Efficient MW-Assisted Synthesis of Some New Isoquinolinone Derivatives With *In Vitro* Antitumor Activity

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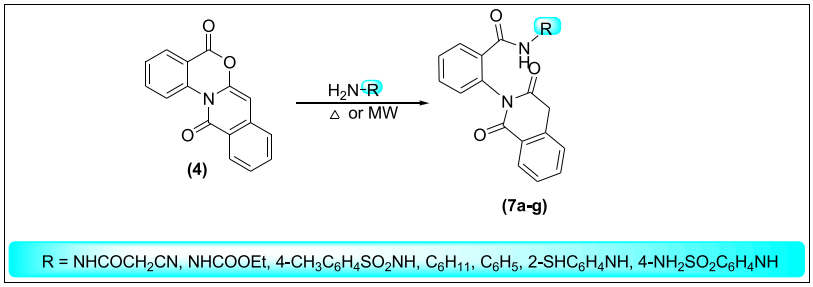

An efficient and convenient synthesis of novel [1,3]oxazino[3,2-b]isoquinoline-5,12-dione derivative **4** was achieved by the reaction of anthranilic acid with homophthalic anhydride under microwave irradiation, followed by cyclization with acetic anhydride. Some new isoquinolinone and fused isoquinolinone derivatives were prepared via reaction of compound **4** with different nitrogen nucleophiles by using reflux and a focused microwave reactor. Microwave irradiation favored the formation of the desired products with improved yields and shortened reaction times. This is a simple and green method for the synthesis of isoquinolinone derivatives. The structures of the prepared compounds were elucidated by IR, ^{1}H-NMR, and mass spectroscopy. Some of the newly prepared compounds were tested *in vitro* against a panel of three human tumor cell lines, namely, hepatocellular carcinoma (liver) HepG2, colon cancer HCT-116, and mammary gland breast MCF-7. Almost all of the tested compounds showed satisfactory activity.

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INTRODUCTION

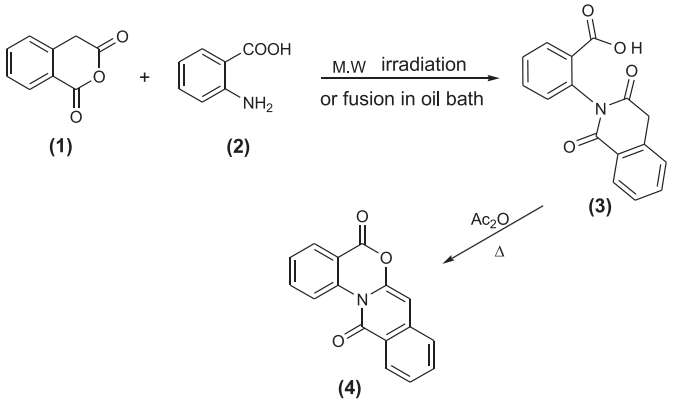
Microwave (MW)-assisted synthesis is a branch of green chemistry that has gained much attention in recent years. Moreover, it is considered pollution-free and eco-friendly and typically offers high yields, together with simplified processing and handling [1] as compared with conventional synthesis methods. In this method, reactions occur more rapidly, safely, and with higher chemical yields, often far better than conventional methods, which require longer reaction times and larger quantities of solvents and reagents, cause environmental pollution, and contribute to health hazards [2]. Many heterocyclic compounds have been synthetized by using MW irradiation [3]. Six-membered heterocycles and their fused derivatives have been reported among the compounds synthetized via MW irradiation, using cyclocondensation, cycloaddition, and multicomponent reactions, generating good yield and short reaction time in an easy and rapid way [4].

Isoquinolinones and 1,2-dihydroisoquinolines have received a big amount of interest in medicinal chemistry research [5]. Their structures are incorporated in several alkaloids [6] and other pharmacologically important compounds [7]. Many compounds containing the isoquinolinone templates have widespread biological applications, such as antidepressant [8,9], antitumor [10], and antimicrobial activities [11–13]. Because of their biological and pharmacological importance, several methods have been reported for the synthesis of isoquinolinones. Most of these methods involve the use of either a preformed isoquinoline or homophthalic acid, which is in turn obtained by a multistep sequence [14–19]. Homophthalic acids are transformed into isoquinolinones via isocoumarins [20,21], isoquinolone-4-carboxylic acid [14–19], or homophthalimide [19]. In continuation of our previous work on isoquinolines [22–24], the present study was designed to synthesize some new derivatives of isoquinoline and fused isoquinoline.

RESULTS AND DISCUSSION

Several isoquinoline and fused isoquinoline derivatives were synthesized as anticancer [12,25], anti-inflammatory [26], antimalarial [27] and anti-HIV [28]. In this research, condensation of homophthalic anhydride **1** with anthranilic acid **2** under MW irradiation or fusion on an oil bath at 170°C for 2 h afforded 2-(1,3-dioxo-3,4-dihydroisoquinolin-2(1*H*)-yl)benzoic acid **3** (Scheme 1). The chemical structure **3** was confirmed by analytical and

Scheme 1. Synthetic route for the preparation of compounds **3** and **4**.



spectroscopic data (IR, ^{1}H-NMR, and MS). Thus, the IR spectrum of **3** showed ν_{OH} (br) at 3393–2547 cm^{-1}, $\nu_{C=O\ (acid)}$ at 1722 cm^{-1} and $\nu_{C=O\ (cyclic\ amide)}$ (br) at 1678 cm^{-1}. Moreover, ^{1}H-NMR spectrum of **3** in dimethyl sulfoxide (DMSO)-d_6 showed the following signals from low to high field at δ (ppm): 12.91 (s, 1H, COOH, exchangeable with D_2O), 8.08–7.36 (m, 8H arom.) and 4.39–4.15 (dd, 2H, CH_2, J = 22.5 Hz). Also, the mass spectrum of compound **3** shows the respective molecular ion peak at *m*/*z* = 281 (11.1%), which upon loss of water molecule afforded the base peak at *m*/*z* = 263.

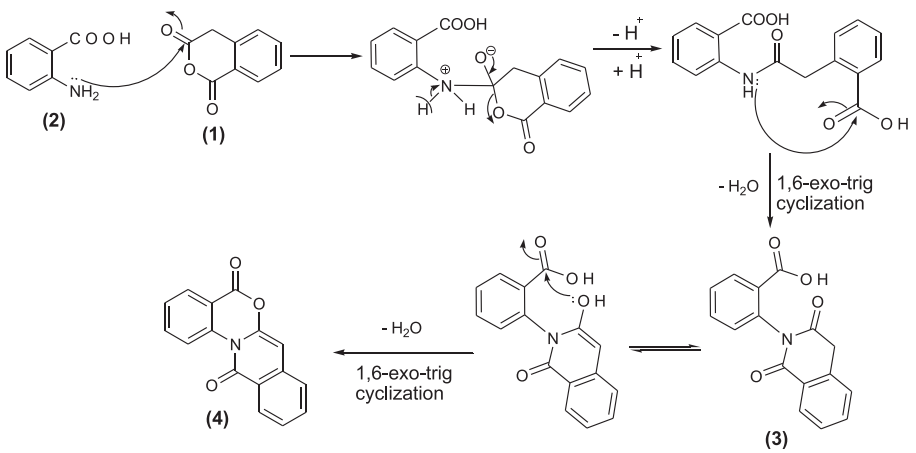
The titled compound 5*H*,12*H*-benzo[4,5][1,3]oxazino[3,2-*b*]isoquinoline-5,12-dione **4** was obtained in fairly good yield upon cyclization of compound **3** by using freshly distilled acetic anhydride (Scheme 1). The synthesis of compound **4** was not possible by MW-assisted synthesis because it involves the use of acetic anhydride, which is not amenable to use under MW irradiation. The assigned structure of **4** was consistent with the study of its IR spectrum which devoid the broad band of OH and displayed $\nu_{C=O\ (\delta\text{-lactone})}$ at 1767 cm^{-1}, $\nu_{C=O\ (cyclic\ amide)}$ at 1677 cm^{-1}. Furthermore, ^{1}H-NMR spectrum ($CDCl_3$) revealed the absence of δ- ^{1}H of CH_2 protons at 4.39 ppm and the presence of δ-^{1}H of C_7-H at 6.34 ppm, which was completely in accord with the assigned structure.

Compound **4** was formed via attack of the nitrogen nucleophile of the amino group of anthranilic acid at the carbonyl group of homophthalic anhydride (tetrahedral mechanism) followed by 1,6-exo-trig cyclization with elimination of 2 mol of water. The synthetic mechanism for the target compound **4** is shown in Scheme 2.

[1,3]Oxazino[3,2-*b*]isoquinolinone derivative **4** was used as key intermediate to synthesize varieties of isoquinoline and fused isoquinoline derivatives via its interaction with different nitrogen nucleophiles with the aim to obtain more precise and interesting pharmaceutical compounds. We have developed a new synthetic method for synthesis of 2-substituted isoquinolinone derivatives by using MW irradiation, which led to improved yields and less reaction time.

All new isoquinolinone and fused isoquinolinone derivatives were synthesized by both conventional

Scheme 2. Proposed mechanism for formation of compound **4**.



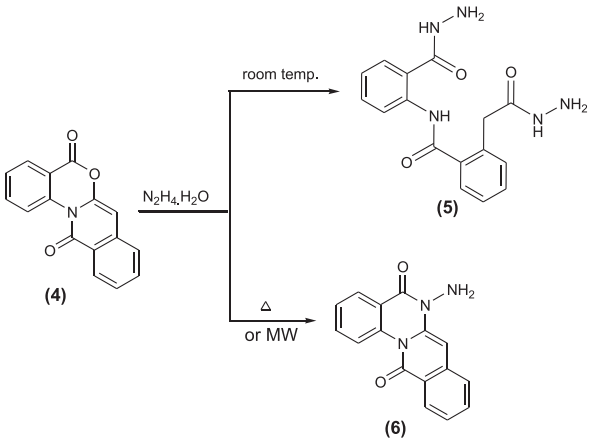
synthesis and MW-assisted synthesis by synthetic Schemes 3–5.

The [1,3]oxazino[3,2-*b*]isoquinolinone derivative **4** stirred with hydrazine hydrate (80%) in dioxane at room temperature afforded the dihydrazide derivative **5**, whereas refluxing dioxane yielded 6-amino-5*H*-isoquinolino[2,3-*a*]quinazoline-5,12(6*H*)-dione **6** as the sole product with 60% yield. Compound **6** was also synthesized by using MW irradiation in fairly good yield (82%) (Scheme 3).

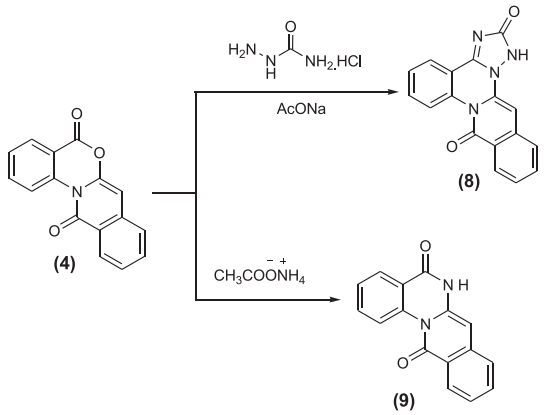
Some new isoquinolinone derivatives **7a**–**g** were obtained from the reaction of [1,3]oxazino[3,2-b] isoquinolinone derivative **4** with some nitrogen nucleophiles such as cyanoacetohydrazide, ethyl carbazate, *p*-toluenesulfonohydrazide, cyclohexylamine, aniline, 2-aminothiophenol, and sulfanilamide (Scheme 4). These nucleophilic substitution reactions were performed under reflux and MW irradiation to determine which method gives the best results. Reaction conditions, substituents, yields, and reaction times are listed in Table 1.

A comparative analysis of percentage yields and total reaction time for all synthesized isoquinolinone derivatives by both conventional method and MW-assisted method was carried out to find out if the MW-assisted synthesis of isoquinolinone derivatives adds any advantage or not. It was found that there is improvement in percentage yields and reduction in the reaction time of the synthesized isoquinolinone derivatives. By using MW irradiation, reaction is possible within few minutes, and it also improves the yield. This would be highly advantageous for drug discovery laboratories where small amounts of different analogues have to be synthesized in short periods of time. MW-assisted synthesis is quicker, high yielding, environment-friendly, and shows cleaner chemistry [1,2]

Scheme 3. Synthetic route for the preparation of compounds **5** and **6**.



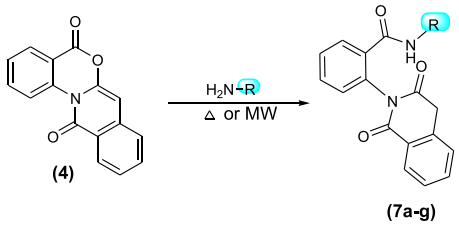
Scheme 5. Synthetic route for the preparation of compounds **8** and **9**.



After spectral analysis of all synthesized isoquinolinone derivatives, it was observed that for all synthesized isoquinolinone derivatives, the IR spectra value of N─H stretching ranges between 3141 and 3371 cm^{-1} and the value of C═O stretches for amides of 2-substituted isoquinolinone ranges between 1660 and 1721 cm^{-1}. After analyzing the ^{1}H-NMR data, the presence of dd δ-^{1}H value of CH_2 protons of isoquinolinone ring (H_a, H_b) between 3.59 and 4.42 ppm for all 2-substituted isoquinolinone derivatives was observed (Fig. 1).

Furthermore, new fused isoquinolinone derivatives were obtained when compound **4** was reacted with semicarbazide hydrochloride in the presence of anhydrous sodium acetate under reflux in glacial acetic acid or MW irradiation to yield the annelated triazoloquinazoline derivative **8**. The structure of

Scheme 4. Synthetic route for the preparation of compounds 7a–g. [Color figure can be viewed at wileyonlinelibrary.com]

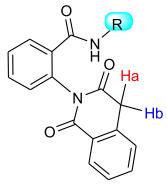


R = $NHCOCH_2CN$, NHCOOEt, 4-$CH_3C_6H_4SO_2NH$, C_6H_{11}, C_6H_5, 2-SHC_6H_4NH, 4-$NH_2SO_2C_6H_4NH$

Table 1

Comparative study of conventional versus MW method.

Compound	*R*	Reflux		Microwave (MW)		
		Time reaction (h)	Yield (%)	700 W		
				Time reaction (min)	Yield (%)	mp °C
7a	$NHCOCH_2CN$	8	62	3	85	264–266
7b	NHCOOEt	7	69	4	89	188–190
7c	$4\text{-}CH_3C_6H_4SO_2NH$	10	45	6	90	234–236
7d	C_6H_{11}	6	53	3	92	90–92
7e	C_6H_5	7	61	5	96	251–253
7f	$2\text{-}SHC_6H_4NH$	8	42	3	82	124–126
7g	$4\text{-}NH_2SO_2C_6H_4NH$	9	66	4	93	322–324



(7a-g)

Figure 1. General structure for isoquinolinone derivatives. [Color figure can be viewed at wileyonlinelibrary.com]

compound **8** was confirmed by spectroscopic data. The IR spectrum showed that bands at 3310, 1686, and 1625 cm^{-1} attributed to NH, C═O, and C═N groups, respectively. The ^{1}H-NMR spectrum of compound **8** showed the following signals from low to high field at δ (ppm): 10.9 (s, 1H, NH, exchangeable with D_2O), 9.04–7.43 (m, 8H arom.), 6.61 (s, 1H, C_4─H). The recorded peak in the mass spectrum of compound **8** at m/z = 302 (5.22%) indicates the removal of two molecules of water under the reaction conditions via ring opening followed by double cyclization in situ (Scheme 5). However, fusion of compound **4** with ammonium acetate on an oil bath at 170°C or under MW irradiation afforded 5*H*-isoquinolino[2,3-*a*]quinazoline-5,12(6*H*)-dione **9** (Scheme 5).

Pharmacological activity. ***Antitumor activity using* in vitro *Ehrlich ascites assay.*** We assessed the cytotoxic activity of the compounds (listed in Table 2 and shown in Fig. 2) against three human tumor cell lines, namely, hepatocellular carcinoma (liver) HePG2, colon cancer HCT-116, and mammary gland breast MCF-7.

In general, the cytotoxic activity of the tested compounds ranged from very strong to weak activity. Compound **7e** showed approximately equal activity to the 5-FU as a standard for HePG-2 cell line with IC_{50} 7.6 ± 0.7 and 7.9 ± 0.5 µg/mL, respectively. The optimal results were observed for compounds **5** and **7e** (very strong activity) with IC_{50} 9.0 ± 0.8 and 7.6 ± 0.7 µg/mL for HePG-2 cell line, respectively. Compounds **7e** and **7c** exhibited very strong activity with IC_{50} 7.2 ± 0.5 and 9.3 ± 0.9 µg/mL for HCT-116 cell line, respectively. Compound **7e** showed very strong activity with IC_{50} 8.3 ± 0.8 µg/mL for MCF-7 cell line. Compounds **7a** and **7c** showed strong activity toward HePG-2 cell IC_{50} 17.9 ± 1.4 and 13.3 ± 1.2 µg/mL, while compounds **4** and **5** showed strong activity toward HCT-116 cell IC_{50} 15.4 ± 1.4 and 11.3 ± 1.1 µg/mL, respectively. Also, strong activity toward MCF-7 cell line was observed with compounds **7b**, **7c**, and **9** with IC_{50} 17.0 ± 1.8, 20.4 ± 2.0, and 10.6 ± 1.3 µg/mL, respectively. Moderate activity toward HePG-2, HCT-116, and MCF-7 cell lines was observed with compounds **3**, **4**, **5**, **7a**, **7b**, **7d**, **7g**, and **9**.

Table 2

Cytotoxicity (IC_{50}) of the tested compounds on different cell lines.

Compd. no.	IC_{50} (µg/mL)[a]		
	HePG2	HCT-116	MCF-7
3	49.4 ± 2.9	69.4 ± 3.3	74.5 ± 3.9
4	25.9 ± 1.8	15.4 ± 1.4	36.5 ± 2.8
5	9.0 ± 0.8	11.3 ± 1.1	25.8 ± 2.5
6	86.2 ± 4.3	80.1 ± 4.1	90.2 ± 4.9
7a	17.9 ± 1.4	39.4 ± 2.3	41.3 ± 2.9
7b	58.1 ± 3.2	33.0 ± 2.0	17.0 ± 1.8
7c	13.3 ± 1.2	9.3 ± 0.9	20.4 ± 2.0
7d	64.0 ± 3.6	47.8 ± 2.6	65.4 ± 3.7
7e	7.6 ± 0.7	7.2 ± 0.5	8.3 ± 0.8
7f	80.3 ± 3.9	74.3 ± 3.6	81.3 ± 4.2
7g	32.5 ± 2.1	51.6 ± 2.9	54.4 ± 3.2
8	69.7 ± 3.8	89.7 ± 4.6	92.1 ± 4.5
9	43.0 ± 2.7	23.2 ± 1.7	10.6 ± 1.3
5-FU	7.9 ± 0.5	5.3 ± 0.2	5.4 ± 0.3

5-FU, 5-fluorouracil.

[a]IC_{50} (µg/mL): 1–10 (very strong), 11–20 (strong), 21–50 (moderate), 51–100 (weak), and above 100 (noncytotoxic).

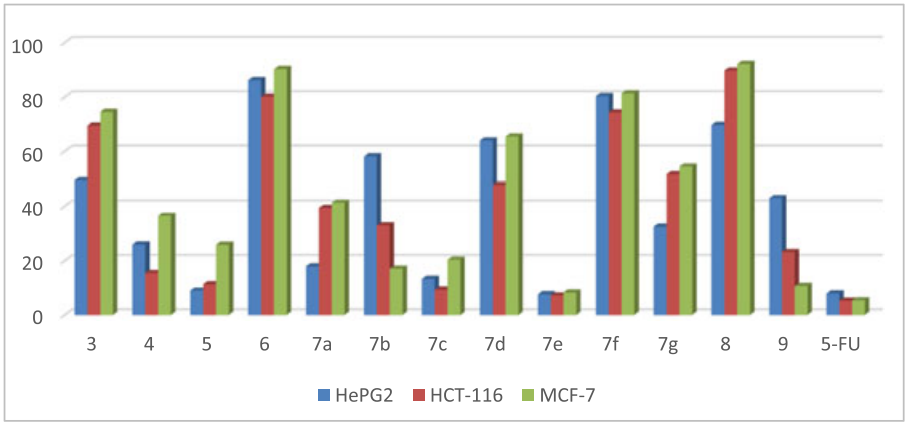
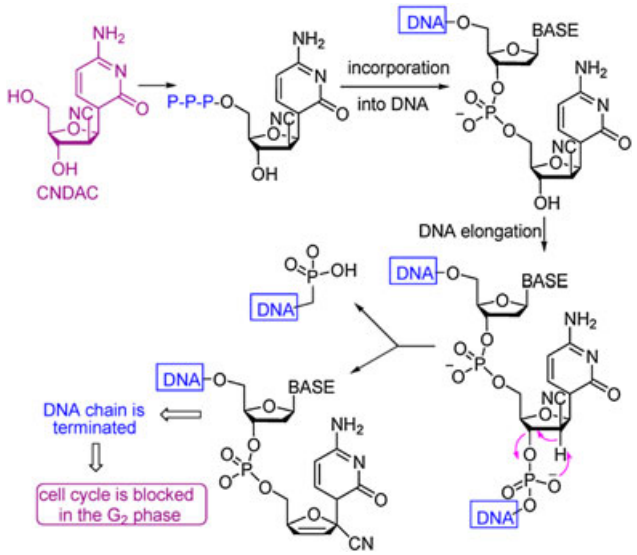


Figure 2. Cytotoxic activity of the tested compounds on different cell lines. [Color figure can be viewed at wileyonlinelibrary.com]

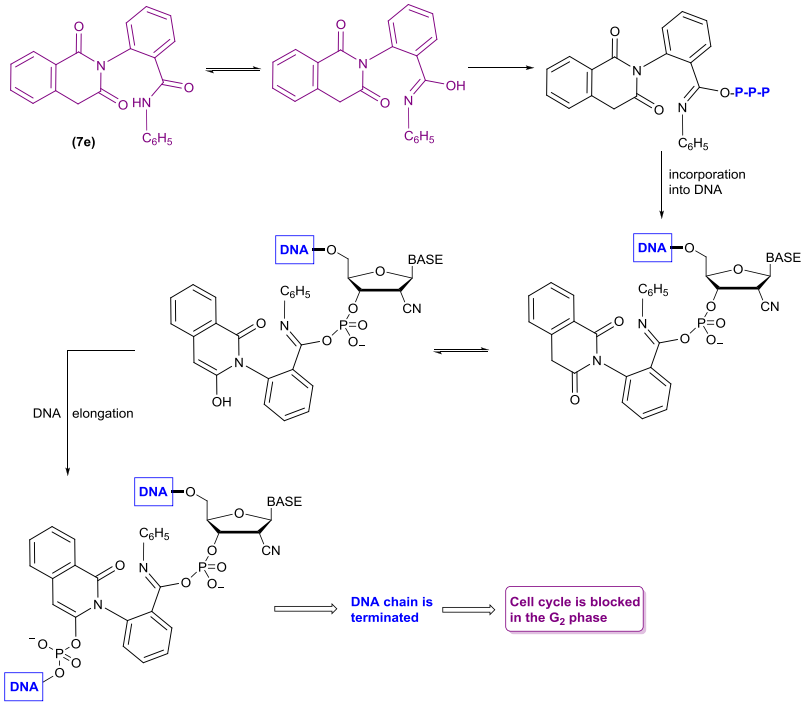
Structure–activity relationship. DNA is made up of chemical building blocks called nucleotides. The four types of nitrogen bases found in nucleotides are as follows: adenine (A), thymine (T), guanine (G), and cytosine (C). The base adenine always pairs with thymine, while guanine always pairs with cytosine through hydrogen bond. The cytotoxic activity of the tested compounds toward different cell lines depends on two factors [29,30]: (i) the formation of intermolecular hydrogen bond with DNA bases and (ii) the positive charge on the tested compounds attracted to the negative charge on the cell wall. By comparing the experimental cytotoxicity of the compounds reported in this study to their structures, the following structure-activity relationship was postulated.

- Compound **7e** showed very strong activity; this is due to the presence of the NH group which may be added to any unsaturated moiety in DNA or forming hydrogen bond with either one of the nucleobases of the DNA and causes it damage.
- Compound **5** showed very strong activity; this is due to the presence of 3 NH and 2 NH_2 groups available to form hydrogen bond with either one of the nucleobases of the DNA and causes it damage.
- Compound **7c** showed very strong activity; this is due the presence of 2 NH which can form hydrogen bond with either one of the nucleobases of the DNA and causes it damage. Also, the presence of the SO_2Ph group as a strong electron attracting group rendered the molecule positively charged forming electrostatic attraction with the DNA nucleobases. Moreover, the SO_2 group acts on the mitotic spindle [31].
- Compounds **7a**, **7b**, and **9** contain NH group forming hydrogen bond with either one of the nucleobases of the DNA and causes it damage.

Scheme 6. Mechanism of the antitumor action of CNDAC. [Color figure can be viewed at wileyonlinelibrary.com]



Scheme 7. Mechanism of the antitumor action of compound **7e**. [Color figure can be viewed at wileyonlinelibrary.com]



2′-C-Cyano-2′-deoxy-1-β-D-arabinopentofuranosylcytosine (CNDAC) [32] is a nucleoside analogue with a novel mechanism of action that is being evaluated in clinical trials. Incorporation of CNDAC triphosphate into DNA and extension during replication lead to single-strand breaks directly caused by β-elimination. These breaks, or the lesions that arise from further processing, cause cells to arrest in G_2. The electron withdrawing effect [33] of the cyano group at the arabinose 2′-β-position increases the acidity of the 2′-α proton and facilitates a β-elimination reaction involving an oxygen of the phosphate group at the 3′-β position that leads to single strand break that affords a DNA molecule lacking a 3′-hydroxyl, which prevents its repair by ligation and leads to inhibition of the cell cycle at the G_2 phase (Scheme 6).

Also, the presence of NH and OH groups enhances the cytotoxicity of compound **7e**. The proposed mechanism of DNA interaction with compound **7e** as compared with CNDAC is shown in Scheme 7.

CONCLUSION

The objectives of the present study were to synthesize some new isoquinolinone and fused isoquinolinone derivatives by using reflux and a focused MW reactor and study their cytotoxic activity. MW irradiation favored the formation of the desired products with improved yields and shortened reaction times. This is a simple and green method for the synthesis of isoquinolinone derivatives. The tested compounds showed very strong to weak cytotoxic activity against three anticancer cell lines. The best results were observed for compounds **5**, **7e**, and **7c** (very strong activity). Compound **7e** showed approximately equal activity to the 5-FU as a standard against HePG2 cell line.

EXPERIMENTAL

All melting points were taken on a Griffin and George melting-point apparatus (Griffin & George Ltd., Wembley, Middlesex, UK) and are uncorrected. IR spectra were recorded on Pye Unicam SP1200 spectrophotometer (Pye Unicam Ltd., Cambridge, UK) by using the KBr wafer technique. ^{1}H-NMR spectra were determined on a Varian Gemini 300 MHz (Santa Clara, CA) by using tetramethylsilane as internal standard (chemical shifts in δ scale). EI-MS was measured on a Shimadzu GC-MS (Columbia, MD) operating at 70 eV. Elemental analyses were carried out at the Microanalytical Unit, Faculty of Science, Ain Shams University, using a Perkin-Elmer 2400 CHN elemental analyzer (Waltham, MA), and satisfactory analytical data (±0.4) were obtained for all compounds. MW experiments were carried out by using a CEM Discover Labmate microwave apparatus (300 W with CHEMDRIVER software; Matthews, NC, USA). The homogeneity of the synthesized compounds was

controlled by thin layer chromatography (TLC), using aluminum sheet silica gel F_{254} (Merck).

Synthesis of 2-(1,3-dioxo-3,4-dihydroisoquinolin-2(1H)-yl) benzoic acid 3. A mixture of homophthalic anhydride (1.62 g, 10 mmol) and anthranilic acid (1.37 g, 10 mmol) was exposed to MW at 900 W for 5 min and undergoes fusion on an oil bath at 170°C for 2 h. After cooling, the reaction mixture was treated with ethanol and the solid formed was filtered, dried, and recrystallized from EtOH to give **3** as pale yellow crystals (yield 72% with MW and 53% with fusion), mp 224–226°C. IR (υ/cm^{-1}): br 3393–2547 (OH_{acid}), 1722 ($C{=}O_{acid}$), 1678 ($C{=}O_{cyclic\ amide}$). ^{1}H-NMR (DMSO-d_6) δ (ppm): 12.91 (s, 1H, OH, exchangeable with D_2O), 8.08–7.36 (m, 8H arom.), 4.39–4.15 (dd, 2H, CH_2, J = 22.5 Hz). ms *m*/*z* (%): 281 (M^+; 11.1), 263 (100), 236 (78.97), 207 (52.42), 118 (61.49), 90 (92.39), 77 (9.65). *Anal*. Calcd. for $C_{16}H_{11}NO_4$ (281.26): C, 68.32; H, 3.94; N, 4.98. Found: C, 68.29; H, 3.82; N, 4.93.

Synthesis of 5H,12H-benzo[4,5][1,3]oxazino[3,2-b] isoquinoline-5,12-dione 4. A mixture of compound **3** (2.81 g, 10 mmol) and acetic anhydride (10 mL) was heated on water bath for 15 min. The deposited solid on hot was collected by filtration, washed with petroleum ether (bp 80–100°C), dried, and recrystallized from dioxane to give **4** as yellow crystals, yield 82%, mp 226–228°C. IR (*v*): 1767 ($C{=}O_{\delta\text{-lactone}}$), 1677 cm^{-1} ($C{=}O_{cyclic\ amide}$). ^{1}H-NMR ($CDCl_3$) δ (ppm): 9.36–7.42 (m, 8H arom.), 6.34 (s, 1H, C_7-H). ms *m*/*z* (%): 263 (M^+; 67.1), 235 (58.6), 207 (100), 179 (47.4), 89 (54.2), 77 (9.5). *Anal*. Calcd. for $C_{16}H_9NO_3$ (263.25): C, 73.00; H, 3.45; N, 5.32. Found: C, 73.07; H, 3.37; N, 5.35.

Synthesis of N-(2-(hydrazinecarbonyl)phenyl)-2-(2-hydrazinyl-2-oxoethyl)benzamide 5. Hydrazine hydrate (0.5 mL, 10 mmol) was added dropwise to a solution of compound **4** (2.63 g, 10 mmol) in dioxane (30 mL) with stirring at room temperature for 0.5 h. The separated solid was filtered off, dried, and recrystallized from ethanol to give **5** as white crystals, yield 43%. mp 196–198°C. IR (υ/cm^{-1}): 3325, 3250, 3203 (NH, NH_2), 1675, 1659 (C═O). ^{1}H-NMR (DMSO-d_6) δ (ppm): 11.81 (s, 1H, NH, exchangeable with D_2O), 10.11 (s, 1H, NH, exchangeable with D_2O), 9.13 (s, 1H, NH, exchangeable with D_2O), 8.50–7.14 (m, 8H arom.), 4.42 (br s, 4H, $2NH_2$, exchangeable with D_2O), 3.71 (s, 2H, CH_2). MS *m*/*z* (%): 327 (M^+; 52.53), 277 (10.97), 264 (5.82), 236 (10.17), 120 (100), 115 (14.3), 89 (86.73), 77 (30.12). *Anal*. Calcd. for $C_{16}H_{17}N_5O_3$ (327.34): C, 58.71; H, 5.23; N, 21.39. Found: C, 58.82; H, 5.15; N, 21.28.

Synthesis of 6-amino-5H-isoquinolino[2,3-a]quinazoline-5,12(6H)-dione 6. ***Method 1.*** A mixture of compound **4** (2.63 g, 10 mmol) and hydrazine hydrate (0.5 mL, 10 mmol) in dioxane (30 mL) was heated under reflux for 2 h. The solid formed after cooling was filtered off, dried, and then recrystallized from dioxane to give **6** as yellow crystals, yield 60%. mp 230–232°C. IR (υ/cm^{-1}): 3302, 3201 (NH_2), 1669 (C═O). ^{1}H-NMR (DMSO-d_6) δ (ppm): 9.09–7.36 (m, 8H arom.), 7.01 (s, 1H, C_7─H), 5.71 (s, 2H, NH_2, exchangeable with D_2O). MS *m*/*z* (%): 277 (M^+; 100), 248 (54.7), 220 (10.8), 205 (4.6), 190 (4.6), 115 (8.9), 89 (14.76), 77 (7.3). *Anal*. Calcd. for $C_{16}H_{11}N_3O_2$ (277.28): C, 69.31; H, 4.00; N, 15.15. Found: C, 69.21; H, 3.92; N, 14.99.

Method 2. A mixture of compound **4** (2.63 g, 10 mmol) and hydrazine hydrate (0.5 mL, 10 mmol) was exposed to MW at 700 W for 3 min. After cooling, the reaction mixture was treated with ethanol and the solid formed was filtered, dried, and recrystallized from dioxane to give **6** as yellow crystals, yield 82% (identity mp mixed mp, TLC, and IR).

General procedure for synthesis of isoquinolinone derivatives (7a–g). ***Method A (reflux).*** A mixture of compound **4** (10 mmol) and hydrazine derivatives (10 mmol) or 1°ry amines (10 mmol) in dioxane (20 mL) was heated under reflux for 6–10 h. The precipitated solid product was filtered, washed with ethanol, dried, and finally recrystallized from the appropriate solvent.

Method B (MW). A mixture of compound **4** (10 mmol) and hydrazine derivatives (10 mmol) or 1°ry amines (10 mmol) was subjected to MW irradiation (Table 1). After cooling, the reaction mixture was treated with ethanol and the solid formed was filtered, dried, and recrystallized from the appropriate solvent.

N′-(2-Cyanoacetyl)-2-(1,3-dioxo-3,4-dihydroisoquinolin-2(1H)-yl)benzohydrazide 7a. Recrystallized from dioxane as white crystals, mp 264–266°C. IR (υ/cm^{-1}): 3274 (NH), 2250 (C≡N), 1721, 1667 (C═O). ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.40 (s, 1H, NH, exchangeable with D_2O), 10.21 (s, 1H, NH, exchangeable with D_2O), 8.04–7.37 (m, 8H arom.), 4.33–4.12 (dd, 2H, CH_2, J = 22.5 Hz), 3.67 (s, 2H, CH_2). MS *m*/*z* (%): 362 (M^+; 1.43), 337 (100), 322 (48.7), 207 (5.8), 115 (66.9), 89 (83). *Anal*. Calcd. for $C_{19}H_{14}N_4O_4$ (362.34): C, 62.98; H, 3.89; N, 15.46. Found: C, 63.00; H, 3.91; N, 15.50.

Ethyl 2-(2-(1,3-dioxo-3,4-dihydroisoquinolin-2(1H)-yl) benzoyl)hydrazinecarboxylate 7b. Recrystallized from benzene as buff crystals, mp 188–190°C. IR (υ/cm^{-1}): 3256, 3185 (NH), 1729, 1677 (C═O). ^{1}H-NMR ($CDCl_3$) δ (ppm): 8.21–7.24 (m, 8H arom.), 7.90 (s, 1H, NH, exchangeable with D_2O), 6.66 (s, 1H, NH, exchangeable with D_2O), 4.42–4.15 (dd, 2H, CH_2, J = 24 Hz), 4.05 (q, 2H, CH_2), 1.16 (t, 3H, CH_3). MS *m*/*z* (%): 349 (M^+; −18, 1.23), 330 (1.07), 264 (7.06), 236 (26.58), 180 (28.91), 152 (16.33), 90 (100). *Anal*. Calcd. for $C_{19}H_{17}N_3O_5$ (367.36): C, 62.12; H, 4.66; N, 11.44. Found: C, 62.02; H, 4.53; N, 11.40.

N′-(2-(1,3-Dioxo-3,4-dihydroisoquinolin-2(1H)-yl)benzoyl)-4-methylbenzene sulfonohydrazide 7c. Recrystallized from ethanol as yellow crystals, mp 234–236°C. IR (υ/cm^{-1}):

3325, 3141 (NH), 1718, 1684, 1660 (C═O). ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.49 (s, 1H, NH, exchangeable with D_2O), 9.79 (s, 1H, NH, exchangeable with D_2O), 8.02–7.16 (m, 12H arom.), 4.25–3.95 (dd, 2H, CH_2, J = 22.2 Hz), 2.28 (s, 3H, CH_3). MS *m*/*z* (%): 449 (M^+; 4.54), 431 (46.47), 384 (7.17), 247 (64.05), 219 (37.05), 191 (31.98), 165 (87.90), 115 (44.93), 40 (100). *Anal*. Calcd. for $C_{23}H_{19}N_3O_5S$ (449.48): C, 61.46; H, 4.26; N, 9.35; S, 7.13. Found: C, 61.31; H, 4.33; N, 9.22; S, 6.96.

***N*-Cyclohexyl-2-(1,3-dioxo-3,4-dihydroisoquinolin-2(1H)-yl) benzamide 7d**. Recrystallized from petroleum ether 80–100°C as white crystals, mp 90–92°C. IR (υ/cm^{-1}): 3326 (NH), 2930, 2853 (aliph. CH_2), 1723, 1677 (C═O). ^{1}H-NMR ($CDCl_3$) δ (ppm): 8.23–7.23 (m, 8H arom.), 5.85 (s, 1H, NH, exchangeable with D_2O), 4.35–4.12 (dd, 2H, CH_2, J = 22 Hz), 3.66 (m, 1H, $CH_{cyclohexane}$), 1.65–1.07 (m, 10H, $CH_{2cyclohexane}$). MS *m*/*z* (%): 362 (M^+; 8.60), 264 (100), 263 (37.25), 236 (89.37), 208 (18.75), 90 (46.17), 77 (11.88). *Anal*. Calcd. for $C_{22}H_{22}N_2O_3$ (362.42): C, 72.91; H, 6.12; N, 7.73. Found: C, 73.00; H, 5.95; N, 7.66.

***2*-(1,3-Dioxo-3,4-dihydroisoquinolin-2(1H)-yl)-*N*-phenylbenzamide 7e.** Recrystallized from dioxane as pale yellow crystals, mp 251–253°C. IR (υ/cm^{-1}): 3276 (NH), 1721, 1674 (C═O). ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.17 (s, 1H, NH, exchangeable with D_2O), 8.07–6.61 (m, 13H arom.), 4.34–4.02 (dd, 2H, CH_2, J = 22.5 Hz). *Anal*. Calcd. for $C_{22}H_{16}N_2O_3$ (356.37): C, 74.15; H, 4.53; N, 7.86. Found: C, 74.03; H, 4.42; N, 7.79.

***2*-(1,3-Dioxo-3,4-dihydroisoquinolin-2(1H)-yl)-*N*-(2-mercaptophenyl) benzamide 7f**. Recrystallized from ethanol as yellow crystals, mp 124–126°C. IR (υ/cm^{-1}): 3358 (NH), 1721, 1682 (C═O). ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.38 (s, 1H, NH, exchangeable with D_2O), 8.08–6.42 (m, 12H arom.), 5.53 (s, 1H, SH, exchangeable with D_2O), 4.33–4.055 (dd, 2H, CH_2, J = 22.5 Hz). *Anal*. Calcd. for $C_{22}H_{16}N_2O_3S$ (388.44): C, 68.02; H, 4.15; N, 7.21; S, 8.25. Found: C, 67.94; H, 4.01; N, 7.18; S, 8.23.

***2*-(1,3-Dioxo-3,4-dihydroisoquinolin-2(1H)-yl)-*N*-(4-sulfamoylphenyl) benzamide 7g**. Recrystallized from dioxane as pale yellow crystals, mp 322–324°C. IR (υ/cm^{-1}): 3371, 3303, 3259 (NH, NH_2), 1719, 1670 (C═O). ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.70 (s, 1H, NH, exchangeable with D_2O), 8.04–7.40 (m, 12H arom.), 7.20 (s, 2H, NH_2, exchangeable with D_2O), 4.34–4.16 (dd, 2H, CH_2, J = 21 Hz). MS *m*/*z* (%): 435 (M^+; 1.73), 421 (1.52), 364 (8.99), 278 (19.40), 265 (89.64), 237 (100), 125 (96.12), 90 (86.95). *Anal*. Calcd. for $C_{22}H_{17}N_3O_5S$ (435.45): C, 60.68; H, 3.93; N, 9.65; S, 7.36. Found: C, 60.55; H, 4.09; N, 9.47; S, 7.22.

Synthesis of 9H-isoquinolino[2,3-a][1,2,4]triazolo[1,5-c] quinazoline-2,9(1H)-dione 8. ***Method 1.*** A mixture of compound **4** (2.63 g, 10 mmol), semicarbazide hydrochloride (1.11 g, 10 mmol), and anhydrous sodium acetate (1 g) in glacial acetic acid (20 mL) was refluxed for 10 h. The deposited solid on hot was filtered off, washed with ethanol, dried, and recrystallized from dioxane to give **8** as pale yellow crystals, yield 56%, mp 290–292°C. IR (υ/cm^{-1}): 3310 (NH), 1686 (C═O), 1625 (C═N). ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.9 (s, 1H, NH, exchangeable with D_2O), 9.04–7.43 (m, 8H arom.), 6.61 (s, 1H, C_4─H). MS *m*/*z* (%): 302 (M^+; 5.22), 277 (46.7), 234 (19.8), 190 (85.1), 179 (52), 115 (83.3), 90 (63.7), 77 (48). *Anal*. Calcd. for $C_{17}H_{10}N_4O_2$ (302.29): C, 67.55; H, 3.33; N, 18.53. Found: C, 67.45; H, 3.29; N, 18.50.

Method 2. A mixture of compound **4** (2.63 g, 10 mmol), semicarbazide hydrochloride (1.11 g, 10 mmol), and anhydrous sodium acetate (1 g) was exposed to MW at 800 W for 3 min. After cooling, the reaction mixture was treated with boiling water and the solid formed was filtered, dried, and recrystallized from dioxane to give **8** as pale yellow crystals, yield 85% (identity mp mixed mp, TLC, and IR).

Synthesis of 6H-isoquinolino[2,3-a]quinazoline-5,12-dione 9. ***Method 1.*** A mixture of compound 4 (2.63 g, 10 mmol) and ammonium acetate (10 g) was fused in an oil bath at 170°C for 1 h. The deposited solid on hot was filtered off, washed with water for several times, dried, and recrystallized from DMF to give **9** as yellow crystals, yield 78%, mp > 320°C. IR (υ/cm^{-1}): 3148 (NH), 1674 (C═O). ^{1}H-NMR (DMSO-d_6) δ (ppm): 11.83 (s, 1H, NH, exchangeable with D_2O), 9.27–7.31 (m, 8H arom.), 6.21 (s, 1H, C_7─H). *Anal*. Calcd. for $C_{16}H_{10}N_2O_2$ (262.26): C, 73.27; H, 3.84; N, 10.68. Found: C, 73.31; H, 3.81; N, 10.65.

Method 2. A mixture of compound 4 (2.63 g, 10 mmol) and ammonium acetate (10 g) was exposed to MW at 900 W for 4 min. After cooling, the reaction mixture was treated with boiling water and the solid formed was filtered, dried, and recrystallized from DMF to give **9** as pale yellow crystals, yield 93% (identity mp mixed pm, TLC, and IR).

Pharmacological activity. ***Cytotoxicity assay.*** The cytotoxic activity of 13 compounds was tested against three human tumor cell lines, namely, hepatocellular carcinoma (liver) HePG-2, colon cancer HCT-116, and mammary gland (breast) MCF-7. The cell lines were obtained from the ATCC via the Holding Company for Biological Products and Vaccines (Cairo, Egypt). 5-Fluorouracil was used as a standard anticancer drug for comparison. The reagents used were RPMI-1640 medium, MTT, DMSO, and 5-fluorouracil (Sigma Co., St. Louis, MO, USA) and fetal bovine serum (GIBCO, Paisley, UK).

The different cell lines [34,35] mentioned in the preceding texts were used to determine the inhibitory effects of compounds on cell growth by 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. The cells were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/mL of penicillin and 100 μg/mL of streptomycin at 37°C in a 5% CO_2 incubator. The cell lines were seeded [36] 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 incubator. After incubation, the cells were treated with different concentration 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 in volume of 100 μL was added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at absorbance of 570 nm by using a plate reader (EXL 800, BioTech, Winoosky, VT, USA).

The relative cell viability in percentage was calculated as (A_{570} of treated samples/A_{570} of untreated sample) × 100.

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