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Synthesis, antioxidant and toxicological study of novel pyrimido quinoline derivatives from 4-hydroxy-3-acyl quinolin-2-one

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ABSTRACT

A series of novel pyrimido and other fused quinoline derivatives like 4-methyl pyrimido [5,4-*c*]quinoline-2,5(1*H*,6*H*)-dione (**4a**), 4-methyl-2-thioxo-1,2-dihydropyrimido [5,4-*c*]quinoline-5(6*H*)-one (**4b**), 2-amino-4-methyl-1,2-dihydropyrimido [5,4-*c*]quinolin-5(6*H*)-one (**4c**), 3-methylisoxazolo [4,5-*c*]quinolin-4(5*H*)-one (**4d**), 3-methyl-1*H*-pyrazolo [4,3-*c*]quinoline-4(5*H*)-one (**5e**), 5-methyl-1*H*-[1,2,4] triazepino [6,5-*c*]-quinoline-2,6(3*H*,7*H*)-dione (**5f**), 5-methyl-2-thioxo-2,3-dihydro-1*H*-[1,2,4]triazepino [6,5-*c*]quinolin-6(7*H*)-one (**5g**) were synthesized regioselectively from 4-hydroxy-3-acyl quinolin-2-one **3**. They were screened for their in vitro antioxidant activities against radical scavenging capacity using DPPH$^{\cdot}$, Trolox equivalent antioxidant capacity (TEAC), total antioxidant activity by FRAP, superoxide radical ($O_2^{\cdot -}$) scavenging activity, metal chelating activity and nitric oxide scavenging activity. Among the compounds screened, **4c** and **5g** exhibited significant antioxidant activities.

Nitrogen containing heterocycles are indispensable structural units for medicinal chemists. Among the various heterocyclic compounds, quinolines[1,2] occur predominately in nature ascribable to their stability and ease of generation. They exhibit pronounced biological activities such as antioxidant,[3] antiproliferation,[4] antiinflammation,[5] anticancer activities,[6] etc. Our group initiated a research program on the synthesis of a series of novel quinoline containing heterocycles[7] employing VHRs (Vilsmeier-Haack reactions) and screened them for cytogenetic activities. In this connection, our recent findings have disclosed that hybrid heterocycles incorporating quinoline ring system displayed moderate to good antibacterial activities.[8] This prompted us to synthesize pyrimido,[9] triazepine,[10] isoxazole,[11] pyrazole[12,13] fused quinolines and facilitating for further transformation as well. Free radicals play an important role in the pathogenesis of many diseases necessitating for the continuing interest in the recognition and development of novel antioxidants that prevents radical induced damage. There is now increasing experimental and clinical evidence showing the involvement of oxidative stress-induced by reactive oxygen and nitrogen species in a variety of disorders including cancer,[14] atherosclerosis,[15] neuro degeneration and aging.[16] The production of free radicals and various reactive oxygen species [ROS] derived exogenously from oxidative injuries related to pollution, radiation[17] or food constituents or generated endogenously in the human body from the metabolic reactions[18,19] is responsible for oxidative damage to DNA, proteins and lipids. It is recognized to play a key role in the pathogenesis of many chronic diseases.[20] Several lines of evidence in vitro and in vivo show that antioxidants can be effective in preventing or suppressing such disorders.[21] The free radical scavenging activity of the synthesized quinoline derivatives arise either from phenolic hydroxyl groups or from the imine unit of the quinoline moiety. A reactive free radical can undergo electron transfer or abstract H atom from either of these two sites. Recently there are some reports published in the literature supporting the two possible sites of attack viz. NH or OH by the free radicals. But several reports have attributed it to the phenolic hydroxyl group[22] which is preferred over 'NH' group. A lot of work has been done to synthesize quinoline analogues[23] with the aim to increase activity and stability and possibly to get better insight into structure–activity relationships. In our laboratory molecular combinations of quinolines and other antioxidants have been designed and tested for their radical scavenging activities. Such a compound has been reported to display potent antioxidant properties. In the present work DPPH, super oxide ($O^{\cdot -}$), nitric oxide ($NO^{\cdot}$) were used to evaluate the radical scavenging activity of quinoline moieties belonging to different structural classes followed by the screening of their reducing ability (Ferric Reducing Antioxidant Power), metal chelating activity and total antioxidant activity. Such experimental parameters were determined to assess reactivity toward the above said evaluation and used together with calculated structural parameters and antioxidant activities to establish structure–activity relationships which would help understand the molecular mechanisms of antioxidant action.

In the present investigation the reaction of 4-hydroxy-3-acetyl quinolin-2-one[24] **3** with a series of nitrogen bases (urea, thiourea, guanidine nitrate, hydroxylamine hydrochloride) and a catalytic

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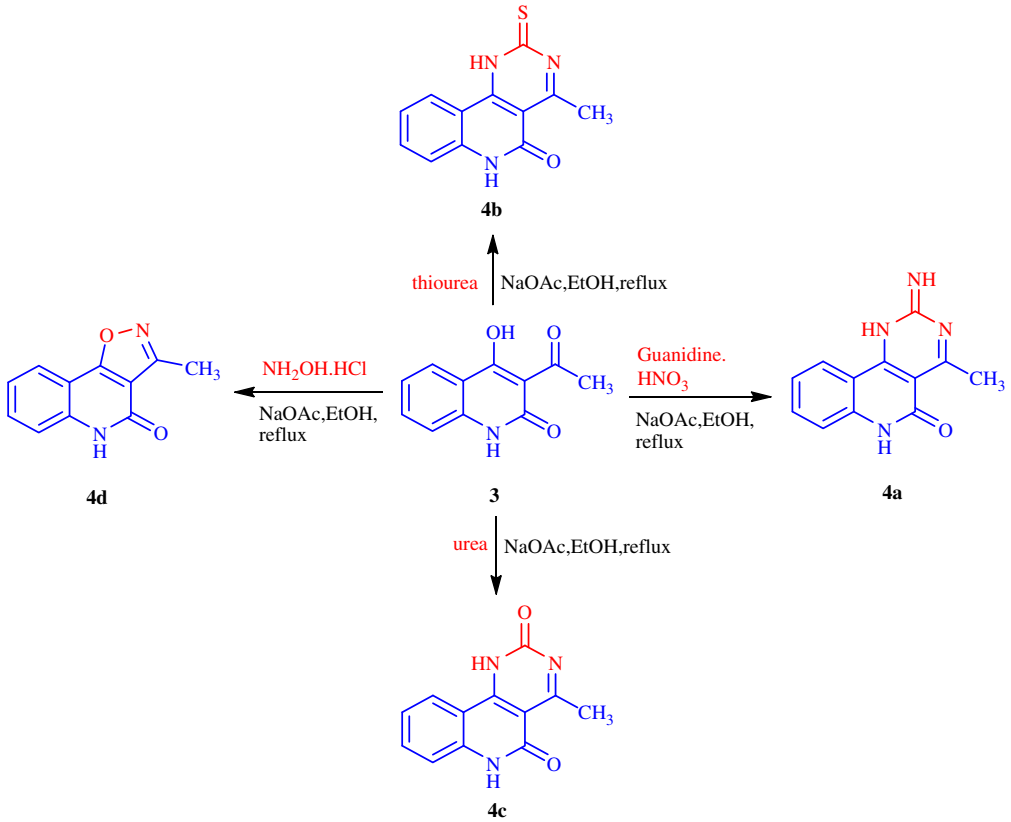
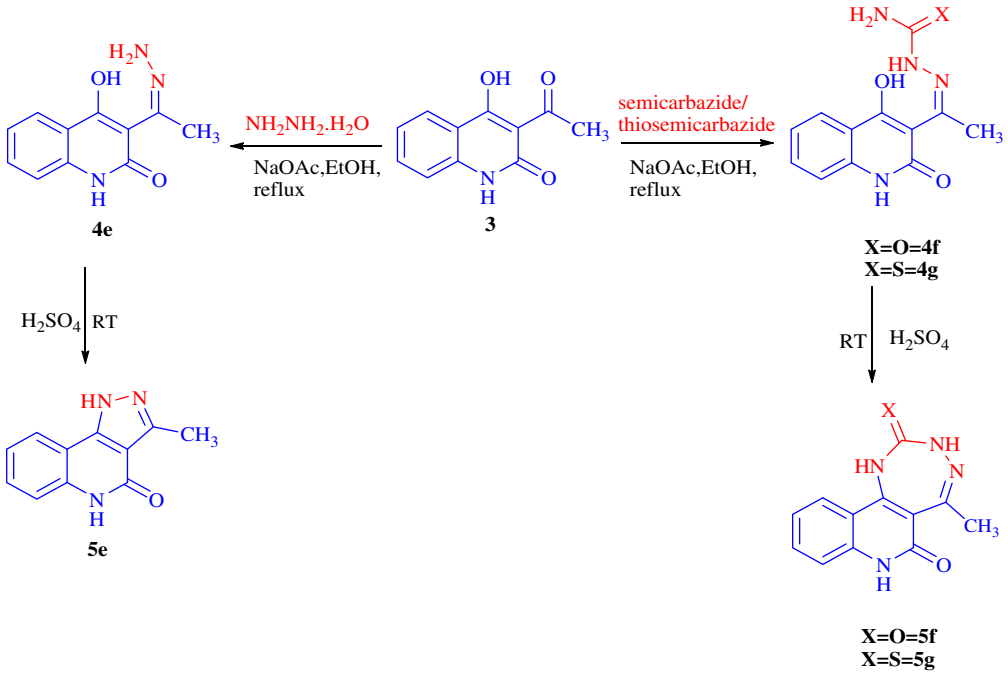
amount of sodium acetate in refluxing ethanol was carried out. After completion of the reaction as inferred by TLC the residue was subjected to column chromatography which afforded the product **4a–d** (Scheme 1). The appearance of a singlet peak from the NH proton at δ 8.5 ppm at the expense of an OH proton which appeared around nearly δ 17 ppm confirmed the cyclization of the compound. Although compound **3** possesses two C=O functionalities, the initial addition of nitrogen nucleophiles proceeds regioselectively at the side chain C=O function. This was supported by its IR spectrum where carbonyl stretching frequency appeared around 1670 cm^{-1}. Therefore it is pertinent to note that the condensation occurred at the carbonyl carbon of the acetyl functionality leads to the formation of the C=N bond which proved the formation of the product **4c**. This was factual for all other compounds of the series (**4a, 4b, 4d**, etc.). Similarly compound **5g** was not accomplished directly by the condensation of 4-hydroxy-3-acyl quinoline-2-one **3** with thiosemicarbazide and as uncyclized product **4g** was formed (Scheme 2) as evidenced by its NMR spectrum. A two proton singlet (NH_2 proton) which appeared at δ 6.3 ppm confirmed the structure of the compound **4g**. The product **4g** was cyclized with concentrated H_2SO_4 to afford **5g** (similarly the other compounds of the series **5e** and **5f** were achieved by the same route). For all compounds we exclusively obtained the regioisomer based on the mechanisms described either by Sweet and Fissekis[25] or Kappe.[26] All the compounds were obtained in moderate to good yields ranging from 75% to 92% and by recrystallization with ethanol. Scheme 2 reports cyclization for the formation of compounds **5e–g** from **4e–g** with quantitative yields ranging from 75% to 82%.

Free radical scavenging is one of the best known mechanisms by which antioxidant inhibit lipid peroxidation. Antioxidant studies viz. DPPH, superoxide and nitric oxide radical scavenging activity evaluation are standard assays and offer rapid techniques for screening the radical scavenging activity (RSA) of specific compounds. The RSAs of compounds **4a–5g** were estimated against these six methods. It depicts the inhibitory effect of the quinoline moieties on various free radicals. The inhibitory effect of the compound is marked and overall suppression ratio is in the order **5g** > **4c** > **5f**. The antioxidant experiments testify that these compounds exhibit very good antioxidant activities which were much better than that of the standards used. The plot of % $DPPH^{\cdot}$ scavenging capacity against a range of concentrations for each antioxidant gave the IC_{50} values. The IC_{50} values of synthesized molecules were tested for the $DPPH^{\cdot}$ radical scavenging capacities and are shown in Figure 1. As is clear from the figure, the scavenging effect decreased in the order **4d** > **5f** > **5g** > **4b** > **5e** > **4a** > **4c**. However the samples did not show scavenging capacities as effective as the standards BHA and BHT. Compound **4d** exhibited highest scavenging capacity with IC_{50} value 465.5 μg/ml concentration. The reason for the higher radical scavenging capacity of the compound **4d** can be explained by looking into the mechanism of radical scavenging by quinolines. It has been reported that the presence of methyl group adjacent to an atom that can stabilize an unpaired electron in the quinoline moiety in general boosts the antioxidant capacity of the molecule.[27] The results from the above experiments thus confirm that both H-donating ability and the antioxidant activity are more pronounced in quinoline systems and in addition to that the presence of isoxazole ring system seems to increase the DPPH radical scavenging activity of the compound. As can be seen in the Table 1, all the compounds showed reasonable activity against ABTS assay with the antioxidant capacity ranging from 1228.5 to 4012.9 μmol Trolox/g. In this experiment as well, compound **4d** possessed the highest antioxidant activity (4012.9 μmol Trolox/g) although not as active as BHA (8052.7 μmol Trolox/g).

In superoxide radical scavenging assay compounds **5g** and **4a** showed higher scavenging ability and at comparable level to the standard BHA, BHT as shown in Figure 2. Thus the superoxide



Scheme 1. Synthesis of compounds **4a–d**.



Scheme 2. Synthesis of compounds **4e–g** and **5e–g**.

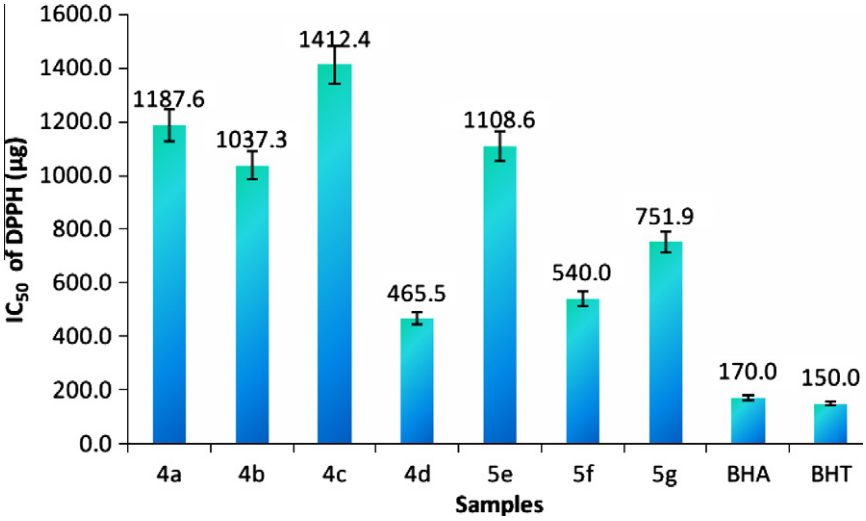


Figure 1. Effects of BHA, BHT and the synthetic compounds against DPPH radical.

Table 1
The ferric reducing/antioxidant power (FRAP), total antioxidant activity (TAA) and metal chelating activity of BHA, BHT and synthetic compounds

Sample	FRAP (mmolFe (II)/g)	TEAC (µmol Trolox/g)	Metal chelating activity (mg EDTA/g)
4a	156.3 ± 4.1[d,*]	2486.7 ± 148.5[d]	1.2 ± 0.3[e]
4b	144.9 ± 56.8[d]	3631.5 ± 460.6[c]	9.6 ± 2.5[c,d]
4c	205.2 ± 14.9[b]	3879.2 ± 110.7[d]	10.7 ± 0.3[b]
4d	168.6 ± 5.2[c]	4012.9 ± 181.5[c]	4.9 ± 0.5[b,c]
5e	149.6 ± 23.1[d]	1228.5 ± 543.5[e]	10.4 ± 0.8[d]
5f	355.3 ± 14.8[d]	2587.5 ± 789.5[d]	12.3 ± 1.0[b,c]
5g	147.7 ± 7.7[d]	2646.0 ± 766.6[d]	9.1 ± 1.0[b,c]
BHA	748.7 ± 15.5[a]	8052.7 ± 739.1[b]	16.1 ± 1.2[a]
BHT	134.2 ± 16.2[c]	13106.7 ± 229.9[a]	13.2 ± 2.3[b]

[*] Mean ± SD means with same letter were not significant different (p <0.01).
[a–e] Indicates significant differences at a significance level of (p <0.01).

radical scavenging property decreases as **5g** > **4a** > **5f** > **4c**. The results clearly pointed out that the 'S' atom present in the triazepine moiety seems to induce the activity of the compound **5g** and it act as a good radical scavengers. Therefore it is assumed that in addition to the quinoline skeleton, the presence of triazepine ring might have considerable role in enhancing the activities to certain extent. The nitric oxide scavenging capacity helps to arrest the chain of reactions initiated by excess generation of $NO^{\cdot}$ that are detrimental to human health. Nitric oxide is also implicated for inflammation, cancer and other pathological conditions. It is clear from Figure 3 that the $NO^{\cdot}$ scavenging effects was compared with that of BHA and BHT. Compounds **4a, 5f** and **5e** showed very low $NO^{\cdot}$ scavenging effect and scavenged only 12%, 16.1% and 18.5% as against 62.3% **4c**, 61.6% **5g** and 47.7% **4a** of $NO^{\cdot}$, respectively. In this assay compound **4c** showed highest activity which might be due to the presence of 'oxygen' functionality and two nitrogens.

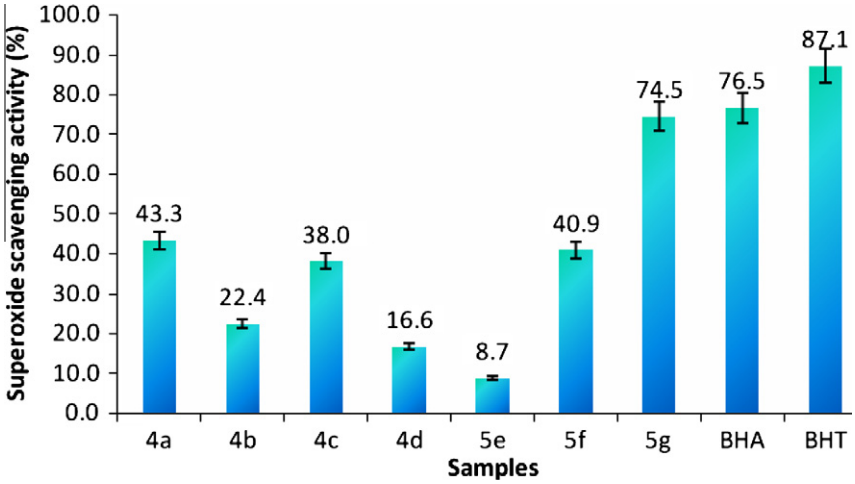


Figure 2. Effects of BHA, BHT and the synthetic compounds against super oxide radical.

Overall the nitric oxide scavenging capacity decreases in the order **4c** > **5g** > **4a** > **4d**. Thus we have shown that the quinoline moieties **4c** and **5g** possessed considerable nitric oxide scavenging antioxidant capacity and its efficiency as an anti oxidative agent will be confirmed using various assays in future.

FRAP assay is often used to measure the reduction of ferric ion Fe^{3+} to ferrous ion Fe^{2+} in the presence of antioxidants. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.[28] From the value (Table 1) of FRAP experiments it is clear that compound **5f** showed higher ferric reducing power than compounds **4c**, **4d**, and **4a**. The reducing power of the compound **5f** was much higher than that of the standard BHT. The reducing power of **4c** was also relatively higher than BHT. The remaining compounds **4d, 4a**, and **5g** also showed good to moderate activity than that of the standard antioxidants used. From the values it is assumed that in this assay as well the triazepine ring (comprising more no. of nitrogen atoms) of the quinoline moiety might play substantial role in suppressing the radicals. Metal chelating capacity was significant since it reduces the concentration of the catalyzing transition metal in lipid peroxidation (thus delaying metal-catalyzed oxidation).[29] Since ferrous ions constitute the most effective pro-oxidants in food and biological systems, the good chelating effect would be beneficial and removal of free iron from circulation and it is a corrective approach to prevent oxidative stress-induced disorder. As seen in Table 1. Compounds **5f** (12.3 mg EDTA/g), **4c** (10.4 mg EDTA/g), 5e (10.7 mg EDTA/g) showed comparable activity with BHA (13.2 mg EDTA/g) and slightly lesser than that of BHT (16.1 mg EDTA/g). Other compounds exhibited lower to moderate metal chelating activity. It was reported that the compounds with structures containing two or more functional groups such as –OH, –SH, –COOH, –N, –S–, –O– can show metal chelating activity (Yuan et al.[11]). The strongest activity of quinoline **5f** is believed to be due to the ferrous ion chelating effects by 'oxygen' containing moiety. As a result, from structural point of view **5f, 4c** and **5e** demonstrate a marked capacity for iron binding, suggesting their role as hydroxyl radical protector.

The toxicological study was carried out according to the OECD guidelines 423. Female Wistar rats (180–220 g weight) were taken for the study and kept for overnight fasting. Three animals were taken for each group. A day after, body weight was taken and test compounds **4c** and **5g** were administered orally at a dose of 2000 mg/kg in carboxy methyl cellulose. The mortality and morbidity of the animals were observed after 0, ½, 1, 2, 4, 6, 8, 12, and 24 h. Morbidity like convulsions, tremors, grip strength and pupil dilatation were observed. Feed (artificial pellets) was given to the animals after 4 h of the dosing and the body weight was checked at 6 h after dosing. The experiment was replicated. The animals were observed twice daily for 14 days and body weight

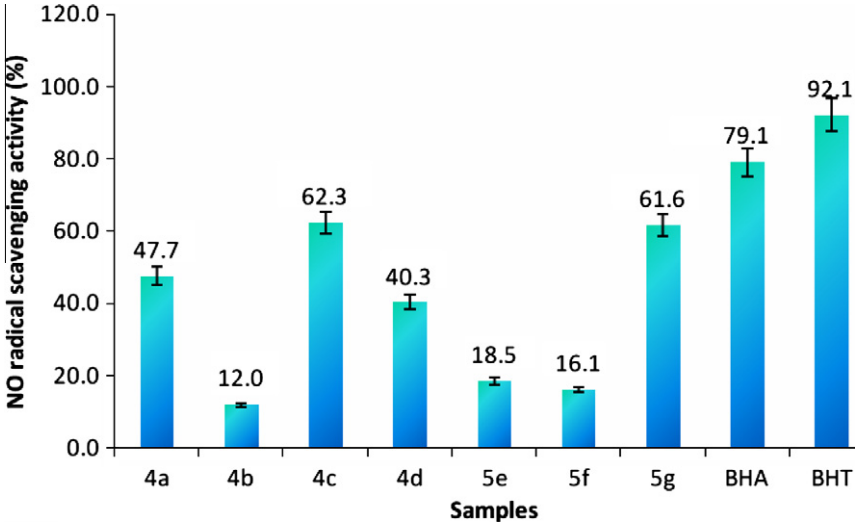


Figure 3. Effects of BHA, BHT and the synthetic compounds against nitric oxide radical.

was taken. Acute toxicity was carried out and none of the rats showed observable signs of toxicity upon single administration of test substance I and II (2 g/kg, po) on day one. The study was repeated with another set of animals for 14 days and no signs of toxicity were observed.

The present investigation describes the synthesis of various new pyrimido, triazepine and other fused quinoline derivatives prepared through conventional approach in near quantitative yield from 4-hydroxy-3-acytyl quinoline-2-one **3**. The antioxidant power of these compounds were screened against six methods. Among them compounds **5g** and **4c** exhibited moderate to exceptional activities. The simplicity of these tests offers an easy method to characterize radical scavengers acting on various quinoline moieties and the toxicity of compounds **4c** and **5g** of the sequence was tested for female Wistar rats. The tolerated dose was 2000 mg/kg dilution in carboxy methyl cellulose for animals weighing 20–25 g, where lethal doses were given. There were no characteristic lesions in any organ.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.018.

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