Synthesis of novel substituted 4H-chromenes and their antioxidant screening

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ABSTRACT

Novel substituted 4H-chromenes were synthesized via O-alkylation of 2-amino-7-hydroxy-4-phenyl-4H-chromene-3-carbonitrile (1) followed by hydrazinolysis to give the hydrazide 3. This key intermediate 3 was reacted with aromatic aldehydes, aryldeneiminonitriles and substituted isothiocyanates to yield compounds 4a&b and 5a&b, respectively. In addition, cyclization of 5a&b in piperidine yielded the target thiazolo derivatives 6a&b. All newly synthesized compounds were evaluated for their in vitro antioxidant activity. Compound 5a was found to be the most potent antioxidant and the least active among the series being compound 1.

Key Words: Heterocycles, substituted chromenes, thiazoles, Schiff bases, antioxidant activity.

INTRODUCTION

4H-Chromenes exhibit a remarkable array of biochemical and pharmacological activities. They constitute the basic structural back bone of many types of tannin and polyphenols widely present in plants e.g. green tea, fruits and vegetables [1]. The presence of the chromene-containing structure has been associated with the capability to prevent several diseases [2]. Synthetic analogues have attracted considerable attention due to their useful biological and pharmacological properties including antimicrobial [3-13], antioxidant [14,15], anticancer [16-19], hypotensive [20], local anesthetic [21] and central nervous system activities [12,22]. As well as treatment of Alzheimer’s disease [23] and Schizophrenia disorder [24].

The aim of the present work is the synthesis of novel compounds bearing 4-aryl 4H-chromene and evaluation of their antioxidant activities.

For this purpose the 2-amino-4-(4-phenyl)-7-hydroxy-4H-chromene-3-carbonitrile 1 [25], alongside with its ester and hydrazide derivatives 2&3 were synthesized (Scheme 1).

EXPERIMENTAL SECTION

Chemistry

Reactions were routinely monitored by thin-layer chromatography (TLC) on silica gel sheets that precoated with UV fluorescent silica (MERCK 60 F 254) and spots were developed using I2vapour / UV light as visualizing agents. Solvent system was chloroform: methanol (in different ratio). 1H NMR spectra were determined in CDCl3, or DMSO-d6 solvent with Varian Gemini 300 MHZ Spectrometer. Peak positions were given in parts per million (δ) downfield the tetramethylsilane as internal standard. IR spectra were recorded on a Shimadzu 435 Spectrometer, using KBr discs and values were represented in cm−1. GC Mass spectra were run on Shimadzu QP-2010 spectrometer and Mass spectra were run on Hewlett Packard 5988 spectrometer at the Microanalytical Center, Cairo University, Egypt. Melting points were determined on a Griffin instrument and are uncorrected. All reported products showed 1H NMR spectra in agreement with the assigned structures. Elemental analyses were performed at
the Micro-analytical Center, Cairo University, Egypt. Compound 1 was prepared adopting a reported procedure [25].

Reagents & conditions: 

a: ClCH₂COOC₂H₅, K₂CO₃ / DMF, reflux 2h  
b: N₂H₄ / EtOH, reflux 4h  
c: ArCHO, TEA / EtOH, reflux 2-4h  
d: ArCH=C(CN)₂, piperidine/EtOH, reflux 2h  
e: RCNS/EtOH, reflux 4h  
f: piperidine/H₂O, reflux 6h

Scheme 1
(RS)-Ethyl 2-(2-amo3-cyano-4(phenyl)-4H-chromen-7-yloxy) acetate (2)

To a well-stirred mixture of I (2.64 g, 0.01 mol) and anhydrous potassium carbonate (5.52 g, 0.04 mol) in dry dimethylformamide (30 ml), ethyl chloroacetate (1.22 g, 0.01 mol) was added dropwise. After completion of the addition, the reaction mixture was heated under reflux for 2 h then cooled and poured into ice-cold water. The precipitated solid was filtered, washed with water, dried and crystallized from absolute ethanol to afford compound 2 in 3.25 g (93%) yield. mp 130-132 °C, IR (KBr): 3428.81, 3343.96 (forked, NH2), 2983.34 (CH aliph.), 2184.95 (C=O), 1747.19 (C=O) cm⁻¹; ¹H NMR (DMSO-d6): δ 1.20 (t, Jvalue = 7.2 Hz, 3H, CH₂CH₃); 4.15 (q, Jvalue = 7.2 Hz, 2H, CH₂CH₃); 4.66 (s, 1H, ArH); 4.77 (s, OCH3) 6.55 (s, 1H, ArH); 6.67-7.33 (m, 7 H, Ar H and 2H, NH₂, D₂O exchangeable) ppm; EIMS: m/z (%) = 455 (M+1 18, 5.81%), 437 (M+1 18, 100%); Anal. Calcd. for C₂₂H₂₃NO₅: C 66.93, H 4.99, N 11.56; Found: C, 66.80, H 4.90, N 11.50%.

(RS)-2-(2-Amino-3-cyano-4H-chromen-7-yloxy)acetohydrazide (3)

A mixture of compound 2 (3.50 g, 0.01 mol) and hydrazidine hydroxide (0.055 g, 0.011 mol) in absolute ethanol (30 ml) was heated under reflux for 4 h. The solid formed while hot was filtered and crystallized from absolute ethanol to give 3.02 g (90%) of compound 3. mp 215-217 °C, IR (KBr): 3323.71, 3180.04 (2NH₂, NH), 2922.59 (CH aliph.), 2185.92 (C=O), 1652.34 (C=O); ¹H NMR (CDCl₃-d6): δ 3.79 (s, 3H, OCH₃) 4.66 (d, 2H, OCH₂); 5.10 (s, 1H, C=H); 6.52-7.65 (m, 12H, ArH and 2H, NH); 6.58-7.33 (m, 8 H, Ar H and 2H, NH₂, D₂O exchangeable) ppm; EIMS: m/z (%) = 350 (M⁺ 18, 13.61%), 273 (100%); Anal. Calcd. for C₂₉H₁₃N₂O₅ (350.40): C 68.56, H 5.18, N 16.80%.

General procedures for synthesis of compounds 4a&b

A mixture of compound 3 (1.68 g, 0.005 mol) and the appropriate aromatic aldehyde (0.005 mol) in absolute ethanol (25 mL) was treated with triethyl amine (2-3 drops) and heated under reflux for 2-4 h. The solid formed on hot was filtered, dried and crystallized from the absolute ethanol to give compounds 4 a&b.

(2-Amino-4(phenyl)-3-cyano-4H-chromen-7-yloxy)-N'-(ZE)-4-methoxy benzylideneacetohydrazides (4a)

Yield: 70% mp 213-215 °C. IR (KBr): 3430.74, 3343.01, 3211.86 (NH, NH₂), 3083.62 (CH aliph.), 2926.45(CH aliph.), 2185.92 (C=O), 1698.16 (C=O) cm⁻¹; ¹H NMR (CDCl₃-d6): δ 3.79 (s, 3H, OCH₃) 4.66 (d, 2H, OCH₂); 5.10 (s, 1H, C=H); 6.52-7.65 (m, 12H, ArH and 2H, NH₂, D₂O exchangeable); 7.94 (s, 1H, CH=CN); 11.42 (d, 1H, NH, D₂O exchangeable) ppm; EIMS: m/z (%) = 455 (M⁺ 18, 14.57%), 454 (M⁺ 17, 86.14 %), 133.1 (100%); Anal. Calcd. for C₂₉H₂₂N₂O₅ (454.49): C 67.81, H 4.88, N 12.33; Found: C 67.80, H 4.70, N 12.32%.

(2-Amino-4-(phenyl)-3-cyano-4H-chromen-7-yloxy)-N'-(ZE)-2,3-dimethoxybenzylideneacetohydrazides (4b)

Yield: 60% mp 203-205 °C. IR (KBr): 3430.74, 3317.93, 3231.15 (NH, NH₂), 3066.26 (CH aliph.), 2936.09 (CH aliph.), 2187.85 (C=O), 1685.48 (C=O) cm⁻¹; ¹H NMR (CDCl₃-d6): δ 3.77 (s, 3H, OCH₃) 3.78 (s, 3H, OCH₃); 4.66 (d, 2H, OCH₂); 5.13 (s, 1H, C=H); 6.52-7.45 (m, 11H, ArH and 2H, NH₂, D₂O exchangeable); 8.29 (s, 1H, CH=CN); 11.57 (d, 1H, NH, D₂O exchangeable) ppm; EIMS: m/z (%) = 486 (M⁺ 18, 13.96%), 484 (M⁺ 17, 77.76%), 259.05 (100%); Anal. Calcd. for C₃₀H₂₂N₂O₅ (484.52): C 66.93, H 4.99, N 11.56; Found: C 66.80, H 4.90, N 11.50%.

General procedure for the preparation of compounds 5a&b

To a solution of the acid hydrazide 3 (1.68 g, 0.005 mol) in absolute ethanol (20 mL), was added the appropriate isothiocyanate (0.005 mol). The reaction mixture was heated under reflux for 4 h; the solid formed while hot was collected by filtration, washed with ethanol and crystallized from the suitable solvent to afford 5a&b.

(RS)-2-(2-Amino-3-cyano-4-phenyl-4H-chromen-7-yloxy) acetyl) -N'-ethylhydrazincarbothioamides (5a)

Crystallized from methanol: YIELD: 70%, mp 175-177 °C. IR (KBr): 3430.74, 3323.71, 3191.61 (3NH, NH₂), 3051.8 (CH aliph.), 2929.34 (CH aliph.), 2186.88 (C=O), 1651.73 (C=O), 1285.23, 1232.11 (C=S) cm⁻¹; ¹H NMR (DMSO-d6): δ 1.23 (t, Jvalue = 7.2 Hz, 3H, CH₂CH₃); 3.98 (q, Jvalue = 7.2 Hz, 2H, CH₂CH₃); 4.69 (s, 1H, C=H); 5.22 (s, 2H, OCH₂); 6.75-7.33 (m, 8H, ArH and 2H, NH₂, D₂O exchangeable) ppm; but 3NH not observed; EIMS: m/z (%) = 425 (M⁺ 17, 0.55%), 424 (M⁺ 16, 0.79 %), 423 (M⁺ 15, 2.94 %), 187 (100%); Anal. Calcd. for C₂₉H₂₂N₂O₅S (423.51): C 59.56, H 5.00, N 16.54; Found: C 59.46, H 4.90, N 16.50%.

(RS)-2-(2-Amino-3-cyano-4-phenyl-4H-chromen-7-yloxy) acetyl)-N-substituted hydrazinocarbothioamides (5b)

Crystallized from methanol:acetone (1:1), YIELD: 60%, mp 200-202 °C. IR (KBr): 3479.92, 3379.64, 3164.61 (3NH, NH₂), 3059.51 (CH aliph.), 2964.05 (C=O), 2196.52 (C=O), 1292.51, 1242.43 (C=S) cm⁻¹; ¹H NMR (DMSO-d6): δ 4.35 (s, 1H, NH, D₂O exchangeable); 4.64 (s, 1H, C=H); 4.82 (s, 2H, OCH₂); 6.53-7.42
General procedure for the preparation of compounds 6a&b
A mixture of the appropriate thiosemicarbazide derivative 5a&b (0.005 mol) was heated under reflux for 6 h in a mixture of piperidine (2mL) and water (5mL). The reaction mixture was poured into ice-cold water and the mixture was adjusted to pH 7 with acetic acid (20%). The solid obtained was filtered, dried and crystallized from absolute ethanol to give 6a&b.

(RS)-2-(2-Amino-3-cyano-4-phenyl-4H-chromen-7-yloxy)-N'-(4-oxo-3-ethylthiazolidin-(ZE)-2-ylidene) acetohydrazides (6a)

Yield: 80%, mp 158-160 °C. IR (KBr): 3325.64, 3174.26 (NH, NH$_2$), 2936.09 (CH aliph.), 2186.88 (C≡N), 1646.64 (C=N), 1362.13, 1269.81 (C=S) cm$^{-1}$; $^1$H NMR (DMSO-d$_6$): $\delta$ 1.21 (t, $J$ value = 7.2 Hz, 3H, CH$_2$CH$_3$); 3.97 (q, $J$ value = 7.2 Hz, 2H, CH$_2$CH$_3$); 4.69 (s, 1H, C4H); 5.20 (s, 2H, OCH$_2$); 6.75-7.33 (m, 8H, ArH and 2H, NH$_2$, D$_2$O exchangeable) ppm; but NH not observed; EIMS: m/z (%) = 405 (M$^+$, 1.04 %), 187 (100%); Anal. Calcd. for C$_{21}$H$_{19}$N$_5$O$_2$S (405.48): C 62.21, H 4.72, N 17.27; Found: C 62.20, H 4.90, N 17.20%.

Antioxidant activity
Materials and methods
2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was purchased from Sigma–Aldrich (Missouri, USA). Ascorbic was purchased from Merck (New Jersey, USA). All the used chemicals and solvents were of analytical grade.

DPPH radical scavenging activity
DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of each compound was determined as mentioned before [27-29]. Briefly, 2 mL of DPPH solution (0.2 mmol/L, in ethanol) was incubated with different concentrations of the test compounds. All reactions were performed in triplicates and the reaction mixtures were shaken and wrapped in aluminum foil and kept at room temperature for 30 min in dark. The Spectrophotometric measurements were done under dim light at 517 nm. All data are depicted as mean ± SD (n=3). The IC$_{50}$ (the concentration of sample necessary to cause 50% inhibition of DPPH radical scavenging activity) was calculated for each compound. Ascorbate (Vitamin C), an antioxidant, was used as a positive control.

Yeast based antioxidant screening assay
The yeast based biological assay detects antioxidant activities of samples against physiologically relevant oxidants. Since DPPH radical scavenging activity method provides only an indication of the ability of a compound to scavenge oxidants we used yeast-based method which can also measure the ability of a compound to induce cellular resistance to the damaging effects of oxidants [30]. Antioxidant activity screening was done in a 96-well microplate high throughput assay using Saccharomyces cerevisiae as described before [31]. Briefly, S. cerevisiae (BY4743) were cultured overnight then diluted to an optical density of 0.2 at 600 nm (OD600) in the media. 180 µL of the diluted culture was added per well in a 96-well plate then 10 µL of test compound was added to a final concentration of 8 mM. H$_2$O$_2$ (10 µL) was added into each well to a final concentration of 4 mM. The OD600 reading as an indicator of yeast growth was taken using a microplate reader before and after the incubation at 30°C with shaking for 20 hours. Ascorbic acid was used as a positive control. The net growth of yeast after the treatment with H$_2$O$_2$ and test compounds was measured using the following equation:

\[
\text{Growth} = \frac{OD_{final} - OD_{initial}}{OD_{control} - OD_{initial}}
\]

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RESULTS AND DISCUSSION

Chemistry
One pot three-component reaction of resorcinol, malononitrile and benzaldehyde with few drops of piperidine base afforded aminonachromone 1 which upon alkylation using ethyl chloroacetate in dry dimethyl formamide in the presence of anhydrous potassium carbonate as a catalyst yielded the ester derivative 2 in an excellent yield (Scheme 1). There is no conflict of interest between both the hydroxyl group and the amino group during the alkylation process of compound 1 since; the amino group is very weak to an extent that could not undergo a simple condensation reaction with an aldehyde or benzene sulphonyl chloride [26]. The key intermediate 3 was achieved by refluxing both the substrate 2 and hydrazine hydrate in ethanol for 4 hours. The IR spectrum of 3 revealed band at 3323.71, 3180.04 cm\(^{-1}\) for (NH, NH) groups and at 1662.34 cm\(^{-1}\) for (amidic C=O) group. \(^1\)H NMR spectrum of compound 3, showed singlet signals derived from hydrazide structure appeared at \(\delta\) 4.30 (NH\(_2\) ) and \(\delta\) 9.36 (NH) with the integration for two protons and one proton, respectively. Heating equimolar amounts of the hydrazide 3 with the corresponding aromatic aldehyde in absolute ethanol containing catalytic amount of triethyl amine yielded smoothly Schiff bases 4a&b (Scheme 1).

The structure of the synthesized compounds was confirmed using microanalyses and spectral data. \(^1\)H NMR spectra of compounds 4a&b indicated the presence of the two isomers (ZE) in equimolar amounts, this is obviously showed by presence of two CH\(_2\) groups as the peak is forked at its apex and a doublet signal which was D\(_2\)O exchangeable indicating NH proton (Scheme 1).

In the present work, hoping to synthesize o-Aminocyanopyrazoles A (Figure 1), the acid hydrazide 3 was allowed to react with arylidene malononitrile in ethanol. Actually, the products in hand were neither A nor the starting material 3. Instead, the products were identified as Schiff bases 4a&b.

For the preparation of the thiosemicarbazides 5a&b; the respective isothiocyanate was reacted with the acid hydrazide 3 in refluxing ethanol (Scheme 1). The \(^1\)H NMR spectra of 5a displayed the appearance of a triplet and a quartet signals at \(\delta\) 1.20-1.25 and \(\delta\) 3.95-4.01 due to ethyl group.

The triazolothio derivatives 6a&b were prepared by refluxing the corresponding thiosemicarbazide derivatives 5a&b in piperidine (Scheme 1). The structure of compounds 6a&b was confirmed with the aid of spectroscopic data and element analysis.

Antioxidant activity
In the current study, the antioxidant activities of all newly synthesized compounds were evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method and yeast based antioxidant screening assay. Compound 5a was found to be the most potent antioxidant with the least values of IC\(_{50}\) (inhibition concentration) and the least active compound among the series being compound 1 with the largest values of IC\(_{50}\) (Table 1 and Figure 2). Antioxidant activities of test compounds based on their ability to inhibit the H\(_2\)O\(_2\) induced yeast oxidative stress are listed in Table 2 and illustrated in Figure 3.
Table 1 Free radical scavenging capacities of the test compounds measured by DPPH assay.

Values of IC₅₀ are expressed as means ± SD (n= 3)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Mean IC₅₀ ±SD (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>805.00 ± 26.63</td>
</tr>
<tr>
<td>3</td>
<td>701.67 ± 25.54</td>
</tr>
<tr>
<td>4a</td>
<td>432.33 ± 22.90</td>
</tr>
<tr>
<td>4b</td>
<td>404.67 ± 18.15</td>
</tr>
<tr>
<td>5a</td>
<td>55.67 ± 12.90</td>
</tr>
<tr>
<td>5b</td>
<td>267.33 ± 19.40</td>
</tr>
<tr>
<td>6a</td>
<td>199.00 ± 36.51</td>
</tr>
<tr>
<td>6b</td>
<td>85.33 ± 6.43</td>
</tr>
</tbody>
</table>

Figure 2 Free radical scavenging capacities of the test compounds measured by DPPH assay

Figure 3 Antioxidant activity screening in a 96-well microplate high throughput assay using Saccharomyces cerevisiae.
In conclusion, we have successfully reported the synthesis of some novel compounds bearing 4-aryl 4H-chromene and evaluation of their antioxidant activities. The antioxidant activities of the 8 newly prepared compounds were measured using DPPH radical scavenging method and yeast based antioxidant screening assay. The reactivity of the tested compounds as antioxidant was increased upon hydrazinolysis of 2 to give 3. Also, conversion of 3 to 4a&b was accompanied with increasing of activity which also increased significantly upon reaction of 3 with ethyl isothiocyanate to give the most potent compound 5a but this activity was decreased upon cyclization of 5a to 6a. On contrast the antioxidant activity was increased upon cyclization of 5b to 6b.

REFERENCES