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Original article

Synthesis and evaluation of novel chromone analogs for their inhibitory activity against interleukin-5

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ABSTRACT

A novel series of chromone analogs were synthesized and evaluated for their inhibitory activity against interleukin-5. Among them compounds 5-Cyclohexylmethoxy-3-(4-hydroxybenzyl)-4*H*-chromen-4-one (**6a**, 98% inhibition at 30 μM, $IC_{50} < 3.0$ μM) and 5-Cyclohyxylmethoxy-3-(hydroxymethyl)-4*H*-chromen-4-one (**8a**, 84% inhibition at 30 μM, $IC_{50} = 7.6$ μM) showed most potent activity. The structural requirement of chromone analogs possessing the inhibitory activity against IL-5 could be summarized as: (i) importance of hydrophobic group such as cyclohexylmethoxy at 5th position of ring A, (ii) requirement of ring B with small size of hydrogen bonding group with electron donating property such as phenolic hydroxyl group at 4th position and (iii) planarity of the chromen-4-one ring.

1. Introduction

Eosinophilic inflammation in the airway, along with hyper-responsiveness and tissue injury, is a characteristic feature in the pathogenesis of bronchial asthma [1,2]. There is a strong evidence for a major role of T helper type 2 (Th2) cytokines in diseases such as asthma and, the therapeutic strategies that target Th2 cytokines are of potential benefit in such allergic disease [3,4]. Several studies indicate the role of eosinophil as a major effector cell in allergic inflammation, capable of causing most of the morphological and functional alterations observed in asthma. Several cytokines can affect eosinophils but despite possible redundancy with IL-3 and granulocyte-macrophage colony stimulating factors (GM-CSF), IL-5 seems to be the primary cytokine involved in the production, differentiation, maturation and activations of the eosinophils under *in vivo* conditions [5]. It was observed that IL-5 transgenic mice developed lifelong eosinophilia, whereas GM-CSF transgenic showed increased numbers of mononuclear cells and neutrophils, but only a minimal increase in the number of eosinophils [6,7]. Similar findings have emerged from knockout mice studies [8–10]. It has also been shown that IL-5, is present in increased amounts in the mucosa of asthmatic airways and the expression of IL-5 mRNA is directly related to severity of the disease [11,12]. In addition, inhalation of IL-5 has been shown to increase the percentage of eosinophils in induced sputum and to augment airway hyper-responsiveness in asthmatics [13]. These observations suggest that eosinophils are the major cell type in allergic reactions and thus the action of IL-5 emerges as one of the new immunomodulatory therapeutic strategy in the treatment of allergic diseases like bronchial asthma. The major advantage of this strategy is the specificity of reducing eosinophillic inflammation, without any side effects.

Recently, some small organic compounds have been found to inhibit the activity of IL-5. Isothiazolones synthesized by modification of Cys 66 residue in IL-5, have been identified as IL-5 antagonists [14]. Naturally occurring sophoricoside (**1a**, 92.1% inhibition at 50 μM, $IC_{50} = 1.4$ μM) and its analogs have been isolated and identified as inhibitors of IL-5 [15], besides acting as differential inhibitors of IL-3 and GM-CSF [16]. Considering the activity of budesonide (70.3% inhibition at 50 μM, $IC_{50} = 26.8$ μM), which is used for the treatment of chronic asthma, the activity of sophoricoside appears to be very potent. Isoflavonoid analogs of sophoricoside [17,18] and chalcones [19,20] have been demonstrated to be novel inhibitors of IL-5.

Structure–activity relationship of sophoricoside analogs **1a–d** [17] revealed the necessity for the planar chromen-4-one ring and a phenolic hydroxyl group at 4th position of ring B. More potent activity of sophoricoside (Chart 1) than its aglycon, genistein (79.1% inhibition at 50 μM, $IC_{50} = 10.6$ μM) indicates the necessity for

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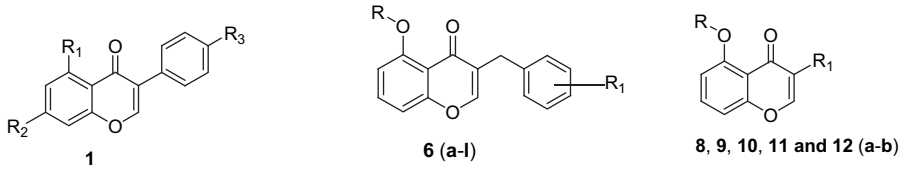

1a: Sophoricoside R_1,R_2 = OH, R_3 = OGlu
1b: R_1 = benzyloxy, R_2 = H, R_3 = OH
1c: R_1 = H, R_2 = benzyloxy, R_3 = OH
1d: R_1 = cyclohexylmethoxy, R_2 =H, R_3 = OH

Chart 1. Interleukin-5 inhibitors **1** and chromone analogs.

a glucopyranosyl moiety. Introduction of benzyloxy, cylohexylmethoxy or isopentyloxy groups at 5th position of isoflavone increased the activity and eliminated the necessity of glucopyranosyl moiety of sophoricoside. However, the role of B ring of isoflavone analogs for the inhibition of IL-5 has not been explored. For continuation of our effort to find the more detail structure–activity relationship of isoflavones as IL-5 inhibitor, we designed and prepared the derivatives **6**–**12** shown in Chart 1 structurally varied around B ring of isoflavones and evaluated them for their inhibitory activity against interleukin-5.

2. Chemistry

In continuation of our previous work [17,18] on the modification of B ring of compound **1**, we have synthesized a series of new chromone analogs starting from commercially available 2, 6-dihydroxyacetophenone as shown in Fig. 1. The preparation of chalcones **4** was accomplished by the aldol condensation of substituted 2-hydroxyacetophenone and appropriate commercially available benzaldehyde **3** in the presence of potassium hydroxide in ethanol at 50 °C for 12 h [17–20]. Trans-stereochemistry of propenone moiety of **4** was confirmed by coupling constants of vinyl hydrogens (15–16 Hz). Acetophenones **2** used in the preparation of **4** were prepared by the partial alkylation of 2, 6-dihydroxyacetophenone [17]. Most benzaldehydes **3** used in the preparation of **4** are commercially available. The 4-formyl benzenesulfonamide used for the preparation of **4b** by van Es's procedure [21]. The catalytic hydrogenation of **4** gave the intermediates **5** [20]. The cyclization of **5** to the desired compound **6** was achieved by treating with *N*, *N*-dimethylformamide, methanesulfonyl chloride and boron trifluoride diethyl etherate at reflux. The substituents of **6a**–**l** are listed in Table 1.

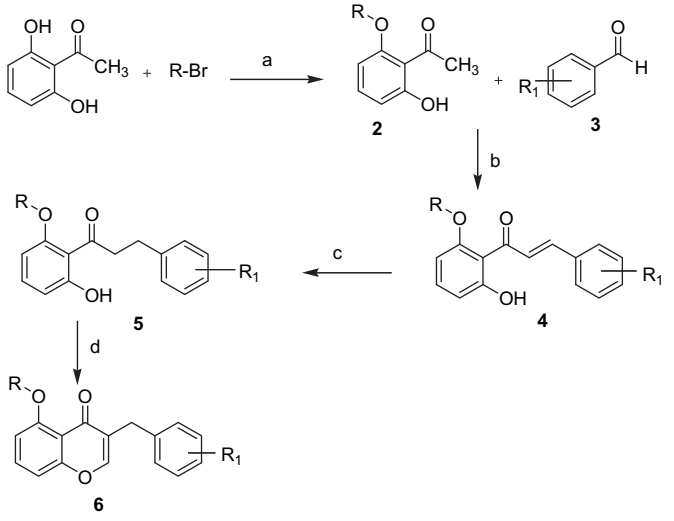

Fig. 1. Synthesis of chromone analogs. (a) CH_3CN, aqueous 95% KOH, reflux, overnight, (b) KOH/90% ethanol, overnight reflux, (c) H2-Pd/methanol, 20–30 psi, 2 h, (d) BF_3–Et_2O, CH_3SO_2Cl, DMF, 2 h reflux. Note = substitutions are located in Table 1.

The synthetic route used to synthesize aliphatic substituted chromone analogs is outlined in Fig. 2. Preparation of **7** was accomplished by the cyclization of **2** using *N*, *N*-dimethylformamide and phosphoryl chloride at ambient temperature [22,23]. Compound **7** was then oxidized to acid **10** by the treatment of Jones reagent [24]. Reduction of **7** using isopropanol and basic alumina under refluxing condition gave alcohol **8** [23], which was then treated with thionyl chloride to produce the corresponding halide **9** [25]. One pot reaction of **7** and cyclic malonate in the presence of triethylamine and formic acid (1:1) under reflux for 2 h [26] gave carboxylic acid **11**. Oxime **12** was prepared by the treatment of **7** with hydroxylamine hydrochloride at ambient temperature for 1 h [27].

3. Pharmacology

Inhibitory activity of the chromone analogs against IL-5 was evaluated using the IL-5-dependent pro-B Y16 cell line according to the known procedure [15]. The cells were incubated with 3 units/mL mIL-5 for 48 h, in the presence or absence of sample, and then measured cell metabolism as an index of proliferation, using

Table 1
Substituents and inhibitory activity of chromone analogs against interleukin-5.

Compd.	R	R_1	Inhibition at 30 μM [%]	IC_{50} [μM][b]
6a	Cyclohexylmethyl	OH	98	3.0
6b	Cyclohexylmethyl	SO_2NH_2	31	>30
6c	Cyclohexylmethyl	COOH	77	12.3
6d	Isobutyl	COOH	73	11.4
6e	Isopentyl	OH	36	>30
6f	Isopentyl	CH_3	35	>30
6g	Cyclohexylmethyl	H	56	22.9
6h	Cyclohexylmethyl	CH_3	86	14.8
6i	Cyclohexylmethyl	C_2H_5	45	>30
6j	Cyclohexylmethyl	Cl	36	>30
6k	Cyclohexylmethyl	CF_3	47	>30
6l	Cyclohexylmethyl	3,4,5-OCH_3	85	12.5
8a	Cyclohexylmethyl	CH_2OH	84	7.6
8b	Isobutyl	CH_2OH	56	21.6
9a	Cyclohexylmethyl	CH_2Cl	45	>30
9b	Isobutyl	CH_2Cl	43	>30
10a	Cyclohexylmethyl	COOH	43	>30
10b	Isobutyl	COOH	33	>30
11a	Cyclohexylmethyl	CH_2CH_2COOH	<10	>30
11b	Isobutyl	CH_2CH_2COOH	<10	>30
12a	Cyclohexylmethyl	C(=N) OH	<10	>30
12b	Isobutyl	C(=N) OH	<10	>30
Sophoricoside (**1a**)			93.8[a]	10.3
Budesonide			70.3[a]	26.2

[a] Inhibition at 50 μM.
[b] IC_{50} is taken as a mean value from 3 independent experiments.

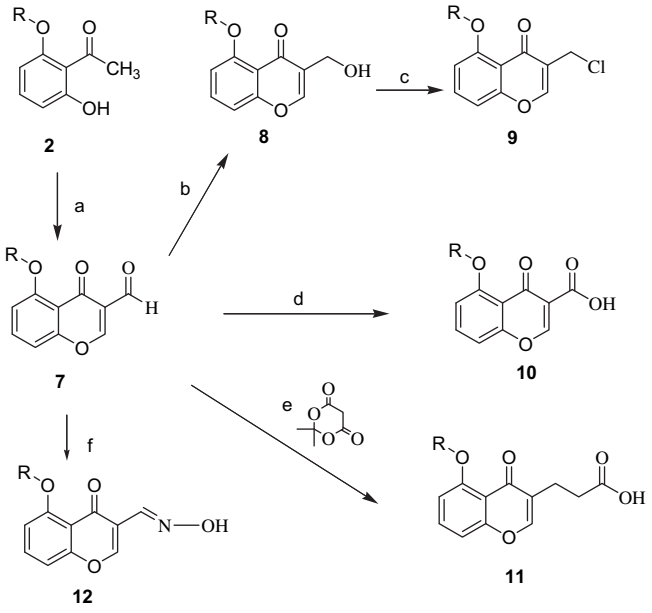

Fig. 2. Preparation of aliphatic substituted chromone analogs. (a) $POCl_3$, DMF, overnight, rt; (b) Alumina (Basic), IPA, reflux, 4 h; (c) SO_2Cl_2, reflux, 2 h; (d) Jone's reagent, 0 °C, 1 h; (e) TEA: HCOOH 1/1 reflux, 2 h; (f) NH_2OH, ethanol, rt 1 h. Note = substitutions are located in Table 1.

2-(4-iodophenyl)-3-(nitrophenyl)-5-(2,4-disulphophenyl)-2*H* tetrazolium sodium salt (WST-1). Effect on the IL-5 bioassay by each test compound is represented as inhibition % at 30 μM samples and also IC_{50} values (Table 1). Data were collected from three independent experiments.

4. Results and discussion

As we have reported earlier [17] that the isoflavones (**1c**, **1d**) with 4-hydroxyl on the ring B and benzyloxy (or cyclohexylmethoxy) group on the ring A plays an important role in modulating inhibitory activity of IL-5 [18]. To further confirm the role of ring B of isoflavone for their inhibitory activity, we synthesized a series of chromone analogs.

The analogs **6a** (98% inhibition at 30 μM, $IC_{50} < 3.0$ μM) and **8a** (84% inhibition at 30 μM, $IC_{50} = 7.6$ μM) had much better activity than others. The level of activity of **6a** is comparable to that of **1d** (91.7% inhibition at 50 μM, $IC_{50} = 5.8$ μM) [18]. This indicate that ring B of isoflavone analog [18] needs a strong electron donating group with hydrogen acceptor property and is not necessary to attach directly on chromone ring.

Since the hydroxyl group at 4th position of ring B showed good activity as shown in **6a**, the larger hydrogen bond acceptor such as $-SO_2NH_2$ or carboxylic acid on ring B was introduced in **6b** (31% inhibition at 30 μM, $IC_{50} > 30$ μM), **6c** (77% inhibition at 30 μM, $IC_{50} = 12.3$ μM) and **6d** (73% inhibition at 30 μM, $IC_{50} = 11.4$ μM). Surprisingly, they showed the less activity compared to **6a**. This implies that the small size of hydrogen bonding group like hydroxyl at this position might be important factor in the activity.

In addition to that, the cyclohexylmethoxy group at 5th position on ring A of **6a** and **8a** plays an important role to increase the hydrophobicity and cell permeability for the enhancement of activity. The smaller alkoxy group such as isopentyl decreases the activity as shown in compounds **6e** (36% inhibition at 30 μM, $IC_{50} = 38$ μM) and **6f** (35%inhibition at 30 μM, $IC_{50} > 30$ μM) compared to **6a**. Thus the optimum alkoxy group at this position of **6** or **8** such as cyclohexylmethoxy group should be important as was in isoflavone analogs [18]

Compound **6h** (86% inhibition at 30 μM, $IC_{50} = 14.8$ μM) with a cyclohexylmethoxy group on ring A and a methyl group on ring B showed greater activity than **6g** (56% inhibition at 30 μM, $IC_{50} = 22.9$ μM) without methyl group. Based on these finding, we increased the hydrophobicity of this position with ethyl group shown in **6i** (45% inhibition at 30 μM, $IC_{50} > 30$ μM), chloro group shown in **6j** (36% inhibition at 30 μM, $IC_{50} = 48$ μM) and trifluoromethyl shown in **6k** (47% inhibition at 30 μM, $IC_{50} = 36.2$ μM) to get the more insight of structure–activity relationship. However, these changes were not much encouraging on activity.

Compound **6a** and **6l** (85% inhibition at 30 μM, $IC_{50} = 12.3$ μM) showed better activity than trifluoromethyl analog **6k** (47% inhibition at 30 μM, $IC_{50} = 36.2$ μM) and **6b**. Since the hydroxyl and methoxy groups are highly electron donating to phenyl ring as compared to trifluoromethyl or sulfonamide groups, the high electron density at B ring would be important for binding to the receptor.

To get more detail about the importance of ring B we have also synthesized some chromone derivatives **8**–**12** without phenyl ring. On introduction of hydroxylmethyl group at chromone ring with cyclohexylmethoxy group at 5th position on ring A as shown in **8a** (85% inhibition at 30 μM, $IC_{50} = 7.6$ μM) the activity becomes moderate in comparison to **6a**. Similarly the chloromethyl substituted analog **9a** (45% inhibition at 30 μM, $IC_{50} > 30$ μM), acid derivatives **10a** (43% inhibition at 30 μM, $IC_{50} > 30$ μM) and **11a** (<10% inhibition at 30 μM, $IC_{50} > 30$ μM) and oxime derivative **12a** (<10% inhibition at 30 μM, $IC_{50} > 30$ μM) did not contribute much on enhancing the activity.

Further, on replacing cyclohexylmethyl group with isobutyl on ring A, compound **8b** (56% inhibition at 30 μM, $IC_{50} = 21.6$ μM) and chloromethyl substituted analog **9b**. The extended side chain of **11b** and the oxime derivatives **12b** dose not turn up with any inhibition against IL-5 activity. These findings further strengthen our view point regarding the importance of ring B with an electron donating group at 4th position is critical for the activity.

5. Conclusion

It can be concluded from the whole study that the structural requirements of chromone analogs possessing their inhibitory activity against IL-5 could be summarized as:

(1) Importance of hydrophobic group such as cyclohexylmethoxy at 5th position of ring A,
(2) Requirement of ring B with small size of hydrogen bonding group such as phenolic hydroxyl group at 4th position,
(3) Planarity of the chromen-4-one ring. Further, the introduction of a methylene unit between chromone ring and ring B did not show much beneficial effect on activity. However, the introduction of aliphatic groups on chromone ring in place of phenyl ring (B ring) decreases the overall activity.

6. Materials and methods

6.1. Chemistry

Melting points (m.p.) were determined on Electro thermal 1A 9100 MK2 apparatus and are uncorrected. All commercial chemicals were used as obtained and all solvents were purified by the standard procedures prior to use [28]. Thin layer chromatography was performed on E Merck silica gel GF-254 precoated plates and the identification was done with UV light and colorization with spray 10% phosphomolybdic acid followed by heating. Flash column chromatography was performed with E Merck silica gel (230–400 mesh).

Infra red spectrum was recorded by using sample (neat sample without solvent or KBr) as such on FT-IR spectrum with Nicolet–380 models. NMR spectra were measured against the peak of tetramethylsilane by JEOL JNM-EX90 NMR (89.45 MHz) and Varain Unity Inova 400 NMR (400 MHz) spectrometers. Elemental analyses were performed using an EA1110 elemental analyzer (CE Instrument).

*6.1.1. Procedures for the preparation of compounds **2**(**a**–**c**), **4** (**a**–**l**) and **5** (**a**–**l**)*

The synthetic procedures for all compounds **2**(**a**–**c**), **4** (**a**–**l**) and **5** (**a**–**l**) are followed according to the same procedures as described previously [17–20].

*6.1.2. General synthetic procedure for the compounds **6** (**a**–**l**)*

Boron trifluoride etherate (4 equiv) was added drop wise to a compound **5**, 0.7 g (0.001 mol) in anhydrous DMF (20 mL) under nitrogen at room temperature. The solution was stirred for 10 min before the drop wise addition of CH_3SO_2Cl (3 equiv). The resulting solution was warmed to 80 °C and stirred for 2 h. The reaction was cooled to room temperature before H_2O (25 mL) was added. The combined organic extracts were washed with saturated aqueous NaCl, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by column chromatography to afford desired compounds.

*6.1.2.1. 5-(Cyclohexylmethoxy)-3-(4-hydroxybenzyl)-4H-chromen-4-one (**6a**).* Yield 40%; Yellow solid; m.p. 88–90 °C; R_f = 0.39 (Ethyl acetate:hexane = 2:3); IR (cm^{-1}): 2905, 2848, 1655, 1628; ^1H NMR ($CDCl_3$) δ 1.06–2.04 (m, 11H), 3.70 (s, 2H), 3.86 (d, J = 6.0 Hz, 2H), 5.72 (s, 1H), 6.73 (d, J = 8.4 Hz, 2H), 6.75 (d, J = 8.4 Hz, 1H), 6.92 (d, J = 8.4 Hz, 1H), 7.08 (d, J = 8.4 Hz, 2H), 7.36 (s, 1H), 7.47 (t, J = 8.4 Hz, 1H). Anal. Calc. for $C_{23}H_{24}O_4$: C, 75.80; H, 6.64. Found: C, 75.65; H, 6.58.

*6.1.2.2. 4-((5-(Cyclohexylmethoxy)-4-oxo-4H-chromen-3-yl)methyl) benzene sulfonamide (**6b**).* Yield 56%; Colorless solid; m.p. 190–191 °C; R_f = 0.10 (Ethyl acetate:hexane = 1:1); IR (cm^{-1}): 2900, 2852, 1650, 1620; ^1H NMR ($CDCl_3$) δ 1.07–2.04 (m, 11H), 3.12 (s, 2H), 3.87 (d, J = 6.0 Hz, 2H), 6.71 (d, J = 8.0 Hz, 1H), 6.93 (d, J = 8.4 Hz, 1H), 7.32 (d, J = 8.4 Hz, 2H), 7.40 (s, 2H), 7.48 (t, J = 8.4 Hz, 1H), 7.82 (d, J = 6.8 Hz, 2H), 8.10 (s, 1H). Anal. Calc. for $C_{23}H_{25}NO_5S$: C, 64.62; H, 5.89. Found: C, 64.55; H, 5.81.

*6.1.2.3. 4-((5-(Cyclohexylmethoxy)-4-oxo-4H-chromen-3-yl)methyl) benzoic acid (**6c**).* Yield 60%; Colorless solid; m.p. 149–151 °C; R_f = 0.12 (Ethyl acetate:hexane = 1:1); IR (cm^{-1}): 2921, 2848, 1647, 1620; ^1H NMR ($CDCl_3$) δ 1.07–1.89 (m, 11H), 3.66 (s, 2H), 3.82 (d, J = 6.0 Hz, 2H), 6.92 (d, J = 8.4 Hz, 1H), 7.05 (d, J = 8.4 Hz, 1H), 7.29 (s, 4H), 7.59 (t, J = 8.4 Hz, 1H), 8.13 (s, 1H). Anal. Calc. for $C_{24}H_{24}O_5$: C, 73.45; H, 6.16. Found C, 73.38; H, 6.10.

*6.1.2.4. 4-((5-Isobutoxy-4-oxo-4H-chromen-3-yl)methyl)benzoic acid (**6d**).* Yield 60%; Colorless solid; m.p. 91–92 °C; R_f = 0.08 (Ethyl acetate:hexane = 1:1); IR (cm^{-1}): 2959, 2873, 1747, 1716, 1646, 1570; ^1H NMR ($CDCl_3$) δ 1.03 (d, 6H), 2.06 (m, 1H), 3.79 (s, 2H), 3.99 (d, J = 5.6 Hz, 2H), 6.80 (d, J = 8.4 Hz, 1H), 7.06 (d, J = 7.6 Hz, 1H), 7.29 (m, 4H), 7.59 (t, J = 8.4 Hz, 1H), 8.15 (s, 1H). Anal. Calc. for $C_{21}H_{20}O_5$: C, 71.58; H, 5.72. Found: C, 71.46; H, 5.65.

*6.1.2.5. 3-(4-Hydroxybenzyl)-5-(isopentyloxy)-4H-chromen-4-one (**6e**).* Yield 30%; Yellow solid; m.p. 85–87 °C; R_f = 0.40 (Ethyl acetate:hexane = 1:1); IR (cm^{-1}): 2960, 2850, 1700, 1619, 1527; ^1H NMR ($CDCl_3$) δ 0.94 (d, J = 6.4 Hz, 6H), 1.82 (t, J = 6.8 Hz, 2H), 1.85–1.90 (m, 1H), 3.66 (s, 2H), 4.11 (t, J = 6.8 Hz), 6.73 (d, J = 8.8 Hz, 2H), 6.81 (d, J = 8.8 Hz, 1H), 6.94 (d, J = 8.8 Hz, 1H), 7.09 (d, J = 8.8 Hz, 2H), 7.43 (s, 1H), 7.49 (t, J = 8.8 Hz, 1H). Anal. Calc. for $C_{21}H_{22}O_4$: C, 74.54; H, 6.55. Found: C, 74.49; H, 6.47.

*6.1.2.6. 5-(Isopentyloxy)-3-(4-methylbenzyl)-4H-chromen-4-one (**6f**).* Yield 40%; Yellow solid; m.p. 65–67 °C; R_f = 0.45 (Ethyl acetate: hexane = 1:1); IR (cm^{-1}): 2848, 1647, 1620, 1464; ^1H NMR ($CDCl_3$) δ 0.99 (d, J = 6.4 Hz, 6H), 1.86 (t, J = 7.2 Hz, 2H), 1.92–1.97 (m, 1H), 3.73 (s, 2H), 4.11 (t, J = 7.2 Hz, 2H), 6.78 (d, J = 8.4 Hz, 1H), 6.91 (d, J = 8.0 Hz, 1H), 7.11 (d, J = 8.4 Hz, 2H), 7.16 (d, J = 8.0 Hz, 2H), 7.31 (s, 1H), 7.47 (t, J = 8.4 Hz, 1H). Anal. Calc. for $C_{22}H_{24}O_3$: C, 78.54; H, 7.19. Found: C, 78.49; H, 7.12.

*6.1.2.7. 3-Benzyl-5-(cyclohexylmethoxy)-4H-chromen-4-one (**6g**).* Yield 60%; Yellow solid; m.p. 93–95 °C; R_f = 0.42 (Ethyl acetate: hexane = 2:3); IR (cm^{-1}): 2910, 2848, 1655, 1628; ^1H NMR ($CDCl_3$) δ 1.09–2.05 (m, 11H), 3.79 (s, 1H), 3.87 (d, J = 6.0 Hz, 2H), 6.76 (d, J = 8.0 Hz, 1H), 6.91 (d, J = 8.8 Hz, 1H), 7.20–7.33 (m, 6H), 7.46 (t, J = 8.4 Hz, 1H). Anal. Calc. for $C_{23}H_{24}O_3$: C, 79.28: H, 6.94. Found: C, 79.19; H, 6.88.

*6.1.2.8. 5-(Cyclohexylmethoxy)-3-(4-methylbenzyl)-4H-chromen-4-one (**6h**).* Yield 50%; Yellow solid; m.p. 80–82 °C; R_f = 0.48 (Ethyl acetate:hexane = 2:3); IR (cm^{-1}): 2912, 2854, 1658, 1615; ^1H NMR ($CDCl_3$) δ 1.05–1.30 (m, 5H), 1,56 (s, 3H), 1.72–1.85 (m, 6H), 3.73 (s, 2H), 3.85 (d, J = 5.6 Hz, 2H), 6.37 (d, J = 8.4 Hz, 1H), 6.49 (d, J = 8.8 Hz, 2H), 6.55 (d, J = 8.4 Hz, 1H), 7.10 (s, 1H), 7.18 (t, J = 8.4 Hz, 1H), 7.32 (d, J = 8.4 Hz). Anal. Calc. for $C_{24}H_{26}O_3$: C, 79.53; H, 7.23. Found: C, 79.44; H, 7.15.

*6.1.2.9. 5-(Cyclohexylmethoxy)-3-(4-ethylbenzyl)-4H-chromen-4-one (**6i**).* Yield 51%; Colorless solid; m.p. 88–89° C; R_f = 0.55 (Ethyl acetate:hexane = 1:4); IR (cm^{-1}): 2920, 2852, 1747, 1645; ^1H NMR ($CDCl_3$) δ 1.095 (t, J = 3.4 Hz, 3H), 1.17–2.0 (m, 1H), 2.62 (q, J = 7.6 Hz, 2H), 3.76 (s, 2H), 3.87 (d, J = 6.4 Hz, 2H), 6.75 (d, J = 8.0 Hz, 1H), 6.90 (d, J = 8.8 Hz, 1H), 7.12–1.24 (m, 4H), 7.28 (d, J = 8.0 Hz, 1H), 7.47 (t, J = 8.4 Hz, 1H). Anal. Calc. for $C_{25}H_{28}O_3$: C, 79.75; H, 7.50. Found: C, 79.60; H, 7.42.

*6.1.2.10. 3-(4-Chlorobenzyl)-5-(cyclohexylmethoxy)-4H-chromen-4-one (**6j**).* Yield 40%; Yellow solid; m.p. 70–72 °C; R_f = 0.29 (Ethyl acetate:hexane = 1:4); IR (cm^{-1}): 2895, 2832, 1630, 1610; ^1H NMR ($CDCl_3$) δ 1.02–1.74 (m, 11H), 3.49 (s, 2H), 3.81 (d, J = 5.6 Hz, 2H), 6.35 (d, J = 8.4 Hz, 1H), 6.56 (d, J = 8.4 Hz, 1H), 7.15 (d, J = 8.4 Hz, 2H), 7.22 (s, 1H), 7.26 (d, J = 8.4 Hz, 2H), 7.31 (t, J = 8.4 Hz, 1H). Anal. Calc. for $C_{23}H_{23}ClO_3$: C, 72.15; H, 6.05. Found: C, 72.05; H, 5.94.

*6.1.2.11. 5-(Cyclohexylmethoxy)-3-(4-(trifluoromethyl)benzyl)-4H-chromen-4-one (**6k**).* Yield 55%; Yellow solid; m.p. 90–91 °C; R_f = 0.42 (Ethyl acetate:hexane = 1:4); IR (cm^{-1}): 2926, 2853, 1650; ^1H NMR ($CDCl_3$) δ 1.05–2.17 (m, 11H), 3.84 (s, 1H), 3.87 (d, J = 6.4 Hz, 2H), 6.71 (d, J = 8.0 Hz, 1H), 6.93 (d, J = 8.4 Hz, 1H), 7.40 (d, J = 8.0 Hz, 3H), 7.48 (t, J = 8.0 Hz, 1H), 7.56 (d, J = 8.4 Hz, 2H). Anal. Calc. for $C_{24}H_{23}F_3O_3$: C, 69.22; H, 5.57. Found: C, 69.13; H, 5.48.

*6.1.2.12. 5-(Cyclohexylmethoxy)-3-(3,4,5-trimethoxybenzyl)-4H-chromen-4-one (**6l**).* Yield 55%; Yellow solid; m.p. 108–110 °C; R_f = 0.73 (Ethyl acetate:hexane = 1:1); IR (cm^{-1}): 2920, 2850, 1645, 1602, 1505; ^1H NMR ($CDCl_3$): δ 1.08–2.04 (m, 11H), 3.72 (s, 2H), 3.85 (m, 9H), 3.81 (d, J = 6.0 Hz, 2H), 6.52 (s, 2H), 6.71 (d, J = 8.0 Hz, 1H), 6.95 (d, J = 8.4 Hz, 1H), 7.31 (s, 1H), 7.50 (t, J = 8.4 Hz, 1H), 13.0 (s, 1H). Anal. Calc. for $C_{26}H_{30}O_6$: C, 71.21; H, 6.90. Found: C, 71.15; H, 6.81.

*6.1.3. General procedure for the preparation of **7**(**a**–**b**)*

To a mono-protected ketone **2a** or **2b** in 50 mL DMF (1 mol) was added drop wise $POCl_3$ (6 mol) for 15 min at 0 °C. After addition was

completed, the reaction was allowed to stand at room temperature for overnight. Reaction mixture was quenched with water and extracted with ethyl acetate. The resulting organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to give a pure compound.

*6.1.3.1. 5-(Cyclohexylmethoxy)-4-oxo-4H-chromene-3-carbaldehyde (**7a**).* Yield 85%; Yellow solid; m.p. 133–134 °C; R_f = 0.54 (Ethyl acetate:hexane = 1:1); IR (cm^{-1}): 2923, 2853, 1696, 1653; ^1H NMR ($CDCl_3$) δ 1.09–2.03 (m, 11H), 3.91 (d, J = 6.4 Hz, 2H), 6.82 (d, J = 8.4 Hz, 1H), 7.01 (d, J = 7.6 Hz, 1H), 7.55 (t, J = 8.0 Hz, 1H), 8.37 (s, 1H), 10.36 (s, 1H). Anal. Calc. for $C_{17}H_{18}O_4$: C, 71.31; H, 6.34. Found: C, 71.26; H, 6.28.

*6.1.3.2. 5-Isobutoxy-4-oxo-4H-chromene-3-carbaldehyde (**7b**).* Yield 78%; Yellow solid; m.p. 97–98 °C; R_f = 0.14 (Ethyl acetate: hexane = 1:9); IR (cm^{-1}): 2954, 2864, 1696, 1662, 1566; ^1H NMR ($CDCl_3$) δ 1.13 (s, 6H), 2.23 (m, 1H), 3.85 (d, J = 5.8 Hz, 2H), 6.87 (d, J = 8.0 Hz, 1H), 7.04 (d, J = 8.4 Hz, 1H), 7.57 (t, J = 8.4 Hz, 1H), 8.37 (s, 1H), 10.3 (s, 1H). Anal. Calc. for $C_{14}H_{14}O_4$: C, 68.28; H, 5.73. Found: C, 68.21; H, 5.66.

*6.1.4. General procedure for the preparation of **8** (**a**–**b**)*

About 2 g of basic alumina and about 100 mg of the corresponding formylchromone **7a** or **7b** (5% of alumina weight), dissolved in 50 mL of isopropanol were placed in a round-bottom flask containing a magnetic bar, the mixture was stirred for 4 h at 75 °C. The mixture was filtered by vacuum filtration through celite, and evaporation of solvent to give crude alcohol. This may be purified by column chromatography using 15% ethyl acetate in hexane.

*6.1.4.1. 5-(Cyclohexylmethoxy)-3-(hydroxymethyl)-4H-chromen-4-one (**8a**).* Yield 87%; Colorless solid; m.p. 84–86 °C; R_f = 0.55 (Ethyl acetate:hexane = 6:4); IR (cm^{-1}): 3402, 3363, 2924, 2859, 1726, 1650; ^1H NMR ($CDCl_3$) δ 1.09–2.17 (m.11H), 3.87 (d, J = 6.0 Hz, 2H), 4.51 (d, J = 4.4 Hz, 2H), 6.80 (d, J = 8.0 Hz, 1H), 6.98 (d, J = 8.4 Hz, 1H), 7.51 (t, J = 8.4 Hz, 1H), 7.76 (s, 1H). Anal. Calc. for $C_{17}H_{20}O_4$: C, 70.81; H, 6.99. Found: C, 70.72; H, 6.85.

*6.1.4.2. 3-(Hydroxymethyl)-5-isobutoxy-4H-chromen-4-one (**8b**).* >Yield 85%; Colorless solid; m.p. 99–101 °C; R_f = 0.52 (100% Ethyl acetate); IR (cm^{-1}): 3354, 2953, 2861, 1726, 1649; ^1H NMR ($CDCl_3$) δ 1.11 (d, J = 7.2 Hz, 6H), 2.25 (m, 1H), 3.86 (d, J = 6.0 Hz, 2H), 4.51 (d, J = 6.8 Hz, 2H), 6.75 (d, J = 8.4 Hz, 1H), 6.90 (d, J = 8.4 Hz, 1H), 7.52 (t, J = 8.4 Hz, 1H), 7.77 (s, 1H). Anal. Calc. for $C_{14}H_{16}O_4$: C, 67.73; H, 6.50. Found: C, 67.64; H, 6.41.

*6.1.5. General procedure for the preparation of **9** (**a**–**b**)*

Thionylchloride 2 mL was added drop wise to 3-hydroxy methyl derivative **8a** or **8b** (100 mg) in methylenechloride solution. After the solution was stirred at 35 °C for 2 h, reaction mixture was quenched by ice water and extracted with methylenechloride. A resulting organic layer was dried over Na_2SO_4 and concentrated to give a crude compound which was purified by column chromatography.

*6.1.5.1. 3-(Chloromethyl)-5-(cyclohexylmethoxy)-4H-chromen-4-one (**9a**).* Yield 60%; Colorless solid; m.p. 130–131 °C; R_f = 0.75 (Ethyl acetate:hexane = 1:1); IR (cm^{-1}): 3354, 2950, 2852, 1727, 1651; ^1H NMR ($CDCl_3$) δ 1.08–2.03 (m, 11H), 3.87 (d, J = 6.0 Hz, 2H), 4.50 (s, 2H), 6.78 (d, J = 8.4 Hz, 1H), 6.98 (d, J = 8.4 Hz, 1H), 7.51 (t, J = 8.0 Hz), 7.9 (s, 1H). Anal. Calc. for $C_{17}H_{19}ClO_3$: C, 66.56; H, 6.24. Found: C, 66.40; H, 6.15.

*6.1.5.2. 3-(Chloromethyl)-5-isobutoxy-4H-chromen-4-one (**9b**).* Yield 65%; Colorless solid; m.p. 139–141 °C; R_f = 0.55 (Ethyl acetate: hexane = 1:1); IR (cm^{-1}): 3350, 2955, 2851, 1729, 1645; ^1H NMR ($CDCl_3$) δ 1.11 (d, J = 7.2 Hz, 6H), 2.21 (m, 1H), 3.85 (d, J = 6.4 Hz, 2H), 4.49 (d, J = 6.8 Hz, 2H), 6.74 (d, J = 8.4 Hz, 1H), 6.91 (d, J = 8.4 Hz, 1H), 7.51 (t, J = 8.0 Hz, 1H), 7.77 (s, 1H). Anal. Calc. for $C_{14}H_{15}ClO_3$: C, 63.04; H, 5.67. Found: C, 62.91; H, 5.58.

*6.1.6. General procedure for the preparation of **10**(**a**–**b**)*

To the solution of 4-oxo-benzopyran-3-carboxaldehyde **7a** or **7b** (0.1 mol) in methylenechloride was added jone's reagent at 0 °C. After addition was completed, the reaction mixture was allowed to stir at room temperature for 2 h. It was poured in to water and extracted with methylenechloride. The resulting mixture was washed with brine, dried over Na_2SO_4 and concentrated to give an acid, which was purified by column chromatography.

*6.1.6.1. 5-(Cyclohexylmethoxy)-4-oxo-4H-chromene-3-carboxylic acid (**10a**).* Yield 80%; Yellow solid; m.p. 166–167 °C; R_f = 0.5 (100% Ethyl acetate); IR (cm^{-1}): 3373, 2924, 2860, 1728, 1649; ^1H NMR ($CDCl_3$) δ 1.13–2.17 (m, 11H), 3.92 (d, J = 6.0 Hz, 2H), 6.91 (d, J = 8.4 Hz, 1H), 7.12 (d, J = 8.0 Hz, 1H), 7.66 (t, J = 8.4 Hz, 1H). Anal. Calc. for $C_{17}H_{18}O_5$: C, 67.54; H, 6.00. Found: C, 67.40; H, 5.88.

*6.1.6.2. 5-Isobutoxy-4-oxo-4H-chromene-3-carboxylic acid (**10b**).* Yield: 81%; Colorless solid; m.p. 115–117 °C; R_f = 0.27 (100% Ethylacete); IR (cm^{-1}): 2964, 2854, 1728, 1624; ^1H NMR ($CDCl_3$) δ 1.09 (d, 6H), 2.24 (m, 1H), 3.91 (d, J = 6.4 Hz, 2H), 6.91 (d, J = 8.4 Hz, 1H), 7.13 (d, J = 7.6 Hz, 1H), 7.69 (t, J = 8.4 Hz, 1H), 8.86 (s, 1H), 14.0 (s, 1H). Anal. Calc. for $C_{14}H_{14}O_5$: C, 64.12; H, 5.38. Found: C, 64.00; H, 5.27.

*6.1.7. General procedure for the preparation of **11** (**a**–**b**)*

A mixture of 4-oxo-benzopyran-3-carboxaldehyde **7a** or **7a** (0.1 mol), meldrum's acid (0.1 mol) and triethylamine formic acid (1:1) 50 mL was refluxed at 95–100 °C for 2–3 h, until the disappearance of starting material. It was cooled to room temperature and poured into ice water. The mixture was acidified to pH 2 with 6 N HCl. The pale yellow solid was filtered washed with water and purified by column chromatography.

*6.1.7.1. 3-(5-(Cyclohexylmethoxy)-4-oxo-4H-chromen-3-yl)propanoic acid (**11a**).* Yield 64%; Colorless solid; m.p. 155–157 °C; R_f = 0.26 (100% Ethyl acetate); IR(cm^{-1}): 3401, 2922, 2853, 1699, 1647; ^1H NMR ($CDCl_3$) δ 1.05–2.04 (m, 11H), 2.78 (s, 4H), 3.87 (d, J = 6.4 Hz, 2H), 6.76 (d, J = 8.0 Hz, 1H), 6.94 (d, J = 8.0 Hz, 1H), 7.48 (t, J = 8.4 Hz, 1H), 7.73 (s, 1H). Anal. Calc. for $C_{19}H_{22}O_5$: C, 69.07; H, 6.71. Found: C, 68.89; H, 6.58.

*6.1.7.2. 3-(5-Isobutoxy-4-oxo-4H-chromen-3-yl)propanoic acid (**11b**).* Yield 65%; Colorless solid; m.p. 123–125 °C; R_f = 0.26 (100% Ethyl acetate); IR(cm^{-1}): 3401, 2954, 2855, 1707, 1642; ^1H NMR ($CDCl_3$): δ 1.14 (d, J = 7.2 Hz, 6H), 2.23 (m, 1H), 2.72 (s, 4H), 3.84 (d, J = 5.8 Hz, 2H), 6.77 (d, J = 8.4 Hz, 1H), 6.96 (d, J = 8.4 Hz, 1H), 7.47 (t, J = 8.4 Hz, 1H), 7.74 (s, 1H), 8.16 (s, 1H). Anal. Calc. for $C_{16}H_{18}O_5$: C, 66.19; H, 6.25. Found: C, 66.08; H, 6.15.

*6.1.8. General procedure for the preparation of **12** (**a**–**b**)*

To a well stirred solution of 4-oxo-benzopyran-3-carboxaldehyde **7a** or **7b** (0.1 mol) in ethanol (30 mL), hydroxylamine hydrochloride (0.1 mol) was added. The solution was stirred vigorously for 1 h. Mixture was concentrated, extracted with ethyl acetate, washed with brine, dried, and evaporated the solvent to give corresponding oxime derivatives. A pure compound was obtained by flash column chromatography.

*6.1.8.1. (E)-5-(Cyclohexylmethoxy)-4-oxo-4H-chromene-3-carbaldehyde oxime (**12a**).* Yield 73%; Yellow solid; m.p. 164–165 °C; $R_f = 0.59$ (Ethyl acetate:hexane = 1:1); IR (cm^{-1}): 2923, 2855, 1712, 1651; ^1H NMR ($CDCl_3$) δ 1.09–2.02 (m, 11H), 3.88 (d, $J = 5.6$ Hz, 2H), 6.79 (d, $J = 8.0$ Hz, 1H), 6.99 (d, $J = 8.4$ Hz, 1H), 7.87 (s, 1H), 8.19 (s, 1H), 9.25 (s, 1H). Anal. Calc. for $C_{17}H_{19}NO_4$: C, 67.76; H, 6.36. Found: C, 67.62; H, 6.25.

*6.1.8.2. (E)-5-Isobutoxy-4-oxo-4H-chromene-3-carbaldehyde oxime (**12b**).* Yield 69%; Yellow solid; m.p. 139–140 °C; $R_f = 0.76$ (Ethyl acetate:hexane = 4:1); IR (cm^{-1}): 3293, 2926, 2876,1641, 1607; ^1H NMR ($CDCl_3$) δ 1.13 (d, $J = 6.4$ Hz, 6H), 2.26 (m, 1H), 3.84 (d, $J = 6.0$ Hz, 2H), 6.81 (d, $J = 7.6$ Hz, 1H), 6.99 (d, $J = 8.4$ Hz, 1H), 7.52 (t, $J = 8.8$ Hz, 1H), 7.85 (s, 1H), 8.15 (s, 1H), 9.21 (s, 1H). Anal. Calc. for $C_{14}H_{15}NO_4$: C, 64.36; H, 5.79. Found: C, 64.25; H, 5.62.

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