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Discovery and synthesis of novel 3-phenylcoumarin derivatives as antidepressant agents ☆

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ABSTRACT

A series of 3-phenylcoumarins were synthesized and screened for potential antidepressant activity by tail suspension test (TST) in mice. Three compounds (**6**, **7** and **13**) exhibited impressive antidepressant activity, measured in terms of percentage decrease in immobility duration (% DID). In addition, the active antidepressant compounds were subsequently studied at their most effective dose and activity of these compounds were confirmed in forced swimming test (FST) animal model, in which the compounds at a low dose of 0.5 mg/kg significantly decreased the immobility time and exhibited greater efficacy than the reference standards fluoxetine and imipramine. The potent compounds did not show any neurotoxicity in the rotarod test and the preliminary results are promising enough to warrant further studies around this scaffold.

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Depression is a serious and burdensome psychiatric illness associated with high rates of chronicity, relapse and that is characterized generally, by pervasive low mood, anxiety, cognitive impairment, loss of interest or pleasure in normally enjoyable activities and suicidal behaviours.[1,2] According to WHO estimation, 121 million people worldwide suffer from mental depression. The high prevalence of suicide in depressed patients (up to 15%) coupled with complications arising from stress and its effect on the cardiovascular system have suggested, that it will become the second leading cause of premature death or disability worldwide by the year 2020.[3] Despite a broad range of antidepressants available today, a significant proportion of these patients will not respond to treatment, or will show only partial response.[4] Clinical limitations and adverse effects of currently used antidepressants necessitate continuous development of novel, efficient and safe drugs for treatment of depression.

Natural as well as synthetic coumarins have recently drawn much attention due to its diverse pharmacological activities. Many coumarins and their derivatives underwent extensive investigations aimed to assess their potential beneficial effects on human health.[5,6] In addition many coumarin derivatives have antidepressant properties.[7–10] The recognition of key structural features within coumarin family is crucial for the design and development of new analogues with improved activity and for the characterization of their mechanism of action and potential side effects. The different substituents in the coumarin nucleus strongly influence the biological activity of the resulting derivatives.

Figure 1 shows the chemical structures of some potent antidepressant molecules that contains a coumarin in its molecular makeup and form the basis of our designed prototype. Capra et al. revealed that the scopoletin (Fig. 1) isolated from Polygala sabulosa produces a specific antidepressant-like effect in the tail suspension test, an animal model predictive of antidepressant activity and was also able to reverse a depressant-like behaviour induced by acute immobility stress.[11] Also, coumarins such as Psoralidin[12] (Furocoumarins) and Esuprone[13] belong to the new generation of monoamine oxidase (MAO) inhibitors and demonstrated to possess potent antidepressant properties. Many synthetic efforts based on this coumarin scaffold are also currently under investigation for antidepressant activity as exemplified by the compounds LU 53439[9] (Fig. 1) and other coumarin derivatives.[14–17]

In continuation of our interest, in this class of compounds,[18] we embarked on the synthesis of novel coumarin derivatives as potential antidepressant agents. Herein, we wish to describe the synthesis and biological evaluation of novel 3-phenylcoumarin derivatives as potential antidepressant agents.

The synthesis of the substituted 3-phenylcoumarins is illustrated in Scheme 1. Commercially available 2-substituted phenols (**1** and **2**) underwent the Duff formylation reaction in presence of hexamethylene tetramine (HMTA) and TFA at 120 °C to furnish substituted salicylaldehydes (**3** and **4**). Subsequent Perkin

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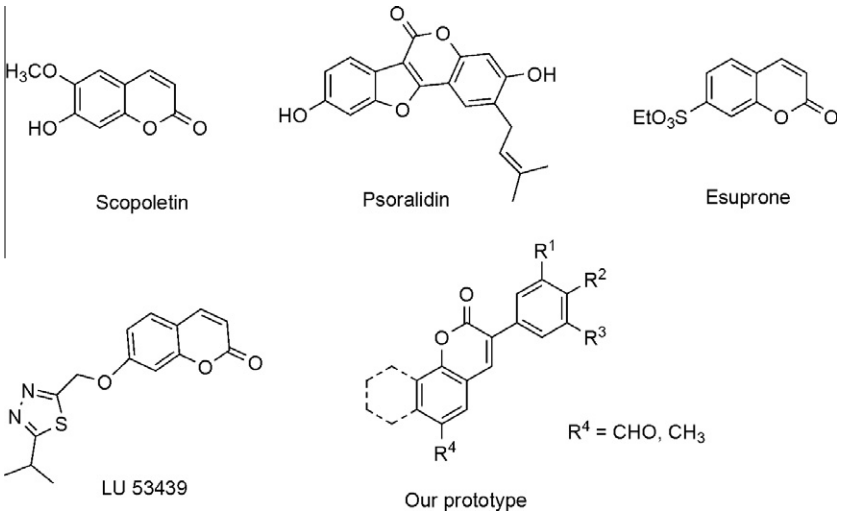
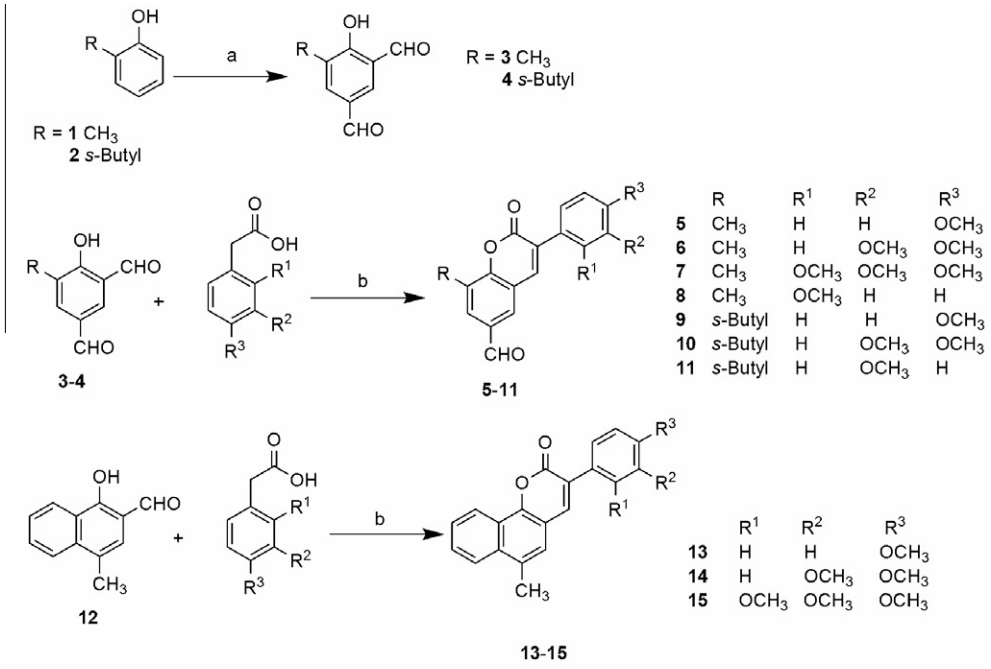


Figure 1. Chemical structure of some coumarin based potent antidepressant agents and general structure of our synthesized compounds.



Scheme 1. Synthesis of novel 3-phenylcoumarin derivatives (**5**–**11** and **12**–**15**). Reagents and conditions: (a) (1) HMTA/TFA, 120 °C, 3 h; (2) 10% H_2SO_4, 90–100 °C, 2 h; (b) DCC, DMSO, 110 °C, 24 h.

condensation[19] reaction of these substituted salicylaldehydes and the appropriately substituted phenylacetic acids, was carried out with *N*,*N*-dicyclohexylcarbodiimide (DCC) as dehydrating agent, in DMSO, at 110 °C reflux, for 24 h, resulted in the formation of coumarinic compounds (**5**–**11**). Similarly, the other substituted salicylaldehyde compound **12** underwent the Perkin reaction with the appropriately substituted phenylacetic acids to furnish another set of coumarinic compounds in good yields (**13**–**15**).[20] Structures of the compounds were substantiated by ^{1}H NMR, ^{13}C NMR, 2D NMR, Mass spectrometry and IR spectroscopy. The purity of these compounds was ascertained by TLC and spectral analysis (Supplementary data).

In the IR spectrum of the prototype compound **13** exhibited absorption band of lactone carbonyl at 1705 cm^{-1}. Presence of aromatic skeleton is confirmed by peak at 3067 cm^{-1} corresponding to aromatic C–H stretching. The ^{1}H NMR of **13** displayed singlets at δ 2.61, 3.82, 7.33, & 7.83 for methyl protons, methoxy protons, proton at C-5 and proton C-4, respectively. Remaining multiplets at δ 6.99–8.62 affirmed the presence of eight aromatic protons. ^{13}C NMR of **13** indicated presence of methyl carbon with peak at δ 19.2, methoxy carbon gave a peak at δ 55.4 and while the carbonyl carbon resonated at δ 161.0. Peaks for eighteen carbons in the aromatic region from δ 114.0 to 160.1, underlined the presence of required aromatic skeleton. Mass spectra (ESI-MS) of **13** showed a molecular ion peak at *m*/*z* 317 $(M+H)^+$.[21]

For the purpose of biological evaluation of synthesized compounds, adult male Swiss Albino mice (20–25 g) were used. Mice were housed in six per cage and maintained in humidity and temperature controlled rooms with day–night cycle. They were allowed to acclimatize with the environment for one week before commencement of the experiments. Free access to food and water was permitted. All synthesized compounds **5**–**11** and **13**–**15** were

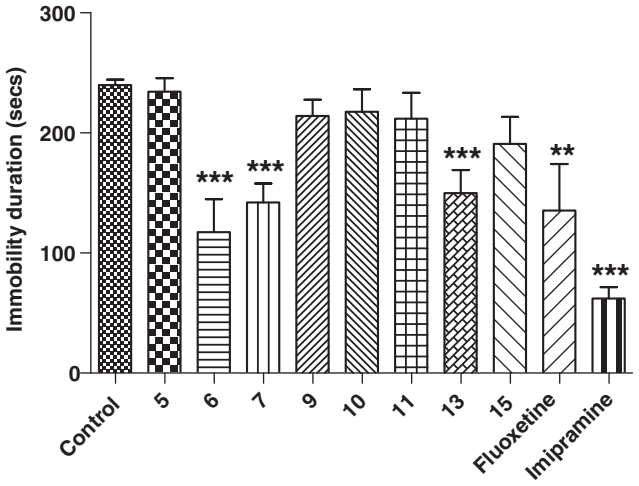
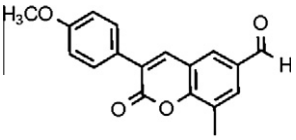
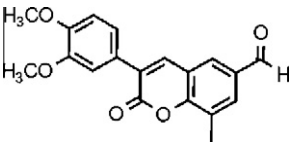
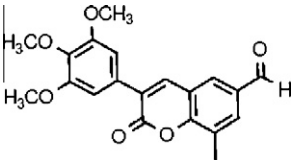
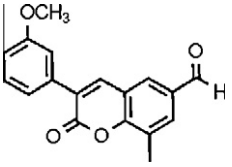
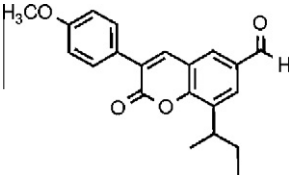
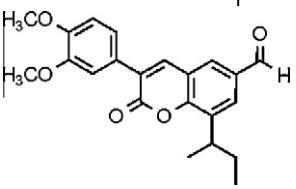


Figure 2. Effect of treatment of mice with novel 3-phenylcoumarin derivatives (at dose of 0.5 mg/kg), fluoxetine (20 mg/kg) and imipramine (30 mg/kg) given intraperitoneally (ip) on the immobility time in the tail suspension test. Results are represented as mean ± S.E.M. with $n = 12$ in each group. Values are significant at $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ when compared with control group.

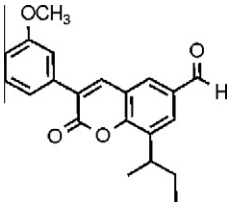
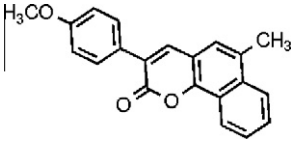
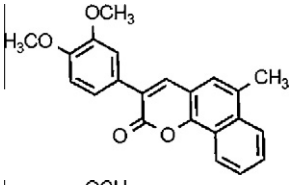
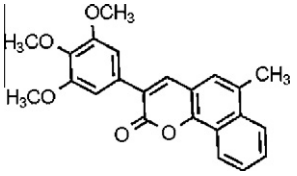
assayed for antidepressant activities at dose of 0.5 mg/kg (ip), in tail suspension test (TST)[22,23] model in mice and the results are shown in Figure 2. Among 10 compounds tested, three compounds (**6**, **7** and **13**) showed potent antidepressant activity. In TST, after initial escape-oriented movements, mice develop an immobile posture when placed in an inescapable stressful situation. This stressful situation involved haemodynamic stress of being hung in an uncontrollable fashion by their tail. Duration of immobility was recorded by a fire-wire camera for a 6 min period and analyzed using ANY-maze video tracking system (Stoelting CO, v.4.72). In this established behavioural model, the ability of a compound to decrease immobility duration is taken as a measure of its antidepressant activity. The solutions of standard drugs (fluoxetine, and imipramine) were prepared in water. The solutions of the synthesized compounds were prepared by dissolving these compounds in DMSO and diluted with water till the concentration became 1.0 mg/mL and administered intraperitoneally (ip) to mice. To control group, 1% DMSO was injected (ip) at a constant volume of 0.1 mL/10 g. The acute treatment of synthesized compounds (0.5 mg/kg, ip) was administered 30 min prior to experiments. Similarly, fluoxetine (20 mg/kg, ip) and imipramine (30 mg/kg, ip), conventional antidepressants were used as a positive control. Test was performed 30 min after the administration of last dose repetition. Percentage decrease in immobility duration (% DID)

Table 1
Evaluation of novel 3-phenylcoumarin derivatives at a dose of 0.5 mg/kg, ip in tail suspension test model in mice

Compound	Structure	Duration of immobility (s) (mean ± SEM)	% Decrease in immobility duration (% DID)
5		234.4 ± 11.19	78.1
6		117.5 ± 27.36	39.1
7		142.0 ± 15.77	47.3
8		ND	
9		214.2 ± 13.43	71.4
10		217.6 ± 18.77	72.5

(*continued on next page*)

Table 1 (*continued*)

Compound	Structure	Duration of immobility (s) (mean ± SEM)	% Decrease in immobility duration (% DID)
11		212.0 ± 21.50	70.6
13		149.8 ± 19.21	49.9
14		ND	
15		191.0 ± 22.50	63.6
Control		239.8 ± 4.47	79.9
Fluoxetine (20 mg/kg)		135.3 ± 38.86	45.1
Imipramine (30 mg/kg)		62.00 ± 7.13	20.6

ND = not done due to solubility problem.

for test and standard drugs was calculated using following formula:

$$\%\text{DID} = [(A - B)/A] * 100$$

Where A is the duration of immobility (s) in control group and B is the duration of immobility (s) in test group.

Majority of the synthesized compounds exhibited considerable antidepressant potential as evident from their duration of immobility values and high % DID values (Table 1). Compounds (**6**, **7** and **13**) that were found most potent in the tail suspension test (Fig. 2), were further evaluated in forced swim test (FST)[24] for confirmation of their antidepressant activity. Forced swimming test in mice, is a behavioural despair test. This experimental model is based on the observation that mice, when forced to swim in an inescapable condition, after an initial period of agitation/flurry, adopt an immobility behavior. Thus, mice were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing approximately 20 cm of water at 25 ± 1 °C. The immobility time, defined as the absence of escape-oriented behaviors, such as swimming, was recorded using a fire-wire camera for duration of 6 min and analyzed ANY-maze video tracking system (Stoelting CO, v.4.72). Each mouse was judged immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. Conventional antidepressants decrease the immobility time in this test. All three compounds (**6**, **7** and **13**), have shown good activity profile at very low dose 0.5 mg/kg compare to standard drug imipramine at the dose of 30 mg/kg ip and the results are shown in Figure 3.

After confirmed antidepressant activities of the compounds (**6**, **7** and **13**), these potent compounds were next tested for their neurological toxicity by rotarod toxicity test as described by Dunham and Miya (1957)[25] and were studied in the Rotamex 4/8 apparatus

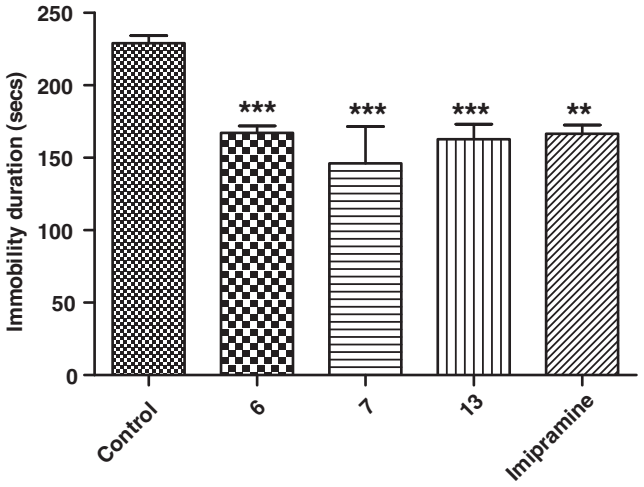


Figure 3. Effect of treatment of mice with novel 3-phenylcoumarin derivatives (at dose of 0.5 mg/kg) and imipramine (30 mg/kg) given intraperitoneally (ip) given on the immobility time in the forced swim test. Results are represented as mean ± S.E.M. with $n = 12$ in each group. Values are significant at $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ when compared with control group.

(M/s Columbus Instruments, USA). Rotarod consisted of a rod which was coated with polypropylene foam to provide friction and to prevent animals from slipping off the rod. The distance between rod and floor was kept 15 cm to avoid intentional jumping of mice. The rod was driven by a motor and the rotational speed was maintained at 8 rpm. Animals were trained on the rotarod for 2 min per trail, with 3 day per trial for two day. On the third day, mice were given trial before and after treatment of coumarin derivatives. The compounds

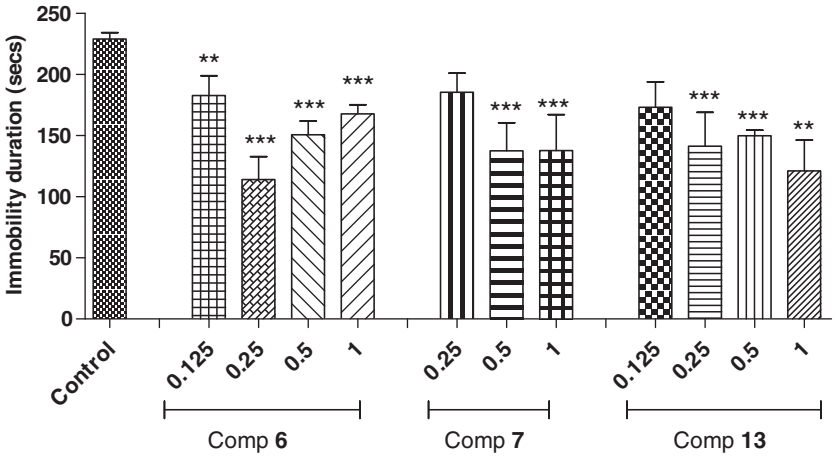


Figure 4. Effect of treatment of mice with compounds **6**, **7**, and **13** given intraperitoneally (ip) at graded doses on the immobility time in the forced swim test. Results are represented as mean ± S.E.M. with $n = 12$ in each group. Values are significant at $^{*}P$ <0.05, $^{**}P$ <0.01, $^{***}P$ <0.001 when compared with control group.

were used at highest dose of 1 mg/kg (ip), to evaluate any defect in motor coordination. All the three compounds did not show any defects in neuro-muscular coordination.

Furthermore, dose dependent studies on the active compounds (**6**, **7**, and **13**) at different doses (Fig. 4) 0.125, 0.25, 0.5, and 1.0 mg/kg, revealed that the compound **6**, **7** and **13** produced their maximal effects at the dose of 0.25, 0.5, and 0.25 mg/kg, respectively.

Among 10 compounds tested, three compounds (**6**, **7** and **13**) showed potent antidepressant activity and the compounds have good activity profile at very low dose compared to the standard drugs fluoxetine (at the dose of 20 mg/kg, ip) and imipramine (30 mg/kg, ip). The compounds **8** and **14** were not screened due to lack of solubility. It is interesting to note that the presence of bulky aliphatic group (*sec*-butyl group) on C-8 position as in the compounds **9**, **10** and **11** tend to decrease the antidepressant activity drastically. Also, the changes on the methoxy substituent position on the phenyl ring in the 3-position of coumarin seem to modulate the pharmacologic potential of the synthesized coumarins. Surprisingly, the substitution at position 6 with either electron withdrawing (compounds **6** and **7**) or by the electron donating group as in the case of compound **13** seem to have no effect, as the activity was conserved in both and further diversification at this point will be interesting.

To conclude, the present work revealed the synthesis of novel 3-phenylcoumarin derivatives and many of these compounds significantly reduced immobility duration at very low dose of 0.5 mg/kg in TST and FST which was comparable to standard drugs such as fluoxetine (20 mg/kg, ip) and imipramine (30 mg/kg, ip), underlying their antidepressant potential. Further, the most potent compound **6** did not show any neurotoxicity in the rotarod test and the preliminary results are promising enough to warrant further detailed mechanistic studies and antidepressant research around this scaffold.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.02.040.

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21. 3-(4-Methoxyphenyl)-6-methyl-2*H*-benzo[*h*]chromen-2-one (**13**). Purified by chromatography using 98:2 hexane/ethyl acetate as eluent. Light yellow solid, yield: 68%; mp 135–137 °C; IR (KBr): 3067, 1705, 1641, 1026 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.62–8.58 (m, 1H), 8.01–7.98 (m, 1H), 7.83 (s, 1H), 7.70 (d, J = 8.8 Hz, 2H), 7.68–7.64 (m, 2H), 7.33 (s, 1H), 6.96 (d, J = 8.8 Hz, 2H), 3.87 (s, 3H), 2.70 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 161.0, 160.1, 149.1, 139.0, 133.7, 130.8, 129.8, 128.3, 127.4, 127.1, 126.8, 124.4, 123.6, 122.9, 122.7, 114.9, 114.0, 55.4, 19.2; DEPT ($CDCl_3$, 75 MHz) δ: 139.0, 129.8, 128.3, 126.8, 124.4, 123.6, 122.7, 114.0, ESI-MS: m/z: 317 $(M+H)^{+}$.
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