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Synthesis and anticancer activity of novel quinazolinone and benzamide derivatives

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Abstract In trying to develop new anticancer agents, a series of quinazolinone and benzamide derivatives were synthesized via reaction of 6-iodo-2-phenyl-4*H*-benzoxazin-4-one with nitrogen nucleophiles, namely, formamide, ammonium acetate, hydrazine hydrate, hydroxylamine hydrochloride, substituted aromatic amines, benzyl amine, and/or thiocarbonohydrazide. All compounds were fully characterized by means of IR, MS, and ^{1}H-NMR spectra. Some of the synthesized compounds were evaluated in vitro for their anti-proliferative activity against HePG-2 and MCF-7 cell lines. 2-(Benzoylamino)-*N*-(4-hydroxyphenyl)-5-iodobenzamide and tetrazino[1,6-c]quinazoline-3(4*H*)-thione derivative were the most potent against the two cancer cells comparable to that of doxorubicin. Most of the synthesized compounds also exhibited good cytotoxic activity.

Keywords Anticancer activity · Quinazolin-4(3*H*)-one · Benzoxazinone · Hepatocellular carcinoma · Michigan Cancer Foundation-7

Introduction

Pharmacologically, quinazolin-4-ones are among the most important classes of heterocyclic compounds. The stability of the quinazolinone nucleus has inspired medicinal chemists to introduce many bioactive moieties to this nucleus to synthesize new potential medicinal agents. The quinazolinone skeleton is a frequently

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encountered heterocycle in medicinal chemistry literature with applications including antibacterial [1], analgesic, antiinflammatory [2], antifungal [3, 4], antimalarial, antihypertensive [5], central nervous system (CNS) depressant [6], anticonvulsant [7], antihistaminic, antiparkinsonism, [8] antiviral and anticancer activities [9, 10].

The discovery and development of novel therapeutic DNA intercalators for the treatment of malignancy is one of the most important goals in modern medicinal chemistry. DNA intercalators including doxorubicin were originally isolated from *Streptomyces peucetius* and are most widely used of the anthracycline antibiotic group of anticancer agents [11, 12]. The precise mechanism of action of doxorubicin is known to intercalate between base pairs in the DNA helix. Intercalation leads to single- and double-strand breakages, thereby preventing DNA replication and ultimately inhibiting protein synthesis. Scission of DNA is believed to be mediated through the action of topoisomerase II or by the iron-catalyzed generation of free radicals, both hydrogen peroxide and hydroxyl, which are highly destructive to cells. Doxorubicin also induces the formation of covalent topoisomerase-DNA complexes, resulting in inhibition of the religation portion of the ligation–religation reaction in replicating DNA. Although it is active throughout the cell cycle, the maximal toxicity occurs during the DNA synthesis (S) phase. At low drug concentrations, cells will continue through the S phase and die in the G2 phase [13].

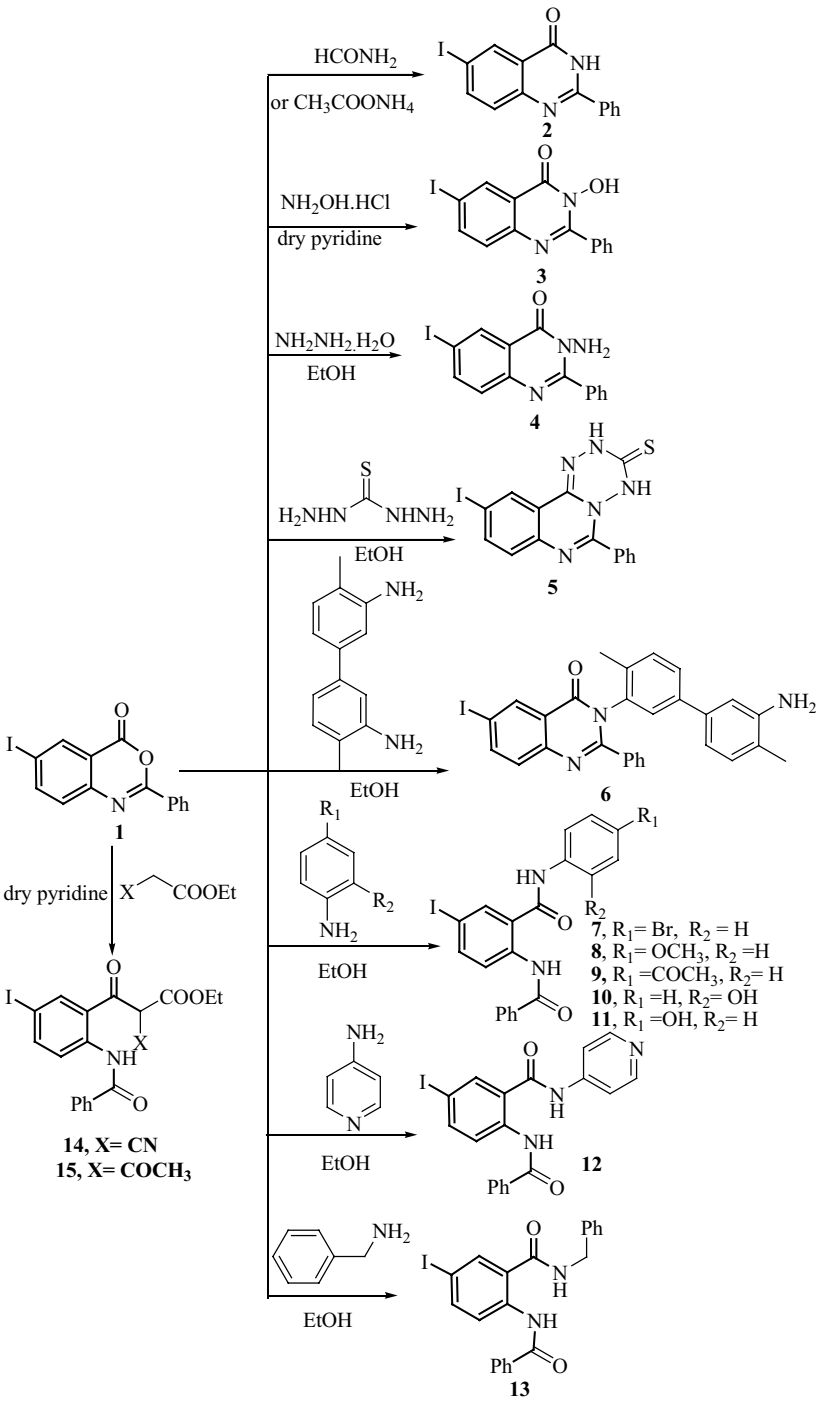
Based on the earlier findings, molecular hybridization between quinazolinone and other effective antitumor moieties was carried out in an attempt to obtain new effective molecules with antitumor activity. Furthermore, in continuation of our previous work, [14–21], the present work aimed at utilization of 6-iodo-2-phenyl-4*H*-benzoxazin-4-one **1** for design and synthesis of different quinazolin-4(3*H*)-ones in order to have the main pharmacophoric features of DNA intercalating agents with the major objective of developing agents with potential anti-proliferative activity that could be highly effective, safe, and devoid of side effects.

Results and discussion

Chemistry

6-Iodo-2-phenyl-4*H*-benzoxazin-4-one **1** was prepared from stirring 5-iodoanthranilic acid with benzoyl chloride in dry pyridine at room temperature [22]. Aminolysis of benzoxazinone **1** was conducted through its reaction with formamide and/or with ammonium acetate to afford quinazolinone **2**. The structure of compound **2** was assigned from its IR spectrum which exhibited strong absorption bands at 3165 and 1675 cm^{-1} corresponding to ν_{NH} and $\nu_{C=O}$, respectively (cf. Scheme 1).

When benzoxazinone **1** reacted with hydroxylamine hydrochloride in pyridine, 3-hydroxy-6-iodo-2-phenylquniazolin-4(3*H*)-one (**3**) was obtained. The structure of compound **3** is confirmed by IR spectrum which showed strong absorption bands at 3425 and 1670 cm^{-1} due to ν_{OH} and $\nu_{C=O}$, respectively. The ^1H NMR spectrum showed the D_2O exchangeable proton for the OH group at 12.07 ppm. Hydrazinolysis of benzoxazinone **1** with hydrazine hydrate in boiling ethanol afforded 3-amino-6-iodo-2-phenylquinazolin-4(3*H*)-one (**4**) [22].

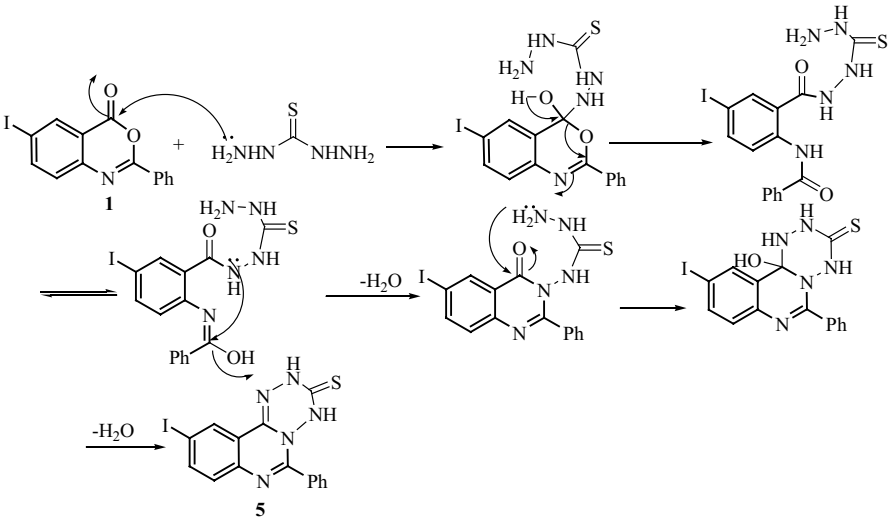


Scheme 1 Reactions of benzoxazinone **1** with different nitrogen and carbon nucleophiles

A new fused tricyclic system containing the tetrazino moiety **5** was obtained through the effect of thiocarbonohydrazide on benzoxazinone **1**. The IR spectrum of tetrazino[1,6-c]quinazolinethione derivative **5** revealed the disappearance of the carbonyl band, and the presence of absorption band at 1141 $\nu_{C=S}$. ^{1}H NMR revealed the appearance of two D_2O exchangeable protons for −2NH signals. According to our speculation, the latter product was formed as a result of nucleophilic attack of the amino group of thiocarbonohydrazide on the electronically deficient carbon atom of benzoxazinone **1** via a tetrahedral mechanism, resulting in ring opening followed by ring closure to afford quinazolinylthiosemicarbazide derivative which was subjected to dehydration to give tetrazino[1,6-c]quinazoline-thione derivative **5** (cf. Scheme 2).

Reaction of benzoxazinone **1** with 3′-amino-4,4′-dimethyl-1,1′-biphenyl-3-ylamine under reflux gave 6-iodo-2-phenyl-*N*-(3′-amino-4,4′-dimethyl-1,1′-biphenyl-3-yl)-quinazolin-4(3*H*)-one **(6)**. On the other hand, treatment of benzoxazinone **1** with nitrogen nucleophiles, namely *p*-bromoaniline, *p*-anisidine, *p*-aminoacetophenone, *p*-aminophenol, *o*-aminophenol, *p*-aminopyridine, and/or benzylamine, in ethanol under reflux afforded 5-iodobenzamide derivatives **7**–**13**. The present investigation was extended to study the behaviour of benzoxazinone **1** towards carbon nucleophiles exemplified by ethyl cyanoacetate and/or ethyl acetoacetate in dry pyridine to yield ethyl 3-(2-benzamido-5-iodophenyl)-2-cyano-3-oxopropanoate **(14)** and ethyl 2-(2-benzamido-5-iodobenzoyl)-3-oxobutanoate **(15)**, respectively (cf. Scheme 1).

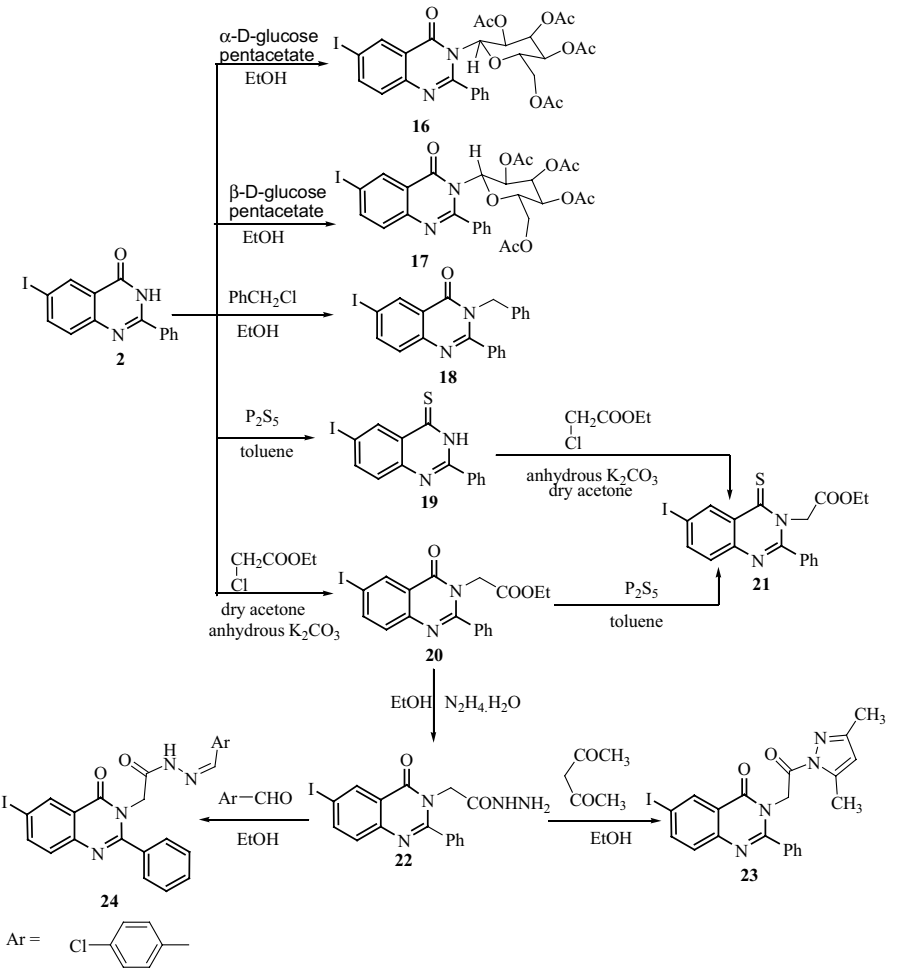
Glycosylation of quinazolinone **2** with *α*-D-glucose pentaacetate and/or *β*-D-glucose pentaacetate in ethanol gave glycosides **16** and **17**, respectively. The IR spectrum for glycosides **16** and **17** showed absorption bands at 1746 and 1751 cm^{-1} for acetoxy carbonyl groups. ^{1}H NMR spectra of glycosides **16** and **17** showed a



Scheme 2 Proposed mechanism of formation of compound **5**

singlet at δ 6.33 and 5.72 ppm characteristic for anomeric protons of α-configuration and β-configuration, respectively. The observation that an equatorial proton on C2 in the diastereomer **16** in consistently found further down field by 0.61 ppm than the axial one on the same carbon in the diastereomer **17** is due to its existence in the deshielding cones of C3–C4 and O–C bonds. Also, when quinazolinone **2** was submitted to react with benzyl chloride in ethanol under refluxing temperature, the 3-benzyl-4(3*H*)-quinazolinone **18** was isolated (cf. Scheme 3).

Thiation of quinazolinone **2** with phosphorus pentasulfide in dry toluene gave 6-iodo-2-phenylquinazoline-4(3*H*)-thione **(19)**. The behavior of quinazolinone **2** and/or quinazolinthione **19** towards carbon electrophile, namely ethyl chloroacetate, in the presence of anhydrous K_2CO_3 in dry acetone has also been investigated to afford the corresponding *N*-alkyl derivatives **20** and **21**, respectively. This reaction



Scheme 3 Reactions of quinazolinone derivative **2** with different electrophile reagents

takes place via a direct nucleophilic displacement mechanism by a nitrogen nucleophile of the predominant lactam of the partially positive saturated carbon on ethyl chloroacetate via the S_N^2 mechanism. The confirmatory evidence of *N*-alkyl derivatives **20** and **21** were obtained from the IR spectrum which exhibited absorption bands at 1754 ν for $_{CO}$ ester, 1680 ν for $_{C=O}$, 1731 ν for $_{CO}$ ester, and 1203 ν for $_{C=S}$, respectively, and disappearance of the NH band. It was found that the thiation proceeded via reaction of *N*-alkyl derivative **20** with phosphorus pentasulfide in dry toluene to afford the corresponding thiated compound **21**.

Hydrazinolysis of *N*-alkyl derivative **20** with hydrazine hydrate in ethanol gave acetohydrazide **22**. The pronounced biological activity [23, 24] of pyrazoles prompted the authors to utilize the amino functionality of acetohydrazide **22** in the design and preparation of new heterocyclic systems incorporating a pyrazole nucleus. Thus, acetohydrazide **22** was allowed to react with acetylacetone to afford pyrazole derivative **23**. On the other hand, condensation of acetohydrazide **22** with *p*-chlorobenzaldehyde in ethanol yielded the acetohydrazide derivative **24** (cf. Scheme 3).

Cytotoxicity and antitumor evaluation

Most of the synthesized compounds were screened for their anticancer activity in vitro against two representative cell lines, hepatocellular carcinoma (HePG-2) and mammary gland breast cancer (MCF-7). The results were expressed as growth inhibitory concentration (IC_{50}) values, which represent the compounds concentrations required to produce a 50% inhibition of cell growth after 72 h of incubation, compared to untreated controls (Table 1). The obtained results revealed that compounds **10** and **5** were the most cytotoxic agents against the two cell lines. As for activity against the HepG2 cell line, compound **10** was the most potent derivative as active as doxorubicin which showed the percentage viability IC_{50} at 5.47 µg/ml, whereas, the highest cytotoxic activities were displayed by compounds **5**, **11**, and **6** with IC_{50} values at 7.78, 10.02, and 10.80 µg/ml, respectively. The cytotoxic activity against HepG2 and MCF-7 cell lines were enhanced in compound **10** when compared to compounds **11** and **8.** The high anticancer activity of compound **10** is due to the presence of di-active groups (OH and NH) in the 1,2 position in its molecular structure. This structure feature has the ability to form an intramolecular hydrogen bonding (iHB). The H atom which is not involved in this bond will then be abstracted by free radicals, resulting in a stable phenoxy radical [25]. On the other hand, compound **5** exhibited cytotoxic activity against HepG2 and MCF-7 cell lines higher than compound **6** because of the presence of two basic centers (2NH groups) in compound **5**, whereas compound **6** has only one center (NH_2 group; cf. Fig. 1).

Compounds **8**, **20**, **21**, and **22** also possessed strong anti-proliferative activities against HepG2 cells. Furthermore, moderate inhibitory activity was also demonstrated by compounds **4**, **7**, **9**, **13**, **15**, and **23.** On the other hand, several compounds showed weak antiproliferative activities as compounds **2**, **3**, **12**, **16**, and **17**. Finally, compound **14** appeared to be inactive.

Table 1 Cytotoxic activity of synthesized compounds against human tumor cells

Compounds	In vitro cytotoxicity IC_{50} (μg/ml)	
	HePG2	MCF-7
2	86.96 ± 3.9	82.26 ± 4.1
3	83.11 ± 3.6	90.39 ± 4.2
4	49.11 ± 2.5	68.29 ± 3.8
5	7.78 ± 0.5	9.26 ± 0.8
6	10.80 ± 1.0	28.78 ± 2.0
7	44.44 ± 2.3	57.97 ± 3.2
8	13.34 ± 1.3	19.91 ± 1.7
9	20.50 ± 1.6	47.56 ± 3.1
10	5.47 ± 0.3	8.94 ± 0.6
11	10.02 ± 0.9	14.05 ± 1.2
12	62.23 ± 2.7	86.81 ± 4.2
13	37.40 ± 2.1	54.76 ± 3.2
14	> 100	92.87 ± 4.6
15	31.36 ± 1.9	43.89 ± 2.8
16	79.60 ± 3.6	65.42 ± 3.5
17	70.91 ± 3.1	71.72 ± 3.9
20	19.48 ± 1.6	33.66 ± 2.3
21	11.68 ± 1.2	26.35 ± 1.8
22	16.29 ± 1.5	18.14 ± 1.5
23	26.34 ± 1.8	39.68 ± 2.6
DOX	4.50 ± 0.2	4.17 ± 0.2

IC_{50} (μg/ml): 1–10 (very strong), 11–20 (strong), 21–50 (moderate), 51–100 (weak), and above 100 (non-cytotoxic)

DOX doxorubicin

On the other hand, compounds **8**, **11**, and **22** have strong cytotoxic activity against MCF-7, but compound **8** has lower cytotoxic activity compared to compound **11**, possibly due to the introduction of an electron-donating group (OCH_3) at position 4 of the benzene ring, suggesting that the electronic influence of the substituents in the benzene ring appears to play an important role in the antiproliferative activity. Compound **22** exhibited strong activity with IC_{50} at 18.14 μg/ml, and has two basic centers (NH and NH_2 groups; cf. Fig. 1).

Furthermore, interpretation of the results revealed that compounds **6**, **9**, **15**, **20**, **21**, and **23** have moderate anticancer activity against the MCF-7 cell line with percentage inhibition IC_{50} at 28.78, 47.56, 43.89, 33.66, 26.35, and 39.68 μg/ml, respectively, whereas, compounds **2, 3, 4**, **7**, **12, 13, 14**, **16**, and **17** displayed weak cytotoxic activities.

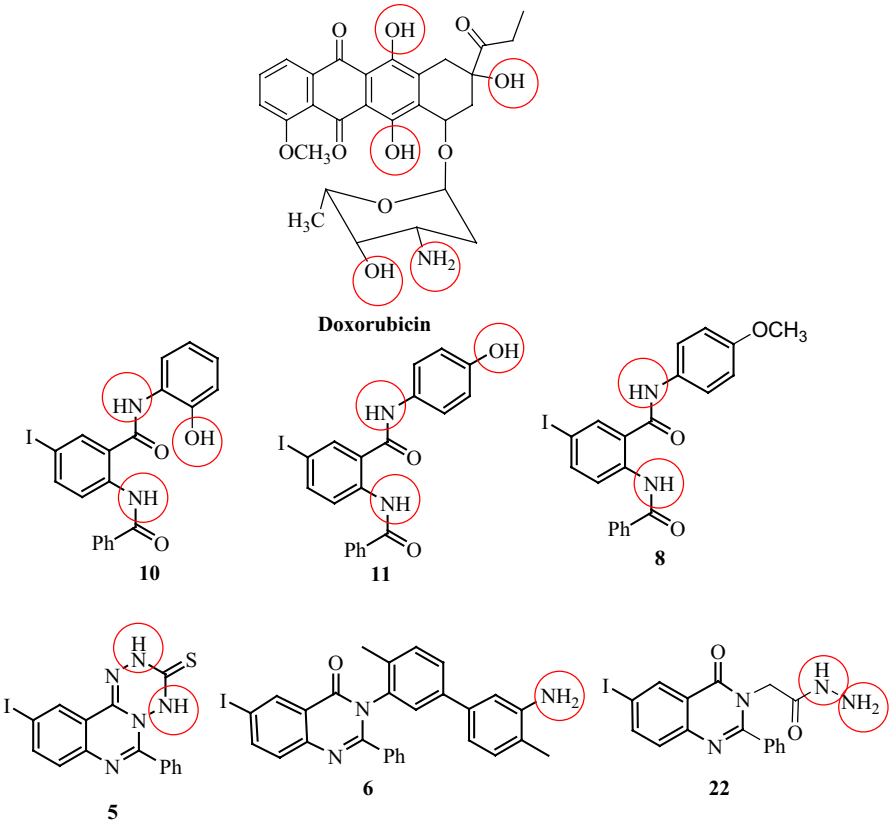


Fig. 1 Structure of doxorubicin and some of the designed target compounds

Experimental

All melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. The IR spectra were recorded using potassium bromide disks on a Pye Unicam SP-3-300 IR spectrophotometer. ^{1}H-NMR experiments were run at 300 and 400 MHz on a Varian Mercury VX-300 NMR spectrometer using tetramethylsilane (TMS) as internal standard in $CDCl_3$ or dimethyl sulfoxide (DMSO-d_6). Chemical shifts are quoted as δ. The mass spectra were recorded on a Shimadzu GCMS-QP-1000EX mass spectrometers at 70 eV. All spectral measurements were carried out at the Central Laboratory of Ain-Shams University and the Main Defense Chemical Laboratory, Egypt. Biological activity was carried in the Pharmacognosy Department, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt. All the newly synthesized compounds gave satisfactory elemental analyses. The purity of the synthesized compounds were checked by thin-layer chromatography (TLC).

Synthesis

*6-Iodo-2-phenylquinazolin-4(3H)-one (**2**)* A solution of benzooxazinone derivative **1** (5 mmol, 1.75 g) in formamide (10 ml) was refluxed for 2 h, left to cool, then poured into ice. The crude solid product was collected by filtration, dried, and recrystallised from ethanol to give **2** as white crystal: mp > 300 °C, yield 76%. FT-IR (KBr, cm^{-1}): 3165 ν_{NH}, 3092 ν_{CH} aromatic, 1675 $\nu_{C=O}$. ^{1}HNMR (300 MHz, DMSO-d_6): 12.66 (s, 1H, NH, D_2O exchangeable), 8.49–7.44 (m, 8H, Ar–H). MS (*m/z* (%)): 348 (M^+, 100.00), 245 (57.14), 193 (7.52), 104 (20.47), 90 (21.97), 77 (23.29).

*3-Hydroxy-6-iodo-2-phenylquniazolin-4(3H)-one (**3**)* A mixture of benzooxazinone derivative **1** (5 mmol, 1.75 g) and hydroxylamine hydrochloride (5 mmol, 0.35 g) in dry pyridine (20 ml) was refluxed for 15 h, left to cool, and neutralized by cold dilute hydrochloric acid. The precipitated solid was collected, washed with cold water, dried, and recrystallised from toluene to afford **3** as orange crystals: mp 250 °C, yield 75%. FT-IR (KBr, cm^{-1}): 3425 ν_{OH}, 3113 ν_{CH} aromatic, 1670 $\nu_{C=O}$. ^{1}HNMR (300 MHz, DMSO-d_6): 12.07 (s, 1H, OH, D_2O exchangeable), 8.53–7.51 (m, 8H, Ar–H). MS (*m/z* (%)): 366 (M + 2]$^+$, 4.08), 364 (M^+, 2.47), 245 (15.56), 217 (2.24), 140 (2.18), 105 (100.00), 77 (63.79).

*10-Iodo-6-phenyl-2H-[1,2,4,5]tetrazino[1,6-c]quinazoline-3(4H)-thione (**5**)* A mixture of benzooxazinone derivative **1** (5 mmol, 1.75 g) and thiocarbonohydrazide (5 mmol, 0.53 g) in ethanol (20 ml) was heated at reflux temperature for 12 h, left to cool, and the precipitated solid was collected, dried, and recrystallised from ethanol to afford **5** as yellow crystals: mp 170 °C, yield 71%. FT-IR (KBr, cm^{-1}): 3304, 3275, 3207 ν_{NH}, 1644 $\nu_{C=N}$, 1141 $\nu_{C=S}$. ^{1}HNMR (300 MHz, DMSO-d_6): 8.66–7.53 (m, 8H, Ar–H), 5.77 (s, 1H, =N–NHCS, D_2O exchangeable), 4.50 ($_S$, 1H, CSNHN-, D_2O exchangeable). MS (*m/z* (%)): 419 (M^+, not observed), 349 (20.60), 256 (44.60), 224 (23.80), 192 (58.10), 160 (68.30), 145 (54.90), 128 (55.10), 105 (83.20), 91 (12.40), 77 (70.60), 64 (100.00).

*6-Iodo-2-phenyl-N-(3′-amino-4,4′-dimethyl-1,1′-biphenyl-3-yl)-quinazolin-4(3H)-one (**6**)* A mixture of benzooxazinone derivative **1** (5 mmol, 1.75 g) and 3′-amino-4,4′-dimethyl-1,1′-biphenyl-3-ylamine (5 mmol, 1.06 ml) in ethanol (20 ml) was heated at reflux temperature for 10 h, left to cool, and the precipitated solid was collected, dried, and recrystallised from DMF to yield **6** as brown crystals: mp > 300 °C, yield 90%. FT-IR (KBr, cm^{-1}): 3432, 3200 ν_{NH2}, 3060 ν_{CH} aromatic, 2867 ν_{CH} aliphatic, 1620 $\nu_{C=O}$. ^{1}HNMR (300 MHz, DMSO-d_6): 7.59–7.30 (m, 14H, Ar–H), 3.60 (s, 2H, NH_2, D_2O exchangeable), 2.49 ($_S$, 6H, $3CH_3$). MS (*m/z* (%)): 544 (M^+, 1.29), 465 (25.90), 334 (33.77), 204 (58.13), 127 (32.99), 104 (60.49), 77 (100.00).

*Reaction of 6-iodo-2phenyl-4H-benzo[d][1,3]oxazine-4-one (**1**) with substituted amines; General procedure*

A mixture of benzooxazinone derivative **1** (5 mmol, 1.75 g) and substituted amines, namely *p*-bromoaniline, *p*-anisidine, *p*-aminoacetophenone, *o*-aminophenol, *p*-aminophenol, *p*-aminopyridine, and/or benzylamine (5 mmol), in ethanol (20 ml) was heated at reflux temperature for 8–12 h, left to cool, and the solid product was collected, dried, and recrystallised to give **7**–**13**, respectively.

*2-(Benzoylamino)-N-(4-bromophenyl)-5-iodobenzamide (**7**)* Yellow crystals, mp 245 °C (toluene), yield 60%. FT-IR (KBr, cm^{-1}): 3278, 3113 ν_{NH}, 3065 ν_{CH} aromatic, 1645 $\nu_{C=O}$. ^{1}HNMR (300 MHz, DMSO-d_6): 11.42 (s, 1H, NH, D_2O exchangeable), 10.66 (s, 1H, NH, D_2O exchangeable), 8.21–7.53 (m, 12H, Ar–H). MS (*m/z* (%)): 520 (M^+, not observed), 502 $(M–H_2O]^+$, 3.00), 349 (68.40), 305 (20.70), 178 (21.10), 105 (100.00), 77 (94.00), 51 (14.60).

*2-(Benzoylamino)-5-iodo-N-(4-methoxyphenyl)benzamide (**8**)* Yellow crystals, mp 190 °C (toluene), yield 81%. FT-IR (KBr, cm^{-1}): 3270, 3135 ν_{NH}, 3065 ν_{CH} aromatic, 2995 ν_{CH} aliphatic, 1646 $\nu_{C=O}$. ^{1}HNMR (300 MHz, DMSO-d_6): 11.80 (s, 1H, NH, D_2O exchangeable), 10.49 (s, 1H, NH, D_2O exchangeable), 8.52–6.63 (m, 12H, Ar–H), 3.70 ($_S$, 3H, OCH_3). MS (*m/z* (%)): 472 (M^+, not observed), 454 $(M–H_2O]^+$, 0.34), 424 (10.20), 349 (26.30), 223 (44.30), 179 (35.10), 105 (100.00), 77 (74.90), 51 (18.40).

*N-(4-Acetylphenyl)-2-(benzoylamino)-5-iodobenzamide (**9**)* Pale yellow crystals, mp 240 °C (ethanol), yield 60%. FT-IR (KBr, cm^{-1}): 3113 ν_{NH}, 3058 ν_{CH} aromatic, 2957 ν_{CH} aliphatic, 1701 $\nu_{C=O}$ ketone, 1683 $\nu_{C=O}$ amide. ^{1}HNMR (300 MHz, DMSO-d_6): 12.12 (s, 2H, 2NH, D_2O exchangeable), 8.53–7.23 (m, 12H, Ar–H), 2.59 ($_S$, 3H, $COCH_3$). MS (*m/z* (%)): 484 (M^+, not observed), 466 $(M–H_2O]^+$, 0.54), 440 (1.00), 349 (60.50), 223 (26.60), 179 (21.60), 105 (100.00), 77 (66.30), 51 (16.90).

*2-(Benzoylamino)-N-(2-hydroxyphenyl)-5-iodobenzamide (**10**)* Black crystals, mp 180 °C (toluene), yield 70%. FT-IR (KBr, cm^{-1}): 3497 ν_{OH}, 3385 ν_{NH}, 3057 ν_{CH} aromatic, 1667 $\nu_{C=O}$. ^{1}HNMR (300 MHz, DMSO-d_6): 10.03 (s, 1H, NH, D_2O exchangeable), 9.57 (s, 1H, NH, D_2O exchangeable), 9.04 (s, 1H, OH, D_2O exchangeable), 8.52–6.42 (m, 12H, Ar–H). MS (*m/z* (%)): 458 (M^+, not observed), 440 $(M–H_2O]^+$, 30.00), 348 (92.50), 286 (84.00), 245 (100.00), 217 (10.20), 193 (14.60), 105 (30.50), 90 (28.90), 77 (40.20), 63 (14.40).

*2-(Benzoylamino)-N-(4-hydroxyphenyl)-5-iodobenzamide (**11**)* Yellow crystals, mp 186–190 °C (ethanol), yield 70%. FT-IR (KBr, cm^{-1}): 3414 ν_{OH}, 3312 ν_{NH}, 3062 ν_{CH} aromatic, 1688 $\nu_{C=O}$. ^{1}HNMR (300 MHz, DMSO-d_6): 11.87 (s, 1H, NH, D_2O exchangeable), 10.41 (s, 1H, NH, D_2O exchangeable), 9.35 (s, 1H, OH, D_2O exchangeable), 8.52–6.56 (m, 12H, Ar–H). MS (*m/z* (%)): 458 (M^+, not observed), 395 (29.80), 282 (2.20), 218 (2.30), 207 (18.40), 191 (2.90), 133 (2.20), 105 (99.90), 91 (4.40), 77 (39.10).

*2-(Benzoylamino)-5-iodo-N-pyridin-4-ylbenzamide (***12***)* Black crystals, mp 140 °C (toluene), yield 60%. FT-IR (KBr, cm^{-1}): 3249 ν_{NH}, 3059 ν_{CH} aromatic, 1688, 1669 $\nu_{C=O}$. ^{1}HNMR (300 MHz, DMSO-d_6): 11.48 (s, 2H, 2NH, D_2O-exchangeable), 8.34–7.57 (m, 12H, Ar–H). MS (*m/z* (%)): 443 (M^+, not observed), 425 $(M–H_2O]^+$, 0.07), 396 (73.00), 349 (43.00), 105 (100.00), 91 (58.00), 77 (50.50), 63 (49.00).

*2-(Benzoylamino)-N-benzyl-5-iodobenzamide (***13***)* Yellow crystals, mp 150 °C (toluene), yield 96%. FT-IR (KBr, cm^{-1}): 3298 ν_{NH}, 2922 ν_{CH} aliphatic, 1675 $\nu_{C=O}$. ^{1}HNMR (300 MHz, DMSO-d_6): 12.45 (s, 2H, 2NH, D_2O-exchangeable), 8.49–7.24 (m, 13H, Ar–H), 4.03 ($_S$, 2H, CH_2-NH). MS (*m/z* (%)): 456 (M^+, not observed), 438 $(M–H_2O]^+$, 2.15), 368 (44.96), 299 (40.29), 285 (73.79), 236 (100.00), 211 (49.32), 171 (21.15), 152 (42.01), 123 (44.79), 104 (24.85).

*Reaction of 6-iodo-2phenyl-4H-benzo[d][1.3]oxazine 4-one (***1***) with ethyl cyanoacetate and/or ethyl acetoacetate; General procedure*

To a solution of benzooxazinone derivative **1** (5 mmol, 1.75 g) in dry pyridine (20 ml), ethyl cyanoacetate and/or ethyl acetoacetate (5 mmol) was added. The mixture was heated at refluxing temperature for 48 h, left to cool and poured into ice; the crude solid product deposited was collected by filtration and washed with water, dried, and recrystallised to afford **14** and *15*, respectively.

*Ethyl 3-(2-benzamido-5-iodophenyl)-2-cyano-3-oxopropanoate (***14***)* Brown crystals, mp 113–115 °C (ethanol), yield 90%. FT-IR (KBr, cm^{-1}): 3248 ν_{NH}, 3057 ν_{CH} aromatic, 2959 ν_{CH} aliphatic, 2215 ν_{CN}, 1757 ν_{CO} ester, 1673 $\nu_{C=O}$. MS (*m/z* (%)): 462 (M^+, not observed), 444 $(M–H_2O]^+$, 3.16), 395 (16.32), 372 (59.44), 305 (13.20), 105 (3.84), 77 (8.64).

*Ethyl 2-(2-benzamido-5-iodobenzoyl)-3-oxobutanoate (***15***)* Black crystals, mp 107 °C (ethanol), yield 89%. FT-IR (KBr, cm^{-1}): 3248 ν_{NH}, 3057 ν_{CH} aromatic, 2985 ν_{CH} aliphatic, 1756 ν_{CO} ester, 1690 $\nu_{C=O}$ ketone, 1669 $\nu_{C=O}$ amide. ^{1}HNMR (300 MHz, DMSO-d_6): 11.48 (s, 1H, NH, D_2O exchangeable), 8.37–7.47 (m, 8H, Ar–H), 4.35 (q, 2H, CH_2 CH_3, $J = 7.2$ Hz), 3.44 ($_S$, 1H, CH–), 2.69 (s, 3H, $COCH_3$), 1.31 (t, 3H, CH_2CH_3, $J = 7.2$ Hz). MS (*m/z* (%)): 479 (M^+, not observed), 461 $(M–H_2O]^+$, 0.04), 395 (28.50), 349 (25.20), 305 (53.00), 105 (100.00), 77 (41.40), 63 (38.00).

*Reaction of 6-iodo-2-phenylquinazolin-4(3H)-one (***2***) with α-d-glucose pentaacetate and/or β-d-glucose pentaacetate; General procedure*

A mixture of quinazolinone **2** (5 mmol, 1.75 g), α-D-glucose pentaacetate, and/or β-D-glucose pentaacetate (5 mmol) in absolute ethanol (20 ml) was heated at reflux temperature for 48 h and left to cool; the crude solid product deposited after cooling was collected by filtration, dried, and recrystallised to give **16** and **17**, respectively.

*(2R,3R,4S,5R,6S)-2-Acetoxymethyl)-6-(6-iodo-4-oxo-2-phenylquinazolin-3(4H)-yl) tetrahydro-2H-pyran-3,4,5-triyltriacetate (***16***)* Black crystals, mp 209–210 °C

(benzene), yield 60%. FT-IR (KBr, cm^{-1}): 3165 ν_{CH} aromatic, 2922 ν_{CH} aliphatic, 1746 ν_{CO} ester, 1676 $\nu_{C=O}$ amide. ^{1}HNMR (400 MHz, $CDCl_3$): 8.65–7.35 (m, 8H, Ar–H), 6.33 (s, 1H, -N–CH–O–), 5.47–5.09 (m, 3H, 3CHOCO), 4.25 (d, 1H, O–CH–CH_2–, J = 2.7 Hz), 4.10 (d, 1H, O–CH–CH_2–, J = 2.4 Hz), 3.73 (t, 1H, O–CH–CH_2–, J = 5.1 Hz), 2.17–2.00 (m, 12H, 4$COCH_3$). MS (*m/z* (%)): 349 (3.00), 331 (39.00), 287 (4.00), 242 (21.30), 115 (100.00).

*(2R,3R,4S,5R,6R)-2-Acetoxymethyl)-6-(6-iodo-4-oxo-2-phenylquinazolin-3(4H)-yl) tetrahydro-2H-pyran-3,4,5-triyltriacetate (***17***)* Brown crystals, mp 122–123 °C (benzene), yield 75%. FT-IR (KBr, cm^{-1}): 3165 ν_{CH} aromatic, 2974ν_{CH} aliphatic, 1751 ν_{CO} ester, 1675 $\nu_{C=O}$ amide. ^{1}HNMR (400 MHz, $CDCl_3$): 8.65–7.35 (m, 8H, Ar–H), 5.72 (s, 1H, –N–CH–O–), 5.27–5.10 (m, 3H, 3CHOCO), 4.30–4.25 (m, 2H, O–CH–CH_2–), 4.12 (t, 1H, O–CH–CH_2–, J = 1.8 Hz), 2.17–2.01 (m, 12H, 4$COCH_3$). MS (*m/z* (%)): 448 (1.00), 434 (69.80), 361(100.00), 349 (33.10), 245 (24.00), 204 (42.80).

*3-Benzyl-6-iodo-2-phenylquinazolin-4(3H)-one (***18***)* A mixture of quinazolinone **2** (5 mmol, 1.75 g) and benzylchloride (5 mmol, 0.63 ml) in absolute ethanol (20 ml) was heated at reflux temperature for 12 h and left to cool; the crude solid product deposited after cooling was collected by filtration, dried, and recrystallised from benzene to give **18** as beige crystals: mp 227–228 °C, yield 70%. FT-IR (KBr, cm^{-1}): 3061 ν_{CH} aromatic, 2923 ν_{CH} aliphatic, 1676 $\nu_{C=O}$ amide. ^{1}HNMR (400 MHz, $CDCl_3$): 8.58–7.25 (m, 13H, Ar–H), 4.46 (s, 2H, –CH_2 Ph). MS (*m/z* (%)): 438 (M^+, 5.15), 395 (100.00), 367 (84.34), 322 (40.54), 286 (30.47), 272 (96.50), 127 (68.34), 105 (18.38), 77 (46.92).

*6-Iodo-2-phenylquinazoline-4(3H)-thione (***19***)* A mixture of quinazolinone **2** (5 mmol, 1.75 g) and phosphorus pentasulfide (5 mmol, 1.11 g) in absolute toluene (20 ml) was heated at reflux temperature for 24 h. The reaction mixture was filtered off while hot and concentrated. The crude solid product deposited after cooling was collected by filtration, dried, and recrystallised from DMF to give **19** as yellow crystals: mp 247–248 °C, yield 65%. FT-IR (KBr, cm^{-1}): 3239 ν_{NH}, 3061 ν_{CH} aromatic, 1200 $\nu_{C=S}$. MS (*m/z* (%)): 364 (M^+, 100.00), 330 (89.13), 204 (27.76), 134 (13.36), 101 (16.37), 77 (22.03).

*Ethyl 2-(6-iodo-4-oxo-2-phenylquinazolin-3(4H)-yl)acetate (***20***)* A mixture of quinazolinone **2** (10 mmol, 3.48 g), ethyl chloroacetate (10 mmol, 1 ml), and anhydrous potassium carbonate 5 g in dry acetone (50 ml) was heated in a water bath for 24 h. The excess solvent was evaporated and the reaction mixture was then poured into water, and the crude solid product was collected by filtration, dried, and recrystallised from ethanol to give **20** as white crystals: mp 122–123 °C, yield 60%. FT-IR (KBr, cm^{-1}): 3065 ν_{CH} aromatic, 2978 ν_{CH} aliphatic, 1754 ν_{CO} ester, 1680 $\nu_{C=O}$. ^{1}HNMR (300 MHz, DMSO-d_6): 8.52–7.52 (m, 8H, Ar–H), 5.28 (s, 2H, CH_2), 4.20 (q, 2H, CH_2CH_3, J = 7.2 Hz), 1.19 (t, 3H, CH_2CH_3, J = 7.2 Hz). MS (*m/z* (%)):

434 (M^{+}, 3.58), 361 (71.27), 331 (21.56), 205 (24.46), 204 (33.52), 104 (15.42), 77 (18.09).

*Ethyl 2-(6-iodo-2-phenyl-4-thioxoquinazolin-3(4H)-yl)acetate (***21***)* *Method A* A solution of compound **20** (5 mmol, 2.2 g) and phosphorus pentasulfide (5 mmol, 1.1 g) in dry toluene (20 ml) was heated at reflux temperature for 1 h, filtered while hot, and left to cool: the solid product was collected by filtration, dried, and recrystallised from dioxane to give **21** as red crystals: mp 120–122 °C, yield 40%. FT-IR (KBr, cm^{-1}): 3062 ν_{CH} aromatic, 2965 ν_{CH} aliphatic, 1731 ν_{CO} ester, 1203 $\nu_{C=S}$. ^{1}HNMR (300 MHz, $CDCl_3$): 8.96–7.36 (m, 8H, Ar–H), 4.70 (s, 2H, CH_2), 4.29 (q, 2H, $\underline{CH_2}CH_3$, J = 6.9 Hz), 1.31 (t, 3H, $CH_2\underline{CH_3}$, J = 6.9 Hz). MS (*m/z* (%)): 450 (M^{+}, 9.11), 377 (36.99), 360 (19.92), 331 (40.18), 250 (100.00), 204 (69.11), 177 (14.36), 104 (14.52), 77 (12.89).

Method B A mixture of quinazolinthione **19** (10 mmol, 3.48 g), ethyl chloroacetate (10 mmol, 1 ml), and anhydrous potassium carbonate 5 g in dry acetone (50 ml) was heated in a water bath for 24 h. The excess solvent was evaporated and the reaction mixture was poured into water; the crude solid product was collects by filtration, dried, and recrystallised from dioxane to give **21** as red crystals: mp 122–123 °C, yield 60%.

*2-(6-Iodo-4-oxo-2-phenylquinazolin-3(4H)-yl)acetohydrazide (***22***)* A mixture of compound **20** (5 mmol 2.2 g) and hydrazine hydrate (5 mmol, 0.25 ml) in absolute ethanol (30 ml) was heated at reflux temperature for 10 h and left to cool: the crude solid product deposited after cooling was collected by filtration, dried, and recrystallised from ethanol to give **22** as yellow crystals: mp > 300 °C, yield 75%. FT-IR (KBr, cm^{-1}): 3400, 3307 $\nu_{NH2,NH}$, 3078 ν_{CH} aromatic, 2989 ν_{CH} aliphatic, 1670 $\nu_{C=O}$. ^{1}HNMR (300 MHz, DMSO-d_6): 9.40 (s, 1H, NH, D_2O exchangeable), 8.39–7.47 (m, 8H, Ar–H), 4.96 (s, 2H, CH_2–CO), 4.29 (s, 2H, NH_2, D_2O exchangeable). MS (*m/z* (%)): 420 (M^{+}, 0.98), 348 (22.58), 313 (100.00), 285 (55.21), 256 (20.70), 245 (27.04), 228 (12.58), 158 (11.62), 95 (15.52), 55 (11.16).

*3-(2-(3,5-Dimethyl-1H-pyrazol-1-yl)-2-oxoethyl)-6-iodo-2-phenylquinazolin-4(3H)-one (***23***)* A mixture of acetohydrazide derivative **22** (2.5 mmol, 1 g) and acetyl acetone (2.5 mmol, 0.25 ml) in ethanol (20 ml) was heated at reflux temperature for 7 h, left to cool, and the crude solid product deposited was collected by filtration, dried, and recrystallised from ethanol to give **23** as yellow crystals: mp 155–157 °C, yield 50%. FT-IR (KBr, cm^{-1}): 3096 ν_{CH} aromatic, 2984 ν_{CH} aliphatic, 1674 $\nu_{C=O}$. ^{1}HNMR (300 MHz, DMSO-d_6): 9.21–7.52 (m, 8H, Ar–H), 6.20 ($_S$, 1H, pyrazolo-H), 4.80 (s, 2H, CH_2–C=O), 2.73 (s, 3H, CH_3), 2.41 (s, 3H, CH_3). MS (*m/z* (%)): 484 (M^{+}, 0.81), 453 (6.41), 331 (34.42), 312 (12.46), 258 (21.18), 204 (20.03), 136 (100.00).

*N'-(4-Chlorobenzylidene)-2-(6-iodo-4-oxo-2-phenylquinazolin-3(4H)-yl)acetohydrazide (***24***)* A mixture of acetohydrazide derivative **22** (2.5 mmol, 1 g) and *p*-chlorobenzaaldehyde (2.5 mmol, 0.35 g) in ethanol (20 ml) was heated at reflux

temperature for 12 h, left to cool, and the crude solid product deposited after cooling was collected by filtration, dried, and recrystallised from dioxane to give **24** as yellow crystals: mp 240–245 °C, yield 66%. FT-IR (KBr, cm^{-1}): 3210 ν_{NH}, 3056 ν_{CH} aromatic, 2977 ν_{CH} aliphatic, 1708, 1665 $\nu_{C=O}$, 1619 $\nu_{C=N}$. ^{1}HNMR (300 MHz, DMSO-d_6): 11.92 (s, 1H, NH, D_2O-exchangeable), 9.20 (s, 1H, CH=N–), 8.54–7.52 (m, 12H, Ar–H), 4.32 (s, 2H, $COCH_2$). MS (*m/z* (%)): 542 (M^+, 3.07), 433 (14.13), 355 (13.19), 139 (19.11), 116 (19.17), 70 (100.00).

Cytotoxicity and antitumor evaluation

Materials and methods

Hepatocellular carcinoma (HEPG-2) and mammary gland breast cancer (MCF-7) cell lines were obtained from ATCC via a holding company for biological products and vaccines (VACSERA), Cairo, Egypt.

Doxorubicin was used as a standard anticancer drug for comparison.

Procedure MTT assay

The cell lines mentioned above were used to determine the inhibitory effects of the tested compounds on cell growth using the MTT assay. This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. Cell lines were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/ml penicillin and 100 µg/ml streptomycin at 37 °C in a 5% CO_2 incubator. The cell lines were seeded in a 96-well plate at a density of 1.0×10^4 cells/well at 37 °C for 48 h under 5% CO_2. After incubation the cells were treated with different concentrations of compounds and incubated for 24 h. After 24 h of drug treatment, 20 µl of MTT solution at 5 mg/ml was added and incubated for 4 h. DMSO (100 µl) was added into each well to dissolve the formed purple formazan. The colorimetric assay was measured and recorded at an absorbance of 570 nm using a plate reader (EXL 800, USA). The relative cell viability in percentage was calculated as: (A570 of treated samples/A570 of untreated sample) × 100 [26, 27].

Calculation of the IC_{50} for each compound

Cytotoxicity was estimated as the concentration that caused approximately 50% loss of the monolayer. The assay was used to examine the newly synthesized compounds. Doxorubicin was used as a standard anticancer drug for comparison. To calculate IC_{50}, you would need a series of dose-response data (e.g., drug concentrations *x*1, *x*2, *xn* and growth inhibition *y*1, *y*2, *yn*). The values of y are in the range of 0–1.

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