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Design and synthesis of new series of coumarin–aminopyran derivatives possessing potential anti-depressant-like activity ☆

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ABSTRACT

A new series of coumarin based aminopyran derivatives were designed, synthesized and evaluated for their preclinical antidepressant effect on Swiss albino mice. Among the series, compounds **21**, **25**, **26**, **27**, **32** and **33** exhibited significant activity profile in forced swimming test (FST). Compound **27** was most efficacious, which at a very low dose of 0.5 mg/kg reduced the time of immobility by 86.5% as compared to the standard drug fluoxetine (FXT) which reduced the immobility time by 69.8% at the dose of 20 mg/kg, ip. In addition, all active compounds were screened in dose dependent manner (at doses of 0.25, 0.5, 1 mg/kg ip) in FST and tail suspension test (TST). Interestingly, all active compounds did not caused any significant alteration of locomotor activity in mice as compared to control, indicating that the hybrids did not produce any motor impairment effects. The results indicate that coumarin–aminopyran derivatives may have potential therapeutic value for the management of mental depression.

Major depression disorder (MDD) is described as a depressive state of mind, which is associated with faulty mood, loss of interest, disruption in sleep patterns, fatigue and sometimes suicidal tendencies. It is a chronic and life-threatening mental illness, which remains hidden and untreated at most of the times.[1] World Health Organisation (WHO) had estimated that 'at least 350 million people live with depression and it is the leading cause of disability worldwide'.[2] Epidemiological studies have indicated that about 2/3 of people who commit suicide are depressed at the time of their death.[3] Exact cause of depression is not clearly known, but it is believed that imbalance of neurotransmitters in brain, genetic vulnerability, stressful life events and medical problems are the main factors leading to depression.[4] Currently available antidepressant treatments are selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs), serotonin-norepinephrine reuptake inhibitors (SNRI) and some nonmedication therapeutic options.[5]

Even though wide range of conventional therapies is available, nearly 15% of depressed people are still refractory to the current existing therapies.[6] In addition, most of the people suffer from relapse and experience serious side effects after treatment with current therapies.[7] In Feb 2007, the USFDA displayed 'black box' label on currently available antidepressants indicating that their use may increase the risk of suicidal thinking behaviour in few cases of children, adolescents and adults.[8] Hence, there is an urgent need to develop new class of prototypes that are more effective, tolerable and safe in depressed individuals against this deadly disorder.

Coumarins are prominent class of benzopyrones, which belong to natural as well as synthetic origin that exhibit diverse biological activities.[9] Many reports suggest that coumarins and their synthetic analogues possess antidepressant properties.[10] Coumarin containing natural product scopoletin, isolated from the *Polygala sabulosa* was found to have significant in vivo antidepressant activity.[11] In addition, xanthotoxin and praeruptorin-A belongs to the new generation of MAOIs exhibiting potent antidepressant properties.[12] Our research group has been involved for past several years in the development of 3-phenylcoumarin based scaffolds as potential antidepressant agents, which significantly decreased the immobility time compared to the standard drug fluoxetine (FXT), (Fig. 1).[13] The promising hit compound may serve as a valuable template to design further analogue to improve activity and efficacy.[14]

During recent years aminopyran containing compounds have shown prominent antidepressant activity.[15] Dutta et al. have

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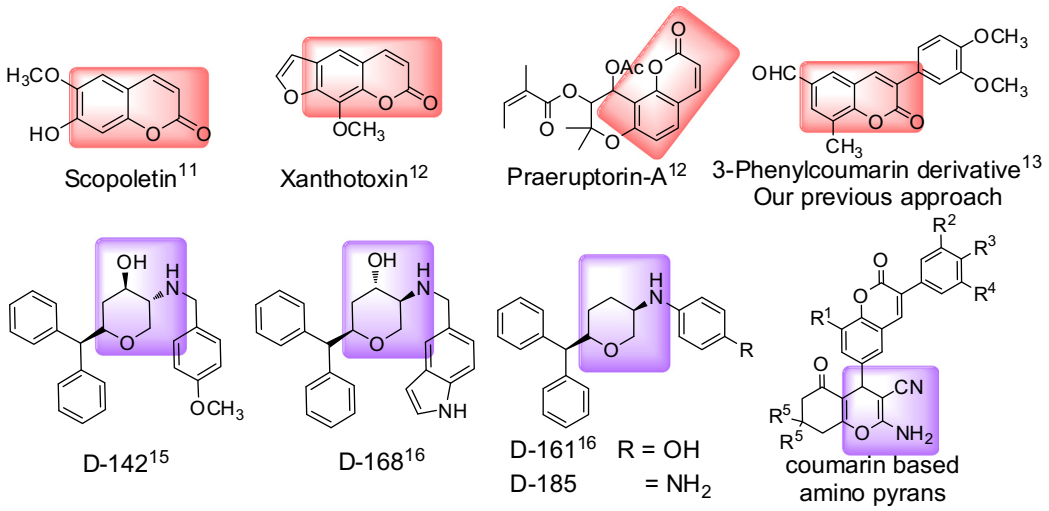
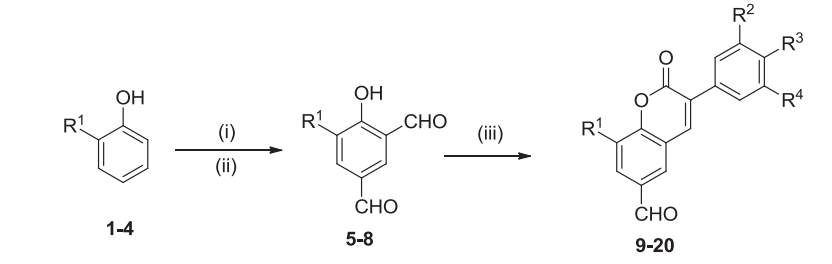


Figure 1. Designing of coumarin–aminopyran hybrids based on coumarin and aminopyran scaffolds showing antidepressant activity.



	R^1
1,5	CH_3
2,6	Isopropyl
3,7	*Sec*-butyl
4,8	*Tert*-butyl

	R^1	R^2	R^3	R^4
9	CH_3	H	OCH_3	H
10	CH_3	H	OCH_3	OCH_3
11	CH_3	OCH_3	OCH_3	OCH_3
12	Isopropyl	H	OCH_3	H
13	Isopropyl	H	OCH_3	OCH_3
14	Isopropyl	OCH_3	OCH_3	OCH_3
15	*Sec*-butyl	H	OCH_3	H
16	*Sec*-butyl	H	OCH_3	OCH_3
17	*Sec*-butyl	OCH_3	OCH_3	OCH_3
18	*Tert*-butyl	H	OCH_3	H
19	*Tert*-butyl	H	OCH_3	OCH_3
20	*Tert*-butyl	OCH_3	OCH_3	OCH_3

	R^1	R^2	R^3	R^4	R^5
21	Isopropyl	H	OCH_3	H	CH_3
22	CH_3	H	OCH_3	OCH_3	CH_3
23	CH_3	OCH_3	OCH_3	OCH_3	H
24	CH_3	OCH_3	OCH_3	OCH_3	CH_3
25	Isopropyl	H	OCH_3	H	H
26	Isopropyl	H	OCH_3	OCH_3	H
27	Isopropyl	H	OCH_3	OCH_3	CH_3
28	Isopropyl	OCH_3	OCH_3	OCH_3	H
29	Isopropyl	OCH_3	OCH_3	OCH_3	CH_3
30	*Sec*-butyl	H	OCH_3	H	H

	R^1	R^2	R^3	R^4	R^5
31	*Sec*-butyl	H	OCH_3	H	CH_3
32	*Sec*-butyl	H	OCH_3	OCH_3	CH_3
33	*Sec*-butyl	OCH_3	OCH_3	OCH_3	H
34	*Sec*-butyl	OCH_3	OCH_3	OCH_3	CH_3
35	*Tert*-butyl	H	OCH_3	H	H
36	*Tert*-butyl	H	OCH_3	H	CH_3
37	*Tert*-butyl	H	OCH_3	OCH_3	H
38	*Tert*-butyl	H	OCH_3	OCH_3	CH_3
39	*Tert*-butyl	OCH_3	OCH_3	OCH_3	H
40	*Tert*-butyl	OCH_3	OCH_3	OCH_3	CH_3

Scheme 1. Synthesis of 3-aryl coumarin based aminopyran derivatives. Reagents and conditions: (i) HMTA, TFA, 120 °C, 4 h. (ii) aq H_2SO_4, 100 °C, 2 h. (iii) appropriate phenylaceticacid, cyanuric chloride, NMM, DMF, 110 °C, 30–90 min, (vi) malononitrile, different 1,3-cyclohexadiones, DMAP, EtOH, reflux, 0.5 h.

synthesized a series of substituted aminopyran derivatives, as new generation of triple reuptake inhibitors (TRI) and observed significant antidepressant effects.[16] In the search of drug like molecules, molecular hybridisation technique is an emerging strategy in which two active pharmacophores are fused in a single frame work.[17] The resultant hybrid molecule may modulate the potency and efficacy, compared to that of parent subunits. Thus inspired from the molecular hybridisation approach,[18–20] we have rationally designed and synthesized some new 3-phenylcoumarin-aminopyran hybrids for a possible antidepressant activity.

The synthesis of intermediate and final compounds is described in the scheme 1. The Duff reaction on *ortho*-substituted phenols

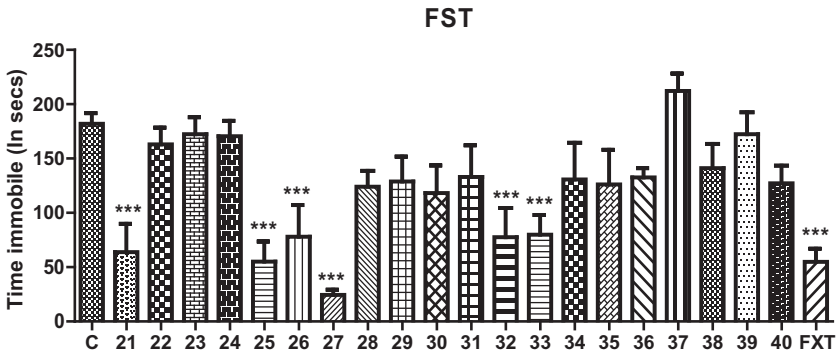


Figure 2. Effect of coumarin–aminopyran derivatives on the immobility duration of mice in the forced swimming test (FST). All values are expressed as mean ± SEM ($n = 8$ each). Mice were intraperitoneally administrated 1% DMSO, compounds (0.5 mg/kg) and fluoxetine FXT (20 mg/kg), respectively, $^{***}P$ <0.001, when compared with the control.

Table 1
Tabular representation of percentage change in immobility duration in forced swimming test (FST) after administration of different compounds and standard drug FXT to mice

Compound	Mean ± SEM	% Decrease of immobility
Control	182.00 ± 9.74	—
21	63.95 ± 25.87	64.86***
22	163.00 ± 15.41	10.44
23	172.50 ± 15.47	5.22
24	170.40 ± 14.26	6.37
25	55.07 ± 18.53	69.74***
26	77.95 ± 29.17	57.17***
27	24.40 ± 4.817	86.59***
28	124.00 ± 14.55	31.87
29	128.80 ± 23.05	29.23
30	118.20 ± 25.54	35.05
31	133.10 ± 29.00	26.87
32	77.60 ± 26.82	57.36***
33	79.93 ± 18.17	56.08***
34	130.70 ± 33.74	28.18
35	126.20 ± 31.87	30.65
36	132.50 ± 8.744	27.20
37	212.30 ± 15.94	−16.64
38	141.10 ± 22.31	22.47
39	172.30 ± 20.36	5.33
40	127.20 ± 16.37	30.10
Fluoxetine (FXT)	54.92 ± 12.11	69.82***

$^{***}P$ <0.001, when compared with the control.

(**1**–**4**) in the presence of hexamethylene-tetraamine (HMTA) and TFA at 120 °C afforded aromatic dicarbaldehydes (**5**–**8**).[21] These dicarbaldehyde intermediates were then allowed to react with different substituted phenyl acetic acids in the presence of cyanuric chloride and *N*-methylmorpholine (NMM) in DMF for 1 h, resulting in a good yield of respective 3-aryl coumarin aldehydes (**9**–**20**).[22] Finally, the multi component reaction[23] with malononitrile and different 1,3-cyclohexadiones gave the desired coumarin–aminopyran hybrids (**21**–**40**).[24] The structures of the compounds were substantiated by ^{1}H NMR, ^{13}C NMR, and mass spectrometry (for further details see the Supporting information).

All the coumarin–aminopyran compounds were screened at a dose of 0.5 mg/kg ip in adult male Swiss albino mice in FST.[25] Among the 20 compounds screened, 6 compounds were found to be active in forced swimming model (Fig. 2). Compounds **21**, **26**, **32** and **33** significantly reduced immobility time by 64.8%, 57.1%, 57.3% and 56.0%, respectively, when compared to control group. Much better activity was shown by compound **25** which reduced immobility time by 69.7% that was comparable to the standard drug FXT (69.8%). However, highest activity was exhibited by compound **27** which reduced immobility time by 86.5% suggesting it to be the most potent among the series (Table 1).

Further, effective compounds were studied at lower and higher doses in FST. Figure 3 reveals that compound **26**, **27** and **32** at

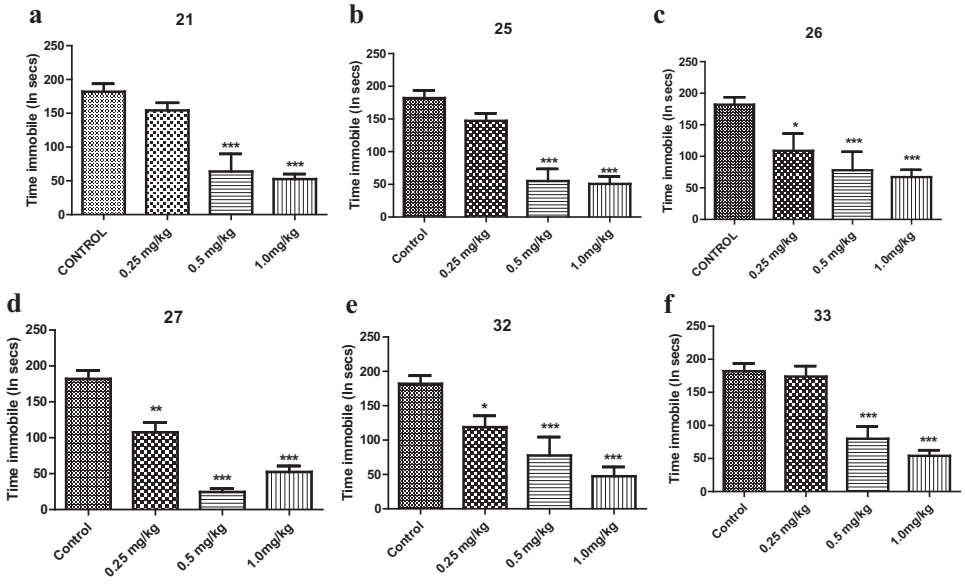


Figure 3. Graded dose response of effective compounds (a) **21**, (b) **25**, (c) **26**, (d) **27**, (e) **32** and (f) **33** in FST. The figure shows significant and dose dependent reduction in immobility time. Results are expressed as mean ± SEM, $^{*}P$ <0.05, $^{**}P$ <0.01 and $^{***}P$ <0.001 when compared with the control group.

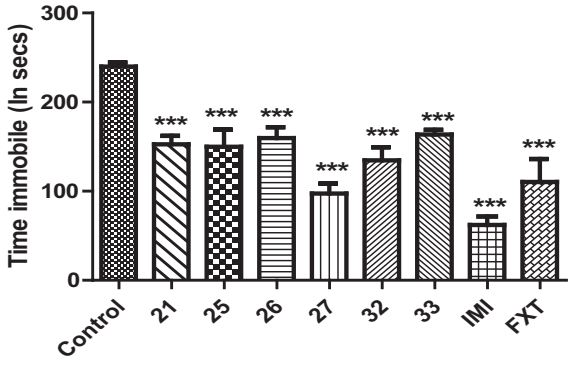


Figure 4. Effect of compounds on immobility duration of mice in TST at a dose of 0.5 mg/kg ip imipramine (IMI), fluoxetine (FXT) were the standard drugs used in this study at a dose of 30 mg/kg and 20 mg/kg, respectively. Results are expressed as mean ± SEM ($n = 8$ each). $^{***}P$ <0.001 as compared to that of control.

0.25 mg/kg, ip significantly attenuated immobility time by 49%, 41% and 35%, respectively, in FST. However, the other active compounds **21**, **25** and **33** at lower dose, were unable to reduce immobility time. Interestingly all effective compounds, when administered at higher doses of 0.5 and 1.0 mg/kg, ip showed significant reduction of immobility duration. As all the compounds showed activity at 0.5 mg/kg, therefore, we selected this dose as the effective dose for further screening in tail suspension test (TST), which is another model for testing antidepressant drugs.[26] Thus, compounds **21**, **25**, **26**, **27**, **32** and **33** were further evaluated in a second test, TST, which also quantifies immobility period. As represented in Figure 4, mice treated with all active compounds showed significant reduction of immobility duration in TST when compared with control mice. However, noteworthy activity was exhibited by compound **27**, which at a very low dose of 0.5 mg/kg ip, produced comparable reduction in immobility time as shown by standard drugs FXT and imipramine (IMI). This confirms that our prototypes exhibited antidepressant type activity.

Next, in order to determine if active compounds exhibit any effect on locomotor activity, open field test was performed at the effective dose of 0.5 mg/kg ip. All active compounds were evaluated for both horizontal activity (Fig. 5a) and total distance travelled (Fig. 5b), which are important parameters to check behavioural response of the mice.[27] During this experiment, mice were treated with compounds at 30 min time point and further examined up to 120 min. However no statistically significant difference was observed between control mice and compounds treated mice in this experiment. Thus, it indicates that the compounds did not induce any anxiety and hyper-activity like effects after administration to the mice. Thereafter, to examine the effect of active compounds on neuromuscular coordination, rotarod test[28]

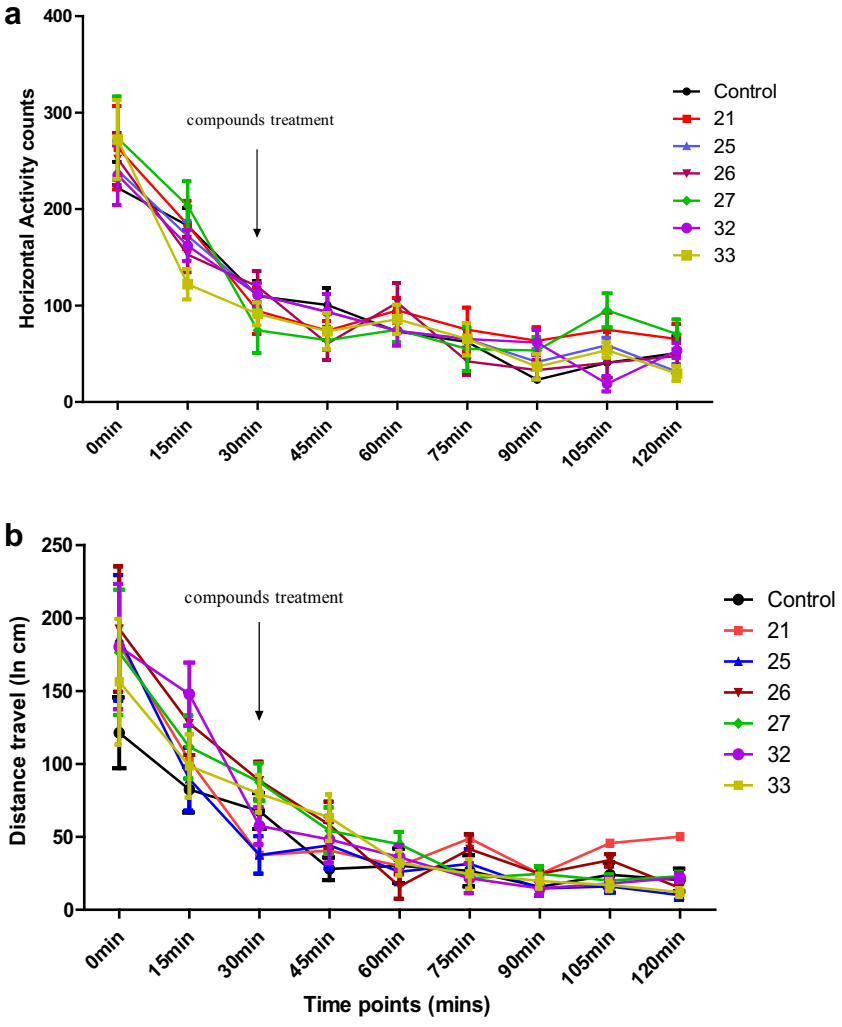


Figure 5. Effect of compounds on locomotor activity (a) horizontal activity and (b) total distance travelled. Compounds were administered at 30 min time point and counts were recorded for total 120 min in digiscan animal activity monitor. All the values are represented as mean ± SEM ($n = 8$ each).

was carried out at the highest dose of 1.0 mg/kg, ip. In this test, the control mice and active compounds treated mice stayed for 120 s on the rotating rod (Supporting information Table S3). Both the above behavioural findings confirmed that active compounds do not have any motor impairment effects.

In terms of structure activity relationship, it is interesting to note that the activity profile differs depending upon the number of methoxy substituents attached to that of 3-phenyl ring and alkyl group attached to the C8 position. In common, compounds containing mono and di methoxy substituted groups (**25**, **27**, **21** and **26**) potentially reduced the percentage of immobility time than compounds containing tri methoxy substituted groups (**23**, **24**, **28**, **29**, **33**, **34**, and **39**). But, the presence of bulky aliphatic groups like *sec*-butyl, *tert*-butyl group on C8 position, tends to decrease the percentage of immobility duration (**30**, **35**, **34**, **36**, **38** and **39**). However, a different trend was observed in the case of compounds **32**, **31**, **22** and **30**.

In conclusion, our drug design and discovery program led us to develop a new series of coumarin–aminopyran derivatives as potential antidepressant agents. The preclinical antidepressant FST test has provided evidence that compound **27** reduced the immobility time by 86.5% at very low dose of 0.5 mg/kg ip, which is more potent than the standard drug FXT (20 mg/kg, ip). The activity of compound **27** was also proved in TST. Additionally, all active compounds including compound **27** did not exhibit any neurotoxicity as confirmed by rotarod test. Our ongoing studies are directed towards the detailed mechanistic and pharmacokinetic studies on compound **27** so as to advance this molecule into a therapeutic option.

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Supplementary data

Supplementary data (details for synthesis and characterization of all compounds together with protocols for biological materials and methods) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.11.036.

References and notes

1. Hampton, T. *JAMA* **2012**, *308*, 4442.
2. World Health Organisation. Available at: http://www.who.int/entity/mental_health/management/depression/flyer_depression_2012.pdf (accessed July 23, 2013).
3. Al-Habeeb, A. A.; Sherra, K. S.; Al-Sharqi, A. M.; Qureshi, N. A. *East Mediterr. Health J.* **2013**, *19*, 248.
4. DeWeerdt, S. *Nature* **2013**, *497*, S14.
5. Chancellor, D. *Nat. Rev. Drug Discov.* **2011**, *10*, 809.
6. Anderson, H. D.; Pace, W. D.; Libby, A. M.; West, D. R.; Valuck, R. J. *Clin. Ther.* **2012**, *34*, 113.
7. (a) Check, E. *Nature* **2004**, *431*, 122; (b) Licinio, J.; Wong, M. L. *Nat. Rev. Drug Discov.* **2005**, *4*, 165.
8. Friedman, R. A.; Leon, A. C. *N. Engl. J. Med.* **2007**, *356*, 2343.
9. Peng, X. M.; Damu, G. L.; Zhou, C. *Curr. Pharm. Des.* **2013**, *19*, 3884.
10. Patil, P. O.; Bari, S. B.; Firke, S. D.; Deshmukh, P. K.; Donda, S. T.; Patil, D. A. *Bioorg. Med. Chem.* **2013**, *21*, 2434.
11. Capra, J. C.; Cunha, M. P.; Machado, D. G.; Zomkowski, A. D.; Mendes, B. G.; Santos, A. R.; Pizzolatti, M. G.; Rodrigues, A. L. *Eur. J. Pharmacol.* **2010**, *643*, 232.
12. Huong, D. T.; Huong, L.; Choi, H. C.; Rho, T. C.; Lee, H. S.; Lee, M. K.; Kim, Y. H. *Arch. Pharm. Res.* **1999**, *22*, 324.
13. (a) Sashidhara, K. V.; Kumar, A.; Chatterjee, M.; Rao, K. B.; Singh, S.; Verma, A. K.; Palit, G. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1937; (b) Sashidhara, K. V.; Rao, K. B.; Singh, S.; Modukuri, R. K.; Aruna Teja, G.; Chandasana, H.; Shukla, S.; Bhatta, R. S. *Bioorg. Med. Chem. Lett.* **2014**, *20*, 4876.
14. Keseru, G. M.; Makara, G. M. *Drug Discov. Today* **2006**, *11*, 741.
15. Dutta, A. K.; Gopishetty, B.; Gogoi, S.; Ali, S.; Zhen, J.; Reith, M. *Eur. J. Pharmacol.* **2011**, *671*, 39.
16. (a) Dutta, A. K.; Ghosh, B.; Biswas, S.; Reith, M. E. *Eur. J. Pharmacol.* **2008**, *589*, 73; (b) Gopishetty, B.; Hazeldine, S.; Santra, S.; Johnson, M.; Modi, G.; Ali, S.; Zhen, J.; Reith, M.; Dutta, A. *J. Med. Chem.* **2011**, *54*, 2924.
17. Viegas-Junior, C.; Danuello, A.; Bolzani, V. D. S.; Barreiro, E. J.; Fraga, C. A. M. *Curr. Med. Chem.* **2007**, *14*, 1829.
18. Sashidhara, K. V.; Kumar, M.; Khedgikar, V.; Kushwaha, P.; Modukuri, R. K.; Kumar, A.; Gautam, J.; Singh, D.; Sridhar, B.; Trivedi, R. *J. Med. Chem.* **2013**, *56*, 109.
19. Sashidhara, K. V.; Kumar, M.; Sonkar, R.; Singh, B. S.; Khanna, A. K.; Bhatia, G. *J. Med. Chem.* **2012**, *55*, 2769.
20. Sashidhara, K. V.; Kumar, M.; Modukuri, R. K.; Srivastava, R. K.; Soni, A.; Srivastava, K.; Singh, S. V.; Saxena, J. K.; Gauniyal, H. M.; Puri, S. K. *Bioorg. Med. Chem.* **2012**, *20*, 2971.
21. Sashidhara, K. V.; Kumar, M.; Modukuri, R. K.; Srivastava, A.; Puri, A. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6709.
22. (a) Sashidhara, K. V.; Palnati, G. R.; Avula, S. R.; Kumar, A. *Synlett* **2012**, *23*, 611; (b) Sashidhara, K. V.; Modukuri, R. K.; Jadiya, P.; Rao, K. B.; Sharma, T.; Haque, R.; Singh, D. K.; Banerjee, D.; Siddiqi, M. I.; Nazir, A. *ACS Med. Chem. Lett.* **2014**, *5*, 1099.
23. Khan, A. T.; Lal, M.; Ali, S.; Khan, M. M. *Tetrahedron Lett.* **2011**, *52*, 5327.
24. *General synthetic procedure for preparation of compounds (**21–40**)*: A mixture of 3-phenylcoumarin intermediates (1 equiv), malononitrile (1 equiv) and DMAP (0.2 equiv) in 4 mL of ethanol was allow to stirred at room temperature for 20–30 min. Then different 1,3-cyclohexadione (1 equiv) was added into the reaction mixture and stirring continued under reflux conditions till the completion of reaction (monitored by TLC). The reaction mixture was concentrated to dryness under reduced pressure. The crude product was purified on a silica gel column (100–200 mesh) using ethylacetate/hexane (50:50, v/v) as eluent to afford compounds **21-40** in good yields.
*(±)2-Amino-8′-tert-butyl-2′,5-dioxo-3′-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydro-2′H,4H-4,6′-bichromene-3-carbonitrile (**39**).*
Light yellow solid, yield: 60%; mp: 230–231 °C; IR (KBr): 3412, 3012, 2970, 2182, 1669, 1582, 1029 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 8.30 (s, 1H), 7.41 (s, 1H), 7.40 (s, 1H), 7.11 (s, 2H), 7.04 (s, 2H), 4.32 (s, 1H), 3.84 (s, 6H), 3.72 (s, 3H), 2.64 (br s, 2H), 2.31 (br s, 2H), 1.98 (br s, 2H), 1.48 (s, 9H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ:195.9, 164.7, 159.2, 158.5, 152.5, 150.1, 141.1, 140.4,137.9, 135.9, 129.9, 128.2, 125.5, 124.7, 119.8, 113.4, 106.1, 60.0, 57.8, 56.0, 36.3, 35.1, 34.3, 29.5, 26.5, 19.8; ESI-MS (m/z): 557 $(M+H)^+$.
25. Petit-Demouliere, B.; Chenu, F.; Bourin, M. *Psychopharmacology (Berl.)* **2005**, *177*, 245.
26. Steru, L.; Chermat, R.; Thierry, B.; Simon, P. *Psychopharmacology (Berl.)* **1985**, *85*, 367.
27. Ishola, I. O.; Chatterjee, M.; Tota, S.; Tadigopulla, N.; Adeyemi, O. O.; Palit, G.; Shukla, R. *Pharmacol. Biochem. Behav.* **2012**, *103*, 322.
28. Chatterjee, M.; Verma, P.; Maurya, R.; Palit, G. *Pharm. Biol.* **2011**, *49*, 477.