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Graphical Abstract

Antioxidant activity

DPPH Assay

Fluorescence Intensity

Wavelength (nm)
1 Introduction

Aromatic heterocyclic compounds are known to show a wide range of ecotoxic effects, e.g. acute toxicity, cytotoxicity, photo-induced toxicity, mutagenicity and carcinogenicity. New efficient methods for the synthesis of heterocycles are a major interest of modern synthetic organic chemistry. Among the heterocycles isocoumarins represent a foremost class of naturally occurring lactones that display a wide range of biological activities such as protease inhibitor properties, antifungal, cytotoxic, anti-allergic, antimicrobial and antimalarial effects. Isocoumarins are class of natural products with a broad spectrum of biological activity including antioxidative, antiangiogenic, antifungal, antiallergic and antimicrobial activities. They are also important in medicinal chemistry as building blocks for the bioactive compounds. In addition to its natural occurrence, various synthetic methodologies for construction of isocoumarins were disclosed. Isocoumarins are useful synthetic precursors to other heterocyclic and carboxyclic compounds.

Free radicals oxidatively damage lipids, proteins and genomic DNA integrity. They are widely recognized as the root cause of numerous degenerative diseases including cancer. Antioxidants are potent scavengers of free radicals and serve as inhibitors of neoplastic processes. Vegetables, fruits and their seeds are rich sources of vitamins C, E, β-carotene and also compounds that might protect the organism against free radical induced injury and diseases. There has been an increasing interest in the use of natural antioxidants, such as tocopherols, flavonoids for the preservation of food materials. These natural antioxidants avoid the toxicity problems which may arise from the use of synthetic antioxidants, such as butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) and propyl gallate (PG). Due to the complexity of antioxidant activity in foods, the evaluation of synthetic heterocyclic compounds recently widely focused.

2 Results and discussion

2.1 Chemistry

In this work, we have reported the synthesis of novel series of 3-acetyl-2-methyl-1-phenylisonochromone [4, 3-b] pyrrol-5(1H)-ones, 4a-f using MK-10 as catalyst. The antioxidant activity and fluorescence properties of these 3-acetyl-2-methyl-1-phenylisonochromene [4, 3-b] pyrrol-5(1H)-ones, 4a-f have also been reported.

The formation of 4a-f was confirmed by FTIR, 1H NMR, 13C NMR and mass spectral analysis. FT-IR data of compound, 4a shows absorption peak at 1462 cm⁻¹ indicates benzene C=C stretching, peak at 1668 cm⁻¹ indicates alkene C=C stretching, peak at 1732 cm⁻¹ indicates C=O stretching, peaks at 3199-3057 cm⁻¹ indicates sp² C-H stretching. 1H-NMR data of compound, 4a confirms the existence of one methyl of pyrrole ring, one methyl of acetyl group and two methyl of substituted amine groups at δ 1.04, 1.04, 2.32, 2.79 ppm respectively, two methylene protons of the ethyl groups at δ 2.26 and the seven aromatic protons exists between δ 6.2 to 8.36 ppm. 13C-NMR data of compound, 4a confirms the existence of six aliphatic carbons at δ 12.26-31.30 ppm, sixteen aromatic carbons at δ 110.4-142.1 ppm and two carbonyl groups at δ 161.99 (C=O of isocoumarin ring) and 193.66 (C=O of acetyl group).

2.2 Fluorescence studies

In this paper synthesis of isochromenopyrrolone analogues using MK-10 under solvent free approach for organic synthesis is described which involves microwave exposure of neat reactants. The synthesis of 3-acetyl-2-methyl-1-phenylisonochromene [4, 3-b] pyrrol-5(1H)-ones, 4a-f were carried out using MK-10 as catalyst was found to be more efficient with high yield compared to the conventional synthesis method.

Fluorescent and antioxidant studies of effectively synthesized isochromenopyrrolone analogues

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

An efficient strategy for the synthesis of 3-acetyl-2-methyl-1-phenylisonochromeno [4, 3-b] pyrrol-5(1H)-ones, 4a-f have been developed using Montmorillonite K10 as catalyst. The reaction proceeded from acetyl acetone, substituted primary amines and ninhydrin via acidic media under solvent free condition. Compounds, 4a-c exhibits green fluorescence under UV light and its fluorescent property in liquid state were investigated. All the synthesized compounds were subjected for the evaluation of free radical scavenging activity. Among all the tested compounds, 4a & 4c exhibited good percentage inhibition in comparison with the standard ascorbic acid.
For the fluorescence studies, we analyzed that the compound, 4c exhibits a strong emission at 464 nm, the compound, 4b shows emission at 462 nm which is on par to that of 4c and the compound, 4a shows weak emission at 456 nm.

2.3 Hydrogen peroxide scavenging assay
In the present study, Hydrogen peroxide scavenging assay was performed for isochromeno pyrrolones, 4a-f. The absorbance of the standard ascorbic acid and the compounds, 4a-f was measured at 230 nm and the results were tabulated in Table-1. The percentage inhibition of the target molecules, 4a-f have been plotted against the various concentrations of the standard ascorbic acid and the test solutions (Fig. 1). All readings were triplicate to check the accuracy.

2.4 DPPH assay
In the present study, DPPH assay was performed for the isochromeno pyrrolones, 4a-f. The absorbance of the standard ascorbic acid, compounds, 4a-f were measured at 520 nm and the results were tabulated in Table-2. The percentage inhibition of the target molecules, have been plotted against the various concentrations of the standard ascorbic acid and the test solutions 4a-f (Fig. 2). All readings were triplicate to check the accuracy.

3 Experimental
Solvents and reagents were commercially sourced and used without further purification. Thin layered chromatography (TLC) was performed on preparative plates of silica gel. Visualization was made with UV chamber. Column chromatography was performed by using silica gel (60-120 mesh). Melting points were measured on Elchem Microprocessor based DT apparatus using an open capillary tube and are corrected with standard benzoic acid. IR spectrum was obtained on a Nicolet infrared spectrophotometer (KBR disc). UV-vis spectrum was obtained on UV-2550, Shimadzu Corporation, Kyoto, Japan. The NMR spectrum was recorded on a Bruker Avance III- 400 & 500 MHz spectrometer with Tetrachloromethane (TMS) as internal standard. Chemical shifts were reported in δ units (ppm). GC-MS spectrum was recorded on a Perkin Elmer spectrophotometer, GC model: clarus 680, Mass Spectrometer model: clarus 600 (EI).

The fluorescence spectra were obtained on Hitachi F-7000 FL Spectrophotometer.


Method A: (E)-2-hydroxy-2-(2-oxo-4-(phenylimino)pentan-3-yl)-1H-indene-1,3(2H)-dione, 3a
A mixture of acetyl acetone, 1 (1.1 mmol) and aniline, 2f (1.1 mmol) were heated at 85 °C for 15-30 min. Then the reaction mixture was poured into a solution of ninhydrin (1.1 mmol) in acetic acid (10 mL), stirred at room temperature until the disappearance of the reactants. The completion of the reaction was monitored by TLC. After the completion, the reaction mixture was cooled to room temperature and then quenched this mixture into crushed ice. The solid formed was allowed to stand for 30 min and filtered off to dryness. Compound 3f was recrystallized by mixture of aceton and hexane (2:8), gave crystals of intermediate compound, 3.

3-acetyl-2-methyl-1-phenylisochromeno[4,3-b]pyrrol-5(1H)-one, 4f.
About 0.5 mL of Conc. H2SO4 was added to a solution of 200 mg of compound, 3 in 10 mL of acetic acid. The reaction mixture was heated on water bath at 85 °C for about 30-45 min. The initial pale green color of the solution was intensified to brown which indicates the conversion of product, 4a. The completion of the reaction was monitored by TLC. Then the reaction mixture was cooled to room temperature and poured into crushed ice with continuous stirring. Precipitated solid was allowed to stand for 30 min and filtered off to dryness. Then the solid obtained was purified by column chromatography using petroleum ether: ethyl acetate (8:2) as eluent to give the reddish brown crystals of isochromeno pyrrolones, 4a.

Method-B: 3-acetyl-2-methyl-1-phenylisochromeno[4,3-b]pyrrol-5(1H)-one, 4f.
Appropriated acetyl acetone, 1 (1.1 mmol), aniline, 2f (1.1 mmol), ninhydrin (1.1 mmol) followed by adding of MK-10 (10 mg) were introduced in an Erlen-meyer flask. This was subjected to microwave irradiation for sufficient intervals of time using resting interval of 1 min after every 30 s of irradiation. The reaction was monitored by TLC. After cooling, the reaction mixture became sticky and the sticky solid was then triturated to afford the product. The product was recrystallized by mixture of acetone and hexane (2:8), gave crystals of compound, 4f.
Optimization of 3-acetyl-2-methyl-1-phenylisochromeno[4,3-b]pyrrol-5(1H)-one, 4a-f has been done by comparing the yield of conventional and microwave irradiation methods. The results of the comparison were tabulated in Table – 3.

3-acetyl-1-(2,6-diethylphenyl)-2-methylisochromeno[4,3-b] pyrrol-5(1H)-one, (4a)
Pale brown solid, Mol. formula: C23H19NO5. Exact Mass: 373.1678, Mol.Wt.: 373.4443, yield 82 %, M.pt. 209-211 °C. FT-IR (KBr pellets, cm-1): 3388.93, 3199.91, 3057.17, 2966.52, 1732.08, 1668.43, 1462.04. 1H NMR (500MHz, CDCl3): δ= 8.35-8.36 (1H, dd, J = 1.5 Hz J2 = 1 Hz, -CH), 7.55-7.57 (1H, t, -CH3), 7.34 (2H, m, -CH2), 7.30 (1H, d, -CH), 7.28 (1H, d, -CH), 6.27-6.28 (1H, d, J = 6 Hz -CH), 2.79 (3H, s, -CH3), 2.32 (3H, s, -CH3), 2.23 (2×2H, m, 2×CH2) of Et, 1.01-1.05 (2×3H, t, -CH3) of Et. 13C NMR (125MHz, CDCl3): δ= 193.6, 161.9, 142.1, 141.0, 138.8, 134.9, 133.8, 131.7, 130.6, 130.5, 2×127.1, 2×126.5, 117.8, 117.7, 111.5, 110.4, 31.3, 2×23.7, 2×13.7, 12.2. Mass: m/z 373.21.
3-acetyl-1-benzyl-2-methylisochromeno[4,3-b]pyrrol-5(1H)-one (4e). Pale brown solid, Mol. formula: C_{22}H_{20}N_{2}O_{2}. Exact Mass: 331.2087, M. p. 192-194 °C. FT-IR (KBr pellets, cm^{-1}): 3199.91, 2966.52, 1786.08, 1670.35, 1606.70. 1H NMR (500MHz, CDCl_3): δ= 2.60 (3H, s, -CH_3), 2.55 (3H, s, -CH_3), 1.92 (1H, m, VCH), 7.38 (1H, m, -CH), 7.65 (1H, s, VCH), 7.92 (1H, s, -CH), 3.18 (3H, s, VCH). Mass: m/z 331.18.

3-acetyl-2-methyl-1-tolylosichromeno[4,3-b]pyrrol-5(1H)-one (4d). Pale brown solid, Mol. formula: C_{22}H_{20}N_{2}O_{2}. Exact Mass: 331.2087, M. p. 192-194 °C. FT-IR (KBr pellets, cm^{-1}): 3199.91, 2966.52, 1786.08, 1670.35, 1606.70. 1H NMR (500MHz, CDCl_3): δ= 2.60 (3H, s, -CH_3), 2.55 (3H, s, -CH_3), 1.92 (1H, m, VCH), 7.38 (1H, m, -CH), 7.65 (1H, s, VCH), 7.92 (1H, s, -CH), 3.18 (3H, s, VCH). Mass: m/z 331.18.

3-acetyl-2-methyl-1-p-tolylosichromeno[4,3-b]pyrrol-5(1H)-one (4e). Pale brown solid, Mol. formula: C_{22}H_{20}N_{2}O_{2}. Exact Mass: 331.2087, M. p. 192-194 °C. FT-IR (KBr pellets, cm^{-1}): 3199.91, 2966.52, 1786.08, 1670.35, 1606.70. 1H NMR (500MHz, CDCl_3): δ= 2.60 (3H, s, -CH_3), 2.55 (3H, s, -CH_3), 1.92 (1H, m, VCH), 7.38 (1H, m, -CH), 7.65 (1H, s, VCH), 7.92 (1H, s, -CH), 3.18 (3H, s, VCH). Mass: m/z 331.18.

3-acetyl-2-methyl-1-phenylisochromeno[4,3-b]pyrrol-5(1H)-one (4f). Pale brown solid, Mol. formula: C_{22}H_{20}N_{2}O_{2}. Exact Mass: 331.2087, M. p. 192-194 °C. FT-IR (KBr pellets, cm^{-1}): 3199.91, 2966.52, 1786.08, 1670.35, 1606.70. 1H NMR (500MHz, CDCl_3): δ= 2.60 (3H, s, -CH_3), 2.55 (3H, s, -CH_3), 1.92 (1H, m, VCH), 7.38 (1H, m, -CH), 7.65 (1H, s, VCH), 7.92 (1H, s, -CH), 3.18 (3H, s, VCH). Mass: m/z 331.18.

3.2. Antioxidant assay

Preparation of stock solution

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer saline (PBS) at pH 7.4. A stock solution (1 mg/ml) was prepared for the synthesized compounds, 4a-f in methanol and subsequently diluted as 10, 20, 30, 50 & 100 µL.

Hydrogen peroxide scavenging anti-oxidant activity

Antioxidant activity was measured by reported methodology. For the present study the samples were prepared by taking 1 mg of the compounds, 4a-f dissolved in 1 mL of AR grade methanol. The samples 10, 20, 30, 50 & 100 µL were taken in different test tubes to which 20 µL of hydrogen peroxide was added and made up to 2 mL using phosphate buffer and incubated at room temperature for 10 min. The absorbance of the incubated solutions and the blank solution (phosphate buffer without hydrogen peroxide) were recorded against ascorbic acid. The absorbance was measured at 230 nm using a UV-vis Spectrophotometer. Radical Scavenging capacity (RSC) in percent was calculated by the following equation:

\[
\% \text{ of RSC} = \left( \frac{[A_s - A_b]}{A_s} \right) \times 100
\]

Where, RSC = Radical Scavenging Capacity
A_s = Absorbance of sample
A_b = Absorbance of blank

DPPH radical scavenging assay

Radical scavenging activity of compounds against stable DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) was determined spectrophotometrically. When DPPH reacts with an antioxidant compound, which can donate hydrogen, DPPH reduced. The changes in colour (from deep-violet to light yellow) were measured at 520 nm on a UV-vis spectrophotometer. A solution of DPPH was mixed with the synthesized compounds, 4a-f gives rise to the reduced form with the loss of violet color.

\[
\text{DPPH} + \text{RH} = \text{DPPH} + \text{R}^·
\]

Where RH is the reduced form and R· is free radical

The DPPH radical scavenging activities of all the synthesized compounds, 4a-f and ascorbic acid were determined according to the reported method. Initially, 1 mg/mL of the test material solutions and the standard (ascorbic acid), at a concentration of 10, 50 and 100 µg/ml, respectively, were added to 1 mL of 0.2 mM freshly prepared DPPH ethanol solution. The reaction mixture were shaken vigorously and allowed to stand for 30 min at room temperature, under dark conditions. The absorbance of each reaction mixture was measured at 520 nm. The percentage of DPPH radical scavenging activity (%) of the sample was calculated as follows:

\[
\% \text{ Inhibition} = \left( \frac{[A_s - A_b]}{A_s} \right) \times 100
\]

Where A_s= absorption of blank sample
A_b= absorption of sample

3.3 Fluorescence studies

UV-vis Spectra

The UV absorption spectrum of compound, 4a in various solvents such as DMF, ethanol and DMSO solution were recorded in the range of 200-800 nm. The excitation peaks appears at 314 nm and 365 nm respectively (Fig. 5a).

Fluorescence spectra

The fluorescence emission spectra of the synthesized compounds, 4a-c were recorded in DMSO with excitation...
wavelength at 365 nm. The fluorescence emission spectrum of the compounds, 4a-c were depicted in Fig. 5b.

Table 1 Percentage inhibition of H$_2$O$_2$ assay of 3-acetyl-2,5-methyl-1-phenylisochromeno [4, 3-b] pyrrol-5(1H)-ones, 4a-f

<table>
<thead>
<tr>
<th>Conc. µL</th>
<th>4a</th>
<th>4b</th>
<th>4c</th>
<th>4d</th>
<th>4e</th>
<th>4f</th>
<th>%Inhibition</th>
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<td>2.07</td>
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Table 2 Percentage inhibition of DPPH assay of 3-acetyl-2,5-methyl-1-phenylisochromeno [4, 3-b] pyrrol-5(1H)-ones, 4a-f

<table>
<thead>
<tr>
<th>Conc. µL</th>
<th>4a</th>
<th>4b</th>
<th>4c</th>
<th>4d</th>
<th>4e</th>
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<td>1.90</td>
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Table 3 Reaction parameters of 3-acetyl-2,5-methyl-1-phenylisochromeno [4, 3-b] pyrrol-5(1H)-ones, 4a-f

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<td>1.10 82</td>
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Notes and references

Conclusions

In present study, we report an efficient one pot MK-10 catalysed synthesis of fused isochromenopyrrole-5(1H)-ones gave an enhanced yield with lesser time than the conventional heating. Out of the synthesized compounds 4c & 4b exhibits strong fluorescence emission. The synthesized compounds were successfully screened for the free radical scavenging activity by H$_2$O$_2$ and DPPH assay method. Compounds 4a, 4b & 4c showed a high percentage of inhibition similar to that of standard Ascorbic acid.

Notes and references

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FIGURE CAPTIONS

Fig.1: Comparison of percentage inhibition of H$_2$O$_2$ assay of 3-acetyl-2-methyl-1-phenylisochromeno [4, 3-b] pyrrol-5(1H)-ones, 4a-f

Fig.2: Comparison of percentage inhibition of DPPH assay of 3-acetyl-2-methyl-1-phenylisochromeno [4, 3-b] pyrrol-5(1H)-ones, 4a-f

Fig.3: [a] Comparison of UV–Vis spectrum (DMF, ethanol and DMSO) of 4a. [b] Fluorescence emission spectra of compounds, 4a-c

Scheme 1: Synthesis of 3-acetyl-2-methyl-1-phenylisochromeno [4, 3-b] pyrrol-5(1H)-ones, 4a-f
Fig. 1

\[ \text{H}_2\text{O}_2 \text{ Assay} \]

<table>
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<tr>
<th>Stnd.</th>
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<th>4b</th>
<th>4c</th>
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% of Inhibition

CONC. µL

Fig. 1
DPPH Assay

% of Inhibition

CONC. µL

Stnd. 4a 4b 4c 4d 4e 4f

Fig. 2
Fig. 3
Scheme 1

(i) glacial AcOH, 85 °C; (ii) MK-10 catalyst; (iii) conc. H₂SO₄

R = (a) 2,6-diethylphenyl; (b) 2,3-dimethylphenyl; (c) benzyl; (d) m-tolyl; (e) p-tolyl; (f) phenyl.