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Original Article

Synthesis and antioxidant evaluation of 4-(furan-2-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate esters

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Abstract

Biginelli-type pyrimidines contain an interesting moiety which has attracted considerable attention of medicinal chemists in the last few decades. Despite the very diverse pharmacologic effects ascribed to this kind of pyrimidines, there are few reports on the antioxidant evaluation of Biginelli pyrimidines. In this study synthesis of some novel Biginelli-type pyrimidines is reported. The prepared compounds are ester derivatives of 6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate with a simple hetaryl group, furan, at C-4 position of the pyrimidine ring. These compounds were evaluated for free radical and H_2O_2 scavenging activities. The reducing power of these compounds was also determined. Compound **3c** was the most potent one in diphenyl picrylhydrazine scavenging activity assay with the IC_{50} of 0.6 mg/ml. The results of reducing power assays proved that **3d** and **3e** are moderate reducing agents. All of the studied compounds were very weak in scavenging hydrogen peroxide compared with gallic acid.

Keywords: Synthesis; Biginelli pyrimidines; DPPH scavenging activity; Reducing power assay; H_2O_2 scavenging activity

INTRODUCTION

2-Oxo (thioxo)-1,2,3,4-tetrahydropyrimidine is an interesting moiety which has attracted considerable attention of medicinal chemists in the last few decades (1). This chemical entity which is also called 3,4-dihydropyrimidine-2(1*H*)-one (thione) (DHPM) was introduced to chemistry at the beginning of 1890s by the Italian chemist Pietro Biginelli. A broad range of biological effects, including calcium channel modulation (2,3), adrenoceptor blocking (4), antitumor (5), antiviral (6), anti-inflammatory (7) and antimicrobial (8) activities have been attributed to this class of heterocyclic compounds.

Free radicals with a singlet electron in their structure play an important role in the pathogenesis of various disorders such as cancers, atherosclerosis, diabetes, Alzehimer, Parkinson and diseases related to aging process (9,10).

Several free radicals are formed through the aerobic metabolism in human. Most of them have a central oxygen atom in their structure hence are also called reactive oxygen species (ROS). A large number of reactive species are formed from the reaction of ROS with biological molecules such as polyunsaturated lipids, thiol containing proteins and nitric oxide (NO). Peroxynitrite anion ($ONOO^-$) is an example which is readily formed in the reaction of O_2 with NO. This anion is unstable at physiological pH and rapidly decomposes to form potent nitrating and oxidizing species (11,12).

Endogenous antioxidant mechanisms and exogenous antioxidant compounds are capable of deactivating or neutralizing these reactive species to prevent the irreversible adverse effects that free radicals exert on cells. Gluthation reductase (GSH), superoxide dismutase (SOD) and catalase are three important enzymes in these endogenous mechanisms. Radical scavenging compounds such as α-tocopherol, vitamin C, carotenoids,

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and poly phenols with herbal origin are some examples of exogenous antioxidant compounds. These enzymes and antioxidant compounds equilibrate the generation and neutralization of free radicals in the normal physiologic conditions. In case of overproduction of free radicals in the body they become insufficient or defective and design of potent and safe antioxidant molecules seems to be necessary to hold this balance again (13,14). This accounts for continuing interest in the identification and development of novel antioxidant agents.

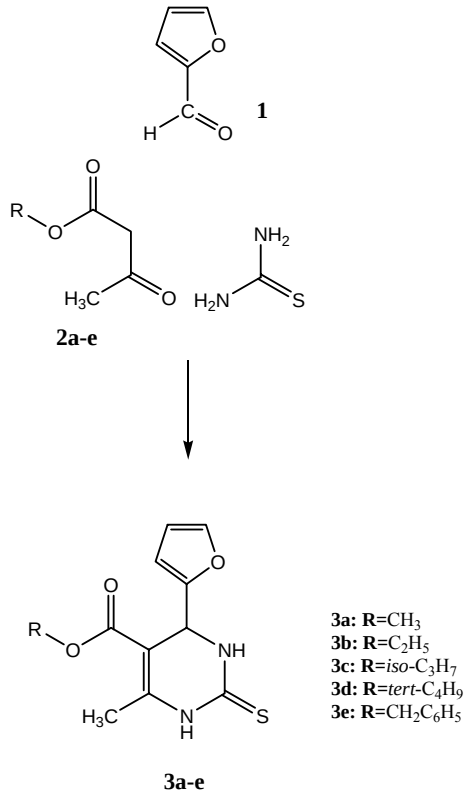
Molecules with free radical scavenging abilities are the most-studied antioxidant agents. Despite the extensive studies on the pharmacology of Biginelli pyrimidines during the last decades, there are few reports on their antioxidant evaluation. Stefani and coworkers have described the antioxidant activity of ester derivatives of DHPMs containing phenyl or *meta*-nitrophenyl ring at the C-4 position of the pyrimidine scaffold. All of the provided compounds were antioxidants to some degree according to lipid peroxidation, spectro-fluorimetry using 2′,7′-dichlorofluorescein diacetate (DCHF-DA), and thiol-peroxidase activity assay methods (15). Another report of the antioxidant evaluation of Biginelli pyrimidines describes the ester derivatives of DHPMs substituted by methyl, phenyl, 2-hydroxy-5-bromophenyl, 2-hydroxy-phenyl, and 1-naphthyl at the C-4 position which were subjected to antioxidant evaluations. These compounds were elucidated to be potent antioxidants in terminating cumene oxidation chains by the reaction with cumylperoxy radicals (16). The third report is on the antioxidant power of the fused-ring derivatives of DHPMs. These compounds which cannot be considered as usual Biginelli pyrimidines were studied by diphenyl picrylhydrazine (DPPH) and hydroxyl radical scavenging activity evaluation methods (17).

Here, we report the synthesis of some novel Biginelli pyrimidines. They are ester derivatives of 6- methyl -2- thioxo -1 2, 3, 4- tetrahydro pyrimidine-5-carboxylate which have a simple hetaryl group, furan, at the C-4 position of the pyrimidine ring. All of these compounds have at least one labile hydrogen atom in their structure which provides them a radical scavenging potential. The conjugated system present in the structure of these compounds would help the stabilization of the radical formed after reaction with ROS. These compounds are evaluated for DPPH free radical scavenging ability. Reducing power and H_2O_2 scavenging activity of them are also determined.

MATERIALS AND METHODS

Chemistry

All chemicals used for the synthesis of compounds were supplied by Merck or Sigma. Melting points were determined on a Mettler capillary melting point apparatus and were uncorrected. The IR spectra were recorded with a WQF-510 Ratio Recording FTIR spectrometer as a KBr disc (γ, cm^{-1}). The ^{1}HNMR spectra (DMSO-d_6) were recorded on a Bruker 400 MHz spectrometer. Chemical shifts (δ) are reported in ppm downfield from the internal standard tetramethylsilane (TMS). Mass spectra were acquired with a Platform II Mass Spectrometer from Micromass. Electron-impact ionization was performed at an ionizing



Scheme 1. Synthesis of 1,2,3,4-tetrahydropyrimidine-5-carboxylate esters

energy of 70 eV. The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel plate using chloroform and methanol. The procedure for the synthesis of the desired compounds is depicted in Scheme 1.

General procedure for the synthesis of 4-(furan -2- yl) -6- methyl -2- thioxo -1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate esters ***3a-e***

A mixture of furan-2-carbaldehyde **1** (1 mmole), appropriate acetoacetate ester **2a-e** (1 mmole) and thiourea (1.3 mmole) was refluxed in 4 ml of absolute ethanol for 6 h. Ferric chloride (0.2 mmole) was used as a Lewis' acid. The reaction mixture was filtered off and the solvent was removed under reduced pressure. The residue was purified using salting out method with acetone and petroleum ether as solvents (**3a,b**) or by column chromatography using chloroform/ methanol as eluent (**3c-e**).

Antioxidant evaluation

DPPH free radical scavenging assay

This assay was performed after modification of the method described by Blois and coworkers (18). Briefly, 0.1 ml of different concentrations of methanolic solution of standard (0.0005-1.25 mg/ml) or test compounds (0.156, 0.312, 0.625 and 1.25 mg/ml) were added to 2 ml of DPPH methanolic solution (60 mM). The mixture was shaken vigorously and allowed to react at room temperature and in darkness for 5 h. The absorbance of the resulting solution was measured at 517 nm using a UV/Vis spectrophotometer after 5 hours incubation. Scavenging of DPPH free radicals was calculated as:

DPPH scavenging activity (%) = [(Ac–At) / Ac] × 100

where, Ac is the absorbance of the control tube (containing all reagents except the test compound), and At is the absorbance of the test tube. Gallic acid was used as the standard at the concentration range of 0.0005-1.25 mg/ml.

Reducing power assay

This assay was done in accordance with SDS modified and pH-optimized ferricyanide method described by Berker and coworkers (19). Briefly, to 0.2 ml of various concentrations (0.125, 0.25, 0.5 mg/ml in DMSO) of test or standard compounds, 1.25 ml of H_2O, 50 µL of 1 M hydrochloric acid, 0.3 mL of 1% potassium ferricyanide solution, 0.1 ml of 1% SDS solution and 0.1 ml of 0.2% w/v $FeCl_3.6H_2O$ (all solutions in water) were added. The mixture was left at room temperature for 30 min and the resulting absorbance was then measured against a blank at 750 nm. The color of the final solution was stable for at least 30 min. The Prussian blue formed in this method did not precipitate due to the stabilizing effect of SDS. Ascorbic acid at the concentration range of 0.006-0.4 mg/ml and gallic acid at the concentration range of 0.0003-0.32 mg/ml were used as standard antioxidant compounds.

Hydrogen peroxide scavenging assay

H_2O_2 scavenging power was determined according to the method of Ruch and coworkers (20).This method is based on the ability of a compound to convert hydrogen peroxide to water. A 40 mM solution of hydrogen peroxide was prepared in saline phosphate buffer (pH 7.4). 100 µl DMSO solutions of the test compounds or standards at the concentrations of 0.125, 0.25 and 0.5 mg/ml were separately added to 2 ml of the prepared hydrogen peroxide solution and the absorbance was measyred at 230 nm after 10 min against a blank solution. The blank solution was composed of 100 µl DMSO solutions of test compounds or standards and 2 ml of saline phosphate buffer. The hydrogen peroxide scavenging activity for compounds and standards was calculated using the following equation:

H_2O_2 scavenging activity (%) = [(Ac-At) / Ac] × 100

where, Ac is the absorbance of the control and At is the absorbance of the tested compounds or standards. Gallic acid at the concentration rang of 0.015-0.25 mg/ml was used as the standard.

RESULTS

Chemistry

1,2,3,4-tetrahydropyrimidine-5-carboxylate esters **3a-e** were prepared in 53-65% with condensing furan-2-carbaldehyde **1**, appropriate acetoacetate esters **2a-e** and thiourea. The

condensation reactions were performed in boiling ethanol using ferric chloride as a Lewis' acid catalyst (Scheme 1). Structural details and some of the characterization data of the final compounds are summarized in Table 1.

The structures of title compounds were confirmed by FTIR, and ^{1}HNMR spectroscopy.

The details of the spectral data for the prepared compounds are provided in the following. All atoms are numbered sequentially to facilitate the assignment of protons in ^{1}HNMR (Table 1).

*Methyl 4-(furan-2-yl)-6-methyl-2-thioxo-1, 2, 3, 4-tetrahydropyrimidine -5- carboxylate (**3a**)*

IR: (KBr) γcm^{-1}: 3313, 3184 (NH), 3107 (C-H, Aromatic), 3001, 2984 (C-H, Aliphatic), 1662 (C=O, ester), 1572, 1442 (C=C, aromatic); ^{1}HNMR (DMSO-D_6): δ 2.34 (s, 3H, C_6-CH_3), 3.65 (s, 3H, OCH_3), 5.29 (d, *J*=3.6 Hz, 1H, C4-H), 6.20 (d, *J*=3.2 Hz, 1H, C5′-H), 6.40 (dd, 1H, *J*=3.2 Hz, 1.6 Hz, 1H, C4′-H), 7.64 (m, , 1H, C3′-H), 9.72 (d, *J* = 2.0 Hz, 1H, N3-H), 10.48 (s, 1H, N1-H); EI-MS (+) m/z (%): 252.1 (M^+, 80), 253.1 (M^+ + H^+, 27), 254.08 (M^++2, 12, ^{34}S).

*Ethyl 4-(furan-2-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3b**)*

IR: (KBr) γcm^{-1}: 3317, 3178 (NH), 3120 (C-H, Aromatic), 2964 (C-H, Aliphatic), 1664 (C=O, ester), 1577, 1452 (C=C, aromatic); ^{1}HNMR (DMSO-D_6): δ1.19 (t, *J*=6.8 Hz, 3H, $CH_2C\underline{H_3}$), 2.34 (s, 3H, C_6-CH_3), 4.08-4.14 (m, 2H, $C\underline{H_2}CH_3$), 5.29 (d, *J*=4.0 Hz, 1H, C4-H), 6.20 (d, *J*=2.8 Hz, 1H, C5′-H), 6.44 (m, 1H, C4′-H), 7.64 (m, 1H, C3′-H), 9.69 (bs, 1H, N3-H), 10.45 (s, 1H, N1-H); EI-MS (+) m/z (%): 266.09 (M^+, 68), 267.1 (M^+ + H^+, 15), 268.09 (M^++2, 9, ^{34}S).

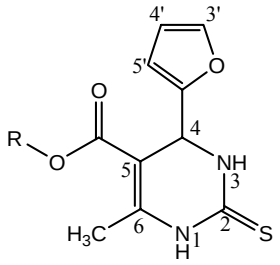
*Iso-Propyl 4-(furan-2-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3c**)*

IR: (KBr) γcm^{-1}: 3321, 3174 (NH), 3111 (C-H, Aromatic), 2985 (C-H, Aliphatic), 1660 (C=O, ester), 1576, 1452 (C=C, aromatic); ^{1}HNMR (DMSO-D_6): δ1.12 (d, *J*=6.0 Hz, 3H, $CH(C\underline{H_3})_2$), 1.23 (d, *J*=6.4 Hz, 3H, $CH(C\underline{H_3})_2$), 2.30 (s, 3H, C_6-CH_3), 4.92 (heptet, , 1H, $C\underline{H}(CH_3)_2$), 5.28 (d, *J*=4.0 Hz, 1H, C4-H), 6.20 (d, *J*=3.2 Hz, 1H, C5′-H), 6.44 (m, 1H, 1H, C4′-H), 7.64 (m, 1H, C3′-H), 9.67 (bs, 1H, N3-H), 10.42 (s, 1H, N1-H); EI-MS (+) m/z (%): 280.04 (M^+, 85), 281.1 (M^+ + H^+, 38), 282.1 (M^++2, 15, ^{34}S).

*Tert-Butyl 4 -(furan-2-yl) -6-methy l- 2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3d**)*

IR: (KBr) γcm^{-1}: 3167 (NH), 3107 (C-H, Aromatic), 2978 (C-H, Aliphatic), 1705 (C=O, ester), 1653 (C=C alkene), 1591, 1471 (C=C, aromatic); ^{1}HNMR (DMSO-D_6): δ1.35 (s, 9H, $CH(C\underline{H_3})_3$), 2.24 (s, 3H, C_6-CH_3), 5.17 (s, 1H, C4-H), 6.13 (bs, 1H, C5′-H), 6.39 (m, 1H, 1H, C4′-H), 7.58 (bs, 1H, C3′-H), 9.55 (bs, 1H, N3-H), 10.28 (s, 1H, N1-H); ESI EI-MS (+) m/z (%): 294.0 (M^+, 100), 295.1 (M^+ + H^+, 60), 296.1 (M^++2, 26, ^{34}S).

Table 1. Structural details and some of the characterization data of the final compounds



Compound	R	Mol. formula	M.P (ºC)	MW	Yield (%)
3a	CH_3	$C_{11}H_{12}N_2O_3S$	255-256	252.29	53
3b	C_2H_5	$C_{12}H_{14}N_2O_3S$	225-227	266.32	65
3c	*iso*-C_3H_7	$C_{13}H_{16}N_2O_3S$	191-192	280.34	62
3d	*tert*-C_4H_9	$C_{14}H_{18}N_2O_3S$	196-198	294.37	58
3e	$CH_2C_6H_5$	$C_{17}H_{16}N_2O_3S$	168-169	328.39	55

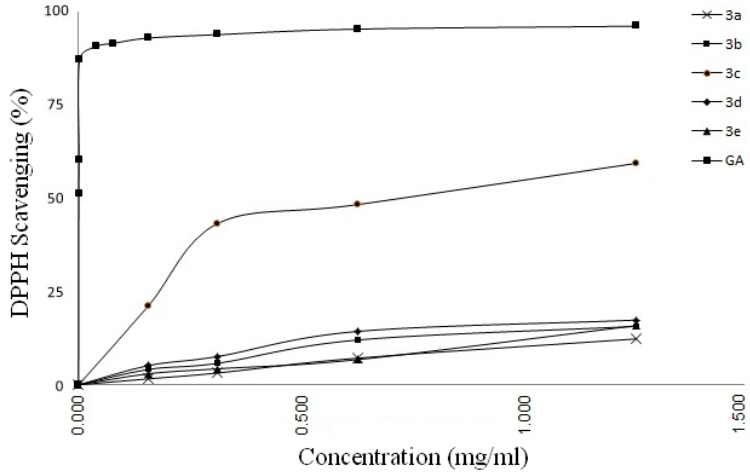


Fig. 1. % DPPH scavenging activity of compounds **3a-e**

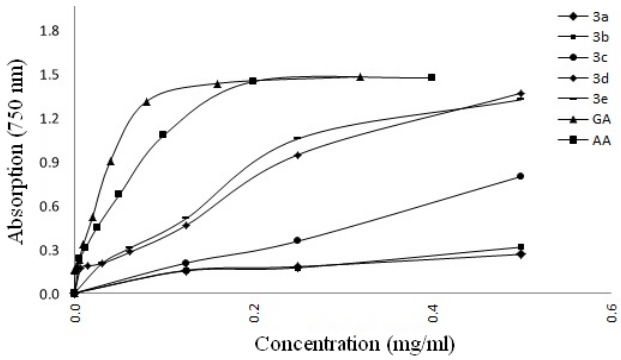


Fig. 2. Reducing power activity values for compounds **3a-e**

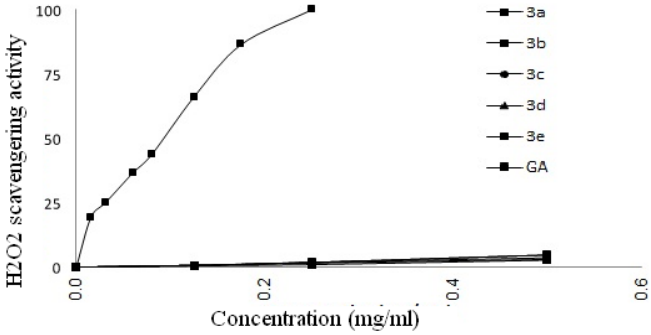


Fig. 3. Values for hydrogen peroxide scavenging power of compounds **3a-e**

*Benzyl 4- (furan-2-yl) -6- methy l- 2- thioxo-1, 2,3,4-tetrahydropyrimidine-5-carboxylate (**3e**)*

IR: (KBr) γcm^{-1}: 3298, 3178 (NH), 3120 (C-H, Aromatic), 2999 (C-H, Aliphatic), 1699 (C=O, ester), 1645 (C=C alkene), 1566 (C=C, aromatic); ^{1}HNMR (DMSO-D_6): δ 2.30 (s, 3H, C_6-CH_3), 5.06 (doublet, *J*=12.8 Hz, H_a, $C\underline{H}_aH_bC_6H_5$), 5.12 (doublet, *J*=12.8 Hz,H_b, $CH_a\underline{H}_bC_6H_5$), 5.27 (d, *J*=3.6 Hz, 1H, C4-H), 6.11 (d, *J*=3.2 Hz, 1H, C5′-H), 6.38 (dd, 1H, *J*=3.2 Hz, 1.6 Hz, 1H, C4′-H), 7.22-7.33 (m, 5H, C_6H_5), 7.59 (dd, *J*=1.6, *J*=0.8, 1H, C3′-H), 9.66 (m, 1H, N3-H), 10.46 (s, 1H, N1-H).

Antioxidant evaluation

The antioxidant potential of the synthesized compounds was assessed using three different methodologies, namely DPPH free radical scavenging, reducing power, and hydrogen peroxide scavenging assays.

DPPH free radical scavenging assay

The DPPH free radical scavenging activity of methanolic solutions of Biginelli derivatives **3a-e** was determined in terms of DPPH scavenging percentage. Results of this assay are provided as the percentage of scavenging/concentration in Fig. 1 and as IC_{50} (mg/ml) in Table 2, respectively.

Reducing power assay

Reducing power values for compounds **3a-e** are provided diagrammatically in Fig. 2.

Hydrogen peroxide scavenging assay

The values for hydrogen peroxide scavenging power of compounds **Va-f** are provided in Fig. 3.

Table 2. IC_{50} values for DPPH scavenging ability of the compounds **3a-e**

Compound	IC_{50} (mg/ml)
3a	4.92
3b	2.69
3c	0.6
3d	2.24
3e	3.62
3a	4.92
Gallic Acid	0.0008

DISCUSSION

Chemistry

All compounds showed in the IR spectra an absorption band at 1660-1705 cm^{-1}, typical of the stretch vibrations of the carboxylate ester C=O group.

The ^{1}HNMR spectra of all final compounds contained a singlet in the δ 2.24-2.34 ppm region belonging to the CH_3 protons at the C-6 position. The characteristic peak for the C-4 proton of the 1,2,3,4-tetrahydropyrimidine ring appeared at 5.17-5.29 ppm confirming the formation of this heterocycle. These two peaks are indicatives of the presence of 1,2,3,4-tetrahydropyrimidine ring. A signal with a multiplicity of twelve appeared at 4.08-4.14 ppm belonging to the hydrogens of the methylen part of the ethyl ester group in compound **3b**. This multiplicity can be explained by considering the fact that these two hydrogens are prochiral. Two other prochiral hydrogens were observed in compound **3e**. In this compound, benzylic hydrogens were the prochiral atoms which appeared as two doublets at 5.06 and 5.12 ppm with large *J* values of 12.8 and 14.4 Hz, confirming the germinal coupling of the two prochiral hydrogens. The prochiral hydrogens in **3b** and **3e** are depicted in Fig. 4. Other ^{1}HNMR spectral signals were in accordance with the proposed structures.

Antioxidant evaluation

DPPH free radical scavenging assay

A freshly prepared DPPH solution exhibits a deep purple color with an absorption maximum at 517 nm. This purple color generally disappears when an antioxidant is present in the medium. Thus, antioxidant molecules can quench DPPH free radicals, by providing hydrogen atoms or by electron donation via a free radical attack on the DPPH molecule, and convert them to colorless product (21,22).

From the analysis of results for the DPPH free radical scavenging activity of the studied compounds summarized in Fig. 1 and Table 2, we can conclude that the studied compounds were all weaker than the standard antioxidant, gallic acid, in DPPH scavenging activity. Compound **3c** was the most potent antioxidant with the IC_{50} of 0.6 mg/ml.

Reducing power assay

SDS modified and pH optimized ferricyanide method was used to evaluate the reducing power of the compounds. Ferricyanide and ferric chloride ($FeCl_3$) were used as reagents in this method. The reactions provided in the following equation occur in this assay. The production of $Fe[Fe(CN)_6]^-$ complex results in a blue color whose intensity depends directly on the reducing power of the compounds.

$$Fe^{3+} + \text{antioxidant} \rightleftharpoons Fe^{2+} + \text{oxidized antioxidant}$$

$$Fe^{2+} + Fe(CN)_6^{3-} \rightleftharpoons Fe[Fe(CN)_6]^-$$

or

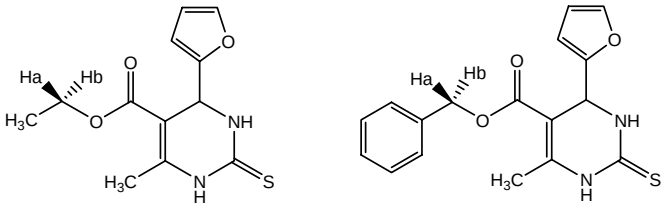
$$Fe(CN)_6^{3-} + \text{antioxidant} \rightleftharpoons Fe(CN)_6^{4-} + \text{oxidized antioxidant}$$

$$Fe(CN)_6^{4-} + Fe^{3+} \rightleftharpoons Fe[Fe(CN)_6]^-$$

The results of reducing power assays proved **3d** and **3e** as the most potent compounds. Both were weaker than the standards, gallic acid and ascorbic acid and could be considered as moderate reducing agents.

Hydrogen peroxide scavenging assay

The method provided by Ruch and coworkers which is based on the ability of a compound to convert hydrogen peroxide to water was used for determination of H_2O_2 scavenging power. Hydrogen peroxide is a weak oxidizing agent capable of oxidizing the essential thiol (-SH) group of proteins, thus inactivating a few enzymes. It rapidly passes through the cell membranes and inside the cell reacts with Fe^{2+} to form hydroxyl radical which exerts several toxic effects (23). It is therefore biologically advantageous for cells to



3b **3e**

Fig. 4. Prochiral hydrogens in **3b** and **3e**

control the amount of hydrogen peroxide to prevent oxidative stress conditions. It can be concluded from the results summarized in Figure 3 that all of the studied compounds were very weak compared with gallic acid in scavenging hydrogen peroxide.

CONCLUSION

Biginelli pyrimidines possessing a 2- oxo(thioxo) -1,2,3,4- tetrahydropyrimidine moiety, has attracted considerable attention of medicinal chemists. There are few reports on the antioxidant evaluation of these compounds. In the current study, the synthesis of some novel 4- (furan-2-yl) -6-methyl -2- thioxo-1,2,3,4- tetrahydropyrimidine -5- carboxylate esters is reported. The antioxidant activity of the synthesized compounds was determined. Compound **3c** was the most potent antioxidant with the IC_{50} of 0.6 mg/ml. The results of reducing power assays proved **3d** and **3e** as the moderate reducing agents. All of the studied compounds were very weak compared with gallic acid in scavenging hydrogen peroxide.

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