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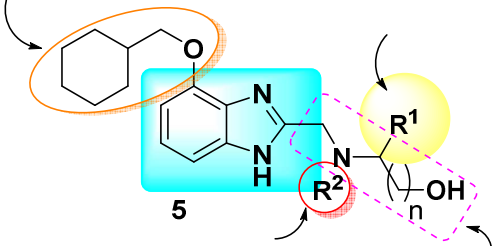
Cyclohexymethoxy: essential group for cell permeability

Hydrophobic unit crucial. (R^1 = ethyl or cyclohexylmethyl, R^2 = H)



5

Hydrophobic substitution on nitrogen did not improve the activity

Hydroxyethylaminomethyl motif n = 1 critical for the activity.

5e; R^1 = -CH_2CH_3, R^2= H, IL-5 inhibition IC_{50} = 3.5 µM

5k; R^1 = -CH_2Cy, R^2= **-H,** IL-5 inhibition IC_{50} = 5.0 µM

Discovery of benzimidazole analogs as a novel interleukin-5 inhibitors

Pulla Reddy Boggu[a,b], Youngsoo Kim[c], Sang-Hun Jung[a]*

[a] College of Pharmacy and Institute of Drug Research and Development, Chungnam National University, Daejeon 34134, Republic of Korea

[b] School of Pharmacy, Sungkyunkwan University, Suwon, Gyeonggi-do 16419, Republic of Korea

[c] College of Pharmacy, Chungbuk National University, Chinju 19421, Republic of Korea

Abstract

A series of novel hydroxyethylaminomethylbenzimidazole analogs **5a-y** were synthesized and evaluated for their IL-5 inhibitory activity using pro-B Y16 cell line. Among them, 2-(((4-(cyclohexylmethoxy)-1*H*-benzo[*d*]imidazol-2-yl)methyl)amino)butan-1-ol (**5e,** 94.3% inhibition at 30 μM, IC_{50} = 3.5 μM, cLogP = 4.132) and 3-cyclohexyl-2-(((4-(cyclohexylmethoxy)-1*H*-benzo[*d*]imidazol-2-yl)methyl)amino) propan-1-ol (**5k,** 94.7% inhibition at 30 μM, IC_{50} = 5.0 μM, cLogP = 6.253) showed the most potent inhibitory activity. The essential feature of SAR (Fig. 5) indicated that the chromenone ring can be replaced by benzimidazole ring to maintain the inhibitory activity. In addition, the hydroxyethylaminomethyl group was suitable for the IL-5 inhibitory activity. Moreover, the hydrophobic substituents on carbon play an important role in the IL-5 inhibitory activity of these analogs. However, *N*-substituted analogs did not improve the inhibitory activity. In addition, MTT assay of **5e** and **5k** with normal B lymphoblasts revealed that they had no significant effects on cell viability.

Key words: Benzimidazole, Interleukin-5, Inhibitor, SAR

*Corresponding author: E-mail: jungshh@cnu.ac.kr

1. Introduction

In the past decades have noticed a worldwide increase in allergic diseases, including asthma, atopic dermatitis, allergic rhinitis, and food allergy [1,2]. It is believed that several environmental factors interacting with genetic factors contribute to sensitization to environmental allergens and to suffering from allergic diseases [3,4]. The pivotal roles of T helper type 2 (Th2) cytokines have been well defined in these diseases and therefore therapeutic strategies that target the Th2 cytokines are of potential benefit in allergic disease [5–7]. In these regards, glucocorticoids with immunosuppressive activities such as dexamethasone have been used as the most fruitful treatment accessible for hypersensitivity diseases including asthma, atopic dermatitis and food allergy [8]. Nevertheless, during long term treatment, systemic side effects are identified [9]. Consequently, this issue resulted in many efforts to discover novel potential therapies, which is safer than corticoids.

In the 1990s, eosinophilic inflammation is well established as fundamental causes of allergic diseases. Excessive release of eosinophils from the bone marrow into circulation by the stimulation of allergens results in rapid accumulation in the tissue, where they produce lipid mediators, enzymes and proteins to cause tissue damage and eventually adverse allergic reactions such as nasal rhinitis, asthma, and atopic dermatitis. Thus selective inhibition of eosinophil is an ideal target for reducing tissue damage as well as allergic diseases without inducing entire immunosuppressive consequences. [10–14].

In these considerations, the IL-5 antagonist with small molecular weight has been investigated. The small isothiazolone molecules by the modification of Cys 66 residue in IL-5 was originally tested as its antagonists [15]. In an effort to screening the natural compounds had resulted in the discovery of sophoricoside (SOP, **1**) [16] isolated from *Sophora japonica*, which showed specific inhibition in the mIL-5-dependent Y16 proliferation over IL-3 and GM-CSF stimulation [17]. However, SOP (**1**) is chemically and metabolically unstable glycoside and therefore the formation of stable analogs as a candidate was inevitable. Thus, the structural necessities of this isoflavone for its inhibitory effect against IL-5 were investigated [18,19]. The structural requirements of these isoflavone analogs comprise a planar chromen-4-one ring, the existence of hydroxyl group at the 4^{th} position of B ring, and introduction of hydrophobic units at 5^{th} position, which might regulate the permeability of these isoflavones. Fortunately, the metabolically unstable glycopyranosyl group of SOP is not needed for the inhibitory activity [18,19]. Our further studies explored the exact role of B ring and the optimum connection between the chromenone core and phenyl ring B. Thus, a series of hydroxyethylaminomethylchromen-4-one (**2** and **3,** Fig. 1) [20–22] were discovered as IL-5 inhibitor.

Recently, our efforts focused to find a novel bioisostere of the chromen-4-one ring of SOP. Thus, a novel 2-benzyl-1-indanone scaffold (**4**, Fig. 1) was tested as IL-5 inhibitor [23]. However, the 1-indanone compounds showed similar activity as compared to chromen-4-one compounds. Therefore, to get more potency than chromenone and 1-indanone analogs we synthesized a novel hybrid scaffold **5** (Fig. 2), which is designed from the chromen-4-one (**2** & **3**) and 1-indanone (**4**) scaffolds, and evaluated the IL-5 inhibitory effect on IL-5 bioassay.

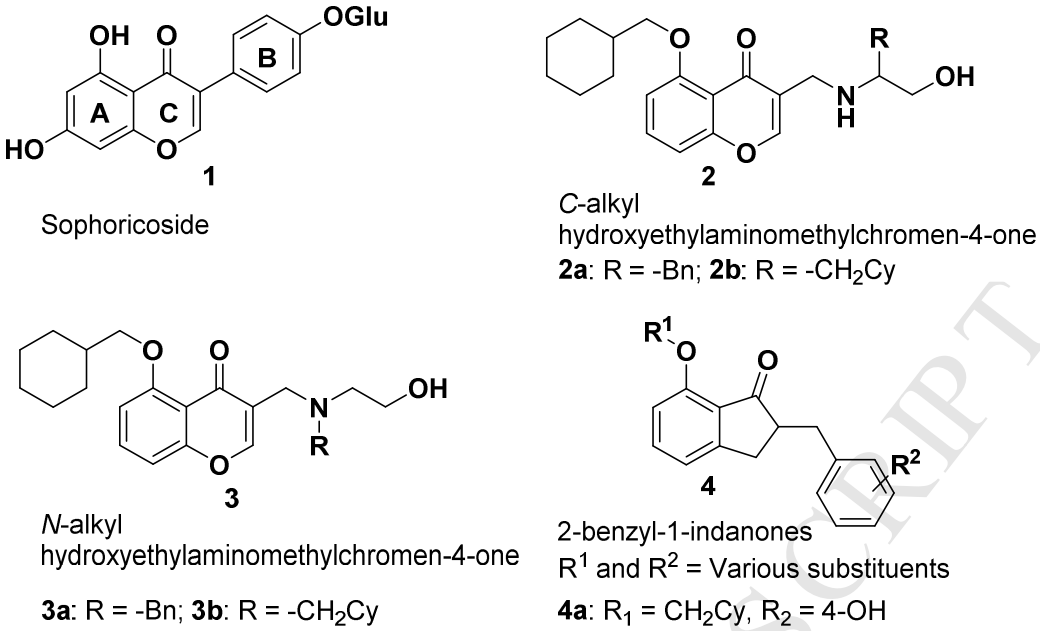


Figure 1. Representative IL-5 inhibitors.

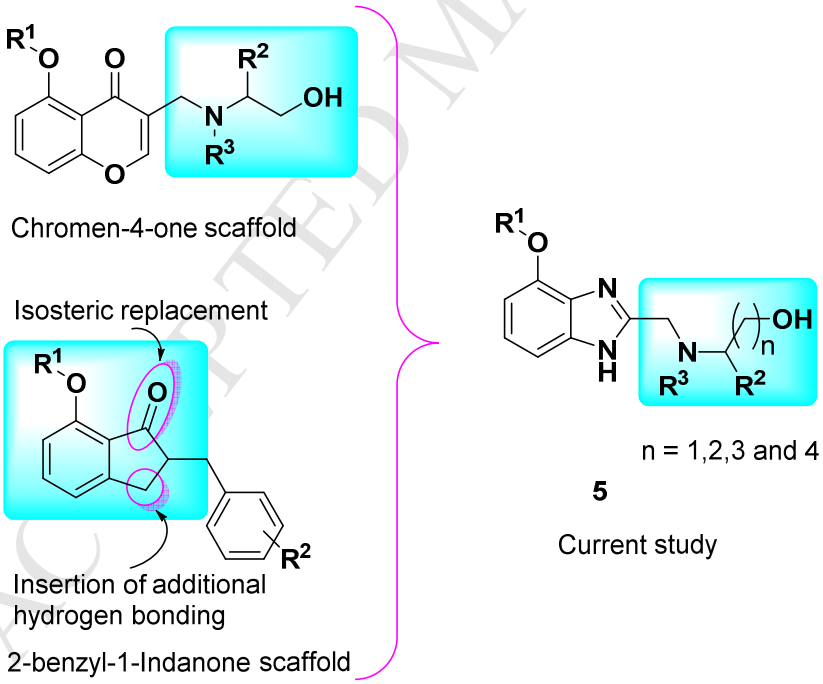


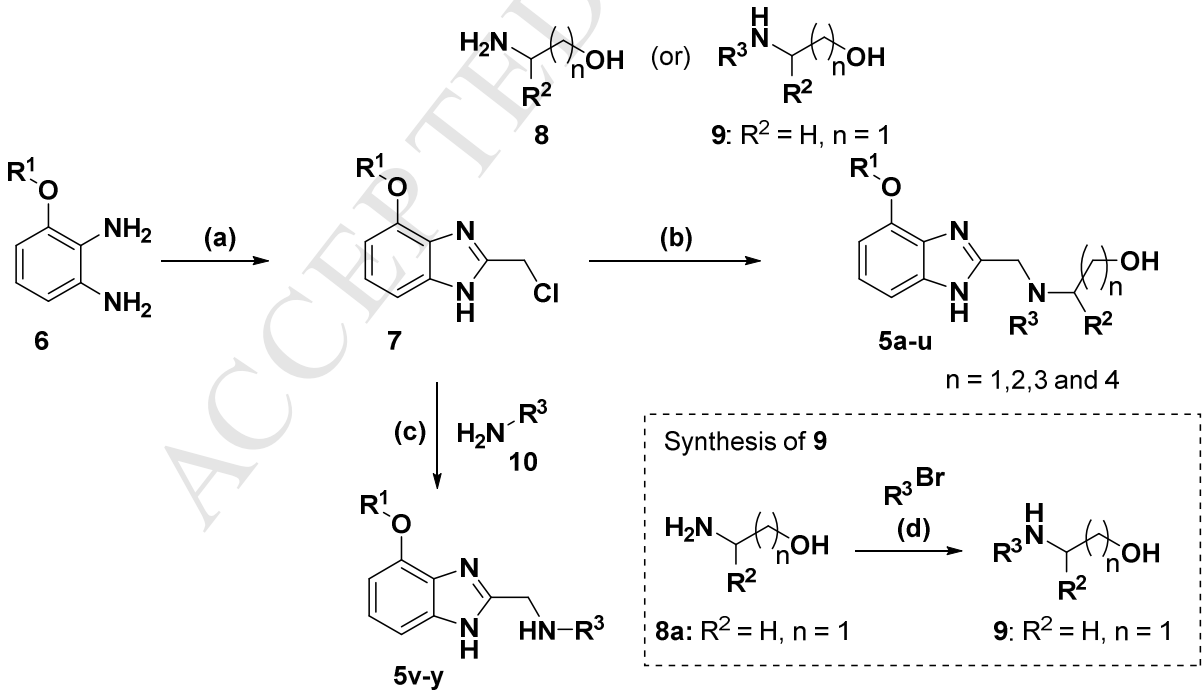
Figure 2. Design of novel benzimidazole analogs as IL-5 inhibitor.

2. Chemistry

The synthetic routes to obtain the target benzimidazole derivatives **5a-y** by cyclization reactions of 1,2-diamines and 2-chloroacetic acid followed by nucleophilic substitution with various substituted amino alcohols are outlined in Scheme 1, and substituents are denoted in Table 1. The synthesis of 1,2-diamine **6** was adopted by previously reported literature [24]. The intermediate **6** was reacted with 2-chloroacetic acid in the presence of aq. 6*N* HCl to afford the corresponding cyclized chloro intermediate **7**. The final compounds **5a-y** were obtained from the nucleophilic substitution reaction of chloro intermediate **7** with the appropriate amino alcohol in the presence of potassium carbonate (K_2CO_3) in *N*,*N*-dimethylformamide (DMF). The intermediate **9** was synthesized from the ethanolamine by reaction with alkyl bromide in the presence of K_2CO_3 in DMF in excellent yields. All the synthesized compounds were mainly characterized by ^{1}H and ^{13}C NMR spectroscopy techniques.

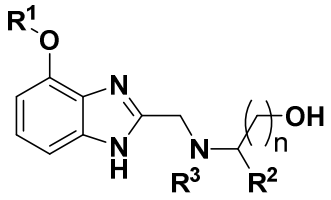
Interestingly, on comparing the ^{1}H NMR spectra of linear chain benzimidazoles **5a-d**, *C*-substituted benzimidazoles **5e**, **5f**, and **5h-k** and *N*-substituted benzimidazoles **5l-u**, we observed *doublet of doublet (dd)* signals of benzylic methylene (–CH_2-NH) protons and *dd* or *multiplet (m)* signals of alcoholic methylene (–CH_2-OH) protons for the *C*-substituted benzimidazoles, however, the linear chain and *N*-substituted benzimidazoles showed *singlet (s)* of benzylic methylene and *triplet (t)* of alcoholic methylene protons at room temperature (see the supplementary material). Distinct features of ^{1}H NMR spectra for the benzylic methylene protons and alcoholic methylene protons of the benzimidazoles of **5a**, **5e**, **5g** and **5m** are illustrated in Fig. 3 and their chemical shift values are listed in Table 2. These data clearly indicate that the chiral center as well as tautomerism of *C*-substituted benzimidazole **5e** is effecting the splitting pattern (geminal and vicinal coupling) of the benzylic methylene and alcoholic methylene protons as shown in Fig. 3a & 3b, which can be detected as separate sets of signals in ^{1}H NMR. However, the linear chain benzimidazole **5a**, *C*-disubstituted

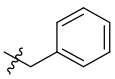
benzimidazole **5g**, and *N*-substituted benzimidazole **5m** did not detect separate sets of signals. Moreover, on comparing the ^{1}H NMR spectra of linear chain benzimidazoles **5a-d**, *C*-substituted benzimidazoles **5e-k** and *N*-substituted benzimidazoles **5l-u**, we observed *doublet (d)* of *o*- proton and *triplet* (t*)* of *m*- proton and *broad singlet (br. s.)* of *p*- proton of the *N*-substituted benzimidazoles, however, the linear chain and *C*-substituted benzimidazoles showed *doublet (d)* of *o*- proton and *multiplet* (*m)* of *m*- and *p*- protons at room temperature (see the supplementary material). As shown in Fig. 3c, the aromatic *p*- proton of the compound **5m** showed broad singlet pattern. This outcome clearly indicates that the tautomerism of *N*-substituted benzimidazole **5m** is causing the splitting pattern of the aromatic *p*- proton. Additionally, we also observed that some of the quaternary carbon signals in ^{13}C NMR spectra of benzimidazoles are not observable possibly due to tautomerism [24] (see the supplementary material).

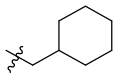
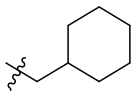
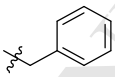
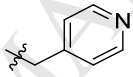
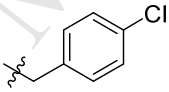
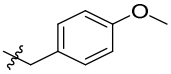
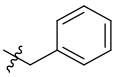
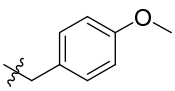
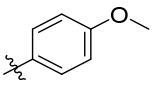


Scheme 1. Synthesis of **5a-y** (substituents are denoted in Table 1). Reagents and Conditions: (a) Chloroacetic acid, 6*N* HCl, reflux, 16 h, 72%; (b) Amine **8** (or) **9**, K_2CO_3, DMF, 60 °C, 3 h, 52 - 76%; (c) Amine **10**, K_2CO_3, DMF, 60 °C, 3 h, 71 - 78%; (d) R^3-Br, K_2CO_3, DMF, 60 °C, 3 h, 81 - 89%.

Table 1. Substituents, cLog P values and IL-5 activity results of synthesized hydroxyethylaminomethylbenzimidazoles **5a-y**



Comp. No.	Substituents				[a] CLogP	IL-5	
	R^1	R^2	R^3	n		% Inhibition at 30 µM [b]	[b] IC_{50} (µM)
5a	CH_2Cy [c]	H	H	1	3.294	63.3	20.0
5b	CH_2Cy	H	H	2	3.624	52.7	28.5
5c	CH_2Cy	H	H	3	3.487	44.3	30.0
5d	CH_2Cy	H	H	4	4.016	23.7	>30.0
5e	CH_2Cy		H	1	4.133	94.3	3.5
5f	CH_2Cy		H	1	4.662	73.3	7.5
5g	CH_2Cy	$(CH_3)_2$	H	1	4.003	54.7	27.5
5h	CH_2Cy		H	1	4.339	77.7	20.0
5i	CH_2Cy		H	2	4.644	64.7	22.0
5j	CH_2Cy		H	1	5.172	81.0	12.0

5k	CH_2Cy		H	1	6.254	94.7	5.0
5l	CH_2Cy	H		1	4.894	63.0	20.0
5m	CH_2Cy	H		1	4.365	34.7	>30.0
5n	CH_2Cy	H	CH_3	1	3.836	42.0	>30.0
5o	CH_2Cy	H		1	4.674	10.0	>30.0
5p	CH_2Cy	H		1	5.867	24.0	>30.0
5q	CH_2Cy	H		1	6.486	76.7	21.0
5r	CH_2Cy	H		1	5.552	62.3	25.0
5s	CH_2Cy	H		1	4.055	43.3	>30.0
5t	CH_2Cy	H		1	6.265	96.0	13.0
5u	CH_2Cy	H		1	5.471	73.3	19.0
5v	CH_2Cy	-		-	5.045	49.3	>30.0
5w	CH_2Cy	-		-	4.965	43.3	>30.0
5x	CH_2Cy	-		-	5.466	25.0	>30.0
5y	CH_2Cy	-		-	5.568	20.3	>30.0
2a[20]					4.791	72.0	25.7
2b[20]					5.873	93.0	17.3
3a[21]					5.172	94.8	16.1

3b[21]	6.106	94.7	12.4
4a [23]	5.856	100.0	4.0
Sophoricoside (**1**)		79.1[e]	10.6
Budesonide		70.2[e]	26.2

[a] ClogP calculated by Chemdraw® (ver. 15.1); [b] % of inhibition and IC_{50} values are taken as a mean from three independent experiments; [c] Cy = Cyclohexyl.

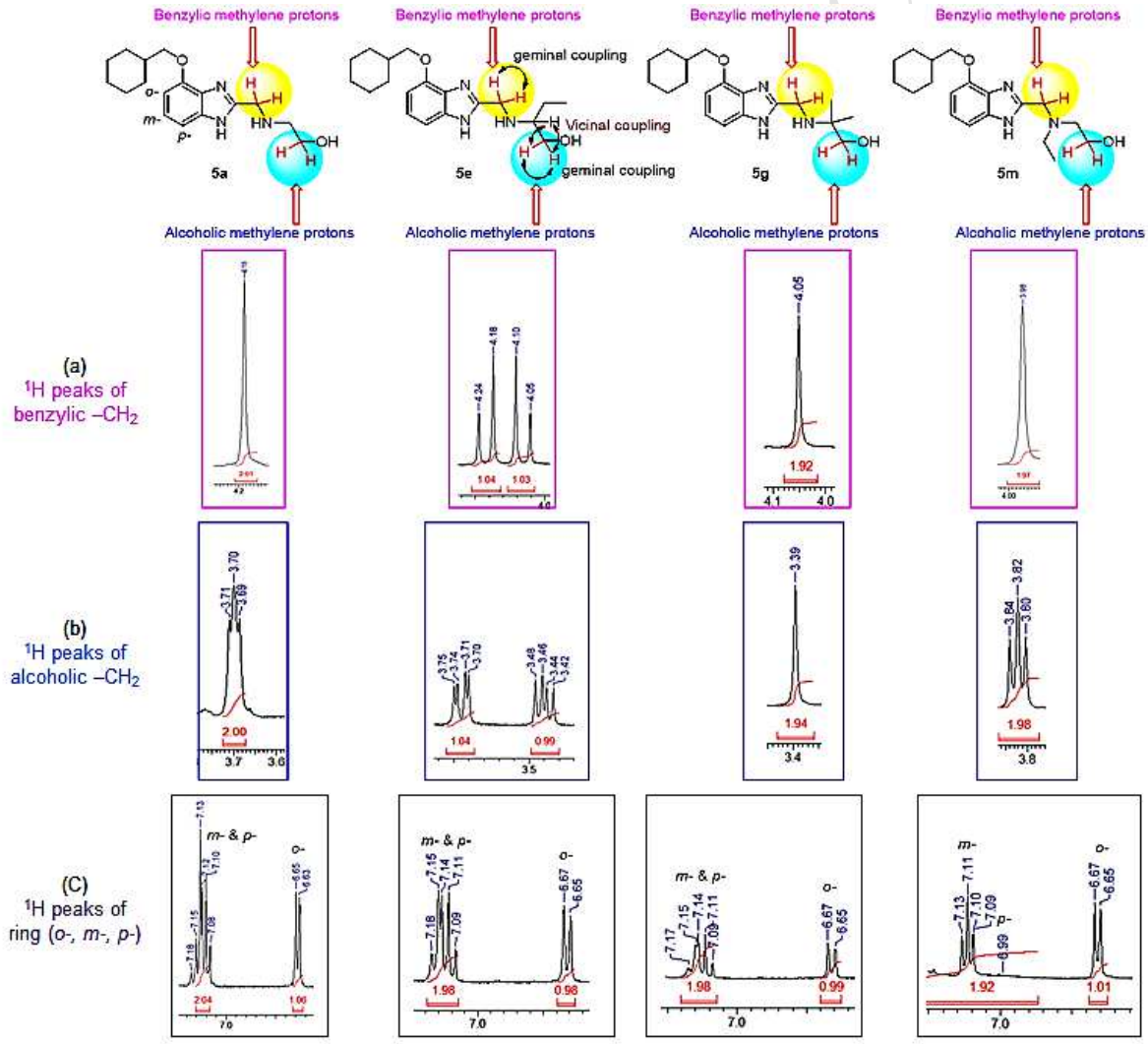


Figure 3. Comparison among ^{1}H NMR data of compound **5a** (linear chain benzimidazole), **5e** (*C*-substituted benzimidazole), and **5g** (*C*-disubstituted benzimidazole), **5m** (*N*-substituted

benzimidazole). (a) Proton peaks of benzylic methylene group, (b) Proton peaks of alcoholic methylene group, (c) Proton peaks of aromatic ring (*o-*, *m-, and p-*).

Table 2. ^{1}H NMR chemical shifts (δ) of the benzylic methylene, alcoholic methylene, and aromatic ring protons of **5a**, **5e**, **5g**, and **5m**

Chemical shift		**5a**	**5e**	**5g**	**5m**
Benzylic –CH_2		4.18 (s, 2H)	4.21 (d, *J* = 15.65 Hz, 1H) 4.08 (d, *J* = 15.55 Hz, 1H)	4.05 (s, 2H)	3.98 (s)
Alcoholic –CH_2		3.70 (t, *J* = 4.0 Hz, 2H)	3.72 (dd, *J* = 3.59, 11.13 Hz, 1H) 3.45 (dd, *J* = 6.80, 11.18 Hz, 1H)	3.39 (s, 2H)	3.73 (t, *J* = 5.12 Hz, 2H)
Aromatic ring	*m-* & *p-*	7.07 - 7.17 (m, 2H),	7.07 - 7.19 (m, 2H),	7.07 - 7.20 (m, 2H),	7.11 (t, *J* = 8.0 Hz, 1H, *m-*), 6.87-7.26 (br.s., 1H, *p-*)
	o-	6.64 (d, *J* = 7.56 Hz, 1H)	6.66 (d, *J* = 7.54 Hz, 1H)	6.66 (d, *J* = 8.38 Hz, 1H),	6.66 (d, *J* = 7.80 Hz, 1H)

3. Pharmacology

Inhibitory activity of the benzimidazole derivatives **5** against IL-5 was evaluated using the IL-5 dependent pro-B Y16 cell line according to the previously described procedure [16]. The Y16 cells were incubated with 3 units/mL mIL-5 for 48 h in the presence or absence of the compound, and then cell metabolism was calculated as an index of proliferation, using WST-1. The outcomes of biological screening of benzimidazoles against IL-5 are noted in Table 1 as % inhibition at 30 µM and also IC_{50} values. To exclude any cytotoxic effects, the most potent compounds **5e** and **5k** were subjected to MTT assay with normal B lymphoblasts such as HCC1395 BL and NCI-BL 1184 that were incubated in the presence of serum but not IL-5 (Table 3). The detailed assay protocols are designated in the experimental section.

Table 3. MTT assay in normal B lymphoblasts

Comp. No.	% Inhibition at 30 μM	
	HCC1395 BL	NCI-BL1184
5e	7.5 ± 4.6	10.2 ± 8.8
5k	12.5 ± 9.3	9.8 ± 5.1

Each value represents the mean ± SD of the three independent measurements.

4. Conformational analysis and alignment studies of 5k and 5q

Molecular models of the benzimidazole analogs **5k** and **5q** were constructed using SYBYL®-x2.0 program package (Tripos Associates Inc.)[25] and their geometry were optimized (Powell conjugate gradient minimization, termination at a gradient of 0.0005 kcal/mol)[26] 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 4-cyclohexymethoxybenzimidazole structure as a template as represented. The 3D structures of the analyzed compounds were assumed to be a bioactive conformation and were aligned according to a 4-cyclohexylmethoxybenzimidazole template as shown in **Fig. 4**. The selected dihedral angles and atomic distances are listed in **Table 4** and **5** respectively.

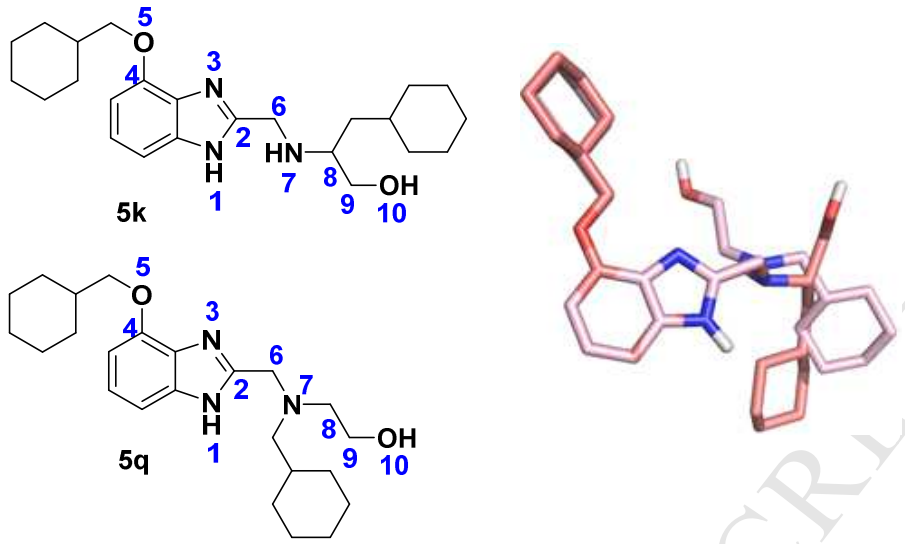


Figure 4. Alignment of **5k** (Salmon color) and **5q** (Light pink color)

Table 4. Torsion angle (°) and total energy (kcal/mol) of **5k** and **5q**

Compound **5k** [a] (27.138 kcal/mol)		Compound **5q** [a] (25.967 kcal/mol)	
∠N_3-C_2-C_6-N_7	252.9	∠N_3-C_2-C_6-N_7	179.6
∠C_2-C_6-N_7-C_8	199.5	∠C_2-C_6-N_7-C_8	359.8
∠C_6-N_7-C_8-C_9	295.3	∠C_6-N_7-C_8-C_9	66.3
∠N_7-C_8-C_9-O_{10}	66.2	∠N_7-C_8-C_9-O_{10}	173.9

[a] Numbers on the atoms of **5k** and **5q** are presented in **Fig. 4**.

Table 5. Distance (Å) in **5k** and **5q**

Compound **5k** [a]		Compound **5q** [a]	
O_5-O_{10}	7.976	O_5-O_{10}	5.362
N_3-O_{10}	5.133	N_3-O_{10}	4.225

[a] Numbers on the atoms of **5k** and **5q** are presented in **Fig. 4**.

4. Results and discussion

We designed and synthesized a novel series of benzimidazole derivatives **5a-y** (Table 1) with aminoalcohol group to evaluate which moiety plays an important role in the IL-5 inhibitory activity. Initially, analog **5a** (63.3% inhibition at 30 µM, IC_{50} = 20.0 µM, cLogP = 3.294) showed similar inhibitory activity as compared to budesonide (IC_{50} = 26.2 µM) and chromenone **2a** (IC_{50} = 25.7 µM). This result suggested that the chromenone ring can be replaced by benzimidazole ring.

The above result encouraged us to find a potent bezimidazole analog as IL-5 inhibitor. Thus to find out the optimum length of aminoalcohol moiety between amino (NH) and hydroxyl (OH) function in **5a,** methylene linkage was increased. As a result, decrement in the inhibitory activity was observed in the case of three and four methylene unit analogs **5b** (n = 2, 52.7% inhibition at 30 µM, IC_{50} = 28.5 µM, cLogP = 3.624) and **5c** (n = 3, 44.3% inhibition at 30 µM, IC_{50} = 30.0 µM, cLogP = 3.487), and complete loss of activity was observed in five methylene unit analog **5d** (n =4, 23.7% inhibition at 30 µM, IC_{50} = >30.0 µM, cLogP = 4.016). This result supports that chain length of amino alcohol plays an important role in the inhibitory activity of benzimidazole analogs and therefore hydroxyehylamino group is optimum.

Interestingly, branched aminoethanol analogs as shown in **5e** (n = 1, R^1 = ethyl, R^2 = H, 94.3% inhibition at 30 µM, IC_{50} = 3.5 µM, cLogP = 4.132) and **5f** (n = 1, R^1 = propyl, R^2 = H, 73.3% inhibition at 30 µM, IC_{50} = 7.5 µM, cLogP = 4.661) showed 5.7-fold and 2.7-fold more active than unbranched analog **5a**, respectively. However, dimethyl branched analog **5g** (54.7% inhibition at 30 µM, IC_{50} = 27.5 µM, cLogP = 4.002) did not improve the inhibitory activity. Thus, only straight chain hydrophobic group as a branch in amino ethanol should be more favourable for the inhibitory activity.

In the next set of experiments, phenyl substituted aminoethanol analog **5h** (77.7% inhibition at 30 µM, IC_{50} = 20.0 µM, cLogP = 4.339) was prepared and exhibited moderate inhibitory

activity as compared to **5a** (IC_{50} = 27.0 µM). This result suggested that the planar group as a branch is still applicable to maintain the IL-5 inhibitory activity. In addition, we increased methylene unit between amino and hydroxyl function of **5h**. As a result, decrement in the activity was observed in **5i** (64.7% inhibition at 30 µM, IC_{50} = 22.0 µM, cLogP = 4.64367). This result again confirmed that aminoethanol group is optimum for the activity. However, insertion of methylene unit (one methylene unit) between amino alcohol chain and the phenyl group in **5h** as shown in **5j** (81.0% inhibition at 30 µM, IC_{50} = 12.0 µM, cLogP = 5.171) enhanced the inhibitory activity. Interestingly, replacement of phenyl group of **5j** with cyclohexyl moiety as shown in **5k** (94.7% inhibition at 30 µM, IC_{50} = 5.0 µM, cLogP = 6.253) led to slight enhancement of inhibitory activity. This implied that the influence of bulkiness of the hydrophobic group in this region should be marginal.

For determining more detail SAR of **5a**, hydrophobic substituents at the amino group were introduced. Accordingly, we prepared n-propyl analog **5l** (63.0% inhibition at 30 µM, IC_{50} = 20.0 µM, cLogP = 4.894), ethyl analog **5m** (34.7% inhibition at 30 µM, IC_{50} = >30.0 µM, cLogP = 4.365)**,** and methyl analog **5n** (42.0% inhibition at 30 µM, IC_{50} = >30 µM, cLogP = 3.836), which did not turn up the IL-5 inhibitory activity. In another set of experiments, the bulky hydrophobic groups on the nitrogen of **5a** were introduced. The resulted isopropyl **5o** (10.0% inhibition at 30 µM, IC_{50} = >30.0 µM, cLogP = 4.674) and cyclohexyl **5p** (24.0% inhibition at 30 µM, IC_{50} = >30.0 µM, cLogP = 5.867) analogs showed almost diminished the inhibitory activity. Insertion of a methylene unit between amino nitrogen and bulky cyclohexyl group as in analog **5q** (76.7% inhibition at 30 µM, IC_{50} = 21.0 µM, cLogP = 6.486) showed better inhibitory activity than **5p** and **5a**. Therefore, directly attached bulky hydrophobic groups on amino nitrogen are not suitable for the IL-5 inhibitory activity.

Replacement of cyclohexyl group in **5q** with planar phenyl ring as shown in benzyl analog **5r** (62.3% inhibition at 30 µM, IC_{50} = 25.0 µM, cLogP = 5.552) showed similar activity as

compared to **5q**. Installing basic function with pyridine-4-ylmethyl as shown in **5s** (43.3% inhibition at 30 µM, IC_{50} = >30.0 µM, cLogP = 4.055) gave the adverse effect on the activity. To further investigate the effect of hydrophobic substituents at position 4 of the phenyl ring of **5r**, analogs **5t** and **5u** were prepared. Slight increment in the inhibitory activity was observed in 4-chlorobenzyl **5t** (96.0% inhibition at 30 µM, IC_{50} = 13.0 µM, cLogP = 6.265) and 4-methoxybenzyl **5u** (73.3% inhibition at 30 µM, IC_{50} = 19.0 µM, cLogP = 5.471). These efforts marginally variated the activity.

In another set of experiment, we removed hydroxyethyl group of **5r** and **5u**. The resulted compounds **5v** (49.3% inhibition at 30 µM, IC_{50} = >30.0 µM, cLogP = 5.045) and **5w** (43.3% inhibition at 30 µM, IC_{50} = >30.0 µM, cLogP = 4.964) much decreased the activity. Further, we reduced the methylene unit between amino and phenyl ring of **5v** and **5w** as shown in analog **5x** (25.0% inhibition at 30 µM, IC_{50} = >30 µM, cLogP = 5.467) and **5y** (20.3% inhibition at 30 µM, IC_{50} = >30.0 µM, cLogP = 5.568) which did not improve the IL-5 inhibitory activity. These results imply that the hydroxyethyl group is essential for the activity.

Lipophilicity of analogs **5a-y** as indicated with cLogP does not correlate with the activity. However, increasing the hydrophobic binding ability of substituent on terminal carbon 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 active *C*-substituted analog **5k** and less active *N*-substituted analog **5q** (Fig. 4 and Table 4, 5) and observed a dramatic difference in the region of the side chain at position 2 of benzimidazoles. As shown in Table 4, the dihedral angle ($\angle C_2$-C_6-N_7-C_8) of analog **5k** is 199.5°, which indicates that the hydroxyethylaminomethyl group at position 2 of **5k** is located out of the plane of the benzimidazole ring. In addition, the longer distance (O_5-O_{10} = 7.976 Å, Table 5) from the oxygen (O_5) of the benzimidazole ring to the oxygen (O_{10})

of hydroxyethylaminomethyl group of **5k** also supports this stretched conformation. The corresponding dihedral angle ($\angle C_2$-C_6-N_7-C_8, Table 4) of **5q** is 359.8°, which depicts that the hydroxyethanolaminemethyl chain oxygen (O_{10}) is close to cyclohexylmethoxy oxygen (O_5) of benzimidazole ring. Therefore, the shorter distance (O_5-O_{10} = 5.362 Å, Table 5) from the oxygen (O_5) of benzimidazole ring to the oxygen (O_{10}) of hydroxyethylaminomethyl group of **5q** compared to that of **5k** clearly indicates the folded conformation. Therefore, these studies indicate that the *C*-substituted analog **5k** could be much closer to the effective conformation for binding to the putative receptor.

For confirmation of IL-5 activity related to the cytotoxicity, an MTT assay of the active compounds was carried out with normal B lymphoblasts. As shown in Table 3, none of the compounds **5e** and **5k** showed significant effects on cell viability. These results implied that the IL-5 inhibitory activity of **5e** and **5k** could be originated from the inhibition of proliferation of Y-16 cells by blocking the stimulatory activity of IL-5 rather than cytotoxic effect. Thus this could be effective and safe as drug candidate for the treatment of eosinophilia and other allergic disorders.

5. Conclusion

A series of novel hydroxyethylaminomethylbenzimidazole analogs **5a-y** were synthesized and evaluated for their IL-5 inhibitory activity. Among them, *C*-substituted benzimidazole analogs **5e** (IC_{50} = 3.5 μM) and **5k** (IC_{50} = 5.0 μM) showed the most potent inhibitory activity than the previously reported chromenone analog **2b** (IC_{50} = 17.3 μM) and Budesonide (IC_{50} = 26.2 μM). The essential feature of SAR (Fig. 5) indicated that the chromenone ring can be replaced by benzimidazole ring to maintain the inhibitory activity. In addition, the hydroxyethylaminomethyl group was suitable for the IL-5 inhibitory activity. Moreover, the hydrophobic substituents on carbon play an important role in the IL-5

inhibitory activity of these analogs. However, *N*-substituted analogs did not improve the inhibitory activity. In addition, MTT assay of **5e** and **5k** with normal B lymphoblasts revealed that they had no significant effects on cell viability. Taken together, current study identifies the benzimidazole molecules as a novel scaffold for inhibiting the IL-5 receptor and makes them potential therapeutic agents against eosinophilia and other allergic disorders.

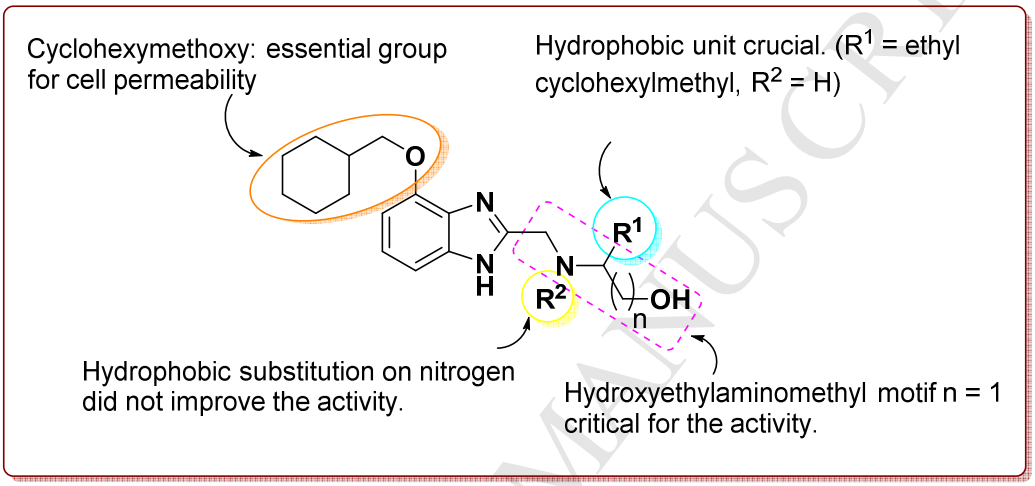


Figure 5. SAR analysis of the novel benzimidazole analogs **5**

6. Materials and methods

6.1. Chemistry

All commercial chemicals were used as obtained and all solvents were purified by distillation prior to use applying the standard procedures [27]. Thin layer chromatography (TLC) was performed on E Merck silica gel GF-254 precoated plates, visualization was performed under UV illumination (λ = 254 nm), and colorization with $KMnO_4$ and I_2. All derivatives were purified by flash column chromatography which was done on E Merck silica gel (230–400 mesh). ^1H and ^{13}C NMR spectra was measured against the peak of tetramethylsilane (TMS) using a JEOL, JNM-AL400 NMR (400 MHz) and Bruker Fourier 300 NMR (300 MHz)

spectrometer. Melting points (mp) of the synthesized compounds were determined on an Electro thermal 1A 9100 MK2 apparatus and are uncorrected. Infrared (IR) spectra was noted on a Nicolet 380 model FTIR. HRMS was measured in ESI ionization by using AB Sciex Triple TOF 5600 LCMS instrument.

*6.1.1. General procedure for the synthesis of compounds **5a-y***

To a solution of 2-(chloromethyl)-4-(cyclohexylmethoxy)-1*H*-benzo[*d*]imidazole **7** (0.72 mmol) in DMF (5 mL), K_2CO_3 (0.79 mmol) and appropriate amine **8** or **9** or **10** (0.79 mmol) were added. The resulting solution was stirred at 60 °C for 3 h. The mixture was cooled, diluted with water, and then extracted with EtOAc. The combined organic extracts were washed with water, brine solution, dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure. The crude mixture was subjected to flash silica gel (230-400 mesh) column chromatography (eluting with 0 - 2% MeOH in dichloromethane) to afford the title compounds **5**.

*6.1.1.1. 2-(((4-(Cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methyl)amino)ethan-1-ol (**5a**).* Yield 52%; Off white solid; mp 116 – 118 °C; IR (neat) 3400 - 2600 (br., peak), 2920, 2850, 1597, 1537, 1445, 1425, 1244, 1189, 1094, 1079, 984, 881, 781, 728 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.07 - 7.17 (m, 2H), 6.64 (d, J = 7.56 Hz, 1H), 4.18 (s, 2H), 3.89 (d, J = 6.34 Hz, 2H), 3.70 (t, J = 4.0 Hz, 2H), 2.84 - 2.91 (t, J = 4.0 Hz, 2H), 1.62 - 1.88 (m, 6H), 0.96 - 1.28 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.6, 148.2, 140.2, 128.6, 122.8, 107.7, 103.7, 73.8, 60.6, 51.2, 46.9, 37.5, 29.8, 26.3, 25.6; HRMS (ESI) calculated for $C_{17}H_{25}N_3O_2$ $[M+H]^+$ 304.2025, found 304.2046.

*6.1.1.2. 3-(((4-(Cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methyl)amino)propan-1-ol (**5b**).* Yield 56%; Colorless sticky oil; IR (neat) 3500 - 2500 (br., peak), 2921, 2849, 1595, 1541, 1443, 1325, 1255, 1240, 1102, 1065, 995, 781, 725 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$)

δ 7.08 - 7.20 (m, 2H), 6.62 - 6.70 (m, 1H), 4.12 (s, 2H), 3.92 (d, *J* = 6.15 Hz, 2H), 3.82 (t, *J* = 5.54 Hz, 2H), 2.92 (t, *J* = 6.01 Hz, 2H), 1.61 - 1.92 (m, 8H), 1.00 - 1.30 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.9, 148.3, 139.9, 128.8, 122.8, 107.5, 103.6, 73.7, 61.6, 47.7, 46.9, 37.4, 31.0, 29.7, 26.3, 25.5; HRMS (ESI) calculated for $C_{18}H_{27}N_3O_2$ $[M+H]^+$ 318.2181, found 318.2202.

6.1.1.3. 4-(((4-(Cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methyl)amino)butan-1-ol ***(5c)***. Yield 63%; Light yellow sticky oil to solid; mp 55 – 57 °C; IR (neat) 3500 - 2500 (br., peak), 2922, 2849, 1623, 1594, 1541, 1443, 1325, 1256, 1241, 1102, 1065, 781, 725 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.09 - 7.20 (m, 2H), 6.66 (dd, *J* = 1.12, 7.45 Hz, 1H), 4.15 (s, 2H), 3.92 (d, *J* = 6.15 Hz, 2H), 3.63 (t, *J* = 4.84 Hz, 2H), 2.74 (t, *J* = 5.45 Hz, 2H), 1.63 - 1.95 (m, 10H), 1.02 - 1.30 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.2, 148.3, 140.0, 128.8, 122.9, 107.6, 103.6, 73.7, 62.0, 48.9, 46.7, 37.4, 31.1, 29.7, 27.0, 26.3, 25.6; HRMS (ESI) calculated for $C_{19}H_{29}N_3O_2$ $[M+H]^+$ 332.2338, found 332.2361.

6.1.1.4. 5-(((4-(Cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methyl)amino)pentan-1-ol ***(5d)***. Yield 63%; Colorless sticky oil; IR (neat) 3500 - 2500 (br., peak), 2920, 2851, 1625, 1593, 1446, 1330, 1243, 1102, 1045, 1006, 780, 721 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.08 - 7.22 (m, 2H), 6.67 (d, *J* = 7.64 Hz, 1H), 4.11 (s, 2H), 3.93 (d, *J* = 6.15 Hz, 2H), 3.65 (t, *J* = 6.19 Hz, 2H), 2.69 (t, *J* = 6.85 Hz, 2H), 1.82 - 1.98 (m, 3H), 1.64 - 1.80 (m, 3H), 1.36 - 1.62 (m, 6H), 0.97 - 1.35 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.1, 148.1, 140.3, 128.5, 122.8, 107.9, 103.7, 73.8, 61.9, 49.1, 47.2, 37.5, 32.0, 29.8, 28.9, 26.3, 25.6, 23.1; HRMS (ESI) calculated for $C_{20}H_{31}N_3O_2$ $[M+H]^+$ 346.2494, found 346.2518.

6.1.1.5. 2-(((4-(Cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methyl)amino)butan-1-ol ***(5e)***. Yield 61%; White solid; mp 84 – 86 °C; IR (neat) 3500 - 2500 (br., peak), 2920, 2851, 1626, 1594, 1531, 1447, 1331, 1246, 1102, 1046, 1007, 781, 727 cm^{-1}; ^{1}H NMR (300 MHz,

$CDCl_3$) δ 7.07 - 7.19 (m, 2H), 6.66 (d, *J* = 7.54 Hz, 1H), 4.21 (d, *J* = 15.65 Hz, 1H), 4.08 (d, *J* = 15.55 Hz, 1H), 3.91 (d, *J* = 6.15 Hz, 2H), 3.72 (dd, *J* = 3.59, 11.13 Hz, 1H), 3.45 (dd, *J* = 6.80, 11.18 Hz, 1H), 2.60 - 2.70 (m, 1H), 1.62 - 1.94 (m, 6H), 1.38 - 1.61 (m, 2H), 1.00 - 1.36 (m, 5H), 0.93 (t, *J* = 7.54 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.2, 148.2, 140.0, 128.6, 122.7, 107.5, 103.6, 73.8, 62.8, 60.6, 44.7, 37.4, 29.8, 26.3, 25.6, 24.1, 10.3; HRMS (ESI) calculated for $C_{19}H_{29}N_3O_2$ $[M+H]^+$ 332.2338, found 332.2359.

*6.1.1.6. 2-(((4-(Cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methyl)amino)pentan-1-ol (**5f**)*. Yield 58%; Colorless oil; IR (neat) 3500 - 2500 (br., peak), 2921, 2850, 1625, 1593, 1530, 1446, 1245, 1102, 1046, 1007, 781, 727 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.08 - 7.20 (m, 2H), 6.66 (d, *J* = 7.92 Hz, 1H), 4.21 (d, *J* = 15.65 Hz, 1H), 4.08 (d, *J* = 15.55 Hz, 1H), 3.92 (d, *J* = 6.05 Hz, 2H), 3.72 (dd, *J* = 3.40, 11.13 Hz, 1H), 3.43 (dd, *J* = 6.61, 11.18 Hz, 1H), 2.67 - 2.78 (m, 1H), 1.81 - 1.96 (m, 3H), 1.63 - 1.80 (m, 3H), 1.02 - 1.50 (m, 9H), 0.86 - 0.97 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.2, 148.2, 140.2, 128.4, 122.7, 107.7, 103.7, 73.8, 63.2, 59.0, 44.7, 37.5, 33.7, 29.8, 26.3, 25.6, 19.2, 14.1; HRMS (ESI) calculated for $C_{20}H_{31}N_3O_2$ $[M+H]^+$ 346.2494, found 346.2515.

*6.1.1.7. 2-(((4-(Cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methyl)amino)-2-methylpropan-1-ol (**5g**)*. Yield 65%; White solid; mp 121 – 123 °C; IR (neat) 3500 - 2500 (br., peak), 2915, 2848, 1600, 1548, 1450, 1425, 1330, 1276, 1248, 1102, 1055, 995, 852, 781, 727 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.07 - 7.20 (m, 2H), 6.66 (d, *J* = 8.38 Hz, 1H), 4.05 (s, 2H), 3.91 (d, *J* = 6.24 Hz, 2H), 3.39 (s, 2H), 1.62 - 1.93 (m, 6H), 0.95 - 1.34 (m, 11H); ^{1}H NMR (400 MHz, CD_3OD) δ 7.07 - 7.12 (m, 2H), 6.66 - 6.72 (m, 1H), 3.97 (s, 2H), 3.92 (d, *J* = 6.34 Hz, 2H), 3.41 (s, 2H), 1.84 - 2.00 (m, 3H), 1.69 - 1.82 (m, 3H), 1.24 - 1.37 (m, 3H), 1.09 - 1.19 (m, 9H); ^{13}C NMR (100 MHz, CD_3OD) δ 154.9, 149.7, 124.3, 108.6,

105.1, 75.1, 69.4, 55.6, 41.0, 39.3, 31.1, 27.8, 27.1, 23.7; HRMS (ESI) calculated for $C_{19}H_{29}N_3O_2$ $[M+H]^+$ 332.2338, found 332.2362.

6.1.1.8. *2-(((4-(Cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methyl)amino)-2-phenylethan-1-ol (**5h**).* Yield 75%; White solid; mp 85 – 87 °C; IR (neat) 3500 - 2500 (br., peak), 2919, 2850, 1596, 1538, 1444, 1246, 1097, 1061, 1021, 729, 699 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.28 - 7.37 (m, 5H), 7.08 - 7.23 (m, 2H), 6.67 (d, *J* = 8.38 Hz, 1H), 3.97 - 4.09 (m, 2H), 3.92 (d, *J* = 5.96 Hz, 2H), 3.78 - 3.90 (m, 2H), 3.64 - 3.73 (m, 1H), 1.63 - 1.96 (m, 6H), 1.00 - 1.31 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.7, 139.9, 128.7, 127.8, 127.3, 122.8, 103.8, 73.8, 66.7, 64.5, 45.1, 37.5, 29.8, 26.4, 25.6; HRMS (ESI) calculated for $C_{23}H_{29}N_3O_2$ $[M+H]^+$ 380.2338, found 380.2359.

6.1.1.9. *3-(((4-(Cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methyl)amino)-3-phenylpropan-1-ol (**5i**).* Yield 72%; Off white solid; mp 77 – 79 °C; IR (neat) 3500 - 2500 (br., peak), 2920, 2850, 1596, 1538, 1504, 1445, 1247, 1097, 1061, 1020, 992, 781, 729, 699 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.27 - 7.36 (m, 4H), 7.22 - 7.26 (m, 1H), 7.09 - 7.21 (m, 2H), 6.67 (d, *J* = 8.38 Hz, 1H), 3.77 - 4.05 (m, 7H), 1.67 - 2.06 (m, 8H), 0.96 - 1.35 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.7, 142.9, 128.6, 127.3, 126.8, 122.7, 103.7, 73.7, 61.5, 60.9, 44.7, 39.2, 37.4, 29.7, 26.3, 25.6; HRMS (ESI) calculated for $C_{24}H_{31}N_3O_2$ $[M+H]^+$ 394.2494, found 394.2515.

6.1.1.10. *2-(((4-(Cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methyl)amino)-3-phenylpropan-1-ol (**5j**).* Yield 71%; Colorless sticky oil; IR (neat) 3500 - 2500 (br., peak), 2920, 2850, 1594, 1536, 1503, 1446, 1246, 1096, 1062, 1021, 781, 729, 698 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.27 - 7.35 (m, 2H), 7.01 - 7.25 (m, 5H), 6.65 (d, *J* = 8.75 Hz, 1H), 4.16 (d, *J* = 16.11 Hz, 1H), 4.02 (d, *J* = 16.11 Hz, 1H), 3.91 (d, *J* = 6.33 Hz, 2H), 3.72 (dd, *J* = 3.54, 11.08 Hz, 1H), 3.49 (dd, *J* = 5.59, 10.99 Hz, 1H), 2.94 (td, *J* = 5.62, 10.83 Hz, 1H),

2.78 (d, *J* = 6.98 Hz, 2H), 1.69 - 1.94 (m, 6H), 0.90 - 1.30 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.2, 138.8, 129.4, 128.6, 126.5, 122.8, 103.7, 73.8, 62.9, 60.7, 44.9, 38.3, 37.5, 29.9, 26.4, 25.6; HRMS (ESI) calculated for $C_{24}H_{31}N_3O_2$ $[M+H]^+$ 394.2494, found 394.2515.

*6.1.1.11. 3-Cyclohexyl-2-(((4-(cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methyl)amino)propan-1-ol (**5k**).* Yield 63%; Colorless sticky oil; IR (neat) 3500 - 2500 (br., peak), 2920, 2848, 1623, 1590, 1535, 1444, 1243, 1101, 1046, 1007, 781, 727 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.07 - 7.21 (m, 2H), 6.66 (d, *J* = 8.48 Hz, 1H), 4.20 (d, *J* = 15.65 Hz, 1H), 4.07 (d, *J* = 15.65 Hz, 1H), 3.93 (d, *J* = 6.15 Hz, 2H), 3.71 (dd, *J* = 3.49, 11.04 Hz, 1H), 3.40 (dd, *J* = 6.47, 11.13 Hz, 1H), 2.82 (dq, *J* = 3.73, 6.40 Hz, 1H), 1.59 - 1.95 (m, 11H), 0.86 - 1.40 (m, 13H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.2, 122.7, 103.7, 73.8, 63.6, 56.5, 44.7, 39.6, 37.5, 34.3, 33.5, 33.5, 29.8, 26.4, 26.1, 26.1, 25.6; HRMS (ESI) calculated for $C_{24}H_{37}N_3O_2$ $[M+H]^+$ 400.2964, found 400.2987.

*6.1.1.12. 2-(((4-(Cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methyl)(propyl)amino)ethan-1-ol (**5l**).* Yield 61%; Light brown solid; mp 107 – 109 °C; IR (neat) 3500 - 2500 (br., peak), 2921, 2849, 1621, 1597, 1537, 1446, 1423, 1326, 1248, 1099, 1058, 892, 782, 729 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.35 (br. s., 1H), 7.22 (br. s., 1H), 7.12 (t, *J* = 7.93 Hz, 1H), 6.66 (d, *J* = 8.29 Hz, 1H), 3.98 (s, 2H), 3.93 (d, *J* = 6.34 Hz, 2H), 3.74 (t, *J* = 5.12 Hz, 2H), 2.76 (t, *J* = 5.12 Hz, 2H), 2.53 - 2.60 (m, 2H), 1.92 (br. s., 3H), 1.66 - 1.80 (m, 3H), 1.52 (sxt, *J* = 7.41 Hz, 2H), 1.17 - 1.36 (m, 3H), 0.99 - 1.13 (m, 2H), 0.88 (t, *J* = 7.32 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.6, 122.5, 103.6, 73.8, 59.5, 56.9, 56.5, 52.6, 37.4, 29.9, 26.4, 25.6, 19.9, 11.5; HRMS (ESI) calculated for $C_{20}H_{31}N_3O_2$ $[M+H]^+$ 346.2494 found 346.2517.

*6.1.1.13. 2-(((4-(Cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methyl)(ethyl)amino)ethan-1-ol (**5m**).* Yield 68%; Light yellow solid; mp 99-102 °C; IR (neat) 3500 - 2500 (br., peak),

2919, 2847, 1596, 1538, 1446, 1426, 1324, 1259, 1245, 1099, 1056, 994, 891, 867, 781, 728 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.36 (br. s., 1H), 6.87 - 7.26 (m, 2H), 6.66 (d, *J* = 7.80 Hz, 1H), 3.98 (s, 2H), 3.92 (d, *J* = 6.34 Hz, 2H), 3.73 (t, *J* = 5.12 Hz, 2H), 2.77 (t, *J* = 5.12 Hz, 2H), 2.71 (q, *J* = 7.07 Hz, 2H), 1.81 - 2.02 (m, 3H), 1.64 - 1.80 (m, 3H), 1.17 - 1.35 (m, 3H), 0.99 - 1.13 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.5, 122.6, 103.6, 73.8, 59.4, 55.8, 52.0, 48.4, 37.4, 29.9, 26.4, 25.6, 11.3; HRMS (ESI) calculated for $C_{19}H_{29}N_3O_2$ $[M+H]^+$ 332.2338 found 332.2359.

6.1.1.14. *2-(((4-(Cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methyl)(methyl)amino)ethan-1-ol (**5n**).* Yield 76%; Colorless sticky oil; IR (neat) 3500 - 2500 (br., peak), 2920, 2846, 1595, 1538, 1445, 1426, 1258, 1245, 1099, 1055, 867, 781, 728 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (br. s., 1H), 7.12 (t, *J* = 7.93 Hz, 1H), 6.67 (d, *J* = 8.78 Hz, 1H), 3.89 - 3.95 (m, 4H), 3.75 - 3.80 (m, 2H), 2.67 - 2.73 (m, 2H), 2.38 (s, 3H), 1.92 (d, *J* = 12.44 Hz, 3H), 1.65 - 1.79 (m, 3H), 1.16 - 1.35 (m, 3H), 0.98 - 1.12 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.8, 148.2, 122.6, 108.1, 103.6, 73.7, 59.4, 58.9, 55.8, 42.5, 37.4, 29.8, 26.3, 25.6; HRMS (ESI) calculated for $C_{18}H_{27}N_3O_2$ $[M+H]^+$ 318.2181, found 318.2205.

6.1.1.15. *2-(((4-(Cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methyl)(isopropyl)amino)ethan-1-ol (**5o**).* Yield 61%; Light yellow solid; mp 75 – 77 °C; IR (neat) 3500 - 2500 (br., peak), 2919, 2848, 1622, 1598, 1538, 1445, 1424, 1326, 1248, 1099, 1057, 892, 782, 729 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.48 (br. s., 1H), 7.11 (t, *J* = 8.05 Hz, 1H), 6.99 (br. s., 1H), 6.65 (d, *J* = 8.05 Hz, 1H), 3.88 - 3.98 (m, 4H), 3.68 (t, *J* = 5.24 Hz, 2H), 2.98 - 3.09 (m, 1H), 2.76 (t, *J* = 5.00 Hz, 2H), 1.92 (br. s., 3H), 1.66 - 1.80 (m, 3H), 1.19 - 1.34 (m, 3H), 1.01 - 1.11 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.7, 122.5,

103.6, 73.8, 59.7, 52.6, 51.4, 48.1, 37.4, 29.9, 26.4, 25.6, 17.9; HRMS (ESI) calculated for $C_{20}H_{31}N_3O_2$ $[M+H]^+$ 346.2494 found 346.2517.

*6.1.1.16. 2-(Cyclohexyl((4-(cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methyl)amino)ethan-1-ol (**5p**).* Yield 61%; White solid; mp 159 – 161 °C; IR (neat) 3500 - 2500 (br., peak), 2921, 2849, 1619, 1598, 1541, 1500, 1449, 1426, 1356, 1324, 1241, 1101, 1070, 1015, 994, 890, 870, 781, 730 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 10.47 (br. s., 1H), 7.10 (t, *J* = 8.05 Hz, 1H), 6.98 (br. s., 1H), 6.65 (d, *J* = 8.29 Hz, 1H), 4.00 (br. s., 2H), 3.93 (br. s., 2H), 3.67 (t, *J* = 5.12 Hz, 2H), 2.82 (t, *J* = 5.24 Hz, 2H), 2.47 - 2.62 (m, 1H), 1.59 - 2.00 (m, 11H), 1.04 - 1.32 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.1, 122.4, 103.6, 73.9, 60.9, 60.1, 53.4, 48.7, 37.4, 29.9, 28.8, 26.4, 26.0, 25.9, 25.7; HRMS (ESI) calculated for $C_{23}H_{35}N_3O_2$ $[M+H]^+$ 386.2807 found 386.2828.

*6.1.1.17. 2-(((4-(Cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methyl)(cyclohexylmethyl)amino)ethan-1-ol (**5q**).* Yield 66%; Off white solid; mp 83 – 85 °C; IR (neat) 3500 - 2500 (br., peak), 2920, 2848, 1619, 1599, 1543, 1501, 1448, 1426, 1356, 1324, 1241, 1102, 1069, 1015, 782, 730 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.23 (br. s., 1H), 7.12 (t, *J* = 7.93 Hz, 1H), 6.67 (d, *J* = 8.05 Hz, 1H), 3.97 (s, 4H), 3.73 (t, *J* = 5.00 Hz, 2H), 2.73 (br. s., 2H), 2.39 (d, *J* = 7.32 Hz, 2H), 1.56 - 2.03 (m, 12H), 0.90 - 1.32 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.6, 122.6, 103.7, 73.9, 62.2, 59.8, 57.1, 53.3, 37.5, 35.9, 31.6, 29.9, 26.6, 26.5, 25.9, 25.7; HRMS (ESI) calculated for $C_{24}H_{37}N_3O_2$ $[M+H]^+$ 400.2964 found 400.2987.

*6.1.1.18. 2-(Benzyl((4-(cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methyl)amino)ethan-1-ol (**5r**).* Yield 68%; Colorless sticky oil; IR (neat) 3500 - 2500 (br., peak), 2920, 2850, 1619, 1595, 1538, 1508, 1448, 1355, 1324, 1242, 1100, 1065, 1030, 783, 730 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.28 - 7.38 (m, 5H), 7.22 - 7.27 (m, 1H), 7.11 (t, *J* = 8.01 Hz, 1H), 6.66

(d, *J* = 8.57 Hz, 1H), 3.99 (s, 2H), 3.93 (d, *J* = 5.96 Hz, 2H), 3.73 - 3.80 (m, 4H), 2.82 (t, *J* = 5.03 Hz, 2H), 1.85 - 1.99 (m, 3H), 1.67 - 1.81 (m, 3H), 1.03 - 1.34 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.3, 138.1, 128.9, 128.4, 127.3, 122.6, 103.7, 73.8, 59.4, 58.9, 56.1, 52.0, 37.4, 29.8, 26.3, 25.6; HRMS (ESI) calculated for $C_{24}H_{31}N_3O_2$ $[M+H]^+$ 394.2494, found 394.2515.

*6.1.1.19. 2-(((4-(Cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methyl)(pyridin-4-ylmethyl)amino)ethan-1-ol (**5s**).* Yield 56%; Light brown sticky oil; IR (neat) 3500 - 2500 (br., peak), 3079, 2920, 2855, 1633, 1594, 1535, 1509, 1448, 1375, 1354, 1235, 1105, 1069, 1031, 981, 782, 729 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (d, *J* = 6.10 Hz, 2H), 7.29 (d, *J* = 6.20 Hz, 2H), 7.19 (br. s., 1H), 7.09 - 7.14 (m, 1H), 6.66 (d, *J* = 8.54 Hz, 1H), 4.01 (s, 2H), 3.91 (d, *J* = 6.10 Hz, 2H), 3.81 (t, *J* = 5.00 Hz, 2H), 3.77 (s, 2H), 2.85 (t, *J* = 5.00 Hz, 2H), 1.87 (d, *J* = 11.22 Hz, 3H), 1.65 - 1.74 (m, 3H), 1.13 - 1.29 (m, 4H), 0.97 - 1.08 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.7, 149.4, 148.4, 123.7, 122.8, 103.8, 73.8, 59.5, 57.9, 56.7, 52.0, 37.5, 29.8, 26.3, 25.6; HRMS (ESI) calculated for $C_{24}H_{31}N_3O_2$ $[M+H]^+$ 395.2447, found 395.2468.

*6.1.1.20. 2-((4-Chlorobenzyl)((4-(cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methyl)amino)ethan-1-ol (**5t**).* Yield 62%; Yellow solid; mp 78 – 81 °C; IR (neat) 3500 - 2500 (br., peak), 2921, 2851, 1594, 1538, 1446, 1252, 1090, 1014, 986, 783, 729 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 - 7.29 (m, 1H), 7.23 - 7.27 (m, 4H), 7.17 (d, *J* = 6.83 Hz, 1H), 7.09 - 7.14 (m, 1H), 6.66 (d, *J* = 8.78 Hz, 1H), 3.97 (s, 2H), 3.92 (d, *J* = 6.10 Hz, 2H), 3.75 - 3.80 (m, 2H), 3.72 (s, 2H), 2.77 - 2.82 (m, 2H), 1.83 - 1.96 (m, 3H), 1.66 - 1.79 (m, 3H), 1.12 - 1.34 (m, 3H), 0.98 - 1.12 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.1, 136.7, 132.9, 130.2, 128.4, 122.7, 103.7, 73.8, 59.3, 58.2, 56.1, 51.8, 37.4, 29.7, 26.3, 25.6; HRMS (ESI) calculated for $C_{24}H_{30}ClN_3O_2$ $[M+H]^+$ 428.2105, found 428.2126.

*6.1.1.21. 2-(((4-(Cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methyl)(4-methoxybenzyl)amino)ethan-1-ol (**5u**).* Yield 67%; Light yellow crystals; mp 68 – 70 °C; IR (neat) 3500 - 2500 (br., peak), 2920, 2850, 1595, 1538, 1509, 1446, 1243, 1099, 1033, 988, 783, 729 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.14 (br. s., 1H), 7.18 - 7.27 (m, 3H), 7.11 (t, *J* = 8.01 Hz, 1H), 6.82 - 6.86 (m, 2H), 6.66 (d, *J* = 8.66 Hz, 1H), 3.98 (s, 2H), 3.94 (d, *J* = 5.59 Hz, 2H), 3.78 (s, 3H), 3.73 - 3.77 (m, 2H), 3.70 (s, 2H), 2.80 (t, *J* = 4.89 Hz, 2H), 1.93 (d, *J* = 11.18 Hz, 3H), 1.70 - 1.85 (m, 3H), 1.05 - 1.33 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.9, 158.9, 152.3, 130.2, 130.0, 129.9, 122.6, 113.8, 113.8, 103.7, 73.8, 59.5, 58.3, 56.0, 55.1, 55.1, 51.9, 37.4, 29.8, 26.4, 25.6; HRMS (ESI) calculated for $C_{25}H_{33}N_3O_3$ $[M+H]^+$ 424.2600, found 424.2621.

*6.1.1.22. N-benzyl-1-(4-(cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methanamine (**5v**).* Yield 76%; Light brown sticky oil; IR (neat) 3500 - 2500 (br., peak), 2918, 2850, 1603, 1506, 1445, 1244, 1097, 1016, 993, 782, 729 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 (m, 2H), 7.18 - 7.26 (m, 3H), 7.02 - 7.18 (m, 2H), 6.61 (d, *J* = 8.20 Hz, 1H), 4.07 (s, 2H), 3.88 (d, *J* = 5.96 Hz, 2H), 3.80 (s, 2H), 1.87 (d, *J* = 10.43 Hz, 3H), 1.62 - 1.79 (m, 3H), 0.97 - 1.29 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.5, 139.3, 128.6, 128.2, 127.3, 122.7, 103.7, 73.8, 53.5, 46.8, 37.5, 29.9, 26.4, 25.6; HRMS (ESI) calculated for $C_{22}H_{27}N_3O$ $[M+H]^+$ 350.2232, found 350.2255.

*6.1.1.23. 1-(4-(Cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)-N-(4-methoxybenzyl)methanamine (**5w**).* Yield 78%; Colorless oil; IR (neat) 3500 - 2500 (br., peak), 2921, 2849, 1616, 1595, 1510, 1446, 1234, 1096, 1033, 987, 817, 782, 729 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (d, *J* = 8.47 Hz, 2H), 7.19 (br. s., 1H), 7.10 - 7.16 (m, 1H), 6.87 (d, *J* = 8.57 Hz, 2H), 6.67 (d, *J* = 8.01 Hz, 1H), 4.11 (s, 2H), 3.94 (d, *J* = 6.15 Hz, 2H), 3.80 (s, 5H), 1.70 - 1.96 (m, 6H), 1.07 - 1.34 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.9,

131.5, 129.4, 113.9, 103.7, 73.8, 55.3, 53.1, 46.9, 37.5, 30.0, 26.5, 25.8; HRMS (ESI) calculated for $C_{23}H_{29}N_3O_2$ $[M+H]^+$ 380.2338, found 380.2362.

*6.1.1.24. N-((4-(Cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methyl)aniline (**5x**).* Yield 71%; Off white solid; mp 78 – 80 °C; IR (neat) 3500 - 2500 (br., peak), 3396, 2918, 2850, 1602, 1505, 1445, 1422, 1313, 1245, 1097, 1016, 992, 891, 870, 782, 732 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.11 - 7.22 (m, 4H), 6.79 (t, *J* = 7.36 Hz, 1H), 6.62 - 6.72 (m, 3H), 4.64 (s, 2H), 4.44 (br. s., 1H), 3.93 (d, *J* = 6.33 Hz, 2H), 1.91 (d, *J* = 9.97 Hz, 3H), 1.65 - 1.78 (m, 3H), 0.99 - 1.33 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.2, 147.5, 129.4, 122.9, 118.5, 113.2, 103.8, 73.9, 42.9, 37.4, 29.8, 26.3, 25.6; HRMS (ESI) calculated for $C_{21}H_{25}N_3O$ $[M+H]^+$ 336.2076, found 336.2099.

*6.1.1.25. N-((4-(Cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methyl)-4-methoxyaniline (**5y**).* Yield 74%; Yellow solid; mp 70 – 72 °C (shrinking), 147 – 150 °C; IR (neat) 3500 - 2500 (br., peak), 2920, 2849, 1618, 1590, 1510, 1444, 1312, 1234, 1097, 1033, 987, 816, 782, 732 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.65 (br.s., 1H), 7.21 (br. s., 1H), 7.11 - 7.17 (m, 1H), 6.73 - 6.80 (m, 2H), 6.59 - 6.72 (m, 3H), 4.60 (s, 2H), 4.18 (br.s., 1H), 3.89 - 3.97 (m, 2H), 3.73 (s, 3H), 1.92 (d, *J* = 9.59 Hz, 3H), 1.67 - 1.84 (m, 3H), 1.02 - 1.33 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.9, 152.5, 141.5, 122.9, 114.9, 114.6, 114.5, 103.8, 73.9, 55.6, 43.9, 37.4, 29.9, 29.8, 26.3, 25.6; HRMS (ESI) calculated for $C_{22}H_{27}N_3O_2$ $[M+H]^+$ 366.2181, found 366.2202.

6.1.2. Synthesis of 2-(chloromethyl)-4-(cyclohexylmethoxy)-1H-benzo[d]imidazole (7). This compound was prepared from 2-chloroacetic acid and 3-(cyclohexylmethoxy)benzene-1,2-diamine (**6**) in a manner similar to the preparation of **5**. Yield 72%; Light yellow solid; mp 125 – 127 °C; 1H NMR (300 MHz, CDCl3) δ 7.14 - 7.25 (m, 2H), 6.68 - 6.74 (m, 1H), 4.85 -

4.89 (m, 2H), 3.94 (d, J = 6.15 Hz, 2H), 1.64 - 1.96 (m, 6H), 0.96 - 1.36 (m, 5H); HRMS (ESI) calculated for $C_{15}H_{19}ClN_2O$ $[M+H]^+$ 279.1264, found 279.1285.

6.2. Biology

6.2.1. In vitro study: IL-5 bioassay, mIL-5-dependent Y16 proliferation [28].

Y16 cell line was originated from a murine early B cell. The cell line was grown in RPMI-1640 media containing 8% 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 2-times with Hanks' solution, and resuspended in a lesser volume of RPMI-1640 media containing 8% FBS. Numbers of the cells were counted after trypan blue exclusion and the diluted to 1 x 10^4 cells/mL with RPMI-1640 media containing 8% FBS. Viability of the cells were more than 95% in all preparations. One hundred µL of (1 x 10^4 numbers) Y16 cells were allotted to each well of a 96-well microplate, and 50 µL of 3 units/mL mIL-5 and 50 µL of sample was 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. Absorbance at wavelength 450 nm (A_{450}) was measured by using a microplate reader after incubation at 37 °C with 5% CO_2 for 2 - 4 h.

6.2.2. MTT assay

Normal B lymphocytes such as HCC1395 BL and NCI-BL1184 were cultured in RPMI-1640 medium or IMDM (Iscove's modified Dulbecco's medium) containing 10% FBS at 37 °C with 5% CO_2. The cells were incubated with the compound **5e** or **5k** for 48 h and reacted with 0.5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) for another 3 h. Formazan crystals were dissolved in 99% DMSO, and absorbance values were measured at 590 nm.

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Highlights

- Novel benzimidazole scaffold has been discovered as potent interleukin-5 inhibitor
- Essential features of SAR were explored
- IC_{50} values of **5e** and **5k** on IL-5 bioactivity show 3.5 and 5.0 μM, respectively
- Compounds **5e** and **5k** did not show significant effects on cell viability of normal B lymphoblasts