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# SYNTHESIS AND EVALUATION OF ANTICANCER ACTIVITY OF NOVEL DERIVATIVES BASED ON 5,10-DIOXO-BENZO[*g*]QUINOLINE MOIETY

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**ABSTRACT:** The reaction of diazotization of 2,4-diamino-5,10-dioxo-1,5,10,10a-tetrahydrobenz[g]quinoline-3-carbonitrile **(1)** afforded 4-amino-2-(chlorodiazenyl)-5,10-dioxo-1,5,10,10a-tetrahydrobenzo[g]quinoline-3-carbonitrile **(2)** as a versatile building block for the design and synthesis of some novel benzo[g]quinolone systems. The structure of the newly synthesized compounds has been confirmed by IR, $^{1}$H NMR, $^{13}$C NMR, mass spectral and elemental analysis. Furthermore, some selected compounds were screened for their in vitro anti-cancer activity against A-549, HT-29 and MKN-45 cancer cells. The results declared that most of the synthesized compounds are endued with high anti-tumor activity; compounds **(4)**, **(6)** and **(7)** presented the highest cytotoxic activity against lung cancer A-549, gastric cancer MKN-45 and colon adenocarcinoma cell lines, respectively.

**KEYWORDS:** Benzo[g]quinolone; Azine; Pyrimidine; Triazine; MTT assay.

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## 1. INTRODUCTION

Cancer is considered as one of the leading causes of death throughout the world, a disease known by uncontrolled multiplication of cells at any part of the human body, metastasis, and invasion [1,2]. Since cancer causes about 13% of all the death [3] and its diagnose at earlier stages is difficult, the discovery and design of potent and selective anticancer agents with promising inhibition action against cancer cells proliferation has become utterly important in medicinal chemistry. The synthesis of heterocyclic systems has undergone a renaissance in recent decades due to their intriguing bioactive properties. Nitrogen-containing heterocycles, in particular conjugates with quinoid structure, constitute an attractive class of privileged heterocycles with auspicious pharmacological properties [4]. In recent years, substantial research has been oriented towards the discovery of new quinones derivatives. The chemistry of quinones was found to be extensively dependent on the derivatives being either on the quinoid or on adjacent rings. This finding was reflected in their chemical reactivity and antitumor effects [5,6]. Furthermore, literature research indicated that pyrimidine and triazine analogues were essential in the structure of various bioactive compounds. Pyrimidine (1,3-diazine), an essential nucleus in DNA and RNA, was found to have wide spectrum of biological activities [7]. Pyrimidine derivatives have gained interest in the field of drug research since they exhibited a broad range of biological activities including antimicrobial [8], antitumor [9], bronchodilator [10], antihistaminic [11], antihypersentensive [12], anti-psoriasis [13] and antipyretic [14] activity. Besides, the pyridopyrimidine are a critical class of annulated uracils with biological importance due to their linking with the purine and pyridine system [15]. Triazine moiety have also attracted a tremendous deal of attention among chemists due to its wide biological activities such as antimicrobial, anticancer, antimalarial, antiviral and antiprotozoal activity [16-18] . Besides, triazine moiety has a large dipole moment, making its hydrogen bond donor and acceptor abilities better than those of amide. This could also be the reason to its expanded activity in biological systems [19]. Medicinal applications associated with piperazine heterocycle render them as useful structural moieties in drug research [20,21] while the piperazine-quinoline combination was found in the structure of many well-known antimicrobial drugs such as ciprofloxacin, norfloxacin, ofloxacin and pefloxacin [22]. The development of more versatile strategies to design and synthesize quinoline-based compounds continue to represent a challenge from an academic perspective. Based upon the aforesaid chemistry and biological significance of quinones based azines, and as a part of our research interest towards developing new synthetic routes of a variety of heterocyclic systems possessing promising biological and pharmacological activities [23-26], herein, we describe an economic and easy synthesis of 2, 4-diamino-5,10-dioxo-1,5,10,10a-tetrahydrobenz [*g*] quinoline-3-carbonitrile (**1**) [27] and 4-amino-2-(chlorodiazenyl)-5,10-dioxo-1,5,10,10a-

tetrahydrobenzo[*g*]quinoline-3-carbonitrile (**2**) as reactive intermediates, for the synthesis of *N*-heterocycles based on benzo[*g*]quinoline-5,10-dione moiety of potential cytotoxic activities.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Reagents were purchased from Sigma Aldrich and used without further purification. Reaction progress was monitored by TLC on silica gel pre-coated F254 Merck plates. Spots were visualized by ultraviolet irradiation. Melting points were determined on a Gallenkamp electro thermal melting point apparatus and are uncorrected. IR spectra were recorded as potassium bromide discs using Bruker-Vector 22 FTIR Spectrophotometer. The NMR spectra were recorded with a Varian Mercury VXR-300 NMR spectrometer at 300 and 75 MHz for $^{1}H$ and $^{13}C$ NMR spectra, respectively, using DMSO-$d_6$ as solvents. Chemical shifts were related to that of the solvent. Mass spectra were recorded on a Hewlett Packard MS−5988 spectrometer at 70 eV. Elemental analyses were carried out at the Micro-analytical Center of Cairo University, Giza, Egypt.

### 2.2. General procedure for the synthesis of 2,4-Diamino-5,10-dioxo-1,5,10,10a-tetrahydrobenz[g]quinoline-3-carbonitrile (1)

This compound has been prepared according to a previous method [27].

### 2.3. General procedure for the synthesis of 4-amino-2-(chlorodiazenyl)-5,10-dioxo-1,5,10,10a-tetrahydrobenzo[g]quinoline-3-carbonitrile (2)

A solution of compound **(1)** (0.01 mol) in EtOH 20 mL was treated with $HCl_{Conc.}$ (0.01 mol), The mixture was cooled at 0-5 ºC, then a cooled solution of sodium nitrite was added so that diazonium salt formed in *situ* under cooling condition.

#### 2.3.1. Reaction of diazotized compound (2) with active hydrogen reagents

A solution of the diazotized compound (**2)** (0.01 mol) was added portion-wise with stirring at 0-5 ºC over a period of 30 min. to a cold solution of the appropriate, *β*-naphthol, resorcinol, malononitrile and ethyl cyanoacetate in EtOH (50 mL) containing 5 g of AcONa. After complete addition, the reaction mixture was stirred for further 4 h, then kept in an ice chest for 12 h, and finally diluted with $H_2O$. The precipitated solid was collected, washed with $H_2O$, dried and finally recrystallized from the proper solvent to afford the corresponding arylazo **(3)**,( **5)**, (**7)** and (**8)**, respectively.

### 2.4. General procedure for the synthesis of 4-Amino-2-((2-hydroxynaphthalen-1-yl)diazenyl)-5,10-dioxo-1,5,10,10a-tetrahydro-benzo[g]quinoline-3-carbonitrile (3)

(55%) yield as red crystals; mp145−147 ºC; IR (KBr): ν ($cm^{-1}$), 3400 (OH), 3315 ($NH_{2str.}$), 2215 ($C{\equiv}N_{str.}$), 1652 ($C{=}O_{str.}$), 1670 ($N{=}N_{str.}$); $^{1}H$ NMR (300 MHz, DMSO−$d_6$): $\delta$ 6.76 (s, $NH_2$), 4.23 (s, CH), 8.1-7.2 (m, 11H, Ar-H), 9.56 (s, OH exchangeable with $D_2O$); $^{13}C$ NMR (75 MHz, DMSO−$d_6$): $\delta$ 115 (CN), 60.41, 116.03, 21.07, 122.18, 125.03, 126.04, 128.23, 133.64,

134.36 (11CH), 108.23, 142.65, 152.42 (C, naphthalene, ethylene) 183.07, 196.62 (2 C=O); MS (*m/z*, %): 421.0 ($M^+$, 50%). Anal. Calcd for: $C_{24}H_{15}N_5O_3$ (421.41): C, 68.40; H, 3.59; N, 16.62; %. Found: C, 68.43; H, 3.61; N, 16.65%

**2.5. General procedure for the synthesis of 4-Amino-2-((2,4-dihydroxyphenyl)diazenyl)-5,10-dioxo-1,5,10,10a-tetrahydrobenzo[g]-quinoline-3-carbonitrile (5)**

(69%) yield as reddish brown crystals; mp155−157ºC; IR (KBr): $\nu$ ($cm^{-1}$), 3400 (2 OH), 3215 ($NH_2$), 22115 (CN), 1652 (CO $_{str.}$), 1670 (N=N); $^1$H NMR (300 MHz, DMSO−$d_6$): $\delta$ 6.76 (s, $NH_2$), 4.23 (s, CH), 8.1-7.2 (m, 8H, Ar-H), 9.05 (s, OH exchangeable with $D_2O$), 9.12 (s, OH exchangeable with $D_2O$); $^{13}$C NMR (75 MHz, DMSO−$d_6$): $\delta$ 115 (CN), 60.41, 116.03, 121.07, 122.18, 125.03, 126.04, 128.23, 133.64, 134.36 (11CH), 108.23, 142.65, 152.42 (C naphthalene, ethylene, 183.07, 196.62 (2CO); MS (*m/z*, %): 387.0 ($M^+$, 50%). Anal. Calcd for: $C_{20}H_{13}N_5O_4$ (387.35): C, 62.01; H, 3.38; N, 18.08 %. Found: C, 62.04; H, 3.39; N, 18.12 %.

**2.6. General procedure for the synthesis of 1,6-Diamino-7,12-dioxo-12,12a-dihydro-7H-benzo[g][1,2,4]triazino[4,3-a]quinoline-2,5-dicarbonitrile (7)**

(57%) yield as reddish brown crystals; mp150−152 ºC; IR (KBr): $\nu$ ($cm^{-1}$) 3215 ($2NH_2$), 2216, 2218 (2 CN), 1652 (CO $_{str.}$); $^1$H NMR (300 MHz, DMSO−$d_6$): $\delta$ 6.79 (s, $NH_2$), 8.99 (s, $NH_2$), 4.23 (s, CH), 8.1-7.2 (m, 4H, Ar-H); $^{13}$C NMR (75 MHz, DMSO−$d_6$): $\delta$ 115.67 (2 CN), 60.68, 126.32, 126.83, 126.04, 131.23, 133.04, 134.47 (5 CH), 92.23, 110.18, 114.52, 130.82, 136.37, 146.36, 156.57, 167.38 (C=C, benzene), 183.07, 196.62 (2 CO); MS (*m/z*, %): 343.0 ($M^+$, 55%). Anal. Calcd for : $C_{17}H_9N_7O_2$ (343.30): C, 59.48; H, 2.64; N, 28.56%. Found: C, 59.50; H, 2.67; N, 28.58%.

**2.7. General procedure for the synthesis Ethyl 2-(2-(4-amino-3-cyano-5,10-dioxo-1,5,10,10a-tetrahydrobenzo[g]quinolin-2-yl)hy-drazono)-2-cyanoacetate (8)**

(80%) yield as brownish red crystals; mp180−182 ºC; IR (KBr): $\nu$ ($cm^{-1}$), 3100-3315 (NH, $NH_2$), 2215-2220 (2 CN), 1652 ($CO_{str.}$); $^1$H NMR (300 MHz, DMSO−$d_6$): $\delta$ 1.36 (t, $J$ = 7.01 *Hz*, 3H, $CH_3$), 4.23 (s, 1H, Quinon.$_{(C1)}$-H), 4.36 (q, $J$ = 7.01 *Hz*, 2H, $CH_2$), 6.76 (s, $NH_2$), 8.1-7.2 (m, 4H, Ar-H), 11.21 (s, 1H, NH-$_{Pyridi.}$), 11.25 (s, 1H, NH-$_{Hydrazi.}$); $^{13}$C NMR (75 MHz, DMSO−$d_6$): $\delta$ 14.4 ($CH_3$), 60.91 ($CH_2$), 115-115.9 (2CN), 60.41, 126.36, 126.48, 134.51, 133.83 (5 CH), 72.64, 110.35, 110.73, 130.81, 136.39, 156.58, 172.61 (C, naphthalene, ethylene), 160.02 (CO ester), 183.07, 196.62 (2CO); MS (m/z, %): 390.00 ($M^+$, 60%). Anal. Calcd for: $C_{19}H_{14}N_6O_4$ (390.35): C, 58.46; H, 3.61; N, 21.53%. Found: C, 58.49; H, 3.67; N, 21.57%.

**2.7.1. General procedure for the cyclization of arylazo (3), (5), and (8)**

A solution of the appropriate aryl azo (**3), (5)** and **(8)** (5 mmol) in AcOH (30 mL) was refluxed for 4 h. The solvent was then evaporate rated *in vacuo* and the remaining product was collected by filtration and dried, recrystallized from EtOH afforded the corresponding fused ring systems

**(4), (6)** and **(9)** respectively.

**2.8. General procedure for the synthesis of 8-Amino-9,14-dioxo-14,14a-dihydro-9H-benzo[g]naphtho[2',1':5,6][1,2,4]triazino[4,3-a]-quinoline-7-carbonitrile ( 4)**

(85%) yield as deep red crystals; mp175–177ºC; IR (KBr): ν ($cm^{-1}$) 3315 ($NH_2$), 2215 (CN), 1652 (CO); $^1$H NMR (300 MHz, DMSO–$d_6$): $\delta$ 6.76 (s, $NH_2$), 4.23 (s, CH), 8.1-7.2 (m, 10H, Ar-H); $^{13}$C NMR (75 MHz, DMSO–$d_6$): $\delta$ 115 (CN), 68.42, 116.03, 121.07, 122.18, 125.03, 126.04, 128.23, 133.64, 134.36 (11CH), 108.23, 142.65, 152.42 (C, naphthalene, ethylene), 183.07, 196.62 (2 CO); MS (*m/z*, %): 403.0 ($M^+$, 50%). Anal. Calcd for: $C_{24}H_{13}N_5O_2$ (403.39): C, 71.46; H, 3.25; N, 17.36%. Found: C, 71.49; H, 3.27; N, 17.37%.

**2.9. General procedure for the synthesis of 8-Amino-2-hydroxy-9,14-dioxo-14,14a-dihydro-9H-benzo[g]benzo[5,6][1,2,4]triazino-[4,3-a]quinoline-7-carbonitrile (6)**

(78%) yield as red crystals; mp165–167ºC; IR (KBr): $\nu$ ($cm^{-1}$) 3215(NH2), 3116 (OH), 2115 (CN), 1652 ($CO_{str.}$); $^1$H NMR (300 MHz, DMSO–$d_6$): $\delta$ 6.76(s, $NH_2$), 4.23 (s, CH), 8.1-7.2 (m, 7H, Ar-H), 9.56 (s, OH exchangeable with $D_2O$); $^{13}$C NMR (75 MHz, DMSO–$d_6$): $\delta$ 115.67 (CN), 68.30, 98.03, 104.07, 126.32, 126.83, 131.23, 133.04, 134.47 (8CH), 92.24, 100.08, 110.52, 130.82, 136.37, 145.24, 152.47, 156.57, 159.36 (C, ethylene, benzene), 183.07, 196.62 (2CO); MS (*m/z*, %): 369.0 ($M^+$, 55%); Anal. Calcd for: $C_{20}H_{11}N_5O_3$ (369.33): C, 65.04; H, 3.00; N, 18.96%. Found C, 65.08; H, 3.05; N, 18.98%.

**2.10. General procedure for the synthesis of 6-Amino-1- hydroxy- 7 ,12 -dioxo-12,12a-dihydro-7H-benzo[g][1,2,4]triazino[4,3-a]quinoline-2,5-dicarbonitrile (9)**

(65%) yield as red crystals; mp 215–217 ºC; IR (KBr): $\nu$ ($cm^{-1}$) 3531 ($OH_{str.}$), 3315 ($NH_{2str.}$), 2215, 2220 (2 $CN_{str.}$), 1652 (2 $CO_{str.}$); $^1$H NMR (300 MHz, DMSO–$d_6$): $\delta$ 6.76 (s, $NH_2$), 4.33 (s, CH), 7.2-8.1 (m, 4H, Ar-H), 9.06 (s, OH exchangeable with $D_2O$); $^{13}$C NMR (75 MHz, DMSO–$d_6$): $\delta$ 115.67, 114.51 (2CN), 60.45, 126.10, 126.04, 134.31, 133.30 (5CH), 92.24, 108.18, 110.25, 130.81, 136.34, 145.57, 152.42, 156.57, 156.52 (C, ethylene, benzene), 183.07, 196.62 (2CO); MS (*m/z*, %): 344.00 ($M^+$, 55%). Anal. Calcd for: $C_{17}H_8N_6O_3$ (344.28): C, 59.31; H, 2.34; N, 24.41 %. Found: C, 59.38; H, 2.39; N, 24.46 %.

**2.11. General procedure for the synthesis of 5-Amino-3-benzyl-4-imino-2-mercapto-3,4,11a,12-tetrahydrobenzo[g]pyrimido[4,5-b]-quinoline-6,11-dione (11)**

A mixture of compound **(1)** (2.66 g, 10 mmol), benzyl isothiocyanate (1.49 g, 10 mmol) and anhydrous $K_2CO_3$ (1.4 g, 10 mmol) in acetonitrile (30 mL) was heated under reflux for 15h. The reaction mixture was left to cool and filtered off, then the obtained potassium salt was dissolved in $H_2O$, neutralized with AcOH then the obtained crude product was filtered washed with $H_2O$, dried and recrystallized from EtOH to afford **(11)**, (75%) yield as reddish-brown crystals; mp 215–217 ºC; IR (KBr): $\nu$ ($cm^{-1}$) 3150, 3315 ($NH_{str}$, $NH_{2str}$), 2515 (S-$H_{str.}$), 1652 (2$CO_{str.}$); $^1$H NMR (300 MHz, DMSO–$d_6$): $\delta$ 4.36 (s, CH), 4.47 (s, $CH_2$), 6.76 (s, $NH_2$), 7.81-

7.20 (m, 10H, Ar-H), 8.01 (s, =NH exchangeable with $D_2O$), 12.23 (s, SH exchangeable with $D_2O$); $^{13}C$ NMR (75 MHz, DMSO–$d_6$): $\delta$ 44.89 ($CH_2$), 68.45, 104.03, 117.24, 126.31, 126.80, 129.55, 132.23, 133.56, 134.43 (9CH), 92.24, 108.18, 110.25, 130.81, 136.34, 145.57, 152.42, 156.57, 156.52 (C, ethylene, benzene), 183.07, 196.62 (2 CO); MS (*m/z*, %): 415. 0 ($M^+$, 55%). Anal. Calcd for: $C_{22}H_{17}N_5O_2S$ (415.47): C, 63.60; H, 4.12; N, 16.86; S, 7.72%. Found: C, 63.63; H, 4.14; N, 16.89; S, 7.75%.

**2.12. General procedure for the synthesis of 5-Amino-3-benzyl-4-imino-2-(methylthio)-3,4,11a,12-tetrahydrobenzo[g]pyrimido[4,5-b] quinoline-6,11-dione (12)**

A stirred mixture of compound **(11)** (0.41 g, 1 mmol), methyl iodide (2 mmol) and anhydrous potassium carbonate (0.27 g, 2 mmol) in dry acetone (10 mL) was heated under reflux for 4h. the reaction mixture was cooled, poured into ice-cold water; the formed precipitate was dried and recrystallized from DMF to afford (**12**) (66%) yield as green crystals; mp 225−227 °C; IR (KBr): $\nu$ ($cm^{-1}$) 3150, 3215 (NH, $NH_2$), 1652 (2 $CO_{str.}$); $^1H$ NMR (300 MHz, DMSO–$d_6$): $\delta$ 2.58 (s, 3H, $CH_3$), 6.76 (s, $NH_2$), 4.36 (s, CH), 4.47 (s, $CH_2$), 8.1-7.2 (m, 11H, Ar-H), $^{13}C$ NMR (75 MHz, DMSO–$d_6$): $\delta$ 15.02 ($CH_3$), 45.04 ($CH_2$), 68.45, 104.03, 117.24, 126.31, 126.80, 129.55, 132.23, 133.56, 134.43 (9CH), 92.24, 108.18, 110.25, 130.81, 136.34, 145.57, 152.42, 156.57, 156.52 (C, ethylene, benzene), 183.07, 196.62 (2CO); MS (*m/z*, %): 429.0 ($M^+$, 55%). Anal. Calcd for: $C_{23}H_{19}N_5O_2S$ (429.49): C, 64.32; H, 4.46; N, 16.31; S, 7.47%. Found: C, 64.35; H, 4.48; N, 16.34; S, 7.49%.

**2.13. General procedure for the synthesis of 5-Amino-3-benzyl-2-hydrazinyl-4-imino-3,4,11a,12-tetrahydrobenzo[g]pyrimido[4,5-b]-quinoline-6,11-dione (13)**

To a suspension of compound **(12)** (4.29 g, 10 mmol) in EtOH (50 mL), hydrazine hydrate 99% (4.0 mL) was added, the reaction mixture was refluxed for 3 h, during which a precipitate was formed. After cooling, the product was filtered, washed with $H_2O$, dried and recrystallized from DMF/ ETOH (4:1) to afford (**13**) (56%) yield as reddish brown crystals; mp 235−237 °C; IR (KBr): $\nu$ ($cm^{-1}$) 3150, 3215 (NH, $NH_2$), 1652 (2 $CO_{str.}$); $^1H$ NMR (300 MHz, DMSO– $d_6$): $\delta$ 2.33($NH_{2\text{-hydrazo}}$),6.76 (s, $NH_2$), 4.76 (s, CH), 4.57 (s, $CH_2$), 8.1-7.2 (m, 12H, Ar-H); $^{13}C$ NMR (75 MHz, DMSO– $d_6$): $\delta$ 44.04 ($CH_2$); 68.45, 104.03, 117.24, 126.31, 126.80, 129.55, 132.23, 133.56, 134.43 (9CH), 92.24, 108.18, 110.25, 130.81, 136.34, 145.57, 152.42, 156.57, 156.52 (C, ethylene, benzene), 183.07, 196.62 (2CO); MS (m/z, %): 413.00 ($M^+$, 55%). Anal. Calcd for: $C_{22}H_{19}N_7O_2$ (413.43): C, 63.91; H, 4.63; N, 23.72 %. Found: C, 63.94; H, 4.65; N, 23.74 %.

**2.14. General procedure for the synthesis of N-(6-Amino-4-benzyl-5-imino-7,12-dioxo-4,5,7,12,12a,13-hexahydro-[1,2,4]triazolo[3',4':2,3]pyrimido[4,5-b]benzo[g]quinolin-1-yl)benzamide (14)**

A solution of compound **(13)** (0.01 mol ), benzoyl chloride (0.01 mol ) and ammonium thiocyanate (0.01 mol ) in acetone 30 mL as a solvent, then the reaction mixture was refluxed

for 10 h, cooled the separated product filtered, dried and recrystallized from EtOH to afford (**14**) in (70%) yield as yellow crystals; mp 250–252 °C; IR (KBr): $\nu$ ($cm^{-1}$) 3150, 3315(NH, $NH_2$), 1652-1685 ($3CO_{str.}$); $^1H$ NMR (300 MHz, DMSO–$d_6$): $\delta$ 2.58 (s, 3H, $CH_3$), 6.76 (s, $NH_2$), 4.36 (s, CH), 4.47 (s, $CH_2$), 8.1-7.2 (m, 14H, Ar-H); $^{13}C$ NMR (75 $MH_Z$, DMSO-$d_6$): $\delta$ 50.04 ($CH_2$), 68.45, 104.03, 117.24, 126.31, 126.80, 129.55, 132.23, 133.56, 134.43 (9CH), 92.24, 108.18, 110.25, 130.81, 136.34, 145.57, 152.42, 156.57, 156.52 (C, ethylene, benzene), 165.78, 183.07, 196.62 (3CO); MS (m/z, %): 542.00 ($M^+$, 55%). Anal. Calcd for: $C_{30}H_{22}N_8O_3$ (542.55): C, 66.41; H, 4.09; N, 20.65; %. Found: C, 66.44; H, 4.22; N, 20.68; %.

**2.15. Cytotoxic activity- MTT Assay**

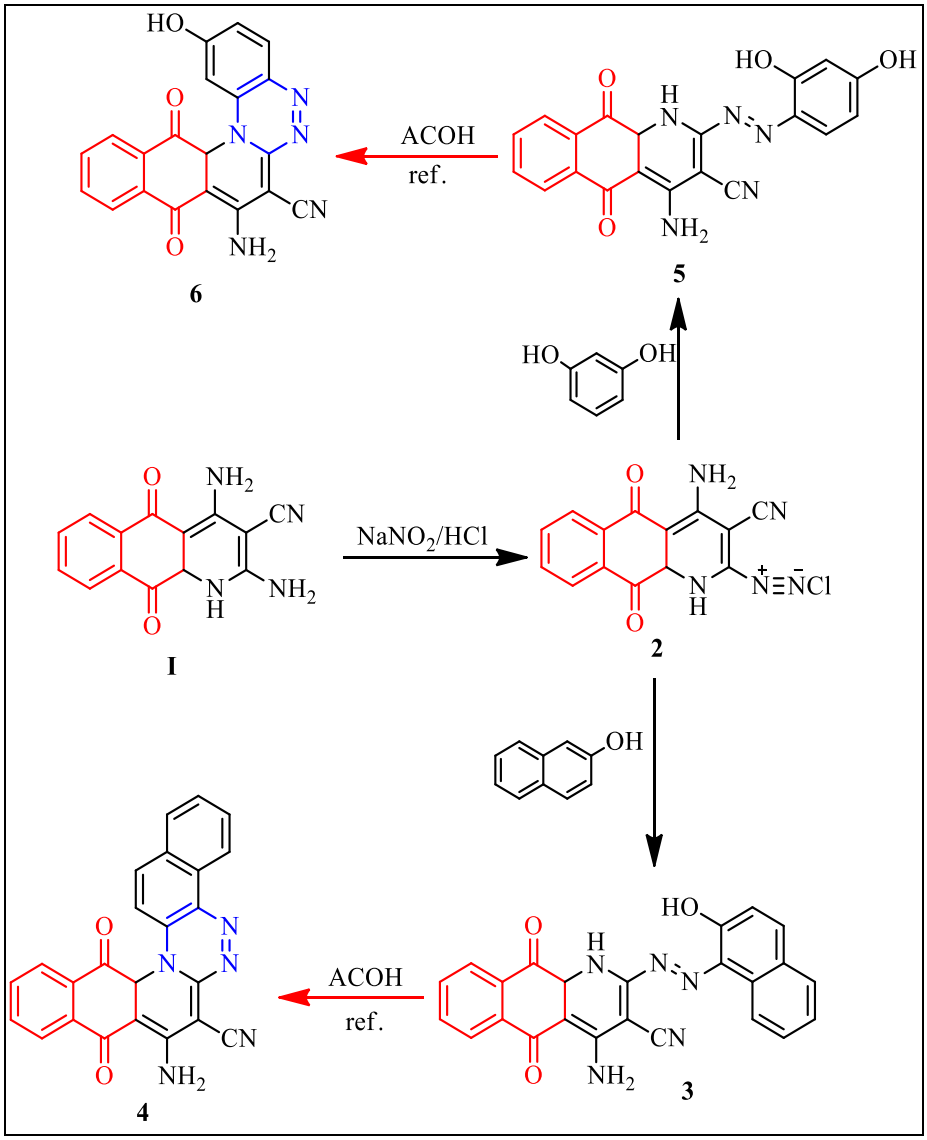
The cytotoxic activity of the synthesized compounds was assessed using the MTT assay [28]. $1\times10^4$ cells/well of each cell line were seeded and maintained in triplicate in 96-well microplate in DMEM, fortified with 10 % FBS and treated with the synthesized compounds **(4)**, **(6)**, **(7)** and **(9)** at 0.1µg/mL, 1 µg/mL ,10µg/mL then incubated at 37°C for 48 h in a $CO_2$ incubator. After incubation, the different plates were washed three times with phosphate buffered solution and 100 µL of the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution (0.5 mg/mL) was added to each well. The cells were further incubated for 1 h at 37 °C. Subsequently, MTT was converted to blue formazan crystals *via* mitochondrial succinate dehydrogenase. The supernatant from each well was cautiously removed, the plates were then washed with PBS, the formazan crystals were dissolved in 100 µL of DMSO, and absorbance at 540 nm wavelength was recorded. The plates were then washed with PBS and solubilized in 100 mL of dimethyl sulfoxide (DMSO) per well. The absorbance at 540 nm was determined using an enzyme-linked immunosorbent assay (ELISA) microplate reader. The concentration of the synthesized compounds needed to inhibit 50% of the growth of the cell lines ($IC_{50}$) was calculated through the analysis of the relationship between concentrations and percent (%) cell growth.

**3. RESULTS AND DISCUSSION**

**3.1. Chemistry**

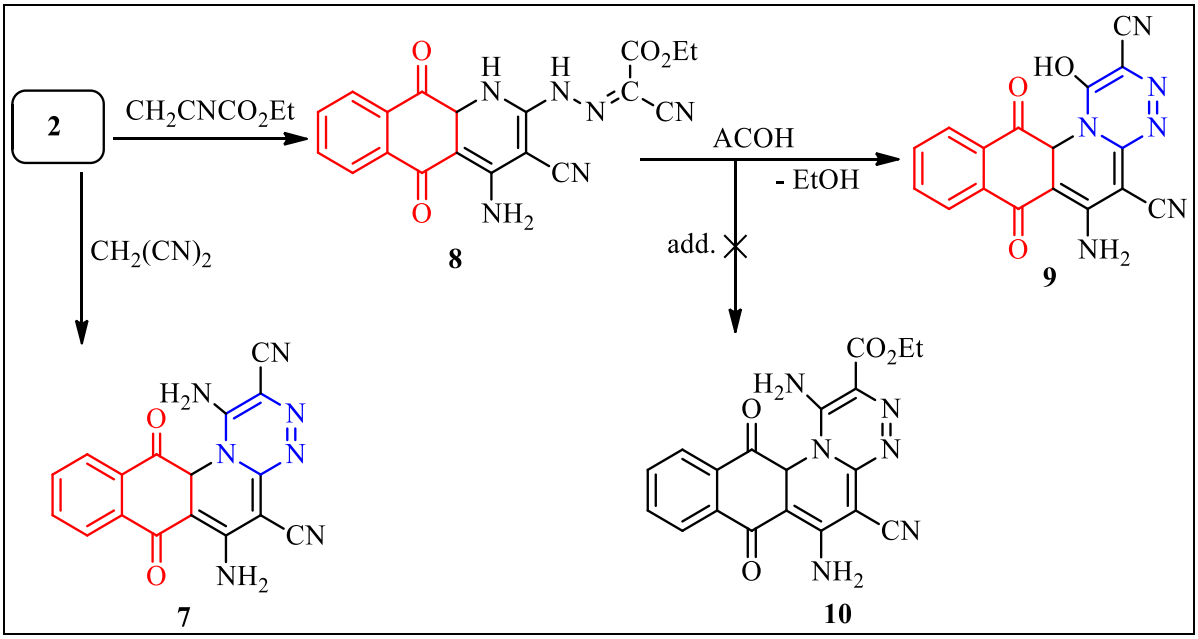
Diazotized aminopyrazoles have been reported [29,30] to react with active methylene reagents to yield hydrazones which readily cyclize into pyrazolo[5,1-*c*][1,2,4]triazines or directly into the cyclic product *via* a cycloaddition reaction under the coupling condition. Thus, diazotization of 2,4-diamino-5,10-dioxo-1,5,10,10a-tetrahydrobenz[*g*]quinoline-3-carbonitrile (**1**) [27] with concentrated HCl and $NaNO_2$ afforded the diazonium salt (**2**), which easily coupled with *β*-naphthol afford 4-amino-2-((2-hydroxynaphthalen-1-yl)diazenyl)-5,10-dioxo-1,5,10,10a-tetrahydro-benzo[*g*]quinoline-3-carbonitrile (**3**). Compound **(3)**, subsequently cyclized through elimination of one molecule of $H_2O$ by refluxing in AcOH, provided 8-amino-9,14-dioxo-14,14a-dihydro-9*H*-benzo[*g*]naphtha[2',1' :5,6][1,2,4]triazino[4,3-*a*]quinoline-7-

carbonitrile (**4**), as shown in Scheme 1. This finding was found to be in contrast compared to the reported direct formation of cyclic pyrazolo[5,1-*c*][1,2,4]triazines on coupling of diazotized amino pyrazoles with naphthols [31]. Similarly, diazonium salt (**2**) was easily coupled with resorcinol to afford the arylazo derivative (**5**), which, endure cyclization to give 8-amino-2-hydroxy-9,14-dioxo-14,14a-dihydro-9*H*-benzo[*g*]benzo[5,6][1,2,4]triazino[4,3-*a*]-quinoline-7- carbonitrile (**6**) upon refluxing in AcOH (Scheme 1). The structures of these compounds were confirmed based upon their elemental and spectral data. For instance, the mass spectrum of (**4**) displayed an intense peak at *m/z* 403.00 corresponding to the molecular formula $C_{24}H_{13}N_5O_2$. Its, IR spectrum revealed the presence of ($NH_2$) stretching band at 3315 $cm^{-1}$, (C≡N) stretching band at 2215 $cm^{-1}$ and two equivalent (C=O) stretching bands at 1652 $cm^{-1}$. The ($^1$H NMR) spectrum of (**4**) declared the lack of the indo cyclic imino originally observed in compound (**3**) at 11.21 ppm, with one $D_2O$-exchangeable broad singlet at 6.76 ppm for the $NH_2$ group.



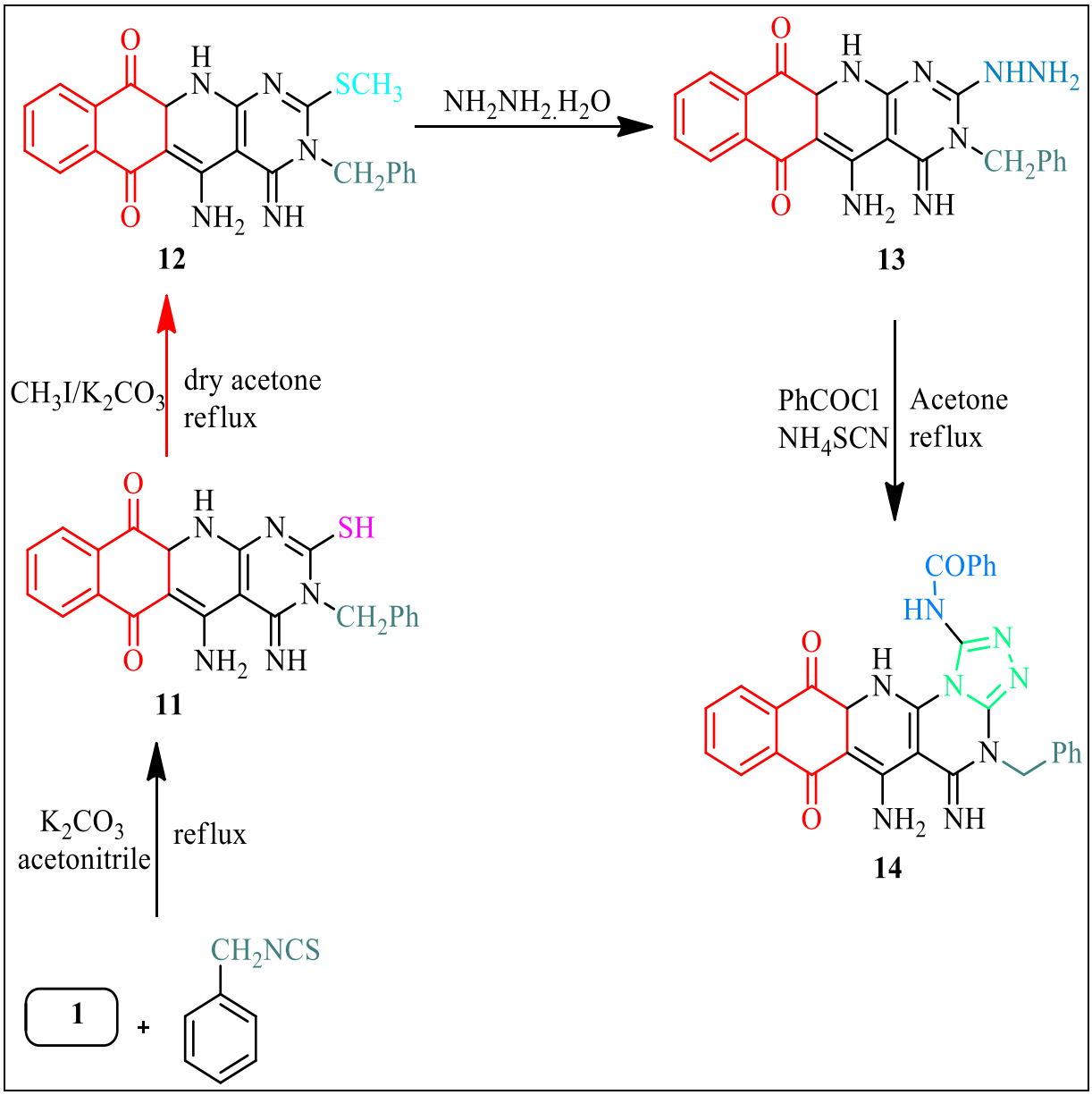

**Scheme 1.** Synthesis of new triazine derivatives **(4)** and **(6)**.

On the other hand, compound (**2**) reacted with malononitrile to yield 1,6-diamino-7,12-dioxo-12,12a-dihydro-7*H*-benzo[*g*][1,2,4]triazino[4,3-*a*]quinoline-2,5-dicarbonitrile**(7)**. The structure of compound **(7)** was confirmed based on its spectral data. The IR spectrum of **(7)** declared the presence of both amino and cyano functions at 3315, 2216 and 2218 $cm^{-1}$, respectively. While, its $^{1}H$ NMR spectrum revealed the presence of two broad singlets, one at 8.99 ppm integrating for two protons ($NH_{2\text{-Triazi.}}$), the down field shift of this amino group was ascribed to the anisotropic effect of ring nitrogen, whereas, the other ($NH_{2\text{-Pyridi.}}$) appeared at 6.79 ppm. Similarly, diazonium salt (**2**) was easily coupled with ethyl cyanoacetate to give 2-(2-(4-amino-3-cyano-5,10-dioxo-1,5,10,10a-tetrahydrobenzo[*g*]quinolin-2-yl)hydrazono)-2-cyano-acetate (**8**). The IR spectrum of compound (**8**) declared the presence of intense absorption bands at 3100-3215 $cm^{-1}$, 2215-2220 $cm^{-1}$ and 1652 $cm^{-1}$ corresponding to imino, amino, cyano and carbonyl groups, respectively. Its corresponding $^{1}H$-NMR spectrum showed signals appearing at $\delta$ 1.36 (t, $J$ = 7.01 *Hz*, 3H, $\underline{CH_3}CH_2$), 4.23 (s, 1H, $Quinon._{(C1)}$-H), 4.36 (q, $J$ = 7.01 *Hz*, 2H, $CH_3\underline{CH_2}$) and 6.76 (s, 2H, $NH_2$), 8.1-7.2 (m, 6H, Ar-H), 11.21 (s, 1H, $NH\text{-}_{Pyridi.}$), 11.25 (s, 1H, $NH\text{-}_{Hydrazo.}$). The mass spectrum of compound (**8**) showed a peak corresponding to the molecular ion at *m/z* 390.00 ($M^+$, 60%) and corresponding to the molecular formula $C_{19}H_{14}N_6O_4$. Compound (**8**) underwent further cyclization *via* refluxing in AcOH to afford 6-amino-1-hydroxy-7,12-dioxo-12,12a-dihydro-7*H*-benzo[*g*][1,2,4]triazino [4,3-*a*]quinoline-2,5-dicarbonitrile (**9**) in 65% yield, (Scheme 2). It is worth mentioning that this reaction may provide two possible products (**10**) and (**9**) but structure of (**10**) was discarded based on the elemental and spectral data. The formation of compound **(9)** was assumed to proceed *via* elimination of EtOH molecule, as shown in the mass spectrum, which declares a molecular ion peak at *m/z* 344.00 corresponding to the molecular formula $C_{17}H_8N_6O_3$. The signals of the ethyl protons originally observed in (**8**) ($^{1}H$ NMR) at 1.36 and 4.41 ppm were disappeared, while the OH signal was observed at 14.56 ppm and its stretching band (IR) was observed at 3531 $cm^{-1}$.




**Scheme 2.** Synthesis of new triazines **(7)** and **(9)**

In this study, the reactivity of the enaminonitrile moiety in compound (**1**) was investigated aiming to annulate the fused pyrimidine analogues. As follows, the enaminonitrile derivative (**1**) was treated with benzyl isothiocyanate in acetonitrile containing $K_2CO_3$, under refluxing to provide 5-amino-3-benzyl-4-imino-2-mercapto-3,4,11a,12-tetrahydrobenzo[*g*] pyrimido[4,5-*b*]quinoline-6,11-dione (**11**) in 75% yield, (Scheme 3). The mass spectrum of (**11**) showed a molecular ion peak at *m/z* 415.00 corresponding to the molecular formula $C_{22}H_{17}N_5O_2S$. Its, IR spectrum showed an intense absorption band at 2515 $cm^{-1}$ due to S-$H_{str.}$, besides the originally observed bands for the quinone carbonyls, N-H and $NH_2$ groups, while, the C≡N group was not observed in the IR spectrum. The $^1H$ NMR spectrum displayed two $D_2O$-exchangeable broad singlets at 8.01 and 12.23 ppm for the exocyclic NH and SH groups, respectively, in addition to the up filed singlet of the methylene protons appearing at 4.47 ppm. Mercapto-pyrimidine tagged intermediate (**11**) was incorporated in further investigations in order to explore the reactivity of its thiol group. Hence, methylation of compound **(11)** *via* treating with MeI in dry acetone containing anhydrous $K_2CO_3$ under reflux condition yielded 5-amino-3-benzyl-4-imino-2-(methylthio)-3,4,11a,12-tetrahydrobenzo[*g*]pyrimido[4,5-*b*]quinoline-6,11-dione (**12**), (Scheme 3).




**Scheme 3.** Synthesis of triazole derivative **(14)**.

The recorded mass spectrum at *m/z* 429.00 corresponds to the formula $C_{23}H_{19}N_5O_2S$, besides its ($^1$H NMR) at $\delta$ 2.58 (s, 3H, $CH_3$) further supported the formation of methylthio derivative (**12**). The synthetic efficiency of the methylthio compound (**12**) was scrutinized in this work. Thusly, compound (**12**) was treated with hydrazine hydrate in EtOH to produce the hydrazide derivative (**13**), (Scheme 3). The latter compound underwent dehydrogenation involving ring closing on treatment with benzoyl chloride and ammonium thiocyanate in acetone to afford *N*-(6-amino-4-benzyl-5-imino-7,12-dioxo-4,5,7,12,12a,13-hexahydro[1,2,4]triazolo[3',4':2,3] pyri-mido[4,5-*b*]benzo[*g*]quinolin-1 yl)benzamide (**14**), (Scheme 3). The structure of (**14**) was in agreement with all analytical and spectroscopic data. The IR spectrum of (**14**) showed the presence of absorption bands appearing at 1652-1685 and 3150-3315 cm$^{-1}$ corresponding to three C=O, NH, $NH_2$ groups respectively, while the mass spectrum of compound (**14**) showed

*m/z* at 542.00 ($M^+$, 55%), corresponding to the molecular formula $C_{30}H_{22}N_8O_3$. The structures of the designed target compounds **(4)**, **(6)**, and **(7)**, **(9)**, **(14)** are presented in Figure1.

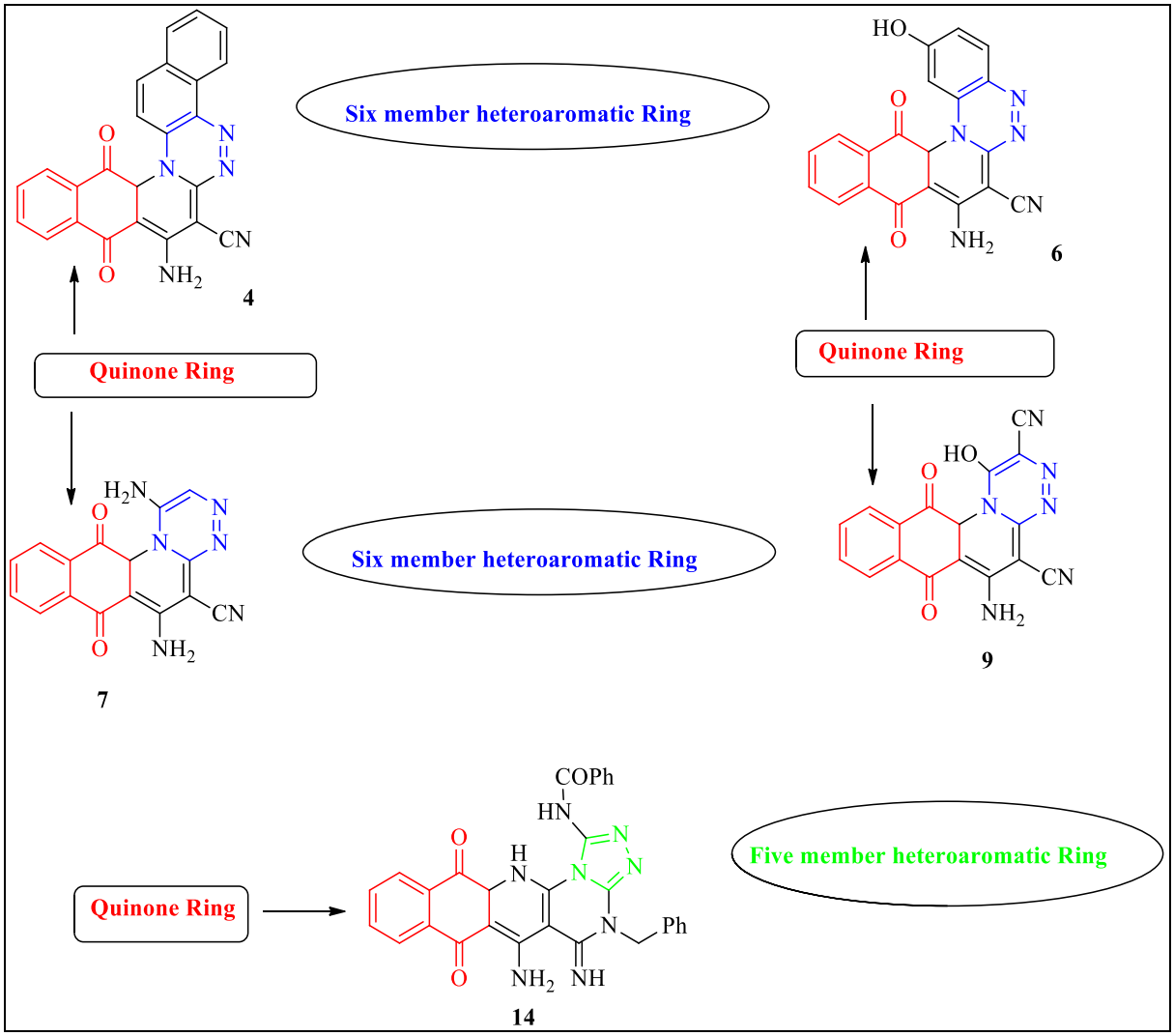



**Figure 1:** Structures of the designed target compounds **(4)**, **(6)**, **(7)**, **(9)** and **(14)**.

### 3.2. In vitro cytotoxic activity- MTT assay

The present study focused on investigating the *in-vitro* cytotoxicity of some selected synthesized target molecules **(4)**, **(6)**, **(7)** and **(9)** against A-549 (human lung adenocarcinoma cell lines), HT-29 (human colorectal cancer cell lines) and MKN-45 (human gastric cancer cell lines) using Foretinib as a standard. The viability rate towards each cell-line was experimentally assessed using a MTT assay (See experimental section) after the incubation of the cancer cell lines with different concentrations (0.1, 1 and 10 µg/mL) of compounds **(4)**, **(6)**, **(7)** and **(9)** for 48 h. A curve corresponding to the percentage of cell viability versus the concentration of each compound versus was plotted. The $IC_{50}$ values of compounds **(4)**, **(6)**, **(7)** and **(9)** against A-549, HT-29 and MKN-45 cancer cells were calculated and presented in Table 1. The criteria used to classify the cytotoxicity of the selected synthesized compounds against A-549, HT-29 and MKN-45 cancer cells, based on US National Cancer Institute, were

as follows: $IC_{50} < 20$ µg/ml (high cytotoxic activity), $IC_{50}$: 20-100 µg/ml (moderate anti-cancer activity), $IC_{50}$: 201-500 µg/ml (weak anti-cancer activity) and $IC_{50}>500$ µg/ml (no anti-cancer activity) [32]. It was exciting to discover that all the synthesized compounds were endued with a strong cytotoxic activity ($IC_{50}< 20$µg/mL) against the tested cancer cells. Besides, the cytotoxicity of all compounds against the tested cancer cells were higher than that of Foretinib (0.032-0.11 µg/ mL). Among the synthesized compounds, compound **(7)** demonstrated potent inhibitory effects on cell viability of A-549 ($IC_{50}= 0.23$ µg/mL) and HT-29 cancer cell lines ($IC_{50}=0.84$ µg/mL). Besides, compound **(4)** demonstrated excellent anti-cancer activity toward A-549 cells ($IC_{50}= 0.43$ µg/mL). Compound **(6)** was endued with high cytotoxic activity against A-549 ($IC_{50}= 5.18$ µg/mL) and HT-29 ($IC_{50}= 8.29$ µg/mL). However, the latter compound revealed the highest anti-cancer activity against MKN-45 ($IC_{50}= 2.36$ µg/mL). Compound **(9)** exhibited high cytotoxic activity against A-549 and HT-29 cells and moderate activity against MKN-45 cancer cells. Structure–activity relationship (SAR) describes the relationship between the chemical or 3D structure of the synthesized compounds and their cytotoxic activity against the three cancer cell lines, which facilitates the determination of the specific chemical groups responsible for invoking target biological effect in the organism. Previous studies revealed that compounds containing electron-withdrawing functional groups displayed more potent cytotoxic effects against the tested cancer cells compared to the electron donor functional groups [33]. Interestingly, a decrease in the cytotoxic activity of compound **(6)** against A-549 ($IC_{50}= 5.18$ µg/L), HT-29 ($IC_{50}= 8.29$ µg/L) and MKN-45 ($IC_{50}= 2.36$ µg/L) cancer cells was observed and attributed to the presence of electron donating OH group in para position at phenyl group attached to triazine ring. Similarly, a decrease in the anti-cancer activity against all the tested cancer cells was observed in compound **(9)**. This finding was ascribed to the presence of electron donating OH group beside electron withdrawing CN group in triazine ring, which led to an increase in $IC_{50}$ values in comparison with other tested compounds. Compounds **(4)** and **(7)** showed the highest cytotoxic activity against A-549 and HT-29 cell lines; these results were attributed to the presence of electron withdrawing groups like phenyl and cyano groups in their structures. The decreasing in anti-cancer activity observed in compound **(6)** against HT-29 cancer cells was associated to the presence of electron donating group (OH). Based on these results, we concluded that the substituents incorporated in the triazine ring of all tested compounds play a crucial role in the inhibition activities and are a considerable determinant of the cytotoxicity.

**Table 1: Antitumor activity of the newly synthesized compounds**

| Compound | A-549 | | HT-29 | | MKN-45 | |
|---|---|---|---|---|---|---|
| | $IC_{50}$ (µg/mL) | Cytotoxic activity | $IC_{50}$ (µg/mL) | Cytotoxic activity | $IC_{50}$ (µg/mL) | Cytotoxic activity |
| **4** | 0.43 | highly cytotoxic | 4.33 | highly cytotoxic | 10.37 | highly cytotoxic |
| **6** | 5.18 | highly cytotoxic | 8.29 | highly cytotoxic | 2.36 | highly cytotoxic |
| **7** | 0.23 | highly cytotoxic | 0.84 | highly cytotoxic | 8.39 | highly cytotoxic |
| **9** | 2.18 | highly cytotoxic | 3.29 | highly cytotoxic | 14.27 | highly cytotoxic |
| Foretinib | 0.11 | | 0.19 | | 0.032 | |

## 4. CONCLUSION

Because of the cytotoxic potential demonstrated by benzo[g]quinoline, pyrimidine and triazine derivatives, new synthetic routes for synthesizing substituted derivatives containing 5,10-dioxo-benzo[g]quinoline moiety were developed, and the cytotoxic activities of the synthesized compounds on three cancer cell lines (A-549, HT-29 and MKN-45) were evaluated. Among the synthesized compounds, compound **(7)** appeared to be the most active compound displaying potent anti-cancer activity against lung cancer cells A549 and colon cancer cell lines HT-29 with the less values of $IC_{50}$. The cytotoxic activity of the latter compound towards the tested cancer lines was ascribed to the presence of withdrawing groups like phenyl group and cyano group in its structure. Besides, compound **(4)** displayed also an excellent cytotoxic activity against the cancer cells A-459 due to the presence of phenyl electron withdrawing group in its structure. Furthermore, compound **(6)** showed a good cytotoxic effect among the tested products against the gastric cancer cell lines MKN-45. Based on our results, compounds **(4), (7)** and **(6)** might have the potential to be the leading compounds as potent anti-lung cancer, anti-colon cancer and anti-gastric cancer candidates and can find promising applications in medicinal chemistry.

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## CONFLICT OF INTEREST

The authors declare no conflict of financial, academic, commercial, political, or personal interests.

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