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Original article

Synthesis and antioxidant activity evaluation of new hexahydropyrimido[5,4-*c*]quinoline-2,5-diones and 2-thioxohexahydropyrimido[5,4-*c*]quinoline-5-ones obtained by Biginelli reaction in two steps

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Abstract

New hexahydropyrimido[5,4-*c*]quinoline-2,5-diones and 2-thioxohexahydropyrimido[5,4-*c*]quinoline-5-ones were prepared in two steps from ethyl 4-phenyl-6-methyl-2-oxo tetrahydropyrimidine-5-carboxylates or 4-phenyl-6-methyl-2-thioxotetrahydropyrimidine-5-carboxylates, previously prepared by Biginelli reaction using appropriate aldehyde, urea derivatives and ethyl acetoacetate.

Their antioxidant properties were evaluated by two methods: scavenging effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and scavenging effect on hydroxyl radicals. The results show that the compounds containing thiourea moiety have better activity.

Keywords: Quinoline; Biginelli reaction; Antioxidants; DPPH; Hydroxyl radical

1. Introduction

Free radicals play an important role in the pathogenesis of many diseases, accounting for continuing interest in the identification and development of novel antioxidants that prevent radical-induced damage.

In humans, several pathologies involve the overproduction of reactive oxygen species (ROS): These species such as the superoxide radical anion ($O_2^{-\bullet}$) and hydrogen peroxide (H_2O_2), are formed in biological system by the partial reduction of molecular oxygen. Formation of the hydroxyl radical ($HO^{\bullet}$), another ROS, is thought to occur through the one-electron reduction of H_2O_2, a reaction that is facilitated by transition metals that are in a reduced valence state (e.g. reduced copper or iron). Additionally, there are a large number of other reactive species that are formed from the reaction of ROS with biological molecules [e.g. polyunsaturated lipids, thiols and nitric oxide (NO)]. For example, $O_2^{-\bullet}$ readily reacts with NO to form peroxynitrite anion ($ONOO^-$), which is unstable at physiological pH and rapidly decomposes to form potent nitrating and oxidizing species [1,2].

In addition a lot of heterocyclic compounds either natural (phytoestrogens) or obtained by synthesis, having coumarin or quinoline rings, were studied for their biological activities. They are used specially as radicals scavengers like quercetol

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or coumestrol [3,4] or the copper or iron chelating molecules such as clioquinol [5,6].

After first biological studies realized in our laboratory [7–9] on new compounds with quinoline and coumarin structures, we report the synthesis of new hexahydropyrimido[5,4-*c*]quinoline-2,5-diones and 2-thioxohexahydropyrimido[5,4-*c*]quinoline-5-one. Their antioxidant properties were evaluated by hydroxyl radical $^{\bullet}OH$ scavenging activity and by their reducing power 2,2-diphenyl-1-picrylhydrazyl radical (DPPH).

2. Results and discussion

2.1. Synthesis

Differently substituted 4-methyl 1,2,3,5,6,10*b*-hexahydropyrimido[5,4-*c*]quinoline-2,5-diones and 4-methyl-2-thioxo-1,2,3,5,6,10*b*-hexahydropyrimido[5,4-*c*]quinoline-5-ones were obtained easily in two steps. The first step (Scheme 1) consists of a Biginelli reaction [10,11] and the second step is the cyclisation in the presence of ammonia [12–14].

As shown in Scheme 1, the condensation of the derivatives of 2-chlorobenzaldehyde **1**, ethyl acetoacetate **2**, urea or thiourea and their derivatives **3** and boric acid according to Biginelli reaction gives ethyl 4-phenyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylates **4a–f** or 4-phenyl-6-methyl-2-thia-1,2,3,4-tetrahydropyrimidine-5-carboxylates **4g–j**. For all compounds prepared, we exclusively obtained the *N*1-substituted regioisomer 4-phenyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate or *N*1-substituted regioisomer 4-phenyl-6-methyl-2-thia-1,2,3,4-tetrahydropyrimidine-5-carboxylate according to the mechanisms described by Sweet and Fissekis [15] or described by Kappe [16]. The measurement around 3 ppm of H doublet coupling constant for the C4 of compounds **4a–j** and C10*b* of compounds **5a–j** confirmed this regiochemistry. All the compounds summarized in Table 1 were obtained in moderate to good yields ranging from 56% to 94%. All these products were isolated from reaction mixture by recrystallisation from ethanol, and their structures were characterized by ^{1}H NMR, IR spectra and elementary analysis.

Scheme 2 reports cyclisation procedure for the formation of **5a–j** from compounds **4a–j**. This cyclisation is realized with ammonia under pressure.

Products **5a–j** obtained with good yields from 65% to 90% are presented in the (Table 2).

All new products were characterized by ^{1}H NMR, IR spectra and elementary analysis. The ^{1}H NMR spectra of compounds **5a–j** show the disappearance of the triplet and quartet of ester group and appearance of additional NH signal.

2.2. Antioxidant activity studies

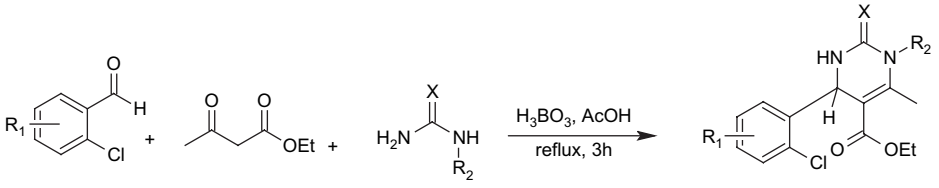
Free radical scavenging is one of the best known mechanisms by which antioxidants inhibit lipid oxidation. DPPH and hydroxyl radical scavenging activity evaluation are standard assays in antioxidant activity studies and offer rapid techniques for screening the radical scavenging activity (RSA) of specific compounds. The RSAs of hexahydropyrimido[5,4-*c*]quinoline-2,5-diones **5a–f** and 2-thioxohexahydropyrimido[5,4-*c*]-quinoline-5-ones **5g–i** were estimated using these two methods.

2.2.1. DPPH radical scavenging

A freshly prepared DPPH solution exhibits a deep purple colour with an absorption maximum at 517 nm. This purple colour generally disappears when an antioxidant is present in the medium as shown in the Scheme 3. Thus, antioxidant molecules can quench DPPH free radicals (by providing hydrogen atoms or by electron donation, conceivably via a free radical attack on the DPPH molecule) and convert them to colourless/bleached product [17,18].

The RSA DPPH values of methanolic solutions of pyrimido[5,4-*c*]quinoline-2,5-diones **5a–f** and 2-thioxopyrimido[5,4-*c*]quinoline-5-ones **5g–j** were examined and compared (Table 3). Results are expressed as a percentage of the ratio of the decrease in absorbance at 517 nm, to the absorbance of DPPH solutions in the absence of compounds **5a–j** at 517 nm.

From analysis of Table 3, we can conclude that the pyrimidoquinoline **5a–j** scavenging effects on DPPH radicals increase with the concentration and were suitable for 2-thioxopyrimido[5,4-*c*]quinoline-5-ones **5g–j** with a thiourea moiety ($\geq$25% at 250 μmol L^{-1}) comparable with RSA DPPH values for the standard ascorbic acid ($\geq$24.6% at 25 μmol L^{-1}). The RSA DPPH value decreased between 8 and 18% at 250 μmol L^{-1} for pyrimido[5,4-*c*]quinoline-2,5-diones **5a–f** with a urea moiety. The presence of a Cl group in 7 or 8 position seems to increase this activity (compounds **5d**, **5e** and **5h**).



Scheme 1.

Table 1

Compounds	R_1	R_2	X	Yields (%)
4a	H	CH_3	O	70
4b	H	C_2H_5	O	73
4c	6-F	C_2H_5	O	75
4d	3-Cl	CH_3	O	90
4e	3-Cl	C_2H_5	O	56
4f	6-Cl	CH_3	O	88
4g	H	CH_3	S	58
4h	4-Cl	CH_3	S	60
4i	6-F	CH_3	S	64
4j	6-Cl	CH_3	S	71

Table 2

Compounds	R_1	R_2	X	Yields (%)
5a	H	CH_3	O	70
5b	H	C_2H_5	O	73
5c	10-F	C_2H_5	O	80
5d	7-Cl	CH_3	O	81
5e	7-Cl	C_2H_5	O	73
5f	10-Cl	CH_3	O	90
5g	H	CH_3	S	82
5h	8-Cl	CH_3	S	65
5i	10-F	CH_3	S	72
5j	10-Cl	CH_3	S	88

2.2.2. $OH^{\bullet}$ radical scavenging

We have used the benzoic acid hydroxylation method [19]. The benzoic acid is hydroxylated by $OH^{\bullet}$ formed by Fenton reaction at C3 or C4 position of the aromatic ring and the fluorescence was measured at 407 nm emission with excitation at 305 nm. This fluorescence generally decreases when an antioxidant is present in the medium. Antioxidant molecules by providing hydrogen atom prevent the hydroxylation of benzoic acid.

The RSA $OH^{\bullet}$ result of molecules **5a–j** are summarized in (Table 4), the results are expressed as:

RSA $OH^{\bullet}$% = Absorbance in the presence of sample/absorbance in the absence of sample × 100 and compared to quercetol. We can conclude that the $OH^{\bullet}$ radical scavenging activity for all compounds increase with the concentration and were excellent for **5h** and **5j** with thiourea moiety (90.2% and 92.3% at 200 μmol L^{-1}) and comparable to quercetol at 100 μmol L^{-1} (93.5%).

3. Conclusion

Several new hexahydropyrimido[5,4-*c*]quinoline-2,5-diones and 2-thioxohexahydropyrimido [5,4-*c*]quinoline-5-ones were prepared by Biginelli reaction of ethyl 4-phenyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylates or 4-phenyl-6-methyl-2-thia-1,2,3,4-tetra hydropyrimidine-5-carboxylates, respectively, in moderate to good yields.

The antioxidant properties of these molecules were evaluated by two methods. The compounds with thiourea moiety seem to give good results.

4. Experimental

4.1. General

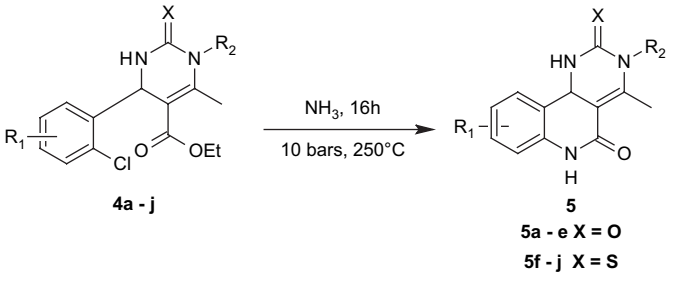
All compounds were characterized using the methods of elementary analyses, IR spectroscopy and NMR spectroscopy. Infrared spectra were recorded on a Shimadzu FTIR-8201 PC spectrometer in KBr (ν in cm^{-1}). Proton NMR spectra were recorded on a Bruker AC 300 spectrometer. Chemical shift values and IR data for all compounds are summarized in Section 4 and are in agreement with the proposed structures.

Melting points (mp) were obtained with a Kofler apparatus and were not corrected. All reactions were monitored with Thin Layer Chromatography (TLC) and carried out on Alugram Sil G/UV_{254} plate with appropriate solvents. Microanalyses were carried out by the service Central d'Analyses, Centre National de la Recherche Scientifique, Vernaison, France.

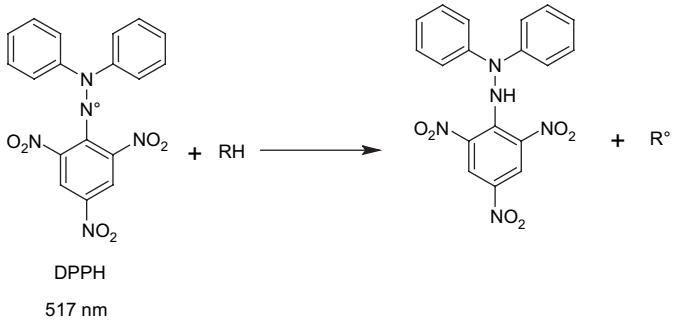
4.2. Synthesis

4.2.1. Ethyl 4-(2-chlorophenyl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (*4a*)

A solution of *o*-chlorobenzaldehyde (3 mmol), ethyl acetoacetate (3 mmol), urea derivatives (3.6 mmol) and H_3BO_3 (0.6 mmol), in glacial acetic acid (10 mL) is heated at 100 °C, while stirring for 2 h. Then it is cooled to rt, and poured into ice water (50 mL). The product collected by filtration was recrystallised from EtOH (95%), to give the pure product, **4a** in 70% yield, mp 215 °C, IR ν(NH) 2990, ν(C=O) ester 1708, ν(C=O) urea 1615. ^{1}H NMR (DMSO-d_6)



Scheme 2.



Scheme 3.

Table 3
Decreasing absorbance (%) DPPH of compounds **5a–j**

Compounds	Concentration (μmol L^{-1})				
	50	100	150	200	250
5a	2.5	6.6	9.1	9.8	12.0
5b	3.4	4.8	6.7	9.1	10.5
5c	3.0	4.6	6.0	8.7	9.8
5d	3.1	5.4	10.2	16.2	17.4
5e	1.9	2.3	4.3	8.1	11.8
5f	1.5	2.1	5.0	5.4	7.6
5g	4.0	8.2	14.1	17.9	26.2
5h	9.5	11.2	17.8	23.8	30.6
5i	3.3	8.6	12.4	17.4	25.4
5j	7.7	8.2	9.5	15.3	24.9

Ascorbic acid: 25 μmol L^{-1}, 24.6%; 50 μmol L^{-1}, 36.9%.

1.15 ppm (3H, t, CH_3 ester), 2.6 ppm (3H, s, CH_3), 3.2 ppm (3H, s, N−CH_3), 3.9 ppm (2H, q, CH_2 ester), 5.7 ppm (1H, d, $J = 2.9$ Hz, CH), 7.3−7.5 ppm (4H, m, aromH), 7.9 ppm (1H, s, NH). Anal. Calcd for $C_{15}H_{17}ClN_2O_3$: C, 58.35; H, 5.55; Cl, 11.48; N, 9.07. Found: C, 58.24; H, 5.59; Cl, 11.49; N, 9.12.

*4.2.2. Ethyl 4-(2-chlorophenyl)-1-ethyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**4b**)*

Compound **4b** was prepared with the same procedure as **4a** in 52% yield, mp 210 °C, IR ν(NH) 3300, ν(C=O) ester 1705, ν(C=O) urea 1670. ^{1}H NMR (DMSO-d_6) 1.1 ppm (3H, t, CH_3 ethyl), 1.2 ppm (3H, t, CH_3 ester), 2.6 ppm (3H, s, CH_3), 3.8 ppm (1H, m, CH ethyl), 3.9 ppm (1H, m, CH ethyl), 3.95 ppm (2H, q, CH_2 ester), 5.7 ppm (1H, d, $J = 2.9$ Hz, CH), 7.3−7.4 ppm (4H, m, aromH), 7.9 ppm (1H, s, NH). Anal. Calcd for $C_{16}H_{19}ClN_2O_3$: C, 59.54; H, 5.93; Cl, 10.98; N, 8.68. Found: C, 60.03; H, 5.87; Cl, 10.92; N, 8.60.

*4.2.3. Ethyl 4-(2-chloro-6-fluorophenyl)-1-ethyl-6-methyl-2-oxo-1,2,3,4-tetrahydro pyrimidine-5-carboxylate (**4c**)*

Compound **4c** was prepared with the same procedure as **4a** in 75% yield, mp 158 °C, IR ν(NH) 3342, ν(C=O) ester 1703, ν(C=O) urea 1670. ^{1}H NMR (DMSO-d_6) 1.0 ppm (3H, t, CH_3 ethyl), 1.2 ppm (3H, t, CH_3 ester), 2.6 ppm (3H, s, CH_3), 3.7 ppm (1H, m, CH ethyl), 3.8 ppm (1H, m, CH ethyl), 3.9 ppm (2H, q, CH_2 ester), 5.7 ppm (1H, d, $J = 3.1$ Hz, CH), 7.2−7.4 ppm (2H, m, aromH), 7.5 ppm (1H, m, aromH), 8 ppm (1H, s, NH). Anal. Calcd for $C_{16}H_{18}ClFN_2O_3$: C, 56.39; H, 5.32; Cl, 10.40; F, 5.58; N, 8.22. Found: C, 56.42; H, 5.27; Cl, 10.37; F, 5.52; N, 8.27.

Table 4
Decreasing fluorescence of **5a–j**

Compounds	Concentration (μmol L^{-1})				
	20	50	100	150	200
5a	58.4	71.8	80.8	83.7	89.7
5b	58.1	70.9	81.1	86.5	89.1
5c	12.5	58.4	72.4	80.2	83.1
5d	66.0	77.3	82.9	87.3	89.2
5e	63.8	74.1	80.6	84.4	86.7
5f	67.1	72.8	80.9	83.2	83.3
5g	56.3	69.4	77.2	82.3	88.2
5h	68.7	76.5	87.5	88.5	90.2
5i	70.6	78.8	80.2	81.5	83.3
5j	68.0	82.7	87.4	89.8	92.3

Quercetol: 50 μmol L^{-1}, 87.9%; 100 μmol L^{-1}, 92.3%.

*4.2.4. Ethyl 4-(2,3-dichlorophenyl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**4d**)*

Compound **4d** was prepared with the same procedure as **4a** in 90% yield, mp 118 °C, IR ν(NH) 3350, ν(C=O) ester 1672, ν(C=O) urea 1621. ^{1}H NMR (DMSO-d_6) 0.9 ppm (3H, t, CH_3 ester), 2.5 ppm (3H, s, CH_3), 3.1 ppm (3H, s, N−CH_3), 3.9 ppm (2H, q, CH_2 ester), 5.6 ppm (1H, d, $J = 3.2$ Hz, CH), 7.2−7.5 ppm (3H, m, aromH), 7.9 ppm (1H, s, NH). Anal. Calcd for $C_{15}H_{16}Cl_2N_2O_3$: C, 52.49; H, 4.70; Cl, 20.66; N, 8.16. Found: C, 52.37; H, 4.73; Cl, 20.69; N, 8.19.

*4.2.5. 4.2.5. Ethyl 4-(2,3-dichlorophenyl)-1-ethyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**4e**)*

Compound **4e** was prepared with the same procedure as **4a** in 56% yield, mp 116 °C, IR ν(NH) 3344, ν(C=O) ester 1678, ν(C=O) urea 1624. ^{1}H NMR (DMSO-d_6) 1.0 ppm (3H, t, CH_3 ethyl), 1.2 ppm (3H, t, CH_3 ester), 2.5 ppm (3H, s, CH_3), 3.7 ppm (1H, m, CH ethyl), 3.8 ppm (1H, m, CH ethyl), 3.9 ppm (2H, q, CH_2 ester), 5.6 ppm (1H, d, $J = 3.0$ Hz, CH), 7.3−7.4 ppm (2H, m, aromH), 7.6 ppm (1H, d, aromH), 7.9 ppm (1H, s, NH). Anal. Calcd for $C_{16}H_{18}Cl_2N_2O_3$: C, 53.79; H, 5.08; Cl, 19.85; N, 7.84. Found: C, 53.75; H, 5.12; Cl, 19.77; N, 7.88.

*4.2.6. Ethyl 4-(2,6-dichlorophenyl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**4f**)*

Compound **4f** was prepared with the same procedure as **4a** in 88% yield, mp 130 °C, IR ν(NH) 3322, ν(C=O) ester 1683, ν(C=O) urea 1630. ^{1}H NMR (DMSO-d_6) 0.9 ppm (3H, t, CH_3 ester), 2.4 ppm (3H, s, CH_3), 3.2 ppm (3H, s, N−CH_3), 3.9 ppm (2H, q, CH_2 ester), 6.2 ppm (1H, d, $J = 3.0$ Hz, CH), 7.2−7.6 ppm (3H, m, aromH), 7.8 ppm (1H, s, NH). Anal. Calcd for $C_{15}H_{16}Cl_2N_2O_3$: C, 52.49; H, 4.70; Cl, 20.66; N, 8.16. Found: C, 52.34; H, 4.74; Cl, 20.48; N, 8.22.

*4.2.7. Ethyl 4-(2-chlorophenyl)-1,6-dimethyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**4g**)*

Compound **4g** was prepared with the same procedure as **4a** in 58% yield, mp 116 °C, IR ν(NH) 3186, ν(C=O) ester 1705, ν(C=S) thiourea 1635. ^{1}H NMR (DMSO-d_6) 1.1 ppm (3H, t, CH_3 ester), 2.6 ppm (3H, s, CH_3), 3.6 ppm (3H, s, N−CH_3), 4 ppm (2H, q, CH_2 ester), 5.8 ppm (1H, d, $J = 2.9$ Hz, CH), 7.3−7.5 ppm (4H, m, aromH), 10 ppm (1H, s, NH). Anal. Calcd for $C_{15}H_{17}ClN_2O_2S$: C, 55.46; H, 5.28; Cl, 10.91; N, 8.62; S, 9.87. Found: C, 55.40; H, 5.31; Cl, 10.95; N, 8.54; S, 9.95.

*4.2.8. Ethyl 4-(2,4-dichlorophenyl)-1,6-dimethyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**4h**)*

Compound **4h** was prepared with the same procedure as **4a** in 60% yield, mp 125 °C, IR ν(NH) 3205, ν(C=O) ester 1708, ν(C=S) thiourea 1651. ^{1}H NMR (DMSO-d_6) 1.0 ppm

(3H, t, CH_3 ester), 2.6 ppm (3H, s, CH_3), 3.6 ppm (3H, s, N–CH_3), 3.9 ppm (2H, q, CH_2 ester), 5.6 ppm (1H, d, $J = 2.6$ Hz, CH), 7.2 ppm (1H, d, aromH), 7.4 ppm (1H, d, aromH), 7.6 ppm (1H, d, aromH), 9.8 ppm (1H, s, NH). Anal. Calcd for $C_{15}H_{16}Cl_2N_2O_2S$: C, 50.15; H, 4.49; Cl, 19.74; N, 7.80; S, 8.93. Found: C, 50.22; H, 4.41; Cl, 19.28; N, 7.91; S, 8.90.

4.2.9. Ethyl 4-(2-chloro-6-fluorophenyl)-1,6-dimethyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**4i**)

Compound **4i** was prepared with the same procedure as **4a** in 64% yield, mp 173 °C, IR ν(NH) 3193, ν(C=O) ester 1710, ν(C=S) thiourea 1643. ^{1}H NMR (DMSO-d_6) 0.9 ppm (3H, t, CH_3 ester), 2.5 ppm (3H, s, CH_3), 3.6 ppm (3H, s, N–CH_3), 3.9 ppm (2H, q, CH_2 ester), 5.8 ppm (1H, d, $J = 2.8$ Hz, CH), 7.1–7.5 ppm (3H, m, aromH), 9.7 ppm (1H, s, NH). Anal. Calcd for $C_{15}H_{16}ClFN_2O_2S$: C, 52.55; H, 4.70; Cl, 10.34; F, 5.54; N, 8.17; S, 9.35. Found: C, 52.70; H, 4.65; Cl, 10.31; F, 5.48; N, 8.05; S, 9.28.

4.2.10. Ethyl 4-(2,6-dichlorophenyl)-1,6-dimethyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**4j**)

Compound **4j** was prepared with the same procedure as **4a** in 70% yield, mp 166 °C, IR ν(NH) 3202, ν(C=O) ester 1702, ν(C=S) thiourea 1665. ^{1}H NMR (DMSO-d_6) 1.0 ppm (3H, t, CH_3 ester), 2.6 ppm (3H, s, CH_3), 3.5 ppm (3H, s, N–CH_3), 3.9 ppm (2H, q, CH_2 ester), 5.7 ppm (1H, d, $J = 2.9$ Hz, CH), 7.2–7.4 ppm (3H, m, aromH), 9.8 ppm (1H, s, NH). Anal. Calcd for $C_{15}H_{16}Cl_2N_2O_2S$: C, 50.15; H, 4.49; Cl, 19.74; N, 7.80; S, 8.93. Found: C, 50.20; H, 4.42; Cl, 19.70; N, 7.91; S, 8.90.

4.2.11. 3,4-Dimethyl-1,2,3,5,6,10b-hexahydropyrimido[5,4-c]quinoline-2,5-diones (**5a**)

A solution of *o*-chloro-3,4-dihydropyrimidin-2-(1*H*)-one, **4a** (1 g, 3.2 mmol) in 80 mL of ammonia at 32% was heated at 250 °C under 10 bars for 16 h. The solvent was removed in vacuum to afford a solid which was dissolved in water and neutralized with 5 M hydrochloric acid. The precipitate obtained was recuperated by filtration and recrystallised from ethanol to give the pure product **6a** in 70% yield, mp 236 °C, IR ν(NH) 3120, ν(C=O) lactam 1680, ν(C=O) urea 1636. ^{1}H NMR (DMSO-d_6) 2.6 ppm (3H, s, CH_3), 3.1 ppm (3H, s, N–CH_3), 5.6 ppm (1H, d, $J = 3.2$ Hz, CH), 7.2–7.6 ppm (4H, m, aromH), 7.9 ppm (1H, s, NH), 12 ppm (1H, s, NH). Anal. Calcd for $C_{13}H_{13}N_3O_2$: C, 64.19; H, 5.39; N, 17.27. Found: C, 64.25; H, 5.34; N, 17.21.

4.2.12. 3-Ethyl-4-methyl-1,2,3,5,6,10b-hexahydropyrimido[5,4-c]quinoline-2,5-diones (**5b**)

Compound **5b** was prepared with the same procedure as **5a** in 73% yield, mp 244 °C, IR ν(NH) 2990, ν(C=O) lactam 1679, ν(C=O) urea 1622. ^{1}H NMR (DMSO-d_6) 1.1 ppm (3H, t, CH_3 ethyl), 2.6 ppm (3H, s, CH_3), 3.6 ppm (1H, m, N–CH ethyl), 3.8 ppm (1H, m, N–CH ethyl), 5.7 ppm (1H, d, $J = 3.3$ Hz, CH), 7.2–7.5 ppm (4H, m, aromH), 7.8 ppm (1H, s, NH), 12 ppm (1H, s, NH). Anal. Calcd for $C_{14}H_{15}N_3O_2$: C, 65.35; H, 5.88; N, 16.33. Found: C, 65.31; H, 5.91; N, 16.35.

4.2.13. 3-Ethyl-10-fluoro-4-methyl-1,2,3,5,6,10b-hexahydropyrimido[5,4-c]quinoline-2,5-diones (**5c**)

Compound **5c** was prepared with the same procedure as **5a** in 80% yield, mp 228 °C, IR ν(NH) 3100, ν(C=O) lactam 1683, ν(C=O) urea 1630. ^{1}H NMR (DMSO-d_6) 1.1 ppm (3H, t, CH_3 ethyl), 2.5 ppm (3H, s, CH_3), 3.7 ppm (1H, m, N–CH ethyl), 3.8 ppm (1H, m, N–CH ethyl), 5.9 ppm (1H, d, $J = 3.6$ Hz, CH), 7.3–7.5 ppm (3H, m, aromH), 7.6 ppm (1H, s, NH), 11.9 ppm (1H, s, NH). Anal. Calcd for $C_{14}H_{14}FN_3O_2$: C, 61.08; H, 5.13; F, 6.90; N, 15.26. Found: C, 61.12; H, 5.06; F, 6.94; N, 15.18.

4.2.14. 7-Chloro-3,4-dimethyl-1,2,3,5,6,10b-hexahydropyrimido[5,4-c]quinoline-2,5-diones (**5d**)

Compound **5d** was prepared with the same procedure as **5a** in 81% yield, mp 254 °C, IR ν(NH) 2990, ν(C=O) lactam 1673, ν(C=O) urea 1622. ^{1}H NMR (DMSO-d_6) 2.6 ppm (3H, s, CH_3), 3.2 ppm (3H, s, N–CH_3), 5.6 ppm (1H, d, $J = 3.5$ Hz, CH), 7.2–7.6 ppm (3H, m, aromH), 7.8 ppm (1H, s, NH), 11.9 ppm (1H, s, NH). Anal. Calcd for $C_{13}H_{12}ClN_3O_2$: C, 56.22; H, 4.36; Cl, 12.77; N, 15.13. Found: C, 56.16; H, 4.39; Cl, 12.70; N, 15.24.

4.2.15. 7-Chloro-3-ethyl-4-methyl-1,2,3,5,6,10b-hexahydropyrimido[5,4-c]quinoline-2,5-diones (**5e**)

Compound **5e** was prepared with the same procedure as **5a** in 73% yield, mp 250 °C, IR ν(NH) 2998, ν(C=O) lactam 1671, ν(C=O) urea 1618. ^{1}H NMR (DMSO-d_6) 1.0 ppm (3H, t, CH_3 ethyl), 2.5 ppm (3H, s, CH_3), 3.7 ppm (1H, m, N–CH ethyl), 3.8 ppm (1H, m, N–CH ethyl), 5.6 ppm (1H, d, $J = 3.3$ Hz, CH), 7.2–7.4 ppm (3H, m, aromH), 7.6 ppm (1H, s, NH), 11.9 ppm (1H, s, NH). Anal. Calcd for $C_{14}H_{14}ClN_3O_2$: C, 57.64; H, 4.84; Cl, 12.15; N, 14.40. Found: C, 57.42; H, 4.88; Cl, 12.10; N, 14.51.

4.2.16. 10-Chloro-3,4-dimethyl-1,2,3,5,6,10b-hexahydropyrimido[5,4-c]quinoline-2,5-diones (**5f**)

Compound **5f** was prepared with the same procedure as **5a** in 90% yield, mp 258 °C, IR ν(NH) 3005, ν(C=O) lactam 1666, ν(C=O) urea 1619. ^{1}H NMR (DMSO-d_6) 2.5 ppm (3H, s, CH_3), 3.2 ppm (3H, s, N–CH_3), 6.1 ppm (1H, d, $J = 3.2$ Hz, CH), 7.2–7.5 ppm (3H, m, aromH), 7.6 ppm (1H, s, NH), 11.6 ppm (1H, s, NH). Anal. Calcd for $C_{13}H_{12}ClN_3O_2$: C, 56.22; H, 4.36; Cl, 12.77; N, 15.13. Found: C, 56.25; H, 4.30; Cl, 12.65; N, 15.22.

4.2.17. 3,4-Dimethyl-2-thioxo-1,2,3,5,6,10b-hexahydropyrimido[5,4-c]quinoline-5-one (**5g**)

Compound **5g** was prepared with the same procedure as **5a** in 82% yield, mp 262 °C, IR ν(NH) 3190, ν(C=O) lactam 1672, ν(C=S) thiourea 1646. ^{1}H NMR (DMSO-d_6) 2.5 ppm (3H, s, CH_3), 3.6 ppm (3H, s, N–CH_3), 5.6 ppm (1H, d, $J = 2.9$ Hz, CH), 7.3–7.5 ppm (4H, m, aromH), 9.6 ppm (1H, s, NH), 12.4 ppm (1H, s, NH). Anal. Calcd for

$C_{13}H_{13}N_3OS$: C, 60.21; H, 5.05; N, 16.20; S, 12.36. Found: C, 60.25; H, 5.01; N, 16.18; S, 12.45.

4.2.18. 8-Chloro-3,4-dimethyl-2-thioxo-1,2,3,5,6,10b-hexahydropyrimido[5,4-c]quinoline-5-one (**5h**)

Compound **5h** was prepared with the same procedure as **5a** in 65% yield, mp 222 °C, IR ν(NH) 3001, ν(C=O) lactam 1670, ν(C=S) thiourea 1639. ^{1}H NMR (DMSO-d_6) 2.4 ppm (3H, s, CH_3), 3.4 ppm (3H, s, N–CH_3), 5.5 ppm (1H, d, $J = 2.7$ Hz, CH), 7.1–7.4 ppm (3H, m, aromH), 7.7 ppm (1H, s, NH), 9.6 ppm (1H, s, NH). Anal. Calcd for $C_{13}H_{12}ClN_3OS$: C, 53.15; H, 4.12; Cl, 12.07; N, 14.30; S, 10.91. Found: C, 53.11; H, 4.18; Cl, 12.12; N, 14.15; S, 10.85.

4.2.19. 10-Fluoro-3,4-dimethyl-2-thioxo-1,2,3,5,6,10b-hexahydropyrimido[5,4-c]quinoline-5-one (**5i**)

Compound **5i** was prepared with the same procedure as **5a** in 72% yield, mp 244 °C, IR ν(NH) 3108, ν(C=O) lactam 1682, ν(C=S) thiourea 1649. ^{1}H NMR (DMSO-d_6) 2.5 ppm (3H, s, CH_3), 3.6 ppm (3H, s, N–CH_3), 5.8 ppm (1H, d, $J = 2.6$ Hz, CH), 7.1–7.4 ppm (3H, m, aromH), 7.5 ppm (1H, s, NH), 9.6 ppm (1H, s, NH). Anal. Calcd for $C_{13}H_{12}FN_3OS$: C, 56.30; H, 4.36; F, 6.85; N, 15.15; S, 11.56. Found: C, 56.25; H, 4.40; F, 6.91; N, 15.21; S, 11.38.

4.2.20. 10-Chloro-3,4-dimethyl-2-thioxo-1,2,3,5,6,10b-hexahydropyrimido[5,4-c]quinoline-5-one (**5j**)

Compound **5j** was prepared with the same procedure as **5a** in 88% yield, mp 274 °C, IR ν(NH) 3091, ν(C=O) lactam 1673, ν(C=S) thiourea 1627. ^{1}H NMR (DMSO-d_6) 2.4 ppm (3H, s, CH_3), 3.7 ppm (3H, s, N–CH_3), 6.2 ppm (1H, d, $J = 2.9$ Hz, CH), 7.0–7.4 ppm (3H, m, aromH), 7.5 ppm (1H, s, NH), 9.5 ppm (1H, s, NH). Anal. Calcd for $C_{13}H_{12}ClN_3OS$: C, 53.15; H, 4.12; Cl, 12.07; N, 14.30; S, 10.91. Found: C, 53.20; H, 4.09; Cl, 12.15; N, 14.21; S, 10.87.

4.3. Antioxidant activity studies

4.3.1. Assay of hydroxyl radical (OH$^\bullet$) scavenging activity

In a screw-capped test tube, 0.2 mL of sodium benzoate (10 mmol) and 0.2 mL of $FeSO_4 \cdot 7H_20$ (10 mmol) and EDTA (10 mmol) were added. Then the sample solution and a phosphate buffer (pH 7.4, 0.1 mol) were mixed to give a total volume of 1.8 mL. Finally, 0.2 mL of H_2O_2 solution (10 mmol) was added, and the whole incubated at 37 °C for 2 h. After incubation, the fluorescence was measured at 407 nm emission with excitation at 305 nm.

4.3.2. DPPH radical scavenging activity

The capacity of compounds to scavenge the "stable" free radical DPPH was monitored according to the method of Hatano et al. [20]. Various concentrations of methanolic compounds solutions (0.3 mL) were mixed with methanolic solution containing DPPH radicals (1.5×10^{-4} mol L^{-1}, 2.7 mL). The mixture was shaken vigorously and left to stand for 2 h in the dark (until stable absorption values were obtained). The reduction of the DPPH radical was determined by measuring the absorption at 517 nm. The RSA was calculated as a percentage of DPPH discolouration using the equation:

$\%RSA = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the compound has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution. Mean values from three independent samples were calculated for each compound and standard deviations were less than 5%. Ascorbic acid was used as standard.

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