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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and biological evaluation of novel 2′,4′,5′-trimethoxyflavonol derivatives as anti-inflammatory and antimicrobial agents

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Abstract A series of novel 3-hydroxy-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one (flavonol) derivatives (**2a–u**) of biological interest have been prepared via CLAISEN–SCHMIDT condensation followed by ALGAR–FLYNN–OYAMADA reaction and to search for the potent nonsteroidal anti-inflammatory agents from this novel series. All the synthesized compounds have been screened for their in vitro proinflammatory cytokines tumor necrosis factor (TNF-α) and interleukin-6 (IL-6) inhibitory activity along with antimicrobial activity. As many as three compounds viz. **2h**, **2l**, and **2q** from this novel series were found to be potent TNF-α and IL-6 inhibitor (up to 72–81 % TNF-α and 86–92 % IL-6 inhibitory activity) but at 10 μM concentration as compared with the standard dexamethasone (71 % TNF-α and 84 % IL-6 inhibitory activities at 1 μM concentration). While the compounds **2d**, **2m**, **2n**, and **2s** were found to be potent antimicrobial agent showing even 2–2.5-fold more potency than that of standard ciprofloxacin and miconazole at the same MIC value of 10 μg/mL.

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Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been recognized as a vital therapeutic agents for the alleviation of pain and inflammation associated with a numerous pathologic conditions viz. arthritis, bursitis, and tendinitis. However, chronic administration of NSAIDs has been associated with clinically significant complications such as gastrointestinal (GI) symptoms including mucosal damage, bleeding, nausea, heartburn, dyspepsia, abdominal pain, and renal toxicity, etc. Nowadays available polytherapy for inflammatory conditions associated with microbial infections increases the risk for developing NSAID-related complications especially in elderly, patients with prior history of peptic ulcer disease, patients with impaired liver or kidney functions, and patients taking anticoagulants, corticosteroids, etc. Hence, there is a pressing need for the drugs having both anti-inflammatory and antimicrobial activities, both from the pharmacoeconomic as well as the patient compliance point of view. (Allison *et al*., 1992; Flower, 2003; Bekhit and Abdel-Azeim, 2004).

The flavonoids are important bioactive molecules having a group of natural products present in a wide variety of plants reported to have potent anti-inflammatory activity in both (in vitro and in vivo) although not fully understood (Harborne and Williams, 2000; Middleton *et al*., 2000). The several action mechanisms are proposed to explain in vivo anti-inflammatory action of flavonoids depending on their chemical structures. The unique action mechanisms and significant in vivo activity make flavonoids a

reasonable candidate for new anti-inflammatory drugs. Flavonoids exhibit a broad range of biological activities, including antiviral, anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic and antitumoral actions (Manthey *et al*., 2001; Rice-Evans and Miller, 1996; Hollman *et al*., 1996).

However, the naturally found flavonoids like baicalein, quercetin, and myricetin have the –OH group in their structure, but in recent studies it is noteworthy that the O-alkylation of these phenolic –OH has considerable attention. Because of converting the trihydroxyl groups to the alkoxy groups on the "A" ring of baicalein resulting in increased activity on P-gp 170 inhibition (Lee *et al*., 2004). Fernández *et al*. analyzed inhibition of aldose reductase by 33 baicalein derivatives with modifications on the "A" and "B" rings, leading to the conclusion that compounds with either three hydroxyl or methoxyl functionalities on the "A" ring are effective inhibitors of aldose reductase (Fernandez *et al*., 2005). Furthermore, Morita *et al*. showed that the increased lipophilicity of phenolic hydroxyls via alkylation may enhance the therapeutic potential to X-linked adrenoleukodystrophy (X-ALD) (Morita *et al*., 2005).

Based on the aforementioned information, the combination of the biologically effective –O alkylation with a flavonol skeleton in continuation with our previous study (Hatnapure *et al*., 2012), herein, we reporting a novel series of 3-hydroxy-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one derivatives and have been screened for their in vitro proinflammatory cytokines TNF-α and IL-6 inhibitory activity.

The cytokines are intercellular messengers responsible for host defense mechanisms as inflammatory, immune and hematogenic responses. One of the key proinflammatory cytokine tumor necrosis factor-α (TNF-α) is mainly produced by the activated macrophages and monocytes, which further induces the production of the several inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and granulocyte–macrophage colony-stimulating factor (GM-CSF). It is also a multitude of biological activities linked to pathology of autoimmune diseases such as rheumatoid arthritis (RA) (Macnaul *et al*., 1990; Brennan *et al*., 1992), Crohn's disease (van Dullemen *et al*., 1995), systemic lupus erythematosus (Maury and Teppo, 1989), multiple sclerosis (Sharief and Hentges, 1991; Beck *et al*., 1988; Gallo *et al*., 1989), septic shock (Lechner *et al*., 1992), and AIDS (Makonkawkeyoon *et al*., 1993). On the other hand, cytokine IL-6 (from the series of cytokine-signaling pathway) contributes to the initiation and extension of the inflammatory process and considered as a central mediator in a range of inflammatory diseases but has not received the desired attention in drug discovery (Dominic and Raj, 2009). TNF-α and IL-6 are thus pharmaceutically important molecular targets for the treatment of the above-mentioned diseases. The available biopharmaceuticals [TNF-soluble receptor (Enbrel ™) and TNF antibody (Remicade ™)] are expensive, difficult to administer orally and have major side effects on prolonged clinical use. Therefore, there is an urgent medical need to discover small-molecule agents to deal with higher levels production of TNF-α.

On going through the literature, we found some flavonoids such as luteolin, galangin, etc., inflammatory inhibitors, when their structural activity relationships were compared (Fig. 1), the important moieties are the C-2, 3-double bond, A-ring 5, 7-hydroxyl groups, and B-ring 4′- or 3′,4′-hydroxyl groups. The C-3 hydroxyl group as in flavonol is favorable for lipoxygenase (LOX) inhibition and oral anti-inflammatory activity (Bauman *et al*., 1980; Landolfi *et al*., 1984).

In flavones and flavonol of the same type, flavonol showed greater inhibition than flavones. The tri methoxy flavonol derivatives have not hitherto tested as anti-inflammatory agents, in particular against the TNF-α and IL-6 and also their antimicrobial activities. In order to improve the anti-inflammatory and antimicrobial activity of the compound based on quercetin and myricetin core (Cho *et al*., 2003). We envisage the new scaffold by incorporating O-alkylation on ring "B" and different substituents on ring "A" hitherto remained untested (Fig. 2). We set out to test our notion by design, synthesis, and biological evaluation of novel flavonol derivatives.

Nowadays, our research group also engaged in anti-inflammatory activity study program of novel urea and thiourea derivatives (Keche *et al*., 2012a, b; Tale *et al*., 2011). Herein, we presenting results on the synthesis and discovery of potent TNF-α and IL-6 inhibitor-based 2′,4′,5′-trimethoxyflavonol scaffold. The libraries of 21 different compounds (**2a**–**u**) are obtained according to Scheme 1 with good yield.

Our synthetic strategy for 3-hydroxy-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one derivatives have been planned by employing the general synthetic path of reaction. The 21 different 3-hydroxyflavones have been prepared by CLAISEN–SCHMIDT condensation followed by ALGAR–FLYNN–OYAMADA reaction (Scheme 1) (Li *et al*., 2008). The key intermediates 1-(2-hydroxyphenyl)-

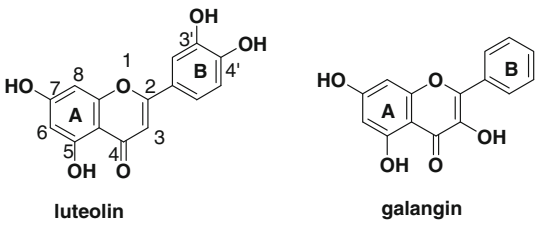
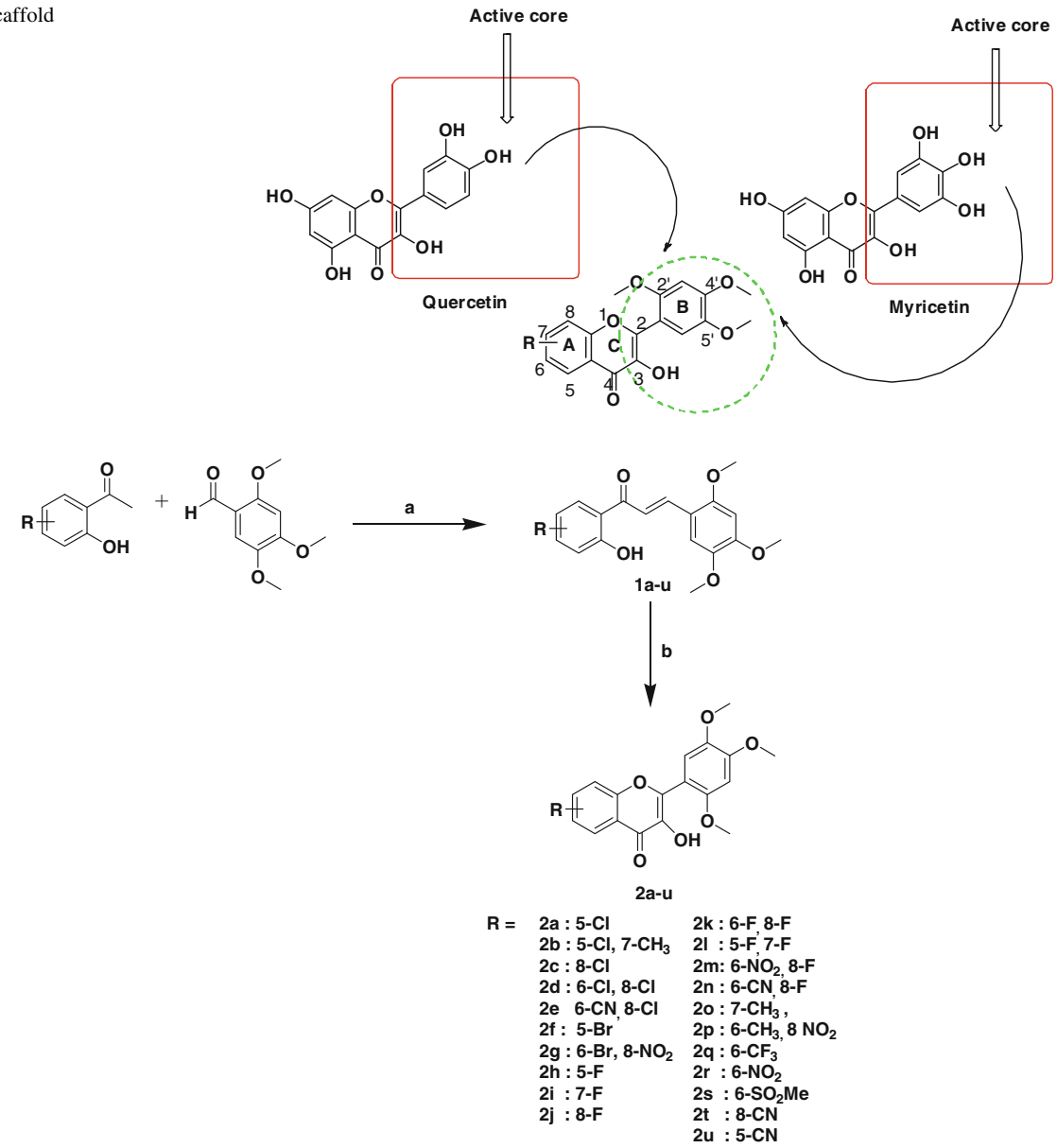


Fig. 1 Structure of luteolin and galangin

Fig. 2 New design scaffold



Reagents and conditions:- (a) EtOH,60% KOH, 0-rt 6-8h: (b) 15% aq NaOH/ 16% H2O2 (v/ v 1:1) 5-6h.

Scheme 1 Synthesis of 3-hydroxy-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one derivatives

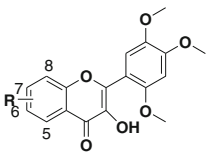
3-(2,4,5-trimethoxyphenyl) prop-2-en-1-one (**1**) derivatives have been synthesized by the CLAISEN–SCHMIDT condensation between substituted 2-hydroxyacetophenones and 2,4,5-trimethoxybenzaldehyde in moderate to good yields (60–70 %). The treatment of **1** with hydrogen peroxide and base in ethanol under ALGAR–FLYNN–OYAMADA conditions afforded the corresponding flavonol (**2a**–**u**) in (50–70 % yields). The desired products were purified by column chromatography (silica gel with 20 % ethyl acetate/hexane). The purity of the compounds was checked by TLC and HPLC. Spectral data ^{1}H, C^{13} NMR, and MS of the newly synthesized compounds have been in full agreement with their proposed structures.

Having secured the structurally diverse novel 3-hydroxy-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one derivatives, next in order to search for their proinflammatory cytokines, TNF-α, and IL-6 production by lipopolysaccharide (LPS) in THP-1 cells was measured according to the method described by Hwang *et al*. (1993). The results of the TNF-α and IL-6 inhibitory activity are shown in Table 1. As many as **3** compounds from this library have been found to be potent TNF-α and IL-6 inhibitor with reference standard

drug dexamethasone. While the compounds **2d**, **2m**, **2n**, and **2s** found to be potent antimicrobial agent showing even 2–2.5-fold more potency than that of standard ciprofloxacin and miconazole at the same MIC value of 10 μg/mL by using agar-well diffusion method (Sridhar *et al*., 2004).

Thus the compounds **2h**, **2l**, and **2q** from the series found to be promising TNF-α and IL-6 inhibitors (up to 72–81 % TNF-α and 82–92 % IL-6 inhibitory activity but at 10 μM concentration), when compared with standard dexamethasone (71 % TNF-α and 84 % IL-6 inhibitory activities at 1 μM concentration). Notably, the compound **2h** exhibited much higher TNF-α or IL-6 (81 and 92 %) inhibitory activity. The compounds **2c**, **2e**, **2i**, **2j**, and **2k** exhibited moderate activity even as remaining compounds found to have low, very low, or no activity at the same level of concentration against the proinflammatory cytokines TNF-α and IL-6. As a result, the most potent compound **2h** could prove to be a promising candidate for drug discovery.

Table 1 Anti-inflammatory activity of 3-hydroxy-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one derivatives



Compound **2**(**a**–**u**)	*R*	% inhibition at 10 μM	
		TNF-α	IL-6
2a	5-Cl	45	39
2b	5-Cl, 7-CH_3	52	46
2c	8-Cl	60	65
2d	6-Cl, 8-Cl	30	28
2e	6-CN, 8-Cl	65	78
2f	5-Br	17	22
2g	6-Br, 8-NO_2	–	–
2h	5-F	81	92
2i	7-F	67	74
2j	8-F	60	75
2k	6-F, 8-F	64	76
2l	5-F, 7-F	72	86
2m	6-NO_2, 8-F	28	41
2n	6-CN, 8-F	27	49
2o	7-CH_3	15	21
2p	6-CH_3, 8-NO_2	–	–
2q	6-CF_3	75	82
2r	6-NO_2	–	–
2s	6-SO_2Me	16	30
2t	8-CN	08	11
2u	5-CN	–	–
Ref-1	Dexamethasone (1 μM)	71	84

In order to search for the potent antimicrobial agent from these newly synthesized compounds (**2a**–**u**) have been evaluated for in vitro antibacterial and antifungal activity against various Gram-positive, Gram-negative bacteria, and fungal strains (Tables 2, 3). As can be seen from obtained results, many compounds from the newly synthesized series found to be potent antibacterial and antifungal agents. Thus the compounds **2d**, **2m**, **2n**, and **2s** exhibited comparable to or even higher antimicrobial activity than the standard ciprofloxacin and miconazole, respectively, almost against all the tested bacteria or fungi. The remaining compounds of this series have been found to be moderate, low, or no activity. Moreover, the compounds **2d** and **2s** have more potent antibacterial agents than the standard drug against some bacteria viz. *Staphylococcus aureus*, *Bacillus subtilis*, and *Salmonella typhimurium*. Also the compound **2d** found to be better antifungal agents as compared with standard miconazole.

The SAR of 3-hydroxy-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one derivatives are shown in Tables 1, 2, and 3, some interesting trend has been observed as the effect of substituent present on ring "A" along with the O-methylation of "B" ring on taken activities that is the nature of the substituent affecting the biological activity of the synthesized analogues. From the TNF-α and IL-6 inhibitory activity data (Table 1), it is observed that a majority of the analogues of this series found to be active as IL-6 inhibitor while very few exhibited TNF-α inhibitory activity. So it is clear from the results that the position of the substituent on "A" ring of flavonol moiety has profound effect on the activity.

The 5-, 6-, and 7-positions are favorable sites for the higher potency. Evidently, the compounds **2h** and **2l** with F at 5 and 7 positions or both as well as CF_3 at 6-positions exhibiting highest TNF-α and IL-6 inhibitory activity, while presence of Cl, Br, CH_3, CN, and NO_2 at same position (compounds **2a**, **2g**, **2p**, **2r**, **2o**, and **2u**) exhibits no TNF-α or IL-6 inhibitory activity. However, surprisingly the compounds **2c** and **2e** have shown moderate activity with chloro and cyano groups at 8- and 6- or 8-positions of "**A**" ring, whereas, the fluoro group at 7- and 6-, 8-positions is moderately potent with (64–67 % TNF-α and 76–77 % IL-6 inhibitory activity). The compound **2h** shows better TNF-α (81 % and IL-6 92 %) activity at 10 μM concentration. None of the compounds exhibits no or very low anti-inflammatory activity. It clears from the SAR fluoro group at 5-, 6-, and 7-positions tolerate the procytokine activity.

The antimicrobial activity data are presented in Tables 2 and 3. As shown in our results, some analogues of this series have been found to be more potent than the standard drug

Table 2 Antibacterial activity of 3-hydroxy-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one derivatives

Compounds	Gram-positive		Gram-negative	
	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Salmonella typhimurium*
2a	35	30	25	40
2b	30	45	35	50
2c	85	–	80	90
2d	10	10	15	10
2e	90	80	75	80
2f	80	65	–	75
2g	40	35	45	35
2h	90	–	75	80
2i	65	55	45	50
2j	–	80	60	90
2k	75	85	–	80
2l	80	90	85	–
2m	20	15	15	20
2n	25	15	10	25
2o	90	85	–	80
2p	–	–	85	–
2q	85	70	90	90
2r	85	90	90	90
2s	15	10	20	10
2t	40	30	25	30
2u	35	45	60	40
Ciprofloxacin	25	20	15	20

MIC values are the average of three reading (μg/mL)

– No activity was observed up to 200 μg/mL

ciprofloxacin, while some of them have comparable potency. Interestingly none of the compounds with high anti-inflammatory activity found to be potent antibacterial or antifungal agents. The compounds **2d**, **2m**, **2n**, and **2s** bearing Cl, NO_2, CN, and SO_2Me groups, respectively, at 6-position with Cl, F, at 8-position or at both have higher potency than the compounds bearing such a group at 5- or 7-positions or at both. It is clear from our results that 6- or 8-position is the favorable site for high-antibacterial activity. The high potency of **2d**, **2m**, **2n**, and **2s** may be attributed to the presence of lipophilic or H-bond acceptor type group's placement such as F, Cl, CN, SO_2Me, and NO_2 at 6- or 8-positions, but unfortunately this functional group presence on 5, 7 or both shows moderate or no activity with respect to standard drug against the tested strains. No activity has been observed in case of compounds **2o**–**2r** up to concentration of 200 μg/mL against some bacteria and fungi.

It is clear from activity data (Table 2 vs. Table 3) that the SAR of 3-hydroxy-2-(2,4,5-dimethoxyphenyl)-4H-chromen-4-one derivatives of antibacterial activity strongly correlates with their SAR of antifungal activity. Again the positions 6 and 8 of ring "**A**" are favorable sites for high activity. The compounds **2d** and **2s** were found to be 2–2.5-fold more potent than the standard drug miconazole, while **2m** and **2n** exhibited comparable antifungal activity similar to the antibacterial activity trend, polar lipophilic groups such as Cl, SO_2Me, NO_2, F at 6- and 8-position or both at same level of concentration 10 μg/mL. Apart from the compounds **2a**, **2b**, **2g**, **2t**, and **2u** have no major effect on the antifungal activity.

It reveals from our SAR studied, the presence of lipophilic F and CF_3 groups with trimethoxy (O-alkylation) tolerates the procytokine activity because of fluorine imparts the special characteristics that enhance therapeutic efficacy and improved pharmacological properties in bioactive molecules. While the fluoro is only slightly more lipophilic than hydrogen and trifluoromethyl is much more lipophilic than methyl or chloro, this factor is often the most significant in improving the pharmacological activity.

In conclusion, we have developed the novel approach for the synthesis of structurally diverse 3-hydroxy-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one derivatives (**2a**–**u**) using simple reaction sequence under mild conditions and their TNF-α and IL-6 inhibitory and antimicrobial activity have been evaluated. This study on the novel flavonol derivative bearing lipophilic fluoro group at 5-, 6-, and 7-positions and presence of trimethoxy group on "B" ring has lead to the discovery of novel and potent TNF-α and IL-6 inhibitors viz. **2h**, **2l**, and **2q** and could prove to be the valuable platform for the search of nonsteroidal anti inflammatory agents based on such a scaffold in vivo. While the presence of chloro, cyano or sulfonyl groups on 6- and 8-positions or both on ring "**A**" found to be an effective potent antimicrobial agents viz. compounds **2d**, **2m**, **2n**, and **2s**.

Experimental

Chemistry

General techniques

All commercial chemicals and solvents are of reagents grade and have been used without further purification. The thin layer chromatography was performed on Merck precoated silica gel 60 F254 plates, with visualization under UV light. ^{1}H NMR spectra have been recorded with Bruker 300 MHz AVANCE instrument and *J* values are in Hertz and chemical shifts (δ) are reported in ppm relative to internal tetramethylsilane. The mass spectra have been measured with Thermo Finnigan-TSQ Quarter Ultra (triple Quad). The purity of all compounds was determined by

Table 3 Antifungal activity of 3-hydroxy-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one derivatives

Compounds	*Candida albicans*	*Aspergillus niger*	*Fusarium solani*	*Aspergillus flavus*
2a	25	40	35	45
2b	30	25	20	35
2c	–	80	90	80
2d	10	10	15	10
2e	75	85	–	90
2f	–	–	90	–
2g	45	30	25	40
2h	–	90	85	70
2i	70	65	85	90
2j	90	–	80	95
2k	–	80	75	80
2l	65	80	–	–
2m	20	15	20	10
2n	25	20	15	25
2o	–	80	90	85
2p	–	90	–	–
2q	60	70	75	85
2r	90	–	80	–
2s	15	10	10	20
2t	40	30	25	30
2u	25	30	15	25
Miconazole	20	20	15	20

MIC values are the average of three reading (μg/mL)

– No activity was observed up to 200 μg/mL

HPLC (Waters 2695 Alliance) using column Kromasil C18, solvent acetonitrile and buffer (0.01 M ammonium acetate + 0.5 % triethylamine, pH 5.0 with acetic acid). Melting points were determined with (PEW-340 MP) melting point apparatus and are uncorrected.

*General procedure for the synthesis of 1-(2-hydroxyphenyl)-3-(2,4,5-trimethoxyphenyl) prop-2-en-1-one derivatives (**1a–u**)*

Different substitutes (2-hydroxyacetophenone) (1 eqi.) and 2,4,5-trimethoxy benzaldehyde (1 eqi.) in EtOH (15 mL) were added and a 60 % of KOH (5 mL) solution was added drop wise at 0 °C. The reaction mixture was stirred at room temperature for 6–8 h. The reaction mixture was cooled and poured into ice water, neutralized using 1 N HCl. The light-yellow solid thus obtained was filtered and purified using silica gel column chromatography (with 1:9 ethyl acetate in hexane) or recrystallized from EtOH, yielded the desired 2,4,5-trimethoxy chalcone analogues (**1a–u**) with an average yield of 60–70 %.

*General procedure for the synthesis of 3-hydroxy-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one derivatives (**2a–u**)*

To a well-stirred solution of 2-hydroxychalcones **1** (1 eqi.) in MeOH (20 mL) and aq. KOH (10 mL, 20 %), cooled at 0–5 °C, 30 % H_2O_2 (10 mL) was added drop wise for 30 min. The reaction mixture was further stirred for 4–5 h. The resulting light-yellow reaction mixture was poured on crushed ice and neutralized with dil. HCl. The light-yellow solid thus obtained was filtered, washed with water and dried. The crude product was purified by chromatography on SiO_2 (100–200 silica gel) (EtOAc/petroleum ether v/v = 2:8) to give the desired compounds as solids. The physical, analytical, and spectral data of new 3-hydroxy-2-(2,4,5-trimethoxyphenyl)-4-H-chromen-4-one analogues are given below.

*5-Chloro-3-hydroxy-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one (**2a**)* White solid: yield 66 %; mp: 191–193 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.05 (bs, 1H), 7.77 (d, J = 8.5 Hz, 1H), 7.51 (d, J = 9 Hz, 1H), 7.35 (m, 1H), 7.10 (s, 1H), 6.61 (s, 1H), 3.90 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz, δ ppm): 172.49(C=O), 165.71(C-2), 164.14 (C-9), 155.19(C-2′), 152.29(C-4′), 151.19(C-5′), 144.25(C-5), 143.31(–OH/C-3), 139.12(C-7), 127. 2 3(C-10), 118.16(C-6), 113.35(C-8), 111.14 (C-6′), 104.54(C-1′), 97.72 (C-3′), 57.16, 56.28, 56.23 (OMe-C); MS (APCI); *m/z* 363.1 $[M+H]^+$; HPLC: 95.96 %.

5-Chloro-3-hydroxy-7-methyl-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one (2b) Off white solid: yield 67 %; mp: 187–189 °C .^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.25 (bs, 1H), 8.00 (s, 1H), 7.63 (s, 1H), 7.05 (s, 1H), 6.79 (s, 1H), 3.84 (s, 3H), 3.77 (s, 3H), 3.69 (s, 3H), 2.96 (s, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz, δ ppm): 173.21(C=O), 157.10(C-2), 152.91 (C-9), 150.31(C-2′), 148.63(C-4′), 147.37(C-5′),143.10(C-7), 141.21(C-5), 140.31(–OH/C-3), 125.41(C-10), 120.51(C-6), 118.12(C-8), 112.33(C-6′), 110.10(C-1′), 98.10(C-3′), 57.33, 56.46, 56.23 (OMe-C), 22.23 (Me/C); MS (APCI); *m/z* 377.1 $[M+H]^+$; HPLC: 96.89 %.

*8-Chloro-3-hydroxy-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one (**2c**)* White solid: yield 64 %; mp: 196–198 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.05 (bs, 1H), 7.77 (d, J = 8.5 Hz, 1H), 7.51 (d, J = 9 Hz, 1H), 7.35 (m, 1H), 7.10 (s, 1H), 6.61 (s, 1H), 3.90 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz, δ ppm): 172.59(C=O), 165.71(C-2), 164.14 (C-9), 155.19(C-2′), 152.29(C-4′), 151.19(C-5′), 143.25(C-8), 142.31(–OH/C-3), 138.12(C-7), 127.33(C-10), 117.16(C-6), 115.35(C-5), 112.14 (C-6′), 105.54(C-1′), 98.72 (C-3′), 56.30, 56.28, 56.23 (OMe-C); MS (APCI); *m/z* 363.1 $[M+H]^+$; HPLC: 93.96 %.

6,8-Dichloro-3-hydroxy-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one (2d) Pale yellow solid: yield 68 %; mp: 199–201 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.19 (bs, 1H), 8.13 (s, 1H), 7.73 (s, 1H), 7.23 (s, 1H), 6.65 (s, 1H), 3.97 (s, 3H), 3.92 (s, 3H), 3.89 (s, 3H); ($CDCl_3$, 300 MHz, δ ppm): 173.03(C=O), 158.13(C-2), 155.47(C-9), 150.86(C-2′), 145.37(C-4′), 144.12 (C-5′), 142.21(–OH/C-3), 136.42(C-8), 133.05(C-6), 130.20(C-10), 123.20(C-7), 121.14(C-5), 117.02 (C6′), 112.32 (C-1′), 97.11(C-3′), 57.22, 56.15, 56.10 (OMe-C); MS (APCI); *m/z* 398.1 $[M+H]^+$; HPLC: 96.06 %.

*8-Chloro-3-hydroxy-4-oxo-2-(2,4,5-trimethoxyphenyl)-4H-chromene-6-carbonitrile (**2e**)* Off white solid: yield 57 %; mp: 203–205 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.27 (bs, 1H), 8.31 (s, 1H), 8.26 (s, 1H), 7.15 (s, 1H), 6.86 (s, 1H), 3.89 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H); ($CDCl_3$, 300 MHz, δ ppm): 172.13(C=O), 157.14(C-2), 154.57(C-9), 150.66 (C-2′), 146.42(C-4′), 143.32(C-5′), 142.31(–OH/C-3), 139.16 (C-8), 132.20(C-10), 124.15(C-5), 123.36(C-7), 119.13(CN), 117.12 (C-6′), 113.16(C-6), 111.33(C-1′), 97.11(C-1′), 57.23, 56.25, 56.14 (OMe-C); MS (APCI); *m/z* 388.1 $[M+1]^+$; HPLC: 94.39 %.

*5-Bromo-3-hydroxy-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one (**2f**)* Off white solid: yield 63 %; mp: 193–195 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.05 (bs, 1H), 8.14 (s, 1H), 7.85 (d, $J = 8.5$ Hz, 1H), 7.57 (d, $J = 9$ Hz 1H), 7.04 (s, 1H), 6.78 (s, 1H), 3.82 (s, 3H), 3.74 (s, 3H), 3.66 (s, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz, δ ppm): 172.49(C=O), 163.72(C-2), 164.14(C-9), 155.19(C-2′), 152.29(C-4′), 151.19(C-5′), 143.25(C-5), 142.31(–OH/C-3), 138.12(C-7), 127. 2 3(C-10), 120.16(C-6), 115.35(C-8), 110.14 (C-6′), 105.54(C-1′), 99.72 (C-3′), 57.17, 56.30, 56.29 (OMe-C); MS (APCI); *m/z* 409.0 $[M+2H]^+$; HPLC: 96.65 %.

*6-Bromo-3-hydroxy-8-nitro-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one (**2g**)* Yellow solid: yield 70 %; mp: 197 –199 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.27 (BS, 1H), 8.31 (s, 1H), 8.26 (s, 1H), 7.15 (s, 1H), 6.84 (s, 1H), 3.89 (s, 3H), 3.83 (s, 3H), 3.78 (s, 3H); ($CDCl_3$, 300 MHz, δ ppm): 174.03(C=O), 157.13(C-2), 155.47(C-9), 150.86(C-2′), 146.37(C-4′), 144.12(C-5′), 14 2.21(–OH/C-3), 141.42(C-8), 133.05(C-6), 129.20(C-10), 123.20(C-7), 121.14(C-5), 117.02 (C-6′), 1 12.32 (C-1′), 97.11(C-3′), 57.24, 56.21, 56.18 (OMe-C); MS (APCI); *m/z* 453.1 $[M+H]^+$; HPLC: 95.03 %.

*5-Fluoro-3-hydroxy-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one (**2h**)* Brown solid: yield 71 %; mp: 186–188 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.05 (bs, 1H), 7.70 (d, $J = 9$ Hz 3H), 7.07 (s, 1H), 6.80 (s, 1H), 3.84 (s, 3H), 3.76 (s, 3H), 3.68 (s, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz, δ ppm):172.60(C=O), 163.71(C-2), 164.14 (C-9), 155.19(C-2′), 152.29(C-4′), 151.19(C-5′), 143.25(C-5), 141.31(–OH/C-3), 139.12(C-7), 127. 2 3(C-10), 118.16(C-6), 113.35(C-8), 111.14 (C-6′), 102.54(C-1′), 95.72(C-3′), 57.28, 56.27, 56. 23 (OMe-C); MS (APCI); *m/z* 347.1 $[M+2H]^+$; HPLC: 97.39 %.

*7-Fluoro-3-hydroxy-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one (**2i**)* Off white solid: yield 69 %; mp: 190–192 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.05 (bs, 1H), 8.26 (d, $J = 8.6$ Hz 1H), 7.21 (d, $J = 9$ Hz, 1H), 7.17 (m, 1H), 7.12 (s, 1H), 6.65 (s, 1H), 3.96 (s, 3H), 3.90 (s, 3H), 3.90 (s, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz, δ ppm): 173.60(C=O), 162.71(C-2), 160.14(C-9), 155.19(C-2′), 152.29(C-4′), 151.19(C-5′), 144.12(C-7), 141.31(–OH/C-3), 137.25(C-5), 129.23(C-10), 118.16(C-6), 113.35(C-8), 111.14 (C-6′), 102.54(C-1′), 95.72 (C-3′), 57.30, 56.28, 56. 23 (OMe-C); MS (APCI); *m/z* 347.1 $[M+2H]^+$; HPLC: 95.33 %.

8-Fluoro-3-hydroxy-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one (2j) Off white solid: yield 65 %; mp: 189–191 °. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.05 (bs, 1H), 8.04 (d, $J = 8.6$ Hz, 1H), 7.44 (d, $J = 9$ Hz, 1H), 7.33 (m, 1H), 7.15 (s, 1H), 6.66 (s, 1H), 3.96(s, 3H), 3.90 (s, 3H), 3.89 (s, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz, δ ppm): 175.60(C=O), 163.71(C-2), 160.14(C-9), 155.19(C-2′), 152.29(C-4′), 151.19(C-5′), 146.12(C-8), 141.31(–OH/C-3), 137.25(C-10), 129.23(C-5), 118.16(C-6), 115.35(C-7), 113.14 (C-6′), 102.54(C-1′), 95.72 (C-3′), 57.27, 56.21, 56. 20 (OMe-C); MS (APCI); *m/z* 347.1 $[M+H]^+$; HPLC: 94.39 %.

*6,8-Difluoro-3-hydroxy-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one (**2k**)* Pale yellow solid: yield 60 %; mp: 206–208 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.19 (bs, 1H), 7.90 (dd, 1H), 7.65 (dd, 1H), 7.10 (s, 1H), 6.85 (s, 1H), 3.88 (s, 3H), 3.80 (s, 3H), 3.72 (s, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz, δ ppm): 173.03(C=O), 158.13(C-2), 155.47(C-9), 150.86(C-2′), 145.37(C-4′), 144.12(C-5′), 142.21(–OH/C-3), 141.42(C-8), 140.05(C-6), 132.20(C-10), 125.20(C-7), 122.14(C-5), 119.02(C-6′), 113.32(C-1′), 97.15(C-3′), 57.23, 56.15, 56.12 (OMe-C); MS (APCI); *m/z* 365.1 $[M+H]^+$; HPLC: 98.57 %.

*5,7-Difluoro-3-hydroxy-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one (**2l**)* Pale yellow solid: yield 66 %; mp: 200–202 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.19 (bs, 1H), 7.09 (s, 1H), 6.76 (dd, 1H), 6.64 (s, 1H), 6.52 (dd, 1H), 3.96 (s, 3H), 3.88 (s, 3H), 3.86 (s, 3H); ($CDCl_3$, 300 MHz, δ ppm): ^{13}C NMR ($CDCl_3$, 300 MHz, δ ppm): 174.03(C=O), 159.14(C-2), 157.47(C-9), 152.86(C-2′), 146.37(C-4′), 145.12(C-5′), 143.21 (–OH/C-3), 142.52(C-7), 141.25(C-5), 130.20(C-10), 127.20 (C-6), 124.14(C-8), 118.02(C-6′), 114.32(C-1′), 98.15(C-3′), 57.21, 56.17, 56. 14 (OMe-C); MS (APCI); *m/z* 365.1 $[M+1]^+$; HPLC: 96.34 %.

*8-Fluoro-3-hydroxy-6-nitro-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one (**2m**)* Off white solid: yield 57 %; mp: 211–213 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.27 (bs, 1H), 8.31 (s, 1H), 8.27(s, 1H), 7.17 (s, 1H), 6.89 (s, 1H), 3.91 (s, 3H), 3.89 (s, 3H), 3.86 (s, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz, δ ppm): 176.03(C=O), 159.13(C-2), 157.47(C-9), 152.86(C-2′), 146.37(C-4′), 145.12(C-5′), 143.21(–OH/C-3), 142.42(C-6), 137.05(C-8), 130.20(C-10), 124.20(C-7), 122.14(C-5), 118.02 (C6′), 113.32 (C-1′), 97.25(C-3′), 57.27, 56.25, 56.21 (OMe-C); MS (APCI); *m/z* 392.1 $[M+H]^+$; HPLC: 95.54 %.

*8-Fluoro-3-hydroxy-4-oxo-2-(2,4,5-trimethoxyphenyl)-4H-chromene-6-carbonitrile (**2n**)* Brown solid: yield 64 %; mp: 209–211 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.27 (bs, 1H), 8.31 (s, 1H), 8.26 (s, 1H), 7.15 (s, 1H), 6.84 (s, 1H), 3.89 (s, 3H), 3.83 (s, 3H), 3.78 (s, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz, δ ppm): 172.13(C=O), 157.14(C-2), 154.57(C-9), 151.66(C-2′), 146.27 (C-4′), 144.32(C-5′), 143.16(C-8), 142.31(–OH/C-3), 132.20(C-10), 124.15(C-5), 123.36(C-7), 120.13(CN), 117.12(C-6′), 115.16(C-6), 112.33(C-1′), 97.20 (C-1′), 57.23, 56.24, 56.17 (OMe-C); MS (APCI); *m/z* 372.1 $[M+H]^+$; HPLC: 93.67 %.

*3-Hydroxy-7-methyl-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one (**2o**)* Brown solid: yield 68 %; mp: 189–191 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.25 (bs, 1H), 8.13 (s, $J = 8$ Hz, 1H), 7.31 (s, 1H),7.23 (d, $J = 8.5$ Hz, 1H), 7.11 (s, 1H), 6.65 (s, 1H), 3.96 (s, 3H), 3.92 (s, 3H), 3.88 (s, 3H), 2.49 (s, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz, δ ppm): 174.21(C=O), 158.10(C-2), 155.91(C-9), 151.31(C-2′), 148.55(C-4′), 147.37(C-5′), 141.10(C-7), 140.31(–OH/C-3), 130.21(C-5), 125.41(C-10), 120.51(C-6), 118.12(C-8), 113.33(C-6′), 111.10(C-1′), 98.30(C-3′), 57.35, 56.53, 56.42 (OMe-C), 21.23 (Me/C); MS (APCI); *m/z* 343.1 $[M+H]^+$; HPLC: 98.64 %.

*3-Hydroxy-6-methyl-8-nitro-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one (**2p**)* Yellow solid: yield 70 %; mp: 181–183 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.25 (bs, 1H), 8.34 (s, 1H), 8.27 (s, 1H), 7.14 (s, 1H), 6.85 (s, 1H), 3.89 (s, 3H), 3.81 (s, 3H), 3.72 (s, 3H), 2.51 (s, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz, δ ppm): 173.03(C=O), 157.13(C-2), 155.47(C-9), 151.86(C-2′), 145.37(C-4′), 143.12(C-5′), 142.21(–OH/C-3), 141.42(C-8), 139.05(C-6), 129.20(C10), 123.20(C-7), 121.14(C-5), 117.02 (C-6′), 114.32(C-1′), 97.14(C-3′), 57.21, 56.23, 56.18 (OMe-C), 21.27 (Me/C); MS (APCI); *m/z* 388.1 $[M+H]^+$; HPLC: 95.04 %.

*3-Hydroxy-6-(trifluoromethyl)-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one (**2q**)* Off white solid: yield 66 %; mp: 216–218 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.05 (bs, 1H), 8.59 (s, 1H), 7.85 (dd, 1H), 7.63 (d, $J = 9$ Hz, 1H), 7.12 (s, 1H), 6.66 (s, 1H), 3.83 (s, 3H), 3.74 (s, 3H), 3.71 (s, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz, δ ppm): 175.01(C=O), 159.13(C-2), 157.47(C-9), 150.86(C-2′), 147.37(C-4′), 145.12(C-5′), 141.21(–OH/C-3), 131.42(C-7), 129.05(C-6), 127.17 (C/CF_3), 130.20(C-10), 123.20(C-8), 121.14(C-5), 118.02(C-6′), 112.32(C-1′), 98.11(C-3′), 57.24, 56.16, 56.12 (OMe-C); MS (APCI); *m/z* 397.1 $[M+H]^+$; HPLC: 95.03 %.

*3-Hydroxy-6-nitro-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one (**2r**)* Yellow solid: yield 66.5 %; mp: 196–198 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.35 (bs, 1H), 8.82 (s, 1H), 8.50 (d, $J = 8.5$ Hz, 1H), 7.87 (d, $J = 9$ Hz, 1H), 7.11 (s, 1H), 6.83 (s, 1H), 3.89 (s, 3H), 3.81 (s, 3H), 3.72 (s, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz, δ ppm): 173.23(C=O), 159.51(C-2), 158.47(C-9), 151.85(C-2′), 146.37(C-4′), 145.30 (C-5′), 142.10(C-6), 141.21(–OH/C-3), 131.42(C-7), 130.20 (C-10), 123.20(C-8), 121.14(C-5), 119.02(C-6′), 112.32(C-1′), 98.20(C-3′), 57.24, 56.18, 56.15 (OMe-C); MS (APCI); *m/z* 374.1 $[M+H]^+$; HPLC: 97.58 %.

*3-Hydroxy-6-(methylsulfonyl)-2-(2,4,5-trimethoxyphenyl)-4H-chromen-4-one (**2s**)* Off white solid: yield 66 %; mp: 220–222 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.24 (bs, 1H), 8.58 (s, 1H), 8.20 (d, $J = 9$ Hz, 1H), 7.86 (d, $J = 8.6$ Hz, 1H), 7.10 (s, 1H), 6.83 (s, 1H), 3.86 (s, 3H), 3.82 (s, 3H), 3.78 (s, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz, δ ppm): 172.45 (C=O), 158.51(C-2), 157.47(C-9), 151.81(C-2′), 146.37(C-4′), 145.30(C-5′), 141.21(–OH/C3), 137.42.(C-6), 131.42 (C-7), 131.20(C-10), 123.20(C-8), 120.14(C-5), 119.02(C-6′), 112.32(C-1′), 96.20(C-3′), 57.25, 56.19, 56.16 (OMe-C), 39.89 (Me/SO_2); MS (APCI); *m/z* 407.1 $[M+H]^+$; HPLC: 94.95 %.

*3-Hydroxy-4-oxo-2-(2,4,5-trimethoxyphenyl)-4H-chromene-8-carbonitrile (**2t**)* Off white solid: yield 58 %; mp: 199–201 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.05 (bs, 1H), 8.22 (d, $J = 8.5$ Hz, 1H), 8.06 (d, $J = 9$ Hz, 1H), 7.41 (m, 1H), 7.21 (s, 1H), 6.75 (s, 1H), 3.81 (s, 3H), 3.77 (s, 3H), 3.67 (s, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz, δ ppm): 172.60(C=O), 163.71(C-2), 161.14(C-9), 156.19(C-2′), 153.29(C-4′), 151.19 (C-5′), 142.31(–OH/C-3), 130.33(C-10), 129.12(C-7), 125.25 (C-8), 117.16(C-6), 115.35(C-5), 112.14 (C-6′), 107.54(C-1′), 98.72 (C-3′), 56.27, 56.25, 56.24 (OMe-C); MS (APCI); *m/z* 372.1 $[M+H]^+$; HPLC: 95.36 %.

*3-Hydroxy-4-oxo-2-(2,4,5-trimethoxyphenyl)-4H-chromene-5-carbonitrile (**2u**)* Off white solid: yield 60 %; mp: 207–209 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.05 (bs, 1H), 8.67 (s, 1H), 8.18 (d, $J = 8.5$ Hz, 1H), 7.59 (d, $J = 9$ Hz 1H), 7.59 (s, 1H), 6.78 (s, 1H), 3.83 (s, 3H), 3.76 (s, 3H), 3.68 (s, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz, δ ppm): 173.16(C=O), 162.20(C-2), 160.10(C-9), 156.21(C-2′),

154.29(C-4′), 151.19(C-5′), 142.31(–OH/C-3), 129.79(C-10), 129.12(C-7), 126.25(C-5), 118.16(C-6), 115.35(C-8), 113.14 (C-6′), 107.54(C-1′), 97.70 (C-3′), 56.22, 56.21, 56.19 (OMe-C); MS (APCI); *m/z* 372.1 $[M+H]^+$; HPLC: 96.76 %.

Biological assay

Anti-inflammatory assay

Proinflammatory cytokine production by LPS in THP-1 cells has been measured according to the method described by Hwang in 1993. During assay, THP-1 cells were cultured in RPMI 1640 culture medium (Gibco BRL, Pasley, UK) containing 100 U/mL penicillin and 100 mg/mL streptomycin containing 10 % fetal bovine serum (FBS, JRH). Cells have been differentiated with phorbol myristate acetate (PMA, Sigma). Following cell plating, the test compounds in 0.5 % DMSO have been added to each well separately and the plate has been incubated for 30 min at 37 °C. Finally, LPS (*E. coli* 0127:B8, Sigma Chemical Co., St. Louis, MO, USA) has been added at a final concentration of 1 μg/mL in each well. Plates have been further incubated at 37 °C for 24 h in 5 % CO_2. After incubation, supernatants have been harvested, and assayed for TNF-α and IL-6 by ELISA as described by the manufacturer (BD Biosciences).

Antibacterial assay

All the newly synthesized compounds have been screened for their antibacterial activity against selected Gram-positive organism's viz. *Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus* (MTCC 96), and Gram-negative organism's viz. *Salmonella typhimurium* (MTCC 98) and *Escherichia coli* (MTCC 443) bacterial strains by agar-well diffusion method with little modification. Different concentrations (10–200 μg/mL) of test compounds have been prepared in DMSO. The bacterial suspension has been spread over nutrient agar plates and the well with of 6 mm diameter has been punched with sterile cork borer. The sample (50 μL) has been added to the well and the plates have been incubated at 36 °C for 24 h. DMSO used as solvent control and ciprofloxacin has been used as standard antibacterial agent. The lowest concentration of compound which completely inhibits the bacterial growth has been taken as minimum inhibitory concentration (MIC).

Antifungal assay

All the newly synthesized compounds have been screened for their antifungal activity against *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 281), *Aspergillus flavus* (MTCC 277), and *Fusarium solani* (MTCC 350) by agar-well diffusion method with little modification. Normal saline has been used to make a suspension of spores of fungal strain, prepared as same as antibacterial assay. Miconazole has been used as standard antifungal agent.

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