DOI: 10.17344/acsi.2025.9335 *Acta Chim. Slov.* **2025,** *72,* 692–706

Scientific paper

Synthesis and Biological Activity of Some Novel Quinolinones Derived from 1-Ethyl-1,2-dihydro-4-hydroxy-2-oxoquinoline-3-carbaldehyde

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Received: 05-21-2025

Abstract

The reactivity of 1-ethyl-1,2-dihydro-4-hydroxy-2-oxoquinoline-3-carbaldehyde (**1**) towards some diaza-nucleophiles has been investigated, under various reaction conditions. 1-Ethyl-1,2-dihydro-4-hydroxy-2-oxoquinoline-3-carbaldehyde (**1**) was reacted with hydrazine, phenylhydrazine, 2,4-dinitrophenylhydrazine, acid hydrazides, semicarbazide, thiosemicarbazides, thiocarbohydrazide, *ortho*-phenylenediamine and *ortho*-aminophenol. It was found that solvents used in these reactions impacted product selectivity. Accordingly, these reactions led to various open-chain and/or cyclic derivatives of quinolinone. The structure of the new compounds was determined using spectroscopic techniques and elemental analyses. All products were screened *in vitro* to determine their antimicrobial, antioxidant and antitumor activities. Compounds **2a** and **2c** are the most active compounds against bacterial and fungal strains. While the most effective compounds against breast cancer (MCF-7) are compounds **2a** and **8a**.

Keywords: 2-Quinolinones, Heterocyclization, Reiemer–Tiemann Reaction, Antimicrobial activity, Antioxidant, Antitumor activity.

1. Introduction

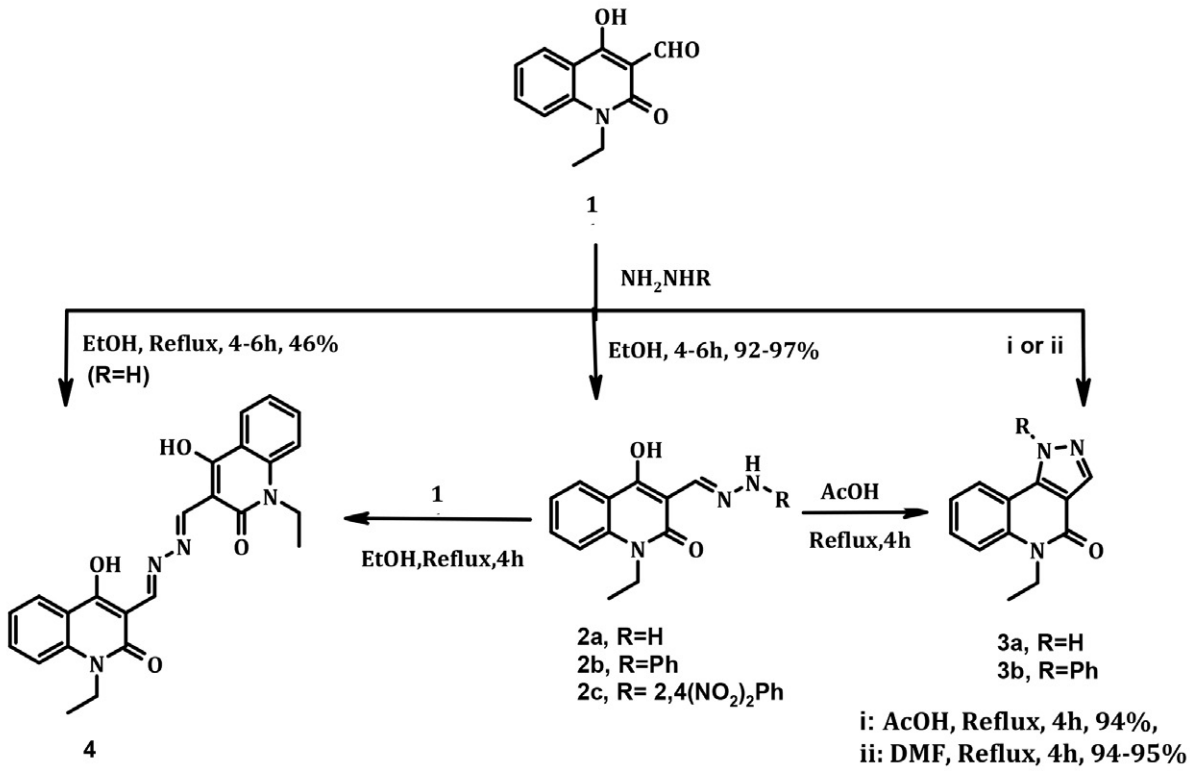
Nowadays, heterocycles containing nitrogen are essential structural building blocks for the synthesis of an enormous number of medicinal drugs. On the other hand, quinolinone derivatives, such as 2- and 4-quinolinones, are among the most important pharmaceutical heterocyclic compounds which are present in various natural sources, including oils, coal tar, herbs, and dyes.[1–7] 2-Quinolinone compounds belong to a class of bioactive heterocyclic systems which have attracted a lot of attention because of their significant medicinal applications such as anti-inflammatory, anti-malarial, antibacterial, and anti-cancer activities.[1,4,8–22] In particular, 4-hydroxyquinolin-2(1*H*)-one and its derivatives are related to a family of biologically active heterocycles. For this reason extensive synthetic research was conducted to prepare an abundance of novel quinolinone compounds belonging to this family.[23–33] The aim of this study is to investigate the chemical behavior of the quinolinone derivatives toward various nucleophilic reagents in a variety of reaction conditions.

The synthesis of bioactive compounds containing heterocycles has been the focus of our recent work. The current study screens the anti-microbial activities, the potential antioxidant activity and anti-tumor activity of these novel heterocycles containing the 2-quinolinone moiety.

2. Results and Discussion

2. 1. Chemistry

The starting compound, 1-ethyl-1,2-dihydro-4-hydroxy-2-oxoquinoline-3-carbaldehyde (**1**) was synthesized according to the literature *via* Reiemer–Tiemann reaction of 4-hydroxyquinolinone.[34] The aldehyde **1** was reacted with some 1,2-dinucleophiles, 1,4-dinucleophiles and 1,5-dinucleophile to construct some different new substituted quinolinone derivatives. Hence, treatment of aldehyde **1** with hydrazine hydrate, in molar ratio 1:2 in ethanol at room temperature, gave hydrazone **2a**, in 92% yield (Scheme 1). IR spectrum of compound **2a** showed a broad vibrational band between 3500 and 3400 cm^{-1} due to the presence of OH group, a notable band at 3282 and 3238 cm^{-1} due to NH_2 group and absorption bands for C=O at 1652 and C=N at 1620 cm^{-1}. ^1H NMR spectrum of compound **2a** displayed three characteristic singlet signals



Scheme 1. Reaction of 1-ethyl-1,2-dihydro-4-hydroxy-2-oxoquinoline-3-carbaldehyde (**1**) with hydrazine derivatives in different conditions.

at δ 6.3, 8.35 and 13.33 ppm assigned to NH_2 due to the presence of hydrazone group; CH=N proton stemming from the condensation of formyl group and hydrazine; and OH protons which refer to the presence of hydrazone functional group and not a pyrazolo compound. ^{13}C NMR spectrum showed two characteristic signals at δ 172.9 and 151.9 ppm, which are attributed to C=O and C=N. Also, the molecular ion peak in MS of compound **2a** appeared at *m/z* 231 and base peak at *m/z* 214 which refers to the loss of OH group.

5-Ethyl-1*H*-pyrazolo[4,3-*c*]quinolin-4(5*H*)-one (**3a**) was obtained in 94% yield by reacting aldehyde **1** with hydrazine hydrate in glacial acetic acid. Also, heating compound **2a** in glacial acetic acid for 30 minutes resulted in compound **3a**, in 90% yield (Scheme 1).[35] IR spectrum of **3a** displayed distinctive absorption bands at ύ 3217 cm^{-1} due to NH group, and 1687 cm^{-1} for C=O group. The protons of CH=N and pyrazole NH of compound **3a** were detected at δ 5.86 and 11.32 ppm in the ^{1}H NMR spectrum, respectively. Aldehyde **1** reacted with hydrazine hydrate, in boiling ethanol for 4 h, to afford compound **4** in 46% yield. The latter compound was also obtained via either one of the two methods: by the reaction of aldehyde **1** with hydrazine hydrate in ethanol at room temperature for 6 h, in 46% yield or by refluxing compounds **2a**–**c** with aldehyde **1** in ethanol for 4 h in 47% yield as depicted in Scheme 1. The IR spectrum of compound **4** showed broad band due to the OH group at ύ 3400 cm^{-1}. In addition, the absence of absorption bands for NH_2 and NH groups confirmed that cyclization did not take place. ^{13}C NMR spectrum of compound **4** revealed the presence of C=N group at δ 120 ppm. The mass spectrum of compound **4** showed a peak at *m/z* 215 corresponding to the formation of 1-ethyl-4-hydroxy-3-(iminomethyl)quinolin-2(1*H*)-one cation radicals.

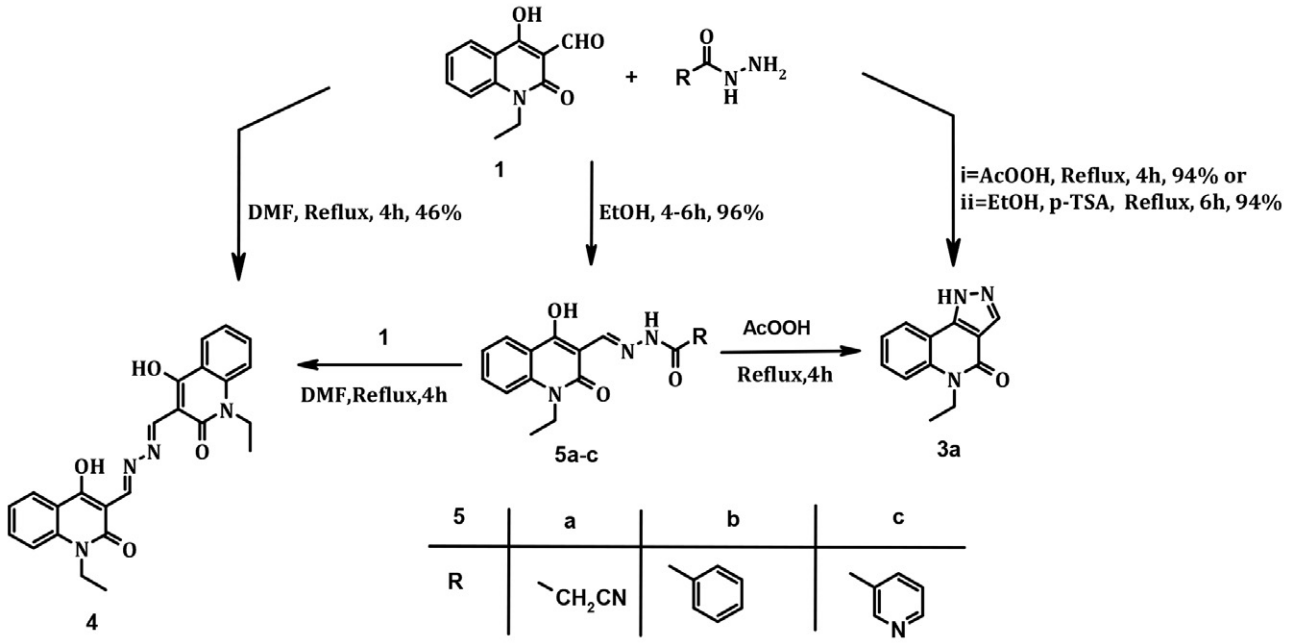
Aldehyde **1** and phenylhydrazine reacted in ethanol for 4 h, to produce phenylhydrazone **2b** in 97% yield (Scheme 1).[36] IR spectrum of compound **2b** revealed characteristics bands at ύ 3400, 3251 and 1635 cm^{-1} due to OH, NH and C=O functional groups. ^{1}H NMR spectrum of compound **2b** showed signals at δ 8.44, 10.64 and 13.45 ppm for CH=N, NH and OH protons. The chemical shift of NH proton appeared at a more down-field value may support the existence of the phenyl group which acts as an electron withdrawing group. In addition, the chemical shifts at aromatic region indicated the presence of 9 protons, at δ 6.8–8.05 ppm. 5-Ethyl-1-phenyl-1*H*-pyrazolo [4,3-*c*]quinolin-4(5*H*)-one (**3b**) was obtained via the reaction of aldehyde **1** with phenylhydrazine in DMF for 4 h, in 97% yield.[36] Refluxing compound **2b** in glacial acetic acid resulted in the same compound **3b** as outlined in Scheme 1. By comparing IR and ^{1}H NMR spectra of compounds **3a** and **3b**, the NH group disappeared in compound **3b**.

Treatment of aldehyde **1** and 2,4-dinitrophenylhydrazine in ethanol, produced 1-ethyl-4-hydroxy- 3-[(2',4'-dinitrophenylhydrazinylidene)methyl]quinolin-2(1*H*)-one (**2c**), in 84% yield (Scheme 1). IR spectrum of compound **2c** showed the presence of absorption bands

due to O–H, N–H, C=O and C=N at ύ 3417, 3276, 1639 and 1609 cm^{-1}, respectively. ^1H NMR spectrum of compound **2c** displayed three singlet signals at δ 9.13, 10.1 and 12.2 ppm due to CH=N, NH and OH protons. Furthermore, the proton at position 3 in 2,4-dinitrophenyl group appeared at 8.79 ppm (Scheme 1).

Moreover, the reactivity of aldehyde **1** towards some carboxylic acid hydrazide was investigated. Thus, reaction of aldehyde **1** with cyanoacetohydrazide at room temperature in ethanol afforded 2-cyano-*N*'-((1-ethyl-1,2-dihydro-4-hydroxy-2-oxoquinolin-3-yl)methylene)acetohydrazide (**5a**), in 96% yield (Scheme 2). IR spectrum of compound **5a** exhibited characteristic absorption band for C≡N group at ύ 2261 cm^{-1}, besides broad bands for O–H and N–H functional groups at ύ 3481, 3198 cm^{-1}. ^1H NMR spectrum displayed three singlet signals at δ 3.88, 12.01 and 14.03 ppm assigned to the protons of CH_2–C≡N, NH and OH, respectively. The ^{13}C NMR spectra revealed three distinctive downfield signals at δ 122, 162, and 166 ppm, attributed to C≡N, C=$O_{quinolone}$ and C=O_{ketone}, respectively.

yield, as shown in Scheme 2. IR spectrum of compound **5b** revealed the existence of O–H, N–H, C=O and C=N stretching bands at ύ 3400, 2233, 1676 and 1633 cm^{-1}, respectively. The ^1H NMR spectrum of **5b** showed three singlet peaks due to OH, NH and CH=N protons appearing at δ 15.50, 12.37 and 8.86 ppm. In addition, in aromatic region 9 protons appeared at δ 7.28–8.08 ppm. Compound **5c** was produced when aldehyde **1** was heated with nicotinohydrazide in ethanol (Scheme 2). IR spectrum of compound **5c** showed characteristic absorption bands at ύ 3400 (OH), 3196 (NH), 1678 (C=O) and 1612 cm^{-1} (C=N). ^{13}C NMR spectrum of compound **5c** demonstrated a chemical shift due to carbonyl group of quinolinone at δ 166 ppm. In addition, two different C=N carbons appeared at δ 161 ppm for quinolinone and δ 153 ppm for nicotinohydrazide. Interestingly, when either hydrazones **5b** or **5c** were refluxed in acetic acid in the presence of sodium acetate, pyrazoloquinolinone **3a** was afforded in high yield. The same compound **3a** was directly produced, in 94% yield, when aldehyde **1** was reacted with benzohydrazide or nico-

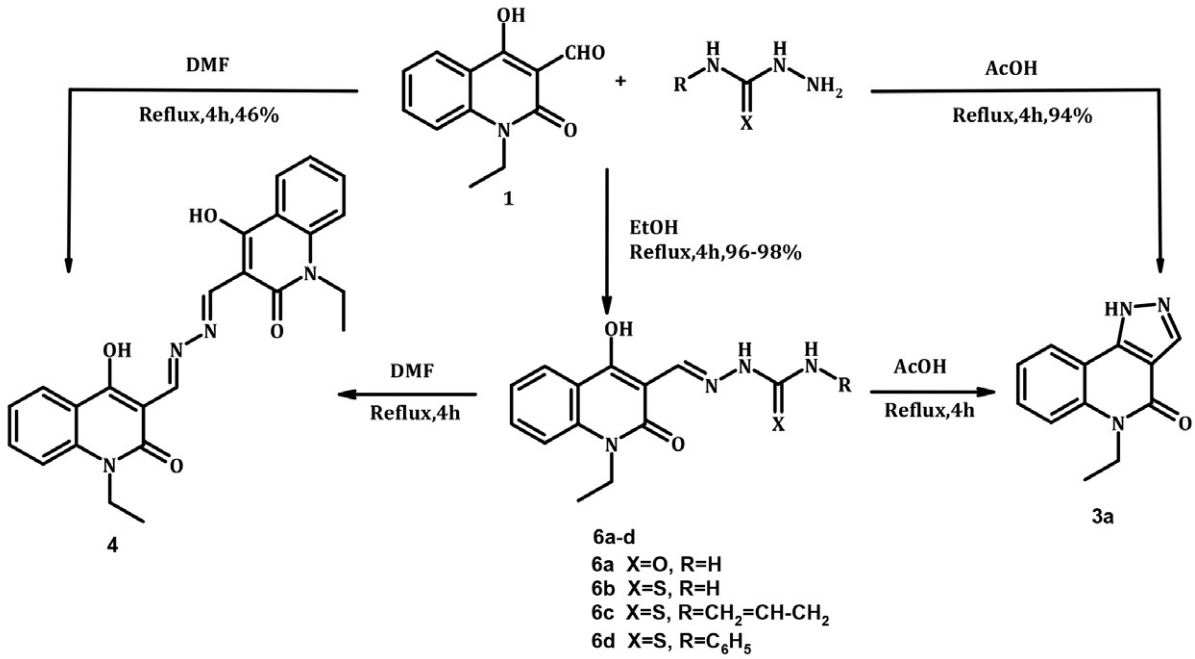


Scheme 2. Reaction of aldehyde **1** with acid hydrazide in different conditions.

The pyrazoloquinolinone **3a** was obtained, in good yield, when the aldehyde **1** reacted with 2-cyanoacetohydrazide in boiling acetic acid. Also, heating compound **5a** in acetic acid afforded compound **3a** in 93% yield (Scheme 2). On the other hand, reaction of aldehyde **1** with 2-cyanoacetohydrazide, benzohydrazide and nicotinohydrazide in DMF, led to bis-hydrazone **4**, in 50% yield. Boiling compounds **5a**–**c** with formylquinolinone **1** in DMF gave the same bis-hydrazone **4** (Scheme 2).

On reacting aldehyde **1** with benzohydrazide in ethanol, *N*'-((1-ethyl-1,2-dihydro-4-hydroxy-2-oxoquinolin-3-yl)methylene)benzohydrazide (**5b**) was obtained in 96% tinohydrazide in boiling ethanol in the presence of *para*-toluenesulphonic acid (*p*-TSA) as catalyst (Scheme 2).

1,4-Binucleophiles such as semicarbazide, thiosemicarbazide and their derivatives were subjected to study their reactivity towards aldehyde **1**. Aldehyde **1** was reacted with these semicarbazides in ethanol to afford the corresponding semicarbazones **6a**–**d**, in good yield (Scheme 3). The structures of the products **6a**–**d** were indisputably confirmed by IR and NMR spectroscopy. ^1H NMR spectrum of compound **6a** exhibited four singlet signals at δ 13.12, 11.32, 8.4 and 4.26 ppm, which were assigned to the OH, NH, CH=N and NH_2 protons, respectively.[36] While



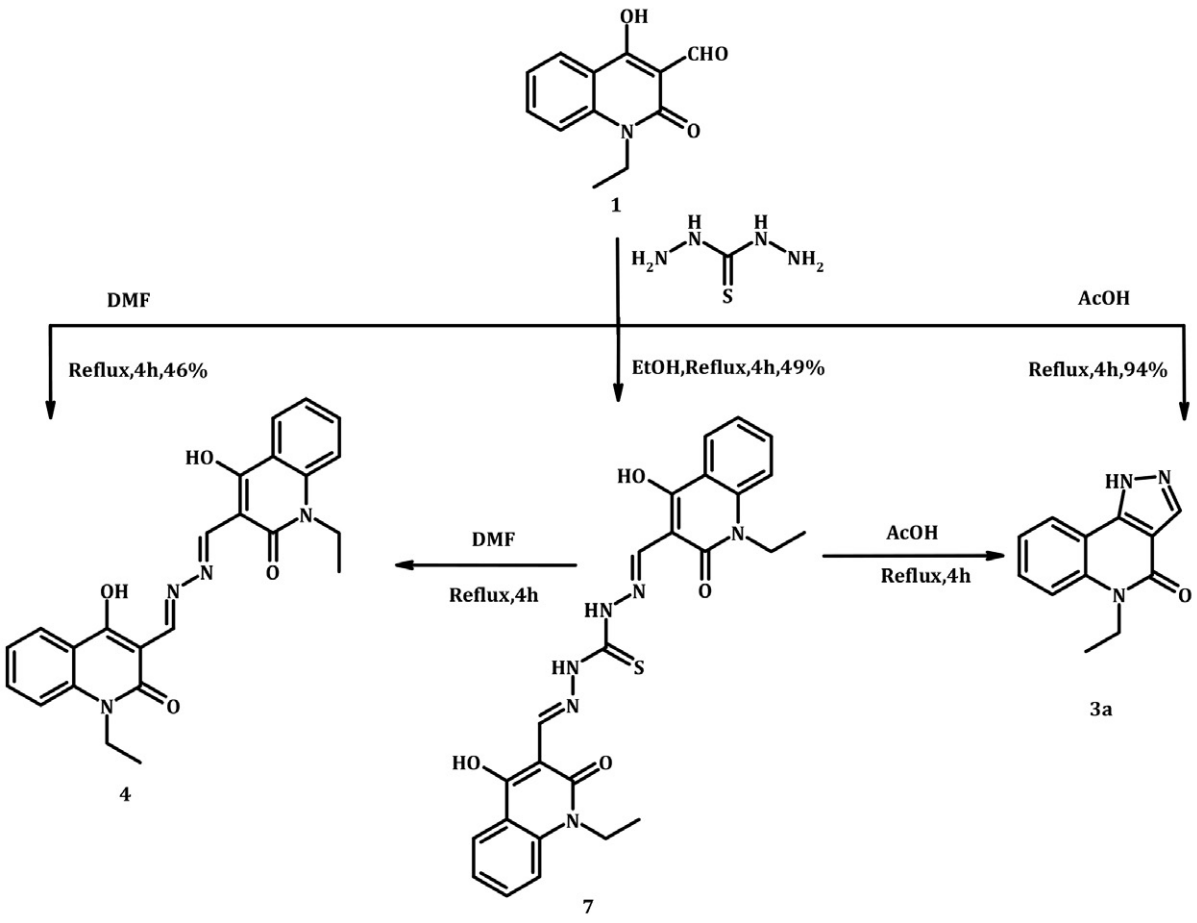
Scheme 3. Reaction of aldehyde **1** with semicarbazide, thiosemicarbazide and its derivatives in different conditions.

^{1}H NMR spectrum of compound **6b** showed four characteristic signals at δ 11.52, 8.61, 8.13 and 5.861 ppm due to protons of OH, CH=N, NH and NH_2 groups. Four distinctive signals, at δ 161, 156, 144 and 139 ppm were observed in the ^{13}C NMR spectrum of compound **6a**, attributed to carbons of $C{=}O_{quinolone}$, $C{=}O_{ketone}$, C=N and $C{-}OH_{quinolone}$, respectively. ^{13}C NMR spectrum of compound **6b** showed four characteristic signals at δ 161, 145, 139 and 133 ppm, due to carbons of $C{=}O_{quinolone}$, C=N, C=S and $C{-}OH_{quinolone}$.[37,38] IR spectrum of compound **6c** showed characteristic absorption bands at ύ 3400 (OH), 3200 (NH), 1633 (C=O), 1615 (C=N) and 1593 cm^{-1} (C=S). The ^{1}H NMR spectrum of compound **6c** showed signals for allylic protons ($CH_2{-}CH{=}CH_2$) at δ 4.29, 5.21, 5.9 ppm. In addition, three singlet signals due to CH=N, NH and OH were observed at δ 8.74, 11.57, 13.5 ppm. In the ^{1}H NMR spectrum of **6d**, the OH, NH, CH=N, and phenyl protons were noticed, at δ 11.87, 10.24, 8.72 and 7.58–7.45 ppm.

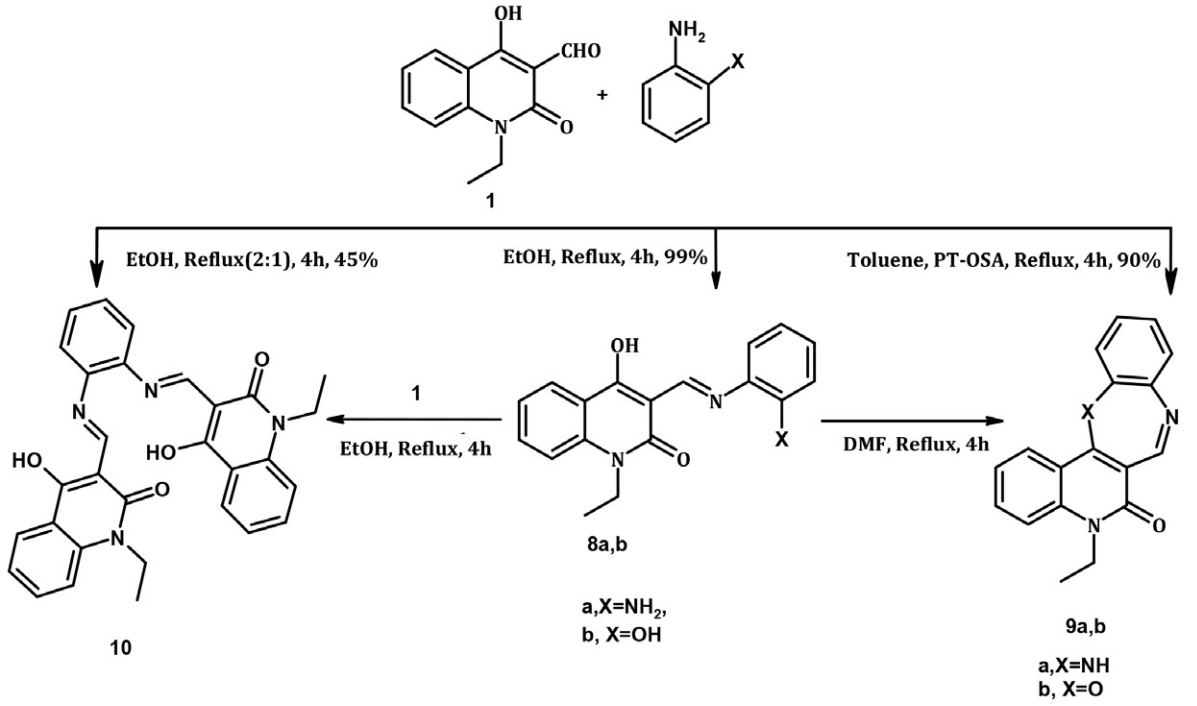
Interestingly, boiling semicarbazones **6a**–**d** in glacial acetic acid gave pyrazoloquinolinone **3a** (Scheme 3). The same product was obtained directly by treatment of aldehyde **1** with substituted semicarbazide, in boiling glacial acetic acid. Furthermore, refluxing semicarbazones **6a**–**d** in DMF gave azine **4**. The same compound was obtained by boiling aldehyde **1** with substituted semicarbazide in DMF (Scheme 3).[39,40]

1,5-Bis((1-ethyl-1,2-dihydro-4-hydroxy-2-oxoquinolin-3-yl)methylene)thiocarbodihydrazide (**7**) was obtained when aldehyde **1** was heated under reflux with thiocarbohydrazides, in ethanol or dioxane. The latter compound was obtained at room temperature, when compound **1** was stirred with a solution of thiocarbohydrazide in ethanol, in molar ratio 1:1 (Scheme 4). IR spectrum of compound **7** showed absorption bands at ύ 3400, 3179, 1629, 1607 and 1247 cm^{-1}, which correspond to the O–H, N–H, C=O, C=N and C=S groups, respectively. The mass spectroscopy and elemental analysis of compound **7** confirmed its molecular formula as $C_{25}H_{26}N_6O_4S$. ^{1}H NMR spectrum of compound **7** showed two singlet signals due to both OH and NH_2 protons at δ 11.52 and 8.13 ppm (disappearing upon addition of D_2O). Refluxing of compound **7** in glacial acetic acid for 4 h led to pyrazoloquinolinone **3a**. The same **3a** was obtained directly by reacting aldehyde **1** with thiocarbohydrazide in boiling glacial acetic acid, in 96% yield. Unexpectedly, heating compound **7** in boiling DMF gave the product **4**. On the other hand, refluxing aldehyde **1** with thiocarbohydrazide directly in DMF produced the same compound **4**, in a good yield (Scheme 4).[41]

Aldehyde **1** was reacted with *ortho*-phenylenediamine or 2-aminophenol in ethanol for 4 h to give the imines **8a**,**b** (Scheme 5).[36] IR spectrum of compound **8a** showed a broad vibrational band at 3400 cm^{-1} due to the presence of OH group, stretching bands at ύ 3380 and 3340 cm^{-1} for NH_2 group, and a signal at ύ 1656 cm^{-1} for C=O. ^{1}H NMR spectrum of compound **8a** revealed three characteristic singlet signals at δ 5.20, 8.78 and 13.78 ppm, assigned to NH_2, CH=N and OH protons. The mass spectrum of compound **8a** showed the molecular ion peak at *m/z* 306 and the base peak at *m/z* 277 due to the loss of ethyl group. IR spectrum of compound **8b** displayed characteristic absorption bands at ύ 3446, 1646 and 1615 cm^{-1}, attributed to OH, C=O and C=N, respectively. In respect to ^{1}H NMR data of compound **8b** at δ 13.94 and 9 ppm two singlet signals for OH and CH=N were observed. Sami *et al.* prepared compounds **8a** and **8b** at 205 and 248 °C in



Scheme 4. Reactions of aldehyde **1** with thiocarbohydrazide in different conditions.



Scheme 5. Reactions of aldehyde **1** with *ortho*-phenylenediamine and 2-aminophenol in different conditions.

71% yield, whereas our work provided the same products in 99% yield and at 212 and 260 °C.[36]

Heterocyclization of both **8a**,**b** was achieved in boiling DMF to produce compounds **9a**,**b** in good yield. Also, refluxing **1** with *ortho*-phenylenediamine or 2-aminophenol in toluene containing a few drops of *para*-toluenesulphonic acid (*p*-TSA) as a catalyst, resulted in the formation of quinolinobenzodiazepines **9a**,**b** (Scheme 5). IR spectrum of the compound **9b** showed characteristic ab sorption bands at ύ 3300 (NH), 1682 (C=O) and 1632 cm^{-1} (C=N). ^{1}H NMR spectrum displayed two singlet signals at δ 9.1 and 12.49 ppm assigned to the protons of CH and NH, respectively.[42] Boiling of the imine compound **8a** with aldehyde **1** in ethanol gave the bis-imine **10**. The same compound **10** was obtained directly by treatment of aldehyde **1** with *ortho*-phenylenediamine, in molar ratio 2:1 in boiling ethanol (Scheme 5). The ^{1}H NMR spectrum of compound **10** showed the disappearance of the characteristic singlet signals attributable to the NH_2 protons.

2. 1. Biological Activity

2. 2. 1. Antimicrobial Activity

The activity of the synthesized compounds against the sensitive organisms was assessed using the standardized disk agar diffusion method. *Proteus vulgaris* [RCMB 004 (1), ATCC13315], *Escherichia coli* [ATCC 25922] and *Pseudomonas aeruginosa* [ATCC 27853] are Gram-negative bacteria, *Staphylococcus aureus* [ATCC 25923], *Staphylococcus epidermidis* [RCMB 009 (2)] and *Bacillus subtilis* [RCMB 015 (1), NRRL B-543] are Gram-positive bacteria, and *Candida albicans* [RCMB 005003 (1), ATCC 10231] and *Aspergillus fumigatus* [RCMB 002008] are fungal strains. Purchased from Egyptian markets, the antibiotics gentamycin and ketoconazole were utilized at 100 μg/mL as references for antibacterial and antifungal activities.[43–46]

The substances were dissolved in dimethyl sulfoxide, an inhibitory solution, to achieve concentration of 100 μg/mL to determine MIC value.

A variety of activities against each species of microorganism are shown in Table 1, suggesting that structural variations have an impact on the growth of the microorganisms. Thus, we can conclude from these results that most of the synthesized compounds showed a low antimicrobial activity towards Gram-positive bacteria, Gram-negative bacteria and the fungal strains (Table 1). Compound **2c** showed activity equal to the activity of the standard ketoconazole against *A. fumigatus* as a fungal strain, while compound **2a** showed higher activity than the standard ketoconazole against *C. albicans* as a fungal strain.[47]

2. 2. 2. Antioxidant Activity

In studies on antioxidant activity, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity evaluation is a common assay. It is a spectrophotometric method that determines an antioxidant molecule's free radical

Table1. The antimicrobial activity of the newly synthesized compounds

Compound No.	Diameter of Inhibition Zone (6 mm) at conc. 100 μg/ml							
	***A. fumigatus* (fungus)**	***C. albicans* (fungus)**	***S. aureus* (G+)**	***B. subtilis* (G+)**	***S. epidermidis* (G+)**	***E. coli* (G–)**	***P. aeruginosa* (G–)**	***P. vulgaris* G–)**
2a	–	25	–	17	15	11	12	–
2b	–	–	–	–	–	–	–	–
2c	17	–	9	14	12	11	10	–
3	–	–	10	10	9	10	–	–
4	–	12	–	–	–	–	11	–
5a	–	–	10	10	12	12	11	–
5b	–	–	–	13	9	–	9	–
5c	–	–	13	11	10	11	–	–
6a	–	–	9	10	9	11	10	–
6b	–	–	10	–	11	9	–	–
6c	–	–	–	–	9	10	–	–
6d	–	–	–	12	12	–	10	–
7a	–	–	–	13	13	–	15	–
8a	–	–	–	–	–	–	–	–
ketoconazole	17	17	–	–	–	–	–	–
gentamycin	–	–	24	26	28	30	27	25

scavenging potential. The main application of the DPPH method is to ascertain the antioxidant potential of plants, edible seeds, seaweed, herbs, plant oils, cereals, honey, and flours.[48–53]

A freshly made DPPH solution has an absorption maximum at 517 nm and is deep purple in color. Usually, when an antioxidant is present in the medium, this purple color fades. Therefore, the DPPH free radical can be neutralized by antioxidant molecules by giving it hydrogen atoms or electrons, which turns the radical into the colorless product (i.e., 2,2-diphenyl-1-picrylhydrazine), which reduces absorbance. That means that the compound's antioxidant activity is stronger the faster the absorbance decreases. This rate is dependent on the solvent (methanol or ethanol) applied during the H-bond acceptance process. But DPPH• has a higher stability in methanol than in ethanol, so it is frequently used as a solvent in DPPH assays. Analysis and comparison were done with the percentage activity of the produced compounds dissolved in ethanol (Table 2).[19] The assessment study was conducted using different concentrations of 3.9, 7.8, 15.6, 31.25, 62.5, 125, 250, 500 and 1000 μg/mL.[54–56]

Table 2. Antioxidant activity of synthesized compounds based on DPPH scavenging activity test.

Compound	IC_{50} (μg/mL)
2a	11.6
2b	16.12
2c	13.82
3a	115.25
4	240.42
5a	40.06
5c	57.03
6a	182.01
6b	182.01
6c	15.39
6d	30.68
7	16.24
8a	19.55
8b	29.35
10	232.33
Ascorbic acid	10.21

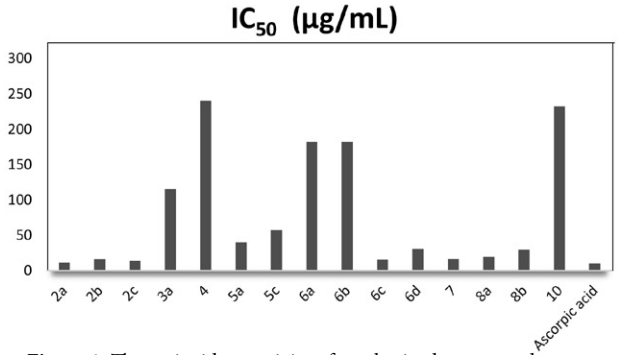


Figure 1. The antioxidant activity of synthesized compounds.

Table **2** shows the calculation of the 50% inhibitory concentration (IC_{50}) for DPPH activity of newly synthesized compounds. The results indicate that, when compared to ascorbic acid, a conventional antioxidant, the synthesized compounds demonstrated promising radical scavenging abilities based on the method employed. Most of the compounds showed good radical scavenging capabilities at lower concentrations, according to the results. However, it was noted that as the test compound concentrations increased, the activity gradually increased in every instance.

Compounds **2a**, **2b** and **2c** exhibit more potent DPPH antioxidant activity compared to other compounds. Order of activity **2a > 2c > 2b** is due to the presence of NH_2 or NH groups as electron donors. Compound **5a** and **5c** have moderate activity, whereas compounds **6c**, **6d** and **7** displayed strong antioxidant qualities; efficient conjugation may be the cause of this.[22] Compound **8a** has better activity than **8b**, most probably due to the OH group in compound **8b** being more electronegative than NH_2 group in compound **8a**, taking into account that both of these two groups are electron donors.

2. 2. 3. Anticancer Activity

The newly synthesized compounds **2a–c**, **5c**, **6c**, **7a** and **8a** were assessed for their anti-proliferative activity toward breast cancer (MCF-7) cell line. The inhibitory activity of the target compounds against tumor cell line was compared with 5-fluorouracil (5-FU) as a reference drug. Cytotoxic evaluation of the tested compounds was monitored and quantified by its ability to cause 50% inhibition of cell growth compared with the unprocessed control cells (IC_{50} value), as presented in Table 3.[19,57–59]

Table 3. Antitumor activity against breast cancer MCF-7 cell line (MTT test, 72 h) of some selected compounds and reference 5-FU.

Compound	IC_{50} (μg/mL)
2a	22.21 ± 1.07
2b	242.19 ± 4.92
2c	62.34 ± 1.46
5c	165.63 ± 11.4
6c	59.70 ± 3.69
7a	101.63 ± 2.87
8a	33.74 ± 1.32
5-FU (standard)	28

The target compounds' antitumor activity in the MCF-7 cell line varies according to the activity results. Compound **2a** demonstrated greater efficacy against the MCF-7 cell line, with an IC_{50} of 22.21 ± 1.07 μg/mL which is relatively close to the value of 5-fluorouracil (IC_{50} = 28 μg/mL) among this series, while other compounds show lesser or no activity.

When comparing compound **8a** (IC_{50} = 33.74 ± 1.32 μg/mL) with **2a** (IC_{50} = 22.21 ± 1.07 μg/mL), we found that

compound **8a** has good growth inhibitory activity but lesser than **2a** due to the amino group in **2a** attached to the aliphatic skeleton, while in **8a** it is attached to the aromatic ring. When replacing hydrogen in **2a** with phenyl group in **2b** or pyridine ring in **5c**, activities are decreased (for **2b** IC_{50} = 242.19 ± 4.92 μg/mL and for **5c** IC_{50} = 165.63 ± 11.4 μg/mL). But when a nitro group is added at the positions 2',4' of the aromatic ring, we found the activity to increase again (for **2c** IC_{50} = 62.34 ± 1.46 μg/mL) due to the electron-withdrawing properties of nitro group. On the other hand, compound **6c** displayed good activity (IC_{50} = 59.70 ± 3.69 μg/mL), when compared to other compound, thus we suspect that the conjugation and electron-withdrawing properties of the substituents are the cause. Compound **7a** showed moderate activity (IC_{50} = 101.63 ± 2.87 μg/mL) in comparison with other previously mentioned compounds (Table 3, Figures 2 and 3).

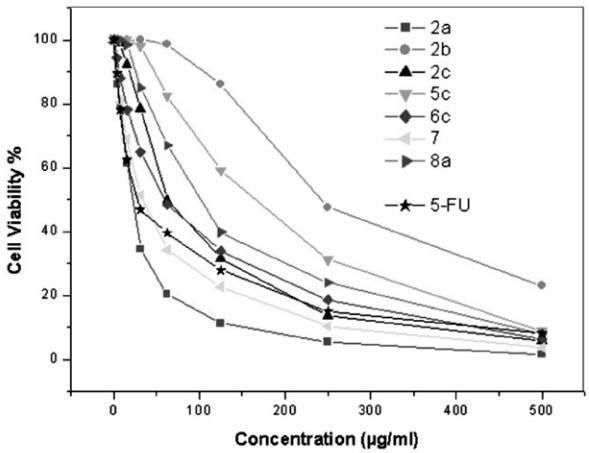


Figure 2. Effect of treatment with various concentrations of compounds **2a**–**c**, **5c**, **6c**, **7a** and **8a** on MCF-7 cell line cytotoxicity (IC_{50})

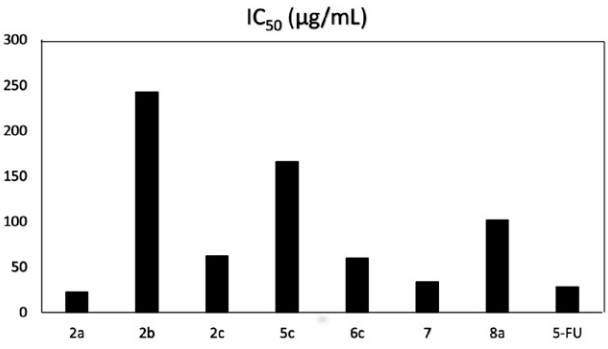


Figure 3. The antitumor activity of selected synthesized compounds.

3. Experimental

3. 1. Chemistry

Melting points were measured using a Stuart SMP3 digital device. Infrared spectra were recorded on a Perkin-Elmer FT-IR 1650 spectrophotometer (cm^{-1}), using KBr disks. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were measured on Bruker AV-400 spectrometer (400 MHz), using DMSOd_6 as a solvent and TMS as the internal standard. Mass spectra were obtained using GC-2010 Shimadzu Gas chromatography instrument mass spectrometer (electron ionization, 70 eV). Elemental microanalyses were performed on a CHNS-932 (LECO) Vario Elemental analyzer at Cairo University's Microanalytical Center.

1-Ethyl-3-[hydrazinylidenemethyl]-4-hydroxyquinolin-2(1*H*)-one (2a). A mixture of aldehyde **1** (2.17 g, 1 mmol) and hydrazine hydrate (1 mL, 2 mmol) in ethanol (25 ml) was stirred at room temperature for 4 h. The pale yellow solid formed was filtered off and washed with ethanol several times to obtain hydrazone **2a**. Yellow crystals, yield 2.1 g (92%). M.p. 186 °C; IR (KBr) ύ 3400 (OH), 3219, 3238 (NH_2), 3100 (CH aromatic), 2982 (CH aliphatic), 1652 (C=O), 1620 (C=N), 1600, 1537 (C=C), 1495, 1474, 946 cm^{-1}; ^{1}H NMR (400 MHz, DMSOd_6) δ 1.18 (t, 3H, J = 6.8 Hz, CH_3), 4.19 (q, 2H, J = 7.2 Hz, CH_2), 6.39 (s, 2H, NH_2, disappeared with D_2O), 7.19 (t, 1H, J = 7.6 Hz, Ar-H6), 7.44 (d, 1H, J = 8.4 Hz, Ar-H8), 7.60 (t, 1H, J = 1.6 Hz, Ar-H7), 7.91 (d, 1H, J = 7.2 Hz, Ar-H5), 8.37 (s, 1H, CH), 13.34 (s, 1H, OH, disappeared with D_2O) ppm; ^{13}C NMR (100 MHz, DMSOd_6) δ 173.0, 152.0, 150.8, 140.2, 138.2, 133.1, 125.6, 121.6, 115.0, 100.2, 39.6, 13.4 ppm; MS *m/z* (I_{rel}, %) 232 $[M]^+$ (12), 231 $[M]^+$ (15), 230 (28), 217 (6), 216 (6), 214 (100), 213 (25), 212 (22), 187 (79), 186 (32), 185 (32), 169 (14), 168 (15), 145 (12), 131 (12), 129 (12), 76 (13). Anal. Calcd for $C_{12}H_{13}N_3O_2$ (231.1): C, 62.33; H, 5.67; N, 18.17. Found: C, 61.93; H, 5.27; N, 17.77.

1-Ethyl-4-hydroxy-3-[(phenylhydrazinylidene)methyl]quinolin-2(1*H*)-one (2b). To a suspension of the aldehyde **1** (2.17 g, 1 mmol) in ethanol (25 mL), phenylhydrazine (1.08 g, 1 mmol) was added, and the mixture was refluxed for 4 h. The canary yellow solid formed was filtered off and crystallized. Canary yellow crystals (ethanol), yield 3 g (97%). M.p. 260 °C; IR (KBr) ύ 3400 (OH), 3252 (NH), 3070 (CH aromatic), 2970 (CH aliphatic), 1635 (C=O), 1615 (C=N), 1602, 1577 (C=C), 1542, 1495, 866 cm^{-1}; ^{1}H NMR (400 MHz, DMSOd_6) δ 1.21 (t, 3H, J = 7.2 Hz, CH_3), 4.29 (q, 2H, J = 7.2 Hz, CH_2), 6.68 (t, 1H, J = 7.2 Hz, Ar-H6), 6.93 (d, 2H, J = 7.6 Hz, Ar-H2',6'), 7.30 (m, 3H, J = 8 Hz, Ar-H3',4',5'), 7.57 (d, 1H, J = 8.8 Hz, Ar-H8), 7.66 (t, 1H, J = 8.4 Hz, Ar-H7), 8.052 (d, 1H, J = 8.0 Hz, Ar-H5), 8.447 (s, 1H, CH), 10.64 (s, 1H, NH, disappeared with D_2O), 13.455 (s, 1H, OH, disappeared with D_2O) ppm; ^{13}C NMR (100 MHz, DMSOd_6) δ 161.0, 160.9, 144.3, 139.2, 138.4, 133.6, 130.0, 124.0, 122.5, 120.2, 116.0, 115.9, 115.1, 112.1, 103.1, 40.1, 37.0, 13.2 ppm; MS *m/z* (I_{rel}, %) 308 $[M]^+$ (29), 307 $[M]^+$ (100), 306 (15), 289 (7), 250 (20), 216 (18), 215 (63), 214 (15), 187 (47), 186 (32), 185 (12), 169 (15), 146 (17), 132 (11), 120 (15), 93 (13). Anal. Calcd for $C_{18}H_{17}N_3O_2$ (307.35): C, 70.34; H, 5.58; N, 13.67. Found: C, 69.94; H, 5.18; N, 13.21.

1-Ethyl-4-hydroxy-3-[(2',4'-dinitrophenylhydrazinylidene)methyl]quinolin-2(1*H*)-one (2c). A mixture of aldehyde **1** (2.17 g, 1 mmol) with 2,4-dinitrophenylhydrazine (1.98 g, 1 mmol) in ethanol (50 mL) was heated under reflux for 4 h. The crystalline material obtained after cooling to room temperature was collected by filtration and recrystallized from ethanol. Orange crystals (ethanol), yield 3.87 g (97%). M.p. 275 °C; IR (KBr) ύ 3400 (OH), 3273 (NH), 3081 (CH aromatic), 2982 (CH aliphatic), 1637 (C=O), 1620 (C=N), 1609, 1539 (C=C), 1517, 1421, 785 cm^{-1}; ^{1}H NMR (400 MHz, DMSOd_6) δ 1.22 (t, 3H, *J* = 6.8 Hz, CH_3), 4.25 (q, 2H, *J* = 7.2 Hz, CH_2), 7.32 (t, 1H, *J* = 7.6 Hz, Ar-H6), 7.58 (d, 2H, *J* = 8.8 Hz, Ar-H5',6'), 7.730 (t, 1H, *J* = 6.8 Hz, Ar-H7), 8.04 (d, 1H, *J* = 6.4Hz, Ar-H8), 8.20 (dd, 1H, *J* = 7.2 Hz, Ar-H5), 8.79 (d, 1H, *J* = 2.4 Hz, Ar-H3'), 9.13 (s, 1H, CH), 10.00 (s, 1H, NH, disappeared with D_2O), 12.30 (s, 1H, OH, disappeared with D_2O) ppm; MS *m/z* (I_{rel}, %) 397 $[M]^+$ (18), 396 (9), 233 (11), 218 (41), 217 (34), 216 (69), 215 (100), 214 (51), 213 (29),188 (82), 187 (56), 186 (77), 173 (15), 160 (33), 168 (11), 146 (72), 132 (49), 130 (20), 120 (30), 119 (42), 118 (29), 91 (41), 77 (24). Anal. Calcd for $C_{18}H_{15}N_5O_6$ (397.34): C, 54.41; H, 3.81; N, 17.63. Found: C, 54.01; H, 3.41; N, 17.23.

5-Ethyl-1*H*-pyrazolo[4,3-*c*] quinolin-4(5*H*)-one (3a)
Procedure *a*: An equal molar mixture of aldehyde **1** (2.17g, 1 mmol) and hydrazine hydrate (0.5 mL, 1 mmol), 2-cyanoacetohydrazide (0.9 g, 1 mmol), semicarbazide hydrochloride (1.11 g, 1 mmol), thiosemicarbazide (0.91 g, 1 mmol), allylthiosemicarbazide (1.31 g, 1 mmol), phenylthiosemicarbazide (1.67 g, 1 mmol) or thiocarbodihydrazide (1.06 g, 1 mmol), was refluxed in acetic acid (25 mL) for 4 h. Afterwards, the mixture was left to cool at room temperature. The canary yellow solid formed was filtered off and recrystallized from acetic acid (92–94%).

Procedure *b*: A mixture of aldehyde **1** (2.17 g, 1 mmol), and benzohydrazide (1.36 g, 1 mmol) or nicotinohydrazide (1.3 g, 1 mmol), in the presence of *para*-toluenesulphonic acid (*p*-TSA) as the catalyst in ethanol (50 mL), was heated under reflux for 4 h. The crystalline material obtained after cooling to room temperature was collected by filtration and recrystallized from acetic acid. Yellow crystals (ethanol), yield 2 g (92–94%). M.p. >300 °C; IR (KBr) ύ 3217 (NH), 3068 (CH aromatic), 2970 (CH aliphatic), 1687 (C=O), 1634 (C=N), 1603, 1565 (C=C), 1550, 1497, 786 cm^{-1}; ^{1}H NMR (400 MHz, DMSOd_6) δ 1.16 (t, 3H, *J* = 6.8 Hz, CH_3), 4.22 (q, 2H, *J* = 7.2 Hz, CH_2), 5.87 (s, 1H, CH), 7.23 (t, 1H, *J* = 6.8 Hz, Ar-H6), 7.52 (d, 1H, *J* = 4.4 Hz, Ar-H8), 7.62 (t, 1H, *J* = 5.6 Hz, Ar-H7), 7.91 (dd, 1H, *J* = 6.4 Hz, Ar-H5), 11.33 (s, 1H, NH, disappeared with D_2O) ppm; ^{13}C NMR (100 MHz, DMSOd_6) δ 161.0, 144.8, 139.1, 133.2, 124.5, 122.5, 115.5, 1152, 102.5, 39.9, 37.1, 13.2 ppm; MS *m/z* (I_{rel}, %) 214 (1), 213 (1), 190 (72), 189 (100), 188 (44), 187 (46), 161 (50), 146 (49), 133 (14), 132 (55), 119 (20). Anal. Calcd for $C_{12}H_{11}N_3O$ (213.09): C, 67.59; H, 5.20; N, 19.71. Found: C, 67.19; H, 4.80; N, 19.31.

5-Ethyl-1-phenyl-1*H*-pyrazolo[4,3-*c*]quinolin-4(5*H*)-one (3b). A mixture of aldehyde **1** (2.17 g, 1 mmol) and phenylhydrazine (1.08 g, 1 mmol) in 30 mL of DMF was heated under reflux for 4 h. The precipitate formed upon standing was filtered, washed with ethanol, dried and recrystallized from acetic acid. yellow crystals (DMF); yield 2.8 g (94%). M.p. >300 °C; IR (KBr) ύ 3070 (CH aromatic), 2979 (CH aliphatic), 1630 (C=O), 1615 (C=N), 1604, 1551 (C=C), 1542, 1495, 769 cm^{-1}; ^{1}H NMR (400 MHz, DMSOd_6) δ 1.21 (t, 3H, *J* = 7.2 Hz, CH_3), 4.29 (d, 2H, *J* = 7.2 Hz, CH_2), 6.70 (t, 1H, *J* = 7.2 Hz, Ar-H6), 6.93 (d, 2H, *J* = 7.6 Hz, Ar-H2',6'), 7.50 (m, 3H, *J* = 8.0 Hz, Ar-H3',4',5'), 7.66 (d, 1H, *J* = 8.8 Hz, Ar-H8), 7.66 (t, 1H, *J* = 8.4 Hz, Ar-H7), 8.05 (d, 1H, *J* = 8.0 Hz, Ar-H5), 8.45 (s, 1H, CH); MS *m/z* (I_{rel}, %) 229 $[M]^+$ (97), 228 (31), 214 (13), 196 (23), 183 (78), 169 (23), 167 (19), 154 (62), 140 (24), 129 (54), 128 (36), 127 (51), 115 (45), 114 (100), 102 (95), 77 (52). Anal. Calcd for $C_{18}H_{15}N_3O$ (289.12): C, 74.72; H, 5.23; N, 14.52. Found: C, 69.54; H, 4.81; N, 14.12.

Bis-(1-ethyl-4-hydroxy-2-oxoquinolin-3-methylene) azine (4).
Procedure *a*: Reaction mixture of aldehyde **1** (2.17 g, 1 mmol) and hydrazine hydrate (0.05 mL, 1 mmol) in ethanol (25 mL) was refluxed for 4 h. The deposited solid was collected by filtration and recrystallized.

Procedure *b*: A mixture of aldehyde **1** (2.17 g, 1 mmol) and cyanoacetohydrazide (0.9 g, 1 mmol), benzohydrazide (1.36 g, 1 mmol), nicotinohydrazide (1.3 g, 1 mmol), semicarbazide hydrochloride (1.11 g, 1 mmol), thiosemicarbazide (0.91 g, 1 mmol), allylthiosemicarbazide (1.31 g, 1 mmol), phenylthiosemicarbazide (1.67g, 1 mmol) or thiocarbodihydrazide (1.06 g, 1 mmol), in 30 mL of DMF was heated under reflux for 4 h. The reaction mixture was allowed to stand, precipitated material was filtered, washed with ethanol, dried and recrystallized from ethanol. Yellow crystals (ethanol), yield 2 g (46%). M.p. >300 °C; IR (KBr) ύ 3400 (OH), 3079 (CH aromatic), 2983 (CH aliphatic), 1633 (C=O), 1628 (C=N), 1601, 1563 (C=C), 1497, 1445, 753 cm^{-1}; ^{1}H NMR (400 MHz, DMSOd_6) δ 1.23 (t, 3H, *J* = 6.8 Hz, CH_3), 4.26 (q, 2H, *J* = 6.0 Hz, CH_2), 7.35 (t, 1H, *J* = 14.0 Hz, Ar-H6), 7.53 (d, 1H, *J* = 7.6 Hz, Ar-H8), 7.76 (t, 1H, *J* = 13.6 Hz, Ar-H7), 8.11 (d, 1H, *J* = 7.6 Hz, Ar-H5), 9.09 (s, 1H, CH), 11.50 (s, 1H, OH, disappeared with D_2O) ppm; ^{13}C NMR (100 MHz, DMSOd_6) δ 210.5, 206.0, 186.3, 159.3, 138.2, 103.3, 100.0, 93.4, 89.9, 74.5, 58.5, 40.1 ppm; MS *m/z* (I_{rel}, %) 430 (1), 323 (18), 268 (33), 267 (27), 240 (24), 231 (18), 222 (12), 216 (71), 215 (94), 214 (87), 193 (19), 189 (74), 188 (75), 187 (72), 173 (23), 161 (41), 133 (42), 132 (100), 131 (31), 120 (99), 119 (64), 105 (43), 93 (49), 92 (46), 91 (39), 83 (39), 69 (47), 57 (40). Anal. Calcd for $C_{24}H_{22}N_4O_4$ (430.16): C, 66.97; H, 5.15; N, 13.02. Found: C, 57.15; H, 4.75; N, 12.62.

2-Cyano-*N*'-((1-ethyl-1,2-dihydro-4-hydroxy-2-oxoquinolin-3yl)methylene)acetohydrazide (5a). A mixture

of aldehyde **1** (2.17 g, 1 mmol) in ethanol (25 mL) with cyanoacetohydrazide was stirred at room temperature for 4 h. The deposited solid was collected by filtration and recrystallized. Yellow crystals (ethanol), yield 2.85 g (96%). M.p. 225 °C; IR (KBr) ύ 3481 (OH), 3198 (NH), 3060 (CH aromatic), 2980 (CH aliphatic), 2261 (CN), 1712 (C=O), 1630 (C=N), 1609, 1594 (C=C), 1577, 1556, 936 cm^{-1}; ^{1}H NMR (400 MHz, DMSOd_6) δ 1.21 (t, 3H, J = 6.8 Hz, CH_3), 3.88 (s, 2H, CH_2), 4.27 (q, 2H, J = 6.8 Hz, $CH_{2ethyl\ quinolone}$), 7.32 (t, 1H, J = 7.6 Hz, Ar-H6), 7.59 (d, 2H, J = 8.4 Hz, Ar-H8), 7.72 (t, 1H, J = 7.2 Hz, Ar-H7), 8.08 (dd, 1H, J = 6.8 Hz, Ar-H5), 8.62 (s, 1H, CH), 12.01 (s, 1H, NH, disappeared with D_2O), 14.03 (s, 1H, OH, disappeared with D_2O) ppm; ^{13}C NMR (100 MHz, DMSOd_6) δ 166.5, 162.6, 144.6, 142.4, 139.1, 135.4, 133.2, 124.5, 122.6, 118.9, 116.7, 102,6, 46.7, 40.5, 13.4 ppm; MS m/z (I_{rel}, %) 298 $[M]^+$ (2), 280 (17), 251 (21), 215 (47), 214 (45), 213 (57), 212 (62), 211 (30), 198 (18), 189 (50), 188 (43), 186 (45), 185 (69), 161 (33), 146 (41), 132 (49), 131 (55), 119 (38), 105 (85), 104 (70), 77 (100), 75 (13). Anal. Calcd for $C_{15}H_{14}N_4O_3$ (298.3): C, 60.40; H, 4.73; N, 18.78. Found: C, 60.00; H, 4.33; N, 18.38.

***N*'-((1-Ethyl-1,2-dihydro-4-hydroxy-2-oxoquinolin-3-yl)methylene)benzohydrazide (5b).** A mixture of aldehyde **1** (2.17 g, 1 mmol) and benzohydrazide (1.36 g, 1 mmol) in 50 mL of ethanol was heated under reflux for 4 h. The reaction mixture was allowed to stand, precipitated material was filtered, washed with ethanol, dried and recrystallized from ethanol. Yellow crystals (ethanol), yield 2.23 g (96%). M.p. 250 °C; IR (KBr): ύ 3400 (OH), 3233 (NH), 3051 (CH aromatic), 2976 (CH aliphatic), 1676 (C=O), 1633 (C=N), 1610, 1570 (C=C), 1536, 1465, 695 cm^{-1}; ^{1}H NMR (400 MHz, DMSOd_6) δ 1.21 (t, 3H, J = 6.8 Hz, CH_3), 4.25 (dd, 2H, J = 6.8 Hz, CH_2), 7.28 (t, 1H, J = 7.2 Hz, Ar-H6), 7.56 (m, 3H, J = 8.4 Hz, Ar-H3',4',5'), 7.626 (d, 1H, J = 7.2 Hz, Ar-H8), 7.67 (t, 1H, J = 8.0 Hz, Ar-H7), 7.97 (d, 2H, J = 7.2 Hz, Ar-H2',6'), 8.08 (d, 1H, J = 7.6 Hz, Ar-H5), 8.86 (s, 1H, CH), 12.37 (s, 1H, NH, disappeared with D_2O), 15.50 (s, 1H, OH, disappeared with D_2O) ppm; ^{13}C NMR (100 MHz, DMSOd_6) δ 166.7, 163.1, 161.2, 148.7, 139.6, 133.5, 132.7, 132.4, 129.1, 128.1, 124.9, 122.3, 116.8, 115.2, 101.9, 40.4, 39.8, 36.9, 13.2 ppm; MS m/z (I_{rel}, %) 335 $[M]^+$ (16), 336 (9), 307 (7), 283 (17), 215 (100), 214 (36), 213 (19), 186 (18), 170 (10), 129 (10), 119 (15), 105 (95), 104 (40), 77. Anal. Calcd for $C_{19}H_{17}N_3O_3$ (335.36): C, 68.05; H, 5.11; N, 12.53. Found: C, 67.65; H, 4.71; N, 12.13.

***N*'-((1-Ethyl-1,2-dihydro-4-hydroxy-2-oxoquinolin-3-yl)methylene)nicotinohydrazide (5c).** A mixture of aldehyde **1** (2.17 g, 1 mmol) and nicotinohydrazide (1.37 g, 1 mmol) in 50 mL of ethanol was heated under reflux for 4 h. The reaction mixture was allowed to stand, precipitated material was filtered, washed with ethanol, dried and recrystallized from ethanol. Canary yellow crystals (ethanol), yield 2.23 g (96%). M.p. 252 °C; IR (KBr) ύ 3400 (OH), 3196 (NH), 3050 (CH aromatic), 2980 (CH aliphatic), 1678 (C=O), 1612 (C=N), 1600, 1594 (C=C), 1539, 1464, 782 cm^{-1}; ^{1}H NMR (400 MHz, DMSOd_6) δ 1.21 (t, 3H, J = 6.8 Hz, CH_3), 4.24 (q, 2H, J = 6.4 Hz, CH_2), 7.29 (t, 1H, J = 7.2 Hz, Ar-H6), 7.53 (d, 2H, J = 8.4 Hz, Ar-H8), 7.58 (t, 1H, J = 5.6 Hz, Ar-H5'), 7.67 (t, 1H, J = 7.6 Hz, Ar-H7), 8.07 (d, 1H, J = 8.0 Hz, Ar-H4'), 8.29 (d, 1H, J = 7.6 Hz, Ar-H5), 8.78 (s, 1H, Ar-H6'), 8.86 (s, 1H, Ar-H2'), 9.10 (s, 1H, CH), 12.49 (s, 1H, NH, disappeared with D_2O), 14.36 (s, 1H, OH, disappeared with D_2O) ppm; ^{13}C NMR (100 MHz, DMSOd_6) δ 166.2, 161.6, 161.1, 153.0, 149.1, 148.9, 139.5, 135.9, 133.6, 128.4, 124.9, 124.2, 122.4, 116.4, 115.2, 101.9, 39.7, 13.3 ppm; MS m/z (I_{rel}, %) 336 $[M]^+$ (20), 318 (49), 317 (25), 289 (8), 217 (18), 215 (40), 213 (45), 212 (57), 97 (13), 189 (80), 188 (60), 187 (26) 161 (34), 147 (100), 146 (25), 106 (61), 90 (20), 78 (55). Anal. Calcd for $C_{18}H_{16}N_4O_3$ (336.34): C, 64.28; H, 4.79; N, 16.66. Found: C, 63.88; H, 4.39; N, 16.46.

General Procedure for the Synthesis of Substituted Semicarbazones 6

To a solution of aldehyde **1** (2.17 g, 1 mmol) in ethanol (15 mL) was added semicarbazide hydrochloride (1.11 g, 1 mmol), thiosemicarbazide (0.91 g, 1 mmol), allylthiosemicarbazide (1.31 g, 1 mmol) or phenylthiosemicarbazide (1.67 g, 1 mmol). The mixture was refluxed at 90 °C for 4 h. The residue was collected by filtration and washed with ether (three times) to give pure products **6a–d**.

1-((1-Ethyl-1,2-dihydro-4-hydroxy-2-oxoquinolin-3-yl) methylene)semicarbazide (6a). Yellow crystals (ethanol); yield 2.65 g (96%). M.p. 295 °C; IR (KBr) ύ 3500 (OH), 3372, 3340 (NH_2), 3192 (NH), 3043 (CH aromatic), 2969 (CH aliphatic), 1705 (C=O), 1628 (C=O), 1610 (C=N), 1600, 1578 (C=C), 1525, 1470, 769 cm^{-1}; ^{1}H NMR (400 MHz, DMSOd_6) δ 1.19 (t, 3H, J = 6.8 Hz, CH_3), 4.24 (q, 2H, J = 7.2 Hz, CH_2), 6.45 (s, 2H, NH_2, disappeared with D_2O), 7.27 (t, 1H, J = 8.0 Hz, Ar-H6), 7.52 (d, 1H, J = 8 Hz, Ar-H8), 7.65 (t, 1H, J = 7.2 Hz, Ar-H7), 8.05 (d, 1H, J = 7.2 Hz, Ar-H5), 8.40 (s, 1H, CH), 10.32 (s, 1H, NH, disappeared with D_2O), 13.12 (s, 1H, OH, disappeared with D_2O) ppm;^{13}C NMR (100 MHz, DMSOd_6) δ 161.2, 156.0, 144.0, 139.1, 133.1, 124.6, 122.3, 116.6, 115.1, 102.1, 40.2, 36.9, 13.2 ppm; MS m/z (I_{rel}, %) 274 $[M]^+$ (17), 273 (14), 257 (5), 216 (14), 215 (100), 214 (16), 113 (34), 187 (45), 169 (11), 77 (4). Anal. Calcd for $C_{13}H_{14}N_4O_3$ (274.28): C, 56.93; H, 5.14; N, 20.43. Found: C, 56.53; H, 4.71; N, 20.03.

1-((1-Ethyl-1,2-dihydro-4-hydroxy-2-oxoquinolin-3-yl) methylene)thiosemicarbazide (6b). Yellow crystals (ethanol); yield 2.8 g (96%). M.p. 243 °C; IR (KBr) ύ 3500 (OH), 3446, 3328 (NH_2), 3204 (NH), 3043 (CH aromatic), 2968 (CH aliphatic), 1617 (C=O), 1610 (C=N), 1600, 1594 (C=C), 1577 (C=S), 1469, 760 cm^{-1}; ^{1}H NMR (400 MHz, DMSOd_6) δ 1.19 (t, 3H, J = 6.8 Hz, CH_3), 4.25 (q, 2H, J =

7.2 Hz, CH_2), 5.80 (s, 2H, NH_2, disappeared with D_2O), 7.31 (t, 1H, *J* = 8.0 Hz, Ar-H6), 7.55 (d, 1H, *J* = 8.0 Hz, Ar-H8), 7.67 (t, 1H, *J* = 7.2 Hz, Ar-H7), 8.03 (dd, 1H, *J* = 7.2 Hz, Ar-H5), 8.13 (s, 1H, NH, disappeared with D_2O), 8.61 (s, 1H, CH), 11.52 (s, 1H, OH, disappeared with D_2O) ppm; ^{13}C NMR (100 MHz, DMSOd_6) δ 160.9, 144.8, 139.1, 133.2, 124.5, 122.5, 115.5, 115.2, 102.5, 40.3, 39.3, 37.0, 13.2 ppm; MS *m/z* (I_{rel}, %) 290 $[M]^+$ (7), 288 $[M]^+$ (11), 287 (8), 248 (13), 231 (20), 216 (52), 215 (100), 214 (50), 201 (15), 188 (53), 187 (72), 186 (85), 185 (96), 171 (21), 161 (24), 146 (40), 119 (34), 89 (14), 77 (36). Anal. Calcd for $C_{13}H_{14}N_4O_2S$ (290.34): C, 53.78; H, 4.86; N, 19.30; S, 11.04. Found: C, 53.38; H, 4.46; N, 18.90; S, 10.64

4-Allyl-1-((1-ethyl-1,2-dihydro-4-hydroxy-2-oxoquinolin-3-yl)methylene)thiosemicarbazide (6c). Yellow crystals (ethanol); yield 3.25 g (98%). M.p. 221 °C; IR (KBr) ύ 3300 (OH), 3200 (NH), 3043 (CH aromatic), 3010 (CH alkene), 2984 (CH aliphatic), 1633 (C=O), 1615 (C=N), 1593 (C=S), 1578, 1570 (C=C), 1529, 1470, 754 cm^{-1}; ^{1}H NMR (400 MHz, DMSOd_6) δ 1.19 (t, 3H, *J* = 6.8 Hz, CH_3), 4.25 (q, 2H, *J* = 7.2 Hz, CH_2), 4.29 (d, 2H, *J* = 8.0 Hz, $CH_{2\ allylic}$), 5.2 (m, 2H, *J* = 5.2 Hz, $=CH_{2\ allylic}$), 5.95 (m, 2H, *J* = 5.2 Hz, $CH_{allylic}$), 7.32 (t, 1H, *J* = 8.0 Hz, Ar-H6), 7.59 (d, 1H, *J* = 8.0 Hz, Ar-H8), 7.68 (t, 1H, *J* = 7.2 Hz, Ar-H7), 8.06 (dd, 1H, *J* = 7.2 Hz, Ar-H5), 8.64 (s, 1H, CH), 8.74 broad (s, 1H, NH, disappeared with D_2O), 11.58 (s, 1H, NH, disappeared with D_2O), 11.7 (s, 1H, OH, disappeared with D_2O) ppm; ^{13}C NMR (100 MHz, DMSOd_6) δ 166.5, 162.6, 161.0, 144.6, 142.4, 139.1, 135.4, 133.2, 124.5, 122.6, 116.0, 102.6, 98.4, 46.6, 40.5, 13.3 ppm; MS *m/z* (I_{rel}, %) 230 $[M]^+$ (2), 257 (62), 256 (100), 255 (47), 216 (20), 215 (61), 214 (66), 213 (22), 173 (19), 169 (15), 161 (38), 146 (37), 133 (15), 132 (31), 126 (39), 119 (16). 106 (14), 99 (14), 77 (15). Anal. Calcd for $C_{16}H_{18}N_4O_2S$ (330): C, 58.16; H, 5.49; N, 16.96; S, 9.70. Found: C, 57.76; H, 5.09; N, 16.56; S, 9.30.

1-((1-Ethyl-1,2-dihydro-4-hydroxy-2-oxoquinolin-3-yl) methylene)-4-phenylthiosemicarbazide (6d). Yellow crystals (ethanol); yield 3.6 g (98%). M.p. 221 °C; IR (KBr) ύ 3300 (OH), 3189 (NH), 3016 (CH aromatic), 2981 (CH aliphatic), 1639 (C=O), 1612 (C=N), 1592 (C=S), 1578, 1571 (C=C), 1530, 1503, 755 cm^{-1}; ^{1}H NMR (400 MHz, DMSOd_6) δ 1.22 (t, 3H, *J* = 6.8 Hz, CH_3), 4.29 (q, 2H, *J* = 7.2 Hz, CH_2), 7.43 (t, 1H, *J* = 8.0 Hz, Ar-H6), 7.58–7.45 (m, 5H, Ar-H 2',3',4',5',6'), 7.62 (d, 1H, *J* = 8.0 Hz, Ar-H8), 7.61 (t, 1H, *J* = 7.2 Hz, Ar-H7), 8.08 (d, 1H, *J* = 7.2 Hz, Ar-H5), 8.72 (s, 1H, CH), 10.24 (s, 2H, 2NH, disappeared with D_2O), 11.87 (s, 1H, OH, disappeared with D_2O) ppm; MS *m/z* (I_{rel}, %) 292 (10), 290 (16), 276 (3), 272 (6), 217 (4), 216 (16), 215 (24), 214 (18), 213 (13), 187 (31), 186 (10), 169 (12), 146 (15), 135 (36), 132 (34), 119 (17), 104 (14), 94 (8). 93 (100), 92 (29), 77 (66), 66 (28). Calcd for $C_{19}H_{18}N_4O_2S$ (366.44): C, 62.28; H, 4.95; N, 15.29; S, 8.75. Found: C, 61.88; H, 4.55; N, 14.89; S, 8.35.

1,5-Bis-(1-ethyl-1,2-dihydro-4-hydroxy-2-oxoquinolin-3-methylene)thiocarbonohydrazide (7).
Procedure *a*: A mixture of aldehyde **1** (2.17 g, 1 mmol) and thiocarbohydrazide (1.06 g, 1 mmol) in 50 mL of ethanol (50%) or dioxane (50%) was heated under reflux for 4 h. The reaction mixture was allowed to stand, precipitated material was filtered, washed with diethyl ether, dried and recrystallized from ethanol.

Procedure *b*: A mixture of aldehyde **1** (2.17 g, 1 mmol) and thiocarbohydrazide (1.06 g, 1 mmol) in 50 mL of ethanol was stirred at room temperature for 6 h. The precipite formed was filtered off and crystallized. Yellow crystals (ethanol); yield 2.3 g (44%). M.p. 235 °C; IR (KBr) ύ 3400 (OH), 3179 (NH), 3026 (CH aromatic), 2979 (CH aliphatic), 1629 (C=O), 1607 (C=N), 1600, 1590 (C=C), 1537, 1509, 1247 (C=S), 731 cm^{-1}; ^{1}H NMR (400 MHz, DMSOd_6) δ 1.19 (t, 3H, *J* = 7.2 Hz, CH_3), 4.258 (q, 2H, *J* = 6.8 Hz, CH_2), 7.310 (t, 1H, *J* = 7.6 Hz, Ar-H6), 7.505 (d, 2H, *J* = 8.8 Hz, Ar-H8), 7.69 (td, 1H, *J* = 7.2 Hz, Ar-H7), 8.04 (dd, 1H, *J* = 6.8 Hz, Ar-H5), 8.14 (s, 1H, NH, disappeared with D_2O), 8.62 (s, 1H, CH), 11.53 (s, 1H, OH, disappeared with D_2O) ppm; ^{13}C NMR (100 MHz, DMSOd_6) δ 163.0, 154.8, 141.9, 136.2, 132.9, 127.5, 125.8, 122.9, 117.8, 115.9, 48.2, 40.3, 13.1 ppm; MS *m/z* (I_{rel}, %) 231 (44), 217 (8), 216 (33), 215 (100), 214 (55), 213 (48), 189 (10), 188 (24), 187 (50), 186 (40), 169 (16), 160 (13), 146 (22), 132 (36), 120 (21), 119 (21), 105 (18), 92 (14), 91 (14), 77 (30). Anal. Calcd for $C_{25}H_{26}N_6O_4S$ (506.58): C, 59.27; H, 5.17; N, 16.59; S, 6.33. Found: C, 58.87; H, 4.77; N, 16.19; S, 5.93.

3-((2-Aminophenylimino)methyl)-1-ethyl-4-hydroxyquinolin-2(1*H*)-one (8a). Aldehyde **1** (2.17 g, 1 mmol) and *ortho*-phenylenediamine (1.08 g, 1 mmol) were dissolved in ethanol (30 mL), and heated under reflux for 4 h. The reaction mixture was allowed to stand, precipitated material was filtered, washed with ethanol, dried and recrystallized from ethanol. Canary yellow crystals (ethanol); yield 3.05 g (99%). M.p. 212 °C; IR (KBr) ύ 3400 (OH), 3380, 3340 (NH_2), 3070 (CH aromatic), 2974 (CH aliphatic), 1656 (C=O), 1615 (C=N), 1602, 1595 (C=C), 1561, 1492, 738 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.21 (t, 3H, *J* = 7.2 Hz, CH_3), 4.21 (d, 2H, *J* = 7.2 Hz, CH_2), 5.21 (s, 2H, NH_2, disappeared with D_2O), 6.77 (d, 2H, *J* = 7.6 Hz, Ar-H3',5'), 7.08 (t, 1H, *J* = 7.2 Hz, Ar-H6), 7.22 (d, 1H, *J* = 8.8 Hz, Ar-H8), 7.43 (m, 2H, *J* = 8.0 Hz, Ar-H4',6'), 7.66 (t, 1H, *J* = 8.4 Hz, Ar-H7), 8.14 (d, 1H, *J* = 8.0 Hz, Ar-H5), 8.79 (s, 1H, CH), 13.78 (s, 1H, OH, disappeared with D_2O) ppm; ^{13}C NMR (100 MHz, DMSO d_6) δ 180.3, 155.2, 141.3, 140.7, 134.5, 128.2, 126.6, 126.5, 122.3, 122.0, 121.5, 129.7, 118.9, 118.1, 115.4, 103.2, 40.2, 13.2 ppm; MS *m/z* (I_{rel}, %) 307 $[M]^+$ (9), 306 (11), 279 (9), 278 (100), 277 (18), 231 (1), 132 (2), 120 (4), 119 (4), 108 (4), 80 (1). Anal. Calcd for $C_{18}H_{17}N_3O_2$ (307.35): C, 70.34; H, 5.58; N, 13.67. Found: C, 69.94; H, 5.18; N, 13.21.

3-((2-Hydroxyphenylimino)methyl)-1-ethyl-4-hydroxyquinolin-2(1*H*)-one (8b). Aldehyde **1** (2.17 g, 1 mmol) and 2-aminophenol (1.09 g, 1 mmol) were dissolved in ethanol (30 mL) and heated under reflux for 4 h. The reaction mixture was allowed to stand, precipitated material was filtered, washed with ethanol, dried and recrystallized from ethanol. Yellow crystals (ethanol); yield 3.05 g (99%). M.p. 260 °C; IR (KBr) ύ 3446 (OH), 3070 (CH aromatic), 2968 (CH aliphatic), 1646 (C=O), 1615 (C=N), 1602, 1596 (C=C), 1543, 1490, 754 cm^{-1}; ^{1} H NMR (400 MHz, DMSO d_6) δ 1.21 (t, 3H, *J* = 7.2 Hz, CH_3), 4.21 (d, 2H, *J* = 7.2 Hz, CH_2), 6.94 (t, 1H, *J* = 7.2 Hz, Ar-H6), 7.04 (d, 1H, *J* = 8 Hz, Ar-H8), 7.13 (d, 3H, *J* = 8Hz, Ar-H5'), 7.22 (t, 1H, *J* = 8.4Hz, Ar-H7), 7.45 (t, 1H, *J* = 8.4 Hz, Ar-H3'), 7.66 (d, 2H, *J* = 7.6 Hz, Ar-H4',6'), 8.12 (d, 1H, *J* = 8.0 Hz, Ar-H5), 9.00 (s, 1H, CH), 13.95 (s, 1H, OH, disappeared with D_2O) ppm; ^{13}C NMR (100 MHz, DMSO d_6) δ 180.7, 152.4, 148.2, 144.9, 134.6, 127.9, 126.5, 122.1, 120.6, 120.1, 119.1, 118.2, 116.9, 116.5, 115.4, 103.5, 40.1, 13.2 ppm; MS *m/z* (I_{rel}, %) 307 $[M]^+$ (9), 306 (11), 279 (9), 278 (100), 277 (18), 231 (1), 132 (2), 120 (4), 119 (4), 108 (4), 80 (1). Anal. Calcd for $C_{18}H_{16}N_2O_3$ (308.33): C, 70.12; H, 5.23; N, 9.09. Found: C, 69.74; H, 4.83; N, 8.69.

5,13-Dihydro-5-ethyl-6*H*-quinolino[4,3-*b*][1,5]benzodiazepin-6-one (9a). A mixture of aldehyde **1** (2.17 g, 1 mmol) and *ortho*-phenylenediamine (1.08 g, 1 mmol) in the presence of *para*-toluenesulphonic acid (*p*-TSA) as the catalyst in toluene (50 mL), was heated under reflux for 6 h. The crystalline material obtained after cooling to room temperature was collected by filtration and recrystallized from ethanol. Pale yellow crystals (ethanol); yield 2.6 g (90%). M.p. >300 °C; IR (KBr) ύ 3300 (NH), 3075 (CH aromatic), 2969 (CH aliphatic), 1682 (C=O), 1632 (C=N), 1601, 1596 (C=C), 1574, 1551, 754 cm^{-1}; ^{1} H NMR (400 MHz, DMSO-d_6) δ 1.21 (t, 3H, *J* = 7.2 Hz, CH_3), 4.24 (d, 2H, *J* = 7.2 Hz, CH_2), 7.29 (t, 1H, *J* = 7.2 Hz, Ar-H6), 7.51–7.65 (m, 3H, *J* = 8.0 Hz, Ar-H3',4',5'), 7.69 (d, 1H, *J* = 8.0 Hz, Ar-H8), 8.07 (t, 1H, *J* = 8.4 Hz, Ar-H7), 8.29 (t, 1H, *J* = 8.4 Hz, Ar-H6'), 8.78 (d, 1H, *J* = 8.0 Hz, Ar-H5), 9.10 (s, 1H, CH), 12.49 (s, 1H, NH, disappeared with D_2O) ppm; MS *m/z* (I_{rel}, %) 289 (1), 278 (77), 277 (100), 175 (13), 187 (3), 159 (7), 145 (3), 119 (10), 77 (4). Anal. Calcd for $C_{18}H_{15}N_3O$ (289.33): C, 74.72; H, 5.23; N, 14.52. Found: C, 74.32; H, 4.83; N, 14.12.

5,13-Dihydro-5-ethyl-6*H*-quinolino[4,3-*b*][1,5]benzoazoxepin-6-one (9b). A mixture of aldehyde **1** (2.17 g, 1 mmol) and *ortho*-phenylenediamine (1.08 g, 1 mmol) or 2-aminophenol (1.09 g, 1 mmol) in the presence of *para*-toluenesulphonic acid (*p*-TSA) as the catalyst in toluene (50 mL), was heated under reflux for 6 h. The crystalline material obtained after cooling to room temperature was collected by filtration and recrystallized from ethanol. Pale yellow crystals (ethanol); yield 2.6 g (90%). M.p. >300 °C; IR (KBr) ύ 3078 (CH aromatic), 2969 (CH aliphatic), 1674 (C=O), 1633 (C=N), 160, 1586 (C=C), 1570, 1497, 757 cm^{-1}; ^{1} H NMR (400 MHz, DMSO-d_6) δ 1.61 (t, 3H, *J* = 7.2 Hz, CH_3), 4.14 (d, 2H, *J* = 7.2 Hz, CH_2), 7.14 (t, 1H, *J* = 7.2 Hz, Ar-H6), 7.21–7.43 (m, 3H, Ar-H3',4',5',6'), 7.49 (d, 1H, *J* = 8.0 Hz, Ar-H8), 7.65 (t, 1H, *J* = 8.4 Hz, Ar-H7), 8.08 (d, 1H, *J* = 8 Hz, Ar-H5), 9.29 (s, 1H, CH) ppm. Anal. Calcd for $C_{18}H_{14}N_2O_2$ (290.11): C, 74.47; H, 4.86; N, 9.65. Found: C, 74.07; H, 4.46; N, 9.25.

1,2-(Bis(1-ethyl-4-hydroxy-2-oxoquinolin-3-methylene)amino)benzene (10).

Procedure *a*: Reaction mixture of aldehyde **1** (2.17 g, 1 mmol) in ethanol (25 mL) and *ortho*-phenylenediamine (1.08 g, 1 mmol) was refluxed for 4 h. The deposited solid was collected by filtration and recrystallized.

Procedure *b*: A mixture of aldehyde **1** (2.17g, 1 mmol) and *ortho*-phenylenediamine (1.08 g, 1 mmol) in DMF was heated under reflux for 4 h. The reaction mixture was allowed to stand, precipitated material was filtered, washed with ethanol, dried and recrystallized from ethanol. Yellow crystals (ethanol); yield 2.3 g (45%). M.p. 280 °C; IR (KBr) ύ 3400 (OH), 3070 (CH aromatic), 2975 (CH aliphatic), 1655 (C=O), 1615 (C=N), 1607, 1596 (C=C), 1555, 1490, 755 cm^{-1}; ^{1} H NMR (400 MHz, DMSO-d_6) δ 1.21 (t, 3H, *J* = 7.2 Hz, CH_3), 4.19 (d, 2H, *J* = 7.2 Hz, CH_2), 7.20 (t, 1H, *J* = 7.2 Hz, Ar-H6), 7.77 (d, 3H, *J* = 8.0 Hz, Ar-H8,3',6'), 7.67 (t, 3H, *J* = 8.4 Hz, Ar-H7,4',5'), 8.07 (d, 1H, *J* = 8.0 Hz, Ar-H5), 8.73 (s, 1H, CH), 13.17 (s, 1H, OH, disappeared with D_2O) ppm; MS *m/z* (I_{rel}, %) 491 (1), 477 (1), 390 (44), 389 (14), 307 (13), 306 (34), 305 (81), 304 (87), 303 (23), 289 (26), 278 (59), 277 (100), 276 (31), 261 (18), 248 (48), 189 (18),188 (26), 187 (25), 161 (15), 118 (30), 117 (19), 91 (17), 77 (12). Anal. Calcd for $C_{30}H_{26}N_4O_4$ (506.55): C, 71.13; H, 5.17; N, 11.06. Found: C, 70.74; H, 4.71; N, 10.66.

3. 1. Biological Activity

3. 1. 1. Anti-microbial Assay

The tested compounds' antimicrobial activity was examined to improve these derivatives' selectivity for the tested bacteria. All microbial strains were supplied by the Regional Center for Mycology and Biotechnology (RCMB), located at Al-Azhar University in Cairo, Egypt.

Following the guidelines provided by the NCCLS (National Committee for Clinical Laboratory Standards, 1993),[47] susceptibility tests were carried out. The inhibition zone screening tests were conducted using the well diffusion method r1. Colonies that had been grown overnight on an agar plate were used to make the inoculum suspension, which was then added to Mueller–Hinton broth (fungi grown in malt broth). Mueller–Hinton agar plates were inoculated using a sterile swab dipped in the suspension (fro fungi malt agar plates were used, for bacteria nutrient agar plates were used). To find the MIC value, the compounds were dissolved in dimethyl sulfoxide

(DMSO) at varying concentrations (10, 5, 2.5, 1.25 mg/mL). For fungi, the inhibition zone was measured after 48 h at 28 °C, and for bacteria, it was measured after 24 h at 37 °C.

3. 1. 2. Antioxidant Assay

The antioxidant activity of extract was determined at the Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University by the DPPH free radical scavenging assay in triplicate and average values were considered.

DPPH Radical Scavenging Activity

A freshly made 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical methanol solution (0.004% w/v) was made and kept at 10 °C in the dark. The test component was dissolved in methanol. A 40 µL aliquot of the methanol solution was combined with 3 mL of the DPPH solution. Using a UV-Vis spectrophotometer, absorbance values were promptly recorded (Milton Roy, Spectronic 1201). Up until the absorbance stabilized (16 minutes), data were continuously recorded at 1-minute intervals to track the decrease in absorbance at 515 nm. The absorbance of the reference compound ascorbic acid and the DPPH radical in the absence of an antioxidant were also measured (control). Each calculation was performed three times, then averaged. Using the following formula, the percentage inhibition (PI) of the DPPH radical was determined:

$$\mathrm{PI} = \frac{A_C - A_T}{A_C} \cdot 100 \quad (1)$$

where A_C is absorbance of the control at t = 0 min and A_T is absorbance of the sample + DPPH at t = 16 min.

GraphPad Prism software (San Diego, CA, USA) was used to estimate the 50% inhibitory concentration (IC_{50}), which is the concentration needed to achieve 50% DPPH radical scavenging activity, from graphic plots of the dose response curve.

3. 1. 3. Anticancer Activity Screening

3. 1. 3. 1. Materials and chemicals

Dimethyl sulfoxide (DMSO), trypan blue dye, and crystal violet stain (1%) (which is made up of 50% methanol and 0.5% [w/v] crystal violet, then made up to the final volume with dd H_2O and filtered through a Whatman No. 1 filter paper) were acquired from Sigma (St. Louis, MO, USA). Additionally, Lonza provided the fetal bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin, and 0.25% trypsin-EDTA.

3. 1. 3. 2. Cell growth and Cell culture

MCF-7 (breast carcinoma), and HePG-2 (hepatocellular carcinoma) were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM), which was enhanced with 50 µg/mL of gentamycin, 10% heat-inactivated fetal bovine serum, 1% L-glutamine, and HEPES buffer. Every cell was sub-cultured two times a week and kept at 37 °C in a humid environment with 5% CO_2.

3. 1. 3. 3. Cell viability assay

To conduct a cytotoxicity assay, 100 µL of growth medium was used to seed $1 \cdot 10^4$ cells per well in 96-well plates. After 24 h of seeding, fresh medium with varying concentrations of the test sample was added. Using a multichannel pipette, confluent cell monolayers were dispensed into 96-well flat-bottomed microtiter plates (Falcon, NJ, USA) and serial two-fold dilutions of the chemical compound under test were added. The microtiter plates were incubated for 24 h at 37 °C in a humidified incubator containing 5% CO_2. For every test sample concentration, three wells were utilized. Control cells were cultured with or without DMSO and without the test sample. The experiment was found to be unaffected by the small amounts of DMSO (maximum 0.1%) in the wells. A colorimetric method was used to determine the viable cell yield following a 24h incubation period at 37 °C.

To sum up, the media were aspirated following the incubation period, and each well received a minimum of 30 minutes of addition of the 1% crystal violet solution. After the stain was eliminated, the plates were thoroughly cleaned with tap water to eliminate any remaining stain. After adding 30% glacial acetic acid to each well and thoroughly mixing them, the plates were gently shaken on a Microplate Reader (TECAN, Inc.) to measure the absorbance of the material using a test wavelength of 490 nm. To account for background absorbance found in wells without additional stain, all results were adjusted. In the absence of the tested compounds, treated samples contrasted with the cell control. All experiments were carried out in triplicate. Every tested compound's cell cytotoxic effect was computed. The number of viable cells was ascertained by measuring the optical density using a microplate reader (SunRise, TECAN, Inc., USA). The percentage of viability was computed as

$$\frac{\mathrm{OD_t}}{\mathrm{OD_c}} \cdot 100\% \quad (2)$$

where OD_t represents the mean optical density of wells treated with the tested sample and OD_c represents the mean optical density of untreated cells. EXCell plots of the relationship between drug concentration and surviving cells were used to determine each tumor cell line's survival curve following treatment with the designated compound. Using GraphPad Prism software (San Diego, CA, USA), the 50% inhibitory concentration (IC_{50}), or the concentration needed to produce toxic effects in 50% of intact cells, was calculated from graphic plots of the dose response curve for each conc.

4. Conclusions

In present work, we have studied the reactivity of 3-formyl-4-hydroxyquinolin-2(1*H*)-one (**1**) towards various nucleophiles, where the reaction conditions have a significant impact on the products formed. In the acidic medium, the ring-closure takes place, while in the presence of DMF, we got a bis compound, and an open-chain structure was produced when ethanol was present. The tested compounds (compounds **2**–**7**) were found to be active against both Gram-positive and Gram-negative bacterial strains. Compounds **2a** and **2c** showed good activity against fungal strain. The antioxidant activity of the prepared compounds showed that quinolinone derivatives **2a**, **2b**, **2c**, **6c**, **6d**, **7**, **8a**, and **8b** have good potent DPPH antioxidant activity. When compared to the other compounds, compounds **2a**, **2b**, and **2c** have stronger DPPH antioxidant activity. Compounds **6c**, **6d**, and **7** demonstrated good antioxidant properties, while compounds **5a** and **5c** have moderate activity. Compound **8a** is more active than compound **8b**. Compounds **2a** and **8a** are the most active against breast cancer (MCF-7). Whereas other selected compounds have lesser or no activity.

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Povzetek

Raziskali smo reaktivnost 1-etil-1,2-dihidro-4-hidroksi-2-oksokinolin-3-karbaldehida (**1**) z izbranimi diaza-nukleofili pod različnimi pogoji. 1-Etil-1,2-dihidro-4-hidroksi-2-oksokinolin-3-karbaldehid (**1**) smo reagirali s hidrazinom, fenilhidrazinom, 2,4-dinitrofenilhidrazinom, kislinskimi hidrazidi, semikarbazidom, tiosemikarbazidom, tiokarbohidrazidom, *orto*-fenilendiaminom in *orto*-aminofenolom. Ugotovili smo, da izbor topila ključno vpliva na rezultat reakcije, posledično so nastali aciklični in/ali ciklični derivati kinolinonov. Strukture novih spojin smo določili s spektroskopskimi tehnikami in s pomočjo elementne analize. Vse produkte smo testirali *in vitro*, da bi določili njihove antimikrobne, antioksidativne in antitumorske aktivnosti. Spojini **2a** and **2c** sta bili najbolj aktivni proti bakterijskim sevom in glivam. Najbolj učinkoviti spojini proti celični liniji raka dojke (MCF-7) pa sta bili **2a** in **8a**.