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Synthesis and evaluation of new 3-phenylcoumarin derivatives as potential antidepressant agents ☆

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ABSTRACT

A series of amine substituted 3-phenyl coumarin derivatives were designed and synthesized as potential antidepressant agents. In preliminary screening, all compounds were evaluated in forced swimming test (FST), a model to screen antidepressant activity in rodents. Among the series, compounds **5c** and **6a** potentially decreased the immobility time by 73.4% and 79.7% at a low dose of 0.5 mg/kg as compared to standard drug fluoxetine (FXT) which reduced the immobility time by 74% at a dose of 20 mg/kg, ip. Additionally, these active compounds also exhibited significant efficacy in tail suspension test (TST) (another model to screen antidepressant compounds). Interestingly, rotarod and locomotor activity tests confirmed that these two compounds do not have any motor impairment effect and neurotoxicity in mice. Our studies demonstrate that the new 3-phenylcoumarin derivatives may serve as a promising antidepressant lead and hence pave the way for further investigation around this chemical space.

Depression, is a widespread and burdensome psychiatric disorder. It affects approximately 350 million people globally.[1] The World Health Organization has indicated that by year 2020, depression will be the second leading cause of disability throughout the world.[2] Pathophysiology of depression is commonly associated with decrease in the synaptic concentration of monoaminergic neurotransmitters. All the available antidepressants increase the synaptic concentration of these neurotransmitters. The commercially available of antidepressants such as tricyclic antidepressants (TCA), monoamine oxidase inhibitors (MAOI), selective serotonin reuptake inhibitors (SSRI) and serotonin noradrenaline reuptake inhibitors (SNRI) require several weeks for onset of action, and are associated with numerous side effects such as, emesis and sexual dysfunction.[3] Moreover approximately 30% of patients do not respond to the currently available antidepressants and the remaining 70% do not experience remission.[4,5] Hence, discovery and development of new antidepressants with greater efficacy is still desirable.

Coumarins (chromen-2-ones or benzopyran-2-ones) are interesting heterocyclic molecules, which are present as a structural motif in numerous natural products. Many coumarins and their derivatives are known to possess a broad range of biological activities depending on their substitution pattern.[6–11] The activity reported for coumarins include anticancer, antioxidant, anti-inflammatory, antimicrobial, and antiviral.[12–15] In particular, several coumarin derivatives have been reported for their significant antidepressant activities (Fig. 1).[16,17]

Several reports have appeared in the literature that 4-methyl and 3,4-dimethyl-7-oxycoumarins incorporated with oxadiazoles, thiadiazoles, triazoles and thiazolidinones preferentially inhibit MAO activity.[17] Psoralen, a major furocoumarin isolated from *Psoralea corylifolia*, displayed antidepressant activity in the FST in mice, a model of 'learned helplessness' that is used in the antidepressant screening.[18] Psoralen has been reported to decrease the immobility time of the animal in FST. In previous work, our research group had reported the design, synthesis and pharmacological evaluation of 3-phenylcoumarin derivatives as potential antidepressant agents.[19] The good results encouraged us to explore the chemical diversity around this pharmacophore. In addition, since the structures of well known antidepressants like nefazodone, aripiprazole bear a piperazine ring in their molecular makeup, and also the drugs like fluoxetine, duloxetine, amitifadine have secondary amine in their structures, it was of interest to synthesize a series of new amine substituted 3-phenylcoumarin derivatives. These derivatives had a number of substituents that could

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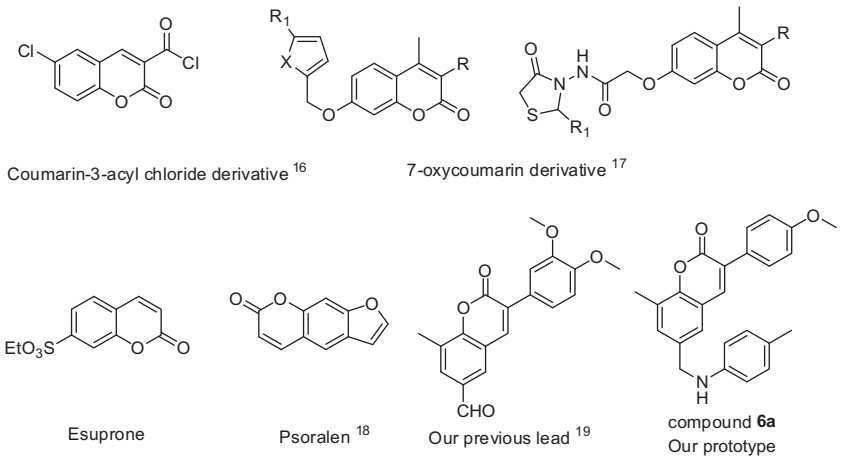
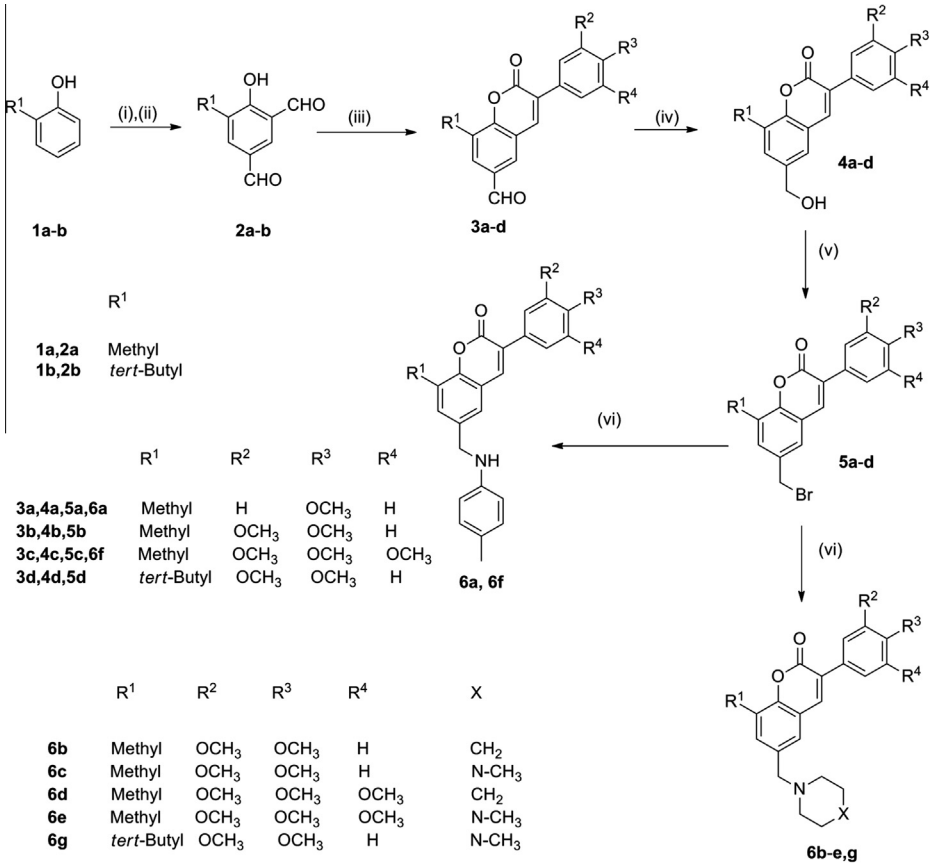


Figure 1. Chemical structures of some biologically important coumarins and our prototype.

influence lipophilic or electronic characteristics (H-bonding properties). Figure 1 shows the chemical structures of some coumarin containing potent antidepressant molecules and our designed prototype.

Synthetic procedure for the preparation of substituted 3-phenylcoumarins is presented in Scheme 1. The 2-alkyl phenols (**1a**,**b**) were subjected to the Duff formylation in the presence of hexamethylenetetramine (HMTA) and trifluoroacetic acid (TFA) at 120 °C to furnish the dicarbaldehyde intermediates (**2a**,**b**).[20] These intermediates were then reacted with different substituted phenyl acetic acids in the presence of cyanuric chloride and *N*-methylmorpholine to give 3-arylcoumarins (**3a–d**).[21] Reduction of 3-arylcoumarins with $NaBH_4$ in methanol gave primary alcohol derivatives (**4a–d**). Nucleophilic substitution of these alcohols with PBr_3 gave alkyl bromides (**5a–d**),[22] which on reaction with amines produced N-substituted products (**6a–g**).[23] All the new synthesized compounds were characterized by using ^1H NMR, ^{13}C NMR, 2D NMR, Mass spectrometry and IR spectroscopy (see Supporting information).

Screening of compounds for antidepressant activity in FST model: To see the antidepressant activity of 3-phenylcoumarins, FST was



Scheme 1. Synthesis of 3-phenylcoumarin derivatives. Reagents and conditions: (i) HMTA, TFA, 120 °C, 4 h; (ii) aq H_2SO_4, 100 °C, 2 h; (iii) phenylacetic acid derivatives, cyanuric chloride, *N*-methylmorpholine, DMF, reflux, 1 h; (iv) $NaBH_4$, rt, 5 min; (v) PBr_3, benzene, reflux, 30 min; (vi) different amines, EtOH, reflux, 1 h.

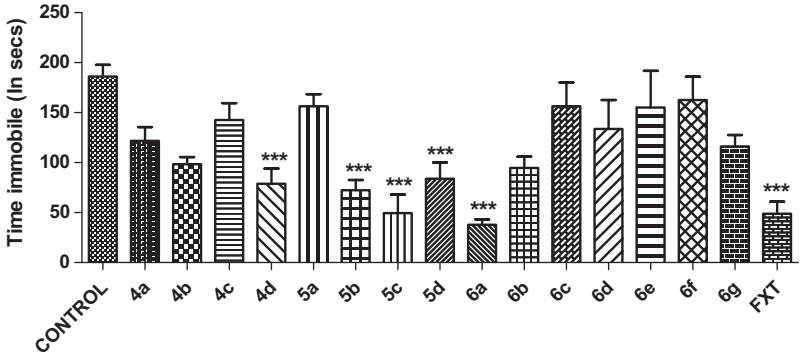


Figure 2. Effect of 3-phenylcoumarin derivatives on the immobility duration of mice in the FST. Results are expressed as mean ± SEM ($n = 8$ each). Mice were intraperitoneally administrated 1% DMSO, 3-phenylcoumarin derivatives (0.5 mg/kg) and FXT (20 mg/kg), respectively. $^{***}p < 0.001$ when compared with the control.

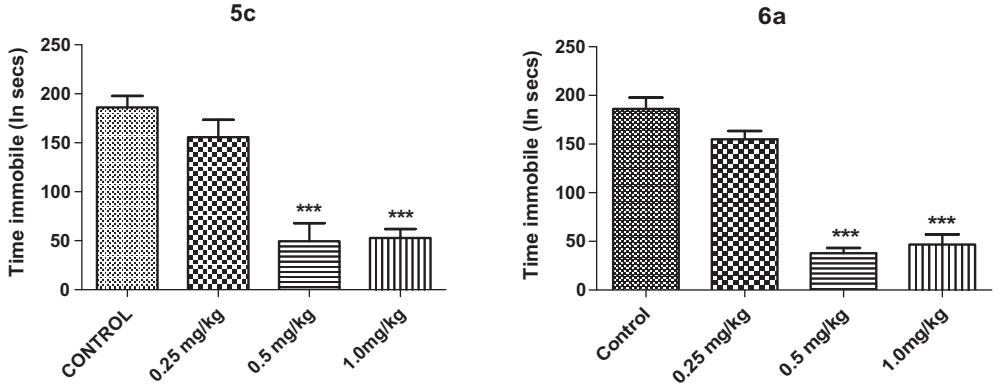


Figure 3. Graded dose response of effective compounds **5c** and **6a** shows significant reduction in immobility time of mice. Data are represented as mean ± SEM ($n = 8$). $^{***}p < 0.001$ versus control.

performed.[24] Compounds were administered intraperitoneally (ip) at a dose of 0.5 mg/kg into Swiss mice. After 30 min, mice were subjected to FST and immobility time was recorded by using ANY-maze software. Results in Figure 2 indicate that compounds **4d**, **5b**, **5c**, **5d** and **6a** significantly reduced the immobility time ($p < 0.001$) of mice, when compared with vehicle treated group. This figure further suggest that compounds **4d**, **5b** and **5d** reduced the immobility time by 58%, 61% and 55%, respectively, while **5c** and **6a** reduced it by 73% and 80%, respectively. The standard drug FXT, however was able to reduce the immobility time by 74% only, at a dose of 20 mg/kg body weight (Fig. 2).

Evaluation of effect of active compounds on immobility time in dose dependant manner in FST model: Further effective compounds (>60% activity) were studied at graded doses (0.25, 0.5 and 1.0 mg/kg, ip) in the FST model. Compounds **5c** and **6a** reduced immobility time by 16% and 17%, respectively at a dose of 0.25 mg/kg, ip. Interestingly, compound **5c** at the higher doses of 0.5 and 1.0 mg/kg significantly reduced the immobility duration by 73% and 71%, respectively. Similarly, compound **6a** reduced the immobility time by 80% and 75% at 0.5 and 1.0 mg/kg doses, respectively (Fig. 3). However, since the difference in percentage between the two doses (0.5 and 1 mg/kg) of both compounds was not statistically significant, we selected the lower dose of both compounds for further screening on TST model of depression.

Effect of active compounds on immobility in tail suspension test (TST): In order to confirm the activity of compounds **5c** and **6a** at the effective dose of 0.5 mg/kg, TST was performed.[25] Compounds **5c** and **6a** significantly reduced the immobility time by 49% and 53% ($p < 0.001$), respectively, which is comparable to 54% reduction in immobility time shown by standard drug FXT (Fig. 4).

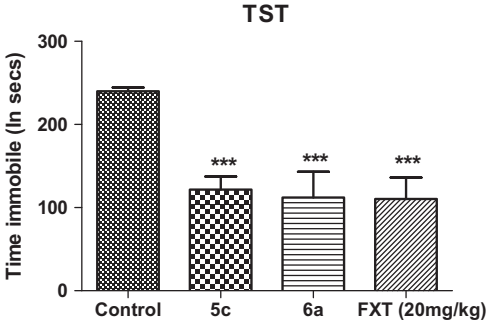


Figure 4. Effect of compounds **5c** and **6a** (0.5 mg/kg, ip) and FXT (20 mg/kg, ip) on immobility duration in TST. Results are expressed as mean ± SEM ($n = 8$). $^{***}p < 0.001$ when compared with the control.

Effect of active compounds on locomotor activity: In order to examine the effect of 3-phenylcoumarins on central nervous system stimulating/depressing effect, we performed open field locomotor activity test. Locomotor activity is a standard test performed during drug discovery phase.[26] Effect of compounds **5c** and **6a** were examined on horizontal activity counts (HC) and total distance travelled (TD) at the dose of 0.5 mg/kg ip. For this, mice were placed into digiscan test chambers and after 30 min, active compounds were administered. The activity of mice was

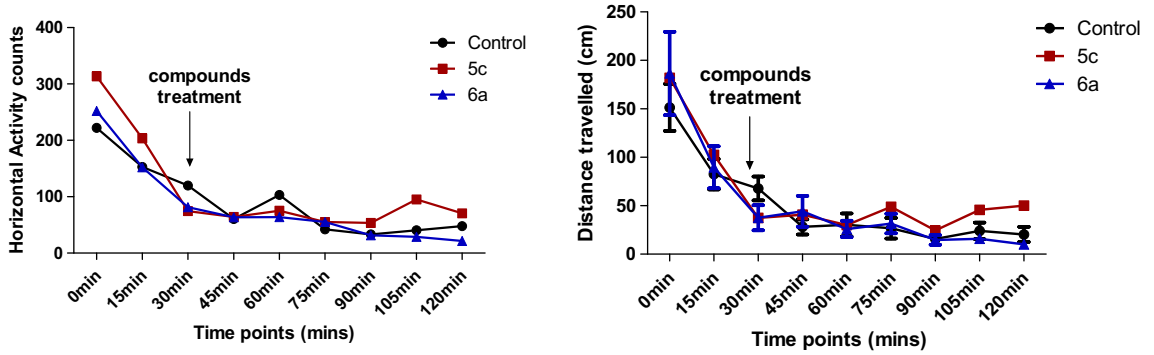


Figure 5. Effect of compounds on locomotor activity (a) horizontal activity (HC) and (b) Total distance travelled (TD). Compounds were administered at 30 min time point and counts were recorded for total 120 min in digiscan animal activity monitor. Values are expressed as mean ± SEM ($n = 8$).

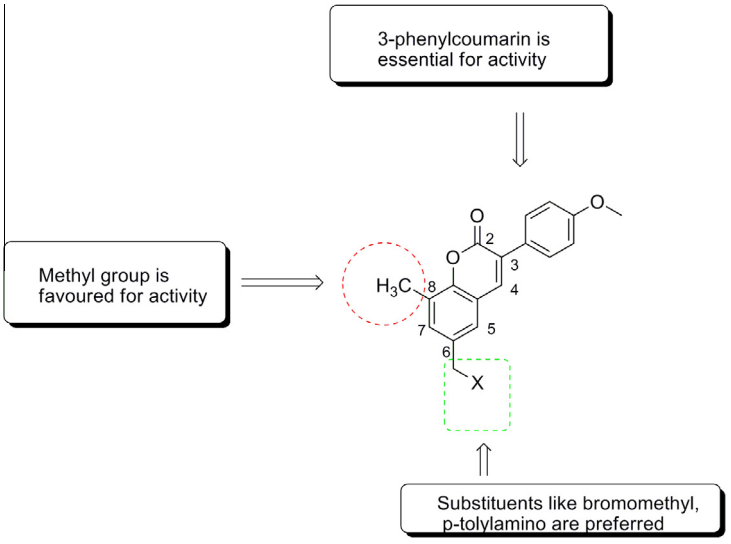


Figure 6. SAR of synthesized hybrid.

monitored up to 120 min. Mice treated with compounds did not show any statistical significant difference from the vehicle-treated control groups in both the parameters (HC and TD). This confirms that the active compounds at their effective dose did not altered locomotor activity in mice as shown in Figure 5. Further, compounds were tested on rotarod[27] to rule out any effect on neuromuscular coordination and no fall was observed in any animal during the entire 120 s duration of the trial (Supporting information Table S1).

Structure–activity relationships (SARs) were inferred from in vivo activity on Swiss albino mice. The initial examination of SAR revealed that the presence of methyl group at position 8 of the phenyl ring and the presence of 3-phenylcoumarin scaffold is crucial for activity. Substituents such as bromomethyl, (*p*-tolylamino)methyl at position 6 are favoured (**5b**, **5c** and **6a**) as these considerably enhanced the antidepressant activity. A pictorial representation of the SAR is depicted in Figure 6.

*Intraperitoneal and intravenous pharmacokinetics of compound **6a** in mice*: The pharmacokinetic studies were conducted in the pharmacological activity model (Swiss albino mice). Compound **6a** shows ~73% bioavailability at 10 mg i.p dose. The pharmacokinetic estimates such as C_{max} were 105.00 ± 9.89 and 256.00 ± 46.66 ng/mL while $AUC_{0-\infty}$ were 315.78 ± 31.48 and 2329.00 ± 440.17 h ng/mL for intravenous and ip, respectively (Fig. 7 and Table 1). Longer half-life after ip administration (7.57 ± 1.27 h) favors the once a day dosing interval. Compound

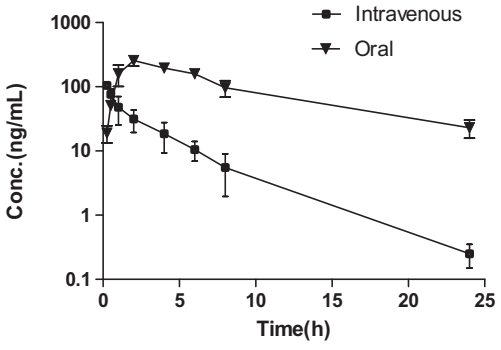


Figure 7. Pharmacokinetic profile of Compound **6a** after ip and iv administration.

6a shows rapid absorption after ip dose helps in the rapid onset of action. This pharmacokinetic study underscores the utility of **6a** as potential antidepressant lead. Positive control FXT bioavailability was 60–90% as reported in the literature.[28]

In conclusion, among all the compounds prepared, compounds **5b**, **5c** and **6a** displayed significant antidepressant-like activity in the FST model. Interestingly compounds **5c** and **6a** exhibited higher potency in reducing immobility time by 73% and 80% at a dose of 0.5 mg/kg, which is comparable to the standard drug FXT.

Table 1
Pharmacokinetic parameters of compound **6a**

Parameters	Intravenous (1 mg/kg)	Intraperitoneal (10 mg/kg)
C_{max} (ng/mL)	105.00 ± 9.89	256.00 ± 46.66
T_{max} (h)		2.00 ± 0.00
$AUC_{0-\infty}$(h ng/mL)	315.78 ± 31.48	2329.00 ± 440.17
V_d (L/kg)	18.01 ± 3.84	30.45 ± 1.64
Cl (L/h/kg)	4.04 ± 1.57	2.83 ± 0.62
$t_{1/2}$ (h)	3.21 ± 0.58	7.57 ± 1.27
F (%)		73.42 ± 6.62

T_{max}, time at which maximum concentration achieved in plasma; C_{max}, maximum concentration achieved in plasma; $AUC_{0-\infty}$, area under the curve from 0 to ∞ h; V_d, volume of distribution; Cl, clearance; $t_{1/2}$, terminal half-life; F, absolute bioavailability.

However, **6a** was more effective than FXT in this antidepressant model. Further, the potency of compound **5c** and **6a** was also confirmed by TST model. In addition, active compounds did not exhibit any motor impairment effect as confirmed by rotarod and digiscan animal activity monitor. Taken together, among the 3-phenylcoumarin derivatives, we identified compound **6a** as a potential antidepressant lead candidate.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.08.037.

References and notes

1. World Health Organisation. Available at: http://www.who.int./mental/management/depression/en/index.html.
2. Murray, C. J.; Lopez, A. D. *Lancet* **1997**, *349*, 1436.
3. (a) Zisook, S.; Rush, A. J.; Haight, B. R.; Clines, D. C.; Rockett, C. B. *Biol. Psychiatry* **2006**, *59*, 203; (b) Papakostas, G. I.; Worthington, J. J.; Iosifescu, D. V.; Kinrys, G.; Burns, A. M.; Fisher, L. B.; Homberger, C. H.; Mischoulon, D.; Fava, M. *Depress. Anxiety* **2006**, *23*, 178.
4. Nierenberg, A. A.; Amsterdam, J. D. *J. Clin. Psychiatry* **1990**, *51*, 39.
5. Berton, O.; Nestler, E. J. *Nat. Rev. Neurosci.* **2006**, *7*, 137.
6. Sashidhara, K. V.; Kumar, A.; Kumar, M.; Singh, S.; Jain, M.; Dikshit, M. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 7034.
7. Sashidhara, K. V.; Kumar, M.; Modukuri, R. K.; Srivastava, A.; Puri, A. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6709.
8. Sashidhara, K. V.; Kumar, A.; Kumar, M.; Sonkar, R.; Bhatia, G.; Khanna, A. K. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4248.
9. Sashidhara, K. V.; Rosaiah, J. N.; Kumar, A.; Bhatia, G.; Khanna, A. K. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3065.
10. Sashidhara, K. V.; Kumar, A.; Kumar, M.; Srivastava, A.; Puri, A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6504.
11. Sashidhara, K. V.; Rosaiah, J. N.; Kumar, M.; Gara, R. K.; Nayak, L. V.; Srivastava, K.; Bid, H. K.; Konwar, R. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7127.
12. (a) Belluti, F.; Fontana, G.; DalBo, L.; Carenini, N.; Giommarelli, C.; Zunino, F. *Bioorg. Med. Chem.* **2010**, *18*, 3543; (b) Riveiro, M. E.; Moglioni, A.; Vazquez, R.; Gomez, N.; Facorro, G.; Piehl, L.; de Celis, E. R.; Shayo, C.; Davio, C. *Bioorg. Med. Chem.* **2008**, *16*, 2665.
13. (a) Fylaktakidou, K. C.; Hadjipavlou-Litina, D. J.; Litinas, K. E.; Nicolaides, D. N. *Curr. Pharm. Des.* **2004**, *10*, 3813; (b) Roussaki, M.; Kontogiorgis, C. A.; Hadjipavlou-Litina, D.; Hamilakis, S.; Detsi, A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3889.
14. Ostrov, D. A.; Hernandez Prada, J. A.; Corsino, P. E.; Finton, K. A.; Le, N.; Rowe, T. C. *Antimicrob. Agents Chemother.* **2007**, *51*, 3688.
15. Neyts, J.; DeClercq, E.; Singha, R.; Chang, Y. H.; Das, A. R.; Chakraborty, S. K.; Hong, S. C.; Tsay, S. C.; Hsu, M. H.; Hwu, J. R. *J. Med. Chem.* **2009**, *52*, 1486.
16. Patil, P. O.; Bari, S. B.; Firke, S. D.; Deshmukh, P. K.; Donda, S. T.; Patil, D. A. *Bioorg. Med. Chem.* **2013**, *21*, 2434.
17. Abdelhafez, O. M.; Amin, K. M.; Ali, H. I.; Abdalla, M. M.; Batran, R. Z. *J. Med. Chem.* **2012**, *55*, 10424.
18. Xu, Q.; Pan, Y.; Yi, L. T.; Li, Y. C.; Mo, S. F.; Jiang, F. X.; Qiao, C. F.; Xu, H. X.; Lu, X. B.; Kong, L. D.; Kung, H. F. *Biol. Pharm. Bull.* **2008**, *31*, 1109.
19. Sashidhara, K. V.; Kumar, A.; Chatterjee, M.; Rao, K. B.; Singh, S.; Verma, A. K.; Palit, G. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1937.
20. Sashidhara, K. V.; Modukuri, R. K.; Sonkar, R.; Rao, K. B.; Bhatia, G. *Eur. J. Med. Chem.* **2013**, *68*, 38.
21. Sashidhara, K. V.; Palnati, G. R.; Avula, S. R.; Kumar, A. *Synlett* **2012**, 611.
22. Nicolaou, K. C.; Lister, T.; Denton, R. M.; Gelin, C. F. *Tetrahedron* **2008**, *64*, 4736.
23. *Preparation of 3-(4-methoxyphenyl)-8-methyl-6-((p-tolylamino) methyl)-2H-chromen-2-one* (**6a**)*:* In 50 mL round-bottomed flask, compound **5a** (0.200 g, 1 mmol) was dissolved with 20 mL of ethanol, then added 4-methylaniline (0.059 g, 1 mmol) successively. The mixed solution was stirred at 75 °C for about 1 h by TLC detected. After the completion of the reaction, the solvent was removed by evaporation and the residue was chromatographed with $CHCl_3$–MeOH (95:5, v/v) to afford the desired compound **6a** as white solid, yield: 72%; mp 121–122 °C; IR (KBr): 3019, 1407, 1071, 757 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 7.70 (s, 2H), 7.66 (d, J = 8.8 Hz, 2H), 7.35 (s, 2H), 7.26 (s, 1H), 7.00–6.95 (m, 4H), 6.56 (d, J = 8.3 Hz, 2H), 4.34 (s, 2H), 3.85 (s, 3H), 2.48 (s, 3H), 2.23 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): 167.9, 161.1, 160.2, 150.9, 145.7, 139.0, 135.7, 132.5, 131.5, 131.0, 129.9, 128.9, 127.6, 127.3, 127.2, 126.2, 123.8, 119.6, 114.0, 113.1, 55.4, 20.5, 15.6; ESI-MS: (m/z): 386 $(M+H)^+$.
24. *Forced swimming test (FST):* Swiss mice were divided into three groups and each group was treated with vehicle, 3-phenylcoumarins (0.5 mg/kg, ip) and standard drug FXT (20 mg/kg, ip). After 30 min of administration, mice were subjected to FST, a behavioural despair model of depression. FST was performed according to the procedure described by Porsolt et al. (Porsolt, R. D.; Le Pichon, M.; Jalfre, M. *Nature* **1977**, *266*, 730.). Briefly, mice were forced to individually swim in water maintained at 25 °C in a transparent glass cylinder (20 cm height, 10 cm diameter) having a height of 10 cm. After 5 min, the animals were removed from water, wiped and returned back to their home cages. They were again placed in the cylinder 24 h later and the total duration of immobility during swimming was recorded by a digital camera during the last 5 min of a 6 min recording session by using ANY-maze software. Initial 1 min was however considered as an acclimatization period..
25. *Tail suspension test (TST):* TST was conducted as previously described by Steru et al. (Steru, L.; Chermat, R.; Thierry, B.; Simon, P. *Psychopharmacology* **1985**, *85*, 367.). Briefly, Swiss mice were individually suspended 60 cm above the surface of a table by their tail with an adhesive tape placed approximately one cm away from the tip of tail. Mice were suspended for 6 min after 1 min acclimatization period and the immobility duration was recorded for the last 5 min of the session by using ANY maze software. Mice were considered immobile only when they were hanging passively or remained completely motionless..
26. *Open-field locomotor activity test (Spontaneous motor activity):* The open-field test to evaluate spontaneous locomotor activity (SLA) is routinely done during drug screening process to rule out any adverse effect of test compound on locomotion. SLA include different types of movements such as distance travelled, rearing, and grooming. Open field activity was performed by using Digiscan Infrared Photocell system, Omnitech Electronics, Columbus, Ohio consisting of 42 cm × 42 cm × 30 cm plexiglas arenas, fitted with infrared beam containing metallic grid (Sanberg, P. R.; Zoloty, S. A.; Willis, R.; Ticarich, C. D.; Rhoads, K.; Nagy, R. P.; Mitchell, S. G.; Laforest, A. R.; Jenks, J. A.; Harkabus, L. J.; Gurson, D. B.; Finnefrock, J. A.; Bednarik, E. J. *Pharmacol. Biochem. Behav.* **1987**, *27*, 569.). Activity of the animals was observed by interruption of infrared beams. During experiments, initially the control and treated animals (Swiss mice) were habituated in the experimental cage for 30 min. Following this, drugs were administered at appropriate doses and activity of animals was monitored for 2 min at regular intervals of 30 min for total 2 h duration. Gross locomotor activity of animals is represented in terms of horizontal activity. All cages were connected with a counting module, which counts the number of interruptions..
27. *Rotarod test:* Rotarod test is commonly used for evaluation of neuromuscular coordination in rodents upon administration of any test compound and the protocol was used as described by Dunham et al. and studied in the Rotamex 4/8 apparatus (M/s Columbus Instruments, USA) (Dunham, N. W.; Miya, T. S. *J. Am. Pharm. Assoc. Am. Pharm. Assoc. (Baltim)* **1957**, *46*, 208.). Basically, the rotarod consists of a rotating rod which is coated with polypropylene foam to provide friction and to prevent animals from slipping off the rod. The distance between the rod and floor is kept 15 cm to avoid intentional jumping of mice. The rod is driven by a motor and the rotational speed was maintained at 8 rpm in our study. Swiss mice were trained for two days on the rotarod for duration of 2 min per trial, with 3 trials per day. On the third day, mice were given trials before and after administration of test compounds..
28. Hui, Y. H.; Huang, N. H.; Ebbert, L.; Bina, H.; Chiang, A.; Maples, C.; Pritt, M.; Kern, T.; Patel, N. *J. Pharmacol. Toxicol. Methods* **2007**, *56*, 256.