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Novel interleukin-5 inhibitors based on hydroxyethylaminomethyl-4*H*-chromen-4-one scaffold

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ABSTRACT

Hydroxyethylaminomethyl-4*H*-chromenones were previously discovered as fairly strong IL-5 inhibitor. For determination of detail structure activity relationship, N-substituted hydroxyethylaminomethyl-chromenones **4a**–**n** were prepared and evaluated for their IL-5 inhibitory activity. Shifting the hydrophobic group to nitrogen from 1-position of hydroxyethylamino moiety of hydroxyethylaminomethyl-4*H*-chromenones enhances the activity. The increment in bulkiness or hydrophobicity of alkyl side chain at amino group increases the activity. The same level of activity of 5-(cyclohexylmethoxy)-3-(*N*-benzyl-2-hydroxyethylaminomethyl)-4*H*-chromenone analogs regardless of hydrophobic or hydrophilic substituents at 4th position of phenyl ring might infer the existence of tunnel structure in the putative receptor for accepting these side chains.

1. Introduction

Asthma is the common chronic inflammatory disease of the airways characterized by reversible airflow blockade and bronchospasm, accompanied with symptoms like coughing, wheezing, shortness of breath or rapid breathing, and chest tightness.[1] The occurrence of asthma has amplified considerably since 1970s.[2] Asthma is linked with intrusion of T cells and eosinophils, increased levels of pro-inflammatory cytokines such as interleukin-4 (IL-4), IL-5 and IL-13, and shedding of bronchial epithelial cells (ECs).[3] The eosinophils are the major effector cells in allergic inflammation and are present in increased numbers in the circulation and sputum depending upon the severity of asthma.[4] IL-5 is the primary cytokine which is involved in the production, differentiation, maturation and activation of the eosinophils.[5] It is present in increased amounts in the mucosa of asthmatic airways and the expression of IL-5 mRNA is directly related to seriousness of the disease.[6,7] Moreover, the inhalation of IL-5 increases the eosinophil percentage in induced sputum and boosts airway hyper responsiveness in asthmatics.[8] Thus targeting IL-5 will be quite effective in the treatment of allergic diseases like asthma.

For decades, a number of small organic compounds have been reported for the inhibition of IL-5. Among them isothiazolones have been synthesized by the modification of Cys 66 residue in IL-5 and reported as its antagonists.[9] Later, Min et al. had identified naturally occurring sophoricoside (**1a**, Fig. 1) and its analogs for their IL-5 inhibitory activity.[10] In addition, sophoricoside has also been identified as differential inhibitor of IL-3 and GM-CSF.[11] As sophoricoside showed promising IL-5 inhibitory activity, various isoflavonoid analogs of sophoricoside[12,13] and chalcone derivatives[14,15] were investigated as potent inhibitors of IL-5. For the SAR studies of these sophoricoside analogs,[12] we initially observed that not only the planar chromen-4-one ring but also phenolic hydroxyl group at *para* position of ring B is essential for their IL-5 inhibitory activity.[12,13] In addition, the introduction of hydrophobic moiety such as cyclohexylmethoxy group at 5-position of isoflavone (**1b** and **1d**, Fig. 1) is also required for the good inhibitory activity. Then the role of ring B was explored and accordingly a number of novel chromenone analogs with insertion of methylene between phenyl and chromenone (**2a**, 98% inhibition at 30 μM, IC_{50} = 3.0 μM, Fig. 1) or without ring B (**2b**, 85% inhibition at 30 μM, IC_{50} = 7.6 μM, Fig. 1) were prepared and evaluated.[16] These variations well retained the level of activity of isoflavones.[16] Along this line, novel 5-(cyclohexylmethoxy)-3-(1-alkyl-2-hydroxyethylaminomethyl)chromones such as **3a–d**[17] were investigated as inhibitors of IL-5 (Fig. 1). Although the chromenone **3a** with 2-hydroxyethylaminomethyl group did not show any activity, introduction of bulky hydrophobic group at 1-position of 2-hydroxyethylamino moiety (**3b–d)** exhibited fairly strong

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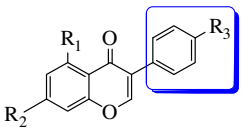
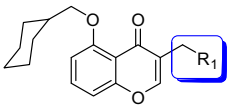
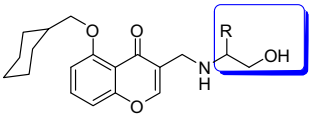


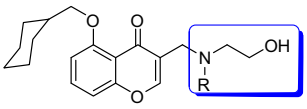
1a: Sophoricoside R_1,R_2 = OH, R_3 = OGlu
1b: R_1 = benzyloxy, R_2 = H, R_3 = OH
1c: R_1 = H, R_2 = benzyloxy, R_3 = OH
1d: R_1 = cyclohexylmethoxy, R_2 =H, R_3 = OH



2a, R_1 = C_6H_5OH
2b, R_1 = OH



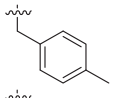
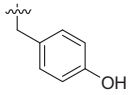
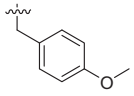
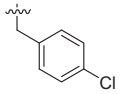
3a-d = substituents are located in Table 1



4a-n, R = substituents are located in Table 1

Figure 1. Interleukin-5 inhibitors **1, 2, 3** and amino alcohol chromone analogs **4**.

Table 1
Structures and *C* log*P* values of **4**(**a**–**n**)

Compd. no.	R	*C* log*P*[a]	Compd. no.	R	*C* log*P*[a]
3a[17]	H	2.9143	**4f**		5.2791
3b[17]		3.9589	**4g**		5.4867
3c[17]		5.8733	**4h**		6.1057
3d[17]		4.7913	**4i**		5.1717
4a	$-CH_3$	3.4557	**4j**		5.6707
4b	$-CH_2CH_3$	3.9847	**4k**		4.5047
4c	$-(CH_2)_2CH_3$	4.5137	**4l**		5.0907
4d	$-(CH_2)_3CH_3$	5.0427	**4m**		5.8847
4e	$-CH(CH_3)_2$	4.2937	**4n**		3.6747

[a] *C* log*P* values were calculated by Chem. draw version 9.0.

activity (Table 1). Thus these results indicated that the ring B of isoflavone **1a** acts as a simple linker between the chromenone and the hydroxyl function.

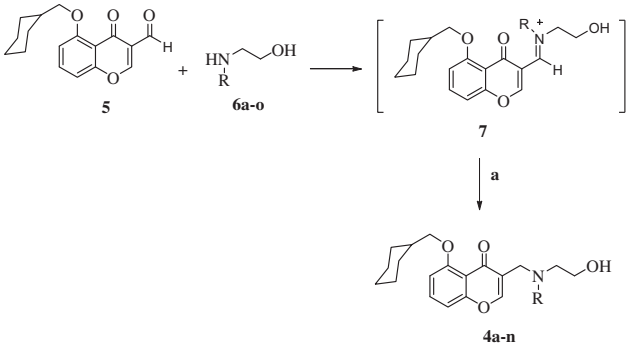
As these hydroxyethylaminomethylchromones **3b–d** (Fig. 1, Table 1) showed fairly good inhibitory activity against IL-5, more detailed structure–activity relationship studies are needed to get the potential drug candidate. Therefore, here in this article we have reported the design and synthesis of a number of N-substituted hydroxyethylaminomethylchromenone derivatives **(4a–n)** and tested for their IL-5 inhibitory activity.

2. Chemistry

The synthesis of the desired compounds **4a–n** was accomplished with reductive amination of **5**[16] as outlined in Scheme 1. Accordingly, 3-formylchromone **5**[16] in 1,2-dichloroethane was treated with secondary amine **6a–n** and sodium triacetoxyborohydride at ambient temperature for 12 h. After purification with flash column chromatography, compounds **4a–n** were obtained with moderate yields. For the reductive amination, treatment of **5** and secondary amines **6** with sodium borohydride or sodium cyanoborohydride in ethanol at reflux did not undergo reaction unlike reductive amination with primary amines.[16] This might be sterically more congested in the iminium intermediate **7** compared to imines formed from primary amine.[18] All these synthesized compounds (Table 1) were characterized by physical and spectral analysis data that confirmed their assigned structures.

3. Conformational analysis and alignment

Molecular model of the compounds **3d**[17] and **4i** were constructed using SYBYL®-X1.3 program package (Tripos Associates Inc.)[19] and their geometry were optimized (Powell conjugate



Scheme 1. Synthesis of choromone analogs **4**(**a**–**n**). Reagents and conditions: (a) $NaBH(OAc)_3$, 1,2-dichloroethane, rt. Note = substituents are located in Table 1.

gradient minimization, termination at a gradient of 0.0005 kcal/mol) using the Tripos standard force field[20] and Gasteiger–Hückel atomic partial charges.[21] The 3D structures of the analyzed compounds were aligned according to a chromenone template, which was assumed to be a bioactive conformation.

4. Pharmacology

Inhibitory activity of the chromenone analogs (**4a**–**n**) against IL-5 was evaluated using the IL-5-dependent pro-B Y16 cell line according to previously reported procedure.[22] The cells were incubated with 3 units/mL mIL-5 for 48 h, in the presence or absence of sample, and then measured cell metabolism as an index of proliferation, using 2-(4-iodophenyl)-3-(nitrophenyl)-5-(2,4-disulphophenyl)-2*H* tetrazolium sodium salt (WST-1). Data were collected from three independent experiments. The effect of test compounds on the IL-5 bioassay is represented as per cent inhibition at 30 μM samples and IC_{50} values were calculated (Table 2).

5. Results and discussion

For determining more detail structure–activity relationship studies of 3-(2-hydroxyethylaminomethyl)chromones, we designed and synthesized a number of analogs of **3a** by introduction of bulky substituents at amino group of **3a** and studied them for their IL-5 inhibitory activity. (Table 2)

In the first set of experiment, methyl analog **4a** (56.0% inhibition at 30 μM, IC_{50} = 30.3 μM, *C* log*P* = 3.4557) showed better inhibitory activity as compared to **3a** (13.2% inhibition at 30 μM, IC_{50} >50 μM).[17] This suggests that the hydrophobic substitution on amino group of **3a** increases the activity of these chromenones. To further confirm the optimum size of substituent at amino group of **3a**, the ethyl group was introduced. As a result the activity improved in analog **4b** (79.2% inhibition at 30 μM, IC_{50} = 19.5 μM, *C* log*P* = 3.9847). This outcome supports that the hydrophobicity or bulkiness at amino alcohol plays a critical role in inhibitory activity against IL-5. To prove our point the chain length and bulkiness at this position was further increased. The propyl analog **4c** (93.7% inhibition at 30 μM, IC_{50} = 15.4 μM, *C* log*P* = 4.5137) and butyl analog **4d** (94.0% inhibition at 30 μM, IC_{50} = 9.2 μM, *C* log*P* = 5.0427) confirmed our viewpoint that hydrophobicity or bulkiness is important for the activity. The bulkier isopropyl analog **4e** (90.2% inhibition at 30 μM, IC_{50} = 10.2 μM, *C* log*P* = 4.2937) maintained the activity. Considering *C* log*P* values of **4a**–**d**, it was observed that increment of hydrophobicity by increasing bulky aliphatic groups or linear alkyl chain is well correlated with the activity.

In another set of experiment the planar phenyl derivatives **4f** (94.4% inhibition at 30 μM, IC_{50} = 14.3 μM, *C* log*P* = 5.2791) and bulky cyclohexyl **4g** (94.5% inhibition at 30 μM, IC_{50} = 9 μM, *C* log*P* = 5.4867) on nitrogen of **3a** were introduced. The results indicated that the bulkier cyclohexyl have better activity than the phenyl one. Thus the bulky hydrophobic group in this region should be more important than the shape or planarity of the phenyl ring.

Insertion of a methyl unit between amino group and bulky substituents slightly decreases the activity as in cyclohexylmethyl analog **4h** (94.7% inhibition at 30 μM, IC_{50} = 12.4 μM, *C* log*P* = 6.1057) and benzyl **4i** (94.8% inhibition at 30 μM, IC_{50} = 16.1 μM, *C* log*P* = 5.1717) compared to the corresponding **4f–g**. Though these derivatives showed slightly less inhibition still they are potent inhibitors of IL-5, which further approves the significance of hydrophobicity in this region.

In next set of experiment, the effect of substituents at 4th position of the phenyl ring of **4i** was investigated. Since phenolic hydroxyl group or electron donating group at 4-position of ring B of **1a** plays an important role for their IL-5 inhibitory activity, methyl (**4j**,94.3% inhibition at 30 μM, IC_{50} = 9.4 μM, *C* log*P* = 5.6707), hydroxyl (**4k**, 93.7% inhibition at 30 μM, IC_{50} = 9.1 μM, *C* log*P* = 4.5047), methoxy (**4l**, 94.1% inhibition at 30 μM, IC_{50} = 9.0 μM, *C* log*P* = 5.0907) and chloro (**4m**, 93.4% inhibition at 30 μM, IC_{50} = 10.3 μM, *C* log*P* = 5.8847) substituents were introduced at 4th position of the phenyl ring of **4i**. The results indicated that all these derivatives have potent inhibitory activity regardless of hydrophobic or hydrophilic nature of these substituents at 4th position of phenyl ring of **4i**. These might infer the existence of tunnel structure in the putative receptor for accepting these side chains. This can be validated by pyridine-4-methyl substituent as shown in analog **4n** (94% inhibition at 30 μM, IC_{50} = 14.2 μM, *C* log*P* = 3.6747).

Comparison of the activity (Table 1) of **4f**, **4h** and **4i** to those of **3b**, **3c** and **3d**, respectively, revealed the potentiation of the activity in compounds **4**. This obviously implies that moving the side chain towards chromenone ring enhances the activity.

To further investigate the detailed structural requirements for the potent IL-5 inhibitor we compared the structural sketches of the compound **3d**[17] and **4i** and observed a dramatic difference in the region of side chain at 3-position of chromenone scaffold (Fig. 2). The dihedral angles ($\angle C_1–C_2–N_3–C_4$ and $\angle C_2–N_3–C_4–C_5$) of compound **3d** are 198.9°and 161.1°, respectively (Table 3). This indicates that the hydroxyethylaminomethyl moiety of **3d** is stretched away from chromenone ring. In addition, the distance from C-1 carbon to hydroxyl group oxygen (6.007 Å, Fig. 2) also confirms this stretch conformation. Meanwhile the corresponding angles ($\angle C_1–C_2–N_3–C_4$ = 307.3° and $\angle C_2–N_3–C_4–C_5$ = 99.7°, Table 3) of **4i** depict that hydroxyl group of hydroxyethylaminomethyl moiety of **4i** is located nearly at right angle position in chromenone ring plane. The much shorter distance from C-1 carbon to oxygen of hydroxyl group (4.469 Å, Fig. 2) in **4i** than in **3d** also implies the folded conformation of hydroxyethylaminomethyl moiety. Such conformational change might cause the enhancement of the activity of analogs **4** compared to those of the corresponding compounds **3**. Therefore, the folded conformation as shown in **4i** could

Table 2
Interleukin-5 inhibitory activity of **4**(**a**–**n**)

Compd. no.	% Inhibition[a] at 30 (μM)	IC_{50} (μM)	Compd. no.	% Inhibition[a] at 30 (μM)	IC_{50} [a] (μM)
3a[17]	13.2	>50	**4f**	94.4	14.3
3b[17]	88.0	18.5	**4g**	94.5	9.0
3c[17]	93.4	17.3	**4h**	94.7	12.4
3d[17]	72.4	25.7	**4i**	94.8	16.1
4a	56.0	30.3	**4j**	94.3	9.4
4b	79.2	19.5	**4k**	93.7	9.1
4c	93.7	15.4	**4l**	94.1	9.0
4d	94.0	9.2	**4m**	93.4	10.3
4e	90.2	10.2	**4n**	94.0	14.2
Sophoricoside	79.1	10.6	Budesonide	55.3	27.1

[a] % Inhibition and IC_{50} values are taken as a mean from 3 experiments.

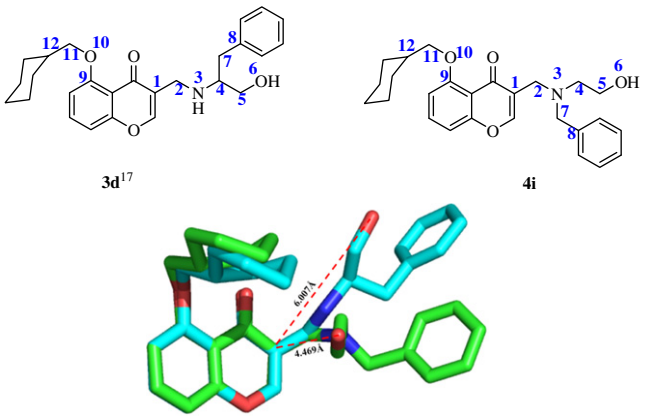


Figure 2. Alignment of compound **3d**[17] (18.0114 kcals/mol) and **4i** (17.5547 kcals/mol). Cyan color (**3d**[17]), Green color (**4i**).

Table 3
Torsion angle(°) of compounds **3d**[17] and **4i**

Compound **3d**[17]		Compound **4i**	
$\angle C_1–C_2–N_3–C_4$	198.9°	$\angle C_1–C_2–N_3–C_4$	307.3°
$\angle C_2–N_3–C_4–C_5$	161.1°	$\angle C_2–N_3–C_4–C_5$	99.7°
$\angle N_3–C_4–C_5–C_6$	189.8°	$\angle N_3–C_4–C_5–C_6$	178.7°
$\angle C_2–N_3–C_4–C_7$	284.8°	$\angle C_1–C_2–N_3–C_7$	102.0°
$\angle N_3–C_4–C_7–O_8$	178.3°	$\angle C_2–N_3–C_7–O_8$	157.5°
$\angle C_9–O_{10}–C_{11}–C_{12}$	56.2°	$\angle C_9–O_{10}–C_{11}–C_{12}$	58.3°

C_{1-12} and N_3 atoms of **3d** and **4i** are mentioned in Figure 2.

be much closer to the effective conformation for binding to the putative receptor.

6. Conclusion

For determination of detailed structure activity relationship of hydroxyethylaminomethylchromenones as IL-5 inhibitor, N-substituted analogs **4a–n** were prepared and evaluated for their IL-5 inhibitory activity. Among these derivatives compounds **4d, 4g** and **4j–l** showed the most potent inhibitory activity. The results indicated that increment in bulkiness or hydrophobicity at amino group increases the activity. The same level of activity of benzyl analogs **4i–m** regardless of hydrophobic or hydrophilic substituents at *para* position of phenyl ring of **4i** might conclude the existence of tunnel structure in the putative receptor for accepting these side chains. Based on the conformational analysis, introduction of hydrophobic group on nitrogen seems to enforce the more effective conformation of hydroxyethylaminomethyl side chain of analogs **4** and thus increase in activity compared to analogs **3**.

Thus the structural requirement of these chromenone analogs possessing the inhibitory activity against IL-5 could be summarized as: (i) importance of hydrophobic group, such as cyclohexylmethoxy at position 5 of ring A, (ii) planarity of the chromen-4-one ring, (iii) hydrophobicity in the ring B region, especially bulky and hydrophobic substituents on nitrogen of hydroxyethylamino moiety as the critical motif for IL-5 inhibitory activity of 3-(hydroxyethylaminomethyl)-4*H*-chromenones.

7. Materials and methods

7.1. Chemistry

Melting points were determined on Electro thermal 1A 9100 MK2 apparatus and are uncorrected. All commercial chemicals were used as obtained and all solvents were purified by the standard procedures prior to use.[23] Thin layer chromatography was performed on E Mercksilica gel GF-254 precoated plates and the identification was done with UV light and colorization with spray 10% phosphomolybdic acid followed by heating. Flash column chromatography was performed with E Merck silica gel (230–400 mesh). Infra red spectrum was recorded by using sample as such on FT-IR spectrum with Nicolet—380 models. NMR spectra were measured against the peak of tetramethylsilane by JEOL JNM-EX90 NMR (89.45 MHz) and Varain Unity Inova 400 NMR (400 MHz) spectrometers. High resolution mass spectra (HRMS) were measured by using Shimadzu LCMS-IT-TOF spectrometer.

7.1.1. Procedure for the preparation of compound 4a–n

To a solution of 3-formylchromone **5** (1 equiv) in 1,2-dichloroethane was added secondary amine **6a–o** (1.2 equiv) and sodium triacetoxyborohydride (1.5 equiv). After stirring at room temperature for 12 h, the reaction mixture was quenched with H_2O and extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash silica gel chromatography to obtained **4a–n**.

7.1.1.1. 5-(Cyclohexylmethoxy)-3-(((2-hydroxyethyl)(methyl)amino)methyl)-4*H*-chromen-4-one (4a). Yield 37%; yellow oil; R_f: 0.32 (MeOH:CH_2Cl_2 = 1:15); IR (KBr) 3407, 2920, 1743, 1650, 1269, 1065 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.05–2.05 (m, 11H), 2.30 (s, 3H), 2.64 (t, J = 5.2 Hz, 2H), 3.43 (s, 2H), 3.71 (t, J = 5.2 Hz, 2H), 3.85 (d, J = 6.0 Hz, 2H), 6.76 (d, J = 8.0 Hz, 1H), 6.96 (d, J = 8.4 Hz, 1H), 7.49 (t, J = 8.4 Hz, 1H), 7.75 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 177.36, 159.86, 158.63, 152.44, 133.72, 121.39, 114.86, 109.62, 107.22, 74.82, 58.74, 58.70, 51.39, 42.12, 37.35, 29.75, 26.46, 25.173; HRMS: calcd. for $C_{20}H_{27}NO_4$: m/z 345.1940, found: 345.1935.

7.1.1.2. 5-(Cyclohexylmethoxy)-3-((ethyl(2-hydroxyethyl)amino)methyl)-4*H*-chromen-4-one (4b). Yield 42%; yellow oil; R_f: 0.33 (MeOH:CH_2Cl_2 = 1:15); IR (KBr) 3442, 2923, 1656, 1269, 1068 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.05 (t, J = 6.4 Hz, 3H), 1.09–2.05 (m, 11H), 2.61 (q, J = 6.8 Hz, 2H), 2.69 (t, J = 5.0 Hz, 2H), 3.53 (s, 2H), 3.67 (t, J = 5.4 Hz, 2H), 3.86 (d, J = 6.4 Hz, 2H), 6.76 (d, J = 8.4 Hz, 1H), 6.96 (d, J = 8.8 Hz, 1H), 7.49 (t, J = 8.4 Hz, 1H), 7.78 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 177.29, 159.80, 158.57, 153.29, 133.80, 120.87, 114.74, 109.70, 107.32, 74.85, 58.65, 54.80, 48.01, 47.85, 37.31, 29.73, 26.44, 25.71, 10.82; HRMS: calcd. for $C_{21}H_{29}NO_4$: m/z 359.2097, found: 359.2090.

7.1.1.3. 5-(Cyclohexylmethoxy)-3-(((2-hydroxyethyl)(propyl)amino)methyl)-4*H*-chromen-4-one (4c). Yield 31%; yellow oil; R_f: 0.34 (MeOH:CH_2Cl_2 = 1:15); IR (KBr) 3390, 2922, 1734, 1657, 1268, 1061 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 (t, J = 7.4 Hz, 3H), 1.05–2.05 (m, 13H), 2.48 (t, J = 7.4 Hz, 2H), 2.69 (t, J = 5.0 Hz, 2H), 3.54 (s, 2H), 3.67 (t, J = 5.2 Hz, 2H), 3.86 (d, J = 6.0 Hz, 2H), 6.76 (d, J = 8.4 Hz, 1H), 6.96 (d, J = 8.0 Hz, 1H), 7.49 (t, J = 8.4 Hz, 1H), 7.80 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 177.45, 159.82, 158.60, 152.04, 133.59, 122.24, 114.82, 109.62, 107.19, 74.83, 59.17, 56.01, 55.61, 48.86, 37.35, 29.72, 26.44, 25.72, 19.80, 11.60; HRMS: calcd. for $C_{22}H_{31}NO_4$: m/z 373.2253, found: 373.2248.

7.1.1.4. 3-((Butyl(2-hydroxyethyl)amino)methyl)-5-(cyclohexylmethoxy)-4*H*-chromen-4-one (4d). Yield 39%; yellow oil; R_f: 0.34 (MeOH:CH_2Cl_2 = 1:15); IR (KBr) 3446, 2922, 1732, 1657, 1268, 1061 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.87 (t, J = 7.4 Hz, 3H), 1.09–2.21 (m, 15H), 2.51 (t, J = 7.4 Hz, 2H), 2.68 (t, J = 5.2 Hz, 2H), 3.53 (s, 2H), 3.67 (t, J = 5.4 Hz, 2H), 3.86 (d, J = 6.0 Hz, 2H), 6.76 (d, J = 8.4 Hz, 1H), 6.96 (d, J = 8.4 Hz, 1H), 7.49 (t, J = 8.4 Hz, 1H), 7.79 (s, 1H);

^{13}C NMR ($CDCl_3$) δ 177.44, 159.82, 158.60, 152.14, 133.61, 122.14, 114.81, 109.65, 107.21, 74.84, 59.12, 55.59, 53.84, 48.84, 37.37, 29.73, 28.72, 26.46, 25.73, 20.39, 13.88; HRMS: calcd. for $C_{23}H_{33}NO_4$: *m*/*z* 387.2410, found: 387.2404.

7.1.1.5. 5-(Cyclohexylmethoxy)-3-(((2-hydroxyethyl)(isopropyl) amino)methyl)-4*H*-chromen-4-one (4e). Yield 41%; yellow oil; R_f: 0.33 (MeOH:CH_2Cl_2 = 1:15); IR (KBr) 3478, 2922, 1737, 1650, 1267, 1061 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.05 (d, *J* = 6.4 Hz, 6H), 1.07–2.05 (m, 11H), 2.65 (t, *J* = 4.8 Hz, 2H), 2.69 (m, 1H), 3.56 (s, 2H), 3.60 (t, *J* = 5.2 Hz, 2H), 3.86 (d, *J* = 6.0 Hz, 2H), 6.76 (d, *J* = 8.4 Hz, 1H), 6.96 (d, *J* = 8.0 Hz, 1H), 7.49 (t, *J* = 8.4 Hz, 1H), 7.80 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 177.67, 159.92, 158.74, 152.17, 133.70, 120.96, 122.98, 114.83, 109.80, 107.24, 74.96, 59.46, 50.62, 50.32, 45.59, 37.49, 29.88, 26.58, 25.85, 17.88; HRMS: calcd. for $C_{22}H_{31}NO_4$: *m*/*z* 373.2253, found: 373.2249.

7.1.1.6. 5-(Cyclohexylmethoxy)-3-(((2-hydroxyethyl)(phenyl) amino)methyl)-4*H*-chromen-4-one (4f). Yield 35%; yellow solid; R_f: 0.27 (MeOH:CH_2Cl_2 = 1:30); IR (KBr) 3447, 2904, 1644, 1273, 1081 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.06–2.07 (m, 11H), 2.69 (m, 2H), 3.59 (t, *J* = 5.6 Hz, 2H), 3.84–3.91 (m, 4H), 4.49 (s, 2H), 6.71–6.78 (m, 4H), 6.92 (d, *J* = 8.4 Hz, 1H), 7.19 (d, *J* = 7.2 Hz, 1H), 7.21 (d, *J* = 7.2 Hz, 1H), 7.46–7.51 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 177.97, 159.67, 158.70, 150.95, 148.11, 133.67, 129.41, 120.39, 117.27, 114.49, 112.81, 109.86, 106.93, 74.75, 60.36, 53.74, 47.48, 37.40, 29.79, 26.46, 25.74; HRMS: calcd. for $C_{25}H_{29}NO_4$: *m*/*z* 407.2097, found: 407.2091.

7.1.1.7. 3-((Cyclohexyl(2-hydroxyethyl)amino)methyl)-5-(cyclohexylmethoxy)-4*H*-chromen-4-one (4g). Yield 32%; yellow solid; R_f: 0.29 (MeOH:CH_2Cl_2 = 1:30); IR (KBr) 3444, 2934, 1735, 1651, 1274, 1061 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.03–2.09 (m, 21H), 2.70 (t, *J* = 5.0 Hz, 2H), 3.57 (t, *J* = 5.2 Hz, 2H), 3.60 (s, 2H), 3.86 (d, *J* = 6.4 Hz, 2H), 6.76 (d, *J* = 8.0 Hz, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 7.49 (t, *J* = 8.2 Hz, 1H), 7.77 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 177.45, 159.85, 158.59, 151.83, 133.83, 122.45, 114.86, 109.62, 107.26, 74.87, 61.37, 59.21, 56.15, 49.56, 37.40, 35.63, 31.59, 29.70, 26.62, 26.45, 25.94, 25.75; HRMS: calcd. for $C_{25}H_{35}NO_4$: *m*/*z* 413.2566, found: 413.2561.

7.1.1.8. 5-(Cyclohexylmethoxy)-3-(((cyclohexylmethyl)(2-hydroxyethyl)amino)methyl)-4*H*-chromen-4-one (4h). Yield 29%; yellow oil; R_f: 0.30 (MeOH:CH_2Cl_2 = 1:30); IR (KBr) 3461, 2927, 1730, 1649, 1273, 1061 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.05–2.06 (m, 22H), 2.25 (d, *J* = 6.4 Hz, 2H), 2.63 (t, *J* = 4.8 Hz, 1H), 3.47 (s, 2H), 3.67 (t, *J* = 5.2 Hz, 2H), 3.86 (d, *J* = 6.4 Hz, 2H), 6.76 (d, *J* = 8.4 Hz, 1H), 6.95 (d, *J* = 8.8 Hz, 1H), 7.49 (t, *J* = 8.6 Hz, 1H), 7.77 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 177.72, 159.92, 158.74, 151.89, 133.66, 114.86, 114.83, 109.80, 107.17, 74.92, 59.70, 59.60, 51.71, 45.82, 37.50, 29.88, 28.79, 26.58, 26.24, 26.11, 25.86; HRMS: calcd. for $C_{26}H_{37}NO_4$: *m*/*z* 427.2723, found: 427.2717.

7.1.1.9. 3-((Benzyl(2-hydroxyethyl)amino)methyl)-5-(cyclohexylmethoxy)-4*H*-chromen-4-one (4i). Yield 27%; yellow oil; R_f: 0.28 (MeOH:CH_2Cl_2 = 1:30); IR (KBr) 3412, 2923, 1730, 1657, 1269, 1066 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.07–2.06 (m, 11H), 2.25 (d, *J* = 6.4 Hz, 2H), 2.69 (t, *J* = 4.0 Hz, 1H), 3.56 (s, 2H), 3.68 (s, 2H), 3.69 (t, *J* = 4.4 Hz, 2H), 3.87 (d, *J* = 6.4 Hz, 2H), 6.76 (d, *J* = 8.0 Hz, 1H), 6.93 (d, *J* = 8.4 Hz, 1H), 7.34–7.19 (m, 5H), 7.48 (t, *J* = 8.2 Hz, 1H), 7.75 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 177.61, 159.96, 158.69, 152.46, 133.78, 129.00, 128.56, 127.36, 115.01, 110.86, 109.73, 107.44, 75.01, 59.47, 58.70, 55.63, 48.94, 37.49, 29.84, 26.58, 25.86; HRMS: calcd. for $C_{26}H_{31}NO_4$: *m*/*z* 421.2253, found: 421.2246.

7.1.1.10. 5-(Cyclohexylmethoxy)-3-(((2-hydroxyethyl)(4-methylbenzyl)amino)methyl)-4*H*-chromen-4-one (4j). Yield 37%; yellow solid; R_f: 0.21 (MeOH:CH_2Cl_2 = 1:30); IR (KBr) 3479, 2904, 1658, 1280, 1066 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.07–2.06 (m, 11H), 2.29 (s, 3H), 2.68 (t, *J* = 5.4 Hz, 2H), 3.55 (s, 2H), 3.62 (s, 2H), 3.67 (t, *J* = 5.2 Hz, 2H), 3.87 (d, *J* = 6.0 Hz, 2H), 6.76 (d, *J* = 8.4 Hz, 1H), 6.93 (d, *J* = 8.4 Hz, 1H), 7.07 (d,, *J* = 7.6 Hz, 2H), 7.17 (d,, *J* = 8.0 Hz, 2H), 7.22 (s, 4H), 7.48 (t, *J* = 8.4 Hz, 1H), 7.75 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 177.51, 159.82, 158.56, 151.92, 136.69, 135.79, 133.55, 129.06, 128.73, 122.41, 114.89, 109.57, 107.21, 74.86, 59.43, 58.27, 55.40, 48.65, 37.37, 29.72, 26.46, 25.75, 20.97; HRMS: calcd. for $C_{27}H_{33}NO_4$: *m*/*z* 435.2410, found: 435.2405.

7.1.1.11. 5-(Cyclohexylmethoxy)-3-(((4-hydroxybenzyl)(2-hydroxyethyl)amino)methyl)-4*H*-chromen-4-one (4k). Yield 32%; yellow solid; R_f: 0.37 (MeOH:CH_2Cl_2 = 1:15); IR (KBr) 3470, 2928, 1733, 1650, 1272, 1066 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.07–2.02 (m, 11H), 2.65 (t, *J* = 5.0 Hz, 2H), 3.53 (s, 2H), 3.57 (s, 2H), 3.68 (t, *J* = 5.2 Hz, 2H), 3.86 (d, *J* = 6.4 Hz, 2H), 6.71 (d, *J* = 8.4 Hz, 2H), 6.77 (d, *J* = 8.0 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 1H), 7.11 (d, *J* = 8.0 Hz, 2H), 7.49 (t, *J* = 8.4 Hz, 1H), 7.75 (s, 1H); ^{13}C NMR ($CDCl_3$+DMSO-d_6) δ 177.58, 159.73, 158.60, 156.44, 152.26, 133.65, 129.97, 129.52, 122.64, 115.36, 114.77, 109.74, 107.24, 74.80, 59.60, 58.22, 55.67, 48.71, 37.42, 29.72, 26.47, 25.78; HRMS: calcd. for $C_{26}H_{31}NO_5$: *m*/*z* 437.2202, found: 437.2196.

7.1.1.12. 5-(Cyclohexylmethoxy)-3-(((2-hydroxyethyl)(4-methoxybenzyl)amino)methyl)-4*H*-chromen-4-one (4l). Yield 34%; yellow solid; R_f: 0.39 (MeOH:CH_2Cl_2 = 1:15); IR (KBr) 3452, 2918, 1734, 1651, 1278, 1067 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.07–2.02 (m, 11H), 2.67 (t, *J* = 5.4 Hz, 2H), 3.54 (s, 2H), 3.60 (s, 2H), 3.67 (t, *J* = 5.2 Hz, 2H), 3.76 (s, 3H), 3.87 (d, *J* = 6.0 Hz, 2H), 6.76 (d, *J* = 8.0 Hz, 1H), 6.81 (d, *J* = 8.4 Hz, 2H), 6.93 (d, *J* = 8.4 Hz, 1H), 7.20 (d, *J* = 8.4 Hz, 2H), 7.48 (t, *J* = 8.4 Hz, 1H), 7.74 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 177.51, 159.84, 158.74, 158.57, 151.93, 133.58, 130.86, 129.93, 122.42, 114.90, 113.74, 109.58, 107.23, 74.87, 59.41, 57.93, 55.32, 55.18, 48.62, 37.38, 29.73, 26.46, 25.75; HRMS: calcd. for $C_{27}H_{33}NO_5$: *m*/*z* 451.2359, found: 451.2355.

7.1.1.13. 3-(((4-Chlorobenzyl)(2-hydroxyethyl)amino)methyl)-5-(cyclohexylmethoxy)-4*H*-chromen-4-one (4m). Yield 40%; yellow oil; R_f: 0.30 (MeOH:CH_2Cl_2 = 1:30); IR (KBr) 3439, 2922, 1735, 1657, 1268, 1062 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.07–2.06 (m, 11H), 2.65 (t, *J* = 5.0 Hz, 2H), 3.53 (s, 2H), 3.62 (s, 2H), 3.70 (t, *J* = 4.8 Hz, 2H), 3.88 (d, *J* = 6.0 Hz, 2H), 6.77 (d, *J* = 8.4 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 1H), 7.22 (s, 4H), 7.49 (t, *J* = 8.4 Hz, 1H), 7.72 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 177.60, 159.96, 158.69, 152.01, 133.80, 133.82, 132.81, 130.13, 128.59, 122.37, 115.02, 109.68, 107.44, 74.99, 59.62, 58.07, 55.58, 49.03, 37.52, 29.84, 26.58, 25.88; HRMS: calcd. for $C_{26}H_{30}ClNO_4$: *m*/*z* 455.1863, found: 455.1859.

7.1.1.14. 5-(Cyclohexylmethoxy)-3-(((2-hydroxyethyl)(pyridin-4-ylmethyl)amino)methyl)-4*H*-chromen-4-one (4n). Yield 35%; yellow oil; R_f: 0.29 (MeOH:CH_2Cl_2 = 1:15); IR (KBr) 3408, 2921, 1744, 1657, 1268, 1064 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.07–2.06 (m, 11H), 2.67 (t, *J* = 4.8 Hz, 2H), 3.56 (s, 2H), 3.67 (s, 2H), 3.75 (t, *J* = 5.2 Hz, 2H), 3.93 (d, *J* = 5.6 Hz, 2H), 6.78 (d, *J* = 8.4 Hz, 1H), 6.94 (d, *J* = 8.8 Hz, 1H), 7.25 (d, *J* = 6.4 Hz, 2H), 7.50 (t, *J* = 8.4 Hz, 1H), 7.73 (s, 1H), 8.47 (d, *J* = 6.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 177.49, 159.86, 158.57, 151.89, 149.79, 148.71, 132.82, 123.50, 122.01, 114.87, 109.53, 107.40, 74.87, 59.53, 57.60, 55.76, 49.09, 37.40, 29.68, 26.43, 25.75; HRMS: calcd. for $C_{25}H_{30}N_2O_4$: *m*/*z* 422.2206, found: 422.2200.

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