclopentanone or cyclohexanone in the presence of elemental sulfur and triethylamine to afford fused thiophene derivatives **4a**,**b**, resp. ^{1}H NMR spectrum of compound **4a** indicated the presence of a multiplet at δ 1.87-2.05 ppm, which could be assigned to three CH_2 groups, a singlet at δ 4.42 ppm, which indicated the presence of the NH_2 group, a singlet at δ 10.65 ppm corresponding to the NH group. Furthermore, ^{13}C NMR spectrum revealed three signals at δ 21.7, 26.2 and 28.9 ppm for the three CH_2 groups in the cyclopentene ring, four signals at δ 130.7, 133.4, 138.7 and 141.5 ppm for the thiophene ring, and two signals at δ 145.6 and 155.3 ppm for the 1,3,4-oxadiazole ring (Scheme 1).

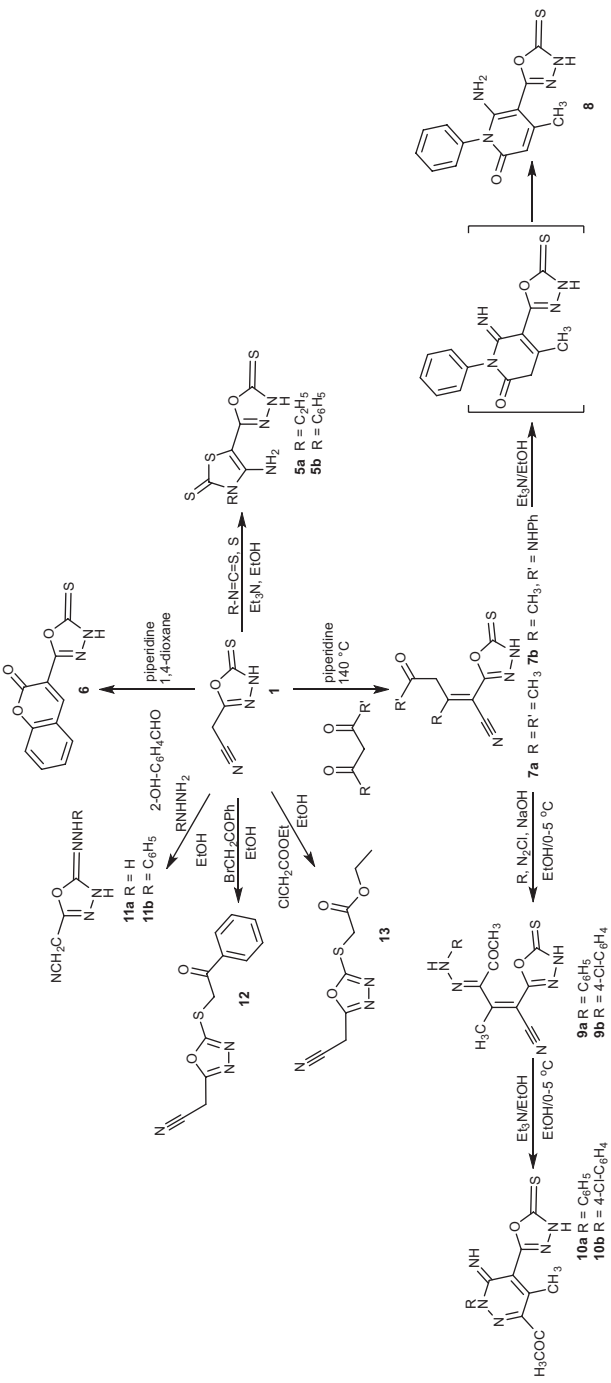
Thiazole derivatives **5a**,**b** were obtained through the reaction of 1,3,4-oxadiazole derivative **1** with either ethylisothiocyanate or phenylisothiocyanate in the presence of elemental sulfur and trimethylamine. Structures of compounds **5a**,**b** were established: in compound **5a**, ^{1}H NMR spectrum showed the presence of a triplet at δ 1.45 ppm corresponding to the CH_3 group, a quartet at δ 4.12 ppm which was assigned to the presence of the CH_2 group, a singlet at δ 4.88 ppm which indicated the presence of the NH_2 group and a singlet at δ 10.28 ppm corresponding to the NH group. In addition, ^{13}C NMR spectrum showed a signal at δ 16.5 ppm for the CH_3 group, a signal at δ 37.8 ppm for the CH_2 group, three signals at δ 135.2, 139.1 and 145.7 ppm were for the thiazole ring and two signals at δ

158.4 and 167.5 ppm for the 1,3,4-oxadiazole ring. Furthermore, the reaction of 1,3,4-oxadiazole derivative **1** with salicylaldehyde afforded coumarin derivative **6**. Also, compound **1** reacted with either acetylacetone or acetoacetanilide to form compounds **7a**,**b**. Their structures were confirmed: ^{1}H NMR spectrum of compound **7a** indicated the presence of two singlets at δ 2.23 and 2.38 ppm assignable to two CH_3 groups, a singlet at δ 4.18 ppm which indicates the presence of the CH_2 group and a singlet at δ 11.13 ppm corresponding to the NH group. Compound **7b** underwent cyclization to afford pyridine derivative **8**. The structure of compound **8** was established according to the IR spectra indicating the presence of two stretching vibration bands for the NH_2 group at ν 3388 and 3316 cm^{-1} in addition to the ^{1}H NMR spectrum indicating the presence of a singlet at δ 1.95 ppm corresponding to the CH_3 group, a singlet at δ 5.23 ppm indicating the NH_2 group, a multiplet at δ 6.92-7.41 ppm which indicated the presence of five H atoms of the benzene ring, and one H of the pyridine ring singlet at δ 9.82 ppm corresponding to the NH group. Moreover, the mass spectrum revealed *m*/*z* at 301 $[M+1]^+$, *m*/*z* at 300 $[M]^+$ and *m*/*z* at 77 $[C_6H_5]^+$ for the phenyl moiety (Scheme 2).

Reaction of compound **7a** with aryldiazonium chlorides afforded compounds **9a**,**b**; the latter products underwent cyclization to produce pyridazine derivatives **10a**,**b**. ^{1}H NMR spectrum of compound **10a** indicated the presence of two singlets at δ 1.88 and 2.34 ppm corresponding to two CH_3 groups, a multiplet at δ 7.17-7.35 ppm which indicated the pres-



Scheme 1


piperidine
1,4-dioxane
6
2-OH-C6H4CHO
RNHNH2
EtOH
11a R = H
11b R = C6H5
BrCH2COPh
EtOH
12
ClCH2COOEt
EtOH
13
R-N=C=S, S
Et3N, EtOH
5a R = C2H5
5b R = C6H5
1
piperidine
140 °C
7a R = R' = CH3
7b R = CH3, R' = NHPh
Et3N/EtOH
8
R, N2Cl, NaOH
EtOH/0-5 °C
9a R = C6H5
9b R = 4-Cl-C6H4
Et3N/EtOH
EtOH/0-5 °C
10a R = C6H5
10b R = 4-Cl-C6H4

Scheme 2

*Table III. Cytotoxicity and antiviral activities of synthesized compounds **1-14b** in CRFK cell cultures against Feline herpes virus and Feline corona virus, in HEL cell cultures against Herpes simplex 1 (KOS), Herpes simplex-2 (G), Vaccinia virus and Herpes simplex-1 (TK-KOS-ACVr) viruses and in HeLa cell cultures against Vesicular stomatitis virus and Coxsackie virus B4*

Compd.	CC_{50} (μmol L^{-1})	IC_{50} (μmol L^{-1})/SI															
		Feline herpes virus		*Feline corona virus*		*Herpes simplex-1* (KOS)		*Herpes simplex-2* (G)		*Vaccinia virus*		*Herpes simplex-1* (TK KOS-ACV)		*Vesicular stomatitis virus*		*Coxsackie virus B4*	
			SI		SI		SI		SI		SI		SI		SI		SI
1	>100	> 100	ND	> 100	ND	> 100	ND	> 100	ND	> 100	ND	> 100	ND	> 100	ND	> 100	ND
2a	> 100	> 100	ND	> 100	ND	> 100	ND	> 100	ND	> 100	ND	> 100	ND	> 100	ND	> 100	ND
2b	> 100	> 100	ND	> 100	ND	> 100	ND	> 100	ND	> 100	ND	> 100	ND	> 100	ND	> 100	ND
2c	44.5	20	2	4	2	> 4	ND	> 4	ND	> 4	ND	> 4	ND	20	2	20	2
3a	57.8	35	3	20	3	4	5	4	5	20	5	20	5	20	2	20	2
3b	39.1	8	3	12	4	4	5	4	5	> 20	ND	> 20	ND	> 20	ND	> 20	ND
4a	18.7	4	4	0.8	2	20	5	20	5	> 20	ND	> 20	ND	> 20	ND	> 20	ND
4b	15.3	20	1	4	3	20	5	20	5	20	5	20	5	20	4	20	4
5a	3.4	0.8	2	0.8	3	2	10	2	10	20	5	20	5	> 0.8	ND	> 0.8	ND
5b	2.2	0.8	2	0.8	3	2	10	2	10	20	5	20	5	> 0.8	ND	> 0.8	ND
6	> 100	> 100	ND	> 100	ND	> 100	> 100	ND	> 100	> 100	ND	> 100	ND	20	4	20	4
7a	> 100	> 100	ND	> 100	ND	> 100	> 100	ND	> 100	> 100	ND	> 100	ND	20	4	20	4
7b	> 100	> 100	ND	> 100	ND	> 100	> 100	ND	> 100	> 100	ND	> 100	ND	20	5	20	5
8	23.9	4	4	12	2	20	5	20	5	20	5	20	5	20	4	20	4
9a	> 100	> 100	ND	> 100	1	> 100	1	> 100	ND	> 100	ND	> 100	ND	> 100	ND	> 100	ND
9b	> 100	> 100	ND	> 100	3	> 100	3	> 100	ND	> 100	ND	> 100	ND	> 100	ND	> 100	ND
10a	21.2	20	3	4	2	20	5	20	5	20	5	20	5	4	25	4	25
10b	25.3	8	3	20	2	20	5	20	5	20	5	20	5	4	25	4	25

11a	30.7	35	2	16	3	> 20	ND	> 20	ND	20	5	20	5	4	4	4	4	4	4
11b	38.3	35	4	12	3	> 20	ND	> 20	ND	4	5	4	5	4	4	4	4	4	4
12	43.4	8	2	20	4	> 20	ND	> 20	ND	> 100	ND	> 100	ND	> 100	ND	ND	> 100	> 100	ND
13	40.8	20	2	20	4	20	5	20	5	4	6	6	5	7.5	10	20	20	20	6
14a	44.9	4	3	12	2	20	5	20	5	4	6	4	6	20	8	20	20	20	5
14b	41.3	20	3	20	4	4	5	4	5	4	5	6	6	20	8	20	20	20	2
GAN	100	5.6	1	100	1	0.03	3333	0.03	3333	100	ND	1	100	100	ND	1	1	1	100
ACY	250	> 100	ND	> 100	1	0.4	625	0.4	625	250	ND	50	5	250	ND	250	250	250	ND
CID	250	> 100	ND	> 100	2	250	2	125	2	10	25	10	25	250	ND	250	250	250	ND
RIB	250	ND	ND	ND	ND	ND	ND	ND	ND	250	ND	50	5	18.4	14	68.2	68.2	68.2	4
DMSO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ACY – acyclovir, CC_{50} – 50 % cytotoxic concentration, CID – cidofovir, DMSO 0.5 % (negative control), GAN – ganciclovir, IC_{50} – 50 % inhibition concentration, ND – not detected, RIB – ribavirin, SI – selectivity index

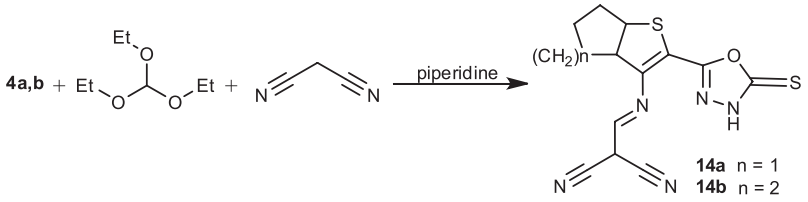
ence of five hydrogens of the benzene ring, two singlets at δ 9.23 and 9.86 ppm corresponding to two NH groups. When 1,3,4-oxadiazole derivative **1** reacted with hydrazine derivatives, hydrazone derivatives **11a**,**b** were obtained. ^{1}H NMR spectrum of compound **11a** indicated the presence of a singlet at δ 3.39-3.44 ppm corresponding to the CH_2 group, a singlet at δ 5.17 ppm which indicated the presence of NH_2, a singlet at δ 9.55 ppm corresponding to the NH group.

Furthermore, compound **1** reacting with either bromoacetophenone or ethyl chloroacetate afforded compounds **12** and **13**, resp. Their structures were in agreement with analytical and spectral data (Scheme 2).

Finally, the reaction of fused thiophene derivatives **4a**,**b** with a mixture of triethyl orthoformate and malononitrile afforded compounds **14a**,**b**. IR spectra indicated the absence of stretching vibration bands of the NH_2 group in compound **14a**. ^{1}H NMR spectrum indicated the presence of a multiplet at δ 1.98-2.17 ppm which could be assigned to three CH_2 groups, two doublets at δ 4.57 and 5.68 ppm which indicated the presence of two hydrogens (CH, CH=), a singlet at δ 10.86 ppm corresponding to the NH group (Scheme 3).

Antiviral activity and QSAR

Compounds **5a**,**b** showed the highest antiviral activities against *Feline herpes virus*, *Feline corona virus*, *Herpes simplex virus-1* and *Herpes simplex virus-2*, which may be due to the presence of the amino thiazole moiety. Compounds **4a**, **10a**,**b** and **14a**,**b** exhibited moderate antiviral activities, while the remaining compounds did not show any appreciable activities comparable with the reference standards, ganciclovir, acyclovir and cidofovir (Table III).



Scheme 3

As far as *Vaccinia virus* and *Herpes simplex virus* (TK-KOS-ACVr), *Coxsackie virus B4* and *Vesicular stomatitis viruses* are concerned, compound **11b** was considered the most active with respect to reference standards ganciclovir, acyclovir, cidofovir and ribavirin. This may be due to the presence of the phenyl hydrazonoyl group. Compounds **13** and **14a**,**b** were considered to be more effective products among the compounds; this may be due to the existence of ethylthioacetate and iminomethyl malononitrile groups, resp. The other tested compounds exhibited very low activity. In regard to cytotoxic activity, compounds **5a**,**b** showed high cytotoxicity, which may due to the presence of the aminothiazole moiety. Further, compounds **4a**,**b** were considered as more cytotoxic agents compared to the other compounds; this may be due to the presence of the amino group on the fused thiophene ring. The other compounds acted as weak or inactive cytotoxic agents (Table III).

CONCLUSIONS

In this article, the synthesized 1,3,4-oxadiazoles containing thiophene, thiazole, coumarin, pyridine and pyridazine moieties were evaluated as cytotoxic and antiviral agents. Among the newly synthesized compounds, thiazole derivatives **5a**,**b** were proven to be the most active antiviral agents against *Feline corona virus*, *Feline herpes virus*, *Herpes simplex virus-1* and *Herpes simplex virus-2*. Compound **11b** was proven as the most effective compound against *Vaccinia virus*, *Herpes simplex virus* (TKKOSACVr), *Vesicular stomatitis viruses* and *Coxsackie virus B4 versus* reference standards (ganciclovir, acyclovir, cidofovir and ribavirin). Among the remaining compounds, compounds **13** and **14a**,**b** were found most effective.

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Supplementary materials are available upon request.

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