

Cytotoxicity, antioxidant, and antimicrobial activities of novel 2-quinolone derivatives derived from coumarin

Ahmed A. Al-Amiery · Redha I. H. Al-Bayati ·
Kawkab Y. Saour · Mahdi F. Radi

Received: 14 August 2011 / Accepted: 23 August 2011 / Published online: 17 September 2011
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Abstract Coumarins are important and useful compounds with diverse pharmacological properties. New coumarin derivatives namely *N*-aminoquinoline-2-one **1**, 1-((4-hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)methyleneamino)quinolin-2(1H)-one **2** and 1,1'-(1E,1'E)-(1,4-phenylenebis(methan-1-yl-1-ylidene))bis(azan-1-yl-1-ylidene)diquinolin-2(1H)-one **3**, were synthesized and characterized by UV–Vis, FT-IR, and NMR spectra in addition of elemental analysis. The synthesized compounds (**2** and **3**) show considerable anticancer activity against HEp-2 cell line. Synthesized compounds (**2** and **3**) were tested against selected types of microbial organisms and showed significant activities. The free-radical scavenging activity of synthesized compounds (**2** and **3**) have been determined by their interaction with the stable free-radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) and all the compounds have shown encouraging antioxidant activities.

Keywords Anticancer · Antioxidant · Antimicrobial · HEp-2 cell line · Quinolone

A. A. Al-Amiery (✉)
Biotechnology Division, Applied Science Department, University of Technology,
Baghdad 10066, Iraq
e-mail: dr.ahmed@eng.ukm.my; dr.ahmed1975@gmail.com

A. A. Al-Amiery
Department of Chemical and Processing Engineering, Faculty of Engineering and Built
Environment, Universiti of Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia

R. I. H. Al-Bayati · M. F. Radi
Department of Chemistry, College of Sciences, Al-Mustansiriyah University, Baghdad, Iraq

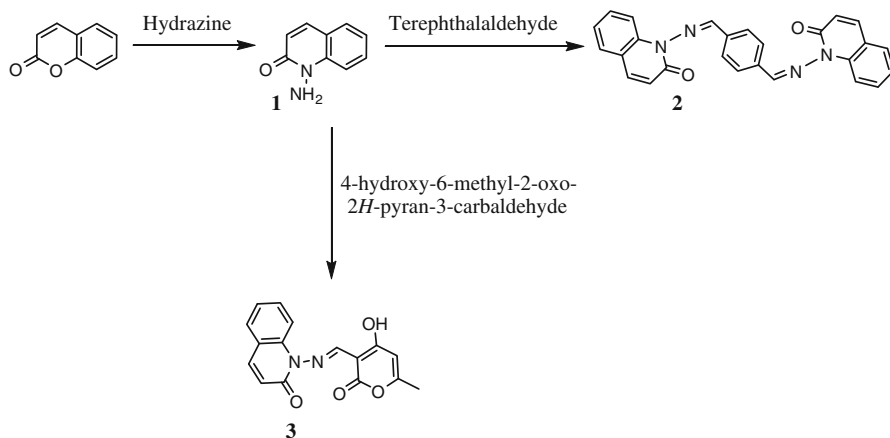
K. Y. Saour
Department of Pharmacology, College of Pharmacy, Baghdad University, Baghdad, Iraq

Introduction

Drug resistance has become a growing problem in the treatment of infectious diseases caused by bacteria and fungi [1]. The serious medical problem of bacterial and fungal resistance and the rate at which it develops has led to increasing levels of resistance to classical antibiotics [2–5]. The discovery and development of effective antibacterial and antifungal drugs with the novel mechanism of action have become urgent tasks for infectious disease research programs [6]. Coumarin (2H-L-benzopyran-2-one) and its derivatives possess a wide range of various biological and pharmaceutical activities. They have a wide range of applications as antitumor [7, 8], anti-HIV [9, 10], anticoagulant [11, 12], antimicrobial [13, 14], antioxidant [15, 16], and anti-inflammatory [17, 18] agents. The antitumor activities of a variety of coumarin compounds have been extensively examined [19, 20]. In addition, coumarins have been shown to inhibit *N*-methyl-*N*-nitrosourea, aflatoxin B1, and 7,12-dimethylbenz(a)anthracene-induced mammary carcinogenesis in rats [21, 22]. Quinolones are a family of synthetic broad-spectrum antibiotics. The term quinolone(s) refers to potent synthetic chemotherapeutic antibacterials [23, 24]. In the current study, we aimed to synthesize new quinolones derived from coumarin, with predictable biological activities. The chemical structures of the synthesized compounds were proved by UV-Vis, IR, NMR spectra, and elemental analysis data.

Results and discussion

The quinolone derivatives are important as biologically active compounds and synthons in organic synthesis due to the introduction of several drug-receptor-binding models, which enable systematic and rational design of novel inhibitor of various enzymes. For the synthesis of new coumarin derivatives, the reaction sequence outlined in Scheme 1, started from coumarin that is commercially available or, alternatively, readily accessible through Pechmann and Perkin condensation.



Scheme 1 Synthesis of compounds 1, 2, and 3

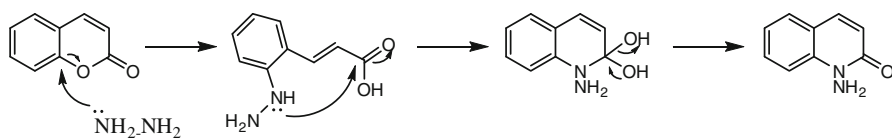
N-amino quinoline-2-one **1** was obtained by heating a mixture of coumarin with large excess of hydrazine hydrate in boiling absolute ethanol for 12 h with yield 91%. The structure of compound **1** was confirmed from its spectral data. In the IR spectrum of compound **1**, the lactam carbonyl stretching frequency was observed at $1,645\text{ cm}^{-1}$, and appearance of N–H amine stretching frequency at $3,290$ and $3,300\text{ cm}^{-1}$ that did not appear in the IR chart of starting material coumarin in addition of $3,045\text{ cm}^{-1}$ (C–H, aromatic) and $1,595, 1,452\text{ cm}^{-1}$ for double bond (C=C). In the $^1\text{H-NMR}$ spectrum of compound **1**, a singlet was observed at 4.11 ppm due to NH_2 protons, triplet 6.70 ppm, doublet 7.10 ppm, and doublet 7.40 ppm due to aromatic hydrogen protons. The C–H (alkene) of coumarin appeared at 5.91 ppm as doublet peaks. The $^{13}\text{C-NMR}$ spectrum analysis for compound **1**, combined with the information from $^1\text{H-NMR}$ experiments, can be considered enough to guide future synthetic work.

The nucleophilic reaction of coumarin with hydrazine hydrate may proceed through the ring opening of the pyrano ring, Scheme 2.

The data obtained for minimizing geometry for compound **1** show that C (8) and C (10) have positive charge in the molecule make the nucleophile ($-\text{NH}_2$) attack these carbons. Then there are two possible of ($-\text{NH}$) attacks, Scheme 3.

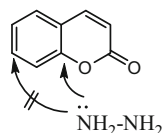
The determined bond angle, twists angle, and 3D geometrical structure (Fig. 1) for compound **1** indicate that this molecule is planar.

Reaction of compound **1** with 4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-carbaldehyde (or terphthaldehyde) in boiling absolute ethanol afforded 1-[(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)methylidene]amino}quinolin-2(1*H*)-one **2** (or 1,1'-(1*Z*,1'*E*)-(1,4-phenylenebis(methan-1-yl-1-ylidene))bis(azan-1-yl-1-ylidene)diquinolin-2(1*H*)-one **3**). In the IR spectrum of compound **2**, the lactam carbonyl stretching frequency was observed at $1,655\text{ cm}^{-1}$, whereas the lactone carbonyl stretching appeared at $1,734\text{ cm}^{-1}$, and appearance of hydroxyl group as broad band at $2,993\text{--}3,161\text{ cm}^{-1}$, the imine band was observed at $1,604\text{ cm}^{-1}$. In the $^1\text{H-NMR}$ spectrum, the C–H (alkene) of coumarin appeared at 6.45 ppm. The protons of the aromatic ring were observed as m (7.6 ppm for aromatic hydrogen) and singlet, 2.82 ppm for methyl group. The $^{13}\text{C-NMR}$ spectrum analysis for compound **2**, combined with the information from $^1\text{H-NMR}$ experiments, can be considered



Scheme 2 Suggested mechanism for synthesis of compound **1**

Scheme 3 Suggested mechanism for nucleophile attack of compound **1**



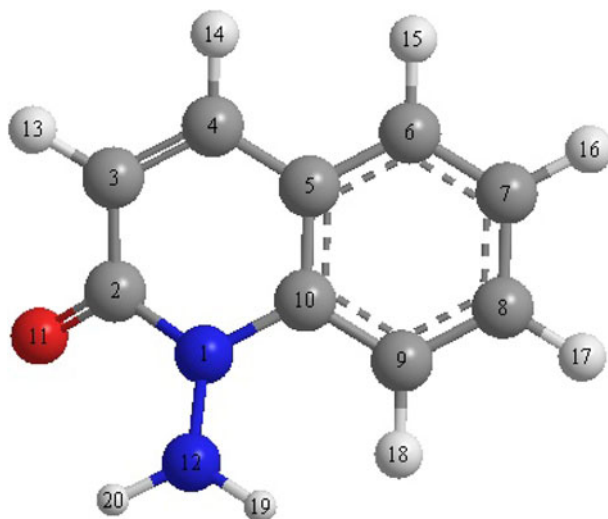


Fig. 1 The 3D structure of compound **1**

enough to prove the structure of compound **2**. The determined bond angle, twists angle, and 3D geometrical structure (Fig. 2) for compound **2** indicate that this molecule is non-planar, and the stereochemistry, C(2)–C(3): (E); C(4)–C(5): (Z); C(7)–N(22): (E); C(13)–C(14): (Z).

For compound **3**, the lactam carbonyl stretching frequency was observed at $1,662\text{ cm}^{-1}$ and the imine group at $1,620\text{ cm}^{-1}$, the $^1\text{H-NMR}$ spectrum, the C–H (alkene) of coumarin appeared at 6.20 and 6.6 ppm as doublet. The protons of the aromatic ring were observed as m at 7.1 ppm for aromatic hydrogen, doublet at 7.39 and 7.7 ppm doublet for proton in imine group. The $^{13}\text{C-NMR}$ spectrum analysis for compound **3**, combined with the information from $^1\text{H-NMR}$ experiments, can be considered enough to prove the structure of compound **3**. The determined bond angle, twists angle, and 3D geometrical structure (Fig. 3) for compound **3** indicate that this molecule is non-planar, and the stereochemistry, C(3)–C(4): (Z); N(12)–C(26): (E); C(15)–C(16): (Z); N(24)–C(25): (E).

Pharmacology

Antimicrobial activities

From the observations of Figs. 4 and 5, the higher inhibition of microbial growth is due to the donor atom (nitrogen atom), which enhances the activity by bonding it with the trace elements present in microorganisms, may combine with azomethine site and may inhibit the growth of microbial [25].

The mode of action of the compounds may involve formation of a hydrogen bond through the azomethine group ($>\text{C}=\text{N}-$) with the active centers of cell constituents [26] resulting in interferences with the normal cell process.

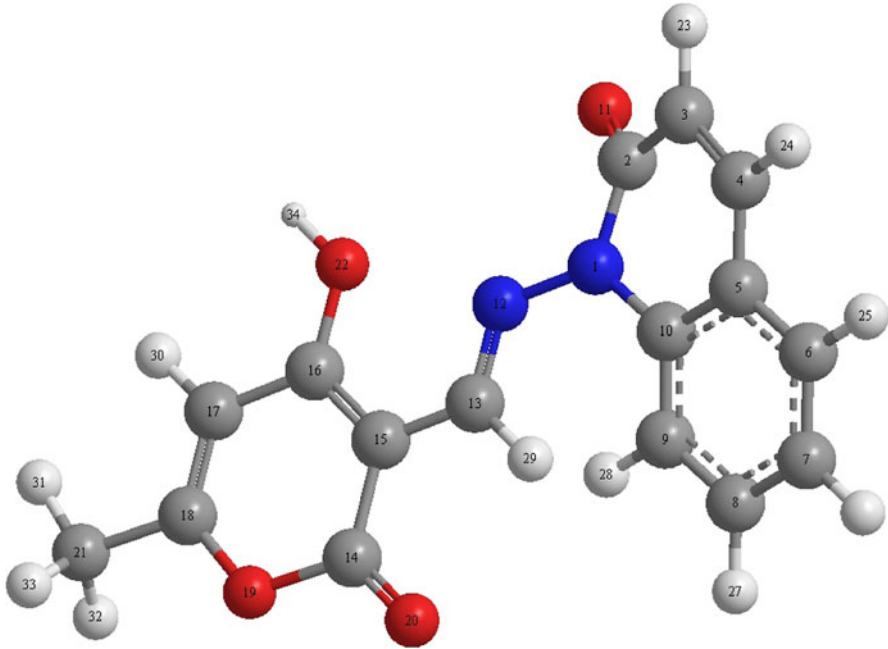


Fig. 2 The 3D structure of compound 2

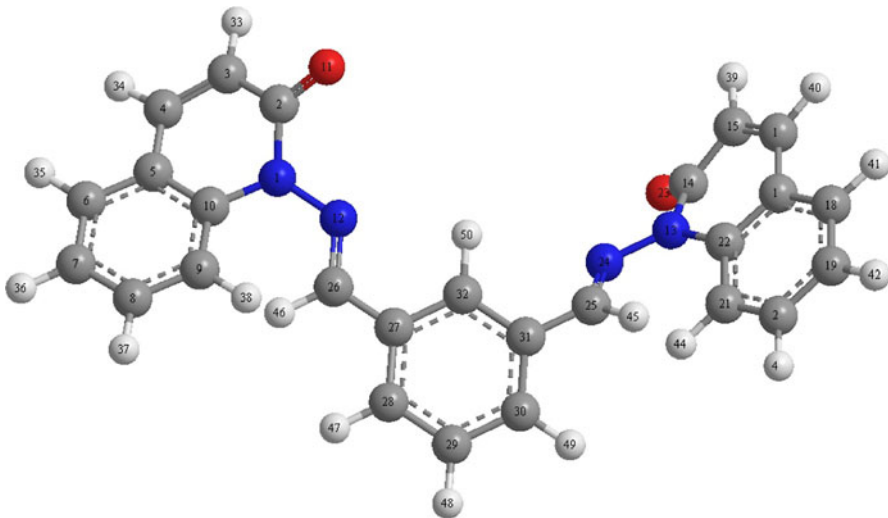


Fig. 3 The 3D structure of compound 3

Antioxidant activity

Many synthetic antioxidant components have shown toxic and/or mutagenic effects, and therefore attention has been given to naturally occurring antioxidants.

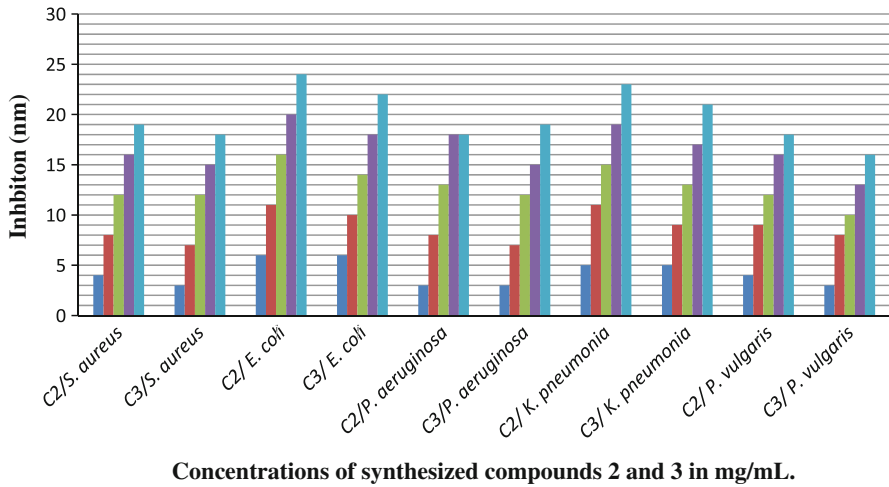


Fig. 4 Effect of synthesized compound 2 and 3 toward bacteria

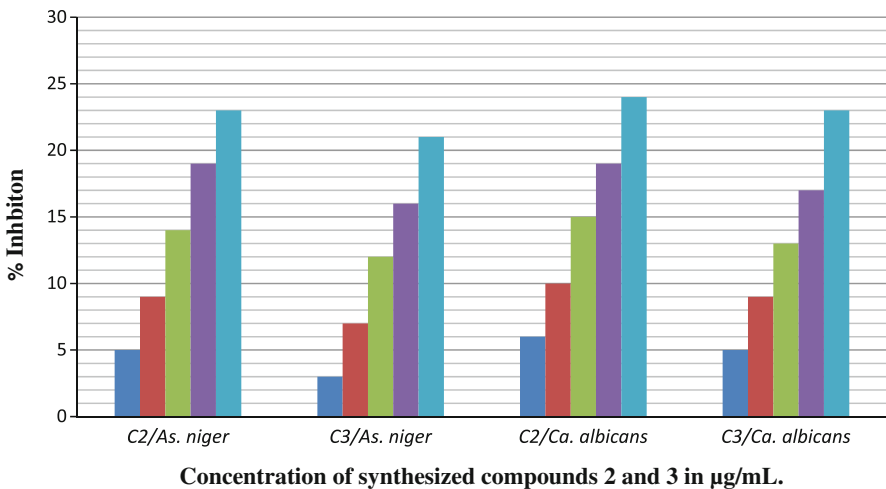


Fig. 5 Effect of synthesized compound 2 and 3 toward *A. niger* and *C. albicans*

Compound 2 and 3 were screened for in vitro antioxidant activity using (1,1-diphenyl-2-picrylhydrazyl) DPPH, scavenging method and they show good antioxidant activity (Fig. 6). The hydrogen-donating activity, measured using DPPH radicals as hydrogen acceptor, showed that significant association could be found between the concentration of novel molecules and the percentage of inhibition. Compounds 2 and 3 were able to reduce the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine. The method was based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H in the reaction [27].

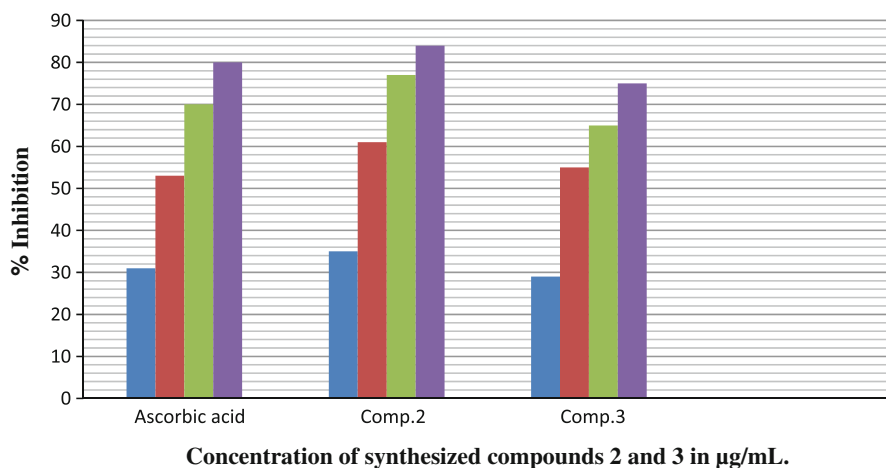
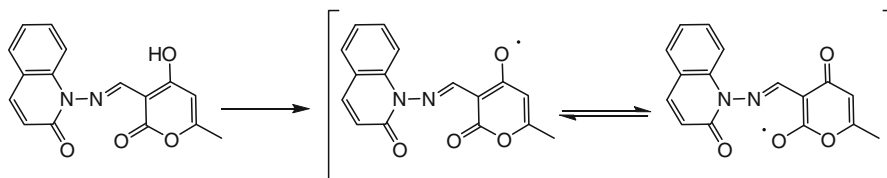


Fig. 6 Effect of compound **2** and **3** toward DPPH



Scheme 4 Suggested mechanism for compound **2** as antioxidant

Table 1 The significant cytotoxic effect for the synthesized compounds **2** and **3**

Treatments	125 µg O.D.	250 µg O.D.	500 µg	Control	IC% 125 µg	IC% 250 µg	IC% 500 µg
Comp. 2	0.325	0.141	0.172	0.515	36.89	72.62	66.60
Comp. 3	0.126	0.148	0.233	0.515	75.53	71.26	54.76

There is a postulated mechanism for the reaction of compound **2** as antioxidant, as shown in Scheme 4. The mechanism depends on the hydrogen of the hydroxyl allylic group, where this atom was under the influence of two effects, namely resonance and inductive. The resonance effect of hydrogen of allylic alcohol group makes the release of hydrogen as a free radical easy while the inductive effect and resonance on lactone ring, oxygen, and nitrogen pushes the electrons toward the carbon free radical, resulting in the molecule becoming stable.

Effect of compounds **2** and **3** on the HEP-2 cell line

Cancer cell line (HEP-2) (cancer of larynx) was treated with the synthesized compounds **2** and **3** and the results showed a significant cytotoxic effect starting from 125 to 500 µg/mL (see Table 1).

Conclusions

In this study, the compounds **2** and **3** were successively synthesized and characterized using various spectroscopic methods and elemental analysis technique. The synthesized compounds were studied theoretically, and the atomic charges, heat of formation, and stereo chemistry were estimated, and it was found that compound **1** was planer while compounds **2** and **3** are not planer. A postulated mechanism has been proposed for the action of compound **2** as an antioxidant. The antioxidant activity of compounds **2** and **3** were initially tested and were shown to have improved properties, as compared to ascorbic acid. Out of these compounds, compound **2** indicated significant antimicrobial activities as compared to other synthesized compounds. In addition, compound **2** is also found to be a superior antioxidant compound as compared to ascorbic acid.

Experimental

All chemicals used in this work were of reagent grade (supplied by either Sigma-Aldrich or Fluka) and used without further purification. The FT-IR spectra were recorded in the (4,000–400) cm^{-1} range on potassium bromide disks using a Shimadzu FTIR 8300 spectrophotometer. The $^1\text{H-NMR}$ spectra were obtained on a Jeol jnm-ECP400 FT-NMR system. The UV-Visible spectra were measured in ethanol using a Shimadzu UV-VIS 160-A spectrophotometer in the range of 200–1,000 nm. Elemental microanalysis was carried out using a CHN elemental analyzer model 5500-Carlo Erba instrument. A Gallenkamp M.F.B.600.010 F melting point apparatus was used to measure the melting points of all the prepared compounds. The compounds were studied by using a theoretical method (Semi empirical AMI module in the CS ChemOffice molecular modeling package) by calculation of atomic charge, bond length, bond angle, and stereochemistry.

Synthesis of *N*-amino quinoline-2-one **1**

Coumarin (1.46 g, 0.01 mol) with excess hydrazine hydrate (99%) (3.2 g, 0.1 mol) in absolute ethanol (25 mL) was refluxed for 12 h and was then cooled and the formed solid was collected and recrystallized from chloroform, yield 91%, m.p. 131–133 °C; $^1\text{H-NMR}$ (CDCl_3 , in ppm): δ 4.110 (s, 2H, $-\text{NH}_2$), δ 5.91 (d, 1H) for $-\text{C}=\text{C}-\text{H}$), δ 6.7 (t, Ar-H), δ 7.4 (d, Ar-H) and δ 7.1 (d, Ar-H); $^{13}\text{C-NMR}$: 126, 127, 127.8, 128, 123.3, 128.5, 129, 155, and 157; IR: 3,290 and 3,300 cm^{-1} (N-H, amine), 3045 cm^{-1} (C-H, aromatic), 1,645 cm^{-1} (C=O, lacton) and 1,595, 1,452 cm^{-1} (C=C); Anal. Calcd. for $\text{C}_9\text{H}_8\text{N}_2\text{O}$: C 67.49%, H 5.03%, N 17.49%. Found: C 66.97%, H 5.83%, N 17.12%.

Synthesis of Schiff bases **2** and **3**

A mixture of compound **1** (0.8 g, 0.005 mol) and 4-hydroxy-6-methyl-2-oxo-2H-pyran-3-carbaldehyde (0.005 mol) or terephthalaldehyde (0.0025 mol) was refluxed

in absolute ethanol (25 mL) for 8 h. The reaction mixture was cooled, filtered, and then recrystallized.

1-((4-Hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)methyleneamino)quinolin-2(1H)-one **2**

Recrystallized from ethanol, yield 84%, m.p. 80–82 °C; $^1\text{H-NMR}$ (CDCl_3): δ 2.82 (s, 3H), δ 6.45 (d, for $-\text{C}=\text{C}-\text{H}$), δ 7.6 (s, 1H) for aromatic ring); $^{13}\text{C-NMR}$: 17, 86, 99, 107, 113, 115, 119, 121, 124, 129, 131, 136, 152, 154, 160, 170; IR: $1,604\text{ cm}^{-1}$ ($\text{C}=\text{N}$), $1,655\text{ cm}^{-1}$ ($\text{C}=\text{O}$, lactam), $1,734\text{ cm}^{-1}$ ($\text{C}=\text{O}$, lactone); λ_{max} , 311 nm at ϵ 1.2 and 354 nm at ϵ 0.8; Anal. Calcd. for $\text{C}_{16}\text{H}_{12}\text{N}_2\text{O}_4$: C 64.86%, H 4.08%, N 9.46%. Found: C 64.25%, H 3.75%, N 9.02%.

1,1'-(1E,1'E)-(1,4-phenylenebis(methan-1-yl-1-ylidene))bis(azan-1-yl-1-ylidene)diquinolin-2(1H)-one **3**

Recrystallized from ethanol, yield 84%, m.p. 280 °C (dec); $^1\text{H-NMR}$ (CDCl_3): δ 6.20 and 6.6 (d, for $-\text{C}=\text{C}-\text{H}$), δ 7.1 (m, 1H) for aromatic ring), δ 7.39 (d, 1H) for aromatic ring), δ 7.70 (d, 1H for $\text{H}-\text{C}=\text{N}$); $^{13}\text{C-NMR}$: 125, 126, 127, 129, 130, 132, 133, 134, 134.5, 144, 145, 159, 162; IR: $1,620\text{ cm}^{-1}$ ($\text{C}=\text{N}$), $1,662\text{ cm}^{-1}$ ($\text{C}=\text{O}$, lactam); λ_{max} , 333 nm at ϵ 2.2 and 227 nm at ϵ 1.9; Anal. Calcd. for $\text{C}_{26}\text{H}_{18}\text{N}_4\text{O}_2$: C 74.63%, H 4.34%, N 13.39%. Found: C 73.90%, H 4.10%, N 12.82%.

Pharmacology

Evaluation of antibacterial activities

The in vitro antibacterial effects of compounds **2** and **3** were evaluated against a species of Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Proteus vulgaris*) by the disc diffusion method [28] using nutrient agar medium. The bacteria were sub-cultured in the agar medium and were incubated for 24 h at 37 °C. The discs (sterile filter paper discs, Whatmans No. 1.0), having a diameter of 5 mm, were then soaked in the test solutions with the appropriate equivalent amount of compounds **2** and **3** dissolved in sterile dimethylsulphoxide (DMSO) at concentrations of (0.1–1.0 mg/disc) and were placed on an appropriate medium previously seeded with organisms in Petri dishes and stored in an incubator for the above-mentioned period of time. The inhibition zone around each disc was measured and the results were recorded in the form of inhibition zones (diameter, mm). To clarify any effect of DMSO on the biological screening, separate studies were carried out using DMSO as a control and it showed no activity against any bacterial strains.

Evaluation of antifungal assay

Antifungal activity was carried out according to Daw et al. [29], based on the measured of the inhibitions of linear growth mycelia of different mold strains (*Aspergillus niger* and *Candida albicans*) in potato dextrose broth medium (PDB). Under aseptic conditions, 1 mL of spore suspension (5×10^6 cfu/mL) of tested fungi was added to 50 mL PDB medium in a 100-mL Erlenmeyer flask. Appropriate volumes of tested compounds **2** and **3** were added to produce concentrations ranging from 10 to 100 $\mu\text{g/mL}$. Flasks were incubated at 27 ± 1 °C in the dark for 5 days and then the mycelium was collected on filter paper. The filter papers were dried to constant weight and the level of inhibition, relative to the control flasks, was calculated from the following formula:

$$\text{Percentage of inhibition} = \frac{C - T}{C} \times 100$$

where T is the weight of mycelium from test flasks and C is the weight of mycelium from control flasks.

Evaluation of antioxidant activity

The stock solution (1 mg/mL) was diluted to final concentrations of 20–100 $\mu\text{g/mL}$. Ethanolic DPPH solution (1 mL, 0.3 mmol) was added to sample solutions in DMSO (3 mL) at different concentrations (50–300 $\mu\text{g/mL}$) [30]. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. The absorbance was then measured at 517 nm in a UV-Vis spectrophotometer. The lower absorbance of the reaction mixture indicates higher free-radical scavenging activity. Ethanol was used as the solvent and ascorbic acid as the standard. The DPPH radical scavenger was calculated using the following equation:

$$\text{Scavenging effect (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the samples or standards. $A_0 - A_1$

Evaluation of cytotoxic activity

3(4,5-Dimethyl-thiazol-2-yl)2,5-diphenyl-tetrazolium bromide (MTT) assay was performed as described previously [31]. Briefly, cells were incubated at 37 °C in a humidified 5% CO₂/95% air mixture and treated with compound **2** and **3** at different concentrations for 72 h. Four hours before the end of the treatment time, 20 μl of 0.5% MTT in phosphate buffer saline (PBS) was added to each microwell. The cells were washed once before adding MTT. After 4 h of incubation at 37 °C, the supernatant was removed and replaced with 100 μl of DMSO. The optical density of each well sample was measured with a microplate spectrophotometer reader.

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