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Efficient one-pot tandem synthesis and cytotoxicity evaluation of 2,3-disubstituted quinazolin-4(3*H*)-one derivatives

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ABSTRACT

Twenty 2,3-disubstituted quinazolin-4(3*H*)-one derivatives **1**–**20** were successfully synthesized in moderate to good yields (25–82%). Their syntheses were based on a one pot tandem ring opening procedure followed by iodine-catalyzed oxidative cyclization of isatoic anhydride with aldehydes, using water as the only solvent under both classical and microwave irradiation conditions. Cytotoxicity assays of the prepared compounds against three human cancer cell lines (HeLa, MCF-7, and A549) indicated that **2**, **3**, and **20** possessed moderate activities against MCF-7 cells (IC_{50} = 47.2 μM, 43.9 μM, and 44.9 μM, respectively). Good cytotoxic activities against A549 cells were observed for **3** and **8** with IC_{50} values of 30.7 μM and 29.8 μM, respectively, which were comparable to the positive control, 5-fluorouracil (5-FU, IC_{50} = 27.9 μM). Furthermore, compound **4** exhibited slightly stronger activity (IC_{50} = 23.6 μM) than the positive control 5-FU against the A549 cell line.

1. Introduction

Cancer continues to be a leading health problem worldwide, and the pursuit of novel clinically useful anticancer agents is therefore one of the top priorities of drug development research. Quinazolinone-based structures have been identified in different classes of cancer chemotherapeutic agents [1]. Several 2,3-disubstituted quinazolin-4(3*H*)-one derivatives reportedly possess antitumor activity [2], and as shown in Fig. 1, the developed derivatives **I** [3], **II** [4], and **III** [5] are representative examples.

Due to the extensive range of applications of 2,3-disubstituted 4(3*H*)-quinazolinone derivatives as anticancer agents, considerable efforts have been made toward the expansion of synthetic approaches. Among the preparation methods for all known 2,3-disubstituted 4(3*H*)-quinazolinone derivatives, the ring opening of isatoic anhydride by nitrogen nucleophiles, followed by the oxidative cyclocondensation by electrophiles, in one pot three component reactions has attracted a great deal of attention [6]. Representative examples of these methodologies include oxidation/cyclization reactions of isatoic anhydride with (1) benzyl halides [7], (2) *N,N*-dialkyl carbodiimides [8], and (3) orthoesters [9–14], especially with aromatic aldehydes (Fig. 2). More recently, different catalytic systems including ceric ammonium nitrate (CAN) in ethanol [15], CuO nanoparticles [16], $Bi(NO_3)_3 \cdot 5H_2O$ [17], thiamine hydrochloride (VB1) [18], $Ga(OTf)_3$ [19], and L-prolinium nitrate inside the meso-channels of (α-Fe_2O_3)-MCM-41 [20] have been reported (Fig. 2 and 4a). These routes, however, generally suffer from low yields of products, prolonged reaction times, high temperatures, and the use of metallic, non-recyclable catalysts.

Iodine is an inexpensive and readily available catalyst for various organic transformations under very mild and convenient conditions, to afford the corresponding products in excellent yields with

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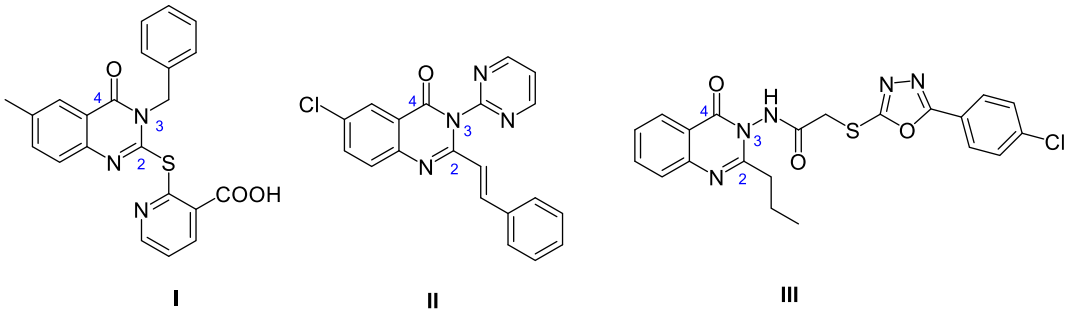


Fig. 1. Relevant 2,3-disubstituted 4(3*H*)-quinazolinones with cytotoxic activities.

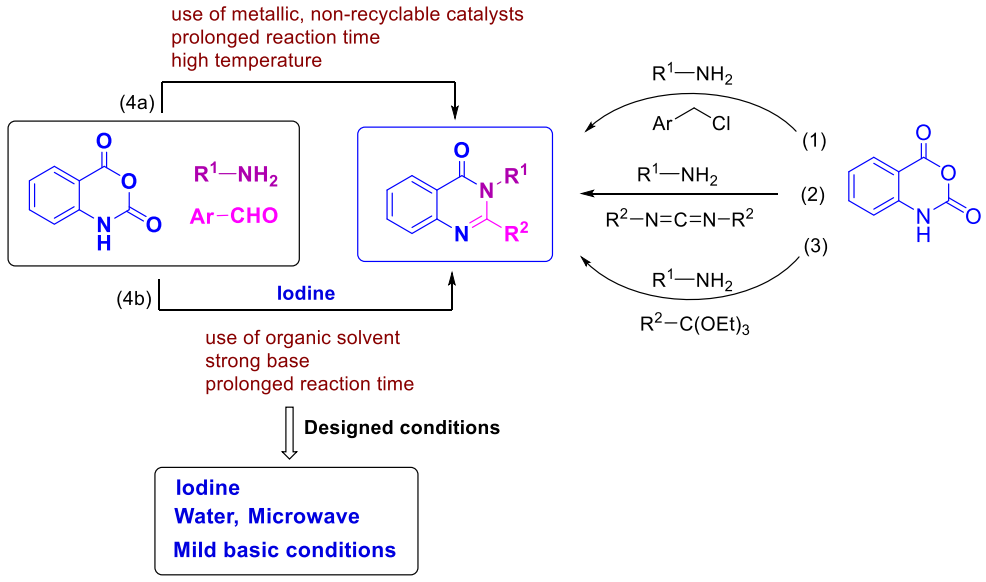


Fig. 2. General three component reaction pathways towards the production of 2,3-disubstituted 4(3*H*)-quinazolinones from isatoic anhydride, and the proposed synthetic procedure.

high selectivity [21]. A three-component condensation of isotoic anhydride, primary amine, and aldehyde in the presence of iodine has been successfully developed for the synthesis of 2,3-substituted 4(3*H*)-quinazolinones [22,23] (Fig. 2 and 4b). However, the use of toxic organic solvents, and the prolonged reaction times under strong basic or acidic conditions are the main drawbacks of these procedures. Thus, the development of a facile and eco-friendly method towards the synthesis of 2,3-disubstituted 4(3*H*)-quinazolinones is highly desirable.

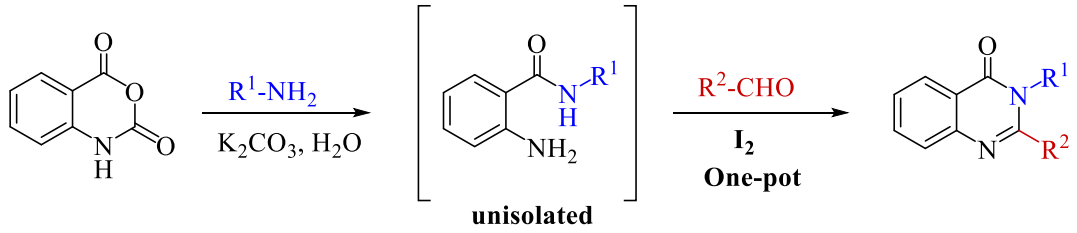
As a solvent, water offers many advantages as it is readily available, inexpensive, non-toxic, and non-flammable, and thus the use of water as substitute for organic solvent is very attractive from both economic and environmental points of view [24]. In addition, the application of microwave dielectric heating for performing organic reactions has many advantages, such as remarkable reductions of reaction time, improved yields, and cleaner reactions than those performed with conventional thermal heating [25]. In an effort to develop an efficient, green synthetic procedure towards 2,3-disubstituted 4(3*H*)-quinazolinones, herein we report a novel one pot tandem ring opening of isatoic anhydride followed by an iodine-catalyzed oxidative cyclization process with water as the only solvent, using microwave heating in the ring forming step. This method offers several advantages, such as omitting toxic solvents or catalysts and requiring a shorter reaction time. The starting materials are also inexpensive and commercially available. Consequently, twenty 2,3-disubstituted 4(3*H*)-quinazolinones have been synthesized and evaluated for their cytotoxicities against three human cancer cell lines, HeLa, MCF-7, and A549.

2. Results and discussion

2.1. Chemistry

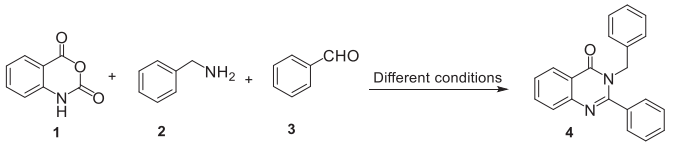
To synthesize 2,3-disubstituted 4(3*H*)quinazolinones, we attempted the one pot tandem ring opening of isatoic anhydride by a primary amine, followed by iodine-catalyzed oxidative cyclization with aldehyde, using water as the only solvent (Scheme 1).

First, the formation of 2,3-disubstituted-3*H*-quinazolin-4-one **4** was explored using isatoic anhydride **1**, benzylamine **2**, and benzaldehyde **3** as the model substrates, in the presence of K_2CO_3 in water and iodine as the oxidant. The reactions were performed using different molar ratios of reactants, as presented in Table 1 (Entries 1–11) [22]. The best yield (80%) of the desired product **4** was obtained by applying the reaction conditions shown in Entry 7. The efficiency of the reaction significantly decreased when K_2CO_3 was replaced with other bases, such as KI or NaOMe (Table 1,



Scheme 1. One pot tandem synthesis of 2,3-disubstituted 4(3*H*)quinazolinones in water.

Table 1
Optimization of the reaction conditions[a].



Entry	Amine (eq)	Aldehyde (eq)	Oxidant (eq)	Base (eq)	Yield (%)[b]
1	1.0	1.0	I_2 (1.0)	K_2CO_3 (1.0)	69
2	1.05	1.05	I_2 (1.5)	K_2CO_3 (2.0)	38
3	1.05	1.10	I_2 (1.5)	K_2CO_3 (2.0)	62
4	1.1	1.1	I_2 (1.5)	K_2CO_3 (2.0)	65
5	1.1	1.1	I_2 (1.0)	K_2CO_3 (1.0)	76
6	1.1	1.1	I_2 (1.1)	K_2CO_3 (1.1)	78
7	**1.1**	**1.1**	**I_2 (1.2)**	**K_2CO_3 (1.2)**	**80**
8	1.5	1.1	I_2 (1.5)	K_2CO_3 (2.0)	46
9	1.3	1.1	I_2 (1.5)	K_2CO_3 (2.0)	60
10	1.1	1.1	I_2 (1.3)	K_2CO_3 (1.2)	64
11	1.1	1.1	I_2 (1.2)	K_2CO_3 (1.3)	51
12	1.1	1.1	I_2 (1.2)	KI (1.2)	68
13	1.1	1.1	I_2 (1.2)	NaOMe (1.2)	53
14	1.1	1.1	I_2 (1.2)	–	51

[a] Reaction conditions: a mixture of isatoic anhydride **1** (0.3 mmol), benzylamine **2a**, and base in water (4 mL) was stirred at RT for 30 min, and then benzaldehyde **3a** was added. The mixture was stirred for 5 additional min, and then the oxidant was added. The resulting mixture was stirred at 80 °C for 4 h.
[b] Isolated yields.

Entries 12, 13) or in the absence of a base (Entry 14). In addition, no reaction occurred when iodine was absent in the reaction or other oxidants including Oxone or $Na_2S_2O_5$ were used as substitutes for iodine.

Having established the optimum reaction conditions, the scope of the reaction was studied by using various substituted benzyl- or alkylamines and substituted aromatic aldehydes without any modifications. As shown in Table 2, the reaction conditions were applicable to a broad range of substituted benzylamines and aromatic aldehydes, leading to the formation of 19 corresponding 2,3-disubstituted-3*H*-quinazolin-4-ones **2**–**19** in moderate to good yields.

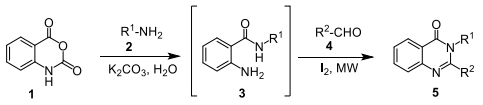
In an effort to shorten the reaction time, microwave heating was applied for the ring forming step. Thus, after treating isatoic anhydride with an amine, followed by an aromatic aldehyde, iodine was subsequently added and the resulting mixture was subjected to microwave irradiation. As a result, almost all the reactions were accomplished within 30 min, and slight increases in the yields of the desired products were observed as compared to the classical heating method (Table 2). The presence of a free hydroxy group is not tolerated by the present synthetic method under both classical and microwave heating conditions (Table 2, Entries 5, 6, and 12). The 2,3-disubstituted quinazolinones have previously been synthesized *via* a one pot condensation of isatoic anhydride, primary amine or ammonium acetate, and aldehydes, followed by an oxidation reaction using iodine as the oxidant [22,23]. However, our present method has several distinguishing features that are worth mentioning: (i) it is environmentally friendly and cost-effective by utilizing water as the solvent under mild basic conditions, (ii) it proceeds faster, within 30 min, under microwave irradiation, (iii) it is applicable to various substituted benzylamine and aromatic aldehydes.

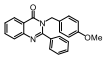
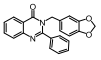
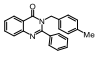
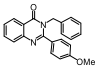
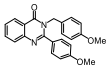
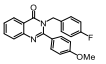
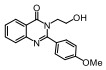
2.2. Cytotoxicity evaluation

The cytotoxicities of the prepared 2,3-disubstituted 4(3*H*)-quinazolinones **1**–**20** against three human cancer cell lines (HeLa, MCF-7, and A549) were examined (Table 3). The results indicated that all tested compounds, except for **1**, **4**–**6**, **12**–**14**, and **17**, showed weak cytotoxicities against the HeLa cell line. The presence of a free hydroxy group at either the N-3 (compounds **5**, **6**, **12**) or the C-2 (compound **14**) position was not effective for enhancing the cytotoxicities against both the MCF-7 and A549 cell lines. Similar results were also observed for the CH_3 (compound **7** and **15**) or 3,4,5-trimethoxyphenyl (compound **13**) substituents. In contrast, the introduction of an electron donating group such as OCH_3 (compound **2**) or an electron withdrawing group such as F (compound **3**) on the benzene ring at the N-3 position of the quinazolinone structure enhanced the activity against both the MCF-7 (IC_{50} = 47.2 ± 1.70 μM and 43.9 ± 0.49 μM, respectively) and A549 cell lines (IC_{50} = 48.3 ± 4.03 μM and 30.7 ± 0.91μM, respectively), as compared to the unsubstituted derivative **1** (IC_{50} > 100 μM). Notably, the presence of fluorine (F) on the benzene ring (compound **3**) resulted in cytotoxicity at a level consistent with that of 5-fluorouracil (5-FU) against the A549 cell line (IC_{50} = 27.9 ± 3.90 μM). Interestingly, although the incorporation of the 1,3-dioxolyl group at this position (compound **4**) decreased the activity on the MCF-7 cells, it considerably increased the cytotoxicity against the A549 cells (IC_{50} = 23.6 ± 5.30 μM), and exhibited slightly stronger activity relative to the positive control 5-FU (IC_{50} = 27.9 ± 3.90 μM). In contrast, further functionalization of **4** by adding a Cl group on the benzene ring at the C-2 position (compound **20**) induced moderate activity against the MCF-7 cells (IC_{50} = 44.9 ± 3.73 μM), but significantly reduced the activity on the A549 cell line.

In general, for the A549 cell line, it seems that the methoxy (compound **2**) or the fluorine (compound **3**), and especially the 1,3-dioxolyl group (compound **4**), in the benzyl moiety at the N-3 position of the quinazolinone system effectively induced the cytotoxicity only when associated with an unsubstituted phenyl group at the C-2 position (Fig. 3). Similarly, the methoxy in the benzene ring at the C-2 position (compound **8**) only achieves the activity when it is in conjunction with the unsubstituted benzyl group at the N-3 position. The simultaneous incorporation of these substituents at both the N-3 and C-2 positions likely leads to the loss of the activity. The structural features of compounds **2** and **3**, or the

Table 2
Syntheses of 2,3-disubstituted 4(3*H*)-quinazolinones *via* one pot tandem reactions in water.



Compound	Amine **2**	Aldehyde **4**	Product **5**	Yields (%)[a]		M.p. (°C) [Ref.]
				Classical	Microwave	
1				80	82	141-143°C
2				25	30	184-186°C [26]
3				42	56	116-118°C [27]
4				45	48	163-165°C [28]
5				15	25	244-246°C [29]
6				56	-[b]	153-155°C [30]
7				40	55	163-165°C [27]
8				61	71	106-108°C [27]
9				45	50	145-147°C [31]
10				60	67	101-102°C
11				46	54	130-132°C
12				45	Trace	152-154°C [32]
13				51	55	145-147°C [33, 27]
14				35	40	197-199°C
15				66	68	125-127°C [22]
16				35	40	124-126°C [34, 27]
17				54	60	152-154°C
18				50	60	118-120°C [35]
19				61	64	136-138°C
20				45	58	150-152°C

[a]Isolated yields; [b]Not detected due to very low yield.

Table 3
Cytotoxic activities of compounds **1**–**20**.

Samples	IC_{50} (μM)		
	HeLa	MCF-7	A549
1	>100	>100	>100
2	85.1 ± 1.00	47.2 ± 1.70	48.3 ± 4.03
3	85.3 ± 0.72	43.9 ± 0.49	30.7 ± 0.91
4	>100	90.8 ± 3.68	23.6 ± 5.30
5	>100	>100	>100
6	>100	>100	>100
7	81.2 ± 1.04	>100	>100
8	93.8 ± 1.06	72.7 ± 1.61	29.8 ± 2.42
9	63.4 ± 7.88	>100	80.8 ± 5.16
10	69.9 ± 2.86	70.1 ± 0.06	43.9 ± 3.60
11	83.2 ± 2.37	50.8 ± 1.25	67.0 ± 2.20
12	>100	>100	>100
13	>100	>100	>100
14	>100	>100	>100
15	88.1 ± 0.66	67.6 ± 1.20	92.1 ± 4.22
16	91.7 ± 1.04	57.5 ± 1.64	86.0 ± 2.07
17	>100	>100	92.4 ± 3.32
18	84.6 ± 0.15	74.4 ± 2.78	84.9 ± 3.60
19	86.0 ± 5.43	>100	84.4 ± 0.75
20	96.3 ± 4.10	44.9 ± 3.73	89.2 ± 3.42
5-FU*	29.9 ± 1.63	35.4 ± 4.56	27.9 ± 3.90

Data are presented as mean ± SD (n = 3).
*5-Fluorouracil: positive control.

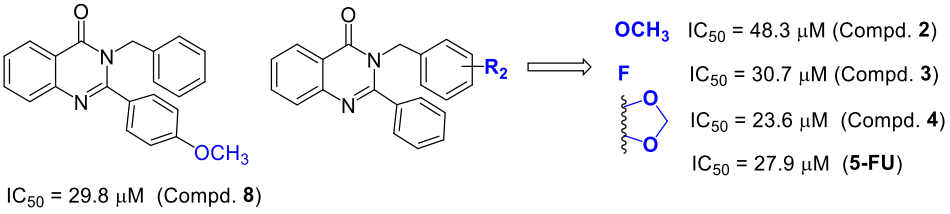


Fig. 3. Important structural features for cytotoxicity against the A549 cell line.

combined structure of the 1,3-dioxolyl group in the benzyl moiety at the N-3 and the Cl group in the benzene ring at the C-2 position (compound **20**), seem to be critical for the cytotoxicity against the MCF-7 cell line. Similar cases were also found in the previous studies, where the presence of either fluorine, chlorine, or methoxy groups in the quinazolinone ring have been indicated to be important for enhancing cytotoxicity of quinazolinones against MCF-7 and/or A549 cell lines [36].

3. Conclusions

A simple, environmentally friendly, one pot tandem procedure has been developed for the synthesis of 2,3-disubstituted-3*H*-quinazolin-4-one under mild conditions *via* the cyclocondensation of isatoic anhydride with a primary amine and aldehyde in water, and iodine as the oxidant, using microwave heating in the ring forming step. This offers several advantages, such as moderate to high yields, simple procedure, low cost, short reaction times, and mild and organic solvent-free conditions. The cytotoxicity evaluations of the prepared compounds against three human cancer cell lines (HeLa, MCF-7, and A549) showed that compounds **2** and **3** both possessed moderate activities against the MCF-7 and A549 cancer cell lines, while compound **20** moderately affected only the MCF-7 cell line. Remarkably, compounds **4** and **8** showed cytotoxicities comparable to that of the positive control on the A549 cell line. Studies ongoing in our lab include monitoring the effects of other pharmacophoric groups at different positions in the quinazolinone system on the cytotoxicity, as well as elucidating the mechanism of the activity, which will be reported in due course.

4. Experimental section

4.1. General information

Reactions were monitored by thin-layer chromatography (TLC) on 0.2 mm pre-coated silica-gel 60 F254 plates (Merck). ^{1}H NMR and ^{13}C NMR spectra were measured with Bruker Avance 500 and 600 MHz spectrometers. HRESI-MS observations were performed on a Sciex OS 1.2 mass spectrometer. FT-IR was conducted using the KBr pellet method on a Thermo Nicolet 6700 spectrometer. Chemical shifts are given in parts per million (ppm) relative to tetramethylsilane (Me_4Si, $\delta = 0$); J values are given in Hertz. All chemicals and solvents used in this study were of analytical grade. Reactions under microwave irradiation were performed in a microwave synthesis system (CEM Discovery).

4.2. General method for the synthesis of 2,3-disubstituted 4(3H)-quinazolinones

4.2.1. Classical conditions

A mixture of isatoic anhydride (0.3 mmol), amine (0.33 mmol), and K_2CO_3 (0.36 mmol) in H_2O (4 mL) was stirred at room temperature for 30 min, and aldehyde (0.33 mmol) was then added. The resulting mixture was stirred for 5 more min, followed by the addition of I_2 (0.36 mmol). The reaction mixture was stirred at 80 °C for 4 h and quenched with a saturated aqueous solution of $Na_2S_2O_3$ (4 mL). The aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with a saturated aqueous solution of NaCl and dried with Na_2SO_4, and then the solvent was evaporated under reduced pressure to obtain the crude product. Purification by column chromatography on silica gel, using a mixture of hexane and ethyl acetate as solvents, afforded the desired products **1**–**20**. All compounds were 95%+ pure according to high resolution 500 MHz ^{1}H NMR, see spectra in Supporting Information.

4.2.2. Microwave conditions

A mixture of isatoic anhydride (0.3 mmol), amine (0.33 mmol), and K_2CO_3 (0.36 mmol) in H_2O (4 mL) was stirred at room temperature for 30 min, and aldehyde (0.33 mmol) was then added. The resulting mixture was stirred for 5 more min, followed by addition of I_2 (0.36 mmol). The reaction mixture was subjected to microwave irradiation conditions for 30 min (CEM Discovery reactor), using the Power Cycling mode with specific parameters as follows: power: 100 W; power interval: 15 s; cooling interval: 2 min; max temp: 250 °C; min temp:100 °C; number of cycles: 20. The workup procedure was the same as that described for the synthesis under classical conditions. Spectral data of compounds **2**–**6** [26–30], **7**–**8** [27], **9** [31], **12** [32], **13** [27,33], **15** [22], **16** [27,34], and **18** [35] were previously described in the literature and are

provided in the Supporting Information.

4.2.3. 3-Benzyl-2-phenylquinazolin-4(3H)-one (**1**)

Yield 82% as white solid; mp 141–143 °C; FT-IR (KBr) ν_{max} (cm^{-1}): 3059, 3033, 2958, 2929, 1677, 1494, 1474, 1378, 1245, 1149, 1075, 966, 773, 704; HRESIMS: *m/z* 313.1341 $[M+H]^+$ (calcd. for $C_{21}H_{16}N_2O$ 313.3720); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.22 (dd, J = 8.0, 1.0 Hz, 1H), 7.87–7.90 (m, 1H), 7.72 (d, J = 8.0 Hz, 1H), 7.58–7.62 (m, 1H), 7.48–7.51 (m, 1H), 7.41–7.46 (m, 4H), 7.19–7.24 (m, 3H), 6.91 (dd, J = 8.5, 2.0 Hz, 2H), 5.19 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 161.4, 156.1, 146.9, 136.7, 135.1, 134.7, 129.6, 128.4, 128.2, 127.9, 127.3, 127.2, 127.0, 126.4, 126.2, 120.3, 48.1.

4.2.4. 3-(4-Fluorobenzyl)-2-(4-methoxyphenyl)quinazolin-4(3H)-one (**10**)

Yield 67% as white solid; mp 101–102 °C; FT-IR (KBr) ν_{max} (cm^{-1}): 3020, 2956, 2927, 2835, 1681, 1607, 1587, 1515, 1379, 1254, 1222, 1034, 770, 691; HRESIMS: *m/z* 361.1355 $[M+H]^+$ (calcd. for $C_{22}H_{17}FN_2O_2$ 361.3884); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.19 (dd, J = 8.0, 1.5 Hz, 1H), 7.86–7.90 (m, 1H), 7.71 (d, J = 8.0 Hz, 1H), 7.57–7.61 (m, 1H), 7.42 (dd, J = 7.0, 2.0 Hz, 2H), 7.07 (t, J = 9.0 Hz, 2H), 6.97–7.01 (m, 4H), 5.20 (s, 2H), 3.82 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 161.5, 160.1, 155.9, 147.0, 134.6, 133.0, 129.6, 128.4, 128.3, 127.4, 127.2, 127.0, 126.3, 120.2, 125.2, 115.1, 113.6, 55.3, 47.6.

4.2.5. 3-(Benzo[d] [1,3]dioxol-5-ylmethyl)-2-(4-methoxyphenyl) quinazolin-4(3H)-one (**11**)

Yield 54% as yellow solid; mp 130–132 °C; FT-IR (KBr) ν_{max} (cm^{-1}): 2962, 2913, 2841, 1676, 1608, 1514, 1500, 1484, 1444, 1244, 1034, 781; HRESIMS: *m/z* 387.1345 $[M+H]^+$ (calcd. for $C_{23}H_{18}N_2O_4$ 387.4070); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.19 (dd, J = 8.0, 1.0 Hz, 1H), 7.84–7.87 (m, 1H), 7.69 (d, J = 8.0 Hz, 1H), 7.55–7.58 (m, 1H), 7.44 (dd, J = 7.0, 2.0 Hz, 2H), 7.09 (dd, J = 6.0, 2.0 Hz, 2H), 6.75 (d, J = 8.0 Hz, 1H), 6.52 (d, J = 1.5 Hz, 1H), 6.35 (dd, J = 8.0, 2.0 Hz, 1H), 5.95 (s, 2H), 5.12 (s, 2H), 3.81 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 161.5, 160.1, 155.9, 147.2, 146.9, 146.2, 134.6, 130.6, 129.7, 127.5, 127.2, 126.9, 126.3, 120.0, 119.6, 113.6, 108.1, 107.1, 100.9, 55.3, 47.9.

4.2.6. 3-Benzyl-2-(4-hydroxyphenyl)quinazolin-4(3H)-one (**14**)

Yield 40% as slightly yellow solid; mp 197–199 °C; FT-IR (KBr) ν_{max} (cm^{-1}): 3306, 3068, 1647, 1611, 1587, 1578, 1566, 1518, 1472, 1431, 1361, 1276, 1208, 1171, 772; HRESIMS: *m/z* 329.1288 $[M+H]^+$ (calcd. for $C_{21}H_{16}N_2O_2$ 329.3710); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 9.89 (s, 1H), 8.18 (dd, J = 8.0, 1.0 Hz, 1H), 7.84–7.87 (m, 1H), 7.69 (dd, J = 8.0, 0.5 Hz, 1H), 7.54–7.58 (m, 1H), 7.28–7.30 (m, 2H), 7.19–7.25 (m, 3H), 6.94 (d, J = 8.5 Hz, 2H), 6.78 (dt, J = 9.5, 3.0 Hz, 2H), 5.23 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 161.6, 158.6, 156.3, 147.0, 136.9, 134.6, 129.7, 128.3, 127.2, 127.0, 126.8, 126.3, 126.2, 125.9, 120.1, 114.8, 48.3.

4.2.7. 3-(Benzo[d] [1,3]dioxol-5-ylmethyl)-2-(4-fluorophenyl) quinazolin-4(3H)-one (**17**)

Yield 60% as white solid; mp 152–154 °C; FT-IR (KBr) ν_{max} (cm^{-1}): 3057, 2919, 1668, 1604, 1583, 1541, 1507, 1471, 1372, 1332, 1245, 1120, 1093, 1037, 891, 777; HRESIMS: *m/z* 375.1145 $[M+H]^+$ (calcd. for $C_{22}H_{15}FN_2O_3$ 375.3714); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.22 (dd, J = 8.0, 1.0 Hz, 1H), 7.86–7.89 (m, 1H), 7.70 (d, J = 8.0 Hz, 1H), 7.58–7.61 (m, 1H), 7.51–7.54 (m, 1H), 7.29 (t, J = 8.5 Hz, 2H), 6.73 (d, J = 8.0 Hz, 1H), 6.50 (d, J = 2.0 Hz, 1H), 6.31 (dd, J = 8.0, 6.0 Hz, 1H), 5.95 (s, 2H), 5.08 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 163.5, 161.6, 161.4, 155.2, 147.3, 146.8, 146.3, 134.7, 131.7, 131.7, 130.6, 130.5, 130.4, 127.3, 127.2, 126.4, 120.4, 119.7, 115.3, 115.1, 108.1, 107.1, 100.9, 47.8.

4.2.8. 2-(4-Chlorophenyl)-3-(4-fluorobenzyl)quinazolin-4(3H)-one (**19**)

Yield 64% as yellow solid; mp 136–138 °C; FT-IR (KBr) ν_{max} (cm^{-1}): 2924, 2852, 1687, 1653, 1601, 1509, 1473, 1380, 1227, 1089, 1027, 861, 775; HRESIMS: *m/z* 365.0858 $[M+H]^+$ (calcd. for $C_{21}H_{14}ClFN_2O$ 365.8044); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.22 (dd, J = 8.0, 1.0 Hz, 1H), 7.87–7.90 (m, 1H), 7.72 (dd, J = 8.0, 0.5 Hz, 1H), 7.59–7.63 (m, 1H), 7.46–7.52 (m, 4H), 7.03–7.07 (m, 2H), 6.96–6.98 (m, 2H), 5.15 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 162.1, 161.3, 160.2, 155.0, 146.8, 134.7, 134.5, 133.9, 132.7, 132.7, 129.9, 128.5, 128.4, 128.3, 127.3, 126.4, 120.4, 115.3, 115.1, 47.4.

4.2.9. 3-(Benzo[d] [1,3]dioxol-5-ylmethyl)-2-(4-chlorophenyl) quinazolin-4(3H)-one (**20**)

Yield 58% as white solid; mp 150–152 °C; FT-IR (KBr) ν_{max} (cm^{-1}): 2923, 2853, 1680, 1601, 1499, 1483, 1446, 1369, 1251, 1088, 1039, 960, 926, 780; HRESIMS: *m/z* 391.0849 $[M+H]^+$ (calcd. for $C_{22}H_{15}ClN_2O_3$ 391.8230); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.22 (dd, J = 8.0, 1.5 Hz, 1H), 7.88 (td, J = 8.5, 1.5 Hz, 1H), 7.71 (d, J = 8.0 Hz, 1H), 7.57–7.62 (m, 1H), 7.49–7.54 (m, 4H), 6.74 (d, J = 8.0 Hz, 1H), 6.51 (d, J = 1.5 Hz, 1H), 6.33 (dd, J = 8.0, 2.0 Hz, 1H), 5.95 (s, 2H), 5.08 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 161.3, 155.0, 147.3, 146.8, 146.3, 134.7, 134.4, 133.9, 130.3, 130.0, 128.3, 127.3, 126.4, 120.4, 119.6, 108.1, 107.1, 100.9, 47.8.

4.3. Cytotoxicity evaluation

4.3.1. Sample preparation

All synthesized compounds were dissolved in DMSO to make 10 mM stock solutions. Serial dilutions were prepared in culture medium. The positive control, 5-FU, was dissolved in DMSO to make a 10 mM stock solution and then stored at −20 °C until use.

4.3.2. MTT proliferation assay

The cytotoxic activities of the synthesized compounds were evaluated against human cancer cell lines (HeLa, MCF-7, and A549), using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay with some modifications [37]. The human cancer cell lines were cultured in α-minimum essential medium (α-MEM), supplemented with 1% antibiotic antimycotic solution and 10% fetal bovine serum, at 37 °C and in a 5% CO_2 atmosphere. Cells at 80–90% confluence were harvested and centrifuged at 3000 rpm for 3 min. The supernatant was discarded and the cell pellet was resuspended in fresh medium. Aliquots (100 μL) of the cells were seeded in 96-well plates (1×10^4 cells/well) and incubated for 24 h. The cells were then washed with phosphate-buffered saline (PBS), and various concentrations of tested compounds, including the positive control, 5-FU (5–100 μM), were added to the wells. After a 72 h incubation, the cells were washed with PBS, and 100 μL aliquots of medium containing MTT solution (5 mg/mL) were added to each well and incubated for 3 h. The absorbance was recorded using a microplate reader at 570 nm. Percent proliferation inhibition was calculated using the following formula:

$$\% \text{ Proliferation cell inhibition} = [(A_t - A_b)/(A_c - A_b)] \times 100.$$

A_t: Absorbance of test compound, A_b: Absorbance of blank, A_c: Absorbance of control.

The concentrations (IC_{50} values) of the compounds required to inhibit 50% of the growth of the human cancer cell lines were calculated based on the relationship between concentrations and percent inhibitions, using the GraphPad Prism 5.0 software. Each experiment was performed three times, and all data are presented as mean ± standard deviation (SD).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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