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Note

Synthesis and antioxidant activity of indolyl chromenes

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A simple and efficient method for the synthesis of indolyl chromenes **4a-h** has been accomplished by the one-pot three-component reaction of salicylaldehyde and malononitrile with indole catalyzed by l-proline in water. All the synthesized compounds are evaluated for antioxidant activity. All of the compounds **4a-h** exhibited excellent free radical scavenging activity and found to be more potent than the standard. Compounds **4b-c** and **4h** showed very high reducing power.

Keywords: Indolyl chromenes, l-proline, antioxidant, reducing power, radical scavenging.

Recent evidence[1] suggests that free radicals, which are generated in any bioorganic redox processes, may induce oxidative damage in various components of the body (e.g., lipids, proteins and nucleic acids) and may also be involved in processes leading to the formation of mutations. Furthermore, radical reactions play a significant role in the development of life-limiting chronic diseases such as cancer, hypertension, cardiac infarction, arteriosclerosis, rheumatism, cataracts and others[2].

Antioxidants may be classified according to their mode of action as being free radical terminators, chelators of metal ions involved in catalyzing lipid oxidation or oxygen scavengers that react with oxygen in closed systems[3]. A number of methods are available for the determination of free radical scavenging activity but the assay employing the stable 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH) has received the maximum attention owing to its ease of use and its convenience[4]. This assay is the most widely used *in vitro* test to assess free radical scavenging capacities[5]. In the DPPH assay, the antioxidant activity of a compound is evaluated spectrophotometrically by monitoring the decrease in absorbance at 517 nm as $DPPH^{\cdot}$ (purple) transformed to the reduced form DPPH-H (yellow).

The Chromene (or benzopyran) moiety often appears as an important structural component in both biologically active and natural compounds. Chromene fragments occur in alkaloids, flavonoids, tocopherols and anthocyanins[6]. Moreover, functionally substituted chromenes have played increasing roles in synthetic approaches to promising compounds in the field of biomedicinal chemistry[7]. The current interest in 4*H*-chromene derivatives bearing a nitrile functionality arises from their potential application as antimicrobial[8], antiviral[9], mutagenicial[10], antiproliferative[11], sex pheromone[12], antitumour[13] and in the treatment of human inflammatory TNF α-medicated diseases, such as rheumatoid and psoriatic arthritis and of cancer therapy[14]. Thus, in view of the diverse therapeutic activity of chromenes and in continuation of our interest in the synthesis of novel biologically active compounds[15], we herein report an efficient eco-friendly one-pot method for the synthesis of indolyl chromenes using a catalytic amount of inexpensive l-proline in water at RT.

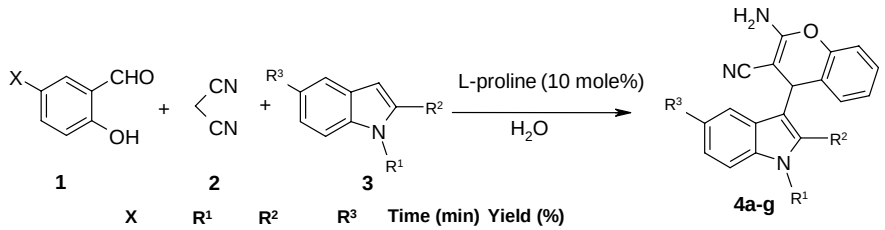
Results and Discussion

Chemistry

The reactions were carried out by first mixing salicylaldehyde **1**, malononitrile **2** and indole **3** in water. Then, a catalytic amount of l-proline (10 mole) was added. The reaction proceeded spontaneously at ambient temperature and was completed within 30-45 min (**Scheme I**). The use of inexpensive catalyst, low catalytic loading, and excellent yields in a shorter span of time makes this methodology superior to our previous report[16].

Similarly, the protocol was extended to synthesize indole with fused chromene **4h** by the reaction of naphth-2-ol-1-carboxaldehyde **5** and malononitrile **2** with indole **3** in 92% yield (**Scheme II**).

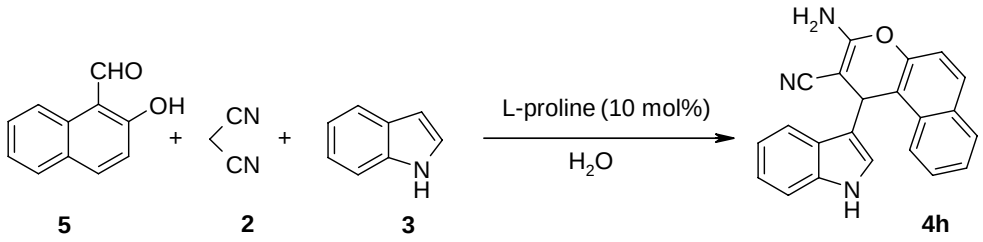
The structures of compounds **4a-h** were confirmed by IR, ^{1}H and ^{13}C NMR spectroscopy, mass spectrometry and elemental analysis and compared with the literature value[16].



1 2 3 4a-g

	X	R^1	R^2	R^3	Time (min)	Yield (%)
4a	H	H	H	H	30	90
4b	H	CH_3	H	H	25	92
4c	H	H	CH_3	H	30	93
4d	H	H	H	OCH_3	35	95
4e	H	H	H	Br	40	85
4f	H	H	H	Cl	45	86
4g	Br	CH_3	H	H	40	86

Scheme I — Synthesis of Indolyl chromanes **4a-g**



5 2 3 4h

Scheme II — Synthesis of indole with fused chromene **4h**

Biological Evaluation

Antioxidant activity

This is the first time where the reducing power and the free radical scavenging activity of indolyl chromenes and their evaluation as *in vitro* anti-oxidants is reported.

Reducing power assay

The reducing power of indolyl chromenes **4a-h** was determined according to Oyaizu's method[17] using ascorbic acid as standard. Mean values from three independent samples were calculated for each compound with standard deviation less than 5%. **Figure 1** shows the reducing power of ethanolic solutions of indolyl chromenes **4a-h** as a function of their concentration. In this assay, the yellow colour of the test solution changes to various shades of green and blue depending upon the reducing power of each compound. The presence of reducers (ie., antioxi-dants) causes the conversion of the Fe^{3+}/ferricyanide complex used in this method to the ferrous form. Therefore, by measuring the formation of Perl's Prussian blue at 700 nm we can monitor the Fe^{2+} concentration; a higher absorbance at 700 nm indicates a higher reducing power. The reducing power of indolyl chromenes in ethanol increases with increase in concentration **Figure 1**. Compounds **4b**, **4c** and **4h** present, at 0.25 g/L, a reducing power even higher than that of ascorbic acid (**4b**, 2.026; **4c**, 1.87; **4h**, 1.78, ascorbic acid, 1.72). Reducing power values for compounds **4a**, **4d-g** were much lower.

Radical scavenging activity assay (RSA)

All compounds were tested for their interaction with the stable free radical DPPH. This interaction indicates their radical scavenging activity in an iron-free system following Hatano's method[18], using 2-tert-butyl-4-methoxyphenol (butylated hydroxyani-sole, BHA) and 2,6-di-tert-butyl-4-methylphenol (butylated hydroxy toluene, BHT) as standards. The radical scavenging activity (RSA) for ethanolic solutions of indolyl chromenes **4a-h** are presented in **Table I** and compared with those of standards BHA and BHT. The results, in percentage, are expressed as the ratio of absorbance decrease at 517 nm, and the

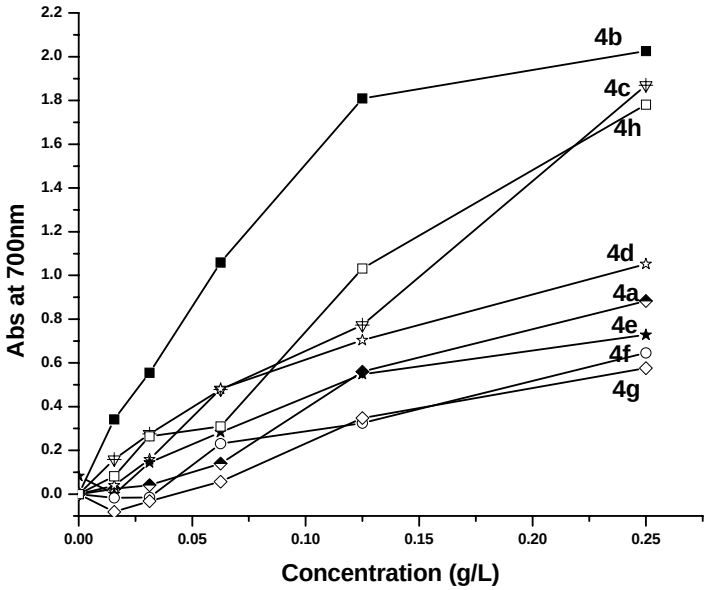


Figure 1 — Reducing power of Indolyl chromenes **4a-h**; Ascorbic acid: Abs_{700nm}= 1.72 at 0.25 g/L.

Table 1- Scavenging activity (%) on DPPH radicals of indolyl chromenes **4a-h** and standards BHA and BHT in ethanol

Compound	Compound concentration (g/L)			
	0.0156	0.0312	0.0625	0.125
4a	26.3	54.7	73.3	81.9
4b	42.8	67.8	84.5	99.0
4c	29.8	48.0	76.5	94.0
4d	31.1	56.2	78.3	96.4
4e	27.2	45.6	71.2	86.8
4f	21.5	44.8	67.8	85.8
4g	37.8	53.2	71.9	92.7
4h	18.7	37.9	67.0	85.0

BHA 0.25 g/L, 84%; BHT 0.25 g/L, 83%

absorbance of DPPH solution in the absence of indolyl chromenes. The analysis of **Table I** leads us to conclude that the RSA of indolyl chromenes on DPPH radicals increases with the increase in concentration. Compounds **4b-h** showed excellent radical scavenging activity (82-99%) at 0.125 g/L concentration and found to be higher than the standards BHA and BHT. Compound **4b** showed highest activity (99%) at 0.125 g/L. The radical scavenging activity of compound **4a** was comparable to or slightly less potent than the standards BHA and BHT. All other compounds **4b-h** exhibited more potent radical scavenging activities than the standards at concentration 0.125 g/L itself. At concentrations greater than 0.125 g/L, the radical scavenging activity for all the compounds exceeded the maximum limit.

Conclusion

In conclusion this work describes for the first time the *in vitro* antioxidant activity of indolyl chromenes as potential antioxidants. Most of the compounds assayed showed excellent reducing power and free radical scavenging activities. Compounds **4b**, **4c** and **4h** are the most active among the series showing high reducing power and radical scavenging activities than the standards. Further work on the activity of these compounds in an expanded panel of organisms and *in vivo* efficacy models will be reported in due course.

Experimental Section

Materials

All melting points are uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 using TMS as an internal standard on a JEOL spectrometer at 500 MHz and 125 MHz respectively. Mass spectra were recorded on a JEOL DX 303 HF spectrometer. Elemental analyses were recorded using a Thermo Finnigan FLASH EA 1112 CHN analyzer. Analytical TLC was performed on precoated plastic

sheets of silica gel G/UV-254 of 0.2 mm thickness (Macherey-Nagel, Germany).

General procedure for the synthesis of indolyl chromenes 4a-h

To a reaction-mixture containing salicylaldehyde (1 mmole), malononitrile (1 mmole) and indole (1 mmole) in water (10 mL), catalytic amount of L-proline (10 mole%) was added and stirred at RT for about 30-45 min. On completion, ethyl acetate was added and the organic layer was separated. The organic layer was distilled off and column chromatographed with a mixture of ethyl acetate and pet. ether (3:7) to afford pure indolyl chromenes.

Procedure for the determination of reducing power

Several concentrations of ethanolic solutions of compounds **4a-h** (**Figure 1**, 2.5 mL) were mixed with 2.5 mL of 200 mmole/L sodium phosphate buffer (*p*H 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Afterwards, 2.5 mL of 10% trichloroacetic acid (w/v) was added and the mixture was centrifuged at 650 rpm for 10 min. The upper layer (5 mL) was mixed with 5 mL of deionised water and 1 mL of 0.1% $FeCl_3$ and the absorbance was measured at 700 nm.

Procedure for the determination of radical scavenging activity

Several concentrations of ethanolic solutions of compounds **4a-h** (**Table I**) were prepared. The compound solution (1 mL) was added to the ethanolic DPPH solution (2 mL) and the mixture was kept in the dark for 60 min. The absorbance at 517 nm was then measured. The RSA was calculated as a percentage of DPPH decolouration using the equation: % RSA = 100 × (1 – A_C/A_D) where A_C is the solution absorbance measured when the compound was added at a particular concentration and A_D is the absorbance of the DPPH solution.

Spectral data

2-Amino-4-(1*H*-indol-3yl)-4*H*-chromene-3-carbonitrile **4a**: yellow solid; m.p. 190-92ºC. (IR, KBr): 3435, 3324, 2189, 1655, 1606, 1400, 1223, 752 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 4.96 (s, 1H), 6.80 (br s, 2H, NH_2, D_2O exchangeable), 6.83 (t, 1H, *J* = 8.45 Hz), 6.96 (m, 5H), 7.20 (d, 1H, *J* = 8.4 Hz), 7.27 (s, 1H), 7.30 (d, 1H, *J* = 8.4 Hz), 10.91 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 33.0, 56.8, 112.3, 116.3, 118.9, 119.0, 119.2, 121.5, 123.6, 124.2, 124.9, 128.4, 129.8, 137.4, 148.9, 160.6; MS (*m*/*z*): 287 (M^+). Anal. Calcd for $C_{18}H_{13}N_3O$: C, 75.25; H, 4.56; N, 14.62. Found: C, 75.20; H, 4.51; N, 14.58%.

2-Amino-4-(2-methyl-1*H*-indol-3yl)-4*H*-chromene-3-carbonitrile **4c**: yellow solid; m.p. 186-89ºC. (IR, KBr): 3476, 3363, 2185, 1645, 1572, 1457, 1226, 750 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 2.44 (s, 3H), 5.05 (s, 1H), 6.75 (br s, 2H, NH_2, D_2O exchangeable), 6.76 (t, 1H, *J* = 7.65 Hz), 6.90 (t, 2H, *J* = 7.4 Hz), 6.94 (d, 1H, *J* = 7.6 Hz), 6.99 (d, 1H, *J* = 8.4 Hz), 7.03 (d, 1H, *J* = 7.65 Hz), 7.15 (t, 1H, *J* = 7.4 Hz), 7.21 (d, 1H, *J* = 8.4 Hz), 10.83 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 11.8, 31.5, 56.7, 111.2, 114.5, 116.2, 118.0, 118.8, 120.6, 121.4, 124.0, 124.9, 127.1, 128.4, 129.9, 132.7, 136.0, 149.1, 160.2; MS (*m*/*z*): 301 (M^+). Anal. Calcd for $C_{19}H_{15}N_3O$: C, 75.73; H, 5.02; N, 13.94. Found: C, 75.66; H, 4.97; N, 13.87%.

2-Amino-4-(5-methoxy-1*H*-indol-3yl)-4*H*-chromene-3-carbonitrile **4d**: yellow solid; m.p. 199-201ºC. (IR, KBr): 3435, 3337, 2186, 1646, 1411, 1209, 755 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.63 (s, 3H), 4.95 (s, 1H), 6.69 (d, 1H, *J* = 8.4 Hz), 6.76 (s, 1H), 6.82 (br s, 2H, NH_2, D_2O exchangeable), 6.96 (t, 1H, *J* = 7.65 Hz), 7.03 (d, 1H, *J* = 7.65 Hz), 7.08 (d, 1H, *J* = 7.65 Hz), 7.15 (t, 1H, *J* = 6.9 Hz), 7.21 (t, 2H, *J* = 8.4 Hz), 10.75 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 33.0, 55.7, 56.8, 101.5, 110.0, 112.8, 116.2, 119.2, 121.5, 124.1, 124.3, 124.9, 126.2, 128.4, 129.8, 132.6, 149.1, 153.3, 160.8; MS (*m*/*z*): 317 (M^+). Anal. Calcd for $C_{19}H_{15}N_3O_2$: C, 71.91; H, 4.76; N, 13.24. Found: C, 71.86; H, 4.71; N, 13.20%.

2-Amino-4-(5-bromo-1*H*-indol-3yl)-4*H*-chromene-3-carbonitrile **4e**: yellow solid; m.p. 160-62ºC. (IR, KBr): 3450, 3345, 2190, 1650, 1407, 1222, 748 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 4.98 (s, 1H), 6.86 (br s, 2H, NH_2, D_2O exchangeable), 6.96 (t, 1H, *J* = 7.65 Hz), 7.02 (t, 2H, *J* = 8.4 Hz), 7.12 (d, 1H, *J* = 8.4 Hz), 7.16 (t, 1H, *J* = 6.9 Hz), 7.30 (d, 1H, *J* = 8.4 Hz), 7.34 (d, 2H, *J* = 6.85 Hz), 11.16 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 32.7, 56.5, 111.8, 114.4, 116.4, 119.1, 121.1, 121.2, 123.9, 124.1, 125.0, 125.4, 127.6, 128.6, 129.8, 136.1, 148.9, 160.6; MS (*m*/*z*): 366 (M^+), 368 (M^++2). Anal. Calcd for $C_{18}H_{12}BrN_3O$: C, 59.04; H, 3.30; N, 11.47. Found: C, 58.98; H, 3.26; N, 11.42%.

2-Amino-4-(5-chloro-1*H*-indol-3yl)-4*H*-chromene-3-carbonitrile **4f**: yellow solid; m.p. 169-71ºC. (IR, KBr): 3452, 3340, 2192, 1654, 1403, 1226, 743 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ 4.98 (s, 1H), 6.84 (m, 10H), 11.13 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 32.7, 56.5, 113.9, 116.4, 118.0, 119.1, 121.6, 123.7, 123.9, 125.0, 125.6, 126.8, 128.6, 129.8, 135.9, 148.9, 160.6; MS (*m*/*z*): 321 (M^+), 323 (M^++2). Anal. Calcd for $C_{18}H_{12}ClN_3O$: C, 67.19; H, 3.76; N, 13.06. Found: C, 67.12; H, 3.72; N, 13.02%.

2-Amino-6-bromo-4-(1-methyl-1*H*-indol-3yl)-4*H*-chromene-3-carbonitrile **4g**: brownish yellow solid; m.p. 196-99ºC. (IR, KBr): 3456, 3343, 2189, 1657, 1608, 1477, 1227, 738 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ 3.71 (s, 3H), 5.0 (s, 1H), 6.90 (br s, 2H, NH_2, D_2O exchangeable), 6.92 (d, 1H, *J* = 7.65 Hz), 7.01 (d, 1H, *J* = 8.4 Hz), 7.08 (d, 1H, *J* = 8.4 Hz), 7.19 (s, 1H), 7.26 (m, 2H), 7.32 (d, 2H, *J* = 8.4 Hz); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 32.6, 32.9, 56.4, 110.6, 116.4, 118.0, 118.8, 118.9, 119.4, 121.0, 121.9, 125.9, 126.9, 131.2, 132.0, 137.7, 148.2, 160.4; MS (*m*/*z*): 380 (M^+), 382 (M^++2). Anal. Calcd for $C_{19}H_{14}BrN_3O$: C, 60.02; H, 3.71; N, 11.05. Found: C, 59.98; H, 3.67; N, 11.01%.

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