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SYNTHESIS OF NOVEL 1,3,4-OXADIAZOLE DERIVATIVES AND THEIR NUCLEOSIDE ANALOGS WITH ANTIOXIDANT AND ANTITUMOR ACTIVITIES

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A series of new (1,3,4-oxadiazol-2-yl)-1H-benzo[h]quinolin-4-one derivatives were synthesized, including glucose and xylose hydrazones that were obtained by the reaction of hydrazides with monosaccharides. Cyclization of the sugar hydrazones with acetic anhydride afforded substituted oxadiazoline derivatives. The newly synthesized compounds were evaluated for their antioxidant properties and cytotoxicity, and showed moderate to high activities.

Keywords: acyclic nucleosides, 1,3,4-oxadiazoles, sugar hydrazones, antioxidant activity, cytotoxicity.

A significant part of drug discovery efforts in the past few years has been focused on prevention or treatment of cancer. This is not surprising because in most developed countries, and to an increasing extent, cancer is among the three most common causes of death. Among the five-membered nitrogen heterocycles, 1,3,4-oxadiazoles are associated with a broad spectrum of biological activities. The 1,3,4-oxadiazole ring has been found in the structures of fungicidal, bactericidal, analgesic, antipyretic, antiphlogistic, anticompulsive, anti-inflammatory, paralytic, hypnotic, and sedative agents [1–3]. Several 1,3,4-oxadiazole derivatives have been shown to possess insecticidal, hypoglycemic, hypotensive, antiviral [4], as well as antitumor activities [5]. Certain 1,3,4-oxadiazoline-2(3*H*)-thiones are known to possess antibacterial, antifungal, antimicrobial, and antiviral activities [4], as well as tyrosinase-inhibiting effect [6]. Nucleosides and their analogs possess a wide range of medicinal properties, including antibiotic, antiviral, and antitumor activity [7–16]. Consequently, and considering the possible enhancement of biological activity resulting from the attachment of carbohydrate moieties to 1,3,4-oxadiazole heterocycles, our attention was turned to the synthesis of new 1,3,4-oxadiazole derivatives and their nucleoside analogs [17, 18].

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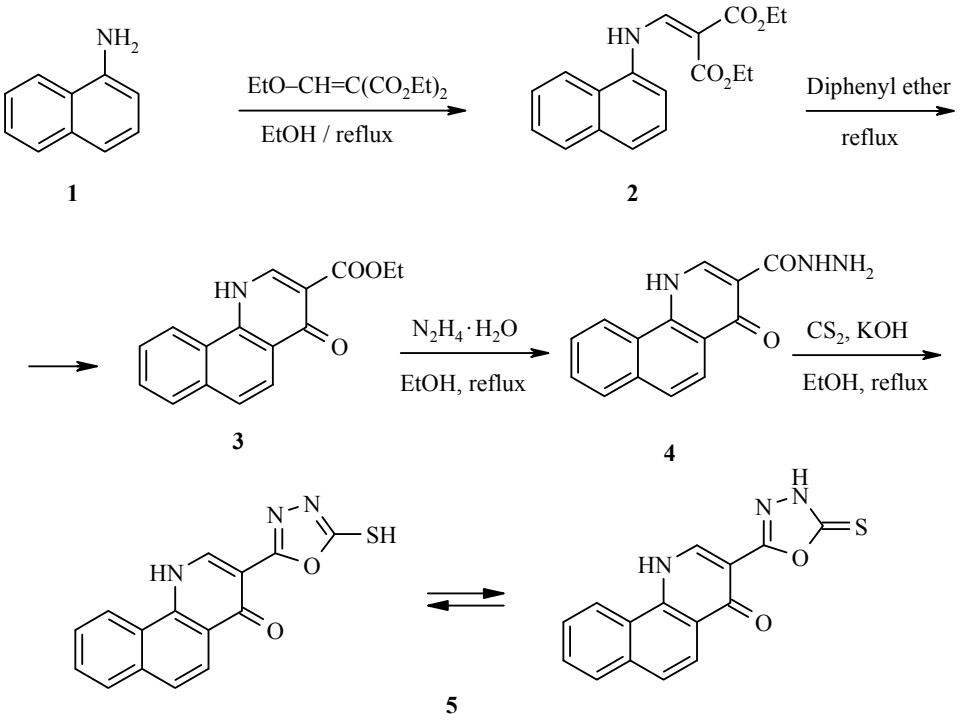
The starting 1-aminonaphthalene (**1**) was refluxed with diethyl ethoxymethylenemalonate in ethanol to give 2-[(naphthalen-1-ylamino)methylidene]malonic acid diethyl ester (**2**) in high yield. Compound **2** underwent intramolecular cyclization in boiling diphenyl ether to give 4-oxo-1,4-dihydrobenzo-[*h*]quinoline-3-carboxylic acid ethyl ester (**3**). Treatment of ester **3** with hydrazine hydrate gave the corresponding acid hydrazide **4**. Its structure was proved by means of IR, ^{1}H NMR, and mass spectra, which all agreed with the assigned structure. Reaction of compound **4** with carbon disulfide in the presence of anhydrous potassium hydroxide gave oxadiazole derivative **5** (Scheme 1).

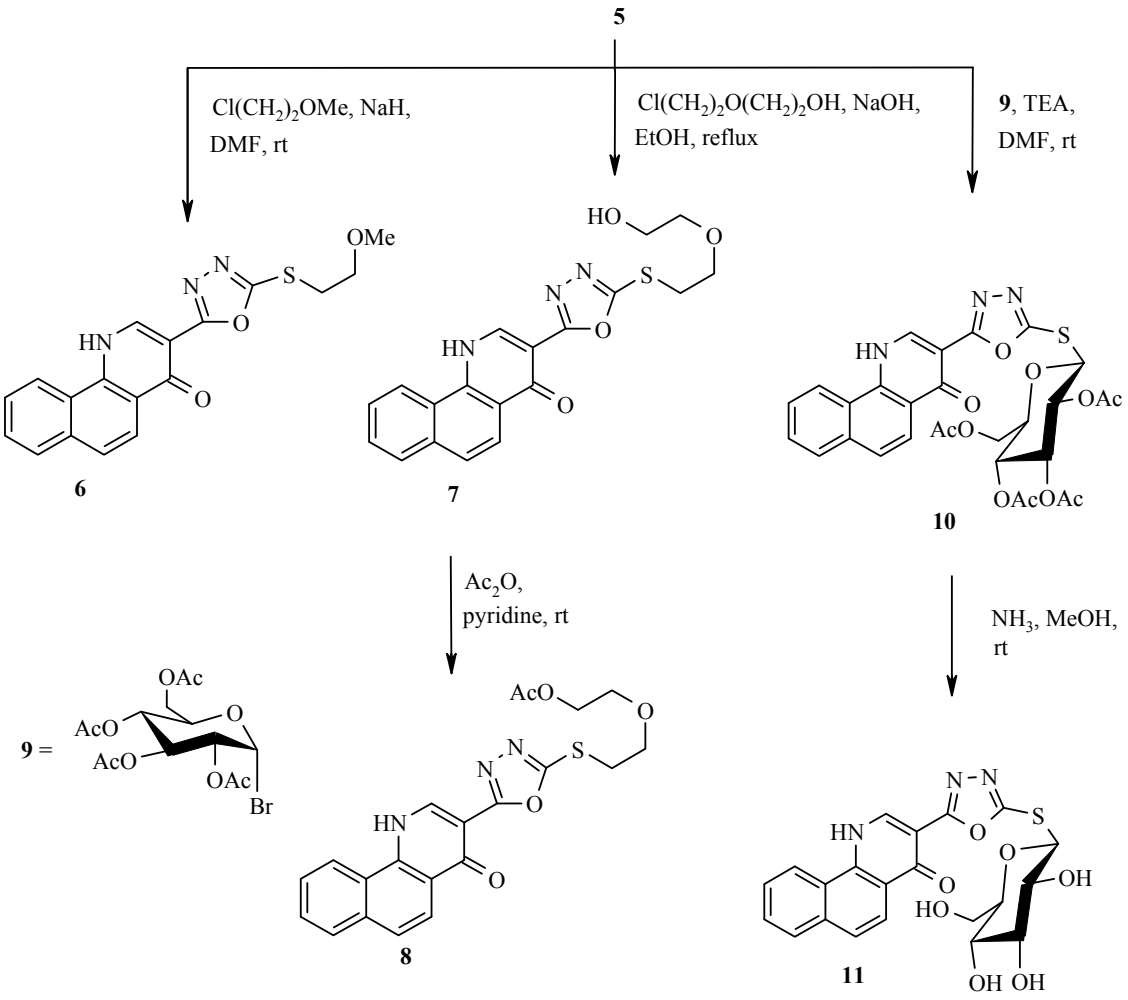
Scheme 1



When compound **5** was deprotonated with sodium hydride in DMF it reacted with 2-chloroethyl methyl ether at room temperature to afford 3-[5-(2-methoxyethylsulfanyl)-1,3,4-oxadiazol-2-yl]benzo[*h*]quinolin-4(1*H*)-one (**6**). The reaction of compound **5** with 2-chloroethoxyethanol in refluxing ethanolic sodium hydroxide solution afforded 3-{5-[2-(2-hydroxyethoxy)ethylsulfanyl]-1,3,4-oxadiazol-2-yl}benzo[*h*]quinolin-4(1*H*)-one (**7**) in 94% yield. Compound **7** was acetylated with acetic anhydride in the presence of pyridine to give compound **8**. Compound **5** also reacted with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**9**) in DMF in the presence of TEA at room temperature. The reaction of the resulting β-thioglycoside **10** with methanolic ammonia solution at room temperature afforded the corresponding deacetylated compound **11** (Scheme 2).

The reaction of oxadiazole derivative **5** with ethyl chloroacetate in DMF in the presence of anhydrous K_2CO_3 at room temperature afforded the corresponding thioacetate **12** (Scheme 3). The ^{1}H NMR spectrum of ester **12** showed the signal of SCH_2 protons at 4.31 ppm. The reaction of the ester **12** with hydrazine hydrate in ethanol at reflux temperature gave the corresponding hydrazide **13**. The hydrazide **13** reacted with monosaccharides galactose and xylose to give sugar hydrazones **14a**,**b**. The IR spectra for hydrazones **14a**,**b** showed characteristic absorption bands at 3410–3415 cm^{-1} corresponding to the free hydroxyl groups of sugar. The sugar hydrazones **14a**,**b** reacted with acetic anhydride in pyridine at room temperature to afford the corresponding acetate derivatives **15a**,**b**. The IR spectra of compounds **15a**,**b** showed characteristic absorption bands at 1737–1743 cm^{-1} corresponding to the acetyl carbonyl groups, indicating the presence of *O*-acetyl group.

Scheme 2



The ^{1}H NMR spectrum of compound **15a** showed the signals of acetyl groups at 1.95–2.16 ppm. Sugar hydrazones **14a**,**b** were cyclized with acetic anhydride at reflux temperature to give 1,3,4-oxadiazoline derivatives **16a**,**b** (Scheme 3).

The synthesized compounds were tested for their antioxidant activity measured as the ability of the newly synthesized compounds to react with the preformed radical cation of 2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS) [19]. L-Ascorbic acid was used as a control. The results of our preliminary screening indicated that compounds **4**, **12**, and **13** showed the highest activity (inhibition of the radical cation) among this series of tested compounds with an effective concentration of 0.2 μM, followed by compounds **5** and **16b**. Compounds **10** and **11** showed moderate activity, while the other tested compounds **2**, **3**, **6–8**, **15a**,**b**, and **16a** exhibited less activity (Table 1).

TABLE 1. Antioxidant Activity of Tested Compounds by ABTS Method

Compound	Inhibition, %	Compound	Inhibition, %	Compound	Inhibition, %
2	0.00	**8**	6.49	**15a**	18.56
3	6.99	**10**	55.89	**15b**	22.95
4	72.05	**11**	52.49	**16a**	7.19
5	68.26	**12**	79.04	**16b**	68.26

By relating of the antioxidant activity to the compound structure, we conclude that the carbohydrazide or thioacetohydrazide residue attached to the benzo[*h*]quinolin-4-one, as well as (1,3,4-oxadiazol-2-yl)-1*H*-benzo[*h*]quinolin-4-one derivatives increase the antioxidant activity.

Cytotoxicity was expressed as the concentration that caused approximately 50% loss of the cell monolayer (IC_{50}). The assay was used to examine the newly synthesized compounds. 5-Fluorouracil as a standard anticancer drug was used for comparison [20–22]. The results of our preliminary screening indicated that compound **5** showed a strong cytotoxicity. Compounds **4**, **12**, **13**, and **16b** showed moderate cytotoxicity, while other compounds showed a weak to very weak cytotoxicity (Table 2).

Scheme 3

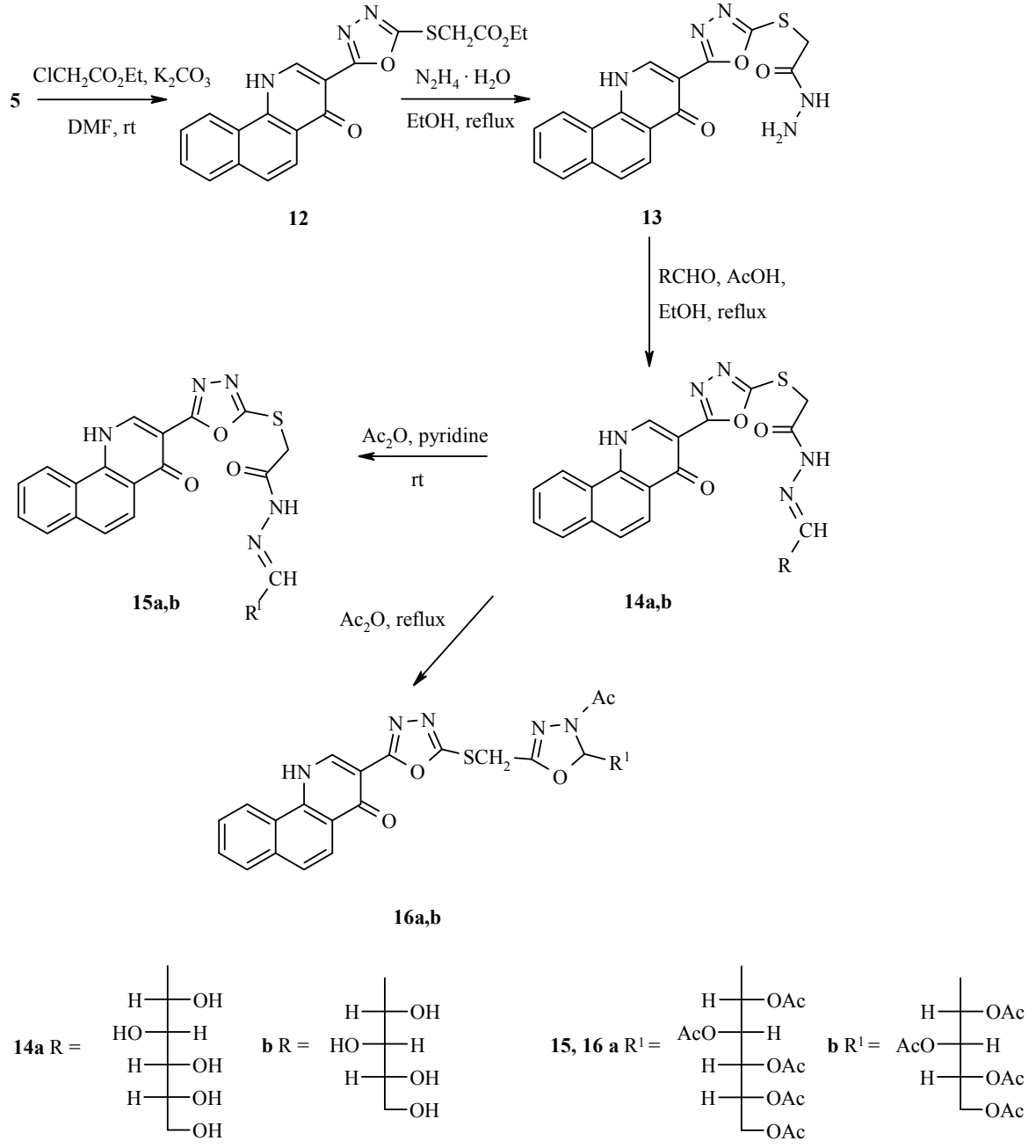


TABLE 2. Cytotoxicity of the Synthesized Compounds **2–8, 10–13, 15a,b, 16a,b** on Different Cell Lines

Compound	IC_{50}, μg/ml*			
	HepG2	WI-38	VERO	MCF-7
2	70.2	84.6	72.4	77.5
3	91.2	82.4	80.3	79.2
4	45.1	48.2	20.5	20.2
5	22.4	27.3	25.8	40.8
6	125.4	98.7	132.4	107.3
7	145.6	175.0	169.8	166.8
8	89.8	100.7	110.2	121.4
10	52.8	70.4	60.7	63.7
11	61.4	60.9	65.4	66.9
12	40.7	39.8	36.9	31.7
13	40.6	39.4	37.8	39.8
15a	106.8	98.9	109.9	170.9
15b	111.2	89.9	100.7	109.9
16a	130.8	130.2	150.8	140.8
16b	39.9	37.5	40.5	42.6
5-Fluorouracil	8.6	3.2	6.5	2.3

* IC_{50}: 1–10 (very strong), 11–25 (strong), 26–50 (moderate), 51–100 (weak), 100–200 (very weak), above 200 μg/ml (noncytotoxic).

By relating the antioxidant activity to the compound structure, we conclude that the carbohydrazide or thioacetohydrazide residue attached to the benzo[*h*]quinolin-4-one, as well as (1,3,4-oxadiazol-2-yl)-1*H*-benzo[*h*]quinolin-4-one derivatives, increase the antioxidant activity.

Cytotoxicity was expressed as the concentration that caused approximately 50% loss of the cell monolayer (IC_{50}). The assay was used to examine the newly synthesized compounds. 5-Fluorouracil as a standard anticancer drug was used for comparison [20–22]. The results of our preliminary screening indicated that compound **5** showed a strong cytotoxicity. Compounds **4**, **12**, **13**, and **16b** showed moderate cytotoxicity, while other compounds showed a weak to very weak cytotoxicity (Table 2).

In conclusion, new 1,3,4-oxadiazole acyclic nucleoside analogs were synthesized. Cyclization of the sugar hydrazones with acetic anhydride afforded substituted oxadiazoline derivatives. The newly synthesized compounds were evaluated for their antioxidant, cytotoxicity, and antitumor activities.

EXPERIMENTAL

The IR spectra were recorded on a Perkin-Elmer model 1720 FTIR spectrometer in KBr disc (compounds **2**, **3**, **5**–**8**, **10**–**14a,b**, **15b**, **16a,b**) or thin film (compounds **4**, **15a**). The ^{1}H NMR spectra were recorded on a Varian Gemini 300 NMR spectrometer at 300 MHz in DMSO-d_6 (internal standard TMS). EI mass spectra were recorded on a Varian MAT 311A spectrometer. Elemental analyses were carried out at the Microanalytical Center of Cairo University, Giza, Egypt. All melting points were determined with a Kofler block apparatus and are uncorrected.The progress of the reactions was monitored by TLC using silica gel plates 60 F 245.

Diethyl 2-[(naphthalen-1-ylamino)methylidene]malonate (**2**) was synthesized by modifying a method described in the literature [23]. Ethyl 4-oxo-1,4-dihydrobenzo[*h*]quinoline-3-carboxylate (**3**) was synthesized following a published method [24].

4-Oxo-1,4-dihydrobenzo[*h*]quinoline-3-carbohydrazide (4). To a solution of compound **3** (2.67 g, 0.01 mol) in ethanol (50 ml), hydrazine hydrate (1.5 g, 0.03 mol) was added. The reaction mixture was refluxed for 12 h and cooled to room temperature. The precipitate was filtered off, dried, and recrystallized from ethanol to give the benzoquinoline derivative **4**. Yield 2.12 g (84%); mp 310–312°C. IR spectrum, ν, cm^{-1}: 3340 (NH_2), 3279 (NH), 3178 (NH), 1677 (CONH), 1654 (C=O). ^{1}H NMR spectrum, δ, ppm: 4.65 (2H, s, NH_2); 7.82–8.27 (6H, m, H Ar); 8.72 (2H, s, NH, H-2); 10.85 (1H, s, NH). Mass spectrum, *m/z* (I_{rel}, %): 253 $[M]^+$ (37), 222 (91), 166 (37), 139 (100), 76 (19). Found, %: C 66.36; H 4.38; N 16.43. $C_{14}H_{11}N_3O_2$. Calculated, %: C 66.40; H 4.38; N 16.59.

3-(5-Sulfanyl-1,3,4-oxadiazol-2-yl)benzo[*h*]quinolin-4(1*H*)-one (5). To a solution of compound **4** (2.53 g, 0.01 mol) in absolute ethanol (50 ml), a solution of potassium hydroxide (0.56 g, 0.01 mol) in water (2 ml) was added. Carbon disulfide (5 ml) was then added, and the reaction mixture was refluxed for 20 h. The solvent was evaporated, and the residue was dissolved in water. The solution was filtered and acidified with dilute HCl. The precipitate was filtered, washed with water, and recrystallized from ethanol. Yield 2.45 g (83%); mp 330–332°C. IR spectrum, ν, cm^{-1}: 3139 (NH), 1629 (C=O), 1376 (C=S). ^{1}H NMR spectrum, δ, ppm: 7.75-8.27 (6H, m, H Ar); 8.55 (1H, s, H-2); 8.84 (1H, s, NH); 13.39 (1H, s, NH). Mass spectrum, *m/z* (I_{rel}, %): 295 $[M]^+$ (2), 267 (65), 221 (100), 193 (25), 149 (43). Found, %: C 61.00; H 3.07; N 14.21. $C_{15}H_9N_3O_2S$. Calculated, %: C 61.01; H 3.07; N 14.23.

3-[5-(2-Methoxyethylsulfanyl)-1,3,4-oxadiazol-2-yl]benzo[*h*]quinolin-4(1*H*)-one (6). To a solution of compound **5** (2.95 g, 0.01 mol) in DMF (15 ml), NaH (0.24 g, 0.01 mol) was added. The mixture was stirred at room temperature for 2 h. Chloroethyl methyl ether (0.95 g, 0.01 mol) was then added. The reaction mixture was stirred at room temperature for 30 h, and then poured into ice water. The product was extracted using ethyl acetate. The solvent was then evaporated under reduced pressure to give compound **6**. Yield 0.96 g (27%); mp 200–202°C. IR spectrum, ν, cm^{-1}: 3191 (NH), 1633 (C=O). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 3.47 (3H, s, CH_3); 3.51 (2H, t, J = 5.5, CH_2); 3.72 (2H, t, J = 5.5, CH_2); 7.79–8.23 (6H, m, H Ar); 8.57 (1H, s, H-2); 8.87 (1H, s, NH). Found, %: C 61.09; H 4.14; N 11.74. $C_{18}H_{15}N_3O_3S$. Calculated, %: C 61.18; H 4.28; N 11.89.

3-{5-[2-(2-Hydroxyethoxy)ethylsulfanyl]-1,3,4-oxadiazol-2-yl}benzo[*h*]quinolin-4(1*H*)-one (7). To a solution of compound **5** (2.95 g, 0.01 mol) in absolute ethanol (15 ml), NaOH (0.4 g, 0.01 mol) was added, and the mixture was stirred at room temperature for 1 h. 2-(2-Chloroethoxy)ethanol (1.24 g, 0.01 mol) was then added, and the reaction mixture was refluxed for 30 h. The solvent was evaporated under reduced pressure. The resulting solid product was recrystallized from ethanol. Yield 3.60 g (94%); mp 80–82°C. IR spectrum, ν, cm^{-1}: 3400 (OH, NH), 1631 (C=O), 1588 (C=N), 1484 (C–O–C). ^{1}H NMR spectrum, δ, ppm: 3.64 (4H, m, $2CH_2$); 3.79 (4H, m, $2CH_2$); 4.70 (1H, s, OH); 7.61–8.26 (6H, m, H Ar); 8.72 (1H, s, H-2); 9.02 (1H, s, NH). Mass spectrum, *m/z* (I_{rel}, %): 383 $[M]^+$ (7), 279 (12), 167 (39), 149 (100). Found, %: C 59.48; H 4.33; N 10.84. $C_{19}H_{17}N_3O_4S$. Calculated, %: C 59.52; H 4.47; N 10.96.

2-{2-[5-(4-Oxo-1,4-dihydrobenzo[*h*]quinolin-3-yl)-1,3,4-oxadiazol-2-ylsulfanyl]-ethoxy}ethyl Acetate (8). To a solution of compound **7** (3.83 g, 0.01 mol) in pyridine (7 ml), acetic anhydride (1.02 g, 0.01 mol) was added, and the reaction mixture was stirred at room temperature for 16 h. The resulting solution was poured into crushed ice, and the product was filtered off, washed with water, and dried. Yield 2.30 g (54%); mp 214–216°C. IR spectrum, ν, cm^{-1}: 3184 (NH), 1735 ($COCH_3$), 1629 (C=O), 1553 (C=N). Found, %: C 59.14; H 4.39; N 9.71. $C_{21}H_{19}N_3O_5S$. Calculated, %: C 59.28; H 4.50; N 9.88.

3-{5-[(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)sulfanyl]-1,3,4-oxadiazol-2-yl}benzo[*h*]quinolin-4(1*H*)-one (10). To a solution of compound **5** (2.95 g, 0.01 mol) in DMF, compound **9** (4.11 g, 0.01 mol) and TEA (0.5 ml) were added. The reaction mixture was stirred at room temperature for 30 h. The solution was poured into crushed ice, and the product was filtered off, washed with water, and dried. Yield 3.94 g (63%); mp 250–252°C. IR spectrum, ν, cm^{-1}: 3226 (NH), 1751 ($COCH_3$), 1635 (C=O), 1554 (C=N). ^{1}H NMR

spectrum, δ, ppm (*J*, Hz): 1.90–2.06 (12H, m, 4$COCH_3$); 4.07–4.19 (2H, m, H-6'); 5.04 (1H, m, H-5'); 5.07 (1H, m, H-4'); 5.20 (1H, m, H-3'); 5.52 (1H, m, H-2'); 5.74 (1H, d, *J* = 9.8, H-1'); 7.69–8.22 (6H, m, H Ar); 8.50 (1H, s, H-2); 8.76 (1H, s, NH). Found, %: C 55.54; H 4.35; N 6.60. $C_{29}H_{27}N_3O_{11}S$. Calculated, %: C 55.68; H 4.35; N 6.72.

3-{5-[β-D-Glucopyranosyl)sulfanyl]-1,3,4-oxadiazol-2-yl}benzo[*h*]quinolin-4(1*H*)-one (11). A solution of compound **10** (6.25 g, 0.01 mol) in methanolic ammonia solution (methanol–34% ammonia, 30:50 ml) was stirred at room temperature for 10 h. The solvent was then evaporated under reduced pressure, and the product was obtained as a solid powder. Yield 4.21 g (92%); mp 224–226°C. IR spectrum, ν, cm^{-1}: 3373 (OH, NH), 1632 (C=O), 1559 (C=N). Found, %: C 55.03; H 4.19; N 9.09. $C_{21}H_{19}N_3O_7S$. Calculated, %: C 55.14; H 4.19; N 9.19.

Ethyl {[5-(4-Oxo-1,4-dihydrobenzo[*h*]quinolin-3-yl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetate (12). To a solution of compound **5** (2.95 g, 0.01 mol) in DMF (10 ml), anhydrous K_2CO_3 (1.38 g, 0.01 mol) was added and the mixture was stirred at room temperature for 2 h. Ethyl chloroacetate (1.23 g, 0.01 mol) was then added, and the reaction mixture was stirred at room temperature for 3 h. The resulting mixture was poured into crushed ice, and the formed precipitate was filtered off, dried, and recrystallized from ethanol. Yield 3.51 g (92%); mp 204-206°C. IR spectrum, ν, cm^{-1}: 3313 (NH), 1739 (COOEt), 1630 (C=O), 1587 (C=N). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.24 (3H, t, *J* = 5.5, CH_3); 4.20 (2H, q, *J* = 5.5, CH_2); 4.31 (2H, s, SCH_2); 7.79–8.28 (6H, m, H Ar); 8.69 (1H, s, H-2); 8.87 (1H, s, NH). Found, %: C 59.73; H 3.84; N 11.02. $C_{19}H_{15}N_3O_4S$. Calculated, %: C 59.83; H 3.96; N 11.02.

2-{[5-(4-Oxo-1,4-dihydrobenzo[*h*]quinolin-3-yl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetohydrazide (13). To a solution of compound **12** (3.81 g, 0.01 mol) in ethanol (50 ml), hydrazine hydrate (1.5 g, 0.03 mol) was added, and the mixture was refluxed for 7 h. The reaction mixture was left to cool. The formed solid product was filtered off, dried, and recrystallized from ethanol. Yield 2.28 g (62%); mp 280–282°C. IR spectrum, ν, cm^{-1}: 3417 (NH_2), 3199 (NH), 1689 (CONH). Mass spectrum, *m/z* (I_{rel}, %): 367 $[M]^+$ (19). Found, %: C 55.44; H 3.43; N 19.06. $C_{17}H_{13}N_5O_3S$. Calculated, %: C 55.58; H 3.57; N 19.06.

Synthesis of Oxadiazole Sugar Hydrazone Derivatives 14a,b (General Method). To a solution of compound **13** (3.67 g, 0.01 mol) in absolute ethanol (30 ml), the solution of the respective monosaccharide (0.01 mol) in water (2 ml) and glacial acetic acid (0.5 ml) were added. The reaction mixture was refluxed for 25 h, concentrated, and left to cool. The formed precipitate was filtered, washed with ethanol, dried, and crystallized from ethanol to give compounds **14a**,**b**.

***N′*-(D-Galactopentitolylmethylidene)-2-{[5-(4-oxo-1,4-dihydrobenzo[*h*]quinolin-3-yl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetohydrazide (14a)**. Yield 4.23 g (80%); mp 320–322 °C. IR spectrum, ν, cm^{-1}: 3415 (OH), 3386 (NH), 1680 (CONH), 1527 (N=C). Found, %: C 52.07; H 4.23; N 13.12. $C_{23}H_{23}N_5O_8S$. Calculated, %: C 52.17; H 4.38; N 13.23.

2-{[5-(4-Oxo-1,4-dihydrobenzo[*h*]quinolin-3-yl)-1,3,4-oxadiazol-2-yl]sulfanyl}-*N′*-(D-xylotetritolylmethylidene)acetohydrazide (14b). Yield 4.39 g (88%); mp 340–342 °C. IR spectrum, ν, cm^{-1}: 3410 (OH, NH), 1633 (CONH). Found, %: C 52.80; H 4.24; N 14.02. $C_{22}H_{21}N_5O_7S$. Calculated, %: C 52.90; H 4.24; N 14.02.

Synthesis of Acetylated Oxadiazole Sugar Hydrazone Derivatives 15a,b (General Method). To a solution of compounds **14a**,**b** (0.01 mol) in pyridine (7 ml), acetic anhydride (0.01 mol) was added, and the reaction mixture was stirred at room temperature for 40 h. The resulting solution was poured into crushed ice, and the product was extracted using chloroform. The chloroform extracts were collected and evaporated under reduced pressure to give compounds **15a**,**b**.

2-{[5-(4-Oxo-1,4-dihydrobenzo[*h*]quinolin-3-yl)-1,3,4-oxadiazol-2-yl]sulfanyl}-*N′*-(2,3,4,5,6-penta-*O*-acetyl-D-galactopentitolylmethylidene)acetohydrazide (15a). Yield 5.40 g (73%); mp 350–352°C. IR spectrum, ν, cm^{-1}: 3448 (NH), 1743 ($COCH_3$). ^{1}H NMR spectrum, δ, ppm: 1.95, 2.02, 2.10, 2.13, 2.16 (15H, 5s, 5CH_3CO); 4.09 (2H, m, OCH_2); 4.50 (1H, m, H-5'); 4.60 (2H, s, SCH_2); 4.71 (1H, m, H-4'); 5.18 (1H, m, H-3');

5.48 (1H, m, H-2'); 7.40–7.79 (7H, m, H Ar, H-1'); 7.94 (1H, s, H-2); 9.35 (2H, s, 2NH). Found, %: C 53.48; H 4.50; N 9.40. $C_{33}H_{33}N_5O_{13}S$. Calculated, %: C 53.58; H 4.50; N 9.47.

2-{[5-(4-Oxo-1,4-dihydrobenzo[*h*]quinolin-3-yl)-1,3,4-oxadiazol-2-yl]sulfanyl}-N'-(2,3,4,5-tetra-*O*-acetyl-D-xylotetritolylmethylidene)acetohydrazide (15b). Yield 5.00 g (75%); mp 325–327°C. IR spectrum, ν, cm^{-1}: 3430 (NH), 1737 ($COCH_3$), 1632 (CONH). Found, %: C 53.86; H 4.33; N 10.35. $C_{30}H_{29}N_5O_{11}S$. Calculated, %: C 53.97; H 4.38; N 10.49.

Synthesis of Oxadiazole–oxadiazoline Derivatives 16a,b (General Method). A solution of **14a,b** (0.01 mol) in acetic anhydride (15 ml) was refluxed for 30 h. The resulting solution was poured into crushed ice, and the product was extracted using chloroform to afford compounds **16a,b**.

3-[5-({[4-Acetyl-5-(1,2,3,4,5-penta-*O*-acetyl-D-galactopentitolyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl]-methyl}sulfanyl)-1,3,4-oxadiazol-2-yl]benzo[*h*]quinolin-4(1*H*)-one (16a). Yield 5.23 g (67%); mp 300-302°C. IR spectrum, ν, cm^{-1}: 3434 (NH), 1745 ($COCH_3$), 1628 (C=O), 1505 (C=N). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.95, 1.96, 2.01, 2.10, 2.19, 2.39 (18H, 6s, $6CH_3CO$); 3.87 (2H, s, SCH_2); 3.98–4.05 (2H, m, OCH_2); 4.96 (1H, m, H-4'); 5.16 (1H, m, H-3'); 5.20 (1H, m, H-2'); 5.37 (1H, dd, $J = 3.2$, $J = 6.2$, H-1'); 5.95 (1H, d, $J = 6.2$, oxadiazoline H-5); 7.04 (1H, s, H-2); 7.71–7.80 (6H, m, H Ar); 8.70 (1H, s, NH). Found, %: C 53.66; H 4.40; N 8.91. $C_{35}H_{35}N_5O_{14}S$. Calculated, %: C 53.77; H 4.51; N 8.96.

3-[5-({[4-Acetyl-5-(1,2,3,4-tetra-*O*-acetyl-D-xylotetritolyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl]methyl}-sulfanyl)-1,3,4-oxadiazol-2-yl]benzo[h]quinolin-4(1*H*)-one (16b). Yield 3.83 g (54%); mp 323–325°C. IR spectrum, ν, cm^{-1}: 3430 (NH), 1739 ($COCH_3$), 1503 (C=N). Found, %: C 54.09; H 4.40; N 9.77. $C_{32}H_{31}N_5O_{12}S$. Calculated, %: C 54.16; H 4.40; N 9.87.

Antioxidant Screening Assay Method [19]. For each of the investigated compounds, 2 ml of ABTS solution (60 mM) was added to MnO_2 suspension (25 mg/ml), all prepared in phosphate buffer (pH 7, 0.1 M). The mixture was shaken, centrifuged, and filtered, and the absorbance ($A_{control}$) of the resulting green-blue solution (ABTS radical solution) was adjusted to ~0.5 at λ 734 nm. Then, 50 μl of (2 mM) solution of the test compound in spectroscopic grade methanol/phosphate buffer (1:1) was added. The absorbance (A_{test}) was measured, and the reduction in color intensity was expressed as % inhibition, which was calculated for each compound from the following equation:

$$\text{Inhibition} = (A_{control} - A_{test}/ A_{control}) \times 100\%$$

Ascorbic acid was used as standard antioxidant (positive control). A blank sample was run without ABTS and using methanol–phosphate buffer (1:1) instead of the sample. A negative control sample was run with methanol–phosphate buffer (1:1) instead of the tested compound.

Cytotoxic Activity [20–22]. RPMI-1640 medium (Sigma Co., St. Louis, USA), Fetal bovine serum (GIBCO, UK) and the cell lines from ATCC were used.

The cytotoxic activity of the synthesized compounds was tested against human hepatocellular liver carcinoma cell line (HepG2), human lung fibroblast cell line (WI 38), human Caucasian breast adenocarcinoma cell line (MCF-7), and normal adult African green monkey kidney cell line (VERO). The stock samples of the compounds were diluted with RPMI-1640 medium to desired concentrations ranging from 10 to 1000 μg/ml. The final concentration of DMSO in each sample did not exceed 1% v/v.

The cells were batch-cultured for 10 days, then seeded in 96-well plates of 10×10^3 cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37°C for 24 h under 5% CO_2 using a water-jacketed carbon dioxide incubator (Shedon.TC2323.Cornelius, OR, USA). The medium (without serum) was added, and cells were incubated either alone (negative control) or with different concentrations of the sample to give final concentrations of 1000, 500, 200, 100, 50, 20, and 10 μg/ml. Cells were suspended in RPMI-1640 medium, 1% antibiotic-antimycotic mixture (10^4 μg/ml penicillin potassium, 10^4 μg/ml streptomycin sulfate and 25 μg/ml amphotericin B) and 1% L-glutamine in 96-well flat bottom microplates at 37°C under 5% CO_2. After 96 h of incubation, the medium was again aspirated; the trays were inverted onto a pad of paper towels, and the

remaining cells rinsed carefully with medium and fixed with 3.7% (v/v) formaldehyde in saline for at least 20 min. The fixed cells were rinsed with water. The % viability of cells was examined visually as described previously [25].

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