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Synthesis, biological evaluation and molecular docking studies of stellatin derivatives as cyclooxygenase (COX-1, COX-2) inhibitors and anti-inflammatory agents

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ABSTRACT

Stellatin (**4**), isolated from *Dysophylla stellata* is a cyclooxygenase (COX) inhibitor. The present study reports the synthesis and biological evaluation of new stellatin derivatives for COX-1, COX-2 inhibitory and anti-inflammatory activities. Eight derivatives showed more pronounced COX-2 inhibition than stellatin and, **17** and **21** exhibited the highest COX-2 inhibition. They also exhibited the significant anti-inflammatory activity in TPA-induced mouse ear edema assay and their anti-inflammatory effects were more than that of stellatin and indomethacin at 0.5 mg/ear. The derivatives were further evaluated for antioxidant activity wherein **16** and **17** showed potent free radical scavenging activity against DPPH and ABTS radicals. Molecular docking study revealed the binding orientations of stellatin and its derivatives into the active sites of COX-1 and COX-2 and thereby helps to design the potent inhibitors.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are of huge therapeutic benefit in the treatment of rheumatoid arthritis and various types of inflammatory conditions. The target for these drugs is cyclooxygenase (COX), a rate limiting enzyme which converts arachidonic acid into inflammatory prostaglandins. COX exists in two isoforms, COX-1 and COX-2. COX-1 is known as a housekeeping enzyme and constitutively expressed in all tissues, while COX-2 is constitutively expressed only in kidney, brain and ovaries. COX-2 is increasingly expressed during inflammatory conditions by pro-inflammatory molecules such as IL-1, TNF-α, LPS and TPA.[1–3]

Chromones constitute an important class of natural products and are reported to exhibit anti-inflammatory,[4–6] anti-arthritic,[6] anti-cancer,[7] immune-stimulation,[7] anti-platelet,[8] uricosuric,[6] anti-allergic,[6] anti-bacterial[9] and antioxidant[4] activities. As anti-inflammatory agents, chromones exert their effects by inhibiting various mechanisms such as COX-1 and COX-2 enzymes,[4] NO production,[7] PKC[10] and O_2^- production in PMA- or f-MLF-stimulated human neutrophils.[10] The structures of some naturally occurring anti-inflammatory chromones, aloesin (**1**)[4], norcimicifugin (**2**)[11], petersinone 1 (**3**)[7], stellatin (**4**)[12], eugenin (**5**)[12] and 5,7-dimethoxy-2-methylchromanone (**6**)[12] are shown in Figure 1.

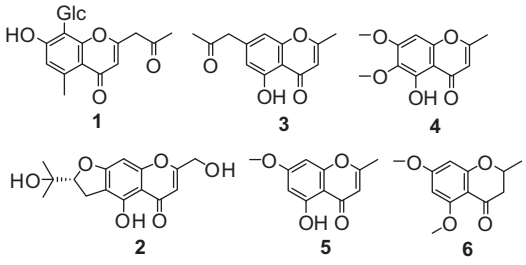

Figure 1. Natural anti-inflammatory chromones of plant origin.

As a part of our continuing program to discover COX-1 and COX-2 inhibitory compounds from Indian medicinal plants,[12–14] the *n*-hexane and EtOAc extracts of *Dysophylla stellata* (Labiatae) were tested in a COX catalyzed prostaglandin biosynthesis assay in vitro and a significant COX-1 and COX-2 inhibitory activity was observed. The chromones **4**–**6** were isolated as the potent COX inhibitory principles and **4** exhibited selectivity towards COX-2 inhibition than COX-1.[12] The HPLC analysis of *D. stellata* revealed **4** as a major constituent[15] and it was concluded that it could be responsible for COX inhibitory activity of *D. stellata*. These findings prompted us to synthesize the derivatives of **4** so as to study the structure–activity relationship (SAR).

The chemical modifications of **4** were carried out at 5-OH to introduce the different functionality. The 5-O-alkylation was

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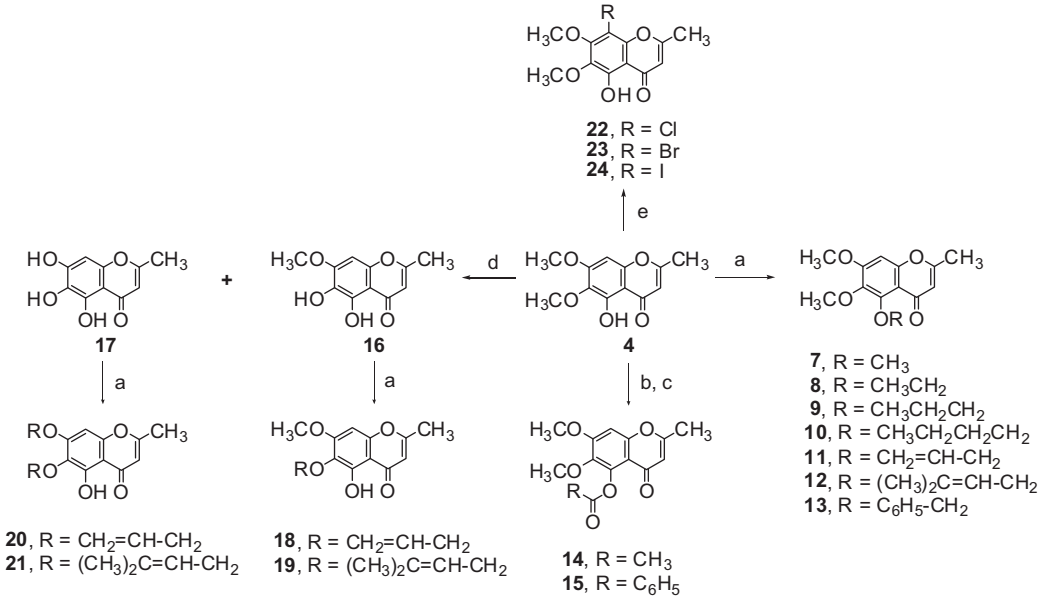

Scheme 1. Reagent and conditions: (a) $K_2CO_3/(CH_3)_2CO$, RX, reflux, 12–24 h, 35–85%; (b) $(CH_3CO)_2O/CH_3COONa$, reflux, 2 h, 75%; (c) $K_2CO_3/(CH_3)_2CO$, PhCOBr, reflux, 24 h, 64%; (d) $AlCl_3/C_7H_8$, reflux, 12 h, 30–70%; (e) NCS (**22**), NBS (**23**), NIS (**24**)/ACN, rt, 4–24 h, 20–75%.

carried out with alkylating agents using anhydrous K_2CO_3 in acetone[16] to furnish compounds **7**–**13** (Scheme 1) in good yields (65–85%), except **10** (35%). The reaction of **4** with $(CH_3CO)_2O$ and anhydrous CH_3COONa afforded **14** in a fairly good yield (75%)[17] while **15** was synthesized in the similar way as *O*-alkyl derivatives. To introduce the free OH, demethylation was performed with anhydrous $AlCl_3$ in toluene.[18] The reaction of **4** with $AlCl_3$ for 2 h resulted in the formation of **16** in a high yield (80%), but, when the reaction time was extended to 12 h, **16** (70%) and **17** (20%) both were formed. Ethers **18**–**21** were synthesized from **16** and **17** respectively, using K_2CO_3 and alkyl bromides in high yields (80–90%). The reaction of **16** with alkyl bromide resulted in the regioselective monoalkylation at 6-position (**18**–**19**). Similarly, the regioselective dialkylation at 6-and 7-positions was observed in **20**, **21**. The reaction of **4** with NCS/NBS/NIS in acetonitrile gave **22**–**24** wherein regioselective halogenation was observed at 8-position. Of the synthetic derivatives, **8**–**13**, **15**, **18** and **20**–**24** were found as new compounds when they were searched on Scifinder and Reaxys databases (The spectral, MS and elemental data of new compounds are submitted as Supplementary data).

The synthesized chromones (**7**–**24**) were evaluated for in vitro COX-1 and COX-2 inhibitory activity in a COX catalyzed prostaglandin biosynthesis assay as described by us previously.[12,13] Initially, the compounds were tested at 30 μM and their effects on COX-1 and COX-2 inhibition are shown in Table 1. The study was further extended to examine the concentration-activity responses at different concentrations to determine the IC_{50} values for COX-1 and COX-2. Five compounds (**16**, **17** and **19**–**21**) showed better COX-1 inhibitory activity than stellatin with IC_{50} values in the range of 14–22 μM; whereas seven compounds (**12** and **16**–**21**) exhibited more COX-2 inhibitory activity than that of stellatin (IC_{50s}, 8–18 μM). Compound **17** (5,6,7-trihydroxy-2-methylchromone) exhibited the highest COX-2 inhibition (IC_{50} 8.87 μM) followed by **21** (IC_{50} 10.35 μM). The COX-2 inhibitory effect of **17** was >2-fold as compared to stellatin (IC_{50} 19.7 μM). The results of the present study suggests that chromones acts as non-selective inhibitors of COX enzymes and showed anti-inflammatory effects.

In case of ether derivatives, the introduction of a chain length up to four carbons (**7**–**11**) and a benzyl group (**13**) resulted in a decreased COX-1 and COX-2 inhibitory activity; however, an isoprenyl group at 5-position (as in **12**) did not alter the COX-1 inhibition whereas it showed improved COX-2 inhibition. The conversion of OH into an ester (**14** and **15**) also decreased the COX-1 and COX-2 inhibitory activity. The conversion of methoxyl groups of **4** into the hydroxyl groups improved both COX-1 and COX-2 inhibitory activity (**16** and **17**) with more pronounced effect towards COX-2 inhibition. Eugenin (**5**), an isolated chromone with one methoxyl group less than stellatin, showed better COX-1 inhibition than stellatin without having much effect on COX-2. The replacement of free OH groups at 6- and 7-position by an allyl or isoprenyl groups (**18**–**21**) improved the COX-1 and COX-2 inhibitory activity (except **18**, in case of COX-1). The halogenated derivatives (**22**–**24**) did not show 'noticeable' effect on COX inhibition as their inhibition was even less than that of stellatin.

The reports on SAR of flavonoids (2-phenylchromones) suggested that 4-oxo functional group of the C-ring is essential for COX inhibitory activity; the C_2–C_3 double bond enhances the activity and reduction of this double bond results in decreased or loss of inhibitory activity.[19,20] The similar trend was also observed in the case of the studied chromones and inhibition was seemed to depend on number and position of hydroxyl residues. The presence of 5-OH is essential for COX inhibitory activity, however, isoprenyl group seems to be ideal replacement. The presence of 6-OH further increased the COX-1 and COX-2 inhibitory activity. Therefore, the SAR study helped us to establish a basic pharmacophore (Fig. 2) responsible for the activity. A chromone with C_2–C_3 double bond, a carbonyl group at 4-position of A-ring and a 5-OH on B-ring are essential features for the COX inhibitory activity.

Ten compounds (**11**, **12** and **16**–**23**) were evaluated further for anti-inflammatory in a TPA-induced mouse ear edema assay as described earlier by us.[12,13] All the compounds showed significant anti-inflammatory activity. Compound **17** exhibited the highest anti-inflammatory activity with 90.7% reduction in the ear edema followed by **21** (85.1% reduction). The anti-inflammatory effects of **17** and **21** were more than that of stellatin (69.4%) and indomethacin (81.4%) at the same dose levels. Compounds **12**, **16**, **19** and **20** showed anti-inflammatory activity comparable to indomethacin (Table 1) and their anti-inflammatory effects were in order: **20** > **16** > **19** > **12**.