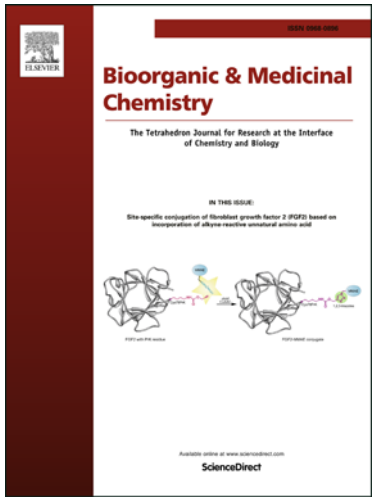

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Rational design of conformationally constrained oxazolidinone-fused 1,2,3,4-tetrahydroisoquinoline derivatives as potential PDE4 inhibitors

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Abstract:

Improvement of subtype selectivity of an inhibitor's binding activity using the conformational restriction approach has become an effective strategy in drug discovery. In this study, we applied this approach to PDE4 inhibitors and designed a series of novel oxazolidinone-fused 1,2,3,4-tetrahydroisoquinoline derivatives as conformationally restricted analogues of rolipram. The bioassay results demonstrated the oxazolidinone-fused tetrahydroisoquinoline derivatives exhibited moderate to good inhibitory activity against PDE4B and high selectivity for PDE4B/PDE4D. Among these derivatives, compound **12** showed both the strongest inhibition activity (IC_{50} = 0.60 μM) as well as good selectivity against PDE4B and good *in vivo* activity in animal models of asthma/COPD and sepsis induced by LPS. The primary SAR study showed that restricting the conformation of the catechol moiety in rolipram with the scaffold of oxazolidinone-fused tetrahydroisoquinoline could lead to an increase in selectivity for PDE4B over PDE4D, which was consistent with the observed docking simulation.

Keywords: conformational restriction; synthesis; tetrahydroisoquinoline derivatives; PDE4 inhibitor; molecular simulation

1. Introduction

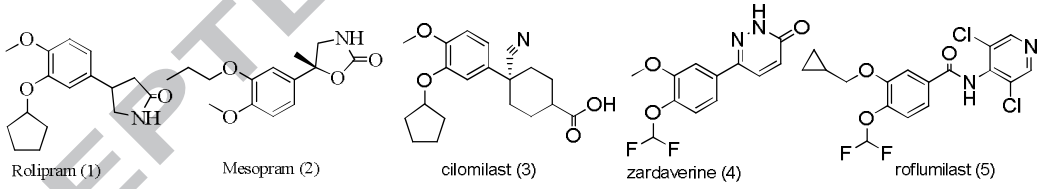
Many biological responses including regulation of important cell functions such as secretion, contraction, metabolism, and growth are mediated by levels of cyclic nucleotides, mainly 3′,5′-adenosine monophosphate (cAMP) and cyclic 3′,5′-guanosine monophosphate (cGMP).[1-2] It is well established that the balance between the levels of the second messengers cAMP and cGMP, plays a critical role in regulating the function of many inflammatory cells, both of which are inactivated by cyclic nucleotide phosphodiesterases (PDEs).[3-4] The PDE4, as one important member of the 11-membered PDEs, specifically targets the second messenger cAMP and is particularly abundant in inflammatory cells, immune cells, airway smooth muscles, and airway epithelium.[5-6] Inhibition of the PDE4 in these cells effectively elevates the intracellular cAMP levels, thereby leading to an activation of specific protein phosphorylation cascades, which in turn inhibits the release of inflammatory mediators such as tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2), interleukin-12 (IL-12), leukotriene B4 (LTB4), as well as activation of inflammatory cells.[5] Since the cellular mediators play a key role in the inflammatory diseases such as asthma and chronic obstructive pulmonary disease (COPD), PDE4 inhibitors are expected to be effective in the treatment of inflammatory disease.[7-10]

The first-generation PDE4 inhibitor rolipram (**1**, Fig.1), belonging to the dialkoxyphenyl (catechol) family, has been the starting point for many medicinal chemistry studies.[11] Further structural modification suggested that the 2-pyrrolidinone ring in rolipram was replaced by some appropriate pharmacophores to derive the most

potent analogues such as mesopram (**2**, Fig.1), cilomilast (**3**, Fig.1), zardaverine (**4**, Fig.1) and roflumilast (**5**, Fig.1).[12-14] A detailed SAR (structure-activity-relation) study about the diether derivative of catechol class suggested that the 4-(3, 4-dialkoxyphenyl) moiety was important for inhibition of PDE4 where the catechol ether oxygens played a key role in binding to the enzyme.[15] The substituent at the 4-position of the phenyl ring was restricted to small lipophilic groups, preferably methoxy or difluoromethoxy while various alkoxy substituents were well tolerated at the 3-position. Although a number of dialkoxyphenyl and its derivatives as PDE4 inhibitors have been reported, roflumilast and apremilast remain the only two marketed drugs in this class.[16] Therapeutic usefulness of the above PDE4 inhibitors was limited by their side effects including gastrointestinal side effects such as nausea and vomiting.[17-18] The PDE4 family consists of four isoforms (PDE4A–D), and each gene has multiple transcripts. Many studies have revealed that the PDE4B plays a key role in both inflammatory cell regulation[19] and its inhibition suppresses TNF-α production, and PDE4D may be responsible for the emetic response.[20] Thus, selective inhibition of PDE4B was expected to achieve efficacy while circumventing the potential side effects of the current PDE4 inhibitors. However, given the apparent structural similarity between PDE4B and PDE4D, only a few PDE4B selective inhibitors have been reported up to now.[21-22]

Conformational constraint is a widely used strategy to maintain biological activity while gaining higher selective activity and reducing side effect.[23-24] Base on this background, we hypothesized that restricting the conformation of the pyrrolidinone

moiety in rolipram (**1**, Fig.1) could be helpful for the selective inhibition of PDE4B with reduced emetic side effects. In the present work, we describe a fruitful approach to conformational constraint with the scaffold of tetrahydroisoquinoline, along with bioisosteric replacement[25] of the pyrrolidinone ring in rolipram to generate new PDE4 inhibitors. Thus, a pentacyclic 2-oxazolidinone ring fusion was incorporated into the 1,2,3,4-tetrahydroisoquinoline skeleton while retaining the promising catechol diether moiety to result in oxazolidinone-fused 1,2,3,4-tetrahydroisoquinoline derivatives, namely 1,5,10,10a-tetrahydro-3*H*-oxazolo[3,4-*b*]isoquinolin-3-one derivatives **6**-**13**. Following a similar strategy, the incorporation of the different substituent in the hexatomic ring was intended to further limit the conformational flexibility to derive the title compounds **14**-**17**. Herein we reported the successful application of two different rigidification strategies, and focused on how to achieve PDE4B selectivity rather than potency.



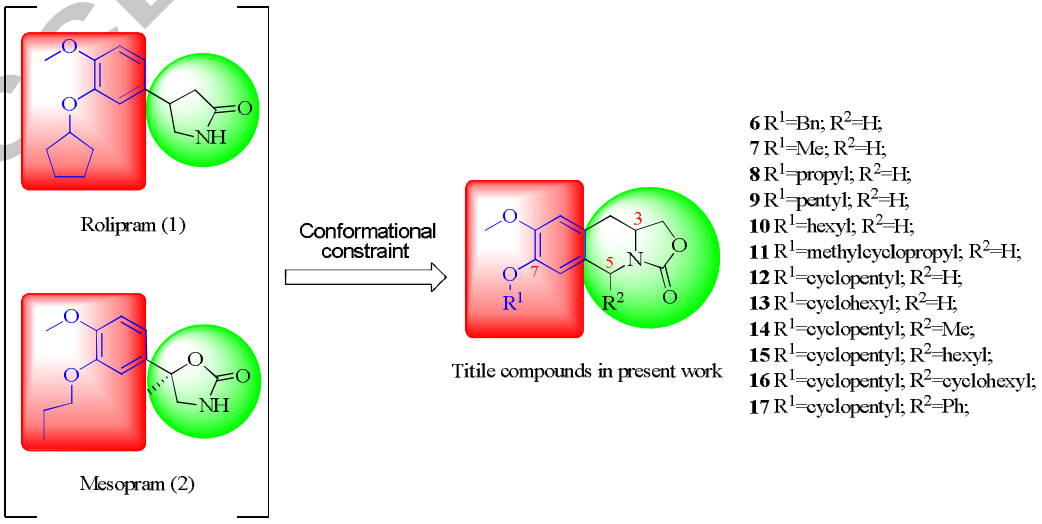


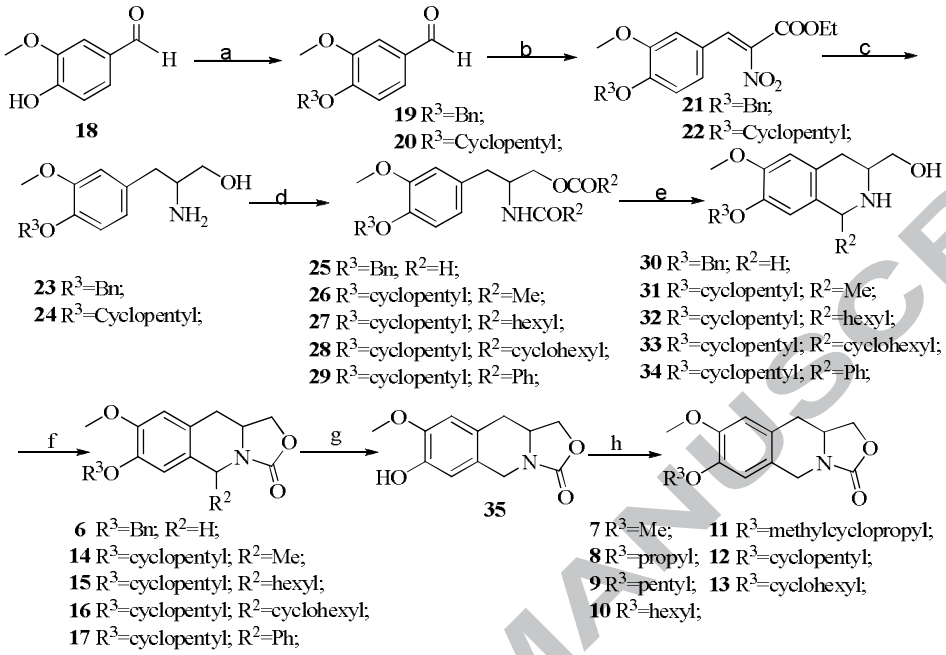
Figure 1. The designed strategy for the title compounds

2. Results and discussion

2.1 Chemistry

As depicted in Scheme 1, treatment of the commercially available vanillin (**18**) and benzyl bromide or bromocyclopentane in the presence of K_2CO_3 afforded the compound **19** or **20**[10], each followed by reaction with ethyl nitroacetate in the presence of dimethylamine hydrochloride and potassium fluoride provided the intermediate **21** or **22** including a pair of *cis-trans* isomerism, respectively. Then reduction of double bond, ester group and nitro group in compound **21** or **22** was simultaneously accomplished by treatment with lithium aluminum hydride to give compound **23** or **24**. Treatment of compound **23** or **24** with different carboxylic acid in the presence of EDC•HCl and DMAP afforded compounds **25**-**26**. Similarly, compounds **27**-**29** were obtained from **24** and different acyl chlorides. Then we applied the typical reaction condition of Bischler-Napieralski reaction[26] to elaborate the intermediates **30**-**34**. Compounds **25**-**29** were treated with phosphorus oxychloride, followed by reduction with sodium borohydride to yield the intermediates **30** and **31**-**34**, which were mainly composed of a pair of enantiomers of racemic compound. The reaction of **30**-**34** with benzyl carbonochloridate under the circumstance of 2 $M•L^{-1}$ NaOH-THF provided title compounds **6** and **14**-**17** as a mixture of isomers possibly in a diastereomeric relation. Finally, hydrogenolysis of compound **6** over palladium/carbon in CH_2Cl_2-MeOH furnished the important intermediate **35**, which was then treated with different haloalkane or

halocycloalkane to afford the corresponding title compounds **7**-**13** as a mixture of a pair of enantiomers of racemic compound, respectively.



Scheme 1. The synthetic route of the title compounds **7-17**. Reagents and conditions: (a) benzyl bromide or bromocyclopentane, K_2CO_3, DMF, 96% for **19**, 97% for **20**; (b) NO_2CH_2COOEt, $(CH_3)_2NH$•HCl, KF, toluene, reflux, 68% for **21**, 55% for **22**; (c) $LiAlH_4$, THF, 69% for **23**, 66% for **24**; (d) carboxylic acid, EDC•HCl, DMAP, CH_2Cl_2, 90% for **25**, 93% for **26** or different chloride, DMAP and pyridine, 88% for **27**, 86% for **28**, 90% for **29**; (e) i: $POCl_3$, toluene, reflux; ii: $NaBH_4$, MeOH, for two steps 56% for **30**, 58% for **31**, 60% for **32**, 55% for **33**, 57% for **34**; (f) CbzCl, 2 M NaOH-THF, 81% for **6**, 80% for **14**, 82% for **15**, 80% for **16**, 81% for **17**; (g) Pb/C, CH_2Cl_2-MeOH, 92%; (h) haloalkane or halocycloalkane, K_2CO_3, DMF, 95% for **7**, 96% for **8**, 97% for **9**, 96% for **10**, 95% for **11**, 93% for **12**, 90% for **13**.

The structures of all the title compounds **6-17** were characterized by NMR and mass spectroscopy (the spectra of ^{1}H NMR and ^{13}C NMR were shown in supplementary materials). The structure of compound **15** was confirmed by X-ray single crystal diffraction (Figure 2, Table S1, and Table S2), which showed that the relative configurations at the two asymmetric centers are in the relative of (3*R*,5*R*), or its antipodal (3*S*,5*S*).

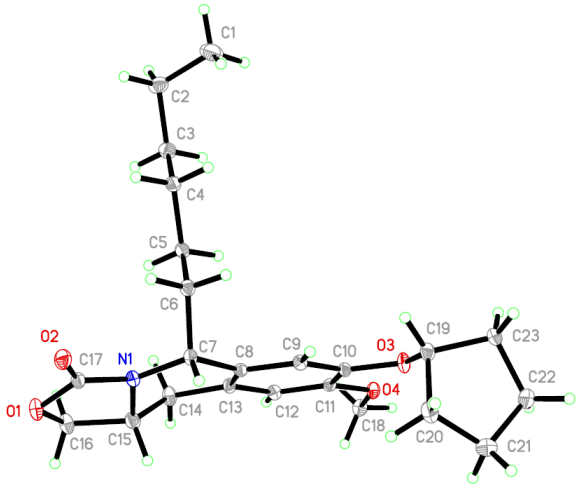


Figure 2. ORTEP structure of the title compound **15**, showing 50% probability ellipsoids; H atoms are shown as small spheres of arbitrary radii.

2.2. Biological evaluation and SAR studies

All title compounds **6-17** prepared and the intermediate **35** were evaluated for their *in vitro* inhibitory activity against PDE4B using the enzymatic assay described previously with rolipram as the positive control.[27] The IC_{50} (The half maximal inhibitory concentration) values were shown in Table 1. In addition, data for the inhibition of TNFα release in human blood mononuclear (HM) [3] were reported for selected compounds **11-17**.

Table 1. Impact on enzymatic potency (PDE4) and inhibition of TNF-α release from human blood mononuclear cells stimulated with lipopolysaccharide [a]

compound	PDE4B IC_{50} (μM)	PDE4D IC_{50} (μM)	TNFα IC_{50} (μM)
35	>100	NT[b]	NT[b]
6	8.18±0.22	NT	NT
7	52.10±0.78	NT	NT
8	22.36±0.33	NT	NT
9	4.10±0.45	23.32±0.65	NT
10	6.05±0.30	NT	NT
11	1.30±0.45	7.15±0.30	9.58±0.20
12	0.60±0.36	5.13±0.52	1.35±0.12
13	2.62±0.15	13.88±0.65	8.40±0.62
14	1.35±0.32	12.83±0.46	2.60±0.22
15	2.10±0.18	23.12±0.30	7.98±0.16
16	2.06±0.26	26.75±0.40	9.05±0.35
17	1.95±0.20	31.65±0.55	6.75±0.30
rolipram	1.22±0.18	1.43±0.23	10.85±0.25

[a] Results are the average of at least three assays.

[b] NT, not tested.

Initially, the effect of substituents at the 7-position of the tetrahydroisoquinoline ring on inhibitory activity and selectivity toward PDE4 was investigated to establish a SAR similar to that previously elucidated for the catechol subunit in rolipram.[28] Briefly, both the alkoxy oxygens were essential for inhibitory activity with a dialkoxy substitution pattern since compound **35** with a free C-7-OH displayed no inhibitory activity. Various alkoxy chains were introduced in which the cyclopentyloxy-substituted compound **12** had excellent activity against PDE4B with the IC_{50} values of

0.60 μM. Introduction of liner alkoxy chains (**7**-**10**), or other cycloalkyl chains such as smaller (**11**) or bigger (**6**, **13**) substituent led to dropped activity. Notably, the representative compounds **11-13** exhibited over 4.5-fold higher selectivity rations for PDE4B over PDE4D than rolipram, although they showed the similar inhibitory activity against PDE4B. These results indicated that the oxazolidinone-fused 1,2,3,4-tetrahydroisoquinoline ring was appropriate for obtaining high affinity and selectivity for PDE4B and compound **12** was therefore selected as a lead compound.

With respect to the 5-alkyl-1,2,3,4-tetrahydroisoquinoline derivatives (**14-17**), the effect of substituents at the 5-position of the tetrahydroisoquinoline ring on inhibitory activity and selectivity toward PDE4 was investigated. In comparison with **12**, the 5-alkyl tetrahydroisoquinoline derivatives **14-17** exhibited slightly decreased inhibitory activity against PDE4B but resulted in the remarkable loss of inhibitory activity against PDE4D, maybe due to differences of the environment around the 5-position of the tetrahydroisoquinoline ring between PDE4B and PDE4D. Accordingly, these results indicated that modification of the benzyl moiety in **12** to form a 5-alkyl tetrahydroisoquinoline ring could lead to improvement of selectivity toward PDE4B over PDE4D by decreasing inhibition for PDE4D. Moreover, selective ratios with respect to substitution at the 5-position of the tetrahydroisoquinoline ring (**14-17**) followed the trend: phenyl > cyclohexyl > hexyl > methyl. We inferred that methyl group could rotate more freely than another three alkyl groups since the size of methyl group was smaller than another alkyl groups such as hexyl, cyclohexyl and phenyl group. Furthermore, compound **17** showed the higher selectivity in

comparison with **14-16**, indicating that an aromatic substituent at the 5-position of the tetrahydroisoquinoline ring was beneficinal to enhance the selectivity toward PDE4B over PDE4D. However, the influence of their stereochemistry of the title compounds on inhibition and selectivity for the PDE4B will be further investigated.

Since PDEs include 11 different isozymes involved in various physiological processes, the selective inhibition of PDE4 is very important. Thus, we determined the selectivity of compound **12** toward the other PDEs isoforms using human PDE1A, PDE2A, PDE3B, PDE5A, PDE6C, PDE7A, PDE8A, PDE9A, PDE10A and PDE11A, respectively. As shown in Table 2, compound **12** displayed much weaker inhibitory against the above other PDEs isoforms than PDE4B at 100 μM, suggesting that compound **12** isexploitable as a potential lead compound for the design of PDE4 inhibitors.

Table 2. Inhibition of various PDEs by compound **12** at 100 μM[a]

PDEs	Inhibition (%)	PDEs	Inhibition (%)
PDE1A	12	PDE7A	5
PDE2A	3	PDE8A	12
PDE3B	5	PDE9A	2
PDE4B	100	PDE10A	1
PDE5A	14	PDE11A	3
PDE6C	8		

[a] Data reported are the mean of three experiments

The ability of selected compounds **11-17** to inhibit the release of HM-TNFα was

consistent well with their relative ability to inhibit PDE4. In the HM-TNFα assay, these compounds displayed good potency, exhibiting IC_{50} values < 10 μM. Notably, compound **12** with an IC_{50} of 1.35 μM, was about 7-fold more potent than rolipram in this assay, indicating that the oxazolidinone-fused 1,2,3,4-tetrahydroisoquinoline moiety had the other beneficial effect on PDE4 inhibition of TNFα release in HM.

LPS induced sepsis model for the measurement of TNF-α inhibition (in female *Swiss Albino* mice) and neutrophilia inhibition for asthma and COPD (in male *Sprague Dawley* rats) with selected compounds **12** and **17** were performed *in vivo*. The details such as oral dosage and number of animals grouped for the experiments were listed in Table 3. The results showed that compound **12** exhibited stronger inhibitory activity against TNF-α release (48%) and LPS induced neutrophilia inhibition (42%) than the positive control rolipram (41% and 32%) and compound **17** (40% and 28%).

Table 3. LPS induced TNF-α in SA mice and neutrophil influx in BALF of SD rats

Compd.	R^1	R^2	*Swiss Albino* mice (n = 6)		*Sprague Dawley* rats (n = 6)	
			Does (mg/kg, po)	TNF-α Inhibition (%)	Does (mg/kg, po)	LPS induced neutrophilia (% inhibition)
12	*cyclopentyloxy*	H	10	48.2	10	42.3
17	*cyclopentyloxy*	Ph	10	40.4	10	28.1
rolipram			10	41.0	10	32.3

2.3. Docking simulation

Considering the inhibitory activity and selectivity of title compounds, it was of interest to explore the binding to the PDE4B structure. Compounds **12** and **17** with strong inhibitory activity and high selectivity, exhibited the great promise as novel

lead compounds for further discovery. Therefore docking simulation of compounds **12** and **17** at PDE4B (PDB ID: 1XMY) was conducted using Surflex-Dock in Sybyl 8.0[3] and the docking contour maps were shown in **Fig 3**. As predicted from the SAR study summarized in Table 1, the catechol residue in compounds **12** and **17** played a key role in the interaction with PDE4B. A phenyl ring structure of the inhibitor **12** or **17** was held tightly in the active site by a pair of hydrophobic residues forming a hydrophobic clamp like rolipram, of which the phenyl ring formed strong π-π stacking interaction with benzene ring (**12**: 3.75 Å and **17**: 3.81 Å, **Fig**. **3B** and **3D**) in the phenylalanine (Phe446). Moreover, the small lipophilic group methoxy in **12** or **17** occupied a small lipophilic pocket while the big cyclopentyloxy group filled a large hydrophobic cavity.

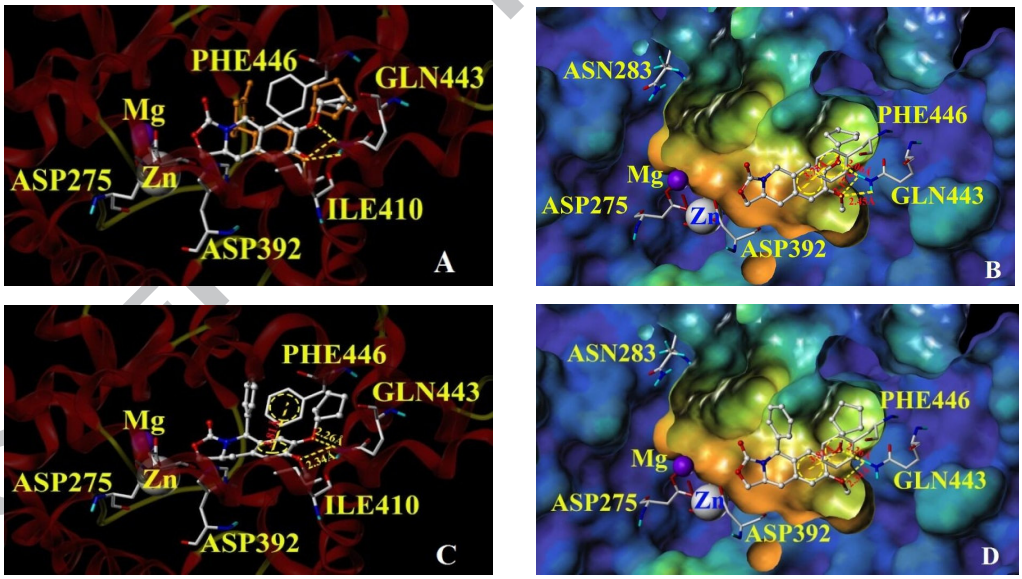


Figure 3. Model of PDE4 and docking of compounds **12** and **17**. The catalytic domain bound to **12** overlaid with rolipram (orange, A). The catalytic domain bound to **12** (B). The catalytic domain bound to **17** (C, D).

The two alkoxy groups in **12** or **17** formed two or three steady hydrogen bonds with

the conserved glutamine residue Gln443 (**Fig. 3**), respectively, suggesting both of the two alkoxy groups in compounds **12** and **17** seemed to be essential for inhibitory activity against PDE4B. Furthermore, the introduction of an additional phenyl ring into the benzyl moiety in **12** to derive **17** resulted in slightly reduced both π-π stacking interaction and hydrogen bonds, probably due to unfavorable steric crash between the binding site of PDE4B and the second phenyl ring observed.

3. **Conclusion**

In the course of our continuing efforts to develop potent PDE4 inhibitors, we designed and synthesized a series of 1,5,10,10a-tetrahydro-3*H*-oxazolo[3,4-*b*] isoquinolin-3-one derivatives structurally related to rolipram using conformational restriction approach as well as bioisosteric replacement strategy. The bioassay results showed oxazolidinone-fused tetrahydroisoquinoline derivative **12** had almost 10-fold higher selectivity toward PDE4B over PDE4D than rolipram, suggesting proper arrangement of the two alkoxy groups in the basic phenyl ring, achieved by conformational restriction of the catechol moiety through formation of a oxazolidinone-fused tetrahydroisoquinoline skeleton, was helpful to enhance selectivity for toward PDE4B over PDE4D. A primary structure-activity relationship study showed that both the alkoxy oxygens were essential for inhibitory activity against PDE4B and introduction of the additional rigid substituents at the benzyl position was helpful to lead to an increase in subtype selectivity, which was consistent well with the observed docking simulation.

Experimental protocols

4.1. Chemistry

Solvents were purified in a conventional manner. Thin layer chromatography (TLC) was performed on precoated E. Merck silica gel 60 F254 plates. Flash column chromatography was performed on silica gel (200-300 mesh, Qingdao, China). Optical rotations were determined with a Perkin-Elmer Model 241 MC polarimeter. ^{1}H NMR and ^{13}C NMR spectra were taken on a JEOL JNM-ECP 600 spectrometer with tetramethylsilane as an internal standard, and chemical shifts are recorded in ppm values. Mass spectra were recorded on a Q-TOF Global mass spectrometer.

*4.1.1 4-(benzyloxy)-3-methoxybenzaldehyde (**19**)*

To a solution of compound **18** (25 g, 0.16 mol) in dry DMF (100 mL) was added K_2CO_3 (33 g, 0.24 mol) and benzyl bromide (25 mL, 0.22 mol), which was then heated at 65 °C under argon. After stirred for 8 h, the mixture was filtrated and concentrated *in vacuo*. The dried residue was dissolved in 500 mL of EtOAc and then washed with H_2O (200 mL × 2), saturated aqueous $NaHCO_3$ (200 mL × 2), and brine (200 mL × 2), dried over Na_2SO_4, and concentrated to dryness. The residue was purified by silica gel column chromatography (5:1, *V*:*V*, petroleum ether-EtOAc) to yield **19** as a white solid (38 g, 96%); ^{1}H NMR ($CDCl_3$): δ 9.84 (s, 1H, C*H*O), 7.45 (dd, 3H, *J* = 7.8, 1.8 Hz, Ar-H), 7.39-7.41 (m, 3H, Ar-H), 7.34 (t, 1H, *J* = 7.3 Hz, Ar-H), 7.00 (d, 1H, *J* = 8.2 Hz, Ar-H), 5.26 (s, 2H, Ar-OCH_2), 3.96 (s, 3H, OCH_3); ^{13}C NMR($CDCl_3$): δ 190.9, 153.6, 150.0, 136.0, 130.3, 128.7 (two), 128.2, 127.2, 126.5, 112.4, 109.3, 70.8, 56.0; ESIMS: calcd for $[M+H]^+$ m/z 243.1; found, 243.2.

*4.1.2 4-(cyclopentyloxy)-3-methoxybenzaldehyde (**20**)*

Compound **20**[11] was obtained from **18** and cyclopentyl bromide as a yellow oil in 97% yield; ^{1}H NMR ($CDCl_3$): δ 9.84 (s, 1H, C*H*O), 7.42 (dd, 1H, *J* = 8.0, 1.8 Hz, Ar-H-6), 7.40 (d, 1H, *J* = 1.8 Hz, Ar-H-2), 6.96 (d, 1H, *J* = 8.4 Hz, Ar-H-5), 4.86-4.89 (m, 1H, H′-1), 3.91 (s, 3H, OC*H*$_3$), 1.98-2.04 (m, 2H, H′-2-1, H′-3-1), 1.91-1.96 (m, 2H, H′-2-2, H′-3-2), 1.82-1.88 (m, 2H, H′-4-1, H′-5-1), 1.61-1.67 (m, 2H, H′-4-2, H′-5-2);

*4.1.3 (Z and E) ethyl -3-(4-(benzyloxy)-3-methoxyphenyl)-2-nitroacrylate (**21**)*

To compound **19** (37.0 g, 0.15 mol) dissolved in anhydrous toluene (250 mL) was added ethyl nitroacetate (20.2 mL, 0.18 mol), dimethylamine hydrochloride (25.0 g, 0.28 mol), potassium fluoride (1.33 g, 23.0 mmol) and then refluxed at 120 °C under argon. The reaction mixture was stirred for 24 h, and then the mixture was concentrated *in vacuo*. The dried residue was dissolved in 300 mL of CH_2Cl_2 and then washed with H_2O (150 mL × 3) and brine (150 mL × 2), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (15:1, *V*:*V*, petroleum ether-EtOAc) to give **21** as a yellow solid (38.8 g, 68%). Compound **21** includes a pair of *Z* and *E* isomers, of which the ratio is about 3:1 by ^{1}HNMR. ^{1}H NMR ($CDCl_3$): δ 8.02 (s, 1 H, *C*H*=), 7.44 (s, 1H, C*H*=C), 7.43 (d, 4H, *J* = 6.9 Hz, Ar′-H-2, Ar′-H-6, *Ar′-H-2, *Ar′-H-6), 7.37-7.41 (m, 4H, Ar′-H-3, Ar′-H-5, *Ar′-H-3, *Ar′-H-5), 7.32-7.35 (m, 2H, Ar′-H-4, *Ar′-H-4), *7.12 (dd, 1H, *J* = 8.4, 2.0 Hz, Ar-H-6), *7.09 (d, 1H, *J* = 2.0 Hz, Ar-H-2), 7.02 (dd, 1H, *J* = 8.5, 2.2 Hz, Ar-H-6), 6.94 (d, 1H, *J* = 2.0 Hz, Ar-H-2), 6.90 (d, 1H, *J* = 8.4 Hz, Ar-H-5), *5.23 (s, 2H, Ar-O-C*H*$_2$), 5.21 (s, 2H, Ar-OC*H*$_2$), *4.45 (q, 2H, *J* = 7.1 Hz,

COOCH_2), 4.38 (q, 2H, J = 7.1 Hz, COOCH_2), 3.90 (s, 3H, OCH_3), *3.88 (s, 3H, OCH_3), *1.38 (t, 3H, J = 7.1 Hz, CH_3), 1.37 (t, 3H, J = 7.1 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ *161.8, 159.5, *152.2, 151.8, 149.8, *149.8, *140.0, 138.4, 136.8, 136.0, *135.9, 132.8, *128.7 (two), 128.7 (two), *128.3, 128.2, *127.2, 127.2, *126.2, 125.1, 121.8, *121.7, 113.3, *113.3, *112.6, 111.9, *70.8, 70.8, *63.0, 62.8, 56.0, *56.0, *29.7, 14.1, *13.8; ESIMS: calcd for $[M+Na]^+$ m/z 380.1; found, 380.2.

*4.1.4 (Z and E)-ethyl-3-(3-(cyclopentyloxy)-4-methoxyphenyl)-2-nitroacrylate (**22**)*

Compound **22** was obtained from **20** as a yellow solid in 55% yield and included a pair of *Z* and *E* isomers, of which the ratio is about 3:1 by ^{1}HNMR. ^{1}H NMR ($CDCl_3$): δ *8.03 (s, 1 H, C*H*=), 7.44 (s, 1 H, C*H*=), *7.15 (dd, 1 H, J = 8.7, 2.3 Hz, Ar-H-6), 7.05 (dd, 1 H, J = 9.2, 2.3 Hz, Ar-H-6), 6.90 (d, 1 H, J = 3.2 Hz, Ar-H-2), *6.89 (d, 1 H, J = 2.8 Hz, Ar-H-2), 6.86 (d, 1 H, J = 8.7 Hz, Ar-H-5), 4.81-4.86 (m, 1 H, H′-1), *4.45 (q, 2 H, J = 6.9 Hz, OCH_2CH$_3$), 4.36 (q, 2 H, J = 6.9 Hz, OCH_2CH$_3$), *3.84 (s, 3 H, OCH_3), 3.82 (s, 3 H, OCH_3), 1.60-2.00 (m, 8 H, 4 × CH_2), *1.39 (t, 3 H, J = 7.3 Hz, OCH$_2$CH_3), 1.36 (t, 3 H, J = 6.9 Hz, OCH$_2$CH_3); ^{13}C NMR ($CDCl_3$): δ 162.1, 159.8, 152.3, 151.9, 150.1, 139.7, 138.1, 137.1, 133.1, 126.6, 125.3, 121.1, 121.0, 113.9, 113.8, 112.3, 80.7, 63.1, 62.9, 56.1, 32.9 (two), 24.2 (two), 14.2, 14.0; ESIMS: calcd for $[M+Na]^+$ m/z 358.1; found, 358.2.

*4.1.5 2-amino-3-(4-(benzyloxy)-3-methoxyphenyl) propan-1-ol (**23**)*

To compound **21** (28.8 g, 76.8 mmol) dissolved in anhydrous THF (200 mL) was added lithium aluminum hydride (19.0 g, 0.50 mol) at 0 °C. The mixture was allowed to stir for 12 h at 70 °C. After that, the mixture was cooled to 0 °C, added H_2O (10

mL) slowly. The mixture was filtered and concentrated *in vacuo*, and diluted with CH_2Cl_2 (300 mL), washed with brine (100 mL × 2), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (100: 2: 1, *V*:*V*:*V*, Chloroform-Methanol-Et_3N) to give **23** (14.9 g, 68%); ^{1}H NMR ($CDCl_3$): δ 7.44 (d, 2H, *J* = 7.4 Hz, Ar′-H-2, Ar′-H-6), 7.37 (t, 2H, *J* = 7.4 Hz, Ar′-H-3, Ar′-H-5), 7.30 (t, 1H, *J* = 7.3 Hz, Ar′-H-4), 6.82 (d, 1H, *J* = 8.2 Hz, Ar-H-5), 6.75 (d, 1H, *J* = 1.7 Hz, Ar-H-2), 6.66 (dd, 1H, *J* = 8.1, 1.7 Hz, Ar-H-6), 5.13 (s, 2H, Ar-OCH_2), 3.88 (s, 3H, OCH_3), 3.64 (dd, 1H, *J* = 10.7, 3.8 Hz, H-1-a), 3.40 (dd, 1H, *J* = 10.7, 7.1 Hz, H-1-b), 3.08-3.12 (m, 1H, N-C*H*), 2.73 (dd, 1H, *J* = 13.6, 5.3 Hz, Ar-CH_2-1), 2.47 (dd, 1H, *J* = 13.6, 8.6 Hz, Ar-CH_2-2), 2.43 (brs, 2H, NH_2); ^{13}C NMR ($CDCl_3$): δ 149.7, 146.8, 137.2, 131.7, 128.5, 127.8, 127.3, 121.2, 114.3, 112.9, 71.2, 66.1, 56.0, 54.2, 40.2; ESIMS: calcd for $[M+Na]^+$ m/z 310.1; found, 310.1.

*4.1.6 2-amino-3-(4-(cyclopentyloxy)-3-methoxyphenyl)-propan-1-ol (**24**)*

Compound **24** was prepared from **22** as a yellow oil in 56% yield; ^{1}H NMR (DMSO-d_6): δ 6.83 (d, 1 H, *J* = 7.8 Hz, Ar-H-5), 6.76 (d, 1 H, *J* = 1.4 Hz, Ar-H-2), 6.68 (dd, 1 H, *J* = 8.3, 1.9 Hz, Ar-H-6), 4.74-4.75 (m, 1 H, H′-1), 3.70 (s, 3 H, OCH_3), 3.29 (dd, 1 H, *J* = 10.5, 4.6 Hz, H-1-a), 3.18 (dd, 1 H, *J* = 10.5, 6.4 Hz, H-1-b), 2.84-2.86 (m, 1 H, NH-C*H*-CH_2), 2.58 (dd, 1 H, *J* = 13.3, 5.9 Hz, Ar-CH_2-1), 2.37 (dd, 1 H, *J* = 13.3, 7.7 Hz, Ar-CH_2-2), 1.80-1.85 (m, 2 H, H′-2-1, H′-3-1), 1.69-1.70 (m, 4 H, H′-2-2, H′-3-2, H′-4-1, H′-5-1), 1.56-1.57 (m, 2 H, H′-4-2, H′-5-2); ^{13}C NMR (DMSO-d_6): δ 148.0, 146.7, 131.8, 121.2, 116.2, 112.3, 79.3, 79.2, 65.2, 55.6, 54.5, 32.3 (two), 23.5 (two); ESIMS: calcd for $[M+H]^+$ m/z 266.2; found, 266.1.

4.1.7 General procedure for the preparation of ***25-26***

To a solution of compound **23** or **24** (1 eq) in dry CH_2Cl_2 (50 mL) was added formic acid or acetic acid (2.4 eq), EDC•HCl (2.6 eq), and DMAP (0.2 eq) at 0 ℃. The reaction mixture was stirred at room temperature for 12 h, then diluted with CH_2Cl_2 (100 mL), washed with 1 mol·L^{-1} HCl (50 mL × 2), saturated aqueous $NaHCO_3$ (50 mL × 2), and brine (50 mL × 2), dried over Na_2SO_4, and concentrated to dryness. The residue was purified by silica gel column chromatography to afford **25-26**, respectively.

*4.1.7.1 3-(4-(benzyloxy)-3-methoxyphenyl)-2-formamidopropyl formate (**25**)*

Compound **25** was synthesized as a white solid in 90% yield; ^{1}H NMR ($CDCl_3$): δ 8.13 (s, 1H, C*H*O), 8.10 (s, 1H, C*H*O), 7.44 (d, 2H, *J* = 7.5 Hz, Ar′-H-2, Ar′-H-6), 7.37 (t, 2H, *J* = 7.3 Hz, Ar′-H-3, Ar′-H-5), 7.31 (t, 1H, *J* = 7.3 Hz, Ar′-H-4), 6.82 (d, 1H, *J* = 8.2 Hz, Ar-H-5), 6.74 (d, 1H, *J* = 1.9 Hz, Ar-H-2), 6.66 (dd, 1H, *J* = 8.1, 2.0 Hz, Ar-H-6), 5.90 (d, 1H, *J* = 8.0 Hz, N*H*), 5.12 (s, 2H, Ar-OCH_2), 4.49-4.55 (m, 1H, N-C*H*), 4.18 (d, 2H, *J* = 4.8 Hz, OCH_2), 3.87 (s, 3H, OCH_3), 2.86 (dd, 1H, *J* = 14.0, 6.7 Hz, Ar-CH_2-1), 2.78 (dd, 1H, *J* = 14.0, 7.9 Hz, Ar-CH_2-2); ^{13}C NMR ($CDCl_3$): δ 160.8, 160.7, 149.8, 147.2, 137.1, 129.5, 128.5 (two), 127.9, 127.3 (two), 121.2, 114.3, 112.8, 71.1, 63.9, 56.1, 48.0, 36.8; ESIMS: calcd for $[M+Na]^+$ m/z 366.1; found, 366.2.

*4.1.7.2 1-acetamido-2-(4-(cyclopentyloxy)-3-methoxyphenyl) propyl acetate (**26**)*

Compound **26** was synthesized as a white solid in 93% yield; ^{1}H NMR ($CDCl_3$): δ 6.80 (d, 1H, *J* = 8.1 Hz, Ar-H-5), 6.71 (d, 1H, *J* = 1.8 Hz, Ar-H-2), 6.67 (dd, 1H, *J* =

8.2, 1.9 Hz, Ar-H-6), 5.67 (d, 1H, J = 8.3 Hz, N*H*), 4.72-4.75 (m, 1H, H-1′), 4.37-4.42 (m, 1H, N-C*H*), 4.09 (dd, 1H, J = 11.4, 5.6 Hz, H-1-a), 4.04 (dd, 1H, J = 11.4, 4.3 Hz, H-1-b), 3.82 (s, 3H, OCH_3), 2.83 (dd, 1H, J = 13.9, 6.2 Hz, Ar-CH_2-1), 2.73 (dd, 1H, J = 13.9, 8.0 Hz, Ar-CH_2-2), 2.09 (s, 3H, COOCH_3), 1.96 (s, 3H, N-CO-CH_3), 1.82-1.92 (m, 6H, H′-2, H′′-3, H′-4-1, H′-5-1), 1.57-1.63 (m, 2H, H′-4-2, H′-5-2); ^{13}C NMR ($CDCl_3$): δ 171.0, 169.7, 150.0, 146.6, 129.3, 121.3, 115.0, 113.0, 80.5, 64.8, 56.1, 49.5, 37.0, 32.8, 24.0 (three), 23.4, 20.8; ESIMS: calcd for $[M+Na]^+$ m/z 372.2; found, 372.2.

4.1.8 General procedure for the preparation of ***27-29***

To a solution of compound **24** (1 eq) in dry pyridine (60 mL) was added different chloride (2.6 eq), and DMAP (0.2 eq) at 0 °C. After stirring at 30 °C for 6 h, the reaction was quenched with methanol. The mixture was concentrated under vacuum to furnish yellow oil, which was subjected to column chromatography on silica gel (EtOAc-petroleum ether, 1:10) to give **27-29**, respectively.

*4.1.8.1 2-(4-(cyclopentyloxy)-3-methoxyphenyl)-1-heptanamidopropyl heptanoate (**27**)*

Compound **27** was prepared as a white solid in 88% yield; ^{1}H NMR ($CDCl_3$): δ 6.79 (d, 1H, J = 8.1 Hz, Ar-H-5), 6.71 (s, 1H, Ar-H-2), 6.67 (d, 1H, J = 8.1 Hz, Ar-H-6), 5.61 (brs, 1H, N*H*), 4.71-4.74 (m, 1H, H-1′), 4.38-4.44 (m, 1H, N-C*H*), 4.11 (dd, 1H, J = 11.4, 5.7 Hz, O-CH_2-1), 4.03 (dd, 1H, J = 11.4, 4.2 Hz, O-CH_2-2), 3.82 (s, 3H, OCH_3), 2.84 (dd, 1H, J = 13.9, 6.1 Hz, Ar-CH_2-1), 2.72 (dd, 1H, J = 13.9, 8.0 Hz, Ar-CH_2-2), 2.34, 2.13 (each t, each 2H, J = 7.7 Hz, each COCH_2), 1.89-1.94 (m, 4H, 2

× CH_2), 1.80-1.85 (m, 2H, CH_2), 1.55-1.66 (m, 6H, 3 × CH_2), 1.27-1.34 (m, 12H, 6 × CH_2), 0.87-0.90 (m, 6H, 2 × CH_3); ^{13}C NMR($CDCl_3$): δ 173.9, 172.7, 150.0, 146.5, 129.4, 121.3, 114.9, 113.0, 80.4, 64.5, 56.0, 49.4, 37.1, 36.9, 34.2, 32.8, 31.5, 31.4, 28.9, 28.8, 25.6, 24.9, 24.0 (three), 22.5, 14.0; ESIMS: calcd for $[M+Na]^+$ m/z 512.3; found, 512.2.

*4.1.8.2 1-(cyclohexanecarboxamido)-2-(4-(cyclopentyloxy)-3-methoxyphenyl) propyl cyclohexanecarboxylate (**28**)*

Compound **28** was prepared as a white solid in 86% yield; ^{1}H NMR ($CDCl_3$): δ 6.79 (d, 1H, *J* = 8.2 Hz, Ar-H-5), 6.71 (d, 1H, *J* = 1.9 Hz, Ar-H-2), 6.66 (dd, 1H, *J* = 8.1, 1.9 Hz, Ar-H-6), 5.62 (d, 1H, *J* = 8.4 Hz, N*H*), 4.72-4.75 (m, 1H, H-1′), 4.37-4.43 (m, 1H, N-C*H*), 4.12 (dd, 1H, *J* = 11.4, 5.9 Hz, O-CH_2-1), 4.01 (dd, 1H, *J* = 11.4, 4.3 Hz, O-CH_2-2), 3.82 (s, 3H, OCH_3), 2.82 (dd, 1H, *J* = 13.9, 6.0 Hz, Ar-CH_2-1), 2.71 (dd, 1H, *J* = 13.8, 8.0 Hz, Ar-CH_2-2), 2.34 (tt, 1H, *J* = 11.3, 3.7 Hz, COC*H*), 2.02 (tt, 1H, *J* = 11.7, 3.2 Hz, COC*H*), 1.75-1.93 (m, 14H, 7 × CH_2), 1.58-1.67 (m, 4H, 2 × CH_2), 1.42-1.49 (m, 2H, CH_2), 1.18-1.38 (m, 8H, 4 × CH_2); ^{13}C NMR($CDCl_3$): δ 176.2, 175.6, 150.0, 146.5, 129.4, 121.3, 115.0, 113.0, 80.5, 64.3, 56.0, 49.3, 45.5, 43.2, 37.1, 32.8, 32.7, 29.7, 29.5, 29.1, 29.0, 25.7, 25.6, 25.6, 25.6, 25.4, 25.3, 24.0 (three); ESIMS: calcd for $[M+Na]^+$ m/z 508.3; found, 508.3.

*4.1.8.3 1-benzamido-2-(4-(cyclopentyloxy)-3-methoxyphenyl) propyl benzoate (**29**)*

Compound **29** was prepared as a white solid in 90% yield; ^{1}H NMR ($CDCl_3$): δ 8.07 (dd, 2H, *J* = 8.4, 1.3 Hz, Ar-H), 7.74 (dd, 2H, *J* = 8.4, 1.3 Hz, Ar-H), 7.58-7.61 (m, 1H, Ar-H), 7.42-7.51 (m, 5H, Ar-H), 6.82 (t, 1H, *J* = 8.4 Hz, Ar-H), 6.78-6.81 (m, 2H,

Ar-H), 6.58 (t, 1H, *J* = 8.1 Hz, N*H*), 4.72-4.78 (m, 2H, H-1′, N-C*H*), 4.49 (dd, 1H, *J* = 11.5, 6.0 Hz, O-CH_2-1), 4.44 (dd, 1H, *J* = 11.5, 4.3 Hz, O-CH_2-2), 3.80 (s, 3H, OCH_3), 3.10 (dd, 1H, *J* = 13.8, 5.6 Hz, Ar-CH_2-1), 2.94 (dd, 1H, *J* = 13.9, 8.2 Hz, Ar-CH_2-2), 1.83-1.95 (m, 6H, 3 × CH_2), 1.58-1.64 (m, 2H, CH_2); ^{13}C NMR($CDCl_3$): δ 167.1, 166.9, 150.1, 146.6, 134.3, 133.3, 131.6, 129.7 (two), 129.2, 128.6 (two), 128.5 (two), 126.9 (two), 121.4, 115.0, 113.1, 80.4, 65.3, 56.0, 50.5, 37.1, 32.9, 32.8, 24.1; ESIMS: calcd for $[M+H]^+$ m/z 474.2; found, 474.2.

*4.1.9 General procedure for the preparation of **30-34***

Compounds **25-29** (1 eq) and phosphorus oxychloride (2.5 eq) was dissolved in dry toluene (80 mL), and the reaction mixture was refluxed for 3 h at 100 °C, then concentrated *in vacuo*. To a solution of the above residue in dry methanol (100 mL), sodium borohydride (2.5 eq) was added at 0 °C. The mixture was stirred for 3 h at room temperature, and then the mixture was filtered and concentrated *in vacuo*. The dried residue was dissolved in 100 mL of CH_2Cl_2 and then washed with H_2O (50 mL × 2), saturated aqueous $NaHCO_3$ (50 mL × 2) and brine (50 mL × 2), dried over Na_2SO_4, and concentrated to dryness. The residue was purified by silica gel column chromatography to afford **30-34**, respectively.

*4.1.9.1 (7-(benzyloxy)-6-methoxy-1,2,3,4-tetrahydroisoquinolin-3-yl)methanol (**30**)*

Compound **30** was obtained as a white solid in 56% yield; ^{1}H NMR ($CDCl_3$): δ 7.43 (d, 2H, *J* = 7.5 Hz, Ar′-H-2, Ar′-H-6), 7.38 (t, 2H, *J* = 7.6 Hz, Ar′-H-3, Ar′-H-5), 7.32 (t, 1H, *J* = 7.6 Hz, Ar′-H-4), 6.71 (s, 1H, Ar-H), 6.66 (s, 1H, Ar-H), 5.00 (s, 2H, Ar-OCH_2), 3.80 (s, 2H, N-CH_2-Ar), 3.71 (s, 3H, OCH_3), 3.46 (dd, 1H, *J* = 10.5, 4.7

Hz, O-CH_2-1), 3.37 (dd, 1H, J = 10.4, 7.0 Hz, O-CH_2-2), 2.77-2.81 (m, 1H, N-CH), 2.55 (dd, 1H, J = 15.9, 3.7 Hz, Ar-CH_2-1), 2.50 (s, 1H, OH), 2.34 (dd, 1H, J = 15.7, 10.7 Hz, Ar-CH_2-2); ^{13}C NMR ($CDCl_3$): δ 148.0, 146.4, 137.9, 128.8 (two), 128.2, 128.1 (three), 127.3, 113.3, 112.1, 70.6, 65.4, 56.1, 55.6, 47.6, 31.3; ESIMS: calcd for $[M+H]^+$ m/z 300.2; found, 300.1.

*4.1.9.2 (7-(cyclopentyloxy)-6-methoxy-1-methyl-1,2,3,4-tetrahydroisoquinolin-3-yl)-methanol (**31**)*

Compound **31** was obtained as a white solid in 58% yield; ^{1}H NMR ($CDCl_3$): δ 6.70 (s, 1H, Ar-H), 6.57 (s, 1H, Ar-H), 4.72-4.77 (m, 1H, H-1′), 4.38 (q, 1H, J = 6.4 Hz, Ar-CH-N), 3.82 (s, 3H, OCH_3), 3.79 (dd, 1H, J = 10.7, 3.7 Hz, O-CH_2-1), 3.53 (dd, 1H, J = 10.7, 7.9 Hz, O-CH_2-2), 3.07-3.11 (m, 1H, N-CH), 2.54-2.60 (m, 2H, Ar-CH_2), 2.42 (brs, 2H, NH, OH), 1.81-1.94 (m, 6H, H′-2, H′-3, H′-4-1, H′-5-1), 1.57-1.65 (m, 2H, H′-4-2, H′-5-2), 1.47 (d, 3H, J = 6.5 Hz, CH_3); ^{13}C NMR($CDCl_3$): δ 148.7, 146.0, 132.2, 126.4, 112.9, 112.6, 80.8, 66.1, 56.1, 55.2, 51.9, 32.8, 32.7, 31.7, 24.0, 24.0, 22.3; ESIMS: calcd for $[M+H]^+$ m/z 292.2; found, 292.2.

*4.1.9.3 (7-(cyclopentyloxy)-1-hexyl-6-methoxy-1,2,3,4-tetrahydroisoquinolin-3-yl)-methanol (**32**)*

Compound **32** was obtained as a white solid in 60% yield and contained a pair of enantiomer, of which the ratio was about 5:1 by ^{1}H NMR. ^{1}H NMR ($CDCl_3$): δ 6.70 (s, 1H, Ar-H), 6.56 (s, 1H, Ar-H), 4.73-4.76 (m, 2H, H-1′, *H-1′), 3.98 (d, 1H, J = 6.3 Hz, NH), 3.82 (s, 3H, OCH_3), *3.81 (s, 3H, OCH_3), 3.79 (dd, 1H, J = 10.6, 3.7 Hz, O-CH_2-1), *3.75 (dd, 1H, J = 10.6, 3.8 Hz, O-CH_2-1), 3.52 (dd, 1H, J = 10.6, 8.1 Hz,

O-CH_2-2), *3.46 (dd, 1H, J = 10.4, 8.5 Hz, O-CH_2-2), *3.21-3.26 (m, 1H, N-CH), 3.02-3.07 (m, 1H, N-CH), *2.62 (dd, 1H, J = 16.0, 4.4 Hz, Ar-CH_2-1), 2.51-2.57 (m, 2H, Ar-CH_2), *2.44 (dd, 1H, J = 16.0, 10.7 Hz, Ar-CH_2-2), 1.95-2.00 (m, 1H, Ar-C-CH_2-1), 1.83-1.90 (m, 12H, 3 × CH_2, *3 × CH_2), *1.72-1.76 (m, 1H, Ar-C-CH_2-1), 1.57-1.68 (m, 4H, 2 × CH_2), 1.29-1.47 (m, 16H, 3 × CH_2, *5 × CH_2), 0.88-0.91 (m, 6H, CH_3, *CH_3); ^{13}C NMR($CDCl_3$): δ *148.6, 148.5, 145.8, *145.7, 131.2, 127.0, *125.6, *113.9, 112.8, 112.5, *112.5, 80.8, *80.6, 66.2, *65.8, *56.1, 56.0, 56.0, 55.0, *54.8, *48.9, *36.6, 36.3, 32.8, *32.8, 32.7, 31.9, 31.9, *30.8, 29.6, *29.3, *27.0, 25.3, 24.0, 24.0, *22.7, 22.6, *14.1, 14.1; ESIMS: calcd for $[M+Na]^+$ m/z 384.3; found, 384.2.

*4.1.9.4(1-cyclohexyl-7-(cyclopentyloxy)-6-methoxy-1,2,3,4-tetrahydroisoquinolin-3-yl) methanol (**33**)*

Compound **33** was obtained as a white solid in 55% yield and contained a pair of enantiomer, of which the ratio was about 4:1 by ^{1}H NMR. ^{1}H NMR ($CDCl_3$): δ 6.69 (s, 1H, Ar-H), 6.56 (s, 1H, Ar-H), 4.71-4.76 (m, 1H, H-1′, *H-1′), 3.94 (brs, 1H, Ar-CH-N), 3.81 (s, 3H, OCH_3), *3.80 (s, 3H, OCH_3), 3.78 (dd, 1H, J = 10.6, 3.7 Hz, O-CH_2-1), *3.71 (dd, 1H, J = 10.7, 3.7 Hz, O-CH_2-1), 3.51 (dd, 1H, J = 10.6, 7.7 Hz, O-CH_2-2), *3.47 (dd, 1H, J = 10.6, 7.6 Hz, O-CH_2-2), *3.33-3.38 (m, 1H, N-CH), 2.98-3.06 (m, 1H, N-CH), 2.51 (d, 1H, J = 7.3 Hz, Ar-CH_2), *2.43-2.47 (m, 1H, Ar-CH_2), 1.83-1.90 (m, 14H, CH, *CH, 3 × CH_2, *3 × CH_2), 1.60-1.72 (m, 6H, 3 × CH_2), *1.43-1.52 (m, 6H, 3 × CH_2), 1.31-1.39 (m, 4H, *CH_2, CH_2), 1.13-1.22 (m, 4H, 2 × CH_2), *1.04-1.09 (m, 4H, 2 × CH_2); ^{13}C NMR($CDCl_3$): δ *184.8, 184.3, 145.8,

*145.0, 130.0, *129.6, *129.5, 127.8, *126.0, *125.9, 115.1, 112.9, 112.5, 112.4, 80.6, 66.2, 65.6, 60.7, *59.3, 56.0, 54.8, *50.0, *47.3, 43.3, *42.4, *32.8, 32.8, *32.7, 32.6, 31.9, *31.2, 30.7, *30.2, *29.2, *29.1, 27.1, 26.7, 26.6, *26.5, *26.4, 25.7, *25.4, *24.0, 24.0, *24.0, 24.0; ESIMS: calcd for $[M+H]^+$ m/z 360.3; found, 360.3.

*4.1.9.5 (7-(cyclopentyloxy)-6-methoxy-1-phenyl-1,2,3,4-tetrahydroisoquinolin-3-yl)-methanol (**34**)*

Compound **34** was obtained as a white solid in 57% yield; ^{1}H NMR ($CDCl_3$): δ 7.28-7.35 (m, 5H, Ar′-H), 6.60 (s, 1H, Ar-H), 6.16 (s, 1H, Ar-H), 5.01 (s, 1H, Ar-C*H*-N), 4.41-4.44 (m, 1H, H-1′), 3.82 (s, 3H, OCH_3), 3.75 (dd, 1H, J = 10.8, 3.5 Hz, O-CH_2-1), 3.52 (dd, 1H, J = 10.9, 7.9 Hz, O-CH_2-2), 3.19-3.26 (m, 1H, N-C*H*), 2.67-2.72 (m, 1H, Ar-CH_2-1), 2.60-2.65 (m, 1H, Ar-CH_2-2), 1.70-1.77 (m, 4H, 2 × CH_2), 1.45-1.63 (m, 4H, 2 × CH_2); ^{13}C NMR($CDCl_3$): δ 148.7, 145.6, 144.4, 130.7, 128.9, 128.5, 127.6, 126.8, 114.7, 112.1, 80.3, 66.2, 62.7, 56.1, 55.7, 46.2, 32.7, 32.3, 31.5, 24.0 (two), 11.4; ESIMS: calcd for $[M+H]^+$ m/z 354.2; found, 354.3.

*4.1.10 General procedure for the preparation of **6** and **14-17***

To a solution of compounds **30-34** (1 eq) in 2 mol•L^{-1} NaOH and THF (*V:V* = 1:1) was added benzyl carbonochloridate (2 eq) , respectively. After stirred at r.t. for 24 h, the mixture was neutralized with 2 mol•L^{-1} HCl until pH = 7, filtered and concentrated under reduced pressure. The residue was dissolved in 100 mL of CH_2Cl_2 and then washed with brine (50 mL × 2), dried over Na_2SO_4, and concentrated to dryness. The residue was purified by silica gel column chromatography to provide different products **6** and **14-17**, respectively.

*4.1.10.1 7-(benzyloxy)-8-methoxy-1,5,10,10a-tetrahydro-3H-oxazolo[3,4-b]isoquinolin-3-one (**6**)*

Compound **6** was prepared as a white solid in 81% yield; ^{1}H NMR ($CDCl_3$): δ 7.44 (d, 2H, J = 7.0 Hz, Ar′-H-2, Ar′-H-6), 7.38 (t, 2H, J = 7.3 Hz, Ar′-H-3, Ar′-H-5), 7.31 (t, 1H, J = 7.3 Hz, Ar′-H-4), 6.64 (s, 1H, Ar-H), 6.63 (s, 1H, Ar-H), 5.12 (s, 2H, Ar-OCH_2), 4.69 (d, 1H, J = 16.4 Hz, N-CH_2-1), 4.57 (t, 1H, J = 8.2 Hz, O-CH_2-1), 4.24 (d, 1H, J = 16.4 Hz, N-CH_2-2), 4.13 (dd, 1H, J = 8.6, 5.0 Hz, O-CH_2-2), 3.91-3.96 (m, 1H, N-CH), 3.87 (s, 3H, OCH_3), 2.78-2.84 (m, 2H, Ar-CH_2); ^{13}C NMR ($CDCl_3$): δ 157.4, 148.7, 147.5, 136.8, 128.6 (two), 128.0, 127.3 (two), 124.2, 123.3, 112.6, 111.9, 71.2, 68.4, 56.1, 51.3, 42.8, 33.6; HRESIMS calcd for $C_{19}H_{19}NO_4Na$ 348.1212; found 348.1230.

*4.1.10.2 7-(cyclopentyloxy)-8-methoxy-5-methyl-1,5,10,10a-tetrahydro-3H-oxazolo [3,4-b]isoquinolin-3-one (**14**)*

Compound **14** was prepared as a white solid in 80% yield; ^{1}H NMR ($CDCl_3$): δ 6.64 (s, 1H, Ar-H), 6.61 (s, 1H, Ar-H), 4.74-4.77 (m, 1H, H-1′), 4.67-4.70 (m, 1H, Ar-CH-N), 4.50 (t-like, 1H, J = 14.2 Hz, O-CH_2-1), 3.94-4.00 (m, 2H, N-CH, O-CH_2-2), 3.83 (s, 3H, OCH_3), 2.76-2.86 (m, 2H, Ar-CH_2), 1.80-1.98 (m, 6H, H′-2, H′-3, H′-4-1, H′-5-1), 1.61-1.66 (m, 2H, H′-4-2, H′-5-2), 1.59 (dd, 3H, J = 6.4, 3.1 Hz, CH_3); ^{13}C NMR($CDCl_3$): δ 157.1, 148.9, 147.0, 130.4, 123.5, 113.4, 112.4, 80.7, 68.2, 56.2, 54.7, 50.6, 33.8, 32.8, 32.7, 24.0 (three); HRESIMS calcd for $C_{18}H_{23}NO_4Na$ 340.1525; found 340.1542.

4.1.10.3 7-(cyclopentyloxy)-5-hexyl-8-methoxy-1,5,10,10a-tetrahydro-3H-oxazolo

*[3,4-b]isoquinolin-3-one (**15**)*

Compound **15** was prepared as a white solid in 82% yield and contained a pair of isomers, of which the ratio was about 5:1 by ^{1}H NMR. ^{1}H NMR ($CDCl_3$): δ *6.64 (s, 1H, Ar-H), 6.62 (s, 1H, Ar-H), 6.61 (s, 1H, Ar-H), *6.55 (s, 1H, Ar-H), *4.79 (dd, 1H, J = 9.6, 3.7 Hz, Ar-C*H*-N), 4.74-4.77 (m, 2H, H-1′, * H-1′), 4.72 (dd, 1H, J = 6.0, 3.0 Hz, Ar-C*H*-N), *4.54 (t, 1H, J = 8.5 Hz, O-CH_2-1), 4.47-4.50 (m, 1H, O-CH_2-1), *4.14 (dd, 1H, J = 8.6, 3.1 Hz, O-CH_2-2), *4.01-4.06 (m, 1H, N-C*H*), 3.91-3.98 (m, 2H, O-CH_2-2, N-C*H*), 2.73-2.83 (m, 4H, Ar-CH_2, *Ar-CH_2), *2.27-2.33 (m, 5H, Ar-C-CH_2-1, 2 × CH_2), 1.78-1.95 (m, 9H, Ar-C-CH_2-1, 4 × CH_2), *1.67-1.76 (m, 6H, 3 × CH_2), 1.61-1.64 (m, 2H, CH_2), *1.26-1.33 (m, 7H, Ar-C-CH$_2$-2, 3 × CH_2), 1.15-1.22 (m, 7H, Ar-C-CH_2-2, 3 × CH_2), *0.88 (t, 3H, J = 7.0 Hz, CH_3), 0.82 (t, 3H, J = 6.9 Hz, CH_3); ^{13}C NMR($CDCl_3$): δ 157.2, *156.9, *149.0, 148.8, 146.6, *146.5, 128.3, *128.2, 124.8, *123.3, 113.9, *113.5, *112.3, 112.1, *80.6, 80.6, *68.2, 68.2, 56.1, *56.0, 54.7, 54.6, *52.5, *48.5, *37.2, 35.1, 33.7, *33.5, 32.8, *32.7, 32.7, 31.7, *29.1, 29.0, *26.0, 24.0 (two), *24.0 (two), 23.0, *22.6, 22.5, *14.0, 14.0; HRESIMS calcd for $C_{23}H_{33}NO_4Na$ 410.2307; found 410.2325.

*4.1.10.4 5-cyclohexyl-7-(cyclopentyloxy)-8-methoxy-1,5,10,10a-tetrahydro-3H-oxazolo[3,4-b]isoquinolin-3-one (**16**)*

Compound **16** was prepared as a white solid in 80% yield and contained a pair of isomers, of which the ratio was about 2:1 by ^{1}H NMR. ^{1}H NMR ($CDCl_3$): δ 6.71 (s, 1H, Ar-H), *6.62 (s, 1H, Ar-H), *6.59 (s, 1H, Ar-H), 6.55 (s, 1H, Ar-H), 4.73-4.78 (m, 2H, H-1′, *H-1′), 4.69 (d, 1H, J = 4.1 Hz, Ar-CH_2-N), 4.58 (d, 1H, J = 3.3 Hz,

Ar-CH_2-N), 4.54 (t, 1H, J = 8.2 Hz, O-CH_2-1), *4.45 (t, 1H, J = 7.3 Hz, O-CH_2-1), 4.11 (dd, 1H, J = 8.6, 2.5 Hz, O-CH_2-2), 4.02-4.06 (m, 1H, N-CH), *3.98 (dd, 1H, J = 11.5, 7.9 Hz, O-CH_2-2), *3.90-3.93 (m, 1H, N-CH), *3.84 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 2.72-2.82 (m, 2H, Ar-CH_2, *Ar-CH_2), 1.82-1.93 (m, 13H, CH, 6 × CH_2), *1.60-1.69 (m, 13H, CH, 6 × CH_2), *1.45-1.47 (m, 2H, CH_2), 1.29-1.33 (m, 2H, CH_2), 1.00-1.07 (m, 4H, 2 × CH_2), *0.88-1.00 (m, 4H, 2 × CH_2); ^{13}C NMR($CDCl_3$): δ 158.0, *156.7, 149.0, *148.8, 146.3, *145.6, 126.6, *126.0, *125.6, 124.3, *115.6, 113.8, 112.2, *112.2, 80.6, *80.6, *68.1, 67.9, *59.9, 57.2, *56.0, 56.0, *55.1, 50.6, 45.2, *41.6, *33.6, 33.3, *32.9, *32.8, 32.7, 32.6, 30.9, *30.4, 28.2, 26.6, *26.5, 26.4, *26.3, 26.3, *26.1, *26.0, *24.1, *24.1, 24.0, 24.0; HRESIMS calcd for $C_{23}H_{31}NO_4Na$ 408.2151; found 408.2173.

*4.1.10.5 7-(cyclopentyloxy)-8-methoxy-5-phenyl-1,5,10,10a-tetrahydro-3H-oxazolo [3,4-b]isoquinolin-3-one (**17**)*

Compound **17** was prepared as a white solid in 81% yield; ^{1}H NMR ($CDCl_3$): δ 7.27-7.34 (m, 5H, Ar′-H), 6.65 (s, 1H, Ar-H), 6.44 (s, 1H, Ar-H), 5.98 (s, 1H, Ar-CH-N), 4.56-4.59 (m, 1H, H-1′), 4.53 (t, 1H, J = 8.3 Hz, O-CH_2-1), 4.12 (dd, 1H, J = 8.6, 4.1 Hz, O-CH_2-2), 4.00-4.05 (m, 1H, N-CH), 3.87 (s, 3H, OCH_3), 2.92-2.95 (m, 2H, Ar-CH_2), 1.80-1.85 (m, 2H, CH_2), 1.70-1.77 (m, 4H, 2 × CH_2), 1.49-1.58 (m, 2H, CH_2); ^{13}C NMR($CDCl_3$): δ 156.6, 149.4, 146.6, 142.1, 128.6 (two), 128.5 (two), 127.9, 125.6, 124.4, 114.8, 112.0, 80.4, 68.5, 56.1, 55.9, 48.1, 33.9, 32.6, 32.5, 24.0 (two); HRESIMS calcd for $C_{23}H_{25}NO_4Na$ 402.1681; found 402.1692.

4.1.11 7-hydroxy-8-methoxy-1,5,10,10a-tetrahydro-3H-oxazolo[3,4-b]isoquinolin-

*3-one (**35**)*

Compound **6** (1.5 g, 4.61 mmol) was dissolved in CH_2Cl_2 and CH_3OH (*V*:*V* = 1:1) and then palladium/carbon (60 mg, 0.56 mmol) was added. After stirred at room temperature under H_2 at atmospheric pressure for 5 h, the mixture was filtered and concentrated *in vacuo*. Then the residue was purified by silica gel column chromatography (10:1, *V*:*V*, Chloroform-Methanol) to afford **35** as a white solid (995 mg, 92%); ^{1}H NMR ($CDCl_3$): δ 8.93 (s, 1H, Ar-O*H*), 6.68 (s, 1H, Ar-H), 6.58 (s, 1H, Ar-H), 4.49 (t, 1H, *J* = 8.2 Hz, O-CH_2-1), 4.44 (d, 1H, *J* = 16.4 Hz, N-CH_2-1), 4.15 (d, 1H, *J* = 16.4 Hz, N-CH_2-2), 4.08 (dd, 1H, *J* = 8.6, 5.2 Hz, O-CH_2-2), 3.93-3.89 (m, 1H, N-C*H*), 3.70 (s, 3H, OCH_3), 2.81 (dd, 1H, *J* = 15.3, 4.0 Hz, Ar-CH_2-1), 2.63 (dd, 1H, *J* = 15.3, 11.1 Hz, Ar-CH_2-2); ^{13}C NMR($CDCl_3$): δ 157.3, 146.9, 145.7, 123.8, 122.9, 113.3, 113.1, 68.6, 56.0, 51.2, 42.5, 33.0; HRESIMS calcd for $C_{12}H_{13}NO_4Na$ 258.0742; found 258.0760.

*4.1.12 General procedure for the preparation of **7-13***

To a solution of compound **35** (1 eq) in dry DMF was added K_2CO_3 (4 eq) and different haloalkane or halocycloalkane (3 eq), which then heated at 55 °C under argon. After stirred for 8 h, the mixture was filtrated and concentrated *in vacuo*. The dried residue was dissolved in 100 mL of CH_2Cl_2 and then washed with H_2O (50 mL × 2), saturated aqueous $NaHCO_3$ (50 mL × 2), and brine (50 mL × 2), dried over Na_2SO_4, and concentrated to dryness. The residue was purified by silica gel column chromatography to give different products **7-13**, respectively.

4.1.12.1 7,8-dimethoxy-1,5,10,10a-tetrahydro-3H-oxazolo[3,4-b]isoquinolin-3-

*one (**7**)*

Compound **7** was obtained as a white solid in 95% yield; ^{1}H NMR ($CDCl_3$): δ 6.61 (s, 1H, Ar-H), 6.60 (s, 1H, Ar-H), 4.76 (d, 1H, J = 16.3 Hz, N-CH_2-1), 4.59 (t, 1H, J = 8.2 Hz, O-CH_2-1), 4.31 (d, 1H, J = 16.4 Hz, N-CH_2-2), 4.15 (dd, 1H, J = 8.5, 5.0 Hz, O-CH_2-2), 3.94-3.99 (m, 1H, N-CH), 3.87 (s, 6H, 2 × OCH_3), 2.83 (d, 2H, J = 7.1 Hz, Ar-CH_2); ^{13}C NMR($CDCl_3$): δ 157.4, 148.3, 147.9, 123.5, 123.3, 111.9, 108.9, 68.4, 56.0, 55.9, 51.3, 42.8, 33.6; HRESIMS calcd for $C_{13}H_{15}NO_4Na$ 272.0899; found 272.0912.

*4.1.12.2 8-methoxy-7-propoxy-1,5,10,10a-tetrahydro-3H-oxazolo[3,4-b]isoquinolin-3-one (**8**)*

Compound **8** was obtained as a white solid in 96% yield; ^{1}H NMR ($CDCl_3$): δ 6.62 (s, 1H, Ar-H), 6.60 (s, 1H, Ar-H), 4.74 (d, 1H, J = 16.4 Hz, N-CH_2-1), 4.58 (t, 1H, J = 8.2 Hz, COO-CH_2-1), 4.29 (d, 1H, J = 16.4 Hz, N-CH_2-2), 4.14 (dd, 1H, J = 8.6, 5.0 Hz, COO-CH_2-2), 3.90-4.00 (m, 3H, N-CH, Ar-OCH_2), 3.84 (s, 3H, OCH_3), 2.82 (d, 2H, J = 6.4 Hz, Ar-CH_2), 1.83-1.89 (m, 2H, CH_2), 1.04 (t, 3H, J = 7.4 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 157.4, 148.3, 147.9, 123.4, 123.3, 112.3, 110.6, 70.6, 68.4, 56.1, 51.3, 42.8, 33.6, 22.4, 10.4; HRESIMS calcd for $C_{15}H_{19}NO_4Na$ 300.1212; found 300.1240.

*4.1.12.38-methoxy-7-(pentyloxy)-1,5,10,10a-tetrahydro-3H-oxazolo[3,4-b]isoquinolin-3-one (**9**)*

Compound **9** was obtained as a white solid in 97% yield; ^{1}H NMR ($CDCl_3$): δ 6.62 (s, 1H, Ar-H), 6.60 (s, 1H, Ar-H), 4.74 (d, 1H, J = 16.4 Hz, N-CH_2-1), 4.58 (t, 1H, J =

8.4 Hz, COO-CH_2-1), 4.29 (d, 1H, J = 16.4 Hz, N-CH_2-2), 4.14 (dd, 1H, J = 8.6, 5.0 Hz, COO-CH_2-2), 3.93-4.00 (m, 3H, N-CH, Ar-OCH_2), 3.84 (s, 3H, OCH_3), 2.80-2.84 (m, 2H, Ar-CH_2), 1.81-1.87 (m, 2H, CH_2), 1.36-1.47 (m, 4H, 2 × CH_2), 0.93 (t, 3H, J = 7.2 Hz, CH_3); ^{13}C NMR($CDCl_3$): δ 157.4, 148.2, 147.8, 123.3, 123.2, 112.3, 110.4, 69.1, 68.4, 56.1, 51.3, 42.8, 33.6, 28.8, 28.0, 22.4, 14.0; HRESIMS calcd for $C_{17}H_{23}NO_4Na$ 328.1525; found 318.1548.

*4.1.12.4 7-(hexyloxy)-8-methoxy-1,5,10,10a-tetrahydro-3H-oxazolo[3,4-b]isoquinolin -3-one (**10**)*

Compound **10** was obtained as a white solid in 96% yield; ^{1}H NMR ($CDCl_3$): δ 6.61 (s, 1H, Ar-H), 6.60 (s, 1H, Ar-H), 4.74 (d, 1H, J = 16.3 Hz, N-CH_2-1), 4.58 (t, 1H, J = 8.3 Hz, COO-CH_2-1), 4.23 (d, 1H, J = 16.4 Hz, N-CH_2-2), 4.14 (dd, 1H, J = 8.6, 5.0 Hz, COO-CH_2-2), 3.94-3.99 (m, 3H, N-CH, Ar-OCH_2), 3.84 (s, 3H, O-CH_3), 2.83 (d, 2H, J = 6.8 Hz, Ar-CH_2), 1.81-1.86 (m, 2H, CH_2), 1.43-1.48 (m, 2H, CH_2), 1.33-1.37 (m, 4H, 2 × CH_2), 0.91 (t, 3H, J = 7.1 Hz, CH_3); ^{13}C NMR($CDCl_3$): δ 157.4, 148.3, 147.9, 123.4, 123.3, 112.3, 110.6, 69.2, 68.4, 56.1, 51.3, 42.8, 33.6, 31.5, 29.1, 25.6, 22.5, 14.0; HRESIMS calcd for $C_{18}H_{25}NO_4Na$ 342.1681; found 342.1705.

*4.1.12.5 7-(cyclopropylmethoxy)-8-methoxy-1,5,10,10a-tetrahydro-3H-oxazolo[3,4-b] isoquinolin-3-one (**11**)*

Compound **11** was obtained as a white solid in 95% yield; ^{1}H NMR ($CDCl_3$): δ 6.61 (s, 1H, Ar-H), 6.60 (s, 1H, Ar-H), 4.73 (d, 1H, J = 16.3 Hz, N-CH_2-1), 4.58 (t, 1H, J = 8.3 Hz, COO-CH_2-1), 4.27 (d, 1H, J = 16.5 Hz, N-CH_2-2), 4.14 (dd, 1H, J = 8.6, 5.0 Hz, COO-CH_2-2), 3.93 (m, 1H, N-CH), 3.85 (s, 3H, OCH_3), 3.82 (d, 2H, J = 7.0 Hz,

Ar-OCH_2), 2.78-2.84 (m, 2H, Ar-CH_2), 1.29-1.36 (m, 1H, CH), 0.65 (tt, 2H, J = 5.0, 1.1 Hz, CH_2), 0.35 (t, 2H, J = 5.0 Hz, CH_2); ^{13}C NMR($CDCl_3$): δ 157.3, 148.7, 148.0, 123.8, 123.4, 112.6, 111.6, 71.3, 68.4, 56.1, 51.3, 42.8, 33.6, 10.3, 3.33, 3.31; HRESIMS calcd for $C_{16}H_{19}NO_4Na$ 312.1212; found 312.1236.

*4.1.12.6 7-(cyclopentyloxy)-8-methoxy-1,5,10,10a-tetrahydro-3H-oxazolo[3,4-b]-isoquinolin-3-one (**12**)*

Compound **12** was obtained as a white solid in 93% yield; ^{1}H NMR ($CDCl_3$): δ 6.62 (s, 1H, Ar-H), 6.60 (s, 1H, Ar-H), 4.73-4.75 (m, 2H, H′-1, H-1-a), 4.58 (t, 1H, J = 8.1 Hz, O-CH_2-1), 4.28 (d, 1H, J = 16.3 Hz, H-1-b), 4.14 (dd, 1H, J = 8.6, 4.9 Hz, O-CH_2-2), 3.98-3.94 (m, 1H, N-CH), 3.82 (s, 3H, OCH_3), 2.82 (d, 2H, J = 6.8 Hz, Ar-CH_2), 1.81-1.94 (m, 6H, H′-2, H′-3, H′-4-1, H′-5-1), 1.59-1.65 (m, 2H, H′-4-2, H′-5-2); ^{13}C NMR($CDCl_3$): δ 157.4, 149.0, 147.0, 123.4, 123.3, 112.6, 112.5, 80.6, 68.5, 56.2, 51.3, 42.8, 33.6, 32.8, 32.7, 24.0 (two); HRESIMS calcd for $C_{17}H_{21}NO_4Na$ 326.1368; found 326.1382.

*4.1.12.7 7-(cyclohexyloxy)-8-methoxy-1,5,10,10a-tetrahydro-3H-oxazolo[3,4-b]-isoquinolin-3-one (**13**)*

Compound **13** was obtained as a white solid in 90% yield; ^{1}H NMR ($CDCl_3$): δ 6.67 (s, 1H, Ar-H), 6.61 (s, 1H, Ar-H), 4.74 (d, 1H, J = 16.3 Hz, N-CH_2-1), 4.58 (t, 1H, J = 8.1 Hz, O-CH_2-1), 4.28 (d, 1H, J = 16.4 Hz, N-CH_2-2), 4.13-4.17 (m, 2H, O-CH_2-2, O-CH), 3.94-4.00 (m, 1H, N-CH), 3.83 (s, 3H, OCH_3), 2.80-2.87 (m, 2H, Ar-CH_2), 1.98-2.06 (m, 2H, CH_2), 1.79-1.87 (m, 2H, CH_2), 1.53-1.61 (m, 2H, CH_2), 1.26-1.38 (m, 4H, 2 × CH_2); ^{13}C NMR ($CDCl_3$): δ 157.4, 149.8, 146.6, 124.2, 123.4, 114.6,

113.1, 77.6, 68.4, 56.2, 51.4, 42.8, 33.7, 32.0, 31.9, 25.6, 24.0, 23.9; HRESIMS calcd for $C_{18}H_{23}NO_4Na$ 340.1525; found 340.1543.

4.2. Assay of human PDE4 activity

A standard PDE assay was conducted as described previously.[3, 29] The enzyme was prepared from U937 cells which was derived from human monocytes, and was stored at -20 ºC after preparation. Measurement of PDE4 activity was performed using this stored enzyme after it was diluted with distilled water containing bovine serum albumin. The substrate solution was prepared by adding [^{3}H]-cAMP (300,000 dpm (5000 Bq)/ assay) and 100 µmol/L cAMP solution to 100 mmol/L Tris-HCl (pH 8.0) containing 5 mmol/L ethylene glycol-bis (*β*-aminoethyl ether) and *O*,*O*'-bis(2-aminoethyl)ethyleneglycol-*N*,*N*,*N*',*N*'-tetraacetic acid. The substrate solution was mixed with the enzyme solution containing a test compound dissolved in DMSO, and incubation was done for 30 min at 30 ºC. Assays were performed in duplicate at different concentrations of each test compound.

4.3. Assay of TNF-*α* release.

The blood is mixed with saline at a ratio of 1:1, and the peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats using Lymphoprep tubes.[30] The PBMCs were suspended in RPMI 1640 with 0.5% human serum albumin, pen/ strep, and 2 mM L-glutamine at 5×10^5 cells/mL. The cells were pre-incubated with the test compounds in 96-well plates for 30 min and stimulated for 18 h with 1 mg/mL lipopolysaccharide. TNF-*α* concentration in the supernatants was measured by homogeneous time-resolved fluorescence resonance (TR-FRET). The assay is

quantified by measuring fluorescence at 665 nm (proportional to TNF-α concentration) and 620 nm (control). Results are expressed as IC_{50} values (μM).

4.4 LPS induced sepsis for measurement of TNF-α inhibition in mice

The LPS induced sepsis model in mice was performed following the literature.[31] Female Swiss albino mice were selected according to the body weights, which were equivalent within each group. The mice were fasted for 20 h with free access to water and dosed for oral administration (po) with the test compounds suspended in vehicle containing 0.5% Tween 80 in 0.25% sodium salt of carboxymethyl cellulose. The control mice were performed the vehicle alone. After 30 min of oral dosing, the mice were injected into intraperitoneal cavity with 500 μg of lipopolysaccharide (*Escherichia coli*, LPS: B4 from Sigma) in phosphate buffer. Then the mice were bled via retro-orbital sinus puncture after 90 min of LPS administration. Serum samples were collected by centrifuging the blood samples at 4000 rpm for 20 min, which were stored overnight at 4 ºC. Immediately, the serum samples were checked for TNF-α levels using commercial mouse TNF-α ELISA kit (Amersham Biosciences) and assay was carried out following the manufacturer instruction.

4.5 LPS induced neutrophilia model for asthma and COPD

LPS induced neutrophilia in Sprague Dawley rats was performed using the protocol described.[31] Male Sprague Dawley rats were acclimatized to laboratory conditions for one week prior to the experiment. According to the body weight, the rats were distributed to various groups randomly. Except normal group, all the rats were exposed to 100 μg/mL lipopolysaccharide (*E. coli*, LPS: B4 from Sigma) for 40 min.

The rats were dosed with the test compound suspended in the vehicle containing 0.25% carboxymethyl cellulose before half an hour of LPS exposure. BAL was performed 6 h after LPS exposure, total cell count and DLC was done and compared with control and the standard drug. Percentage inhibition for neutrophilia was calculated and was shown in Table 2.

4.6 Molecular docking

Molecular docking was performed on Surflex-Dock module of Sybyl 8.0.[3, 25] Crystal structure of PDE4B (PDB ID: 1XMY) obtained from Protein Date Bank was used as the receptor for molecular docking study. The 3D structure of compounds **12** and **17** was drawn and optimized with SYBYL package. The docking procedure was started with the protomol generation, which was created using a ligand-based approach (native ligand for PDE4B structure). Proto threshold was set to 0.5 and proto bloat was kept at 0 as a default parameter. For docking, max conformation and max rotation values were 20 and 100, respectively. Pre-dock and post-dock energy minimization methods were also applied. Docking results were compared by the total score values. The pose with the higher total-score value was considered as the best one. After the end of molecular docking, the interactions of the docked domain with ligand were analyzed.

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References

1. Chen, H.-J.; Wild, C.; Zhou, X.-B.; Ye, N.; Cheng, X.-D.; Zhou, J. *J. Med. Chem.* **2014**, *57*, 3651.

2. Plisson, F.; Hill, T. A.; Mitchell, J. M.; Hoang, H. N.; de Araujo, A. D.; Xu, W. -J.; Cotterell, A.; Edmonds, D. J.; Stanton, R. V.; Derksen, D. R.; Loria, P. M.; Griffith, D. A.; Price, D. A.; Liras, S.; Fairlie, D. P. *Eur. J. Med. Chem*. **2017**, *127*, 703.

3. Li, Y.-S.; Tian, H.; Zhao, D.-S.; Hu, D.-K.; Liu, X.-Y.; Jin, H.-W.; Song, G.-P.; Cui, Z.-N. *Bioorg. Med. Chem. Lett*. **2016**, *26*, 3632.

4. Lipworth, B. J. *Lancet*. **2005**, *365*, 167.

5. Zhou, Z.-Z.; Ge, B.-C.; Zhong, Q.-P.; Huang, C.; Cheng, Y.-F.; Yang, X.-M.; Wang, H.-T.; Xu, J.-P. *Eur. J. Med. Chem*. **2016**, *124*, 372.

6. Bender, A. T.; Beavo, J. A. *Pharmacol. Rev*. **2006**, *58*, 488.

7. Zhang, H.-T. *Curr. Pharm. Des*. **2009**, *15*, 1688.

8. Abdulrahim, H.; Thistleton, S.; Adebajo, A. O.; Shaw, T.; Edwards, C.; Wells, A. *Expert. Opin. Pharmacother*. **2015**, *16*, 1099.

9. Mulhall, A. M.; Droege, C. A.; Ernst, N. E.; Panos, R. J.; Zafar, M. A. *Expert Opin. Invest. Drugs*. **2015**, *24*, 1597.

10. Song, G.-P.; Zhao, D.-S.; Hu, D.-K.; Li, Y.-S.; Jin, H.-W.; Cui, Z.-N. *Bioorg. Med. Chem. Lett*. **2015**, *25*, 4610.

11. Norman, T. R.; Judd, F. K.; Burrows, G. D.; Aust. N. Z. *J. Psychiatry*. **1992**, *26*,

73.

12. Tashkin, D. P. *Expert. Opin. Pharmacother.* **2014**, *15*, 85.

13. Van der Mey, M.; Boss, H.; Hatzelmann, A.; Van der Laan, I. J.; Sterk, G. J.; Timmerman, H. *J. Med. Chem.* **2002**, *45*, 2520.

14. Hatzelmann, A.; Schudt, C. *J. Pharmacol. Exp. Ther*. **2001**, *297*, 267.

15. Kodimuthali, A.; Jabaris, S. S.; Pal. M. *J. Med. Chem*. **2008**, *51*, 5471.

16. Gràcia, J.; Buil, M. A.; Castro, J.; Eichhorn, P.; Ferrer, M.; Gavaldà, A.; Hernández, B.; Segarra, V.; Lehner, M. D.; Moreno, I.; Pagès, L.; Roberts, R. S.; Serrat, J.; Sevilla, S.; Taltavull, J.; Andrés, M.; Cabedo, J.; Vilella, D.; Calama, E.; Carcasona, C.; Miralpeix, M.; *J. Med. Chem*. **2016**, *59*, 10479.

17. Dietsch, G. N.; DiPalma, C. R.; Eyre, R. J.; Pham, T. Q.; Poole, K. M.; Pefaur, N. B.; Welch, W. D.; Trueblood, E.; Kerns, W. D.; Kanaly, S. T. *Toxicol. Pathol*. **2006**, *34*, 39.

18. Houslay, M. D.; Schafer, P.; Zhang, K.-Y. *Drug. Discov. Today*. **2005**, *10*, 1503.

19. Kranz, M.; Wall, M.; Evans, B.; Miah, A.; Ballantine, S.; Delves, C.; Dombroski, B.; Gross, J.; Schneck, J.; Villa, J. P.; Neu, M.; Somers, D. O. *Bioorg. Med. Chem.* **2009**, *17*, 5336.

20. Robichaud, A.; Stamatiou, P. B.; C. Jin, S.-L.; Lachance, N.; MacDonald, D.; Laliberte, F.; Liu, S.; Huang, Z.; Conti. M.; Chan, C.-C.; *J. Clin. Invest*. **2002**, *110*, 1045.

21. Donnell, A. F.; Dollings, P. J.; Butera, J. A.; Dietrich, A. J.; Lipinski, K. K.; Ghavami, A.; Hirst, W. D. *Bioorg. Med. Chem. Lett*. **2010**, *20*, 2163.

22. Kranz, M.; Wall, M.; Evans, B.; Miah, A.; Ballantine, S.; Delves, C.; Dombroski, B.; Gross, J.; Schneck, J.; P. Villa, J.; Neu, M.; O. Somers, D. *Bioorg. Med. Chem.* **2009**, *17*, 5336.

23. Mungalpara, J.; Thiele, S.; Eriksen, Ø.; Eksteen, J.; Rosenkilde, M. M.; Våbenø, J. *J. Med. Chem*. **2012**, *55*, 10287.

24. Yonezawa, S.; Yamamoto, T.; Yamakawa, H.; Muto, C.; Hosono, M.; Hattori, K.; Higashino, K.; Yutsudo, T.; Iwamoto, H.; Kondo, Y.; Sakagami, M.; Togame, H.; Tanaka, Y.; Nakano, T.; Takemoto, H.; Arisawa, M.; Shuto, S. *J. Med. Chem*. **2012**, *55*, 8838.

25. Song, G.-P.; Shen, X.-T.; Li, S.-M.; Li, Y.-B.; Si, H.-Z.; Fan, J.-H.; Li, J.-H.; Gao, E.-Q.; Liu, S.-W. *Eur. J. Med. Chem.* **2016**, *119*, 109.

26. Chrzanowska, M.; Rozwadowska, M. D. *Chem. Rev*. 2004, *104*, 3341.

27. Zhang, M.-Z.; Zhou, Z.-Z.; Yuan, X.; Cheng, Y.-F.; Bi, B.-T.; Gong, M.-F.; Chen, Y.-P.; Xu, J.-P. *Eur. J. Pharmacol*. **2013**, *721*, 56.

28. Marivet, M. C.; Bourguignon, J. J.; Lugnier, C.; Mann, A.; Stoclet, J. C.; Wermuth, C. G. *J. Med. Chem.* **1989**, *32*, 1450.

29. Huang, W.; Zhang, Y.; Sportsman, J. R. *J. Biomol. Screen*. **2002**, *7*, 215.

30. Kleinman, E. F.; Campbell, E.; Giordano, L. A.; Cohan,V. L.; Jenkinson, T. H.; Cheng, J. B.; Shirley, J. T.; Pettipher, E. R.; Salter, E. D.; Hibbs, T. A.; DiCapua, F. M.; Bordner, J. *J. Med. Chem.* **1998**, *41*, 266.

31. Balasubramania, G.; Narayanan, S.; Andiappan, L.; Sappanimuthu, T.; Thirunavukkarasu, S.; Sundaram, S.; Natarajan, S.; Sivaraman, N.; Rajagopal, S.;

Nazumudeen, F. A. A.; Saxena, S.; Vishwakarma, S. L.; Narayanan, S.; Sharma, G. V. R.; Srinivasan, C. V.; Kilambi, N. *Bioorg. Med. Chem.* **2016**, *24*, 5702.

Figure captions

Figure 1. The designed strategy for the title compounds

Figure 2. ORTEP structure of the title compound **15**, showing 50% probability ellipsoids; H atoms are shown as small spheres of arbitrary radii.

Figure 3. Model of PDE4 and docking of compounds **12** and **17**. The catalytic domain bound to **12** overlaid with rolipram (orange, A). The catalytic domain bound to **12** (B). The catalytic domain bound to **17** (C, D).

Scheme 1. The synthetic route of the title compounds **7-17**. Reagents and conditions: (a) benzyl bromide or bromocyclopentane, K_2CO_3, DMF, 96% for **19**, 97% for **20**; (b) NO_2CH_2COOEt, $(CH_3)_2NH$•HCl, KF, toluene, reflux, 68% for **21**, 55% for **22**; (c) $LiAlH_4$, THF, 69% for **23**, 66% for **24**; (d) carboxylic acid, EDC•HCl, DMAP, CH_2Cl_2, 90% for **25**, 93% for **26** or different chloride, DMAP and pyridine, 88% for **27**, 86% for **28**, 90% for **29**; (e) i: $POCl_3$, toluene, reflux; ii: $NaBH_4$, MeOH, for two steps 56% for **30**, 58% for **31**, 60% for **32**, 55% for **33**, 57% for **34**; (f) CbzCl, 2 M NaOH-THF, 81% for **6**, 80% for **14**, 82% for **15**, 80% for **16**, 81% for **17**; (g) Pb/C, CH_2Cl_2-MeOH, 92%; (h) haloalkane or halocycloalkane, K_2CO_3, DMF, 95% for **7**, 96% for **8**, 97% for **9**, 96% for **10**, 95% for **11**, 93% for **12**, 90% for **13**.

Table 1. Impact on enzymatic potency (PDE4) and inhibition of TNF-α release from human blood mononuclear cells stimulated with lipopolysaccharide

Table 2. Inhibition of various PDEs by compound **12** at 100 μM

Table 3. LPS induced TNF-α in SA mice and neutrophil influx in BALF of SD rats

Graphical Abstract

Rational design of conformationally constrained oxazolidinone-fused 1,2,3,4-tetrahydroisoquinoline derivatives as potential PDE4 inhibitors

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