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Synthesis of novel bis(pyrimido[5,4-*c*]quinoline-2,4(1*H*,3*H*)-dione) and its derivatives: Evaluation of their antioxidant properties

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Antioxidant activity

ABSTRACT

One pot cyclocondensation reaction of barbituric/thiobarbituric acid with aromatic aldehydes and *p*-phenylenediamine/2,6-diaminopyridine by refluxing in glacial acetic acid afforded novel bis(pyrimido[5,4-*c*]quinoline-2,4(1*H*,3*H*)-diones)/pyrido bis(pyrimido[5,4-*c*]quinoline-2,4(1*H*,3*H*)-diones. All the synthesized compounds were screened for their antioxidant activities using FRAP and DPPH methods. Compounds with chloro substituents showed relatively good antioxidant properties.

Heterocyclic ring systems remain part of many powerful scaffolds holding several pharmacopores that can act as potent and selective drugs for many diseases.[1,2] Pyrimido[4,5-*b*]quinoline-2,4(1*H*,3*H*,5*H*,10*H*)-dione is one of the important class of heterocyclic moiety that possesses biological properties such as antitumor, anticancer, antihypertensive and antibacterial.[3] It has also been reported to have potential inhibition property against Kaposi's sarcoma-associated herpesvirus (KSHV).[4] Bond et al. studied the inhibitory effect of pyrimido[4,5-*b*]quinoline against topoisomerase and their associated diseases and disorders.[5]

In the last few decades, construction of highly functionalized pyrimido[4,5-*b*]quinoline-2,4(1*H*,3*H*,5*H*,10*H*)-diones have attracted both synthetic and medicinal chemists. Dow et al. have reported the synthesis of 7,8-dimethoxy-5,10-dihydropyrimido[4,5-*b*]quinolin-4(1*H*)-one compounds that act as selective inhibitor of the tyrosine-specific kinase enzyme.[6] Microwave-assisted synthesis of pyrimido[5,4-*b*][4,7]phenanthroline-9,11(7*H*,8*H*,10*H*,12*H*)-dione derivatives, which are obtained by the incorporation of pyrimido-[4,5-*b*]quinoline-2,4(1*H*,3*H*,5*H*,10*H*)-dione and [4,7]-phenanthroline motifs via three-component reactions has been reported by Shi et al.[7] Bazgir et al. have also reported a one-pot synthesis of pyrazolo[4′,3′:5,6]pyrido[2,3-*d*]pyrimidine-dione derivatives.[8] Recently, Aknin et al. have performed one-pot three component reaction involving barbituric acid, aldehydes and anilines to afford functionalized pyrimido-[4,5-*b*]quinoline-2,4(1*H*,3*H*,5*H*,10*H*)-dione as one of the products.[9] Majority of the diseases such as ischemia, cataract, atherosclerosis, inflammation, ageing, carcinogenesis and even AIDS are proved to be associated with oxidative stress arising from the imbalance between the formation and detoxification of free radicals and reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, and hydroxyl radical formed as by-products of a variety of pathways of aerobic metabolism.[10–17] As they are unstable and highly reactive, they can interact with a wide range of biological substrates such as, lipids, DNA and proteins resulting in cell damage.[18–20] Naturally occurring antioxidants such as vitamin C, vitamin E, selenium, β-carotene, lycopene, lutein and other carotenoids could fight with such species and suppress the concentration of free radicals. Apart from these, there are number of literature reports revealing the efficiency of polyphenolic compounds and thiols in the defense against free radicals.[21] Recently, heterocyclic compounds such as quinolines, pyrazoles and isoxazoles, 1,4-thiazepine, Mannich bases and piperamides have been screened for their antioxidant properties.[22]

In our effort of designing novel heterocyclic compounds with potential antioxidant properties, herein we describe a simple and efficient synthesis of some novel bis(dihydropyrimido[5,4-*c*]quinoline-2,4-dione) derivatives (**4**) by one pot multicomponent reaction of barbituric acid/thiobarbituric acid with aromatic aldehydes and *p*-phenylenediamine by refluxing in glacial acetic acid (Scheme 1).

To find the suitable reaction medium for this cyclocondensation, initially we have carried out the one pot cyclocondensation

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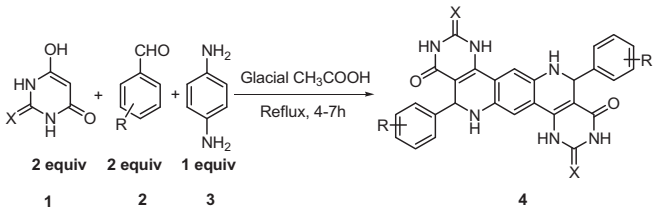


Scheme 1. Synthesis of bis(dihydropyrimido[5,4-*c*]quinoline-2,4-dione).

of barbituric acid, *p*-chlorobenzaldehyde and *p*-phenylenediamine as a model reaction in polar protic solvents such as methanol, ethanol and isopropanol in the presence of L-proline as catalyst under reflux condition.

This cyclocondensation reaction in methanol, ethanol and isopropanol yielded **5** as the only product where, **4c** was not formed in 5 h. The lesser reactivity of barbituric acid might be attributed to the heterogeneity of the reaction mixture. To improve the reactivity and increase the homogeneity, we have carried out the same reaction in high polar protic solvents such as ethyeneglycol and diethyleneglycol at 120 °C in the presence of 30% L-proline where, we observed moderate yields of **4c** (60–65%), (Table 1, entries 4, 5), which may be due to the high polar nature of the solvents that might hold the product by hydrogen bonding. Refluxing in glacial CH_3COOH in the absence of L-proline yielded exclusively **4c** in 5h with 85% yield (Table 1, entry 6).

Though, we got **5** as the product while refluxing in alcoholic solvents for 5 h, it was possible to obtain the Mannich base **6** when the reaction time was extended to 10 h (Table 1, entries 1, 2, 3). ^{1}H NMR spectrum of compound **6** showed two doublets in the region of δ 4.2-4.3 and 4.72–4.73 ppm that correspond to –CH–CH–NH– group, which indicated the formation of C–C bond. Amide protons in barbituric acid moiety appeared as two singlets at δ 11.09 and 11.12 ppm.

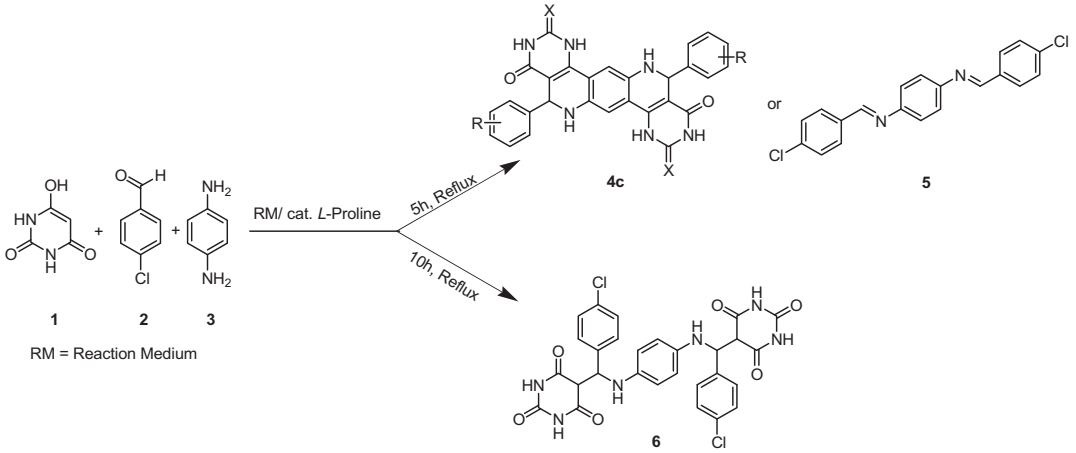
When we tried to recrystalize the Mannich base **6** for further purification in glacial acetic acid, unexpectedly a small amount of compound **4c** was obtained as product, which was confirmed by ^{1}H NMR spectrum, in which, the two singlets appeared at δ 5.01 and 8.75 ppm, corresponding to the methine proton (–CH–) and the cyclic amine proton (–NH). Impressed by this result, compound **6** was refluxed in glacial acid for 7 h expecting the formation of **4c** and its regioisomer **RI** (Scheme 2).

^{1}H and ^{13}C NMR spectra of compound obtained from one pot synthesis and that from acid treatment of Mannich base **6** were found to be similar. Moreover, to elucidate the structure of the compounds obtained by these two routes, the 2D-HMBC and HSQC were recorded from which we observed that, six correlations were found for both compounds instead of nine correlations expected for the regioisomer **RI** (Scheme 3) thereby confirming the structure of both the compounds are identical and **4c** is the formed product.

Perhaps, the formation of **4c** would have taken place via protonation of **6** in acidic medium. This protonated form underwent intermolecular cyclization to afford exclusively compound **4c** as product with 82% yield (Scheme 4).

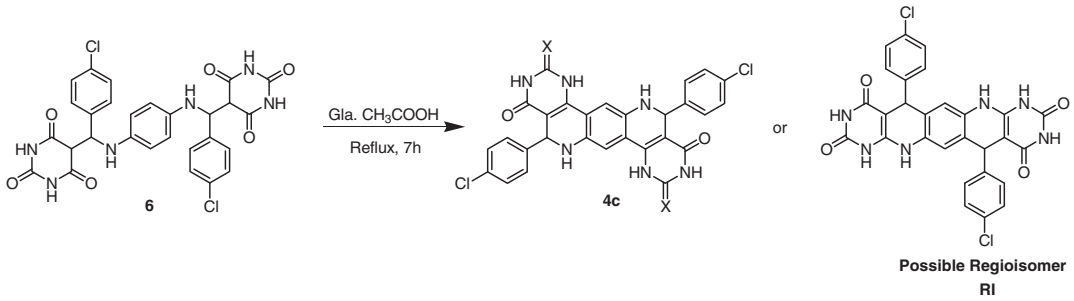
The cylcocondensation reaction involving aldehydes with electron withdrawing group in *para*-position afforded the corresponding products (Table 2, entries 6, 14) in lesser time with better yields. Similarly, chloro substituent in *para*-position also led to lesser reaction time with better yields. In the cases of aldehydes with electron donating groups such as 4-hydroxy and 4-methoxybenzaldehydes, products were not at all formed (Table 2, entries 7, 8, 15, 16). Aromatic aldehydes with either electron releasing groups or electron withdrawing groups in *ortho*-position needed longer reaction times compared to that in *meta*- or *para*-positions which may be due to steric hindrance (Table 2).

Table 1
Cyclocondensation reaction of barbituric acid, *p*-chlorobenzaldehyde and *p*-phenylenediamine in various reaction media

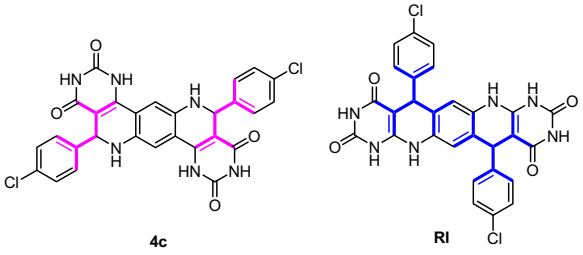


Entry	Reaction medium[a]	Temperature (°C)	Reaction time (h)	Product	Yield[c] (%)
1	Methanol	Reflux	5 (10)	5 (6)	92 (72)
2	Ethanol	Reflux	5 (10)	5 (6)	95 (81)
3	Isopropanol	Reflux	5 (10)	5 (6)	96 (84)
4	Ethyleneglycol	120	5	4c	65
5	Diethyleneglycol	120	5	4c	60
6	Acetic acid[b]	Reflux	5	4c	85

[a] Cyclocondensation reaction of barbituric acid (2 mmol), aromatic aldehydes (2 mmol) and *p*-phenylenediamine (1 mmol) in the presence of 30% L-proline in various reaction media.
[b] Reflux in absence of L-proline.
[c] Isolated yield. Products and yields in parentheses correspond to reaction times given in parentheses.



Scheme 2. Acid treatment of Mannich base **6**.



Scheme 3. Possible HMBC correlation for compound **4c** and **RI**.

The ^{1}H NMR spectrum of each compound in the series **4a**–**f** and **4i**–**n** showed a singlet at δ 5.01–5.26, which revealed the methine proton (–CH) and a singlet at δ 8.72–8.91 ppm due to cyclic amine proton (–NH). The two singlets appeared at δ 10.22–10.50 ppm and 11.44–11.62 ppm, could be accounted for cyclic amide protons (–NH). Aromatic protons appeared as multiplet at δ 6.80–8.43 ppm. The mass spectra of compounds are in agreement with their assigned structures. All the spectra exhibit parent peaks due to molecular ions (M+). The mass spectrum of **4c** showed molecular ion peak (M+) at 574 in accordance with its molecular formula.

Next, we have planned to synthesize another similar heterocylic derivative **8**, where, *o*-phenylenediamine **7** was utilized in the place of *p*-phenylenediamine in the above cyclocondensation. In this case, an unexpected product namely, 4-chlorobenzyl barbituric acid **9** was obtained with 73% yield instead of bis(dihydropyrimido[5,4-*c*]quinoline-2,4-dione) compound **8** (Scheme 5).

The formation of **9** instead of **8** may be attributed to the 1,4-nucleophilic addition of **7** at the –C=C– bond of intermediate **C**, which might have transformed into **9** after hydride transfer (Schemes 5 and 6). The 1,2-nuclephilic addition of **7** might not be possible due to the other amino group present in the next position. The characterization of remaining products is under progress.

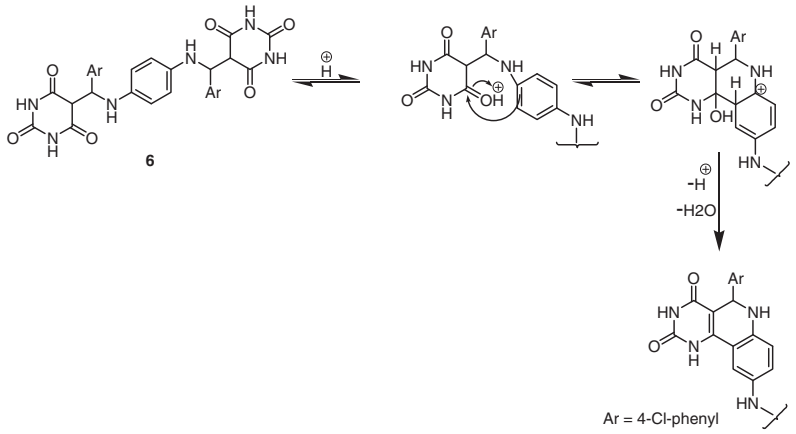
Table 2
One pot synthesis of bis(dihydropyrimido[5,4-*c*]quinoline-2,4-dione) derivatives[23]

Entry	X	R	Product[a]	Time (h)	Yield[b] (%)
1	O	H	4a	6.0	80
2	O	2-Cl	4b	6.5	75
3	O	4-Cl	4c	5.0	85
4	O	2-NO_2	4d	6.0	78
5	O	3-NO_2	4e	5.0	81
6	O	4-NO_2	4f	4.0	87
7	O	4-OH	4g	7.0	NR[c]
8	O	4-OMe	4h	7.0	NR[c]
9	S	H	4i	6.0	78
10	S	2-Cl	4j	6.5	72
11	S	4-Cl	4k	5.0	83
12	S	2-NO_2	4l	6.0	75
13	S	3-NO_2	4m	5.0	82
14	S	4-NO_2	4n	4.0	86
15	S	4-OH	4o	7.0	NR[c]
16	S	4-OMe	4p	7.0	NR[c]

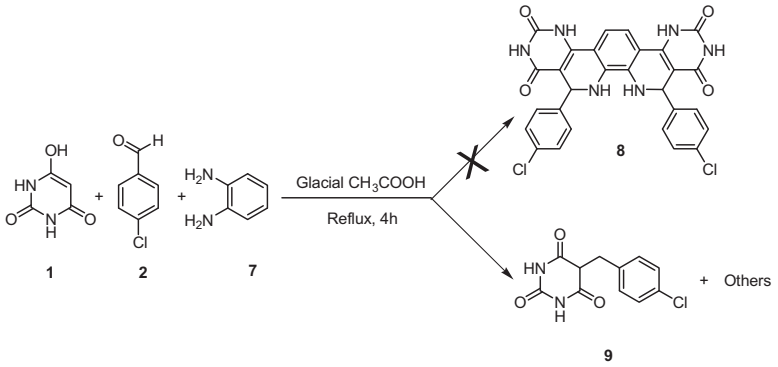
[a] Cyclocondensation reaction of barbituric/thiobarbituric acid (2 mmol), aromatic aldehydes (2 mmol) and *p*-phenylenediamine (1 mmol) refluxing in glacial CH_3COOH.
[b] Isolated yield.
[c] No reaction.

In the ^{1}H NMR spectrum, one doublet appeared at δ 3.24–3.25 ppm for methylene protons (–CH_2) and one triplet at δ 3.95–3.97 ppm for methine proton (–CH), confirming the hydride transfer that has occurred at –C=C– in intermediate **C**. Peak for cyclic amide proton at δ 11.21 ppm also supported the formation of compound **9**.

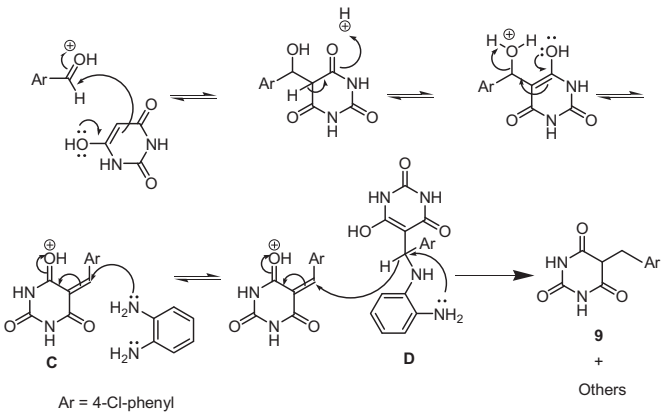
Furthermore, we explored the same reaction conditions for the synthesis of pyrido bis(dihydropyrimido[5,4-*c*]quinoline-2,4-



Scheme 4. Proposed mechanism for the formation of **4c** from **6**.



Scheme 5. One pot reaction of barbituric acid, *p*-chlorobenzaldehyde and *o*-phenylenediamine.



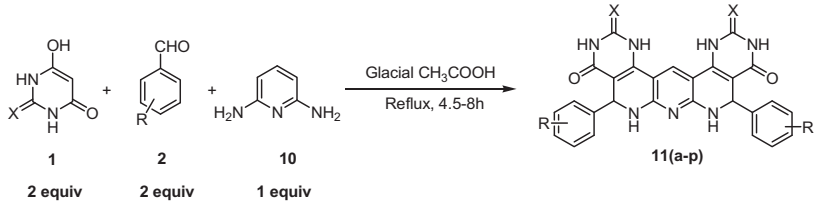
Scheme 6. Proposed mechanism for the formation of **9**.

dione) derivatives using barbituric acid/thiobarbituric acid with aromatic aldehydes and 2,6-diaminopyridine **10** as amine part by refluxing in glacial CH_3COOH,[24] where the corresponding products were obtained in good yields (Table 3).

The ^1H NMR spectrum of each compound in the series **11a–f** and **11i–n** showed a singlet at δ 5.01–5.31 which revealed methine proton (–CH) and a singlet at δ 8.97–9.12 ppm due to cyclic amine proton (–NH). The two singlets appeared at δ 10.51–10.72 ppm and 11.52–11.71 ppm, could be accounted for cyclic amide protons (–NH). Aromatic protons appeared as multiplet at δ 6.94–7.95 ppm. The mass spectrum of **11a** showed the molecular ion peak (M+) at 505, which matches with its molecular formula. The mass spectra of other compounds are in agreement with their assigned structures.

In the present study, antioxidant potential of synthesized bis(pyrimido[5,4-*c*]quinoline-2,4(1*H*,3*H*)-dione compounds (**4a–f**,

Table 3
One pot reaction of barbituric/thiobarbituric acid, aromatic aldehydes and 2,6-diaminopyridine[a]



X = O or S
R = H, 2-Cl, 4-Cl, 2-NO_2, 3-NO_2, 4-NO_2, OH, OMe

Entry	X	R	Product	Time (h)	Yield (%)[b]
1	O	H	11a	6.0	76
2	O	2-Cl	11b	7.0	70
3	O	4-Cl	11c	5.0	83
4	O	2-NO_2	11d	6.5	74
5	O	3-NO_2	11e	6.0	77
6	O	4-NO_2	11f	4.5	85
7	O	4-OH	11g	8.0	NR[c]
8	O	4-OMe	11h	7.0	NR[c]
9	S	H	11i	6.0	74
10	S	2-Cl	11j	7.0	71
11	S	4-Cl	11k	5.0	81
12	S	2-NO_2	11l	6.5	75
13	S	3-NO_2	11m	6.0	78
14	S	4-NO_2	11n	4.5	84
15	S	4-OH	11o	8.0	NR[c]
16	S	4-OMe	11p	8.0	NR[c]

[a] Cyclocondensation reaction barbituric/thiobarbituric acid (2 mmol), aromatic aldehydes (2 mmol) and 2,6-diaminopyridine (1 mmol) by refluxing in glacial CH_3COOH.
[b] Isolated yield.
[c] No reaction.

Table 4
DPPH radical scavenging activity and ferric ion reducing antioxidant power of compounds **4a–f** and **4i–n**

Compounds	DPPH RSA[a] (20 μg/ml)		FRAP IC_{50} value (60 μg/ml)
	(%)	IC_{50} value (μg)	
4a	82.0 ± 2.62	12.15	32.76
4b	84.0 ± 0.82	12.05	24.01
4c	85.2 ± 1.04	11.80	23.21
4d	82.7 ± 1.05	12.07	29.08
4e	81.7 ± 1.30	12.39	33.28
4f	80.9 ± 1.12	12.44	25.01
4i	79.6 ± 1.00	12.62	32.10
4j	84.1 ± 1.08	11.99	24.46
4k	84.4 ± 1.20	11.93	24.11
4l	82.0 ± 0.96	12.15	29.50
4m	79.3 ± 1.00	12.68	33.70
4n	82.3 ± 0.30	12.17	25.21
BHA	87.1 ± 0.89	11.55	20.76

[a] Antioxidant activities were expressed in percentage compared with standard BHA. The data represent mean value (SEM) of three duplicates.

Table 5
DPPH radical scavenging activity and Ferric ion reducing antioxidant power of compounds **11a–f** and **11i–n**

Compounds	DPPH RSA[a] (20 μg/ml)		FRAP IC_{50} value (60 μg/ml)
	(%)	IC_{50} value (μg)	
11a	70.6 ± 1.16	14.21	33.91
11b	80.8 ± 1.50	12.42	25.51
11c	81.1 ± 1.35	12.35	24.41
11d	74.0 ± 1.16	13.56	31.39
11e	70.2 ± 1.23	14.24	34.90
11f	74.2 ± 1.68	13.48	26.06
11i	70.4 ± 1.62	14.25	33.50
11j	80.6 ± 1.21	12.41	25.07
11k	80.9 ± 0.57	12.36	25.00
11l	73.6 ± 1.66	13.60	30.47
11m	70.1 ± 1.96	14.34	34.93
11n	74.0 ± 1.08	13.56	26.25
BHA	87.1 ± 0.89	11.55	20.76

[a] Antioxidant activities were expressed in percentage compared with standard BHA. The data represent mean value (SEM) of three duplicates.

4i–n and **11a–f**, **11i–n**) were studied using DPPH radical scavenging technique by spectrophotometrically according to Shimada et al.[25] and ferric reducing antioxidant power (FRAP) assay.[26]

Radical scavenging activities of all compounds were determined from the interacting ability of compounds with DPPH as stable free radical. Compound **4a**, that is not having any substituents on the phenyl ring, was selected as a model compound for evaluating its DPPH radical scavenging activity. Compound **4a** has been tried in five different concentrations such as 20, 40, 60, 80, 100 μg/mL and sufficient activity was observed in 20 μg/mL concentration. All other compounds in these series were screened for their antioxidant activity in 20 μg/mL concentration. The antioxidant activities were expressed as the percentage of inhibition and 50% inhibitory concentration values in Tables 4 and 5 and compared with that of standard BHA. The results in percentage are expressed as the ratio of absorbance decrease at 517 nm, and the absorbance of DPPH solution in the absence of bis(pyrimido[5,4-*c*]quinoline-2,4(1*H*,3*H*)-dione compounds.

The hydrogen donating ability of cyclic amide groups present as part of all synthesized compounds in the series **4a–p** and **11a–p** might be responsible for their antioxidant properties (Scheme 7).

To investigate the structure activity relationship in the series **4a–p** and **11a–p**, the radical scavenging activity of **4a** was studied and compared with **12** using DPPH radical. The radical scavenging activity of **4a** was found to be higher than **12** (Fig. 1), which may be attributed to the increase in the number of cyclic amide groups capable of donating hydrogen.

The FRAP assay is based on measuring the reducing ability of a compound that reduce the ferric ion to the colored ferrous ion complex. FRAP values are measured spectrophotometrically at 700 nm. To investigate the reducing ability of synthesized compounds, compound **4a** was selected as model compound and to fix optimum concentration required for the reducing ability, this

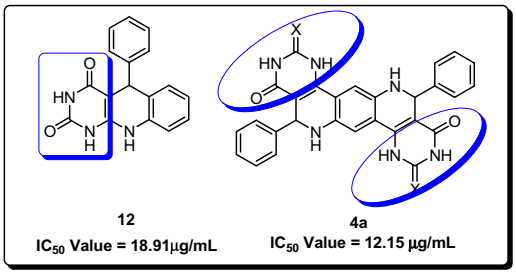
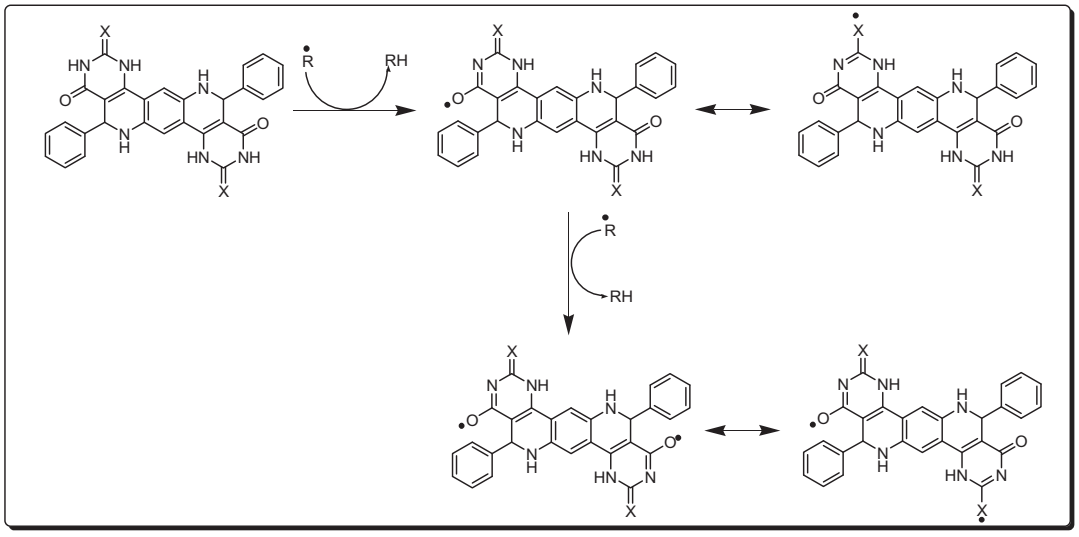


Fig. 1. Structures of compound **12** and **4a**.



Scheme 7. Plausible mechanism for all synthesized compounds interacting with DPPH radical.

compound has been tried in five different concentrations such as 20, 40, 60, 80, 100 μg/mL and sufficient activity was observed in 60 μg/mL concentration. All other compounds in these series were screened for their antioxidant activity in 60 μg/mL concentration. According to the data presented in (Tables 4 and 5), compounds with chloro-substituent on the phenyl ring such as **4b**, **4c**, **4j**, **4k**, **11b**, **11c**, **11j** and **11k** showed better antioxidant power in FRAP assay. Generally, all compounds exhibited comparable or more potent antioxidant potential in FRAP assay in comparison with synthetic antioxidant, BHA.

The analysis of Tables 4 and 5 leads to conclude that the radical scavenging activity of bis(pyrimido[5,4-*c*]quinoline-2,4(1*H*,3*H*)-dione on DPPH radical and ferric ion reducing power has been found to be maximum for all compounds. Especially, compounds with chloro-substituent on the phenyl ring (**4b**, **4c**, **4j**, **4k**, **11b**, **11c**, **11j** and **11k**) have enhanced and exhibited maximum radical scavenging activities (Tables 4 and 5).

In conclusion, bis(pyrimido[5,4-*c*]quinoline-2,4(1*H*,3*H*)-dione derivatives have been successfully synthesized by one pot cyclocondensation of barbituric/thiobarbituric acid, aromatic aldehydes and *p*-phenylenediamine/2,6-diaminopyridine by refluxing in glacial acetic acid. Radical scavenging abilities of all synthesized compounds were evaluated with the stable DPPH radical and FRAP methods and the chloro containing bis(pyrimido[5,4-*c*]quinoline-2,4(1*H*,3*H*)-dione derivatives have enhanced and shown maximum radical inhibitory effect and ferric ion reducing antioxidant power.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.04.068.

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23. *Procedure for the synthesis of Bis(dihydropyrimido[5,4-c]quinoline-2,4(1H,3H)-diones) (**4c**):* A mixture of *p*-phenylenediamine (0.925 mmol), *p*-chlorobenzaldehyde (1.85 mmol) and barbituric acid (1.85 mmol) was refluxed in glacial acetic acid until the completion of reaction as evidenced by TLC. After completion, the reaction mixture was cooled to room temperature. The yellow color solid separated was washed with hot ethanol and dried.
24. *Procedure for the synthesis of pyrido bis(dihydropyrimido[5,4-c]quinoline-2,4(1H,3H)-diones) (**11c**):* A mixture of 2,6-diaminopyridine (0.925 mmol), *p*-chlorobenzaldehyde (1.85 mmol) and barbituric acid (1.85 mmol) was refluxed in glacial acetic acid until the completion of reaction monitored by TLC. After completion, the reaction mixture was cooled to room temperature. The yellow color solid separated was washed with hot ethanol and dried.
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