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PII: S0223-5234(17)30591-3

DOI: 10.1016/j.ejmech.2017.07.069

Reference: EJMECH 9632

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 10 February 2017

Revised Date: 24 July 2017

Accepted Date: 28 July 2017

Please cite this article as: P.R. Boggu, E. Venkateswararao, M. Manickam, Y. Kim, S.-H. Jung, Discovery of novel 3-(hydroxyalkoxy)-2-alkylchromen-4-one analogs as interleukin-5 inhibitors, *European Journal of Medicinal Chemistry* (2017), doi: 10.1016/j.ejmech.2017.07.069.

Graphical abstract

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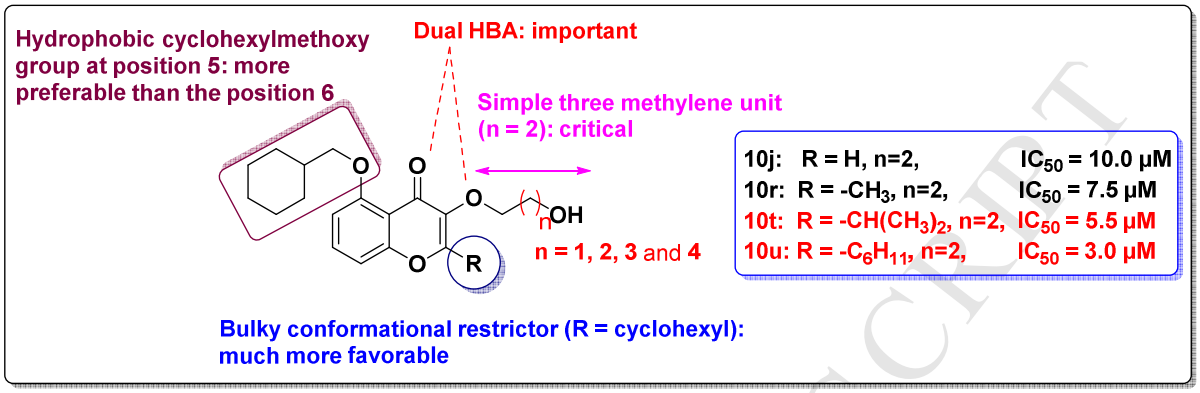

Discovery of novel 3-(hydroxyalkoxy)-2-alkylchromen-4-one analogs as interleukin-5 inhibitors

Pulla Reddy Boggu[a], Eeda Venkateswararao[a], Manoj Manickam[a], Youngsoo Kim[b], Sang-Hun Jung[a*]

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Abstract

A series of novel chromen-4-one analogs **9a-d** and **10a-u** was designed, synthesized and evaluated for their IL-5 inhibitory activity. Most of the chromen-4-one analogs showed strong inhibitory activity in low micro molar potency. Among them, 5-(cyclohexylmethoxy)-3-(3-hydroxypropoxy)-2-isopropyl-4*H*-chromen-4-one (**10t**, 90.0% inhibition at 30 μM, IC_{50} = 5.5 μM, CLogP = 4.76887) and 2-cyclohexyl-5-(cyclohexylmethoxy)-3-(3-hydroxypropoxy)-4*H*-chromen-4-one (**10u**, 95.5% inhibition at 30 μM, IC_{50} = 3.0 μM, CLogP = 5.96187) showed the best inhibition. The structure activity relationship reveals that the hydrophobic cyclohexylmethoxy group at the position 5 of the chromen-4-one ring A is preferable than at position 6 and the dual hydrogen bonding acceptor property on the chromen-4-one ring should be important for the inhibitory activity. In addition, the optimum length of the side chain at position 3 of chromen-4-one ring is critical for the donation of hydrogen to the binding site and the 3-hydroxypropoxy group showed the best activity. Moreover, the conformational restrictor (isopropyl, cyclohexyl group) at position 2 is much more favorable for the formation of effective conformer of side chain with hydrogen bonding donor property of these chromen-4-one analogs.

Key words: Interleukin-5; inhibitor; chromen-4-one; SAR

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1. Introduction

Allergic diseases, such as nasal rhinitis, asthma, idiopathic eosinophilic syndromes and atopic dermatitis, have prominent inflammatory components that are characterized by eosinophilic inflammation [1]. Thus, the major role of chronic pulmonary inflammation in the pathophysiology of asthma has been studied widely in human and in animal models. In asthma, pulmonary inflammation is characterized by edema, decreased mucociliary apparatus, bronchoalveolar eosinophilia [1]. The eosinophil is one of the bone marrow-derived leukocytes and is usually found in large numbers of tissues such as the bone marrow and gastrointestinal tract. Its normal level represents less than 5% of leukocytes in the blood [2]. The unusual level of proliferation of eosinophil causes many diseases including allergies [3], eosinophilic gastrointestinal disorders [4] and hyper-eosinophilic syndrome [5].

The development of eosinophil is being regulated mainly by the three cytokines such as interleukin (IL)-3, IL-5 and granulocyte macrophage colony stimulating factor (GM-CSF) [6–9]. These eosinophilopoietins likely provide permissive proliferation and differentiation signals following the instructive signals specified by the transcription factors GATA-1, PU.1, and C/EBPs. These cytokines are encoded by closely linked genes on chromosome 5q31. The receptors that share a common beta chain and have unique alpha chains were bound to the above cytokines [10,11]. Among them, IL-5 is one of the most specific to the eosinophil lineage and is responsible for selective differentiation of eosinophils [12]. IL-5 also stimulates the release of eosinophils from the bone marrow into the peripheral circulation

[13]. The critical role of IL-5 in the production of eosinophils is best demonstrated by genetic manipulation of mice. Overproduction and deletion of IL-5 in transgenic mice result in profound eosinophilia and a marked reduction of eosinophils in the blood and lungs after allergen challenge [14,15]. The overproduction of one or a combination of these three cytokines occurs in humans with eosinophilia and diseases with selective eosinophilia are often accompanied by overproduction of IL-5 [16]. Several clinical trials with humanized anti-IL-5 antibody demonstrated the critical role of IL-5 in regulating eosinophils in humans. These antibodies can reduce blood and bronchoalveolar eosinophilia caused by allergic challenges or chronic diseases [17–19]. Therefore, exclusively inhibiting the actions of IL-5 can suppress at least one of the alleged causes of asthma, namely tissue damage due to the eosinophil accumulation during pulmonary inflammation. Moreover, various clinical investigations indicate that the IL-5-regulated eosinophilia plays a key role in the pathogenesis of asthma.

The above reports clearly confirm that IL-5 is emerging as an important target for the treatment of asthma. Among them, small organic isothiazolone analogs have been prepared by the modification of Cys 66 residue in IL-5 and reported as its antagonists [20]. Nevertheless, the most significant discovery is the natural product sophoricoside and its analogs (**1**, Figure 1) which showed a strong inhibitory effect in the IL-5 bioassay of mIL-5-dependent Y16 proliferation [21]. Interestingly, it was also reported as a weak inhibition on IL-3 and GM-CSF bioactivities [22]. However, sophoricoside contains the unstable glycoside unit, thereby the structure activity relationship (SAR) studies are needed for the exploration of more potent analog with drug-likeness property. Accordingly, a number of isoflavonoid analogs (**2**, Figure 1) of sophoricoside [23,24] as well as chalcones [25] (**3**, Figure 1) were investigated as potent IL-5 inhibitors by our group. The SAR studies of isoflavonoid analogs revealed that planar chromen-4-one ring and a phenolic hydroxyl group at position 4 on

phenyl ring B particularly play an important role in the inhibitory activity. However, the glucopyranosyl component of sophoricoside may not be an essential motif for the innate activity of these analogs as the weak glycosidic linkage of isoflavone glycoside is chemically cleaved under metabolic conditions [24,26]. To further confirm the role of ring B, a number of novel chromen-4-one analogs (**4**, Figure 1) with the insertion of one methylene motif between chromen-4-one and phenyl ring were investigated for the IL-5 inhibitory activity [27]. These modifications well retained the level of activity of isoflavones. In order to obtain a deeper understanding of the effect of B-ring in isoflavone-type analogs, the amino alcohol group was introduced as shown in **5** (Figure 1) at position 3 instead of phenyl ring [28]. The outcome indicated that the replacement of the 4-hydroxyphenyl ring by hydroxyethylaminomethyl motif was suitable for maintaining the inhibitory activity of isoflavanone analogs. In addition, the results of this study also suggested that the existence of a bulky hydrophobic cyclohexyl methyl group as a side chain in the amino ethanol moiety (**5a**, Figure 1) is crucial for the IL-5 inhibitory activity [28]. Having understood some principal structural characteristics that enhanced the inhibitory activity of IL-5, a series of novel *N*-substituted hydroxyethylaminomethylchromone derivatives have been explored as IL-5 inhibitors [29]. The results suggested that by increasing the hydrophobicity or bulkiness at amino group the activity against IL-5 inhibition was intensified (**6**, Figure 1). In addition, all *N*-benzyl analogs have presented the same level of activity regardless the type of substituents at *para*-position. Furthermore, based on the conformational studies, the insertion of a hydrophobic group on the nitrogen seems to enforce the more effective conformer of hydroxyethylaminomethyl side chain, consequently increasing the activity of these analogs [29]. These results proved that phenyl ring B of **1** is not necessary for the activity. Recently, we investigated chromen-4-ones with a chalcone motif (hybrid chromen-4-one **7**, Figure 1)

and their saturated analogs (**8**, Figure 1). These results indicated that saturated alcohols **8** showed more potent than the unsaturated analogs **7** [30,31].

Therefore, considering all these structural characteristics of isoflavones and amino alcohol motif of chromen-4-ones, we have rationally designed the target molecule by insertion of additional oxygen atom near to the carbonyl group at position 3 of the chromen-4-one ring with conformational restrictor at position 2 which can lead to more active analogs (Figure 2). Additionally, we planned to explore the location of a hydrophobic group in chromen-4-one ring A and study the role of phenyl ring B (Figure 2). Accordingly, a series of novel chromen-4-one analogs **9** and **10** were synthesized and evaluated for their IL-5 inhibitory activity.

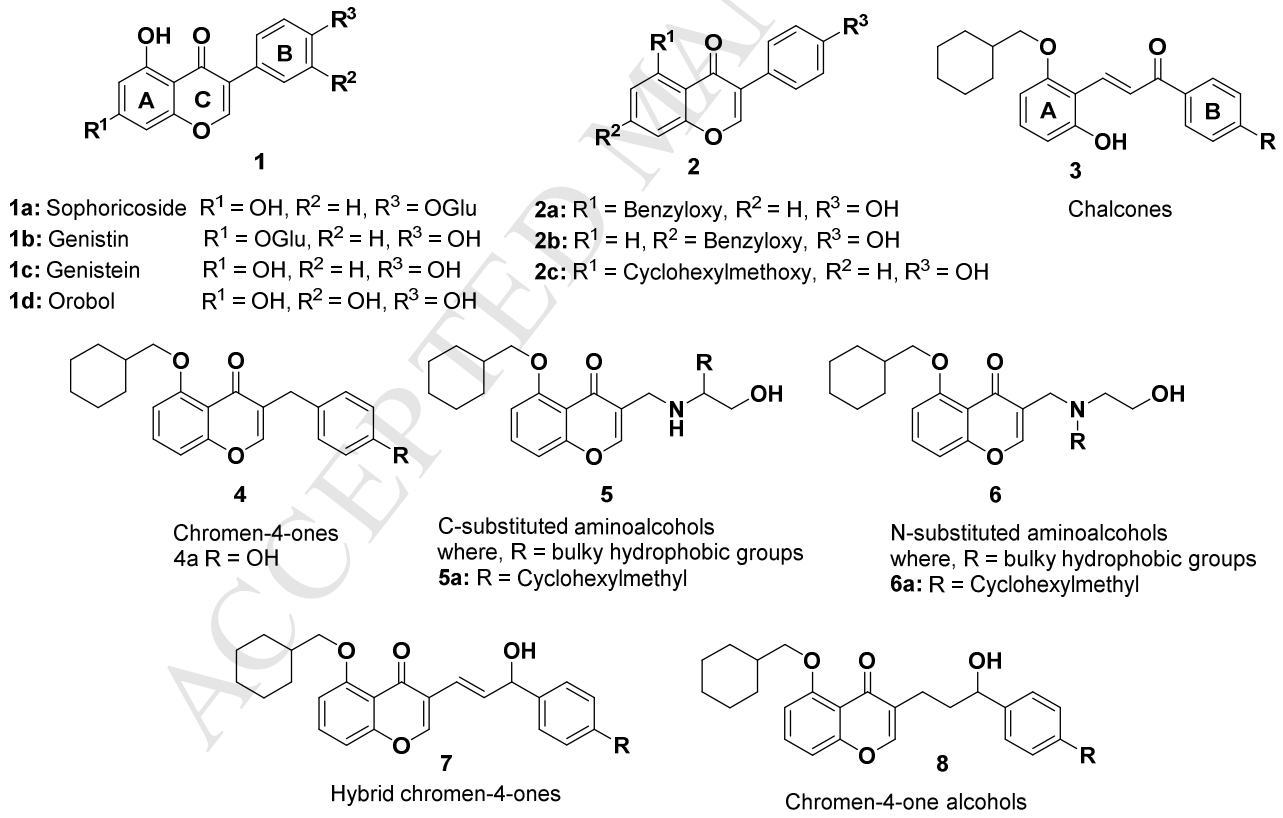


Figure 1. The representative IL-5 inhibitors.

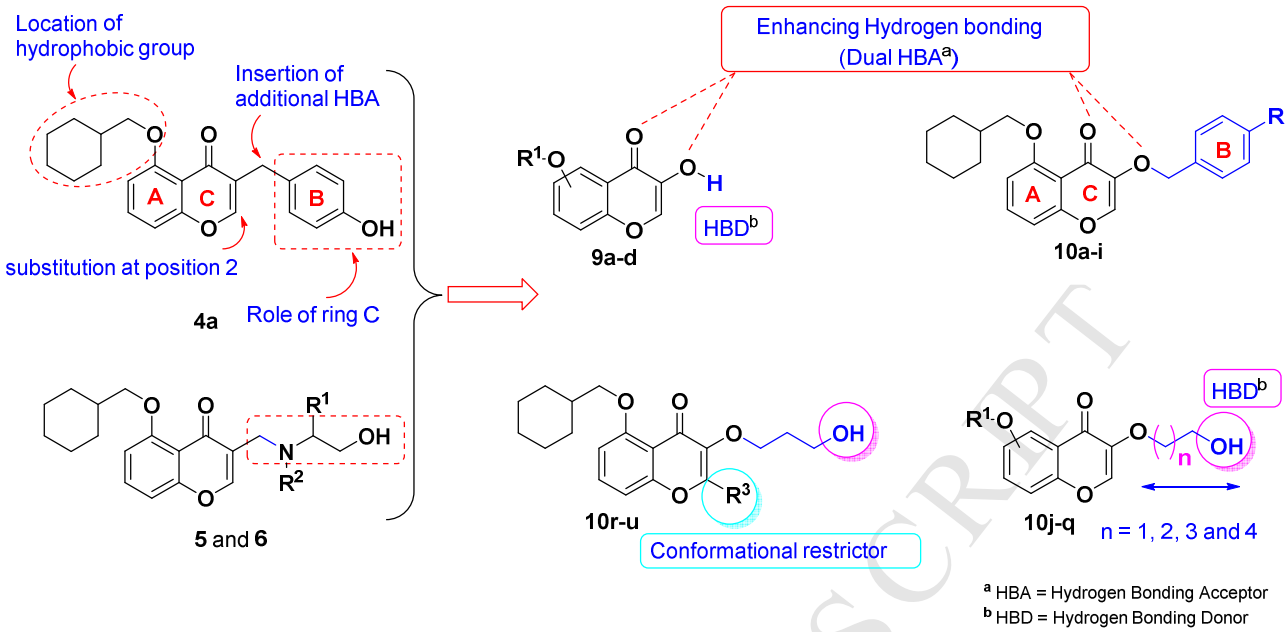


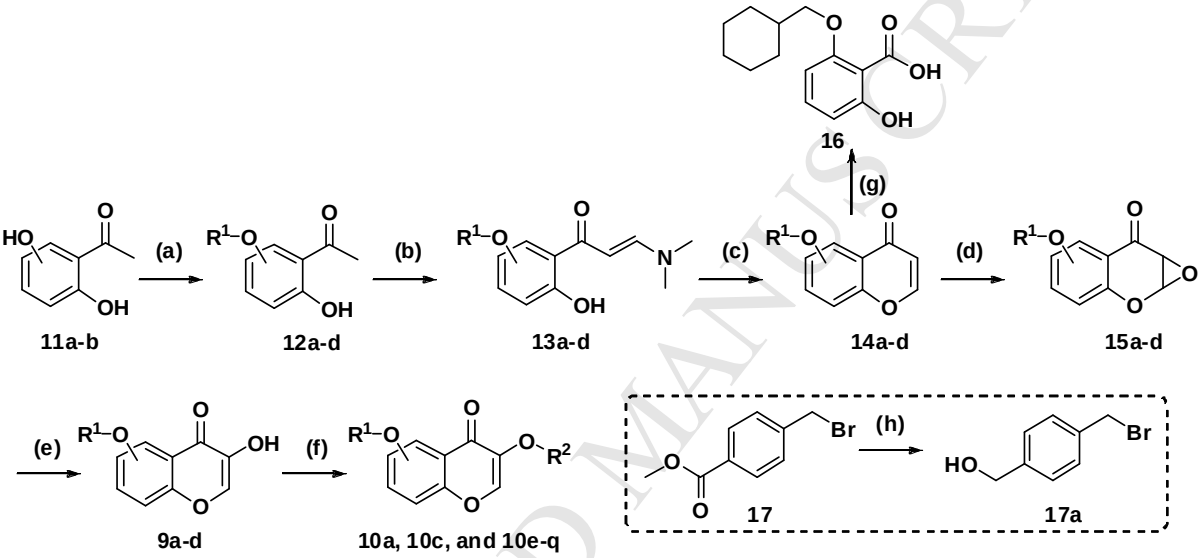
Figure 2. Design of novel chromen-4-one analogs **9** and **10** with dual HBA as IL-5 inhibitors.

2. Chemistry

The novel series of chromen-4-one analogs (**9a-d** and **10a-u,** Table 1) were synthesized from commercially available 2′,6′-dihydroxyacetophenone (**11a**) and 2′,5′-dihydroxyacetophenone (**11b**) in six steps as depicted in Schemes 1 - 5.

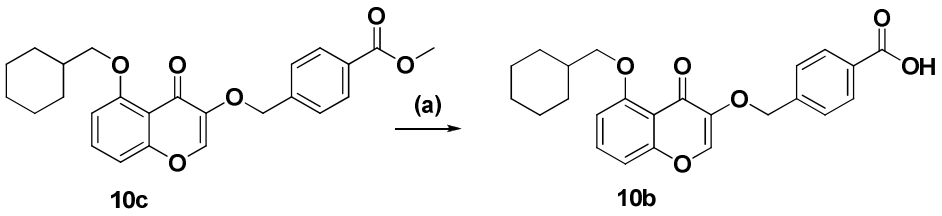
In brief, synthesis of the substituted acetophenone compounds **12** was accomplished by the partial alkylation of **11a** or **11b**. Compounds **13** were prepared with excellent yields by the aldol condensation of appropriate substituted acetophenone **12** with *N*, *N*-dimethylformamide dimethylacetal (DMF-DMA) at reflux temperature, which were being converted to appropriate cyclized chromen-4-one compounds **14** by treatment with concentrated hydrochloric acid in dichloromethane at reflux temperature (Scheme 1). To obtain key intermediate epoxide **15**, initial treatment of **14a** with H_2O_2 using 10% NaOH in ethanol/1,4-dioxane yielded **16** as the result of oxidative cleavage of the chromen-4-one ring [32]. To overcome this problem, mild oxidizing condition, *viz*., *meta*-chloroperbenzoic acid (*m*-CPBA) in ethanol/dichloromethane (EtOH/DCM) (1:1) at room temperature was adopted to

get the desired compounds **15**. The epoxides **15** were reacted with concentrated hydrochloric acid in acetone at room temperature to give 3-hydroxychromenones **9**, which were further reacted with the corresponding alkyl or benzyl halide in the presence of K_2CO_3 in *N*, *N*-dimethylformamide (DMF) at 60 °C to obtain the final chromen-4-one compounds **10** (Schemes 1, 4 and 5). The intermediate bromoalcohol **17a** was prepared from bromo ester **17** by reduction with diisobutylaluminium hydride (DIBAL-H) in high yield (Scheme 1).



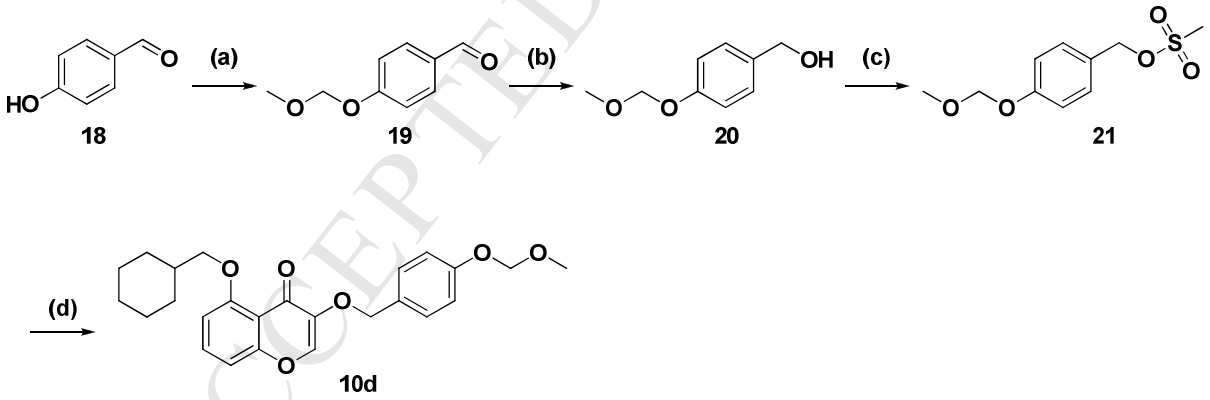
Scheme 1. Synthesis of chromen-4-one derivatives **9a-d**, **10a**, **10c** and **10e-q** (substituents are listed in Table 1). Reagents and Conditions: (a) R^1-Br, K_2CO_3, CH_3CN/DMF (1:1), 80 °C, 30 h, 70 - 76%; (b) DMF-DMA, reflux, 3 h, 85 - 90%; (c) DCM, Conc.HCl, reflux, 2 h, 85 - 92%; (d) mCPBA, EtOH/DCM (1:1), rt, 24 – 72 h, 35 - 45%; (e) Acetone, Conc.HCl, rt, 16 h, 70 - 78%; (f) R^2-X, K_2CO_3, DMF, rt – 60 °C, 4-16 h, 25 - 80%; (g) 10% NaOH, 35% H_2O_2, 1,4-dioxane/EtOH (1:1), 5 °C to rt, 16 h, 47%; (h) DIBAL-H, DCM, 0 °C to rt, 1 h, 75%.

In order to prepare compound **10b**, methyl 4-((5-(cyclohexylmethoxy)-4-oxo-4*H*-chromen-3-yloxy)methyl)benzoate (**10c**) was hydrolyzed with LiOH in THF/H_2O mixture at reflux temperature which gave the acid compound **10b** in good yield (Scheme 2).



Scheme 2. Synthesis of chromen-4-one derivative **10b**. Reagents and Conditions: (a) $LiOH.H_2O$, THF/H_2O (3:1), reflux, 5 h, 72%.

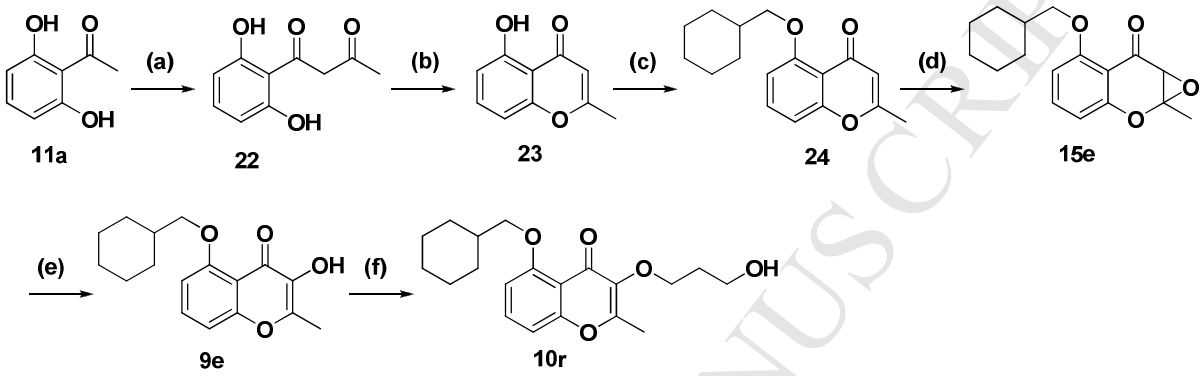
Analog **10d** was prepared from the 4-hydroxybenzaldehyde **18** as depicted in Scheme 3. **18** was converted to intermediate **19** by reacting with chloromethyl methyl ether (MOM-Cl) in the presence of sodium hydride (60%, dispersion in paraffin liquid) in DMF solution at 0 °C for 30 min. Subsequently, **19** was transformed into alcohol **20** by reaction with $NaBH_4$ in methanol solution at 0 °C for 30 min followed by mesylation with methane sulfonyl chloride and triethylamine in dichloromethane at 0 °C to room temperature resulted in **21**. The compound **21** was further reacted with **9a** in the presence of K_2CO_3 and DMF at 20 °C to afford **10d**.



Scheme 3. Synthesis of chromen-4-one derivative **10d**. Reagents and Conditions: (a) MOM-Cl, 60% NaH, DMF, 0 °C, 30 min, 92%; (b) $NaBH_4$, methanol, 0 °C, 30 min, 90%; (c) CH_3SO_2Cl, TEA, DCM, 0 °C to rt, 1 h, 85%; (d) Compound **9a**, K_2CO_3, DMF, 20 °C, 24 h, 55%.

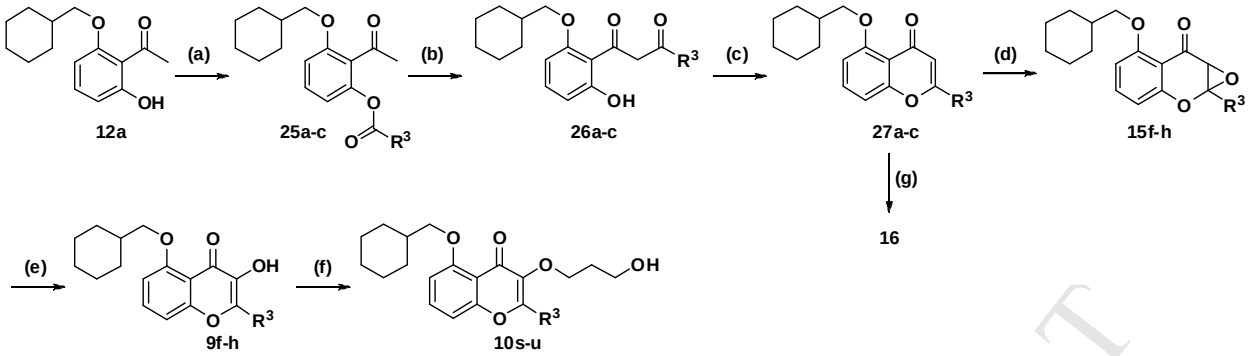
Compound **10r** with methyl substitution at position 2 was prepared as illustrated in Scheme 4. To obtain intermediate **24**, 2′,6′-dihydroxyacetophenone (**11a**) was reacted with sodium

metal in ethyl acetate to give **22**, and then cyclized by treatment with concentrated hydrochloric acid in methanol at reflux temperature followed by alkylation with (bromomethyl)cyclohexane and K_2CO_3 in DMF at 60 °C. Compound **24** was converted to chromen-4-one **10r** in a manner similar to the preparation of **10** from chromen-4-one intermediate **14**.



Scheme 4. Synthesis of 2-methyl chromen-4-one derivative **10r**. Reagents and Conditions: (a) Na, EtOAc, rt, 18 h; (b) MeOH, Conc.HCl, 60 °C, 2 h, 75%; (c) Cy-CH_2-Br, K_2CO_3, DMF, 60 °C, 16 h, 65%; (d) mCPBA, EtOH/DCM (1:1), rt, 24 h, 32%; (e) Acetone, Conc.HCl, rt, 16 h, 68%; (f) OH-$(CH_2)_3$-Br, K_2CO_3, DMF, 60 °C, 16 h, 55%.

2-Substituted chromen-4-one analogs **10s-u** were prepared as shown in Scheme 5. Starting with 1-(2-(cyclohexylmethoxy)-6-hydroxyphenyl)ethanone (**12a**), reaction with appropriate acid chloride gave ester compounds **25a-c** which were subsequently treated with NaH (60%, dispersion in paraffin liquid) in tetrahydrofuran at reflux temperature yields diketone compounds **26a-c** respectively. The diketone compounds **26a-c** were cyclized using concentrated hydrochloric acid in methanol to give cyclized intermediates **27a-c** accordingly, which were in turn used for the preparation of **10s-u** in a manner similar to the preparation of **10** from chromen-4-one intermediate **14**.

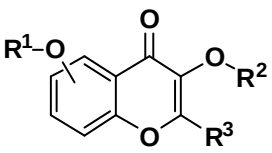


Scheme 5. Synthesis of 2-substituted chromen-4-one derivatives **10s-u** (substituents are listed in Table 1). Reagents and Conditions: (a) R^3-CO-Cl, TEA, MC, 0 °C, 1 h, 90 - 95%; (b) 60% NaH, THF, 60 °C, 2 h, 80 - 85%; (c) MeOH, Conc.HCl, 60 °C, 2 h, 70 - 86%; (d) mCPBA, EtOH/DCM (1:1), rt, 24 – 72 h, 20 - 22%; (e) Acetone, Conc.HCl, rt, 16 h, 50 - 65%; (f) OH-$(CH_2)_3$-Br, K_2CO_3, DMF, 60 °C, 4 -16 h, 45 - 55%; (g) 10% NaOH, 35% H_2O_2, 1,4-dioxane/EtOH (1:1), 5 °C to rt, 16 h, 47%.

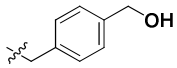
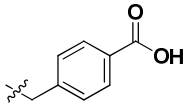
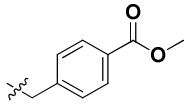
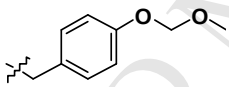
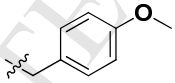
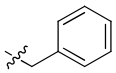
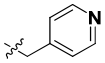
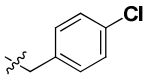
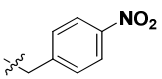
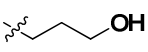
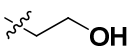
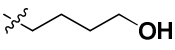
3. Pharmacology

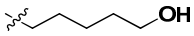
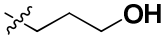
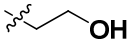
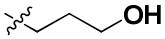
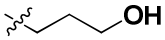
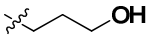
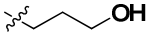
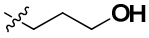
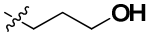
The inhibitory activity of chromen-4-one analogs **9a-d** and **10a-u** against IL-5 was evaluated using the IL-5 dependent pro-B Y16 cell line according to previously reported procedure [21]. These cells were incubated with 3 units/mL mIL-5 for 48 h in the presence or absence of the sample, and then cell metabolism was measured as an index of proliferation, using 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)-2*H*-tetrazolium sodium salt (WST-1). The results of biological screening of chromen-4-one analogs against IL-5 are listed in Table 1 as % inhibition at 30 µM and also IC_{50} values. The detailed assay protocols are described in the experimental section.

Table 1. IL-5 inhibitory activity of chromen-4-one analogs **9a-d** and **10a-u**.



9a-d and **10a-u**

Comp. No.	Substituents			[a]CLogP	IL-5	
	R^1	R^2	R^3		% Inhibition at 30 µM[b]	[b]IC_{50} (µM)
9a	5- CH_2-Cy[c]	H	H	3.91755	68.2	16.0
9b	5-$(CH_2)_2$-$CH(CH_3)_2$	H	H	3.25355	70.0	16.0
9c	6- CH_2-Cy	H	H	3.91755	56.5	29.0
9d	6-$(CH_2)_2$-$CH(CH_3)_2$	H	H	3.25355	62.3	20.0
10a	5- CH_2-Cy		H	4.95087	89.0	11.0
10b	5- CH_2-Cy		H	5.73187	86.4	17.0
10c	5- CH_2-Cy		H	5.95787	84.0	16.0
10d	5- CH_2-Cy		H	5.41447	35.5	>30.0
10e	5- CH_2-Cy		H	5.90787	85.3	20.0
10f	5- CH_2-Cy		H	5.98887	52.0	27.0
10g	5- CH_2-Cy		H	4.49187	60.1	24.0
10h	5- CH_2-Cy		H	6.70187	30.0	>30.0
10i	5- CH_2-Cy		H	5.73187	86.0	13.0
10j	5- CH_2-Cy		H	3.74187	90.6	10.0
10k	5- CH_2-Cy		H	3.34788	79.2	17.0
10l	5- CH_2-Cy		H	3.82088	15.5	>30.0

10m	5- CH_2-Cy		H	4.34987	12.2	>30.0
10n	5-$(CH_2)_2$-$CH(CH_3)_2$		H	3.07788	76.0	14.0
10o	5-$(CH_2)_2$-$CH(CH_3)_2$		H	2.68387	55.4	25.0
10p	6- CH_2-Cy		H	3.74187	36.0	>30.0
10q	6-$(CH_2)_2$-$CH(CH_3)_2$		H	3.07788	34.0	>30.0
10r	5- CH_2-Cy		$-CH_3$	4.24088	90.5	7.5
10s	5- CH_2-Cy		$-(CH_2)_2CH_3$	5.29887	88.5	14.0
10t	5- CH_2-Cy		$-CH(CH_3)_2$	4.76887	90.0	5.5
10u	5- CH_2-Cy		-Cy	5.96187	95.5	3.0
Sophoricoside					79.1[d]	10.6
Budesonide					70.2[d]	26.2

[a] ClogP values are calculated by Chemdraw® (ver. 11).

[b] % Inhibition and IC_{50} values are taken as a mean from three independent experiments.

[c] Cy = Cyclohexyl

[d] Inhibition at 50 µM.

4. Conformational analysis and alignment

Molecular models of the chromen-4-one analogs **10j** and **10u** were constructed using SYBYL®-x2.0 program package (Tripos Associates Inc.) [33] and their geometry were optimized (Powell conjugate gradient minimization, termination at a gradient of 0.0005 kcal/mol) [34] using the Tripos standard force field and Gasteiger-Huckel atomic partial charges. All the molecules were aligned by an atom-by-atom least-square fit and used the chromen-4-one structure as a template as represented. The 3D structures of the analyzed chromen-4-one compounds were assumed to be a bioactive conformation and were aligned

according to a chromen-4-one template as shown in Figure 3. The selected dihedral angles and atomic distances are listed in Table 2 and 3 respectively.

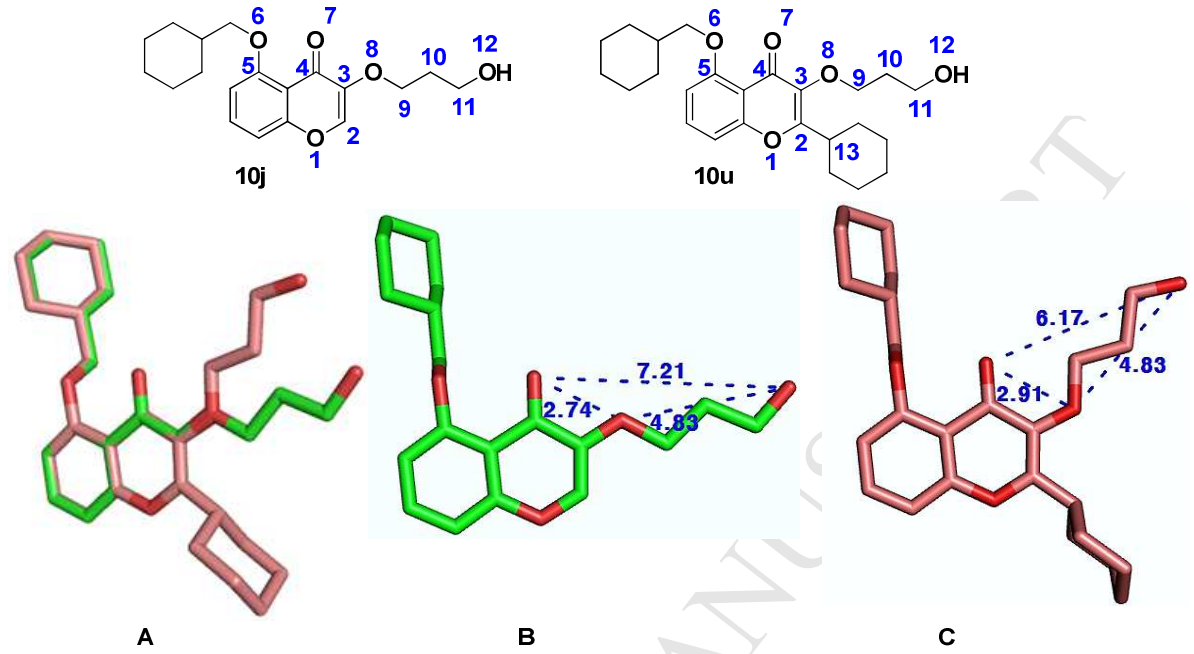


Figure 3. (A) Alignment of **10j (**Green color) and **10u** (Salmon color); (**B**) Distance (Å) calculation in **10j (**Green color); (**C**) Distance (Å) calculation in **10u (**Salmon color).

Table 2. Torsion angle (°) and total energy (kcal/mol) of **10j** and **10u**.

Compound **10j** (14.620 kcal/mol)		Compound **10u** (18.086 kcal/mol)	
∠C_3-O_8-C_9-C_{10}	179.8	∠C_3-O_8-C_9-C_{10}	179.6
∠C_4-C_3-O_8-C_9	180.3	∠C_4-C_3-O_8-C_9	359.8

[a] Numbers on the atoms of **10j** and **10u** are presented in Figure 3

Table 3. Distance (Å) in **10j** and **10u.**

Compound **10j**		Compound **10u**	
O_7-O_8	2.74	O_7-O_8	2.91

O_7-O_{12}	7.21	O_7-O_{12}	6.17
O_8-O_{12}	4.83	O_8-O_{12}	4.83

[a] Numbers on the atoms of **10j** and **10u** are presented in Figure 3

5. Results and discussion

For determining more detail structure activity relationship studies, we designed and synthesized a number of novel chromen-4-one analogs (**9a-d** and **10a-u**) by the introduction of dual hydrogen bonding property on chromen-4-one ring and studied them for their IL-5 inhibitory activity as summarized in Table 1.

Initially, 3-hydroxychromenone analogs were prepared and studied for their IL-5 inhibitory activity. Analog **9a** (68.2% inhibition at 30 µM, IC_{50} = 16.0 µM) showed moderate inhibitory activity. This result indicates that the addition of hydrogen bonding property (HBA and HBD) on chromen-4-one ring should be favorable for the activity. Encouraged by these results, our next effort was to confirm the importance of hydrophobic group on chromen-4-one ring A. Therefore, we replaced cyclohexylmethoxy group with isopentoxy group as shown in analog **9b** (70.0% inhibition at 30 µM, IC_{50} = 16.0 µM) exhibited the same level of activity. However, changing the position of the hydrophobic group on the chromen-4-one ring A from 5 to 6 as shown in analogs **9c** (56.5% inhibition at 30 µM, IC_{50} = 29.0 µM) and **9d** (62.3% inhibition at 30 µM, IC_{50} = 20.0 µM) slightly decreases the activity. These results suggest that both groups (cyclohexylmethoxy and isopentoxy) at position 5 are acceptable for the hydrophobic interactions.

To get the better interactions with the binding site, we introduced alkyl groups with hydrogen bonding property at position 3 of **9a** as shown in analog **10a (**4-hydroxymethylbenzyloxy group, 89.0% inhibition at 30 µM, IC_{50} = 11.0 µM). Surprisingly, **10a** showed the 2.5 fold stronger inhibitory activity against IL-5 as compared to budesonide (70.2% inhibition at 30

μM, IC_{50} = 26.2 μM) and similar activity as compared to sophoricoside (79.1% inhibition at 30 μM, IC_{50} = 10.6 μM). Carboxylic acid compound **10b** (86.4% inhibition at 30 μM, IC_{50} = 17.0 μM) and ester compound **10c** (84.0% inhibition at 30 μM, IC_{50} = 16.0 μM) showed the similar level of the activity. These results suggest that side chain with hydrogen bonding enhances the activity.

Next, to find out the importance of hydrogen bonding property (HBD or HBA) on phenyl ring, hydroxymethyl group in **10a** was replaced with electron donating groups capable to be a mild hydrogen bond acceptor such as methoxymethoxy analog **10d** (35.5% inhibition at 30 μM, IC_{50} = >30.0 μM) and 4-methoxy analog **10e** (85.3% inhibition at 30 μM, IC_{50} = 20.0 μM). Analog **10d** dramatically reduces the activity and analog **10e** showed less activity than **10a**. This indicates that the methoxymethoxy group may be too large to fit into putative binding pocket regardless of its hydrogen bond acceptor property.

Removal of hydroxymethyl group of **10a** as shown in **10f** (52.0% inhibition at 30 μM, IC_{50} = 27.0 μM) largely decreased the activity and the pyridine analog **10g** (60.1% inhibition at 30 μM, IC_{50} = 24.0 μM) did not improve the activity. Chloro analog **10h** (30.0% inhibition at 30 μM, IC_{50} = >30.0 μM) nearly abolished the activity. However, nitro analog **10i** (86.0% inhibition at 30 μM, IC_{50} = 13.0 μM) showed fairly strong inhibition. These results indicate that the hydrogen bonding acceptor at the end of the side chain at position 3 of chromen-4-one **9a** is very much beneficial to the activity.

In the next set of experiments, to confirm the role of phenyl ring B of chromen-4-one analog **10a** for their IL-5 inhibitory activity, we synthesized a series of simple hydroxyalkyl groups having similar hydrogen bonding property. Accordingly, analog **10j** (90.6% inhibition at 30 μM, IC_{50} = 10.0 μM, CLog P = 3.74187) gave an almost similar inhibitory activity as compared to sophoricoside (79.1% inhibition at 30 μM, IC_{50} = 10.6 μM) and **10a** (89.0%

inhibition at 30 µM, IC_{50} = 11.0 µM). This suggests that the phenyl ring B of **10a** can be replaced by simple alkyl group to maintain the activity of chromen-4-one.

Next, to confirm the optimum length of the 3-hydroxypropyl moiety, methylene group units were variated between oxygen (O) at position 3 and hydroxyl (OH) function in **10j**. As a result, decrement in the activity was observed in the case of two methylene unit analog **10k** (79.2% inhibition at 30 µM, IC_{50} = 17.0 µM) and complete loss of activity was observed in four and five methylene unit analogs **10l** (15.5% inhibition at 30 µM, IC_{50} = >30.0 µM) and **10m** (12.2% inhibition at 30 µM, IC_{50} = >30.0 µM). This outcome supports that length of hydroxyalkyl side chain plays a critical role in inhibitory activity against IL-5 of chromen-4-one analogs and hydroxypropyl group is optimum.

Further, to confirm the importance of hydrophobic group on the chromen-4-one ring A with hydroxyalkoxy at position 3, replacement of cyclohexylmethoxy group with the isopentyloxy group as shown in **10n** (76.0% inhibition at 30 µM, IC_{50} = 14.0 µM) and **10o** (55.4% inhibition at 30 µM, IC_{50} = 25.0 µM) was performed. However, these analogs did not improve the IL-5 inhibitory activity. Thus, the cyclohexylmethoxy moiety on ring A of chromen-4-one is an essential group for the activity.

In order to find out the suitable position of the hydrophobic group on the chromen-4-one ring A of **10j** and **10n**, the location of these groups was changed from position 5 to 6 as shown in analogs **10p** (36.0% inhibition at 30 µM, IC_{50} = >30.0 µM) and **10q** (34.0% inhibition at 30 µM, IC_{50} = >30.0 µM). This variation remarkably reduced the activity. Therefore, these results proved that the hydrophobic group at position 5 of chromen-4-one ring is much more preferable for the hydrophobic interactions.

Next objective of our work was to study on position 2 of chromen-4-one ring of **10j**. Introduction of the methyl group as a conformational restrictor at position 2 of **10j** as shown

in **10r** (90.5% inhibition at 30 µM, IC_{50} = 7.5 µM) resulted in remarkable enhancement in the activity. This implies that the conformational restrictor at position 2 should enforce the formation of effective conformer of hydroxypropoxy side chain at position 3 of these chromen-4-one analogs. Considering conformational restrictor at position 2 of the chromen-4-one ring, we further investigated that the size of the group and therefore analogs **10s, 10t** and **10u** were prepared. Among them, n-propyl analog **10s** (88.5% inhibition at 30 µM, IC_{50} = 14.0 µM) decreases the activity. Isopropyl analog **10t** (90.0 % inhibition at 30 µM, IC_{50} = 5.5 µM) and cyclohexyl analog **10u** (95.5% inhibition at 30 µM, IC_{50} = 3.0 µM) showed more potent activity against IL-5 inhibition. These results indicated that the bulky alkyl groups at position 2 as a conformational restrictor is more favorable to IL-5 inhibitory activity compared to a straight chain. Lipophilicity of analogs **10j-u** as indicated with cLogP (Table 1) does not correlate with the activity. Increasing the size of hydrophobic binding ability of substituent at position 2 is important for the enhancement of the activity.

To investigate the effective conformations for the potent IL-5 inhibitor, we compared the 3D structural sketches of unsubstituted analog **10j** and highly active cyclohexyl analog **10u** at position 2 as shown in Figure 3 and Table 3 and observed a remarkable difference in the region of side chain at position 3 of chromen-4-one. As mentioned in Table 2, the dihedral angles ($\angle C_4$-C_3-O_8-C_9) of analog **10j** is 180.3°, which indicates that the side chain of hydroxypropoxy group at position 3 of **10j** is located away from the chromen-4-one carbonyl group. The distance (O_7-O_{12} = 7.21 Å, Figure 3 and Table 3) from the oxygen of chromen-4-one ring carbonyl group to the oxygen of hydroxypropyl group of **10j** also confirms this conformation. The corresponding dihedral angle ($\angle C_4$-C_3-O_8-C_9, Table 2) of **10u** is 359.8°, which depicts that the hydroxypropyl chain is close to carbonyl of chromen-4-one. Therefore the shorter distance (O_7-O_{12} = 6.17 Å, Figure 3 and Table 3) from the oxygen of chromen-4-

one ring carbonyl group to the oxygen of hydroxypropyl group of **10u** compared to that of **10j** obviously indicates the folded conformation. Therefore, introduction of conformational restrictor at position 2 might exert the favorable effect for the formation of more effective conformer of hydrogen bonding capable hydroxypropyl side chain of these chromen-4-one analogs and thus enhance the activity.

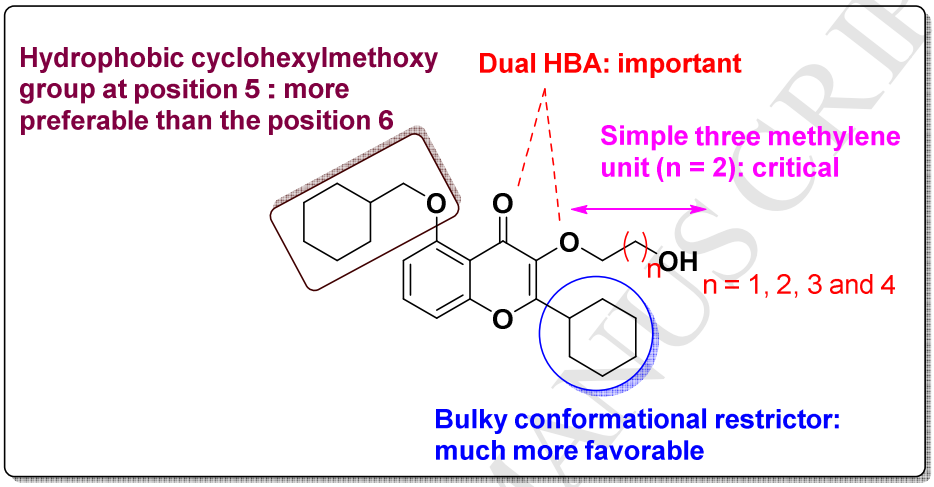


Figure 4. SAR of the novel chromen-4-one analogs.

6. Conclusion

In summary, a novel series of chromen-4-one analogs **9a-d** and **10a-u** were synthesized and evaluated for their IL-5 inhibitory activity. Most of the chromen-4-one analogs showed strong inhibitory activity in low micro molar potency. Among them, 5-(cyclohexylmethoxy)-3-(3-hydroxypropoxy)-2-methyl-4*H*-chromen-4-one (**10r**, 90.5% inhibition at 30 µM, IC_{50} = 7.5 µM, CLogP = 4.24088), 5-(cyclohexylmethoxy)-3-(3-hydroxypropoxy)-2-isopropyl-4*H*-chromen-4-one (**10t**, 90.0% inhibition at 30 µM, IC_{50} = 5.5 µM, ClogP = 4.76887) and 2-cyclohexyl-5-(cyclohexylmethoxy)-3-(3-hydroxypropoxy)-4*H*-chromen-4-one (**10u**, 95.5% inhibition at 30 µM, IC_{50} = 3.0 µM, ClogP = 5.96187) showed highly potent inhibitory activity against IL-5. The structure activity relationship (Figure 4) revealed that the hydrophobic cyclohexylmethoxy group at position 5 of the chromen-4-one ring A is more

preferable than position 6 and the dual hydrogen bonding acceptor property on the chromen-4-one ring should be important for the inhibitory activity. In addition to that, the optimum length of the methylene unit (3-hydroxypropoxy group) is critical for the donation of hydrogen to the binding site. Moreover, the conformational restrictor (isopropyl, cyclohexyl group) at position 2 is much more favorable for the formation of effective conformer of the side chain (3-hydroxypropoxy) hydrogen bonding donor property at position 3 of these chromen-4-one analogs. These results strongly suggest that novel 3-alkoxychromen-4-one analogs can be promising antagonists of IL-5 for the most efficacious treatment of allergic inflammation like asthma.

7. Experimental section

7.1. Chemistry

Melting points (mp) were determined on an Electro thermal 1A 9100 MK2 apparatus and are uncorrected. All commercial chemicals were used as obtained and all solvents were purified by distillation prior to use applying the standard procedures [35]. Thin layer chromatography (TLC) was performed on E Merck silica gel GF-254 pre-coated plates; identification was performed under UV illumination (λ = 254 nm), and colorization with Iodine and $KMnO_4$. All compounds were purified by flash column chromatography which was performed on E Merck silica gel (230–400 mesh). Infrared (IR) spectra were recorded on a Nicolet 380 model FTIR. ^{1}H NMR and ^{13}C NMR spectra were measured against the peak of tetramethylsilane using a Bruker Fourier 300 NMR (300 MHz) and JEOL, JNM-AL400 NMR (400 MHz) spectrometer. High-resolution mass spectra (HRMS) were measured in ESI ionization using AB Sciex Triple TOF 5600 LCMS instrument.

7.1.1. General synthetic procedure for the preparation of compounds **9**.

To a solution of the corresponding epoxide compound **15** (1.46 mmol) in acetone (1 mL), conc. HCl (2 mL) was added and the resulting mixture was stirred for 16 h at room temperature. The reaction mixture was diluted with water, and then extracted with EtOAc. The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, filtered and then concentrated to afford 3-hydroxy chromenone compound **9**. The residue was subjected to flash silica gel (230-400 mesh) column chromatography (eluting with 0-5% ethyl acetate in hexanes) to afford the title compounds **9**.

7.1.1.1. 5-(Cyclohexylmethoxy)-3-hydroxy-4H-chromen-4-one **(9a)**. Yield 75%; White solid; mp 112 - 115 °C; IR (neat) 3296, 2923, 2852, 1620, 1457, 1354, 1236, 1151, 1071, 843, 804, 772 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.85 (s, 1H), 7.52 (t, *J* = 8.41 Hz, 1H), 7.00 (d, *J* = 8.54 Hz, 1H), 6.75 (d, *J* = 8.29 Hz, 1H), 6.42 (br. s., 1H), 3.90 (d, *J* = 6.10 Hz, 2H), 1.93 - 2.06 (m, 3H), 1.69 - 1.85 (m, 3H), 1.31 - 1.43 (m, 3H), 1.12 - 1.30 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.8, 159.6, 158.4, 142.0, 135.5, 133.8, 110.0, 109.5, 105.8, 74.7, 37.5, 29.8, 26.5, 25.8; HRMS (ESI) Calcd. for $C_{16}H_{18}O_4$ $[M+H]^+$ 275.1283, found 275.1303.

7.1.1.2. 3-Hydroxy-5-(isopentyloxy)-4H-chromen-4-one **(9b)**. Yield 78%; White solid; mp 116 - 118 °C; IR (neat) 3282, 2955, 2868, 1621, 1460, 1355, 1241, 1151, 1067, 966, 847, 804 772 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.85 (s, 1H), 7.53 (t, *J* = 8.38 Hz, 1H), 7.01 (d, *J* = 8.57 Hz, 1H), 6.77 (d, *J* = 8.20 Hz, 1H), 6.42 (s, 1H), 4.16 (t, *J* = 6.71 Hz, 2H), 1.92 - 2.08 (m, 1H), 1.86 (q, *J* = 6.71 Hz, 2H), 1.02 (d, *J* = 6.52 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.7, 159.4, 158.4, 142.0, 135.5, 133.8, 112.8, 110.1, 105.9, 67.8, 37.6, 25.0, 22.6; HRMS (ESI) Calcd. for $C_{14}H_{16}O_4$ $[M+H]^+$ 249.1127, found 249.1151.

7.1.1.3. 6-(Cyclohexylmethoxy)-3-hydroxy-4H-chromen-4-one **(9c)**. Yield 71%; off white solid; mp 126 - 129 °C; IR (neat) 3246, 2921, 2855, 1603, 1489, 1403, 1276, 1199, 1163, 982, 850, 800, 784 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.54 (d, *J* = 2.89 Hz,

1H), 7.39 - 7.46 (m, 1H), 7.27 - 7.33 (m, 1H), 6.20 (br. s., 1H), 3.86 (d, *J* = 5.96 Hz, 2H), 1.66 - 1.95 (m, 6H), 1.17 - 1.41 (m, 3H), 1.08 (d, *J* = 11.92 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ173.0, 156.2, 151.3, 141.2, 138.2, 124.7, 122.3, 119.8, 104.5, 74.2, 37.6, 29.8, 26.5, 25.7; HRMS (ESI) Calcd. for $C_{16}H_{18}O_4$ $[M+H]^+$ 275.1283, found 275.1305.

7.1.1.4. 3-Hydroxy-6-(isopentyloxy)-4H-chromen-4-one **(9d)**. Yield 70%; off white solid; mp 118 - 119 °C; IR (neat) 3250, 3082, 2947, 2868, 1602, 1488, 1471, 1407, 1183, 1148, 976, 887, 799 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ8.01 (s, 1H), 7.56 (d, *J* = 2.98 Hz, 1H), 7.38 - 7.46 (m, 1H), 7.30 (m, 1H), 6.22 (s, 1H), 4.09 (t, *J* = 6.61 Hz, 2H), 1.86 (m, 1H), 1.73 (q, *J* = 6.86 Hz, 2H), 0.99 (d, *J* = 6.61 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ173.0, 156.1, 151.3, 141.3, 138.3, 124.7, 122.3, 119.8, 104.4, 67.2, 37.8, 25.1, 22.6; HRMS (ESI) Calcd. for $C_{14}H_{16}O_4$ $[M+H]^+$ 249.1127, found 249.1153.

7.1.1.5. 5-(Cyclohexylmethoxy)-3-hydroxy-2-methyl-4H-chromen-4-one **(9e)**. Yield 68%; Off white solid; mp 147 - 149 °C; IR (neat) 3295, 2921, 2852, 1619, 1457, 1236, 1152, 1071, 843, 804, 772 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ7.48 (t, *J* = 8.38 Hz, 1H), 6.97 (d, *J* = 8.57 Hz, 1H), 6.73 (d, *J* = 8.20 Hz, 1H), 3.89 (d, *J* = 6.05 Hz, 2H), 2.43 (s, 3H), 1.92 - 2.08 (m, 3H), 1.68 - 1.85 (m, 3H), 1.10 - 1.41 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ172.0, 159.5, 157.7, 145.6, 138.8, 133.2, 112.2, 109.7, 105.7, 74.6, 37.4, 29.8, 26.5, 25.7, 14.7; HRMS (ESI) Calcd. for $C_{17}H_{20}O_4$ $[M+H]^+$ 289.144, found 289.1463.

7.1.1.6. 5-(Cyclohexylmethoxy)-3-hydroxy-2-propyl-4H-chromen-4-one **(9f)**. Yield 50%; Off white solid; mp 89 - 92 °C; IR (neat) 3294, 2922, 2852, 1621, 1457, 1236, 1152, 1071, 843, 804, 772 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ7.48 (t, *J* = 8.38 Hz, 1H), 6.99 (d, *J* = 8.57 Hz, 1H), 6.73 (d, *J* = 8.20 Hz, 1H), 6.47 (br. s., 1H), 3.90 (d, *J* = 6.15 Hz, 2H), 2.76 (t, *J* = 7.50 Hz, 2H), 1.93 - 2.10 (m, 3H), 1.72 - 1.88 (m, 5H), 1.28 - 1.46 (m, 3H), 1.07 - 1.25 (m, 3H), 1.02 (t, *J* = 7.40 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ172.2, 159.4, 157.7, 149.2, 138.6,

133.2, 112.2, 109.8, 105.6, 74.6, 37.4, 30.4, 29.7, 26.4, 25.7, 19.9, 13.6; HRMS (ESI) Calcd. for $C_{19}H_{24}O_4$ $[M+H]^+$ 317.1753, found 317.1774.

7.1.1.7. 5-(Cyclohexylmethoxy)-3-hydroxy-2-isopropyl-4H-chromen-4-one **(9g)**. Yield 60%; White solid; mp 104 - 106 °C; IR (neat) 3293, 2921, 2852, 1620, 1457, 1236, 1152, 1072, 842, 804, 772 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.48 (t, *J* = 8.38 Hz, 1H), 7.00 (d, *J* = 7.73 Hz, 1H), 6.72 (d, *J* = 8.10 Hz, 1H), 6.49 (s, 1H), 3.89 (d, *J* = 6.15 Hz, 2H), 3.38 (spt, *J* = 6.91 Hz, 1H), 1.91 - 2.07 (m, 3H), 1.70 – 1.82 (m, 3H), 1.10 - 1.40 (m, 11H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 159.4, 157.7, 152.6, 137.1, 133.1, 112.2, 109.8, 105.6, 74.7, 37.4, 29.8, 27.7, 26.5, 25.7, 19.3; HRMS (ESI) Calcd. for $C_{19}H_{24}O_4$ $[M+H]^+$ 317.1753, found 317.1776.

7.1.1.8. 2-Cyclohexyl-5-(cyclohexylmethoxy)-3-hydroxy-4H-chromen-4-one **(9h)**. Yield 65%; White solid; mp 160 - 162 °C; IR (neat) 3292, 2922, 2852, 1620, 1458, 1235, 1152, 1072, 842, 804, 772 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.48 (t, *J* = 8.38 Hz, 1H), 7.00 (d, *J* = 8.48 Hz, 1H), 6.72 (d, *J* = 8.10 Hz, 1H), 6.52 (s, 1H), 3.89 (d, *J* = 6.24 Hz, 2H), 3.05 (tt, *J* = 3.15, 11.89 Hz, 1H), 1.68 - 2.05 (m, 13H), 1.10 - 1.47 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 159.4, 157.7, 152.2, 137.3, 133.1, 112.2, 109.8, 105.5, 74.6, 37.5, 37.4, 29.8, 29.2, 26.5, 25.9, 25.7, 25.7; HRMS (ESI) Calcd. for $C_{22}H_{28}O_4$ $[M+H]^+$ 357.2066, found 357.2092.

7.1.2. General synthetic procedure for the preparation of compounds **(10a, 10c** *and* **10e-u).**

To a solution of the corresponding 3-hydroxy chromenone compound **9** (0.547 mmol) in DMF (3 mL), K_2CO_3 (0.601 mmol) and alkyl halide (0.601 mmol) were added. The resulting mixture was stirred for 4 – 16 h at 60 °C. After cooling to room temperature, the reaction mixture was diluted with water, and then extracted with EtOAc. The combined organic layers were washed with water, brine solution, dried over anhydrous Na_2SO_4, filtered and then concentrated under reduced pressure. The residue was subjected to flash silica gel (230-400

mesh) column chromatography (eluting with 0-15% ethyl acetate in hexanes) to afford the title compounds.

7.1.2.1. 5-(Cyclohexylmethoxy)-3-(4-(hydroxymethyl)benzyloxy)-4H-chromen-4-one **(10a)**. Yield 48%; White solid; mp 131 - 133 °C; IR (neat) 3435, 2928, 2843, 1613, 1482, 1457, 1166, 1067, 1015, 839, 807, 772 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 (s, 1H), 7.32 - 7.47 (m, 5H), 6.90 (d, *J* = 8.57 Hz, 1H), 6.73 (d, *J* = 8.20 Hz, 1H), 5.10 (s, 2H), 4.70 (br. s., 2H), 3.88 (d, *J* = 6.24 Hz, 2H), 1.90 - 2.10 (m, 3H), 1.64 - 1.84 (m, 3H), 1.04 - 1.43 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3, 160.0, 157.8, 143.4, 141.4, 140.8, 135.8, 133.4, 128.2, 127.1, 115.1, 109.5, 106.3, 74.8, 72.4, 65.0, 37.5, 29.7, 25.8; HRMS (ESI) Calcd. for $C_{24}H_{26}O_5$ $[M+H]^+$ 395.1858, found 395.1886.

7.1.2.2. Methyl 4-((5-(cyclohexylmethoxy)-4-oxo-4H-chromen-3-yloxy)methyl)benzoate **(10c)**. Yield 60%; White solid; mp 117 - 119 °C; IR (neat) 2924, 2852, 1712, 1638, 1602, 1456, 1432, 1267, 1238, 1182, 1068, 1027, 760 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (d, *J* = 8.29 Hz, 2H), 7.44 - 7.56 (m, 4H), 6.92 (d, *J* = 8.47 Hz, 1H), 6.75 (d, *J* = 8.20 Hz, 1H), 5.17 (s, 2H), 3.92 (s, 3H), 3.89 (d, *J* = 6.24 Hz, 2H), 1.91 - 2.10 (m, 3H), 1.66 - 1.85 (m, 3H), 1.04 - 1.44 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 166.9, 160.1, 157.9, 143.5, 141.8, 141.7, 133.5, 129.9, 127.7, 115.2, 109.6, 106.5, 74.8, 72.1, 52.1, 37.4, 29.7, 26.4, 25.7; HRMS (ESI) Calcd. for $C_{25}H_{26}O_6$ $[M+H]^+$ 423.1807, found 423.1834.

7.1.2.3. 5-(Cyclohexylmethoxy)-3-(4-methoxybenzyloxy)-4H-chromen-4-one **(10e)**. Yield 72%; White solid; mp 136 - 139 °C; IR (neat) 2925, 2859, 1650, 1516, 1456, 1232, 1168, 1007, 827, 805, 777 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 - 7.51 (m, 2H), 7.34 (d, *J* = 8.66 Hz, 2H), 6.84 - 6.94 (m, 3H), 6.73 (d, *J* = 8.20 Hz, 1H), 5.05 (s, 2H), 3.88 (d, *J* = 6.24 Hz, 2H), 3.81 (s, 3H), 1.92 - 2.11 (m, 3H), 1.67 - 1.85 (m, 3H), 1.14 - 1.44 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.4, 160.1, 159.6, 157.9, 143.4, 141.8, 133.3, 129.8, 128.6,

124.7, 113.9, 109.6, 106.4, 74.9, 72.5, 55.3, 37.5, 29.8, 26.5, 25.9; HRMS (ESI) Calcd. for $C_{24}H_{26}O_5$ $[M+H]^+$ 395.1858, found 395.1882.

7.1.2.4. 3-(Benzyloxy)-5-(cyclohexylmethoxy)-4H-chromen-4-one **(10f)**. Yield 75%; White solid; mp 118 - 120 °C; IR (neat) 2927, 2844, 1640, 1599, 1456, 1238, 1179, 1067, 1022, 829, 805, 779, 757, 738 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.50 (s, 1H), 7.31 - 7.50 (m, 6H), 6.91 (d, *J* = 8.48 Hz, 1H), 6.73 (d, *J* = 8.20 Hz, 1H), 5.11 (s, 2H), 3.89 (d, *J* = 6.15 Hz, 2H), 1.91 - 2.10 (m, 3H), 1.67 - 1.84 (m, 3H), 1.06 - 1.43 (m, 5H) ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3, 160.1, 157.8, 143.5, 141.5, 136.5, 133.3, 128.5, 128.2, 128.0, 115.2, 109.6, 106.4, 74.8, 72.8, 37.5, 29.8, 26.5, 25.9; HRMS (ESI) Calcd. for $C_{23}H_{24}O_4$ $[M+H]^+$ 365.1753, found 365.1776.

7.1.2.5. 5-(Cyclohexylmethoxy)-3-(pyridin-4-ylmethoxy)-4H-chromen-4-one **(10g)**. Yield 80%; Light yellow solid; mp 106 - 108 °C; IR (neat) 2925, 2851, 1635, 1603, 1477, 1456, 1364, 1241, 1183, 1067, 1035, 836, 803, 772 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.61 (d, *J* = 5.87 Hz, 2H), 7.62 (s, 1H), 7.49 (t, *J* = 8.38 Hz, 1H), 7.37 (d, *J* = 5.59 Hz, 2H), 6.94 (d, *J* = 8.48 Hz, 1H), 6.76 (d, *J* = 8.29 Hz, 1H), 5.14 (s, 2H), 3.89 (d, *J* = 6.15 Hz, 2H), 1.92 - 2.08 (m, 3H), 1.73 - 1.84 (m, 3H), 1.05 - 1.44 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.1, 160.1, 157.9, 149.9, 145.8, 143.5, 142.1, 133.6, 122.0, 115.2, 109.6, 106.6, 74.9, 71.2, 37.4, 29.8, 26.5, 25.8; HRMS (ESI) Calcd. for $C_{22}H_{23}NO_4$ $[M+H]^+$ 366.1705, found 366.1731.

7.1.2.6. 3-(4-Chlorobenzyloxy)-5-(cyclohexylmethoxy)-4H-chromen-4-one **(10h)**. Yield 65%; Off White solid; mp 155 - 157 °C; IR (neat) 3076, 2919, 2851, 1639, 1605, 1474, 1456, 1231, 1179, 1068, 981, 855, 804, 772 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.52 (s, 1H), 7.48 (t, *J* = 8.38 Hz, 1H), 7.31 - 7.40 (m, 4H), 6.92 (d, *J* = 8.01 Hz, 1H), 6.74 (d, *J* = 8.20 Hz, 1H), 5.08 (s, 2H), 3.89 (d, *J* = 6.24 Hz, 2H), 1.92 - 2.10 (m, 3H), 1.67 - 1.84 (m, 3H), 1.05 - 1.44 (m, 5H) ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3, 160.1, 157.9, 143.4, 141.9, 135.0, 134.0, 133.4,

129.5, 128.7, 115.2, 109.6, 106.5, 74.9, 72.1, 37.5, 29.8, 26.5, 25; HRMS (ESI) Calcd. for $C_{23}H_{23}ClO_4$ $[M+H]^+$ 399.1363, found 399.1391.

7.1.2.7. 5-(Cyclohexylmethoxy)-3-(4-nitrobenzyloxy)-4H-chromen-4-one **(10i)**. Yield 56%; Yellow solid; mp 111 – 112 °C; IR (neat) 2930, 2907, 2854, 1650, 1605, 1516, 1456, 1340, 1244, 1182, 1046, 839, 766, 734 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.24 (d, *J* = 8.66 Hz, 2H), 7.64 (t, *J* = 4.19 Hz, 3H), 7.50 (t, *J* = 8.38 Hz, 1H), 6.95 (d, *J* = 8.48 Hz, 1H), 6.77 (d, *J* = 8.29 Hz, 1H), 5.22 (s, 2H), 3.90 (d, *J* = 6.24 Hz, 2H), 2.04 (d, *J* = 11.18 Hz, 3H), 1.67 - 1.85 (m, 3H), 1.06 - 1.44 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.2, 160.1, 159.9, 157.9, 144.0, 143.3, 142.3, 133.7, 128.2, 123.8, 115.2, 109.6, 106.6, 74.8, 71.7, 37.4, 29.8, 26.5, 25.8; HRMS (ESI) Calcd. for $C_{23}H_{23}NO_6$ $[M+H]^+$ 410.1603, found 410.1624.

7.1.2.8. 5-(Cyclohexylmethoxy)-3-(3-hydroxypropoxy)-4H-chromen-4-one **(10j)**. Yield 62%; off white solid; mp 99 - 101 °C; IR (neat) 3456, 2928, 2845, 1624, 1604, 1478, 1458, 1240, 1176, 1067, 1025, 806, 770 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.75 (s, 1H), 7.49 (t, *J* = 8.38 Hz, 1H), 6.96 (d, *J* = 8.10 Hz, 1H), 6.74 (d, *J* = 8.20 Hz, 1H), 4.08 (t, *J* = 5.73 Hz, 2H), 3.83 - 3.96 (m, 4H), 3.55 (t, *J* = 6.10 Hz, 1H), 1.88 - 2.06 (m, 5H), 1.69 - 1.84 (m, 3H), 1.05 - 1.41 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3, 160.1, 158.0, 144.3, 142.3, 133.6, 115.2, 109.6, 106.5, 74.9, 69.3, 59.3, 37.5, 32.0, 29.8, 26.5, 25.8; HRMS (ESI) Calcd. for $C_{19}H_{24}O_5$ $[M+H]^+$ 333.1702, found 333.1723.

7.1.2.9. 5-(Cyclohexylmethoxy)-3-(2-hydroxyethoxy)-4H-chromen-4-one **(10k)**. Yield 25%; off white solid; mp 101 - 103 °C; IR (neat) 3376, 2928, 2849, 1624, 1603, 1478, 1456, 1242, 1177, 1068, 1033, 807, 776 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.51 (t, *J* = 8.38 Hz, 1H), 6.97 (d, *J* = 8.48 Hz, 1H), 6.75 (d, *J* = 8.29 Hz, 1H), 4.01 - 4.10 (m, 2H), 3.85 - 3.93 (m, 4H), 1.88 - 2.06 (m, 3H), 1.65 - 1.84 (m, 3H), 1.03 - 1.42 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.0, 160.1, 158.0, 144.4, 142.1, 133.7, 115.0, 109.6, 106.6, 74.9, 74.4,

61.4, 37.5, 29.8, 26.5, 25.8; HRMS (ESI) Calcd. for $C_{18}H_{22}O_5$ $[M+H]^+$ 319.1545, found 319.1569.

7.1.2.10. 5-(Cyclohexylmethoxy)-3-(4-hydroxybutoxy)-4H-chromen-4-one **(10l)**. Yield 55%; Pale yellow solid; mp 65 – 68 °C; IR (neat) 3397, 2921, 2845, 1635, 1604, 1475, 1455, 1239, 1178, 1067, 826, 805, 768 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.61 (s, 1H), 7.47 (t, *J* = 8.38 Hz, 1H), 6.94 (d, *J* = 8.47 Hz, 1H), 6.72 (d, *J* = 8.20 Hz, 1H), 3.97 (t, *J* = 6.15 Hz, 2H), 3.86 (d, *J* = 6.33 Hz, 2H), 3.73 (t, *J* = 6.19 Hz, 2H), 1.70 - 2.09 (m, 10H), 1.03 - 1.42 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.0, 160.0, 157.8, 144.4, 138.9, 133.3, 109.5, 106.1, 74.7, 70.3, 62.4, 37.4, 29.7, 29.3, 26.5, 25.8, 25.8; HRMS (ESI) Calcd. for $C_{20}H_{26}O_5$ $[M+H]^+$ 347.1858, found 347.1882.

7.1.2.11. 5-(Cyclohexylmethoxy)-3-(5-hydroxypentyloxy)-4H-chromen-4-one **(10m)**. Yield 58%; Pale Yellow solid; mp 55 – 58 °C IR (neat) 3441, 2919, 2851, 1644, 1601, 1476, 1456, 1238, 1177, 1068, 1030, 834, 805, 774 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.59 (s, 1H), 7.47 (t, *J* = 8.38 Hz, 1H), 6.94 (d, *J* = 8.47 Hz, 1H), 6.72 (d, *J* = 8.20 Hz, 1H), 3.92 (t, *J* = 6.47 Hz, 2H), 3.86 (d, *J* = 6.33 Hz, 2H), 3.68 (t, *J* = 6.19 Hz, 2H), 1.51 - 2.09 (m, 12H), 1.04 - 1.42 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.0, 160.0, 157.8, 152.1, 138.7, 133.3, 109.5, 106.0, 74.7, 70.3, 62.6, 37.5, 32.3, 29.7, 28.9, 26.5, 25.8, 22.1; HRMS (ESI) Calcd. for $C_{21}H_{28}O_5$ $[M+H]^+$ 361.2015, found 361.2041.

7.1.2.12. 3-(3-Hydroxypropoxy)-5-(isopentyloxy)-4H-chromen-4-one **(10n)**. Yield 60%; Light yellow oil; IR (neat) 3414 (br. peak, OH), 2954, 2871, 1640, 1605, 1460, 1239, 1179, 1066, 807, 768 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.51 (t, *J* = 8.38 Hz, 1H), 6.98 (d, *J* = 8.57 Hz, 1H), 6.77 (d, *J* = 8.29 Hz, 1H), 4.04 - 4.19 (m, 4H), 3.92 (t, *J* = 5.54 Hz, 2H), 3.57 (br. s., 1H), 1.91 - 2.06 (m, 3H), 1.83 (q, *J* = 6.77 Hz, 2H), 0.99 (d, *J* = 6.61 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.0, 159.9, 158.1, 144.2, 142.9, 133.6, 115.3, 109.8, 106.7, 69.6,

68.1, 59.3, 37.6, 32.0, 25.0, 22.6; HRMS (ESI) Calcd. for $C_{17}H_{22}O_5$ $[M+H]^+$ 307.1545, found 307.1569.

7.1.2.13. 3-(2-Hydroxyethoxy)-5-(isopentyloxy)-4H-chromen-4-one **(10o)**. Yield 40%; Light yellow oil; IR (neat) 3355 (br. peak, OH), 2953, 2871, 1632, 1605, 1461, 1240, 1182, 1068, 807, 768 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.52 (t, *J* = 8.38 Hz, 1H), 6.99 (d, *J* = 8.48 Hz, 1H), 6.78 (d, *J* = 8.29 Hz, 1H), 4.13 (t, *J* = 6.75 Hz, 2H), 4.03 - 4.09 (m, 2H), 3.87 - 3.94 (m, 2H), 1.92 - 2.07 (m, 1H), 1.83 (q, *J* = 6.80 Hz, 2H), 0.99 (d, *J* = 6.52 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.7, 159.9, 158.0, 144.3, 142.5, 133.7, 109.8, 109.4, 106.8, 74.6, 68.0, 61.4, 37.6, 25.0, 22.6; HRMS (ESI) Calcd. for $C_{16}H_{20}O_5$ $[M+H]^+$ 293.1389, found 293.1409.

7.1.2.14. 6-(Cyclohexylmethoxy)-3-(3-hydroxypropoxy)-4H-chromen-4-one **(10p)**. Yield 68%; off white solid; mp 76 – 79 °C; IR (neat) 3401 (br. peak, OH), 2920, 2850, 1644, 1611, 1485, 1460, 1264, 1203, 1182, 1062, 1016, 863, 806 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.91 - 7.97 (m, 1H), 7.57 (d, *J* = 2.89 Hz, 1H), 7.36 - 7.44 (m, 1H), 7.23 - 7.31 (m, 2H), 4.14 (t, *J* = 5.68 Hz, 2H), 3.94 (t, *J* = 5.54 Hz, 2H), 3.85 (d, *J* = 6.05 Hz, 2H), 2.00 (t, *J* = 5.59 Hz, 2H), 1.67 - 1.94 (m, 6H), 1.00 - 1.40 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.3, 156.5, 150.8, 145.5, 143.2, 124.9, 124.6, 119.5, 105.2, 74.2, 69.8, 59.2, 37.6, 32.0, 29.8, 26.5, 25.7; HRMS (ESI) Calcd. for $C_{19}H_{24}O_5$ $[M+H]^+$ 333.1702, found 333.1728.

7.1.2.15. 3-(3-Hydroxypropoxy)-6-(isopentyloxy)-4H-chromen-4-one **(10q)**. Yield 75%; Light yellow solid; mp 86 - 87 °C; IR (neat) 3399, 2929, 2867, 1614, 1487, 1474, 1391, 1267, 1204, 1067, 983, 943, 851, 809 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.59 (d, *J* = 3.07 Hz, 1H), 7.41 (d, *J* = 9.22 Hz, 1H), 7.27 (dd, *J* = 3.03, 9.17 Hz, 2H), 4.14 (t, *J* = 5.68 Hz, 2H), 4.08 (t, *J* = 6.61 Hz, 2H), 3.95 (t, *J* = 5.54 Hz, 2H), 2.61 (br. s., 2H), 2.00 (quin, *J* = 5.61 Hz, 2H), 1.85 (tt, *J* = 6.54, 13.34 Hz, 1H), 1.72 (q, *J* = 6.74 Hz, 2H), 0.98 (d, *J* = 6.52

Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.3, 156.3, 150.8, 145.5, 143.3, 124.9, 124.6, 119.5, 105.1, 69.9, 67.2, 59.2, 37.8, 32.1, 25.1, 22.6; HRMS (ESI) Calcd. for $C_{17}H_{22}O_5$ $[M+H]^+$ 307.1545, found 307.1568.

7.1.2.16. 5-(Cyclohexylmethoxy)-3-(3-hydroxypropoxy)-2-methyl-4H-chromen-4-one **(10r)**. Yield 55%; Off white solid; mp 85 - 87 °C; IR (neat) 3478, 2924, 2850, 1632, 1604, 1456, 1230, 1203, 1055, 809, 770 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (t, *J* = 8.38 Hz, 1H), 6.95 (d, *J* = 8.47 Hz, 1H), 6.74 (d, *J* = 8.29 Hz, 1H), 4.62 (t, *J* = 6.85 Hz, 1H), 4.05 (t, *J* = 5.63 Hz, 2H), 3.97 (q, *J* = 5.62 Hz, 2H), 3.87 (d, *J* = 6.05 Hz, 2H), 2.41 (s, 3H), 1.88 - 2.05 (m, 5H), 1.66 - 1.85 (m, 3H), 1.04 - 1.42 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.3, 159.9, 157.6, 157.4, 140.6, 133.4, 115.0, 109.5, 106.7, 74.9, 68.9, 58.7, 37.3, 32.1, 29.7, 26.5, 25.7, 15.0; HRMS (ESI) Calcd. for $C_{20}H_{26}O_5$ $[M+H]^+$ 347.1858, found 347.1879.

7.1.2.17. 5-(Cyclohexylmethoxy)-3-(3-hydroxypropoxy)-2-propyl-4H-chromen-4-one **(10s)**. Yield 51%; Colorless oil; IR (neat) 3425 (br. peak, OH), 2923, 2852, 1632, 1605, 1475, 1458, 1241, 1189, 1056, 808, 770 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (t, *J* = 8.32 Hz, 1H), 6.96 (d, *J* = 8.54 Hz, 1H), 6.74 (d, *J* = 8.29 Hz, 1H), 4.69 (t, *J* = 7.07 Hz, 1H), 4.04 (t, *J* = 5.24 Hz, 2H), 3.97 (q, *J* = 5.77 Hz, 2H), 3.88 (d, *J* = 5.85 Hz, 2H), 2.73 (t, *J* = 7.44 Hz, 2H), 1.88 - 2.03 (m, 5H), 1.67 - 1.84 (m, 5H), 1.08 - 1.37 (m, 5H), 1.02 (t, *J* = 7.32 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.5, 160.6, 159.9, 157.6, 140.5, 133.3, 115.0, 109.5, 106.7, 74.9, 69.2, 58.7, 37.3, 32.2, 30.4, 29.7, 26.5, 25.7, 20.3, 13.7; HRMS (ESI) Calcd. for $C_{22}H_{30}O_5$ $[M+H]^+$ 375.2171, found 375.2199.

7.1.2.18. 5-(Cyclohexylmethoxy)-3-(3-hydroxypropoxy)-2-isopropyl-4H-chromen-4-one **(10t)**. Yield 55%; off white solid; mp 65 – 69 °C; IR (neat) 3393, 2924, 2851, 1628, 1603, 1476, 1456, 1232, 1195, 1101, 1069, 811, 780 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 (t, *J* = 8.38 Hz, 1H), 6.98 (d, *J* = 8.48 Hz, 1H), 6.74 (d, *J* = 8.29 Hz, 1H), 4.79 (br. s., 1H), 4.04 (t,

J = 5.54 Hz, 2H), 3.97 (t, *J* = 5.40 Hz, 2H), 3.88 (d, *J* = 6.05 Hz, 2H), 1.88 - 2.03 (m, 5H), 1.68 - 1.82 (m, 3H), 1.09 - 1.38 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.6, 164.3, 159.9, 157.7, 138.9, 133.3, 114.9, 109.5, 106.7, 74.9, 69.4, 58.7, 37.3, 32.1, 29.7, 27.3, 26.5, 25.7, 19.8; HRMS (ESI) Calcd. for $C_{22}H_{30}O_5$ $[M+H]^+$ 375.2171, found 375.2198.

7.1.2.19. 2-Cyclohexyl-5-(cyclohexylmethoxy)-3-(3-hydroxypropoxy)-4H-chromen-4-one **(10u)**. Yield 45%; Colorless sticky oil; ; IR (neat) 3438 (br. peak, OH), 2923, 2852, 1626, 1604, 1477, 1456, 1243, 1192, 1077, 1056, 809, 770 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (t, *J* = 8.29 Hz, 1H), 6.96 (d, *J* = 8.54 Hz, 1H), 6.73 (d, *J* = 8.54 Hz, 1H), 4.81 (br. s., 1H), 4.02 (t, *J* = 5.49 Hz, 2H), 3.97 (br. s., 2H), 3.86 (d, *J* = 5.85 Hz, 2H), 3.08 (t, *J* = 12.07 Hz, 1H), 1.82 - 2.00 (m, 9H), 1.62 – 1.74 (m, 5H), 1.09 - 1.44 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.6, 163.9, 159.9, 157.6, 139.1, 133.2, 114.9, 109.5, 106.7, 74.9, 69.6, 58.7, 37.3, 37.3, 32.2, 29.8, 29.7, 26.4, 25.8, 25.7, 25.6; HRMS (ESI) Calcd. for $C_{25}H_{34}O_5$ $[M+H]^+$ 415.2484, found 415.2505.

7.1.3. Preparation of 4-((5-(cyclohexylmethoxy)-4-oxo-4H-chromen-3-yloxy)methyl)benzoic acid **(10b)**.

To a solution of methyl 4-((5-(cyclohexylmethoxy)-4-oxo-4*H*-chromen-3-yloxy)methyl) benzoate (**10c**, 0.36 mmol) in THF (4mL) and H_2O (1 mL), $LiOH.H_2O$ (1.06 mmol) was added at room temperature and then the mixture was refluxed for 5 h. The reaction mixture was cooled and acidified with 1 *N* HCl, and then extracted with ethyl acetate. The combined extracts were dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The residue was subjected to silica gel (230-400 mesh) flash column chromatography (eluting with 0-2 % methanol in dichloromethane) to afford the title compound **10b**. Yield 72%; White solid; mp 193 – 196 °C; IR (neat) 3400-2500 (br. peak, acid OH), 2923, 2851, 1671, 1642, 1603, 1456, 1268, 1237, 1180, 1068, 1031, 845, 804, 758 cm^{-1}; ^{1}H NMR (300 MHz,

$CDCl_3$) δ 8.09 (d, *J* = 8.10 Hz, 2H), 7.45 - 7.61 (m, 4H), 6.93 (d, *J* = 8.47 Hz, 1H), 6.75 (d, *J* = 8.29 Hz, 1H), 5.18 (s, 2H), 3.89 (d, *J* = 6.15 Hz, 2H), 2.04 (d, *J* = 10.71 Hz, 3H), 1.67 - 1.85 (m, 3H), 1.06 - 1.44 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3, 170.9, 160.0, 157.8, 143.4, 142.4, 141.6, 133.6, 130.5, 129.1, 127.7, 115.1, 109.5, 106.4, 74.8, 72.1, 37.4, 29.8, 26.5, 25.8; HRMS (ESI) Calcd. for $C_{24}H_{24}O_6$ $[M+H]^+$ 409.1651, found 409.1673.

7.1.4. Preparation of 5-(cyclohexylmethoxy)-3-(4-(methoxymethoxy)benzyloxy)-4H-chromen-4-one **(10d)**.

7.1.4.1. 4-(Methoxymethoxy)benzaldehyde **(19)**. To a solution of 4-hydroxybenzaldehyde (**18**, 8.18 mmol) in DMF (10mL), NaH (60% dispersion in mineral oil, 12.28 mmol) was added at 0 °C. The reaction was stirred at the same temperature for 5 min and then the chloromethyl methyl ether (10.64 mmol) was added and further stirred for 30 min at the same temperature. The reaction mixture was quenched with ice water and then extracted with ethyl acetate. The combined extracts were washed with water, brine solution and then dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The residue was subjected to silica gel (230-400 mesh) flash column chromatography (eluting with 0-10% EtOAc in n-hexanes) to afford the title compound **19**. Yield 92%; colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.91 (s, 1H), 7.84 (d, *J* = 8.20 Hz, 2H), 7.15 (d, *J* = 8.46 Hz, 2H), 5.26 (s, 2H), 3.50 (s, 3H).

7.1.4.2. (4-(Methoxymethoxy)phenyl)methanol **(20)**. To a solution of 4-(methoxymethoxy)benzaldehyde (**19**, 5.94 mmol) in MeOH (10mL), $NaBH_4$ (8.92 mmol) was added at 0 °C. The reaction was stirred at the same temperature for 30 min. After completion of the reaction, the reaction mixture was quenched with ice water and then concentrated under reduced pressure. The reaction mixture was diluted with water and extracted with EtOAc. The combined extracts were dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to afford the title compound **20**. The crude compound

was used for the next step without further purification. Yield 90%; colorless sticky oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.24 (d, *J* = 8.38 Hz, 2H), 6.81 (d, *J* = 8.47 Hz, 2H), 5.30 (s, 1H), 4.70 (s, 2H), 4.53 (s, 2H), 3.42 (s, 3H).

7.1.4.3. 4-(Methoxymethoxy)benzyl methanesulfonate **(21)**. To a solution of (4-(methoxymethoxy)phenyl)methanol (**20**, 2.97 mmol) in DCM (10mL), TEA (5.94 mmol) and then methane sulfonyl chloride (4.46 mmol) were added at 0 °C. The reaction mixture was allowed to attain ambient temperature and further stirred for 1 h. The reaction mixture was diluted with DCM, washed with water, brine solution, and then dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to afford the title compound **21**. The crude compound was used for the next step without further purification. Yield 85%; Light brown oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (d, *J* = 8.37 Hz, 2H), 7.28 (d, *J* = 8.28 Hz, 2H), 4.72 (s, 2H), 4.61 (s, 2H), 3.42 (s, 3H), 3.14 (s, 3H).

7.1.4.4. 5-(Cyclohexylmethoxy)-3-(4-(methoxymethoxy)benzyloxy)-4H-chromen-4-one **(10d)**. To a solution of 5-(cyclohexylmethoxy)-3-hydroxy-4*H*-chromen-4-one (**9a**, 0.364 mmol) in DMF (3 mL), K_2CO_3 (0.729 mmol) and 4-(methoxymethoxy)benzyl methanesulfonate (**21**, 0.729 mmol) were added and the resulting mixture was stirred for 24 h at 20 °C. The reaction mixture was diluted with water and then extracted with EtOAc. The combined organic layers were washed with water, brine solution, dried over anhydrous Na_2SO_4, filtered and then concentrated under reduced pressure. The residue was subjected to flash silica gel (230-400 mesh) column chromatography (eluting with 0-15% ethyl acetate in hexanes) to afford the title compound **10d**. Yield 55%; Off white solid; mp 76 – 78 °C; IR (neat) 2920, 2852, 1737, 1655, 1601, 1476, 1455, 1234, 1175, 1071, 1000, 834, 811, 765 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 - 7.50 (m, 2H), 7.34 (d, *J* = 8.66 Hz, 2H), 7.02 (d, *J* = 8.64 Hz, 2H), 6.91 (dd, *J* = 0.84, 8.48 Hz, 1H), 6.73 (d, *J* = 7.64 Hz, 1H), 5.18 (s, 2H), 5.05 (s, 2H), 3.88 (d, *J* = 6.24 Hz, 2H), 3.48 (s, 3H), 1.92 - 2.10 (m, 3H), 1.67 - 1.84 (m, 3H), 1.10 - 1.40 (m, 5H); ^{13}C

NMR (100 MHz, $CDCl_3$) δ 173.4, 160.1, 157.9, 157.3, 143.4, 141.7, 133.4, 129.8, 129.8, 116.2, 115.2, 109.6, 106.3, 94.3, 74.8, 72.3, 56.0, 37.4, 29.7, 26.4, 25.8; HRMS (ESI) Calcd. for $C_{24}H_{24}O_6$ $[M+H]^+$ 425.1964, found 425.1925.

7.1.5. General synthetic procedure for the preparation of compounds **12**.

To a stirred solution of the corresponding dihydroxy acetophenone **11** (19.736 mmol) and K_2CO_3 (21.71 mmol) in CH_3CN/DMF (1:1) (30 mL), the corresponding alkyl bromide (21.71 mmol) was added. The resulting solution was heated at 80 °C until complete conversation of the starting material (~30 h). After cooling to ambient temperature, the reaction mixture was concentrated under reduced pressure and diluted with ethyl acetate. The organic phase was washed with water, brine solution, dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The residue was subjected to flash silica gel (230-400 mesh) column chromatography (eluting with 0-5% ethyl acetate in hexanes) to afford the title compounds **12.**

7.1.5.1. 1-(2-(Cyclohexylmethoxy)-6-hydroxyphenyl)ethanone **(12a)**. Yield 76%; Yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.28 (s, 1H), 7.32 (t, *J* = 8.38 Hz, 1H), 6.56 (d, *J* = 8.38 Hz, 1H), 6.38 (d, *J* = 8.29 Hz, 1H), 3.86 (d, *J* = 5.77 Hz, 2H), 2.73 (s, 3H), 1.71 - 1.94 (m, 6H), 1.07 - 1.39 (m, 5H).

7.1.5.2. 1-(2-Hydroxy-6-(isopentyloxy)phenyl)ethanone **(12b)**. Yield 70%; Yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.25 (s, 1H), 7.30 - 7.36 (t, 1H), 6.56 (d, *J* = 8.48 Hz, 1H), 6.39 (d, *J* = 8.29 Hz, 1H), 4.08 (t, *J* = 6.57 Hz, 2H), 2.70 (s, 3H), 1.72 - 1.90 (m, 3H), 1.00 (d, *J* = 6.33 Hz, 6H).

7.1.5.3. 1-(5-(Cyclohexylmethoxy)-2-hydroxyphenyl)ethanone **(12c)**. Yield 75%; Light yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.85 (s, 1H), 7.18 (d, *J* = 2.98 Hz, 1H), 7.11 (dd, *J* =

3.03, 8.99 Hz, 1H), 6.92 (d, *J* = 9.03 Hz, 1H), 3.73 (d, *J* = 6.33 Hz, 2H), 2.63 (s, 3H), 1.66 - 1.93 (m, 6H), 1.17 - 1.40 (m, 3H), 0.98 - 1.17 (m, 2H).

7.1.5.4. 1-(2-Hydroxy-5-(isopentyloxy)phenyl)ethanone **(12d)**. Yield 74%; Light yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.85 (s, 1H), 7.19 (d, *J* = 2.89 Hz, 1H), 7.12 (dd, *J* = 3.03, 8.99 Hz, 1H), 6.92 (d, *J* = 9.03 Hz, 1H), 3.97 (t, *J* = 6.61 Hz, 2H), 2.63 (s, 3H), 1.78 - 1.92 (m, 1H), 1.68 (q, *J* = 6.74 Hz, 2H), 0.98 (d, *J* = 6.61 Hz, 6H).

7.1.6. General synthetic procedure for the preparation of compounds **13**.

A mixture of the corresponding alkyl substituted 2-hydroxy acetophenone **12** (4.02 mmol) in *N, N*-dimethylformamide dimethyl acetate (3 mL) was heated at reflux temperature for 3 h. The resulting reaction mixture was cooled at ambient temperature and triturated with n-hexane to afford the title compound **13**, which was used for the next step without further purification.

7.1.6.1. (E)-1-(2-(cyclohexylmethoxy)-6-hydroxyphenyl)-3-(dimethylamino)prop-2-en-1-one **(13a)**. Yield 90%; Yellow solid; mp 115 – 117 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.70 (s, 1H), 7.97 (d, *J* = 12.20 Hz, 1H), 7.19 (t, *J* = 8.29 Hz, 1H), 6.53 (dd, *J* = 0.73, 8.29 Hz, 1H), 6.41 (d, *J* = 12.44 Hz, 1H), 6.29 - 6.35 (m, 1H), 3.81 (d, *J* = 6.59 Hz, 2H), 3.18 (br. s., 3H), 2.92 (br. s., 3H), 1.67 – 1.96 (m, 6H), 1.02 - 1.33 (m, 5H).

7.1.6.2. (E)-3-(dimethylamino)-1-(2-hydroxy-6-(isopentyloxy)phenyl)prop-2-en-1-one **(13b)**. Yield 87%; Yellow solid; mp 75 – 77 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 14.64 (s, 1H), 7.96 (d, *J* = 12.29 Hz, 1H), 7.20 (t, *J* = 8.29 Hz, 1H), 6.54 (dd, *J* = 0.84, 8.29 Hz, 1H), 6.32 - 6.44 (m, 2H), 4.05 (t, *J* = 6.57 Hz, 2H), 3.18 (br. s., 3H), 2.93 (br. s., 3H), 1.84 - 1.98 (m, 1H), 1.76 (q, *J* = 6.74 Hz, 2H), 0.97 (d, *J* = 6.61 Hz, 6H).

7.1.6.3. (E)-1-(5-(cyclohexylmethoxy)-2-hydroxyphenyl)-3-(dimethylamino)prop-2-en-1-one **(13c)**. Yield 85%; Brown solid; mp 106 – 108 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.38 (s, 1H), 7.89 (d, *J* = 12.11 Hz, 1H), 7.19 (d, *J* = 2.98 Hz, 1H), 6.99 (dd, *J* = 2.93, 8.99 Hz, 1H), 6.86 (d, *J* = 8.94 Hz, 1H), 5.73 (d, *J* = 12.11 Hz, 1H), 3.73 (d, *J* = 6.33 Hz, 2H), 3.20 (br. s, 3H), 2.99 (br. s, 3H), 1.90 – 1.70 (m, 6H), 1.03 - 1.39 (m, 5H).

7.1.6.4. (E)-3-(dimethylamino)-1-(2-hydroxy-5-(isopentyloxy)phenyl)prop-2-en-1-one **(13d)**. Yield 89%; Brown sticky oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.38 (s, 1H), 7.89 (d, *J* = 12.11 Hz, 1H), 7.20 (d, *J* = 2.89 Hz, 1H), 7.00 (dd, *J* = 2.89, 8.94 Hz, 1H), 6.87 (d, *J* = 8.94 Hz, 1H), 5.73 (d, *J* = 12.20 Hz, 1H), 3.96 (t, *J* = 6.66 Hz, 2H), 3.20 (br. s., 3H), 2.97 (br. s., 3H), 1.76 - 1.93 (m, 1H), 1.60 - 1.72 (m, 2H), 0.97 (d, *J* = 6.61 Hz, 6H).

7.1.7. General synthetic procedure for the preparation of compounds **14**.

To a solution of the corresponding unsaturated dimethylamine compound **13** (4.02 mmol) in dichloromethane (10 mL), concentrated HCl (3 mL) was added and then the solution was refluxed for 2 h. After cooling to ambient temperature, the mixture was diluted with water and then extracted with dichloromethane. The combined organic layers were washed with saturated $NaHCO_3$ solution, then with brine solution, dried over anhydrous Na_2SO_4, filtered and evaporated to afford the title compound **14**, which was used for the next step without further purification.

7.1.7.1. 5-(Cyclohexylmethoxy)-4H-chromen-4-one **(14a)**. Yield 92%; Light brown solid; mp 74 – 76 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, *J* = 5.85 Hz, 1H), 7.50 (t, *J* = 8.41 Hz, 1H), 6.96 (dd, *J* = 0.98, 8.54 Hz, 1H), 6.77 (d, *J* = 7.80 Hz, 1H), 6.19 (d, *J* = 6.10 Hz, 1H), 3.87 (d, *J* = 6.10 Hz, 2H), 1.89 - 2.06 (m, 3H), 1.68 – 1.80 (m, 3H), 1.08 – 1.39, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.3, 159.7, 158.6, 153.0, 133.6, 115.8, 114.7, 109.6, 107.3,

74.7, 37.5, 29.7, 26.4, 25.8; HRMS (ESI) Calcd. for $C_{16}H_{18}O_3$ $[M+H]^+$ 259.1334, found 259.1358.

7.1.7.2. 5-(Isopentyloxy)-4H-chromen-4-one **(14b)**. Yield 91%; Light brown oil to solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66 (d, *J* = 5.96 Hz, 1H), 7.51 (t, *J* = 8.38 Hz, 1H), 6.94 - 7.01 (m, 1H), 6.80 (d, *J* = 8.29 Hz, 1H), 6.20 (d, *J* = 5.96 Hz, 1H), 4.12 (t, *J* = 6.71 Hz, 2H), 1.93 – 2.06 (m, 1H), 1.78 - 1.88 (m, 2H), 0.99 (d, *J* = 6.61 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.3, 159.5, 158.6, 153.0, 133.7, 115.8, 114.7, 109.8, 107.5, 67.9, 37.5, 24.9, 22.5; HRMS (ESI) Calcd. for $C_{14}H_{16}O_3$ $[M+H]^+$ 233.1177, found 233.1203.

7.1.7.3. 6-(Cyclohexylmethoxy)-4H-chromen-4-one **(14c)**. Yield 85%; Light brown oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, *J* = 5.96 Hz, 1H), 7.54 (d, *J* = 2.98 Hz, 1H), 7.36 - 7.42 (m, 1H), 7.23 - 7.30 (m, 1H), 6.33 (d, *J* = 5.96 Hz, 1H), 3.85 (d, *J* = 6.15 Hz, 2H), 1.67 - 1.94 (m, 6H), 1.07 - 1.40 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.1, 156.8, 155.4, 151.4, 138.5, 124.5, 119.6, 112.0, 105.4, 74.1, 37.5, 29.7, 26.4, 25.7; HRMS (ESI) Calcd. for $C_{16}H_{18}O_3$ $[M+H]^+$ 259.1334, found 259.1356.

7.1.7.4. 6-(Isopentyloxy)-4H-chromen-4-one **(14d)**. Yield 87%; Light brown oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 (d, *J* = 5.96 Hz, 1H), 7.56 (d, *J* = 2.98 Hz, 1H), 7.36 - 7.43 (m, 1H), 7.22 - 7.29 (m, 1H), 6.33 (d, *J* = 5.96 Hz, 1H), 4.08 (t, *J* = 6.61 Hz, 2H), 1.77 - 1.93 (m, 1H), 1.72 (q, *J* = 6.71 Hz, 2H), 0.98 (d, *J* = 6.52 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.7, 156.6, 155.1, 151.3, 125.5, 124.3, 119.6, 112.1, 105.4, 67.1, 37.7, 25.0, 22.5; HRMS (ESI) Calcd. for $C_{14}H_{16}O_3$ $[M+H]^+$ 233.1177, found 233.1198.

7.1.8. General synthetic procedure for the preparation of compounds **15**.

To a stirred solution of the corresponding chromenone compound **14** (3.097 mmol) in DCM:EtOH (1:1) (10 mL), mCPBA (4.645 mmol) was added and the resulting reaction

mixture was stirred at room temperature for 24 – 72 h. The reaction mixture was diluted with dichloromethane, and then washed with saturated $NaHCO_3$ solution, brine solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was subjected to flash silica gel (230-400 mesh) column chromatography (eluting with 0-10% ethyl acetate in hexanes) to afford the title compounds **15**.

7.1.8.1. 6-(Cyclohexylmethoxy)-1aH-oxireno[2,3-b]chromen-7(7aH)-one **(15a)**. This compound was prepared from 5-(cyclohexylmethoxy)-4*H*-chromen-4-one (**14a**). Yield 37%; Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (t, *J* = 8.41 Hz, 1H), 6.60 - 6.65 (m, 2H), 5.61 (d, *J* = 2.68 Hz, 1H), 3.87 (dd, *J* = 6.22, 8.90 Hz, 1H), 3.77 (dd, *J* = 6.10, 9.02 Hz, 1H), 3.70 (d, *J* = 2.68 Hz, 1H), 1.86 - 2.00 (m, 3H), 1.69 - 1.82 (m, 3H), 1.19 - 1.38 (m, 3H), 1.05 - 1.16 (m, 2H); HRMS (ESI) Calcd. for $C_{16}H_{18}O_4$ $[M+H]^+$ 275.1283, found 275.1307.

7.1.8.2. 6-(Isopentyloxy)-1aH-oxireno[2,3-b]chromen-7(7aH)-one **(15b)**. This compound was prepared from 5-(isopentyloxy)-4*H*-chromen-4-one (**14b**). Yield 45%; Colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 (t, *J* = 8.38 Hz, 1H), 6.55 - 6.72 (m, 2H), 5.61 (d, *J* = 2.61 Hz, 1H), 3.93 - 4.22 (m, 2H), 3.71 (d, *J* = 2.61 Hz, 1H), 1.83 - 1.99 (m, 1H), 1.76 (q, *J* = 6.80 Hz, 2H), 0.98 (d, *J* = 6.52 Hz, 6H); HRMS (ESI) Calcd. for $C_{14}H_{16}O_4$ $[M+H]^+$ 249.1127, found 249.1147.

7.1.8.3. 5-(Cyclohexylmethoxy)-1aH-oxireno[2,3-b]chromen-7(7aH)-one **(15c)**. This compound was prepared from 6-(cyclohexylmethoxy)-4*H*-chromen-4-one (**14c**). Yield 35%; Colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 (dd, *J* = 1.26, 3.12 Hz, 1H), 7.14 - 7.22 (m, 1H), 6.97 - 7.05 (m, 1H), 5.68 (t, *J* = 2.10 Hz, 1H), 3.76 (d, *J* = 6.15 Hz, 2H), 3.72 (t, *J* = 2.10 Hz, 1H), 1.59 - 1.94 (m, 6H), 1.16 - 1.43 (m, 3H), 0.94 - 1.16 (m, 2H); HRMS (ESI) Calcd. for $C_{16}H_{18}O_4$ $[M+H]^+$ 275.1283, found 275.1298.

7.1.8.4. 5-(Isopentyloxy)-1aH-oxireno[2,3-b]chromen-7(7aH)-one **(15d)**. This compound was prepared from 6-(isopentyloxy)-4*H*-chromen-4-one **(14d)**. Yield 41%; Colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (d, *J* = 3.07 Hz, 1H), 7.17 (dd, *J* = 3.07, 9.03 Hz, 1H), 7.01 (d, *J* = 9.13 Hz, 1H), 5.68 (d, *J* = 2.42 Hz, 1H), 3.99 (t, *J* = 6.61 Hz, 2H), 3.72 (d, *J* = 2.51 Hz, 1H), 1.82 (m, 1H), 1.68 (q, *J* = 6.74 Hz, 2H), 0.93 - 1.01 (d, *J* = 6.6 Hz, 6H); HRMS (ESI) Calcd. for $C_{14}H_{16}O_4$ $[M+H]^+$ 249.1127, found 249.1149.

7.1.8.5. 6-(Cyclohexylmethoxy)-1a-methyl-1aH-oxireno[2,3-b]chromen-7(7aH)-one **(15e)**. This compound was prepared from 5-(cyclohexylmethoxy)-2-methyl-4*H*-chromen-4-one (**24**). Yield 32%; Colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 (t, *J* = 8.38 Hz, 1H), 6.56 (d, *J* = 8.29 Hz, 1H), 6.49 (d, *J* = 8.38 Hz, 1H), 4.62 (d, *J* = 2.33 Hz, 1H), 4.06 (d, *J* = 2.70 Hz, 1H), 3.75 - 3.97 (m, 4H), 1.86 - 2.02 (m, 3H), 1.66 - 1.85 (m, 5H), 1.35 - 1.42 (m, 4H), 1.30 - 1.35 (m, 3H), 0.97 - 1.29 (m, 6H), aliphatic protons showed excess; HRMS (ESI) Calcd. for $C_{17}H_{20}O_4$ $[M+H]^+$ 289.1440, found 289.1462.

7.1.8.6. 6-(Cyclohexylmethoxy)-1a-propyl-1aH-oxireno[2,3-b]chromen-7(7aH)-one **(15f)**. This compound was prepared from 5-(cyclohexylmethoxy)-2-propyl-4*H*-chromen-4-one (**27a**). Yield 20%; colorless oil; HRMS (ESI) Calcd. for $C_{19}H_{24}O_4$ $[M+H]^+$ 317.1753, found 317.1778.

7.1.8.7. 6-(Cyclohexylmethoxy)-1a-isopropyl-1aH-oxireno[2,3-b]chromen-7(7aH)-one **(15g)**. This compound was prepared from 5-(cyclohexylmethoxy)-2-isopropyl-4*H*-chromen-4-one (**27b**). Yield 21%; Colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 (t, *J* = 8.34 Hz, 1H), 6.57 (d, *J* = 8.29 Hz, 1H), 6.49 (d, *J* = 8.29 Hz, 1H), 4.87 (d, *J* = 2.61 Hz, 1H), 3.92 - 4.04 (m, 2H), 3.82 - 3.92 (m, 2H), 3.80 (d, *J* = 6.43 Hz, 1H), 2.16 - 2.31 (m, 1H), 1.67 - 1.95 (m, 7H), 1.22 - 1.43 (m, 8H), 1.09 - 1.22 (m, 2H), 1.05 (d, *J* = 6.71 Hz, 4H), 0.89 (d, *J* = 6.71 Hz,

3H), aliphatic protons showed excess; HRMS (ESI) Calcd. for $C_{19}H_{24}O_4$ $[M+H]^+$ 317.1753, found 317.1774.

7.1.8.8. 1a-Cyclohexyl-6-(cyclohexylmethoxy)-1aH-oxireno[2,3-b]chromen-7(7aH)-one **(15h)**. This compound was prepared from 2-cyclohexyl-5-(cyclohexylmethoxy)-4*H*-chromen-4-one **27c**. Yield 22%; colorless oil; HRMS (ESI) Calcd. for $C_{22}H_{28}O_4$ $[M+H]^+$ 357.2066, found 357.2091.

*7.1.9. Procedure for the preparation of compound **16**.* To a solution of 10% aqueous sodium hydroxide solution (3 mL) and 5-(cyclohexylmethoxy)-4*H*-chromen-4-one (**14a,** 3.87 mmol) (or **27a)** in a mixture of 1,4-dioxane and ethanol (10 mL), 10 mL of 35% H_2O_2 was added dropwise at 5 - 10 °C. The reaction mixture was stirred at same temperature for 2 h and the reaction mixture was allowed to attain ambient temperature, and further stirred for 16 h. The yellow suspension was acidified by using 3*N* HCl, the yellow precipitate was filtered and washed with water and dried under vacuum to afford oxidative cleaved product 2-(cyclohexylmethoxy)-6-hydroxybenzoic acid (**16**). Yield 47%; Yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.17 (s, 1H), 11.58 (br. s., 1H), 7.39 (t, *J* = 8.38 Hz, 1H), 6.71 (dd, *J* = 0.84, 8.48 Hz, 1H), 6.48 (d, *J* = 8.29 Hz, 1H), 4.04 (d, *J* = 6.15 Hz, 2H), 1.64 - 2.01 (m, 6H), 1.03 - 1.42 (m, 5H).

7.1.10. Preparation of (4-(bromomethyl)phenyl)methanol **(17a)**.

To a solution of methyl 4-(bromomethyl)benzoate **17** (4.36 mmol) in anhydrous DCM (10 mL), DIBAL-H (10.9 mmol) was added at 0 °C and then the solution was stirred at ambient temperature for 1 h. The reaction mixture was quenched with NH_4Cl solution, diluted with DCM, filtered through celite bed and then the bed was washed with DCM. The organic layer was washed with brine solution, dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to afford tittle compound **17a**, which was used for the next step without

further purification. Yield 75%; White solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 - 7.45 (m, 4H), 4.71 (d, *J* = 5.87 Hz, 2H), 4.51 (s, 2H), 1.69 (t, *J* = 5.91 Hz, 1H).

7.1.11. Preparation of 5-(cyclohexylmethoxy)-2-methyl-4H-chromen-4-one **(24)**.

7.1.11.1. Preparation of 5-hydroxy-2-methyl-4H-chromen-4-one **(23)**. To a solution of 1-(2,6-dihydroxyphenyl)ethanone **11a** (13.14 mmol) in dry EtOAc (20 mL), sodium metal (78.87 mmol) was added and then the solution was stirred at ambient temperature for 18 h. The reaction mixture was diluted with EtOAc and then quenched with cold 0.5 *N* HCl at 0 °C. The aqueous layer was separated, and the remaining organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated in vacuo to give the crude diketone **22**. A solution of the crude diketone **22** with 0.5 mL of concentrated HCl in methanol (10 mL) was stirred at 60 °C for 2 h. The methanol was removed in vacuo to give a residue, followed by adding ethyl acetate and washing with brine solution. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure which was further purified by silica gel (230-400) flash column chromatography (eluting with 0-30% ethyl acetate in hexanes) to afford the title compound **23**. Yield 75%; Light yellow solid; mp 68 -70 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.56 (s, 1H), 7.50 (t, *J* = 8.34 Hz, 1H), 6.86 (d, *J* = 8.38 Hz, 1H), 6.78 (d, *J* = 8.29 Hz, 1H), 6.12 (s, 1H), 2.40 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.6, 167.7, 160.9, 156.8, 135.2, 111.2, 110.4, 109.1, 106.8, 20.6; HRMS (ESI) Calcd. for $C_{10}H_8O_3$ $[M+H]^+$ 177.0551, found 177.0576.

7.1.11.2. Preparation of 5-(cyclohexylmethoxy)-2-methyl-4H-chromen-4-one **(24)**. To a solution of 5-hydroxy-2-methyl-4*H*-chromen-4-one **23** (5.67 mmol) in DMF (3 mL), K_2CO_3 (11.35 mmol) and (bromomethyl)cyclohexane (6.80 mmol) were added. The resulting mixture was stirred for 16 h at 60 °C. After cooling to room temperature, the reaction mixture was diluted with water, and then extracted with EtOAc. The combined organic layers were

washed with water, brine solution, dried over anhydrous Na_2SO_4, filtered and then concentrated under reduced pressure. The residue was subjected to flash silica gel (230-400 mesh) column chromatography (eluting with 0-25% ethyl acetate in hexanes) to afford the title compound **24**. Yield 65%; Light Yellow solid; mp 113 – 115 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (t, *J* = 8.34 Hz, 1H), 6.93 (d, *J* = 8.38 Hz, 1H), 6.75 (d, *J* = 8.20 Hz, 1H), 6.02 (s, 1H), 3.86 (d, *J* = 6.24 Hz, 2H), 2.30 (s, 3H), 1.88 - 2.08 (m, 3H), 1.66 - 1.84 (m, 3H), 1.03 - 1.42 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.1, 163.4, 159.6, 158.6, 133.3, 114.4, 112.1, 109.4, 107.1, 74.7, 37.4, 29.7, 26.4, 25.8, 19.8; HRMS (ESI) Calcd. for $C_{17}H_{20}O_3$ $[M+H]^+$ 273.1490, found 273.1511.

7.1.12. General synthetic procedure for the preparation of compounds **(25a-c)**.

To a solution of the 1-(2-(cyclohexylmethoxy)-6-hydroxyphenyl)ethanone **12a** (8.05 mmol) in DCM (20 mL), trimethylamine (16.1 mmol) and corresponding acid chloride (9.66 mmol) were added at 0 - 5 °C and then stirred for 1 h. The reaction mixture was diluted with DCM and then washed with 1*N* HCl and then brine solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated under reduced pressure to afford the title compounds **25a-c** which was used for the next step without further purification**.**

7.1.12.1. 2-Acetyl-3-(cyclohexylmethoxy)phenyl butyrate **(25a)**. Yield 95%; Light brown oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (t, *J* = 8.34 Hz, 1H), 6.79 (d, *J* = 8.48 Hz, 1H), 6.68 (d, *J* = 8.10 Hz, 1H), 3.81 (d, *J* = 5.87 Hz, 2H), 2.45 - 2.55 (m, 5H), 1.68 - 1.87 (m, 8H), 0.96 - 1.33 (m, 8H).

7.1.12.2. 2-Acetyl-3-(cyclohexylmethoxy)phenyl isobutyrate **(25b)**. Yield 90%; Light brown oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (t, *J* = 8.29 Hz, 1H), 6.79 (d, *J* = 8.05 Hz, 1H), 6.68 (dd, *J* = 0.73, 8.05 Hz, 1H), 3.81 (d, *J* = 5.85 Hz, 2H), 2.70 - 2.80 (m, 1H), 2.50 (s, 3H), 1.69 - 1.84 (m, 6H), 1.23 - 1.31 (m, 9H), 1.00 - 1.11 (m, 2H).

7.1.12.3. 2-Acetyl-3-(cyclohexylmethoxy)phenyl cyclohexanecarboxylate **(25c)**. Yield 92%; Light brown oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (t, *J* = 8.29 Hz, 1H), 6.78 (d, *J* = 8.54 Hz, 1H), 6.66 (d, *J* = 8.29 Hz, 1H), 3.81 (d, *J* = 5.85 Hz, 2H), 2.46 - 2.56 (m, 4H), 1.94 - 2.07 (m, 2H), 1.64 - 1.86 (m, 8H), 1.48 - 1.54 (m, 2H), 0.98 - 1.40 (m, 9H).

7.1.13. General synthetic procedure for the preparation of compound **(27a-c).**

To a solution of the corresponding ester **25** (7.85 mmol) in dry THF (20 mL), NaH (60% dispersion in mineral oil, 11.78 mmol) was added and then heated at 60 °C for 2 h. The mixture was cooled to ambient temperature and then poured into an ice water (100 mL). The aqueous layer was extracted with ethyl acetate, and then the combined organic layers were dried over anhydrous Na_2SO_4, filtered and evaporated in vacuo to give the crude diketones **26**. A solution of the corresponding crude diketone **26** with 0.5 mL of concentrated HCl in methanol (10 mL) was stirred at 60 °C for 2 h. The methanol was removed in vacuo to give a residue, followed by adding ethyl acetate and washing with brine solution. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography (eluting with 0-25% ethyl acetate in hexanes) to afford the title compounds **27a-c**.

7.1.13.1. 5-(Cyclohexylmethoxy)-2-propyl-4H-chromen-4-one **(27a)**. Yield 85%; Light yellow solid; mp 77 – 79 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (t, *J* = 8.34 Hz, 1H), 6.94 (d, *J* = 8.48 Hz, 1H), 6.74 (d, *J* = 8.10 Hz, 1H), 6.02 (s, 1H), 3.86 (d, *J* = 6.24 Hz, 2H), 2.52 (t, *J* = 7.50 Hz, 2H), 1.88 - 2.07 (m, 3H), 1.66 - 1.84 (m, 5H), 1.05 - 1.42 (m, 5H), 1.01 (t, *J* = 7.40 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.2, 166.7, 159.5, 158.7, 133.3, 114.5, 111.5, 109.4, 107.1, 74.7, 37.4, 35.5, 29.7, 26.4, 25.8, 19.9, 13.4; HRMS (ESI) Calcd. for $C_{19}H_{24}O_3$ $[M+H]^+$ 301.1803, found 301.1826.

7.1.13.2. 5-(Cyclohexylmethoxy)-2-isopropyl-4H-chromen-4-one **(27b)**. Yield 82%; Light yellow oil to solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (t, *J* = 8.17 Hz, 1H), 6.95 (d, *J* = 8.29 Hz, 1H), 6.74 (d, *J* = 8.29 Hz, 1H), 6.03 (s, 1H), 3.86 (d, *J* = 6.34 Hz, 2H), 2.78 (sept, *J* = 6.87 Hz, 1H), 1.90 - 2.06 (m, 3H), 1.66 - 1.83 (m, 3H), 1.08 - 1.37 (m, 11H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.5, 171.4, 159.6, 158.7, 133.3, 114.6, 109.5, 109.1, 107.1, 74.8, 37.4, 32.5, 29.7, 26.5, 25.8, 19.9; HRMS (ESI) Calcd. for $C_{19}H_{24}O_3$ $[M+H]^+$ 301.1803, found 301.1826.

7.1.13.3. 2-Cyclohexyl-5-(cyclohexylmethoxy)-4H-chromen-4-one **(27c)**. Yield 80%; White solid; mp 104 – 106 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (t, *J* = 8.29 Hz, 1H), 6.94 (d, *J* = 8.29 Hz, 1H), 6.74 (d, *J* = 8.29 Hz, 1H), 6.01 (s, 1H), 3.86 (d, *J* = 6.34 Hz, 2H), 2.45 (tt, *J* = 3.23, 11.28 Hz, 1H), 1.94 - 2.05 (m, 5H), 1.68 - 1.86 (m, 6H), 1.10 - 1.46 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.5, 170.6, 159.5, 158.6, 133.2, 114.7, 109.5, 109.4, 107.0, 74.7, 42.0, 37.4, 30.2, 29.7, 26.5, 25.8, 25.7; HRMS (ESI) Calcd. for $C_{22}H_{28}O_3$ $[M+H]^+$ 341.2116, found 341.2137.

7.2. Biology

7.2.1. IL-5 bioassay, mIL-5-dependent Y16 proliferation [21].

Y16 cell line was originated from a murine early B cell. The cell line was grown in RPMI-1640 media (10.4 mg/mL RPMI-1640, 24 mM $NaHCO_3$, 100 units/mL benzylpenicillin potassium, 100 µg/mL streptomycin sulfate, pH 7.1) containing 8% fetal bovine serum (FBS) and 3 units/mL mIL-5 at 37 °C with 5% CO_2. The Y16 cells grown were harvested by centrifugation at 250 x g for 10 min at 4 °C, washed twice with Hanks' solution (9.8 mg/mL Hanks' balanced salts, 4 mM $NaHCO_3$, pH 7.1), and resuspended in a small volume of RPMI-1640 media containing 8% FBS. Numbers of the cells were counted after trypan blue exclusion and the diluted to 1 x 10^5 cells/mL with RPMI-1640 media containing 8% FBS.

Viability of the cells was more than 95% in all preparations. One hundred µL of 1 x 10^4 Y16 cells were dispensed to each well of a 96-well microplate (Nunc, Denmark), and 50 µL of 3 units/mL mIL-5 and 50 µL of sample were added. Control group was treated with RPMI-1640 media containing 8% FBS instead of sample, and blank group with RPMI-1640 media containing 8% FBS instead of mIL-5. After incubation at 37 °C with 5% CO_2 for 48 h, Y16 cells in each well were treated with 20 µL of WST-1 solution (3.3 mg WST-1 and 0.7 mg methoxy-PMS per mL of PBS). Absorbance at wavelength 450 nm (A_{450}) was measured by using a microplate reader (Molecular Device, USA) after incubation at 37 °C with 5% CO_2 for 2 - 4 h.

Inhibitory effect on IL-5 bioassay was expressed as % inhibition, [1 – (sample A_{450} – blank A_{450}) / (control A_{450} – blank A_{450})] x 100. The data were collected as the mean of three independent experiments and significance of them was analyzed by Student's t-test.

Acknowledgment

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (grant number: 2015R1D1A3A01020014).

References and notes

[1] A.B. Kay, Asthma and inflammation, J. Allergy Clin. Immunol. 87 (1991) 893–910. http://dx.doi.org/10.1016/0091-6749(91)90408-G.

[2] P.C. Fulkerson, M.E. Rothenberg, Targeting eosinophils in allergy, inflammation and beyond., Nat. Rev. Drug Discov. 12 (2013) 117–129. doi:10.1038/nrd3838.

[3] D. Simon, A. Wardlaw, M.E. Rothenberg, Organ-specific eosinophilic disorders of the skin, lung, and gastrointestinal tract, J. Allergy Clin. Immunol. 126 (2010) 3–13. doi:10.1016/j.jaci.2010.01.055.

[4] M.E. Rothenberg, Eosinophilic gastrointestinal disorders (EGID), J. Allergy Clin. Immunol. 113 (2004) 11–29. doi:10.1016/j.jaci.2003.10.047.

[5] A.D. Klion, How I treat hypereosinophilic syndromes, Blood. 126 (2015) 1069–1077. doi:10.1182/blood-2014-11-551614.

[6] J. Tavernier, R. Devos, S. Cornelis, T. Tuypens, J. Van der Heyden, W. Fiers, G. Plaetinck, A human high affinity interleukin-5 receptor (IL5R) is composed of an IL5-specific α chain and a β chain shared with the receptor for GM-CSF, Cell. 66 (1991) 1175–1184. doi:10.1016/0092-8674(91)90040-6.

[7] Y.H. K. Takatsu, S. Takaki, Interleukin-5 and its receptor system: implications in the immune system and inflammation, Adv. Immunol. 57 (1994) 145–190. doi:10.1016/S0065-2776(08)60673-2.

[8] B. Gregory, A. Kirchem, S. Phipps, P. Gevaert, C. Pridgeon, S.M. Rankin, D.S. Robinson, Differential Regulation of Human Eosinophil IL-3, IL-5, and GM-CSF Receptor -Chain Expression by Cytokines: IL-3, IL-5, and GM-CSF Down-Regulate IL-5 Receptor Expression with Loss of IL-5 Responsiveness, but Up-Regulate IL-3 Receptor Expression, J. Immunol. 170 (2003) 5359–5366. doi:10.4049/jimmunol.170.11.5359.

[9] M.E. Rothenberg, S.P. Hogan, The Eosinophil, Annu. Rev. Immunol. 24 (2006) 147–174. doi:10.1146/annurev.immunol.24.021605.090720.

[10] M. Vadas, A. Lopez, J. Gamble, Y. Khew-Goodall, W. Smith, C. Bernard, G. Cockerill, P. Cockerill, F. Shannon, Q. Sun, Cytokines and allergy., J. Allergy Clin. Immunol. 94 (1994) 1289–1293.

[11] P.L. Ngoc, D.R. Gold, A.O. Tzianabos, S.T. Weiss, J.C. Celedón, Cytokines, allergy,

and asthma., Curr. Opin. Allergy Clin. Immunol. 5 (2005) 161–166. doi:10.1097/01.all.0000162309.97480.45.

[12] C.J. Sanderson, Interleukin-5, Eosinophils, and Disease, Blood. 79 (1992) 3101–3109.

[13] P.D. Collins, S. Marleau, D.A. Griffiths-Johnson, P.J. Jose, T.J. Williams, Cooperation between interleukin-5 and the chemokine eotaxin to induce eosinophil accumulation in vivo., J. Exp. Med. 182 (1995) 1169–1174. doi:10.1084/jem.182.4.1169.

[14] L.A. Dent, M. Strath, A.L. Mellor, C.J. Sanderson, Eosinophilia in transgenic mice expressing interleukin 5, J Exp Med. 172 (1990) 1425–1431. doi:10.1084/jem.172.5.1425.

[15] M. Kopf, F. Brombacher, P.D. Hodgkin, A.J. Ramsay, E.A. Milbourne, W.J. Dai, K.S. Ovington, C.A. Behm, G. Kö, I.G. Young, IL-5-Deficient Mice Have a Developmental Defect in CD5 + B-1 Cells and Lack Eosinophilia but Have Normal Antibody and Cytotoxic T Cell Responses, Immunity. 4 (1996) 15–24.

[16] W.F. Owen, M.E. Rothenberg, J. Petersen, P.F. Weller, D. Silberstein, A.L. Sheffer, R.L. Stevens, R.J. Soberman, K.F. Austen, Interleukin 5 and phenotypically altered eosinophils in the blood of patients with the idiopathic hypereosinophilic syndrome, J Exp Med. 170 (1989) 343–348.

[17] F. Menzella, M. Lusuardi, C. Galeone, S. Taddei, L. Zucchi, Profile of anti-IL-5 mAb mepolizumab in the treatment of severe refractory asthma and hypereosinophilic diseases, J. Asthma Allergy. 8 (2015) 105–114. doi:10.2147/JAA.S40244.

[18] J.C. Cardet, E. Israel, Update on reslizumab for eosinophilic asthma, Expert Opin. Biol. Ther. 15 (2015) 1531–1539. doi:10.1517/14712598.2015.1090972.

[19] M.E. Rothenberg, Humanized Anti-IL-5 Antibody Therapy, Cell. 165 (2016) 509.

doi:10.1016/j.cell.2016.04.020.

[20] R. Devos, Y. Guisez, G. Plaetinck, S. Cornelis, J. Tavernier, J. Van Der Heyden, L.H. Foley, J.E. Scheffler, Covalent modification of the interleukin-5 receptor by isothiazolones leads to inhibition of the binding of interleukin-5., Eur. J. Biochem. 225 (1994) 635–640. doi:10.1111/j.1432-1033.1994.00635.x.

[21] B. Min, S.R. Oh, H.K. Lee, K. Takatsu, I.M. Chang, K.R. Min, Y. Kim, Sophoricoside analogs as the IL-5 inhibitors from Sophora japonica., Planta Med. 65 (1999) 408–412. doi:10.1055/s-1999-14016.

[22] J. Yun, C.-K. Lee, I.-M. Chang, K. Takatsu, T. Hirano, K.R. Min, M.K. Lee, Y. Kim, Differential inhibitory effects of sophoricoside analogs on bioactivity of several cytokines, Life Sci. 67 (2000) 2855–2863.

[23] S.H. Jung, S.H. Cho, T. Hung Dang, J.H. Lee, J.H. Ju, M.K. Kim, S.H. Lee, J.C. Ryu, Y. Kim, Structural requirement of isoflavonones for the inhibitory activity of interleukin-5, Eur. J. Med. Chem. 38 (2003) 537–545. doi:10.1016/S0223-5234(03)00064-3.

[24] H.-M. Yang, H.-R. Shin, S.-C. Bang, K.-C. Lee, L.T.A. Hoang, I.-J. Lee, Y. Kim, S.-H. Jung, The role of alkoxy group on the A ring of isoflavones in the inhibition of interleukin-5., Arch. Pharm. Res. 30 (2007) 950–954. doi:10.1007/BF02993962.

[25] H.M. Yang, H.R. Shin, S.H. Cho, S.C. Bang, G.Y. Song, J.H. Ju, M.K. Kim, S.H. Lee, J.C. Ryu, Y. Kim, S.H. Jung, Structural requirement of chalcones for the inhibitory activity of interleukin-5, Bioorganic Med. Chem. 15 (2007) 104–111. doi:10.1016/j.bmc.2006.10.007.

[26] S.H. Jung, S.H. Cho, T.H. Dang, J.H. Lee, J.H. Ju, M.K. Kim, S.H. Lee, J.C. Ryu, Y.

Kim, Structural requirement of isoflavonones for the inhibitory activity of interleukin-5, Eur. J. Med. Chem. 38 (2003) 537–545. doi:10.1016/S0223-5234(03)00064-3.

[27] P. Thanigaimalai, T.A. Le Hoang, K.C. Lee, V.K. Sharma, S.C. Bang, J.H. Yun, E. Roh, Y. Kim, S.H. Jung, Synthesis and evaluation of novel chromone analogs for their inhibitory activity against interleukin-5, Eur. J. Med. Chem. 45 (2010) 2531–2536. doi:10.1016/j.ejmech.2010.02.041.

[28] P. Thanigaimalai, K.C. Lee, V.K. Sharma, J.H. Yun, Y. Kim, S.H. Jung, Design and synthesis of novel hydroxyalkylaminomethylchromones for their IL-5 inhibitory activity, Bioorganic Med. Chem. 18 (2010) 4625–4629. doi:10.1016/j.bmc.2010.05.028.

[29] C. Joo, E. Venkateswararao, K.-C. Lee, V.K. Sharma, M.-S. Kyung, Y. Kim, S.-H. Jung, Novel interleukin-5 inhibitors based on hydroxyethylaminomethyl-4H-chromen-4-one scaffold, Bioorg. Med. Chem. 20 (2012) 5757–5762. doi:10.1016/j.bmc.2012.08.006.

[30] E. Venkateswararao, M.-S. Kim, V.K. Sharma, K.-C. Lee, S. Subramanian, E. Roh, Y. Kim, S.-H. Jung, Identification of novel chromenone derivatives as interleukin-5 inhibitors, Eur. J. Med. Chem. 59 (2013) 31–38. doi:10.1016/j.ejmech.2012.11.007.

[31] E. Venkateswararao, V.K. Sharma, K.C. Lee, E. Roh, Y. Kim, S.H. Jung, Design and synthesis of novel chromenone derivatives as interleukin-5 inhibitors, Bioorganic Med. Chem. 21 (2013) 2543–2550. doi:10.1016/j.bmc.2013.02.037.

[32] A. Schonberg, N. Badran, N.A. Starkowsky, Furo-chromones and -Coumarins. VII. Degradation of Visnagin, Khellin and Related Substances; Experiments with Chromic Acid and Hydrogen Peroxide; and a Synthesis of Eugenitin, J. Am. Chem. Soc. 75 (1953) 4992–4995. doi:10.1021/ja01116a032.

[33] Tripos Associates. SYBYL-X2.0, Molecular Modeling Software. St. Louis, MO: Tripos Associates., (2014). http://www.tripos.com.

[34] M. Clark, R.D. Cramer, N. Van Opdenbosch, Validation of the general purpose tripos 5.2 force field, J. Comput. Chem. 10 (1989) 982–1012. doi:10.1002/jcc.540100804.

[35] D.D. Perrin, W.L.F. Armarego, D.R. Perrin, Purification of Laboratory Chemicals, second ed., Pergamon Press, Oxford, England, (1982).

Highlights

- Novel 3-alkoxychromen-4-ones were designed and synthesized as IL-5 inhibitor.
- Analog **10u** showed nine fold more active than budesonide.
- The SAR of the synthesized compounds was summarized.
- Conformational studies were performed.