Journal Pre-proof

Design, Synthesis, and Biological evaluation of Tetrahydroisoquinolines Derivatives as Novel, Selective PDE4 Inhibitors for Antipsoriasis Treatment

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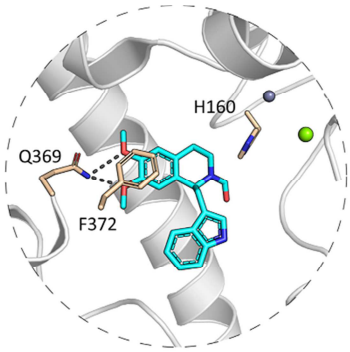
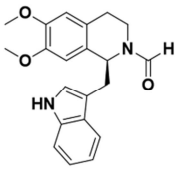
Graphical Abstract



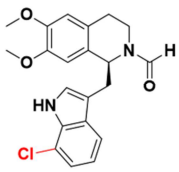


1

PDE4D IC_{50} = 0.14 μM
TNF-α IC_{50} = 33.34 μM
Caco-2 Mean F_{abs} = 100%
Efflux ratio = 1.47

Structure-based SAR

51 new compounds



36

PDE4D IC_{50} = 0.36 μM
TNF-α IC_{50} = 6.76 μM
Caco-2 Mean F_{abs} = 100%
Efflux ratio = 0.44
Selectivity index > 13 over PDE1-3,6
> 55 over PDE5,7-9,11
2%, 4% ointment, effective treatment for psoriasis

Title:

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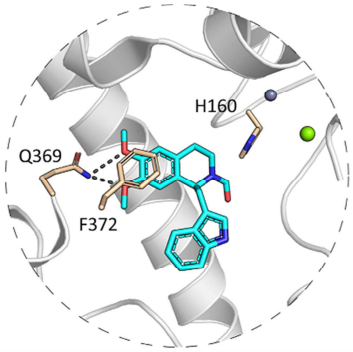

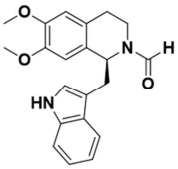

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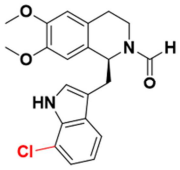

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2%, 4% ointment, effective treatment for psoriasis

Highlights:

- 51 tetrahydroisoquinolines derivatives as novel PDE4 inhibitors were designed, synthesized, and evaluated.
- The structure-activity relationship (SAR) of this novel series of compounds was demonstrated.
- The optimum compound **36** exhibited high safety, potency (PDE4D IC_{50} = 0.36 μM, TNF-α IC_{50} = 6.76 μM), permeability and selectivity *in vitro*.
- The crystal structure of PDE4D in complex with compound **36** was solved.
- Compound **36** exhibited superior therapeutic efficacy compared with calcipotriol against the IMQ-induced murine psoriasis-like skin inflammation.

Design, Synthesis, and Biological evaluation of Tetrahydroisoquinolines Derivatives as Novel, Selective PDE4 Inhibitors for Antipsoriasis Treatment

Rui Zhang[a,b,#], Heng Li[b,c,#], Xianglei Zhang[b,d,#], Jian Li[a], Haixia Su[b,d], Qiukai Lu[b,c], Guangyu Dong[a], Huixia Dou[b,d], Chen Fan[c], Zhanni Gu[a], Qianwen Mu[b,d], Wei Tang*[,b,c], Yechun Xu*[,b,d] and Hong Liu*[,a,b]

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[c] Laboratory of Anti-inflammation and Immunopharmacology, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Shanghai 201203, China.

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[#] These authors contributed equally to this study.

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Abstract

Psoriasis is a kind of chronic inflammatory skin disorder, while the long-term use of conventional therapies for this disease are limited by severe adverse effects. Novel small molecules associated with new therapeutic mechanisms are greatly needed. It is known that phosphodiesterase 4 (PDE4) plays a central role in regulating inflammatory responses through hydrolyzing intracellular cyclic adenosine monophosphate (cAMP), making PDE4 to be an important target for the treatment of inflammatory diseases (e.g. psoriasis). In our previous work, we identified a series of novel PDE4 inhibitors with a tetrahydroisoquinoline scaffold through structure-based drug design, among which compound **1** showed moderate inhibition activity against PDE4. In this study, a series of novel tetrahydroisoquinoline derivatives were developed based on the crystal structure of PDE4D in complex with compound **1**. Anti-inflammatory effects of these compounds were evaluated, and compound **36,** with high safety, permeability and selectivity, exhibited significant inhibitory potency against the enzymatic activity of PDE4D and the TNF-α release from the LPS-stimulated RAW 264.7 and hPBMCs. Moreover, an *in vivo* study demonstrated that a topical administration of **36** achieved more significant efficacy than calcipotriol to improve the features of psoriasis-like skin inflammation. Overall, our study provides a basis for further development of tetrahydroisoquinoline-based PDE4 inhibitors against psoriasis.

Keywords: PDE4 inhibitors, tetrahydroisoquinoline, antipsoriasis

1. Introduction

Psoriasis is a kind of chronic inflammatory skin disorder, the pathogenesis of which is complicated and often linked with multiple factors such as autoimmune activation, genetic susceptibility and environment [1]. It is generally accepted that the disturbed interaction between innate and adaptive immune systems is the main cause of psoriasis, which is closely related to the abnormal activation of keratinocytes, dendritic cells, macrophages, and T cells [2-4]. Conventional treatments of psoriasis consist of topical ointments (corticosteroids, emollients, vitamin D, etc.) and systemic therapies (methotrexate, ciclosporin, acitretin, etc.) [5, 6].

However, the drug-drug interactions and severe adverse effects limit the long-term use of these drugs. Moreover, phototherapy and photochemotherapy are also considered as effective strategies for psoriasis patients, however, they are time-consuming and can only be used for short-term prevention of disease deterioration. Recently, biological agents, including infliximab, adalimumab and secukinumab, have been emerged in clinical as efficacious options for psoriasis treatment [7, 8]. Unfortunately, high cost, poor compliance and tolerance limited their wide applications. Compared with the macromolecular antibodies, small molecules possess several advantages [9, 10]. Apart from being cost-friendly and convenient for administration, small molecules can enter cells and selectively inhibit the inflammatory signaling pathways, thereby providing alternative therapeutic strategies for patients who respond inadequately to conventional treatments or targeted biological agents.

Phosphodiesterase 4 (PDE4), known as one of the 11 members of the PDE super-family, is a cyclic adenosine monophosphate (cAMP) specific hydrolase, which is widely expressed in numerous cells, including keratinocytes, endothelial cells, hematopoietic cells, and nerve cells [11]. PDE4 plays a central role in inflammatory responses through the regulation of intracellular cAMP levels and downstream protein kinase A pathways [12], which makes PDE4 to be a primary target for the treatment of inflammatory diseases like psoriasis [13-17]. Apremilast, approved in 2014 by FDA

and in 2015 by the European Commission, is the first oral PDE4 inhibitor used for treating psoriasis and psoriatic arthritis (PsA), which inspires us to seek a new PDE4 inhibitor with higher safety and potency for anti-psoriasis treatment [18-20].

Berberine (**BBR**) is a kind of tetrahydroisoquinoline alkaloid with a proven medicinal history in Chinese medicinal systems [21]. Clinical investigations on **BBR** have demonstrated its broad spectrum of pharmacological effects, including anti-inflammatory effects [22, 23]. Previously, we have built a chemical library comprising intermediates, precursors and multiple analogues of berberine for discovery of lead compounds. Based on this library, we obtained a series of novel PDE4 inhibitors with tetrahydroisoquinoline skeleton utilizing the structure-based drug design strategy, among which compound **1** showed high inhibitory activity against PDE4D (IC_{50} = 0.14 μM) [24, 25]. In this study, a series of novel tetrahydroisoquinoline derivatives were designed and synthesized on the basis of the crystal structure of PDE4D-**1**. Two commonly used inflammatory cell models, mouse monocyte/macrophage leukemia cell line (RAW 264.7) and human peripheral blood mononuclear cells (hPBMCs), were used for *in vitro* anti-inflammatory activity evaluation. The structure-activity relationship (SAR) of these new compounds was explored and compound **36** was evaluated in the imiquimod-induced murine psoriasis model.

2. Results and discussion

2.1. Design

To seek for a more potent PDE4 inhibitor, we got a deep insight into the binding mode of compound **1** with PDE4D (Figure 1A, B). Based on our previous study, the carbonyl group on the tetrahydroisoquinoline core of compound **1** oriented toward the metal binding (MB) site but had no obvious interaction with the two metal ions (Zn^{2+} and Mg^{2+}) (Figure 1B**,** PDB code: 6IMO) [24, 26]. Thus, we replaced the formamide group with larger groups to give compounds **4–20** (Figure 1C), expecting that the extended groups could form stronger interactions with the two ions or surrounding

residues. In addition, 6,7-dimethyoxyl groups of **1** inserted into a hydrophobic pocket (Q-pocket) and both oxygen atoms formed hydrogen bonds (H-bonds) with Q369 (Figure 1B), playing an indispensable role in binding of **1** to PDE4D. We noticed that the cavity of the Q2 pocket (formed by Q369 and F372) was larger than that of the Q1 (formed by N321 and I336) and it was not fully occupied by the 7-methyoxyl substituent [26]. Thus, we introduced ethyoxyl or benzyloxyl groups and synthesized compounds **21-28** (Figure 1C), in order to occupy the Q2 pocket while retain the H-bond interaction. Finally, we turned our attention to the indole group. Even though the crystal structure of the PDE4D-**1** complex demonstrated that the terminal indole ring was flexible and only formed few hydrophobic interactions with M273 and M357, acting as a hydrophobic clamp (HC, Figure 1B), these interactions were essential for it to maintain the PDE4 inhibitory activity. Therefore, we devoted more attention to evaluating the influence of the "tail" on the binding affinity of the compounds with PDE4D (Figure 1C), including the introduction of heterocycles (compounds **29-30**), the change of the linker between the tetrahydroisoquinoline core and the indole ring (compounds **31-32**), and the substituents on the indole ring (compounds **33-52**).

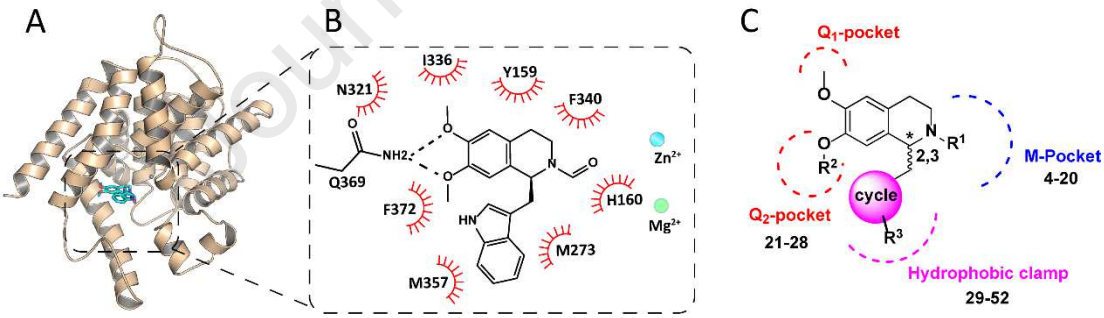


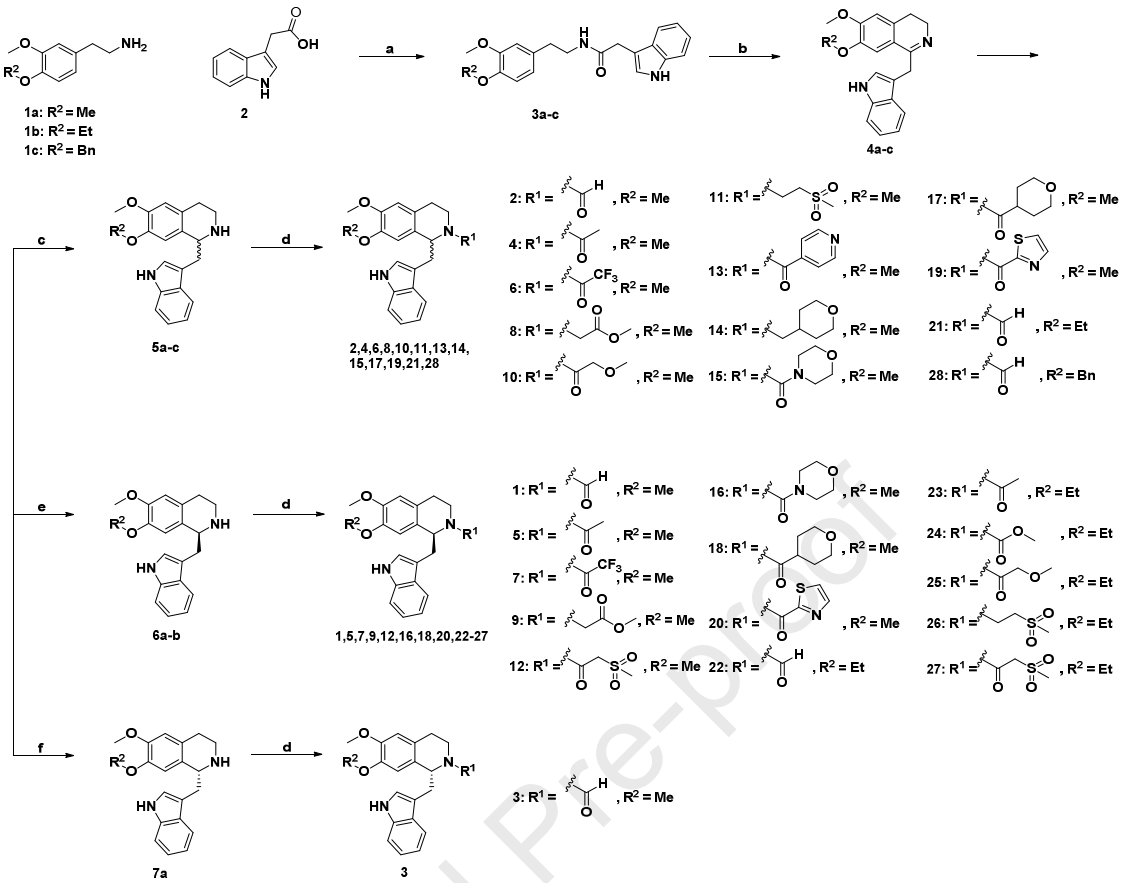
Figure 1. Complex structure of compound 1 with PDE4D catalytic domain. (A) Binding pose of compound **1** in the PDE4D catalytic pocket. Compound **1** and PDE4D are shown as cyan sticks and yellow surface, respectively. (B) Detailed interactions between compound **1** and PDE4D generated by Ligplot. Two H-bonds formed between conserved Q369 and catechol group of compound **1** are shown as black dash lines. (C) Schematic diagram for design of compounds **2-52** based on the analysis of the crystal structure of compound **1** in complex with PDE4D.

It is worth noting that compound **1** possesses a chiral carbon, and our previous study has revealed that the (*S*)-enantiomer is the dominant configuration with higher PDE4 inhibitory activity. Thus, we only investigated the influence of configuration on part of the compounds, while for the others, only (*S*)-enantiomers were synthesized and evaluated. Altogether, 51 new tetrahydroisoquinoline derivatives were synthesized and evaluated as novel PDE4 inhibitors.

2.2. Synthesis of tetrahydroisoquinoline derivatives

The synthetic routes of compounds **1-28** are depicted in Scheme 1. Condensation of 3,4-disubstutituted phenylethylamines (**1a-c**) and 2-(1*H*-indol-2-yl)-acetic acid with HATU afforded the corresponding amide intermediates (**3a-c**). **3a-c** were then treated with $POCl_3$ to afford the dihydroisoquinoline derivatives (**4a-c**). Racemic tetrahydroisoquinolines intermediates **5a-c** were obtained by reduction of **4a-c** with $NaBH_4$. While asymmetric reduction with Noyori catalyst gave the *S*- (**6a-b**) and *R*- (**7a**) isomers respectively. Finally, **5a-c**, **6a-b** and **7a** were reacted with HCOOEt, anhydride, carboxylic acid, halogenide or acyl chloride to afford the target products.

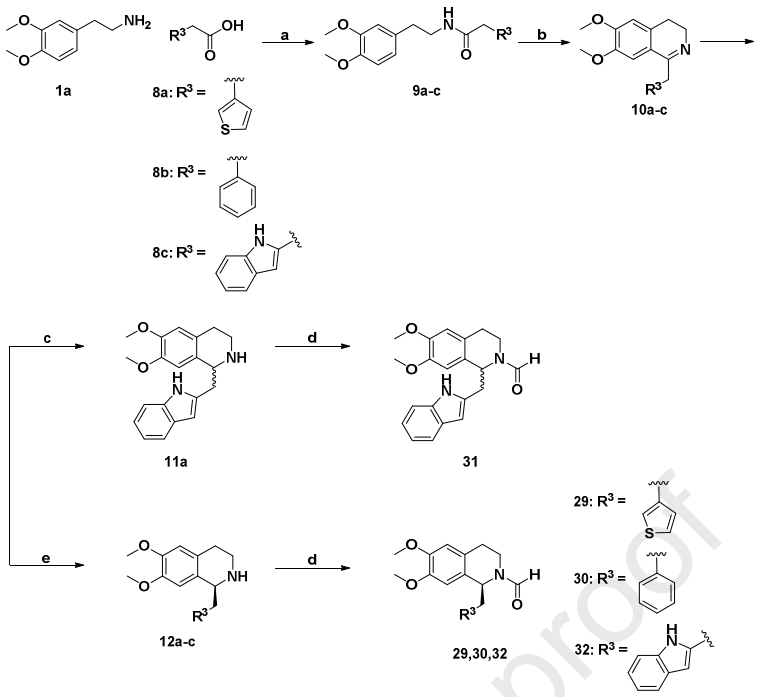
Scheme 1. **Synthetic Methods of Compounds 1-28**[a]



[a]Reagents and conditions: (a) HATU, TEA, DCM, rt, 85%; (b) $POCl_3$, CH_3CN, Ar, 80 °C, 90%; (c) MeOH, $NaBH_4$, 90%; (d) HCOOEt, TEA, 65 °C, or $(RCO)_2O$, TEA, DCM or RX, K_2CO_3, CH_3CN, 80 °C or RCOCl, DIPEA, DCM or RCOOH, HATU, TEA, DCM, 80-88%; (e) $La(OTf)_3$, $AgSbF_6$, (*R*,*R*)-Noyori catalyst, HCOONa, MeOH/H_2O (v/v) = 1:2, Ar, rt; (f) $La(OTf)_3$, $AgSbF_6$, (*S*,*S*)-Noyori catalyst, HCOONa, MeOH/H_2O (v/v) = 1:2, Ar, rt.

The synthetic routes of compounds **29-32** are outlined in Scheme 2. Condensation of 3,4-dimethoxyphenylethylamine with indicated carboxylic acids gave the amide intermediates **9a-c**. The following synthetic steps were similar to that of compound **1**.

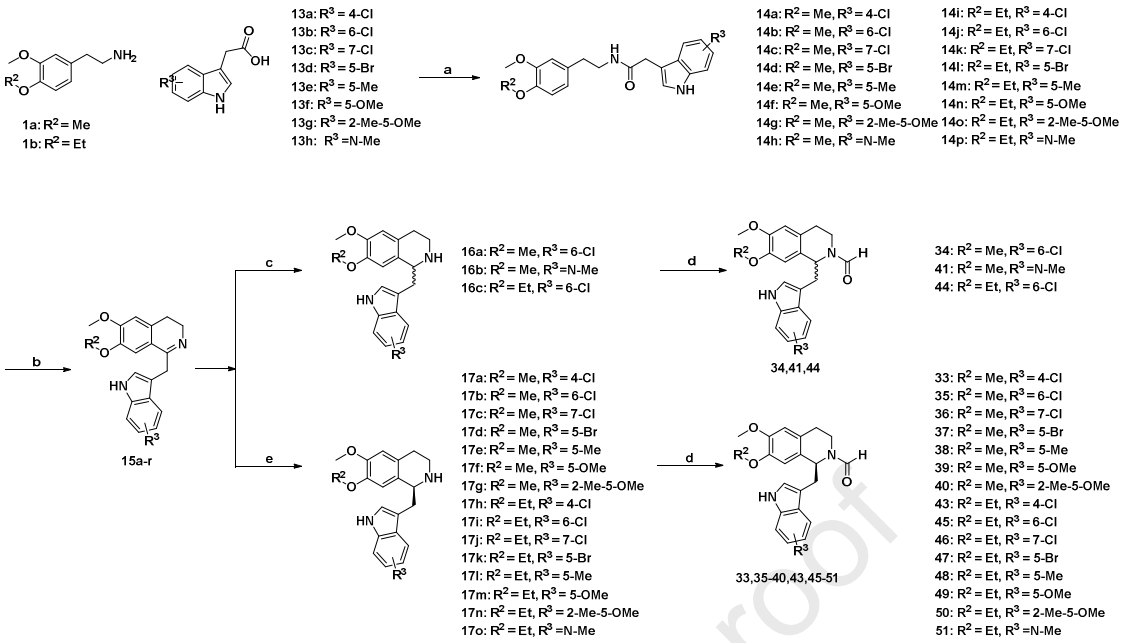
Scheme 2. **Synthetic Methods of Compounds 29-32**[a]



[a]Reagents and conditions: (a) HATU, TEA, DCM, 85%; (b) $POCl_3$, CH_3CN, Ar, 80 °C, 90%; (c) MeOH, $NaBH_4$, 90%; (d) HCOOEt, TEA, 65 °C, 80-88%; (e) $La(OTf)_3$, $AgSbF_6$, (*R*,*R*)-Noyori catalyst, HCOONa, MeOH/H_2O (v/v) = 1:2, Ar, rt.

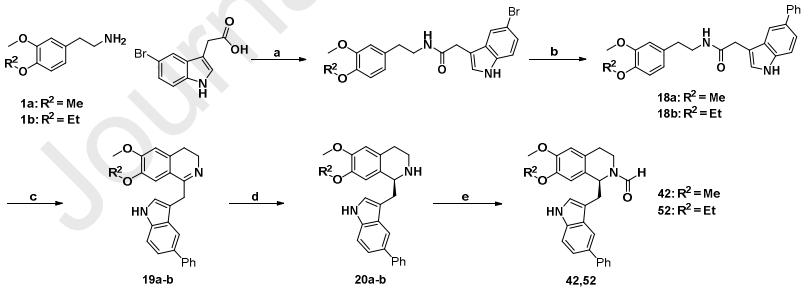
Compounds **33-41**, **43-51** were synthesized using the same methods with compounds **29-32**, only by changing the starting materials (Scheme 3). For compounds **42** and **52**, a benzene ring was introduced through Suziki-coupling [27] to give the amide intermediates **18a-b**. The following steps are showed in Scheme 4.

Scheme 3. **Synthetic Methods of Compounds 33-41, 43-51**[a]



[a]Reagents and conditions: (a) HATU, TEA, DCM, 85%; (b) $POCl_3$, CH_3CN, Ar, 80 °C, 90%; (c) MeOH, $NaBH_4$, 90%; (d) HCOOEt, TEA 65 °C, 80-88%; (e) $La(OTf)_3$, $AgSbF_6$, (*R*,*R*)-Noyori catalyst, HCOONa, MeOH/H_2O (v/v) = 1:2, Ar, rt.

Scheme 4. **Synthetic Methods of Compounds 42, 52**[a]



[a]Reagents and conditions: (a) HATU, TEA, DCM, 85%; (b) Phenylboronic acid, $Pd(dbpf)Cl_2$, K_2CO_3, CH_3CN/H_2O (v/v 1:1), Ar, 60 °C, 90%; (c) $POCl_3$, CH_3CN, Ar, 80 °C, 90%; (d) $La(OTf)_3$, $AgSbF_6$, (*R*,*R*)-Noyori catalyst, HCOONa, MeOH/H_2O (v/v) = 1:2, Ar, rt; (e) HCOOEt, TEA, 65 °C, 80-88%.

2.3. Structure−activity relationships of tetrahydroisoquinoline derivatives

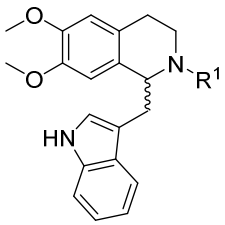
Inhibitory activities against PDE4D of all the synthesized compounds mentioned above were determined using a scintillation proximity assay (SPA) for a preliminary SAR study [28] Meanwhile, TNF-α, known as an important pro-inflammatory

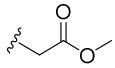
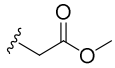
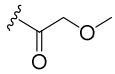
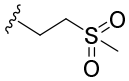
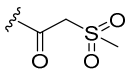
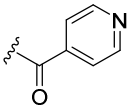
cytokine in the development of inflammation and autoimmune diseases, is mainly produced in monocytes or macrophages. Mouse monocyte/macrophage leukemia cell line (RAW 264.7) is one of the most commonly used inflammatory cell models, which can release a variety of inflammatory factors such as TNF-α after induction and activation by lipopolysaccharide (LPS). Therefore, the inhibition of TNF-α secretion in LPS-stimulated RAW 264.7 cells was measured for further SAR analysis. In addition, their CC_{50} values toward RAW 264.7 were also measured as preliminary safety evaluation indicators.

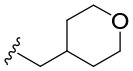
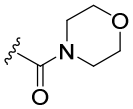
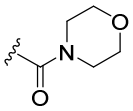
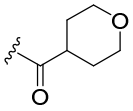
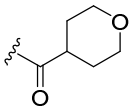
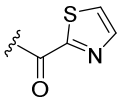
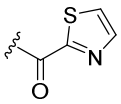
First, the racemic compound **2** and the (*R*)-enantiomer **3** were synthesized to testify the influence of configuration on PDE4D inhibitory activity. As expected, both **2** and **3** showed lower inhibitory activity compared with **1** (**1**, IC_{50} = 0.14 μM; **2**, IC_{50} = 0.39 μM; **3**, IC_{50} = 1.81 μM; Table 1). Then compounds **4-20** were synthesized by replacing the formamide with different amide or alkylamine. Only the compounds with acetamide showed PDE4D inhibitory activity comparable with that of **1** (Table 1). Furthermore, the (*S*)-enantiomer (**5**, IC_{50} = 0.89 μM) exhibited a slightly lower IC_{50} value relative to that of the racemate (**4**, IC_{50} = 1.13 μM). However, the inhibition rate of TNF-α production in RAW 264.7 cells of **4** and **5** (IC_{50} > 50 μM) was not desirable. The analogues **21-28** with 7-ethyoxyl or -benzyloxyl substituents were then prepared in order to to fully fill the Q2 pocket of PDE4D (Table 2). Even though compounds **21-23** (**21**, IC_{50} = 0.075 μM; **22**, IC_{50} = 0.042 μM; **23**, IC_{50} = 0.17 μM) displayed enhanced PDE4D inhibitory compared with the 7-methyoxyl substituted analogues (**2**, **1** and **5**), their inhibitory activities against the TNF-α secretion in RAW 264.7 cells were unexpectedly poor. We speculated that these compounds may degraded rapidly after entering cells, leading to their poor activity at cellular level. Compound **28** with a 7-benzyloxyl substituent showed moderate bioactivity at both enzymatic and cellular levels, with IC_{50} values of 3.57 μM and 36.7 μM respectively. Nevertheless, its cytotoxicity was relatively high (CC_{50} = 53.4 μM), so it was not suitable for a further study. On the other hand, none of compounds **25-27** exhibited satisfactory inhibitory activity against PDE4D. Collectively, formamide or acetamide may be favorable for improving the binding affinity of the compounds with the MB site of

PDE4D, while 7-methyoxyl or 7-ethyoxyl is necessary for keeping the potency of the derivatives.

Table 1. Inhibitory Activities of Compounds 1-20 against PDE4D and TNF-α Secretion in the LPS-stimulated RAW264.7 Cells



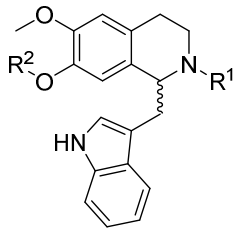
Compds	R^1	Configuration	PDE4D IC_{50} (μM)	RAW264.7 TNF-α IC_{50} (μM)	RAW264.7 CC_{50} (μM)
1		*S*	0.14 ± 0.03	> 50	> 50
2		*Rac*	0.39 ± 0.05	> 50	> 50
3		*R*	1.81 ± 0.18	> 50	> 50
4		*Rac*	1.13 ± 0.21	> 50	> 50
5		*S*	0.89 ± 0.11	> 50	> 50
6		*Rac*	> 1 (-8.2%)[a]	> 50	> 50
7		*S*	> 1 (-4.4%)	> 50	> 50
8		*Rac*	> 1 (-1.8%)	> 50	> 50
9		*S*	> 1 (-3.5%)	> 50	> 50
10		*Rac*	> 1 (34.7%)	> 50	> 50
11		*Rac*	> 1 (42.7%)	> 50	> 50
12		*S*	> 1 (28.0%)	> 50	> 50
13		*Rac*	> 1 (10.4%)	> 50	> 50

14		*Rac*	> 1 (5.0%)	> 50	> 50
15		*Rac*	> 1 (2.8%)	> 50	> 50
16		*S*	> 1 (6.1%)	> 50	> 50
17		*Rac*	> 1 (3.3%)	> 50	> 50
18		*S*	> 1 (10.2%)	> 50	> 50
19		*Rac*	> 1 (-14.7%)	-[b]	-
20		*S*	> 1 (-18.9%)	-	-

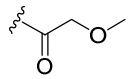
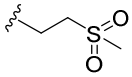
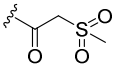
[a] PDE4D inhibition rates at 1 μM were presented in brackets.

[b] Not measured

Table 2. Inhibitory Activities of Compounds 21-28 against PDE4D and TNF-α Secretion in the LPS-stimulated RAW264.7 Cells



Compds	R^1	R^2	Configuration	PDE4D IC_{50} (μM)	RAW264.7 TNF-α IC_{50} (μM)	RAW264.7 CC_{50} (μM)
21		Et	*Rac*	0.075 ± 0.006	> 50	> 50
22		Et	*S*	0.042 ± 0.012	> 50	> 50
23		Et	*S*	0.17 ± 0.02	> 50	> 50
24		Et	*S*	2.40 ± 0.17	> 50	> 50

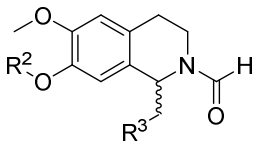
25		Et	*S*	> 1 (22.9%)[a]	> 50	> 50
26		Et	*S*	> 1 (19.4%)	> 50	> 50
27		Et	*S*	> 1 (20.3%)	> 50	> 50
28		Bn	*Rac*	3.57 ± 0.07	36.7	53.4

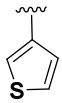
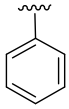
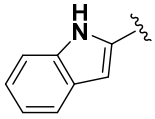
[a] PDE4D inhibition rates at 1 μM were presented in brackets.

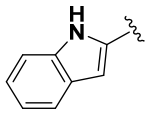
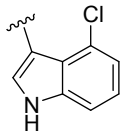
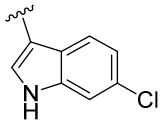
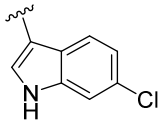
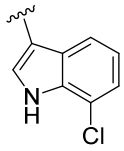
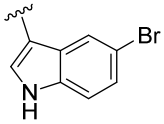
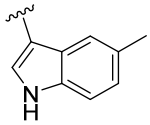
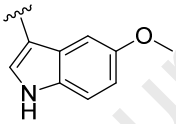
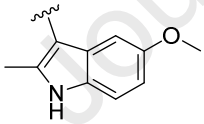
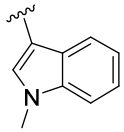
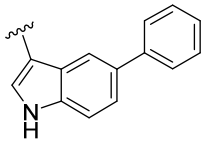
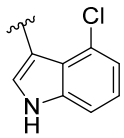
Subsequently, we paid our attention to the tail modification (Table 3). We attempted to replace the indole ring with a benzene or thiophene ring. Unfortunately, the potency of the resulting compounds toward PDE4D almost disappeared. The PDE4D inhibition rate of **29** and **30** at 1 μM were only 6.3 % and 7.3 %, respectively. The inhibitory activity of analogues (**31**, **32**) with a linker between the tetrahydroisoquinoline core and 2-position of indole also decreased drastically. Thus, we retained the original linker and focused on investigating different substitutions on the indole ring. Based on the aforementioned SAR study of the tetrahydroisoquinoline core, we kept the formamide group and synthesized two series of derivatives, compounds **33-42** with the 7-methyoxyl and compounds **43-52** with the 7-ethyoxyl. Compounds **33-37** with a halogen-substituted indole all showed moderate to good potency toward PDE4D. The derivatives with the 6-chloroindole (**34**, IC_{50} = 0.61 μM; **35**, IC_{50} = 0.55 μM) showed comparable inhibitory activity against PDE4D compared with the ones with the 4-chloroindole (**33**, IC_{50} = 0.36 μM) or 7-chloroindole (**36**, IC_{50} = 0.36 μM). Both compounds **33** and **36** also exhibited moderate inhibitory activity in cells (**33**, IC_{50} = 34.9 μM, **36**, IC_{50} = 14.5 μM). By contrast, introduction of 5-bromo gave the less potent derivative (**37**, IC_{50} = 1.03 μM). Even though **37** showed better inhibitory activity against TNF-α release in the RAW264.7 cell than that of **33**, its cytotoxicity was unfortunately higher (CC_{50} = 38.0 μM). Subsequently, we changed the bromine to other groups, like methyl (**38**), methoxyl (**39**), or phenyl (**42**). Among these derivatives, **38** with the 5-methyl group displayed the most potent inhibitory activity against PDE4D, with an IC_{50} value of 0.30 μM, while its inhibition rate of

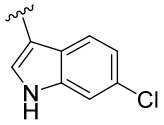
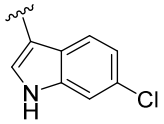
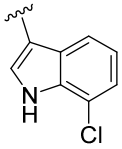
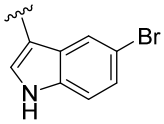
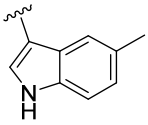
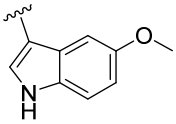
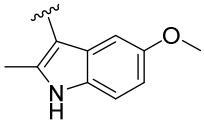
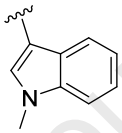
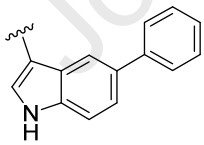
TNF-α production was not satisfactory. Although compound **42** bearing a 5-phenylindole showed lower inhibitory activity at the enzymatic level, its ability to reduce the production of TNF-α was comparable to that of **36**. Meanwhile, the CC_{50} value of **42** was also low. Analogue **40** with 2,5-disubsitituent on the indole ring maintained the PDE4D inhibitory activity yet displayed weak TNF-α inhibition in RAW264.7 cells. When the nitrogen of the indole ring was blocked by a methyl group, the resulting compound **41** exhibited significant decreased ability in inhibiting PDE4D. Among compounds **43-47**, **43** with a 4-chloroindole and **46** with a 7-chloroindole showed moderate inhibitory activity at both the enzymatic and cellular levels, (**43**, PDE4D IC_{50} = 0.81 μM, TNF-α IC_{50} = 37.5 μM; **46**, PDE4D IC_{50} = 0.63 μM, TNF-α IC_{50} = 14.8 μM), but both of them were less potent than their 7-methyoxyl counterparts (**33** and **36**). Replacement of the 5-bromo (**47**) with a 5-methyl (**48**), 5-methoxyl (**49**) or 5-phenyl (**52**) resulted in less potent inhibitors, of which the PDE4D IC_{50} values were more than 1 μM. Compound **50** (IC_{50} = 0.63 μM) with a 2-methyl-5-methoxyl exhibited the PDE4D inhibitory activity more potent than that of **49** but less potent than that of **40** (IC_{50} = 0.61 μM).

Table 3. Inhibitory Activities of Compounds 29-52 against PDE4D and TNF-α Secretion in the LPS-stimulated RAW264.7 Cells



Compds	R^2	R^3	Configuration	PDE4D IC_{50} (μM)	RAW264.7 TNF-α IC_{50} (μM)	RAW264.7 CC_{50} (μM)
29	Me		*S*	> 1 (6.3%)[a]	-[b]	-
30	Me		*S*	> 1 (7.3%)	-	-
31	Me		*Rac*	> 1 (-24.9 %)	> 50	> 50

32	Me		*S*	> 1 (-13.9%)	> 50	> 50
33	Me		*S*	0.36 ± 0.04	34.9	> 50
34	Me		*Rac*	0.61 ± 0.03	> 50	> 50
35	Me		*S*	0.55 ± 0.04	> 50	> 50
36	Me		*S*	0.36 ± 0.02	14.5	> 50
37	Me		*S*	1.03 ± 0.10	29.2	38.0
38	Me		*S*	0.30 ± 0.04	> 50	> 50
39	Me		*S*	1.16 ± 0.19	> 50	> 50
40	Me		*S*	0.61 ± 0.04	> 50	> 50
41	Me		*Rac*	> 1 (23.5%)	> 50	> 50
42	Me		*S*	> 1 (41.5%)	13.8	16.2
43	Et		*S*	0.81 ± 0.07	37.5	> 50

44	Et		*Rac*	> 1 (11.7%)	-	-
45	Et		*S*	> 1 (21.7%)	-	-
46	Et		*S*	0.63 ± 0.08	14.8	45.5
47	Et		*S*	1.41 ± 0.21	> 50	> 50
48	Et		*S*	> 1 (42.8%)	> 50	> 50
49	Et		*S*	> 1 (30.7%)	> 50	> 50
50	Et		*S*	0.63 ± 0.01	> 50	> 50
51	Et		*S*	> 1 (25.3%)	-	-
52	Et		*S*	> 1 (12.3%)	-	-

[a] PDE4D inhibition rates at 1 μM were presented in brackets.

[b] Not measured

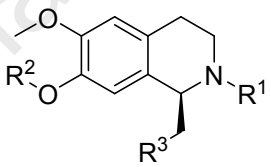
2.4. Inhibition of TNF-α production from hPBMC by the selected compounds

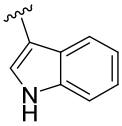
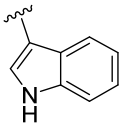
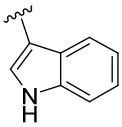
It is known that inhibition of PDE4 would elevate intracellular cAMP levels, which would subsequently reduce the production of TNF-α from human peripheral blood mononuclear cells (hPBMCs) [29]. Consequently, an enzyme-linked immunosorbent assay (ELISA) was used to measure the inhibition of TNF-α

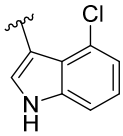
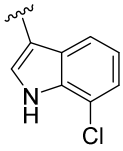
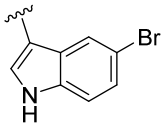
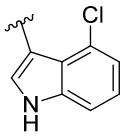
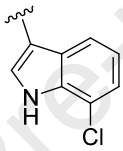
production in the LPS-stimulated hPBMCs, for the compounds with moderate to good inhibitory activity at both the enzymatic and cellular levels.

As shown in Table 4, most of the derivatives exhibited low inhibitory potency on the TNF-α release in hPBMCs, with the inhibition rate lower than 25% at 10 μM. Fortunately, compounds **23** and **36** performed more potently than compound **1** (IC_{50} = 33.34 μM), with IC_{50} values of 7.18 and 6.76 μM, respectively. Moreover, the results of the Caco-2 permeability assay revealed that compound **36** possesses perfect cell-penetrating ability. Its efflux ratio was about 3-fold lower than that of compound **1** (Table 5), which is in accord with its high TNF-α inhibitory activity. Taking a consideration of the PDE4D enzymatic inhibitory activity and the inhibitory activity of the TNF-α release in both RAW 264.7 cells and hPBMCs, we eventually chose compound **36** for a further study of crystal structure determination, selectivity and *in vivo* efficacy.

Table 4. Inhibitory Activities of Selected Compounds against the TNF-α Release from the LPS-stimulated hPBMCs



Compds	R^1	R^2	R^3	hPBMCs TNF-α IC_{50} (μM)
1		Me		33.34 ± 2.91
23		Et		7.18 ± 2.17
28		Bn		> 1 (-6.2 %)[a]

33		Me		> 1 (23.3 %)
36		Me		6.76 ± 2.08
37		Me		> 1 (16.7 %)
43		Et		> 1 (22.8 %)
46		Et		> 1 (21.5 %)

[a] TNF-α inhibition rates at 1 μM were presented in brackets.

Table 5. Caco-2 Permeability of Compounds 1 and 36

Compds	Flow Direction	Mean Recovery (%)	Mean P_{app} [a] (10^{-6} cm/s)	Mean F_{abs} [b] (%)	Efflux Ratio[c]	Permeability class[d]
1	apical to basal	100	14.80	100	1.47	high
	basal to apical	90	21.73			
36	apical to basal	108	34.58	100	0.44	high
	basal to apical	85	15.08			

[a] The P_{app} values in the A to B or B to A were calculated by the following formula: $P_{app} = (V_A/(\text{area} \times \text{time})) \times ([\text{drug}]_{\text{acceptor}}/[\text{drug}]_{\text{initial donor}})$ where, V_A = the volume in the acceptor well (in this assay: 0.3 or 1.0 mL), area = the surface area of the membrane (in this assay: 0.33 cm^2), time = the total transport time in seconds (in this assay: 5400 s). [b] F_{abs}: Human absorbed fraction. [c] Efflux ratio (B to A/A to B ratio) = P_{app} (B to A)/P_{app} (A to B). [d] Permeability class: high, F_{abs} % > 70%; moderate, 30% < F_{abs} % < 70%; poor: F_{abs} % < 30%.

2.5. Crystal structures of PDE4D in complex with compound 36 and apremilast

We solved the crystal structures of the catalytic domain of PDE4D in complex with compound **36** and apremilast (Figure 2). The structure of the PDE4D-**36** complex (PDB code: 7CBJ) showed that the binding conformation of compound **36** in the ligand binding pocket of PDE4D was similar to that of compound **1**. The catechol group formed two H-bonds with the conserved glutamine (Q369) and the π-π stacking interactions between F372 and tetrahydroisoquinoline scaffold were maintained too. While in compared with compound **1**, the installation of a chlorine atom at the 7-position of the indole ring led this ring to flip away and bury into a new hydrophobic pocket formed by residues M273, H276, L319, and I376. As a result, the indole ring of compound **36** was stabilized, as the density map of it was complete. However, the inhibitory activity of compound **36** against PDE4D was weaker than those of compound **1** and apremilast (IC_{50} values of 0.14 μM and 0.077 μM for the two compounds, respectively). It is consisted with our previous finding that interactions between the indole ring and hydrophobic clamp was important to improve the inhibitory activity. In structurally, the binding mode of apremilast with PDE4D (PDB code: 7CBQ) was quite different from that of compound **36.** Except the H-bonds with Q369, a direct H-bond with H160 in the M-pocket and a water molecule mediated H-bond with P356 in the H-pocket were formed (Figure 2D), and the latter may account for the high inhibitory activity of apremilast. In contrast, the carbonyl group of compounds **1** and **36** oriented to the solvent accessible region and made little interactions with residues within the M- and H-pockets.

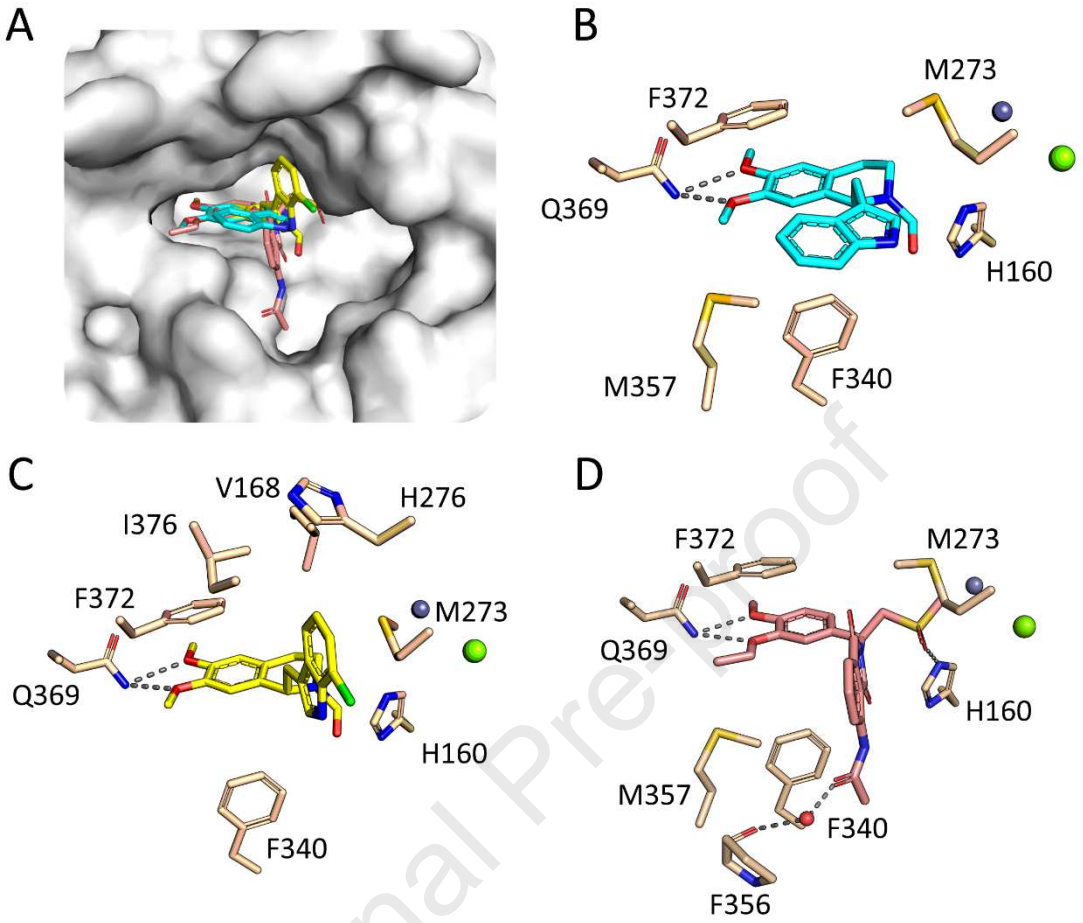


Figure 2. Crystal structures of PDE4D in complex with compounds 1, 36 and apremilast. (A) Superimposed binding modes of three compounds with PDE4D. Compounds **1**, **36** and apremilast are shown as cyan, yellow and pink sticks, respectively. The PDE4D catalytic domain is shown as gray surface. (B-D) Detailed interactions of **1** (B), **36** (C) or apremilast (D) with PDE4D, respectively. H-bonds are shown as grey dash lines.

2.6. Selectivity of compound 36 against other PDE isoforms

Selectivity of compound **36** against other PDE isoforms was examined before the *in vivo* study. As shown in Tables 6 and S2, compound **36** displayed > 13-fold selectivity versus PDE1-3,6, and > 55-fold selectivity versus PDE5,7-9,11, indicating its high selectivity for PDE4.

Table 6. Selectivity of Compound 36 to 11 PDE Enzymes

PDEs	IC_{50} (μM)	Selectivity index
PDE1A[a]	> 5	> 13
PDE2A[a]	> 5	> 13
PDE3A[a]	> 5	> 13
PDE4A[a]	0.36	-
PDE5A[a]	> 20	> 55
PDE6C[a]	> 5	> 13
PDE7A[a]	> 20	> 55
PDE8A[a]	> 20	> 55
PDE9A[a]	> 20	> 55
PDE10A[a]	76.5% [b]	-
PDE11A[a]	> 20	> 55

[a] Commercial recombinant human full-length PDEs (BPS Bioscience, Inc.).

[b] PDE10A inhibition rate at 5 μM

2.7. Compound 36 attenuated IMQ-induced murine psoriasis-like skin inflammation.

Encouraged by the enzymatic inhibitory activity and anti-inflammatory effect of compound **36**, pharmacological effects of compound **36** were further evaluated in the following *in vivo* investigations. Since PDE4 are widely expressed in numerous cells [11], oral administration of PDE4 inhibitors may cause some side effects, the most common ones of which are nausea and diarrhea [20]. Besides, compound **36** displayed poor pharmacokinetics properties after p.o. administration in rats, with the bioavailability of only 0.75% (Table S3). Given the preferable transdermal absorption characteristics (Table S4), compound **36** with a topical treatment strategy was conducted in the imiquimod (IMQ)-induced murine psoriasis model, which could extensively mimic the clinical manifestations of psoriasis patients [30, 31]. During consecutive application of the IMQ cream on BALB/c mice for 7 days, obvious loss of body weight was observed, which could be largely reversed upon the topical

treatment of compound **36** (Figure 3A). Moreover, as presented in Figure 3B, C, severe skin lesions occurred on the back skins following the IMQ application, including scales, thickness and erythema. Therapeutically, compound **36** significantly attenuated the experimental symptoms in a dose-dependent manner during the treatment, which was comparable to the calcipotriol-treated group (Figure 3B, C). Collectively, these results demonstrated that compound **36** exhibited the therapeutic potential to improve the features of psoriasis-like skin inflammation.

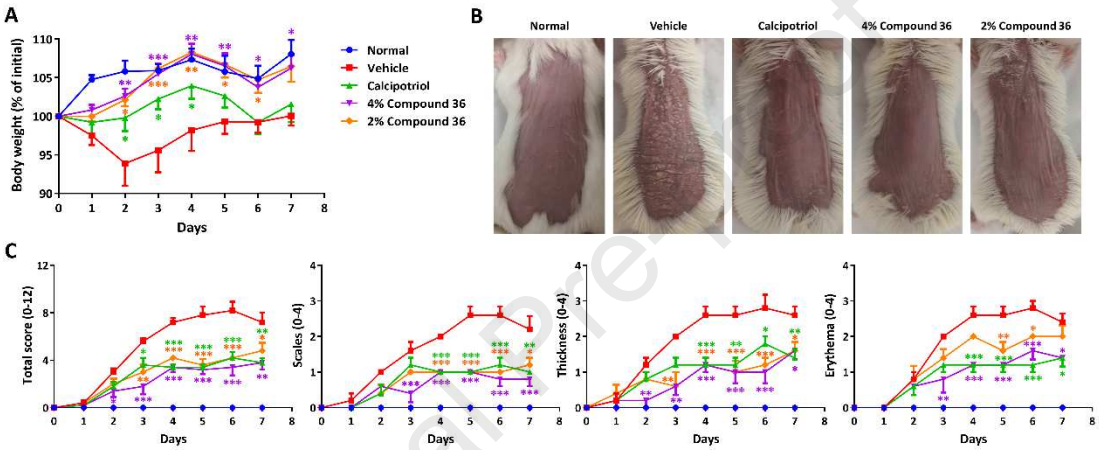


Figure 3. Topical treatment of compound 36 dose-dependently ameliorated the experimental murine psoriatic inflammation. (A) Body weights were monitored daily as the percentage of body weight at day 0. (B) The represent phenotypic pictures of the back skins in each group. (C) The Psoriasis Area and Severity Index (PASI) scores, including scales, thickness, and erythema. Data were shown as mean ± SEM; n = 5 mice per group; * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, compared with the vehicle group.

3. Conclusions

Small molecules with minimal side effects and high potency for anti-psoriasis treatment are greatly needed. Based on the previously reported compound **1** with moderate PDE4D inhibitory activity, a series of novel tetrahydroisoquinoline derivatives were designed and synthesized. Set out from the crystal structure of the PDE4D-**1** complex, we investigated the structure−activity relationship on substituents

supposed to interact with the metal binding site, Q2 pocket and hydrophobic clamp of the enzyme, respectively. The structure-guided optimization resulted in 12 novel PDE4D inhibitors (**2**, **5**, **21-23**, **33**, **36**, **38, 40**, **43**, **46**, and **50**) with IC_{50} values in the sub-micromolar range. Among them, compound **36** was found to be a safe, permeable and selective PDE4 inhibitor with enhanced inhibition of the TNF-α secretion in the LPS-stimulated RAW 264.7 and hPBMCs. The crystal structure of compound **36** in complex with PDE4D was solved and compared with those of PDE4D-**1** and PDE4D-**Apremilast**. Moreover, topical administration of **36** exhibited superior therapeutic efficacy compared with calcipotriol, the first-line medication for psoriasis, against the IMQ-induced murine psoriasis-like skin inflammation. Overall, our study lays a solid foundation for further development of small-molecular PDE4 inhibitors for effective anti-psoriasis treatment.

4. Experimental sections

4.1. Chemistry

4.1.1. Materials and methods. All of the reagents (chemicals) were purchased from commercial chemical reagent company and directly used without further purification. Analytical thin layer chromatography (TLC) was performed on HSGF 254. All products were characterized by their NMR, LR-MS and HR-MS. The purity of the target compounds was determined by HPLC (Agilent Eclipse XDB-C18, 5 μm, 4.6 mm × 150 mm, 30 °C, UV 214/230/254/280 nm, flow rate = 1.0 mL/min) with aqueous CH_3CN or CH_3OH (40−70%) for 20 min, and the peak areas were calculated at 230 nm.

4.1.2. Synthesis of compounds 1-52

(S)-1-((1H-indol-3-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1 H)-carbaldehyde (1). A solution of **6a** (300 mg, 0.93 mmol) and trimethylamine (TEA, 140 μL, 1.02 mmol) in ethyl formate (5.0 mL) was kept stirring for 6 h at 55 °C. The mixture was then diluted with EA. After being washed with saturated NH4Cl and NaCl, the organic layer, the organic layer was dried over Na_2SO_4. The

solvent was removed under vacuum. The residue was purified by column chromatography (PE/EA v: v = 1:1) to give compound **1** (245 mg, 75%) as a white solid. HPLC purity: 98.66 %. m.p.: 88-90 ^{o}C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.94, 10.86 (2 × s, 1H), 8.08, 7.48 (2 × s, 1H), 7.68, 7.57 (2 × d, *J* = 7.9 Hz, 1H), 7.35, 7.33 (2 × d, *J* = 8.2 Hz, 1H), 7.18 – 6.94 (m, 3H), 6.91, 6.67 (2 × s, 1H), 6.73, 6.44 (2 × s, 1H), 5.45, 4.82 (2 × dd, *J* = 10.4, 4.3 Hz, 1H), 4.16, 3.69 (2 × ddd, *J* = 13.0, 6.2, 2.5 Hz, 1H), 3.73, 3.70 (2 × s, 3H), 3.73, 3.49 (2 × s, 3H), 3.43 – 3.37, 3.27 – 3.04 (m, 3H), 2.82 – 2.59 (m, 2H).^{13}C NMR (126 MHz, DMSO-d_6) δ 161.4, 160.6, 147.7, 147.4, 147.2, 146.8, 136.2, 136.1, 128.6, 127.8, 127.6, 127.1, 125.5, 125.4, 124.4, 123.8, 120.9, 120.8, 118.6, 118.4, 118.3, 112.0, 111.9, 111.4, 111.3, 110.9, 110.7, 110.6, 110.4, 56.5, 55.6, 55.5, 55.4, 55.2, 50.1, 33.0, 32.4, 31.4, 28.7, 27.2. ESI-MS *m/z* 351.0 $[M+H]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{21}H_{23}N_2O_3^+$ $[M+H]^+$ 351.1703, found 351.1704.

1-((1H-indol-3-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1H)-carbaldehyde (2). White solid. This compound was prepared using a similar method of compound **1**, by replacing **6a** with **5a**. HPLC purity: 99.17 %. m.p.: 93-95 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92, 10.83 (2 × s, 1H), 8.08, 7.46 (2 × s, 1H), 7.69, 7.58 (2 × d, *J* = 7.8 Hz, 1H), 7.39 – 6.94 (m, 4H), 6.92, 6.67 (2 × s, 1H), 6.73, 6.45 (2 × s, 1H), 5.45, 4.81 (2 × dd, *J* = 10.4, 4.2 Hz, 1H), 4.22 – 4.11, 3.69 – 3.65 (2 × m, 1H), 3.73, 3.70 (2 × s, 3H), 3.73, 3.49 (2 × s, 3H), 3.42 – 3.03 (m, 3H), 2.80 – 2.61 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.6, 161.5, 148.3, 147.8, 147.7, 147.3, 136.4, 136.1, 127.9, 127.9, 127.8, 126.8, 126.1, 125.2, 123.6, 123.0, 122.4, 122.1, 119.8, 119.6, 119.1, 118.1, 112.0, 111.6, 111.4, 111.2, 111.0, 110.6, 110.0, 57.4, 56.1, 56.0, 55.9, 55.6, 51.2, 40.8, 34.0, 33.3, 31.8, 29.2, 27.8. ESI-MS *m/z* 351.1 $[M+H]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{21}H_{23}N_2O_3^+$ $[M+H]^+$ 351.1703, found 351.1697.

(R)-1-((1H-indol-3-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1H)-carbaldehyde (3). White solid. This compound was prepared using a similar method of compound **1**, by replacing **6a** with **7a**. HPLC purity: 99.46 %. m.p.: 159-161 ^{o}C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.89, 10.88 (2 × s, 1H), 8.08, 7.46 (2 × s, 1H), 7.70, 7.57 (2 × d, *J* = 7.9 Hz, 1H), 7.34, 7.33 (d, *J* = 8.1 Hz, 1H), 7.13 – 6.92

(m, 3H), 6.92, 6.67 (2 × s, 1H), 6.73, 6.45 (2 × s, 1H), 5.46 – 5.44, 4.83 – 4.80 (2 × m, 1H), 4.19 – 4.14, 3.71 – 3.65 (2 × m, 1H), 3.73, 3.70 (2 × s, 3H), 3.73, 3.49 (2 × s, 3H), 3.45 – 3.35, 3.29 – 3.04 (m, 3H), 2.84 – 2.61 (2 × m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.4, 160.6, 147.7, 147.4, 147.2, 146.8, 136.2, 136.1, 128.5, 127.8, 127.6, 127.1, 125.5, 125.3, 124.4, 123.8, 120.9, 120.8, 118.6, 118.5, 118.4, 118.3, 112.0, 111.8, 111.4, 111.3, 110.8, 110.7, 110.6, 110.4, 56.5, 55.6, 55.5, 55.4, 55.1, 54.9, 50.1, 33.0, 32.4, 31.4, 28.7, 27.2. ESI-MS *m/z* 351.1 $[M+H]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{21}H_{23}N_2O_3^+$ $[M+H]^+$ 351.1703, found 351.1707.

1-(1-((1H-indol-3-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethan-1-one (4). To a solution of **5a** (150 mg, 0.47 mmol) and N, N-Diisopropylethylamine (DIPEA, 230 μL, 1.40 mmol) in DCM (5 mL) was slowly added acetyl chloride (40 μL, 0.56 mmol) at 0 ^{o}C. The mixture was then stirred at room temperature for 2 h. After being washed with saturated NH_4Cl and brine, the solvent was dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by column chromatography (PE/EA v: v = 1:1) to afford compound **4** as a white solid (150 mg, 89%). HPLC purity: 99.64 %. m.p.: 155-157 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.89, 8.59 (2 × s, 1H), 7.64, 7.59 (2 × d, *J* = 7.7 Hz, 1H), 7.38, 7.31 (2 × m, 1H), 7.22 – 7.04 (m, 2H), 6.87, 6.83 (2 × d, *J* = 2.4 Hz, 1H), 6.64, 6.20 (2 × s, 1H), 6.58 (s, 1H), 5.83 – 5.81, 4.94 – 4.91 (2 × m, 1H), 4.82 – 4.79, 3.24 – 3.17 (2 × m, 2H), 3.87, 3.83 (2 × s, 3H), 3.80, 3.46 (2 × s, 3H), 3.67 – 3.63, 3.51 – 3.47 (2 × m, 1H), 3.34 – 3.27 (2 × m, 1H), 2.93 – 2.79 (2 × m, 1H), 2.74 – 2.64 (2 × m, 1H), 2.15, 1.53 (2 × s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.2, 169.6, 148.1, 147.6, 147.3, 147.0, 136.3, 136.2, 129.1, 128.4, 128.0, 127.2, 126.6, 125.6, 123.7, 123.1, 122.1, 121.8, 119.7, 119.4, 119.2, 117.9, 112.3, 111.7, 111.6, 111.4, 111.1, 110.9, 110.2, 57.9, 56.1, 56.0, 55.9, 55.6, 53.1, 41.7, 34.9, 32.7, 32.0, 28.7, 28.0, 22.2, 21.1. ESI-MS *m/z* 365.1 $[M+H]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{22}H_{25}N_2O_3^+$ $[M+H]^+$ 365.1860, found 365.1863.

(S)-1-(1-((1H-indol-3-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethan-1-one (5). White solid. This compound was prepared using a similar method of compound **4**, by replacing **5a** with **6a**. HPLC purity: 99.83 %.

m.p.: 99-100 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.89, 8.57 (2 × s, 1H), 7.64, 7.59 (2 × d, *J* = 7.7 Hz, 1H), 7.37, 7.31 (2 × m, 1H), 7.21 – 7.05 (m, 2H), 6.87, 6.83 (2 × d, *J* = 2.4 Hz, 1H), 6.64, 6.20 (2 × s, 1H), 6.58 (s, 1H), 5.83 – 5.80, 4.93 – 4.91 (2 × m, 1H), 4.81 – 4.78, 3.24 – 3.17 (2 × m, 2H), 3.87, 3.83 (2 × s, 3H), 3.80, 3.46 (2 × s, 3H), 3.66 – 3.63, 3.51 – 3.46 (2 × m, 1H), 3.33 – 3.27 (2 × m, 1H), 2.92 – 2.78 (2 × m, 1H), 2.73 – 2.68 (2 × m, 1H), 2.14, 1.52 (2 × s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.2, 169.6, 148.1, 147.6, 147.3, 147.0, 136.3, 136.1, 129.0, 128.4, 128.0, 127.2, 126.6, 125.5, 123.7, 123.1, 122.1, 121.8, 119.6, 119.3, 119.1, 117.9, 112.2, 111.7, 111.5, 111.3, 111.2, 110.9, 110.8, 110.2, 57.9, 56.0, 55.9, 55.6, 53.1, 41.6, 34.9, 32.7, 32.0, 28.6, 28.0, 22.1, 21.1. ESI-MS *m/z* 365.1 $[M+H]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{22}H_{25}N_2O_3^+$ $[M+H]^+$ 365.1860, found 365.1867.

1-(1-((1H-indol-3-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-2,2,2-trifluoroethan-1-one (6). To a solution of **5a** (150 mg, 0.47 mmol) and TEA (200 μL, 1.40 mmol) in DCM (5 mL) was added trifluoroacetic anhydride (200 μL, 1.40 mmol) slowly at 0 °C. The mixture was then stirred at room temperature for 2 h. After being washed with water and brine, the solvent was dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by column chromatography (PE/EA v: v = 1:1) to afford compound **4** as a white solid (100 mg, 52%). HPLC purity: 99.44 %. m.p.: 185-187 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.90,10.83 (2 × s, 1H), 7.61, 7.51 (2 × d, *J* = 7.4 Hz, 1H), 7.34 (2 × m, 1H), 7.10 – 6.96 (m, 3H), 6.74, 6.72 (2 × s, 1H), 6.58, 6.43 (2 × s, 1H), 5.59, 5.04 (2 × d, *J* = 5.7 Hz, 1H), 4.40, 3.85 (2 × d, *J* = 12.4 Hz, 1H), 3.72, 3.43 (2 × s, 3H), 3.70 – 3.58 (2 × m, 1H), 3.55, 3.34 (2 × s, 3H), 3.33 – 3.17 (m, 2H), 2.86 – 2.71 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.2, 155.9, 148.4, 148.2, 147.4, 146.8, 136.2, 127.8, 127.6, 127.3, 126.8, 125.0, 123.7, 123.2, 122.5, 122.4, 120.1, 112.0, 119.3, 118.8, 117.9, 115.6, 111.7, 111.5, 111.3, 111.1, 111.0, 110.8, 57.3, 56.0, 55.7, 55.4, 54.7, 40.7, 40.6, 38.0, 33.4, 31.9, 28.8, 27.4. ^{19}F NMR (376 MHz, $CDCl_3$) δ -68.3, -69.2. ESI-MS *m/z* 419.1 $[M+H]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{22}H_{22}F_3N_2O_3^+$ $[M+H]^+$ 419.1577, found 419.1576.

(S)-1-(1-((1H-indol-3-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-2,2,2-trifluoroethan-1-one (7). White solid. This compound was prepared using a similar method of compound **6**, by replacing **5a** with **6a**. HPLC purity: 99.72 %. m.p.: 145-147 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10, 8.05 (2 × s, 1H), 7.61, 7.41 (2 × d, *J* = 7.4 Hz, 1H), 7.35 (2 × m, 1H), 7.18, 7.10 (2 × m, 2H), 6.85, 6.81 (2 × s, 1H), 6.61, 6.58 (2 × s, 1H), 6.17, 5.86 (2 × s, 1H), 5.73 – 5.71, 5.18 – 5.15 (2 × m, 1H), 4.63 – 4.59, 3.94 – 3.90 (2 × m, 1H), 3.85, 3.84 (2 × s, 3H), 3.60 – 3.55, 3.38 – 3.25 (m, 3H), 3.48, 3.25 (2 × s, 3H), 2.94 – 2.69 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.2, 155.9, 148.4, 148.2, 147.4, 146.8, 136.2, 127.8, 127.6, 127.3, 126.7, 125.0, 123.7, 123.2, 122.5, 122.4, 120.1, 119.9, 119.2, 118.8, 117.9, 115.6, 111.7, 111.5, 111.3, 111.1, 111.0, 110.8, 57.3, 56.0, 55.7, 55.4, 54.7, 40.6, 37.9, 33.4, 31.8, 28.8, 27.4. ^{19}F NMR (376 MHz, DMSO-d_6) δ -68.3, -69.3. ESI-MS m/z 419.1 $[M+H]^+$. HR-MS: (ESI, m/z) calcd for $C_{22}H_{22}F_3N_2O_3^+$ $[M+H]^+$ 419.1577, found 419.1587.

methyl 2-(1-((1H-indol-3-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)acetate (8). To a solution of **5a** (150 mg, 0.47 mmol) in acetonitrile (15 mL) were added K_2CO_3 (195 mg, 1.40 mmol) and methyl bromoacetate (88 μL, 0.93 mmol). The resulting mixture was refluxed for 12 h. Then it was extracted with EA and the organic layer was washed with saturated NaCl, dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by column chromatography (PE/EA v: v = 1:1) to afford compound **8** as a white solid (160 mg, 88%). HPLC purity: 99.49 %. m.p.: 105-107 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.59 (d, *J* = 7.7 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 1H), 7.21 – 7.15 (m, 1H), 7.14 – 7.08 (m, 1H), 6.94 (d, *J* = 2.0 Hz, 1H), 6.56 (s, 1H), 5.96 (s, 1H), 4.15 – 4.04 (m, 1H), 3.83 (s, 3H), 3.67 (s, 3H), 3.60 - 3.47 (m, 2H), 3.44 – 3.40 (m, 2H), 3.38 (s, 3H), 3.08 – 3.03 (m, 2H), 2.91 – 2.83 (m, 1H), 2.63 – 2.59 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.9, 147.5, 146.5, 136.3, 129.1, 127.9, 125.5, 123.3, 122.0, 119.5, 119.2, 113.7, 111.6, 111.3, 111.2, 61.5, 55.9, 55.7, 55.5, 51.8, 45.0, 32.5, 24.8. ESI-MS *m/z* 395.2 $[M+H]^+$. HR-MS: (ESI, m/z) calcd for $C_{23}H_{27}N_2O_3^+$ $[M+H]^+$ 395.1965, found 395.1971.

methyl (S)-2-(1-((1H-indol-3-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)acetate (9). White solid. This compound was prepared using a similar method of compound **8**, by replacing **5a** with **6a**. HPLC purity: 99.48 %. m.p.: 106-107 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.59 (d, *J* = 7.9 Hz, 1H), 7.35 (d, *J* = 8.1 Hz, 1H), 7.22 – 7.15 (m, 1H), 7.15 – 7.05 (m, 1H), 6.95 (d, *J* = 2.2 Hz, 1H), 6.56 (s, 1H), 5.98 (s, 1H), 4.11 – 4.09 (m, 1H), 3.83 (s, 3H), 3.67 (s, 3H), 3.59 - 3.48 (m, 2H), 3.44 – 3.40 (m, 2H), 3.40 (s, 3H), 3.09 – 3.04 (m, 2H), 2.90 – 2.83 (m, 1H), 2.64 – 2.59 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.8, 147.6, 146.6, 136.4, 129.0, 127.9, 125.5, 123.3, 122.0, 119.5, 119.2, 113.6, 111.7, 111.3, 111.2, 61.6, 55.9, 55.6, 55.5, 51.9, 45.0, 32.5, 24.8. ESI-MS *m/z* 395.2 $[M+H]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{23}H_{27}N_2O_3^+$ $[M+H]^+$ 395.1965, found 395.1971.

1-(1-((1H-indol-3-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-2-methoxyethan-1-one (10). White solid. This compound was prepared using a similar method of compound **4**, by replacing acetyl chloride with methoxyacetyl chloride. HPLC purity: 98.71 %. m.p.: 173-174 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49, 8.19 (2 × s, 1H), 7.69 – 7.64 (m, 1H), 7.40, 7.33 (2 × d, *J* = 8.0 Hz, 1H), 7.27 – 7.03 (m, 2H), 6.92, 6.88 (2 × d, *J* = 2.2 Hz, 1H), 6.63, 6.55 (2 × s, 1H), 6.57, 6.20 (2 × s, 1H), 5.79, 4.90 (2 × dd, *J* = 9.2, 5.1 Hz, 1H), 4.86 – 4.76, 3.72 – 3.62 (2 × m, 1H), 4.14 (s, 1H), 3.87, 3.84 (2 × s, 3H), 3.79, 3.49 (2 × s, 3H), 3.48 – 3.41 (2 × m, 1H), 3.38, 2.96 (2 × s, 3H), 3.38 – 3.14 (m, 3H), 2.96 – 2.62 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.8, 168.3, 148.6, 148.1, 147.7, 147.4, 136.6, 136.5, 129.1, 128.6, 128.3, 127.6, 126.7, 125.8, 123.9, 123.4, 122.9, 122.4, 120.4, 112.0, 119.7, 118.5, 112.9, 112.2, 112.0, 111.3, 110.6, 72.4, 71.1, 59.5, 59.0, 56.5, 56.4, 56.3, 56.0, 53.5, 40.2, 35.4, 33.1, 32.4, 29.1, 28.3. ESI-MS *m/z* 394.9 $[M+H]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{23}H_{27}N_2O_4^+$ $[M+H]^+$ 395.1965, found 395.1957.

1-((1H-indol-3-yl)methyl)-6,7-dimethoxy-2-(2-(methylsulfonyl)ethyl)-1,2,3,4-tetrahydroisoquinoline (11). White solid. This compound was prepared using a similar method of compound **8**, by replacing methyl bromoacetate with 2-bromoetyl-methylsulfone. HPLC purity: 97.89 %. m.p.: 86-88 ^{o}C. ^{1}H NMR (400

MHz, $CDCl_3$) δ 8.09 (s, 1H), 7.58 (d, *J* = 7.9 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.20 (t, *J* = 7.3 Hz, 1H), 7.13 (t, *J* = 7.3 Hz, 1H), 6.97 (d, *J* = 1.9 Hz, 1H), 6.59 (s, 1H), 6.32 (s, 1H), 3.99 – 3.94 (m, 1H), 3.86 (s, 3H), 3.63 (s, 3H), 3.45 – 3.30 (m, 1H), 3.25 – 3.12 (m, 2H), 3.11 – 2.85 (m, 6H), 2.56 – 2.53 (m, 1H), 2.42 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 147.9, 147.1, 136.3, 129.0, 127.8, 125.5, 122.8, 122.4, 119.8, 118.9, 114.1, 111.6, 111.4, 111.3, 62.3, 56.0, 55.9, 53.5, 48.0, 43.3, 41.7, 32.3, 23.5. ESI-MS *m/z* 429.0 $[M+H]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{23}H_{29}N_2O_4S^+$ $[M+H]^+$ 429.1843, found 429.1843.

(S)-1-(1-((1H-indol-3-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-2-(methylsulfonyl)ethan-1-one (12). To a solution of 2-methylsulfonylacetic acid (65 mg, 0.47 mmol), HATU (265 mg, 0.70 mmol) and TEA (200 μL, 1.40 mmol) was added **6a** (150 mg, 0.47 mmol). The resulting mixture was stirred at r.t. for 8 h. After being washed with saturated NH_4Cl and NaCl, the organic layer was dried over Na_2SO_4. The solvent was removed under vacuum. The residue was purified by column chromatography (PE/EA v: v = 1:1) to afford compound **12** as a white solid (92 mg, 45%). HPLC purity: 97.13 %. m.p.: 105-107 ^{o}C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.98, 10.80 (2 × s, 1H), 7.62, 7.57 (d, *J* = 7.8 Hz, 1H), 7.37, 7.33 (2 × d, *J* = 8.1 Hz, 1H), 7.18 – 6.94 (m, 3H), 6.87, 6.71 (s, 1H), 6.73, 6.22 (2 × s, 1H), 5.65, 5.10 (2 × dd, *J* = 9.6, 4.5 Hz, 1H), 4.54 – 4.46 (m, 1H), 4.46 – 4.41, 4.01 – 3.96 (2 × m, 1H), 3.73, 3.70 (2 × s, 3H), 3.71, 3.38 (2 × s, 3H), 4.01 – 3.96, 3.61 – 3.54 (ddd, *J* = 13.5, 10.7, 4.4 Hz, 1H), 3.30 – 3.08 (m, 3H), 3.02, 2.75 (2 × s, 3H), 2.91 – 2.84, 2.73 – 2.60 (2 × m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.6, 161.0, 148.4, 147.9, 147.8, 147.2, 136.1, 127.9, 127.7, 127.6, 126.9, 126.0, 125.1, 123.4, 123.3, 122.9, 122.2, 120.5, 119.7, 119.1, 118.1, 112.0, 111.7, 111.5, 111.0, 110.9, 110.8, 110.1, 77.3, 77.0, 76.8, 58.8, 58.3, 57.2, 56.1, 55.9, 55.6, 54.0, 42.3, 41.4, 36.2, 32.7, 32.0, 28.5, 27.9. ESI-MS *m/z* 443.2 $[M+Na]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{23}H_{27}N_2O_3^+$ $[M+H]^+$ 443.1635, found 443.1629.

(1-((1H-indol-3-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)(pyridin-4-yl)methanone (13). White solid. This compound was prepared using a similar method of compound **4**, by replacing acetyl chloride with

pyridine-4-carbonyl chloride. HPLC purity: 98.32 %. m.p.: 200-201 ^{o}C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.96, 10.85 (2 × s, 1H), 8.60 (2 × s, 1H), 8.15, 8.14 (2 × s, 1H), 7.68, 7.41 (2 × d, *J* = 8.1 Hz, 1H), 7.36, 7.19 (2 × d, *J* = 7.9 Hz, 1H), 7.15 – 6.80 (m, 4H), 6.77, 6.69 (2 × s, 1H), 6.62 – 6.55 (m, 1H), 6.30, 6.29 (2 × s, 1H), 5.79 – 5.75, 3.49 – 3.36 (2 × m, 2H), 4.68 – 4.54 (m, 1H), 3.74, 3.71 (2 × s, 3H), 3.60, 3.56 (2 × s, 3H), 3.31 – 3.25 (m, 1H), 3.22 – 3.05 (m, 1H), 2.99 – 2.53 (m, 2H).^{13}C NMR (126 MHz, DMSO-d_6) δ 167.7, 167.2, 150.3, 149.5, 148.1, 147.8, 147.4, 147.3, 144.5, 143.6, 136.4, 128.5, 128.4, 128.1, 127.8, 126.0, 125.6, 124.9, 124.3, 121.3, 121.2, 121.0, 120.5, 118.9, 118.8, 118.6, 118.4, 112.4, 112.1, 111.6, 111.3, 111.0, 110.8, 110.6, 58.4, 55.8, 55.6, 52.4, 41.2, 34.8, 32.4, 31.6, 28.6, 27.7. ESI-MS *m/z* 428.1 $[M+H]^+$. HR-MS: (ESI, *m*/*z*) calcd for $C_{26}H_{26}N_3O_3^+$ $[M+H]^+$ 428.1969, found 428.1971.

1-((1H-indol-3-yl)methyl)-6,7-dimethoxy-2-((tetrahydro-2H-pyran-4-yl)methyl)-1,2,3,4-tetrahydroisoquinoline (14). White solid. This compound was prepared using a similar method of compound **8**, by replacing methyl bromoacetate with 4-bromomethyltetrahydropyran. HPLC purity: 99.82 %. m.p.: 89-90 ^{o}C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.71 (S, 1H), 7.57 (d, *J* = 7.8 Hz, 1H), 7.32 (d, *J* = 7.8 Hz, 1H), 7.08 – 7.01 (m, 2H), 6.99 – 6.95 (m, 1H), 6.61 (s, 1H), 6.39 (s, 1H), 3.82 (t, *J* = 7.0 Hz, 1H), 3.69 (s, 3H), 3.54 (ddd, *J* = 11.4, 4.5, 2.1 Hz, 1H), 3.32 (s, 3H), 3.32 – 3.23 (m, 2H), 3.12 – 2.98 (m, 2H), 2.93 (dd, *J* = 14.4, 5.9 Hz, 1H), 2.82 – 2.66 (m, 3H), 2.44 – 2.30 (m, 2H), 2.25 (dd, *J* = 12.5, 8.3 Hz, 1H), 1.26 – 1.21 (m, 2H), 1.17 – 1.10 (m, 1H), 1.00 – 0.72 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 147.1, 146.5, 136.3, 130.3, 127.7, 126.1, 123.8, 120.7, 118.7, 118.1, 113.0, 112.0, 111.8, 111.3, 67.1, 67.0, 61.0, 59.6, 55.5, 55.2, 43.9, 33.3, 31.9, 31.4, 31.2, 23.6. ESI-MS *m/z* 421.1. $[M+H]^+$. HR-MS: (ESI, *m*/*z*) calcd for $C_{26}H_{33}N_2O_3^+$ $[M+H]^+$ 421.2486, found 421.2490.

(1-((1H-indol-3-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)(morpholino)methanone (15). White solid. This compound was prepared using a similar method of compound **4**, by replacing acetyl chloride with 4-morpholinecarbonyl chloride. HPLC purity: 99.47 %. m.p.: 179-180 ^{o}C. ^{1}H NMR

(500 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.67 (d, *J* = 7.6 Hz, 1H), 7.36 (d, *J* = 7.9 Hz, 1H), 7.20 – 7.13 (m, 2H), 6.95 (s, 1H), 6.61 (s, 1H), 6.41 (s, 1H), 5.19 – 5.06 (m, 1H), 3.90 – 3.86 (m, 1H), 3.85 (s, 3H), 3.67 (s, 3H), 3.51 – 3.37 (m, 3H), 3.32 – 3.28 (m, 1H), 3.23 – 3.15 (m, 3H), 3.05 – 2.97 (m, 2H), 2.93 (m, 1H), 2.77 – 2.62 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.2, 148.0, 147.2, 136.4, 129.2, 127.9, 125.9, 123.4, 122.2, 119.7, 118.8, 112.6, 111.6, 111.4, 110.6, 66.4, 56.7, 56.0, 55.9, 47.6, 39.3, 32.5, 28.5. ESI-MS *m/z* 436.1 $[M+H]^+$. HR-MS: (ESI, m/z) calcd for $C_{25}H_{30}N_3O_4^+$ $[M+H]^+$ 436.2231, found 436.2241.

(S)-(1-((1H-indol-3-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl) (morpholino)methanone (16). White solid. This compound was prepared using a similar method of compound **15**, by replacing **5a** with **6a**. HPLC purity: 99.41 %. m.p.: 100-102 ^oC. ^1H NMR (400 MHz, DMSO-d_6) δ 10.79 (s, 1H), 7.70 (d, *J* = 7.8 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.16 – 6.86 (m, 3H), 6.67 (s, 1H), 6.54 (s, 1H), 5.09 – 5.05 (m, 1H), 3.71 (s, 3H), 3.66 (dd, *J* = 13.3, 6.0 Hz, 1H), 3.57 (s, 3H), 3.48 – 3.38 (m, 1H), 3.27 (ddd, *J* = 11.3, 6.4, 2.8 Hz, 2H), 3.18 – 3.04 (m, 4H), 2.87 (ddd, *J* = 13.1, 6.7, 2.8 Hz, 2H), 2.80 – 2.70 (m, 1H), 2.69 -2.59 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.2, 148.0, 147.1, 136.4, 129.2, 127.8, 125.9, 123.5, 122.1, 119.6, 118.7, 112.4, 111.6, 111.4, 110.5, 66.3, 56.7, 56.0, 55.9, 47.5, 39.3, 32.5, 28.5. ESI-MS *m/z* 436.1 $[M+H]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{25}H_{30}N_3O_4^+$ $[M+H]^+$ 436.2231, found 436.2236.

(1-((1H-indol-3-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)(tetrahydro-2H-pyran-4-yl)methanone (17). White solid. This compound was prepared using a similar method of compound **4**, by replacing acetyl chloride with tetrahydro-2H-pyran-4-carbonyl chloride. HPLC purity: 96.82 %. m.p.: 218-220 ^oC. ^1H NMR (400 MHz, DMSO-d_6) δ 10.87, 10.86 (2 × s, 1H), 7.97 – 7.86, 7.40 – 7.33 (2 × m, 1H), 7.60, 7.32 (2 × d, *J* = 8.0 Hz, 1H), 7.14 – 7.08 (m, 2H), 7.07, 6.68 (2 × s, 1H), 7.05 – 6.85 (m, 1H), 6.72, 6.31 (2 × s, 1H), 5.67 – 5.4, 5.11 – 5.07 (2 × m, 1H), 4.52 – 4.47, 3.94 – 3.89 (2 × m, 1H), 3.82, 3.70 (2 × s, 3H), 3.79 – 3.74 (m, 1H), 3.73, 3.43 (2 × s, 3H), 3.58 – 3.55, 3.33 – 3.22 (2 × m, 2H), 3.20 – 3.04 (m, 2H), 2.91 – 2.55 (m, 3H), 2.08 – 1.01 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.3, 173.2, 148.4,

147.7, 147.2, 136.5, 136.1, 129.2, 128.5, 128.1, 127.3, 127., 125.4, 123.8, 123.1, 122.8, 122.0, 120.3, 119.6, 119.3, 117.9, 112.6, 111.9, 111.8, 111.7, 111.0, 110.9, 110.2, 67.5, 67.4, 67.2, 66.9, 56.4, 56.3, 56.1, 56.0, 55.7, 53.1, 40.4, 38.3, 37.7, 34.8, 33.1, 32.0, 29.6, 29.3, 29.1, 28.3, 28.2. ESI-MS *m/z* 436.1 $[M+H]^+$. HR-MS: (ESI, m/z) calcd for $C_{26}H_{31}N_3O_4^+$ $[M+H]^+$ 435.2278, found 435.2290.

(S)-(1-((1H-indol-3-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl) (tetrahydro-2H-pyran-4-yl)methanone (18). White solid. This compound was prepared using a similar method of compound **17**, by replacing **5a** with **6a**. HPLC purity: 99.96 %. m.p.: 114-116 ^oC. ^1H NMR (500 MHz, $CDCl_3$) δ 8.49, 7.77 (2 × d, *J* = 32.8 Hz, 1H), 7.77 – 7.75, 7.60 – 7.58 (2 × m, 1H), 7.40 – 7.38, 7.32 - 7.24 (2 × m, 1H), 7.25 – 7.05 (m, 2H), 6.95, 6.87 (2 × s, 1H), 6.78, 6.57 (2 × s, 1H), 6.65, 6.21 (2 × s, 1H), 5.86 – 5.84, 5.10 – 5.07 (2 × m, 1H), 4.87 – 4.84, 4.02 – 3.96 (2 × m, 1H), 3.95, 3.84 (2 × s, 3H), 3.88, 3.48 (2 × s, 3H), 3.72- 3.69, 3.60 – 3.51 (2 × m, 1H), 3.44 – 3.14 (m, 4H), 2.96 – 2.68 (m, 3H), 2.15 – 1.20 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.4, 173.2, 148.4, 147.8, 147.2, 136.6, 136.2, 129.2, 128.5, 127.3, 127.0, 125.3, 123.8, 123.1, 122.7, 121.0, 120.2, 119.6, 119.3, 117.8, 112.6, 111.9, 111.8, 111.6, 111.1, 110.0, 110.9, 110.3, 67.5, 67.4, 67.2, 66.8, 56.4, 56.3, 56.1, 55.7, 53.2, 40.4, 38.3, 37.7, 34.8, 33.1, 31.9, 29.8, 29.6, 29.2, 29.1, 28.3, 28.2. ESI-MS *m/z* 436.1 $[M+H]^+$. HR-MS: (ESI, m/z) calcd for $C_{26}H_{31}N_3O_4^+$ $[M+H]^+$ 435.2278, found 435.2279.

(1-((1H-indol-3-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)(thiazol-2-yl)methanone (19). White solid. This compound was prepared using a similar method of compound **4**, by replacing acetyl chloride with tetrahydro-2H-pyran-4-carbonyl chloride. HPLC purity: 99.25 %. m.p.: 120-122 ^oC. ^1H NMR (600 MHz, DMSO-d_6) δ 10.81, 10.75 (2 × s, 1H), 8.02 (s, 1H), 7.75, 7.65 (2 × dd, *J* = 3.2, 1.7 Hz, 1H), 7.67, 7.44 (2 × d, *J* = 7.9 Hz, 1H), 7.33, 7.21 (2 × d, *J* = 8.1 Hz, 1H), 7.08 – 6.83 (m, 3H), 6.76, 6.51 (2 × s, 1H), 6.71, 6.36 (2 × s, 1H), 6.61 – 6.57, 5.79 – 5.74 (2 × m, 1H), 5.22 – 5.13, 4.56 – 4.51 (2 × m, 1H), 3.73, 3.71 (2 × s, 3H), 3.69 – 3.65, 3.43 – 3.38 (2 × m, 1H), 3.52, 3.49 (2 × s, 3H), 3.32 – 3.19 (m, 2H), 2.95 – 2.69 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.7, 164.8, 160.4, 159.8, 148.0,

147.8, 147.2, 146.9, 143.3, 142.6, 136.3, 136.2, 128.5, 128.4, 127.9, 127.6, 126.0, 125.9, 124.0, 123.8, 123.3, 123.2, 122.2, 122.1, 119.8, 119.6, 119.5, 119.1, 112.6, 112.2, 111.4, 111.1, 110.7, 56.5, 56.0, 55.9, 55.8, 55.6, 54.6, 41.2, 37.2, 33.4, 32.1, 29.2, 28.3. ESI-MS *m/z* 434.9 $[M+H]^+$. HR-MS: (ESI, *m*/*z*) calcd for $C_{24}H_{24}N_3O_3S^+$ $[M+H]^+$ 434.1533, found 434.1546.

(S)-(1-((1H-indol-3-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl) (thiazol-2-yl)methanone (20). White solid. This compound was prepared using a similar method of compound **19**, by replacing **5a** with **6a**. HPLC purity: 99.34 %. m.p.: 104-105 ^oC. ^1H NMR (400 MHz, $CDCl_3$) δ 8.09, 8.05 (2 × s, 1H), 7.90, 7.67 (2 × d, *J* = 3.1 Hz, 1H), 7.73, 7.34 (2 × d, *J* = 7.8 Hz, 1H), 7.56 – 7.54 (m, 1H), 7.25 – 7.22 (m, 1H), 7.17, 7.03 (2 × t, *J* = 7.0 Hz, 1H), 7.12 – 7.09 (m, 1H), 6.94, 6.82 (2 × s, 1H), 6.65, 6.60 (2 × s, 1H), 6.34, 6.09 (2 × s, 1H), 5.94 – 5.92, 4.79 – 4.75 (2 × m, 1H), 5.30 – 5.25, 3.81 – 3.78 (2 × m, 1H), 3.86, 3.84 (2 × s, 3H), 3.56, 3.40 (2 × s, 3H), 3.52 – 3.41 (m, 2H), 3.31 – 3.27 (2 × m, 1H), 3.09 – 3.02 (2 × m, 1H), 2.86 – 2.75 (2 × m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.0, 165.0, 160.55, 160.0, 148.2, 148.0, 147.4, 147.3, 143.5, 142.8, 136.5, 136.4, 128.7, 128.6, 128.2, 127.8, 126.2, 126.1, 124.2, 124.0, 123.5, 123.4, 122.4, 122.3, 120.0, 119.8, 119.7, 119.3, 112.8, 112.4, 111.6, 111.3, 111.0, 56.9, 56.2, 56.2, 56.0, 55.8, 54.9, 41.4, 37.4, 33.6, 32.3, 29.4, 28.5. ESI-MS *m/z* 434.1 $[M+H]^+$. HR-MS: (ESI, *m*/*z*) calcd for $C_{24}H_{24}N_3O_3S^+$ $[M+H]^+$ 434.1533, found 434.1542.

1-((1H-indol-3-yl)methyl)-7-ethoxy-6-methoxy-3,4-dihydroisoquinoline-2(1H)-carbaldehyde (21). White solid. This compound was prepared using a similar method of compound **2**, by replacing **5a** with **5b**. HPLC purity: 99.60 %. m.p.: 188-190 ^oC. ^1H NMR (500 MHz, $CDCl_3$) δ 8.27, 8.07 (2 × s, 1H), 7.86, 7.28 (2 × s, 1H), 7.34, 7.27 (2 × d, *J* = 7.6 Hz, 1H), 7.08, 7.04 (2 × d, *J* = 8.0 Hz, 1H), 7.10 – 6.76 (m, 2H), 6.62 – 6.60 (m, 1H), 6.64, 6.27 (2 × s, 1H), 6.37, 6.09 (2 × s, 1H), 5.40 – 5.37, 4.43 – 4.35 (2 × m, 1H), 4.27 – 4.17, 3.32 – 3.23 (2 × m, 1H), 3.85 – 3.73, 3.54 – 3.37 (2 × m, 2H), 3.59, 3.55 (2 × s, 3H), 3.12 – 2.83 (m, 3H), 2.69 – 2.50 (2 × m, 1H), 2.50 – 2.30 (2 × m, 1H), 1.19, 1.03 (2 × t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.7, 148.8, 148.2, 147.1, 146.8, 136.6, 136.2, 128.0, 127.9, 126.9, 126.3,

125.3, 123.8, 123.2, 122.3, 122.0, 119.8, 119.6, 119.1 118.1, 112.1, 112.0, 111.9, 111.8, 111.6, 111.3, 111.1, 64.9, 64.3, 57.6, 56.1, 56.0, 51.4, 41.0, 34.0, 33.4, 31.9, 29.3, 27.9, 15.0, 14.8. ESI-MS *m/z* 365.0 $[M+H]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{22}H_{25}N_2O_3^+$ $[M+H]^+$ 365.1860, found 365.1850.

(S)-1-((1H-indol-3-yl)methyl)-7-ethoxy-6-methoxy-3,4-dihydroisoquinoline-2(1H)-carbaldehyde (22). White solid. This compound was prepared using a similar method of compound **21**, by replacing **5a** with **6a**. HPLC purity: 97.69 %. m.p.: 118-120 ^oC. ^1H NMR (400 MHz, DMSO-d_6) δ 10.89, 10.81 (2 × s, 1H), 8.08, 7.46 (2 × s, 1H), 7.69, 7.58 (2 × d, *J* = 7.8 Hz, 1H), 7.35, 7.33 (2 × d, *J* = 8.0 Hz, 1H), 7.17 – 6.94 (m, 3H), 6.92, 6.66 (2 × s, 1H), 6.73, 6.44 (2 × s, 1H), 5.45, 4.80 (2 × dd, *J* = 10.4, 4.3 Hz, 1H), 4.17, 3.66 (2 × ddd, *J* = 13.1, 5.9, 2.6 Hz, 1H), 4.19 – 3.91, 3.72 – 3.70 (2 × m, 2H), 3.73, 3.70 (2 × s, 3H), 3.46 – 3.29 (m, 2H), 3.26 – 2.97 (m, 3H), 1.32, 1.20 (2 × t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.0, 161.9, 149.0, 148.4, 147.3, 147.0, 136.8, 136.4, 128.1, 128.0, 127.1, 126.5, 125.5, 124.0, 123.4, 122.6, 122.3, 120.0, 119.3, 118.4, 112.3, 112.2, 112.0, 111.7, 111.6, 111.3, 65.1, 64.5, 57.8, 56.3, 56.2, 51.6, 41.2, 34.3, 33.6, 32.1, 29.5, 28.1, 15.2, 15.0. ESI-MS *m/z* 365.0 $[M+H]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{22}H_{25}N_2O_3^+$ $[M+H]^+$ 365.1860, found 365.1857.

(S)-1-(1-((1H-indol-3-yl)methyl)-7-ethoxy-6-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethan-1-one (23). White solid. This compound was prepared using a similar method of compound **5**, by replacing **5a** with **5b**. HPLC purity: 98.82 %. m.p.: 85-87 ^oC. ^1H NMR (500 MHz, $CDCl_3$) δ 8.40, 8.15 (2 × s, 1H), 7.64, 7.59 (2 × d, *J* = 7.6 Hz, 1H), 7.39, 7.32 (2 × d, *J* = 8.0 Hz, 1H), 7.24 – 7.16 (m, 2H), 6.90, 6.83 (2 × s, 1H), 6.64, 6.61 (2 × s, 1H), 6.57, 6.26 (2 × s, 1H), 5.81 – 5.78, 4.91 – 4.90 (2 × m, 1H), 4.80 – 4.76, 3.85 – 3.82 (m, 1H), 4.06 – 3.94, 3.76 – 3.71, , 3.48 – 3.42 (m, 2H), 3.86, 3.83 (2 × s, 3H), 3.25 – 3.15 (2 × m, 1H), 3.66 – 3.60 (m, 1H), 3.33- 3.26 (m, 1H), 2.90, 2.79 (2 × ddd, *J* = 17.4, 11.7, 6.1 Hz, 1H), 2.74 – 2.60 (m, 1H), 2.14, 1.52 (2 × s, 3H), 1.44, 1.27 (2 × t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.1, 169.5, 148.6, 147.9, 146.7, 146.4, 136.2, 136.1, 129.1, 128.4, 127.2, 126.7, 125.6, 123.5, 122.9, 122.3, 121.9, 119.8, 119.5, 119.2, 118.0, 112.5, 112.4, 112.0, 111.8,

111.7, 111.6, 111.1, 110.9, 64.7, 64.1, 57.9, 56.0, 53.1, 41.7, 34.9, 32.7, 31.9, 28.6, 28.0, 22.1, 21.0, 14.9, 14.6. ESI-MS *m/z* 379.0 $[M+H]^+$. HR-MS: (ESI, *m*/*z*) calcd for $C_{23}H_{25}N_2O_3^-$ $[M-H]^-$ 377.1871, found 377.1869.

methyl (S)-1-((1H-indol-3-yl)methyl)-7-ethoxy-6-methoxy-3,4-dihydroisoquinoline-2(1H)-carboxylate (24). White solid. This compound was prepared using a similar method of compound **23**, by replacing acetyl chloride with methyl carbonochloridate. HPLC purity: 99.65 %. m.p.: 99-100 ^oC. ^1H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 7.63, 7.61 (2 × d, *J* = 3.4 Hz, 1H), 7.35, 7.33 (2 × d, *J* = 8.5 Hz, 1H), 7.23 – 7.04 (m, 2H), 6.85, 6.80 (2 × s, 1H), 6.61, 6.29 (2 × s, 1H), 6.58, 6.15 (2 × s, 1H), 5.45 – 5.39, 5.35 – 5.27 (2 × m, 1H), 4.15, 3.79 (2 × ddd, *J* = 13.0, 5.9, 3.7 Hz, 1H), 3.84, 3.74 (2 × s, 3H), 3.83, 3.42 (2 × s, 3H), 3.73 – 3.51 (m, 2H), 3.40 – 3.07 (m, 3H), 2.95 – 2.41 (m, 2H), 1.33, 1.25 (2 × t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.3, 148.1, 148.0, 146.3, 146.2, 136.4, 136.2, 129.1, 128.7, 128.0, 127.8, 126.4, 126.2, 123.2, 123.0, 122.1, 122.1, 119.6, 119.4, 119.0, 112.9, 112.7, 112.4, 112.1, 111.6, 111.3, 111.2, 111.0, 64.4, 64.2, 56.0, 55.1, 54.8, 52.7, 52.4, 39.3, 38.2, 33.0, 32.5, 28.3, 28.2, 14.9, 14.8. ESI-MS *m/z* 417.0 $[M+Na]^+$. HR-MS: (ESI, *m*/*z*) calcd for $C_{23}H_{25}N_2O_4^-$ $[M-H]^-$ 393.1820, found 393.1824.

(S)-1-(1-((1H-indol-3-yl)methyl)-7-ethoxy-6-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)-2-methoxyethan-1-one (25). White solid. This compound was prepared using a similar method of compound **23**, by replacing acetyl chloride with methoxyacetyl chloride. HPLC purity: 99.65 %. m.p.: 135-137 ^oC. ^1H NMR (400 MHz, $CDCl_3$) δ 8.42, 8.14 (2 × s, 1H), 7.66, 7.63 (2 × d, *J* = 7.1 Hz, 1H), 7.39, 7.33 (2 × d, *J* = 7.2 Hz, 1H), 7.25 – 7.05 (m, 2H), 6.93, 6.87 (2 × s, 1H), 6.63, 6.57 (2 × s, 1H), 6.60, 6.26 (2 × s, 1H), 5.79 – 5.76, 4.90 – 4.87 (2 × m, 1H), 4.82- 4.77, 3.45 – 3.40 (2 × m, 1H), 4.13 (s, 1H), 4.04 – 3.95 (m, 1H), 3.86, 3.30 (2 × s, 3H), 3.83, 2.95 (2 × s, 3H), 3.78 – 3.57 (m, 2H), 3.38 – 3.17 (m, 3H), 2.69 – 2.74 (2 × m, 1H), 2.74 – 2.64 (2 × m, 1H), 1.44, 1.28 (2 × t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.9, 168.3, 148.9, 148.3, 147.1, 146.8, 136.6, 136.4, 129.0, 128.5, 128.3, 127.5, 126.8, 125.8, 124.0, 123.4, 122.8, 122.3, 120.3, 119.9, 119.6, 118.6, 112.7, 112.6, 112.3, 112.2, 112.0, 112.0, 111.5, 111.3, 72.3, 70.1, 65.1, 64.5, 59.5, 59.0, 56.4, 56.3,

53.5, 40.3, 35.4, 33.1, 32.3, 29.1, 28.3, 15.2, 15.0. ESI-MS *m/z* 409.1 $[M+H]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{24}H_{29}N_2O_4^+$ $[M+H]^+$ 409.2122, found 409.2122.

(S)-1-((1H-indol-3-yl)methyl)-7-ethoxy-6-methoxy-2-(2-(methylsulfonyl)ethyl)-1,2,3,4-tetrahydroisoquinoline (26). White solid. This compound was prepared using a similar method of compound **23**, by replacing acetyl chloride with 2-bromoetyl-methylsulfone. HPLC purity: 98.86 %. m.p.: 78-79 ^oC. ^1H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.58 (d, *J* = 7.8 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.20 (t, *J* = 7.3 Hz, 1H), 7.13 (t, *J* = 7.3 Hz, 1H), 6.98 (d, *J* = 1.8 Hz, 1H), 6.59 (s, 1H), 6.36 (s, 1H), 4.02 – 3.93 (m, 1H), 3.92 – 3.74 (m, 6H), 3.51 – 3.38 (m, 1H), 3.26 – 3.15 (m, 1H), 3.10 – 2.83 (m, 6H), 2.57 – 2.53 (m, 1H), 2.41 (s, 3H), 1.36 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 148.2, 146.6, 136.3, 127.7, 122.8, 122.3, 119.7, 118.8, 112.9, 111.8, 111.5, 64.4, 62.3, 56.0, 47.9, 43.3, 41.7, 32.4, 23.4, 14.9. ESI-MS *m/z* 443.2 $[M+H]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{24}H_{31}N_2O_4S^+$ $[M+H]^+$ 443.1999, found 443.1991.

(S)-1-(1-((1H-indol-3-yl)methyl)-7-ethoxy-6-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)-2-(methylsulfonyl)ethan-1-one (27). White solid. This compound was prepared using a similar method of compound **12**, by replacing **6a** with **6b**. HPLC purity: 99.57 %. m.p.: 101-103 ^oC. ^1H NMR (600 MHz, $CDCl_3$) δ 8.24, 8.02 (2 × s, 1H), 7.61, 7.57 (2 × d, *J* = 7.9 Hz, 1H), 7.40, 7.33 (2 × d, *J* = 8.0 Hz, 1H), 7.26 – 7.16 (m, 2H), 7.02, 6.87 (2 × s, 1H), 6.71, 6.58 (2 × s, 1H), 6.64, 6.24 (2 × s, 1H), 5.81 – 5.78, 5.00 – 4.98 (2 × m, 1H), 4.75 – 4.72, 3.85 – 3.83 (2 × m, 1H), 4.14 – 4.00 (m, 2H), 3.86, 3.83 (2 × s, 3H), 3.77 – 3.49 (m, 1H), 3.34 – 3.19, 2.98 – 2.94 (m, 3H), 2.99, 2.79 (2 × s, 3H), 2.94 – 2.90 (2 × m, 1H), 2.74 – 2.70 (2 × m, 1H), 1.47, 1.28 (2 × t, *J* = 7.0 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 161.6, 161.0, 148.7, 148.2, 147.1, 146.5, 136.1, 127.8, 127.7, 127.5, 126.9, 126.0, 125.1, 123.4, 123.3, 122.9, 122.1, 120.5, 119.7, 119.1, 118.1, 112.2, 112.0, 111.8, 111.7, 111.1, 111.0, 64.8, 64.2, 58.7, 58.3, 57.1, 57.0, 55.0, 54.0, 42.4, 41.5, 41.4, 36.2, 32.7, 32.0, 28.5, 27.9, 14.9, 14.6. ESI-MS *m/z* 457.0 $[M+H]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{24}H_{29}N_2O_5S^+$ $[M+H]^+$ 457.1792, found 457.1785.

(S)-1-((1H-indol-3-yl)methyl)-7-(benzyloxy)-6-methoxy-3,4-dihydroisoquinoline-2(1H)-carbaldehyde (28). White solid. This compound was prepared using a similar method of compound **2**, by replacing **5a** with **5c**. HPLC purity: 99.54 %. m.p.: 108-109 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21, 8.02 (2 × s, 1H), 8.14, 7.51 (m, 1H), 7.54, 7.50 (2 × d, *J* = 7.9 Hz, 1H), 7.54 – 7.27 (m, 5H), 7.28 – 7.07 (m, 3H), 6.86, 6.71 (2 × d, *J* = 2.3 Hz, 1H)6.67, 6.56 (2 × s, 1H), 6.66, 6.30 (2 × s, 1H), 5.57 – 5.54, 4.87 – 4.68 (2 × m, 1H), 5.12 (s, 1H), 4.59 – 4.55, 4.52 – 4.47 (2 × m, 1H), 3.89, 3.85 (2 × s, 3H), 3.57 – 2.99 (m, 3H), 2.93 – 2.77 (2 × m, 1H), 2.75 – 2.59 (2 × m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.0, 161.9, 149.3, 148.8, 147.0, 146.6, 137.5, 137.4, 136.8, 136.4, 129.0, 128.9, 128.4, 128.2, 128.0, 127.9, 127.7, 127.6, 127.2, 127.1, 126.1, 124.0, 123.5, 122.7, 122.4, 120.1, 120.0, 119.5, 118.5, 113.7, 113.6, 112.4, 112.3, 112.1, 112.0, 111.7, 111.4, 71.9, 71.0, 57.8, 56.4, 56.4, 51.6, 41.3, 34.3, 33.6, 32.0, 29.6, 28.2. ESI-MS *m/z* 427.1 $[M+H]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{27}H_{27}N_2O_3^+$ $[M+H]^+$ 427.2016, found 427.2025.

(S)-6,7-dimethoxy-1-(thiophen-3-ylmethyl)-3,4-dihydroisoquinoline-2(1H)-carbaldehyde (29). White solid. This compound was prepared using a similar method of compound **1**, by replacing **6a** with **12a**. HPLC purity: 99.72 %. m.p.: 68-69 ^{o}C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04, 7.64 (2 × s, 1H), 7.49, 7.40 (2 × br, 1H), 7.22, 7.12 (2 × d, *J* = 2.9 Hz, 1H), 7.07, 6.90 (2 × d, *J* = 4.9 Hz, 1H), 6.87, 6.68 (2 × s, 1H), 6.72 (s, 1H), 5.40 – 5.37, 4.81 – 4.78 (2 × m, 1H), 4.20 – 4.16, 3.25 – 3.31 (2 × m, 1H), 3.73, 3.71 (2 × s, 3H), 3.72, 3.66 (2 × s, 3H), 3.14 – 3.00 (m, 3H), 2.73 – 2.63 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.4, 160.7, 147.7, 147.5, 147.2, 147.1, 138.5, 138.3, 129.0, 128.8, 128.1, 127.5, 126.0, 125.5, 125.4, 125.4, 122.9, 122.3, 112.0, 111.9, 110.6, 110.5, 56.8, 55.6, 55.5, 55.4, 55.4, 50.2, 36.6, 35.6, 33.0, 28.7, 27.1. ESI-MS m/z 318.1 $[M+H]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{17}H_{20}NO_3S^+$ $[M+H]^+$ 318.1158, found 318.1154.

(S)-1-benzyl-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1H)-carbaldehyde (30). White solid. This compound was prepared using a similar method of compound **29**, by replacing **12a** with **12b**. HPLC purity: 99.48 %. m.p.: 109-111 ^{o}C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12, 7.65 (2 × s, 1H), 7.38 – 7.03 (m, 5H), 6.62, 6.55

(2 × s, 1H), 6.54, 6.23 (2 × s, 1H), 5.54 – 5.52, 4.61 – 4.58 (2 × m, 1H), 4.50 – 4.46, 3.59 – 3.57 (2 × m, 1H), 3.87, 3.84 (2 × s, 3H), 3.82, 3.62 (2 × s, 3H), 3.39 – 3.33, 3.20 – 3.02 (m, 3H), 2.93 – 2.80 (2 × m, 1H), 2.74 – 2.64 (2 × m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.5, 161.4, 148.4, 148.0, 147.7, 147.4, 137.7, 137.4, 130.0, 129.5, 129.0, 128.4, 127.5, 127.2, 127.1, 126.8, 126.2, 125.3, 111.8, 111.4, 110.5, 110.0, 59.2, 56.2, 56.1, 56.0, 55.8, 52.3, 43.6, 42.2, 40.9, 34.3, 29.2, 27.8. ESI-MS m/z 312.0 $[M+H]^+$. HR-MS: (ESI, *m*/*z*) calcd for $C_{19}H_{22}NO_3^+$ $[M+H]^+$ 312.1594, found 312.1601.

1-((1H-indol-2-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1H)-carbaldehyde (31). White solid. This compound was prepared using a similar method of compound **2**, by replacing **5a** with **11a**. HPLC purity: 98.29 %. m.p.: 184-185 ^{o}C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.72, 8.09 (2 × s, 1H), 8.15, 8.04 (2 × s, 1H), 7.53, 7.50 (2 × d, *J* = 7.7 Hz, 1H), 7.32, 7.28 (2 × d, *J* = 8.0 Hz, 1H), 7.13 – 7.04 (2 × m, 2H), 6.62, 6.58 (2 × s, 1H), 6.46, 6.44 (2 × s, 1H), 6.31, 6.21 (2 × s, 1H), 5.63 - 5.61, 4.82 – 4.80 (2 × m, 1H), 4.51 – 4.48, 3.67 – 3.63 (2 × m, 1H), 3.87, 3.86 (2 × s, 3H), 3.68, 3.63 (2 × s, 3H), 3.38 – 3.30 (m, 2H), 3.28 – 3.21, 3.11 – 3.06 (2 ×m, 1H), 2.90 – 2.85 (2 × m, 1H), 2.74 – 2.62 (2 × m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.2, 148.8, 148.5, 148.2, 136.7, 136.4, 135.6, 134.6, 128.8, 127.3, 126.9, 126.5, 125.3, 122.1, 121.7, 120.5, 120.4, 120.2, 120.0, 112.0, 111.6, 111.3, 111.1, 110.0, 102.8, 102.2, 58.0, 56.3, 56.1, 50.8, 41.3, 37.2, 36.3, 34.9, 29.5, 28.0. ESI-MS *m/z* 351.1 $[M+H]^+$. HR-MS: (ESI, *m*/*z*) calcd for $C_{21}H_{23}N_2O_3^+$ $[M+H]^+$ 351.1703, found 351.1704.

(S)-1-((1H-indol-2-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1H)-carbaldehyde (32). White solid. This compound was prepared using a similar method of compound **31**, by replacing **11a** with **12c**. HPLC purity: 98.64 %. m.p.: 98-99 ^{o}C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.70, 8.10 (2 × s, 1H), 8.16, 8.03 (2 × s, 1H), 7.53, 7.50 (2 × d, *J* = 7.7 Hz, 1H), 7.32, 7.28 (2 × d, *J* = 8.0 Hz, 1H), 7.13 – 7.04 (2 × m, 2H), 6.62, 6.58 (2 × s, 1H), 6.46, 6.44 (2 × s, 1H), 6.32, 6.21 (2 × s, 1H), 5.63 - 5.61, 4.81 – 4.79 (2 × m, 1H), 4.51 – 4.48, 3.67 – 3.63 (2 × m, 1H), 3.87, 3.86 (2 × s, 3H), 3.68, 3.63 (2 × s, 3H), 3.38 – 3.30 (m, 2H), 3.28 – 3.21, 3.11 – 3.06 (2 ×m, 1H),

2.91 – 2.85 (2 × m, 1H), 2.71 – 2.66 (2 × m, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 162.0, 161.7, 148.3, 148.0, 147.8, 136.5, 136.6, 136.2, 135.4, 134.4, 128.7, 128.6, 127.1, 126.7, 125.3, 125.1, 121.9, 121.5, 120.3, 120.0, 119.8, 111.7, 111.4, 111.0, 110.8, 109.7, 102.6, 102.0, 57.8, 56.1, 56.0, 55.9, 50.6, 41.1, 37.0, 36.1, 34.6, 29.2, 27.8.ESI-MS *m/z* 351.1 $[M+H]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{21}H_{23}N_2O_3^+$ $[M+H]^+$ 351.1703, found 351.1710.

(S)-1-((4-chloro-1H-indol-3-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinoline -2(1H)-carbaldehyde (33). White solid. This compound was prepared using a similar method of compound **1**, by replacing **6a** with 17**a**. HPLC purity: 99.89 %. m.p.: 242-243 ^{o}C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.58, 8.43 (2 × s, 1H), 8.10, 7.44 (s, 1H), 7.26 – 7.24 (2 × m, 1H), 7.13 – 7.10 (m, 2H), 7.08 – 7.04, 6.89 – 6.88 (2 × m, 2H), 6.64, 6.57 (2 × s, 1H), 5.78 – 5.75, 4.90 – 4.87 (2 × m, 1H), 5.37 – 5.32, 4.57 – 4.53 (2 × m, 1H), 3.88, 3.64 (2 × s, 3H), 3.86 (s, 3H), 3.80, 3.77 (2 × d, *J* = 3.5 Hz, 1H), 3.21 – 3.15 (2 × m, 1H), 3.10 – 3.05 (2 × m, 1H), 2.96 – 2.89 (2 ×m, 1H), 2.76 – 2.74 (2 × m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.8, 148.1, 147.6, 138.1, 127.8, 125.8, 125.7, 125.5, 123.3, 122.8, 120.7, 111.5, 111.4, 110.6, 109.7, 58.3, 55.9, 33.9, 33.7, 27.8. ESI-MS m/z 385.0 $[M+H]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{21}H_{22}ClN_2O_3^+$ $[M+H]^+$ 385.1313, found 385.1322.

1-((6-chloro-1H-indol-3-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1 H)-carbaldehyde (34). White solid. This compound was prepared using a similar method of compound **2**, by replacing **5a** with 1**6a**. HPLC purity: 95.71 %. m.p.: 144-141 ^{o}C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.52, 8.30 (2 × s, 1H), 8.15, 7.57 (2 × s, 1H), 7.49, 7.45 (2 × d, *J* = 8.5 Hz, 1H), 7.36, 7.32 (2 × d, *J* = 1.9 Hz, 1H), 7.13, 7.04 (2 × dd, *J* = 8.5, 1.8 Hz, 1H), 6.92, 6.88 (2 × d, *J* = 2.0 Hz, 1H), 6.66, 6.55 (2 × s, 1H), 6.64, 6.31 (2 × s, 1H), 5.63 – 5.61, 4.69 – 4.66 (2 × m, 1H), 4.50 – 4.46, 3.40 – 3.32 (2 × m, 1H). 3.88, 3.85 (2 × s, 3H), 3.87, 3.59 (2 × s, 3H), 3.29 – 3.06 (m, 3H), 2.97 – 2.80 (2 × m, 1H), 2.76 – 2.63 (2 × m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.9, 148.7, 148.3, 148.1, 147.7, 137.1, 136.8, 128.6, 128.3, 127.9, 126.9, 126.5, 125.9, 125.6, 124.7, 124.1, 120.9, 120.7, 120.3, 119.4, 112.5, 112.0, 111.9, 111.6, 111.4, 110.8, 110.3, 57.9, 56.6, 56.3, 56.2, 56.1, 51.7, 41.2, 34.4, 33.6, 32.1, 29.6, 28.1.

ESI-MS *m/z* 385.1 $[M+H]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{21}H_{22}ClN_2O_3^+$ $[M+H]^+$ 385.1313, found 385.1325.

(S)-1-((6-chloro-1H-indol-3-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinoline -2(1H)-carbaldehyde (35). White solid. This compound was prepared using a similar method of compound **1**, by replacing **6a** with **17b**.HPLC purity: 99.86 %. m.p.: 103-105 ^oC. ^1H NMR (500 MHz, $CDCl_3$) δ 8.48, 8.27 (2 × s, 1H), 8.15, 7.57 (2 × s, 1H), 7.47, 7.34 (2 × d, *J* = 8.5 Hz, 1H), 7.35, 7.32 (2 × d, *J* = 1.9 Hz, 1H), 7.13, 7.04 (2 × dd, *J* = 8.5, 1.8 Hz, 1H), 6.92, 6.88 (2 × d, *J* = 2.0 Hz, 1H), 6.66, 6.56 (2 × s, 1H), 6.64, 6.32 (2 × s, 1H), 5.63 – 5.61, 4.69 – 4.66 (2 × m, 1H), 4.50 – 4.46, 3.40 – 3.35 (2 × m, 1H). 3.88, 3.85 (2 × s, 3H), 3.87, 3.59 (2 × s, 3H), 3.28 – 3.11 (m, 3H), 2.94 – 2.81 (2 × m, 1H), 2.76 – 2.63 (2 × m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.0, 161.9, 148.7, 148.3, 148.1, 147.8, 137.1, 136.8, 128.7, 128.3, 127.9, 126.9, 126.5, 125.9, 125.6, 124.7, 124.1, 120.9, 120.7, 120.3, 119.4, 112.6, 112.1, 112.0, 111.9, 111.6, 111.4, 110.8, 110.3, 57.9, 56.6, 56.3, 56.2, 56.1, 51.7, 41.3, 34.4, 33.6, 32.1, 29.6, 28.1. ESI-MS *m/z* 385.1 $[M+H]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{21}H_{22}ClN_2O_3^+$ $[M+H]^+$ 385.1313, found 385.1323.

(S)-1-((7-chloro-1H-indol-3-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinoline -2(1H)-carbaldehyde (36). White solid. This compound was prepared using a similar method of compound **1**, by replacing **6a** with **17c**. HPLC purity: 99.43 %. m.p.: 133-135 ^oC. ^1H NMR (500 MHz, DMSO-d_6) δ 11.26. 11.16 (2 × s, 1H), 8.05, 7.47 (2 × s, 1H), 7.68, 7.56 (2 × d, *J* = 7.9 Hz, 1H), 7.27 – 6.97 (m, 3H), 6.95, 6.68 (2 × s, 1H), 6.74, 6.59 (2 × s, 1H), 5.46 – 5.43, 4.83 – 4.80 (2 × m, 1H), 4.18 – 4.14, 3.42 – 3.38 (2 × m, 1H), 3.75, 3.71 (2 × s, 3H), 3.74, 3.58 (2 × s, 3H), 3.28 – 3.09 (m, 3H), 2.74 – 2.64 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.9, 161.1, 148.2, 148.0, 147.7, 147.5, 133.4, 133.3, 130.1, 129.6, 128.9, 128.3, 126.3, 126.0, 125.9, 125.6, 121.0, 120.8, 120.0, 119.8, 118.3, 118.1, 116.3, 116.2, 112.6, 112.5, 112.4, 112.3, 111.3, 111.2, 57.0, 56.2, 56.0, 55.9, 55.8, 50.6, 33.5, 32.7, 31.7, 31.4, 29.2, 27.6. ESI-MS m/z 384.9 $[M+H]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{21}H_{21}ClN_2O_3^+$ $[M+H]^+$ 385.1313, found 385.1320.

(S)-1-((5-bromo-1H-indol-3-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinoline -2(1H)-carbaldehyde (37). White solid. This compound was prepared using a similar method of compound **1**, by replacing **6a** with **17d**. HPLC purity: 99.71 %. m.p.: 113-115 ^{o}C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.11, 11.03 (2 × s, 1H), 8.06, 7.50 (2 × s, 1H), 7.87, 7.69 (2 × d, *J* = 1.9 Hz, 1H), 7.32, 7.29 (2 × d, *J* = 8.6 Hz, 1H), 7.20, 7.08 (2 × d, *J* = 2.3 Hz, 1H), 7.17, 7.14 (2 × dd, *J* = 8.6, 1.9 Hz, 1H), 6.97, 6.68 (2 × s, 1H), 6.73, 6.58 (2 × s, 1H), 5.40, 4.81 (2 × dd, *J* = 10.5, 4.4 Hz, 1H), 4.15, 3.68 (2 × ddd, *J* = 13.1, 6.2, 2.4 Hz, 1H), 3.74, 3.71 (2 × s, 3H), 3.74, 3.56 (2 × s, 3H), 3.39, 3.21 (2 × ddd, *J* = 13.2, 11.5, 4.6 Hz, 1H), 3.18 – 3.02 (m, 2H), 2.79 – 2.59 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.0, 161.9, 148.8, 148.5, 148.2, 148.0, 135.4, 135.0, 130.1, 129.0, 128.0, 127.9, 126.5, 125.8, 125.7, 125.3, 124.7, 122.0, 121.2, 113.5, 113.3, 112.8, 112.2, 112.1, 111.8, 111.5, 110.9, 110.4, 57.7, 56.7, 56.4, 56.3, 56.2, 51.8, 41.3, 34.4, 33.5, 32.1, 29.5, 28.1. ESI-MS m/z 428.8 $[M+H]^+$. HR-MS: (ESI, *m*/*z*) calcd for $C_{21}H_{22}BrN_2O_3^+$ $[M+H]^+$ 429.0808, found 429.0817.

(S)-6,7-dimethoxy-1-((5-methyl-1H-indol-3-yl)methyl)-3,4-dihydroisoquinoline -2(1H)-carbaldehyde (38). White solid. This compound was prepared using a similar method of compound **1**, by replacing 6**a** with **17e**. HPLC purity: 99.30 %. m.p.: 88-89 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.76, 10.67 (2 × s, 1H), 8.09, 7.49 (2 × s, 1H), 7.46 – 7.19 (m, 2H), 7.10 – 6.49 (m, 4H), 5.45 – 5.42, 4.83 – 4.79 (2 × m, 1H),4.18 – 4.14, 3.68 – 3.65 (2 × m, 1H), 3.73, 3.70 (2 × s, 3H), 3.73, 3.50 (2 × s, 3H), 3.21 – 3.02 (m, 3H), 2.74 – 2.62 (m, 2H), 2.40, 2.35 (2 × s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.4, 160.7, 147.7, 147.2, 147.5, 147.2, 146.8, 134.6, 134.5, 128.6, 127.8, 127.3, 126.8, 126.6, 125.6, 125.4, 124.5, 123.9, 122.6, 122.5, 118.2, 112.0, 111.8, 111.1, 111.0, 110.8, 110.0, 109.8, 56.4, 55.7, 55.5, 55.4, 55.2, 50.1, 33.0, 32.5, 31.4, 28.7, 27.2, 21.4. ESI-MS m/z 365.1 $[M+H]^+$. HR-MS: (ESI, *m*/*z*) calcd for $C_{22}H_{25}N_2O_3^+$ $[M+H]^+$ 365.1860, found 365.1858.

(S)-6,7-dimethoxy-1-((5-methoxy-1H-indol-3-yl)methyl)-3,4-dihydroisoquinoli ne-2(1H)-carbaldehyde (39). White solid. This compound was prepared using a similar method of compound **1**, by replacing **6a** with **17f**. HPLC purity: 99.73 %. m.p.: 103-105 ^{o}C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.18, 7.66 (2 × s, 1H), 8.05, 7.92 (2

× s, 1H), 7.28, 7.22 (2 × d, *J* = 8.9 Hz, 1H), 7.00 (2 × s, 1H), 6.94 – 6.79 (m, 2H), 6.65, 6.54 (2 × s, 1H), 6.64, 6.34 (2 × s, 1H), 5.66 – 5.63, 4.72 – 4.69 (2 × m, 1H), 4.49 – 4.45, 3.57 – 3.53 (2 × m, 1H), 3.88, 3.87 (2 × s, 3H), 3.84, 3.83 (s, 3H), 3.80, 3.59 (2 × s, 3H), 3.37 – 3.12 (m, 3H), 2.93 – 2.80 (2 × m, 1H), 2.75 – 2.62 (2 × m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.0, 161.9, 154.6, 148.7, 148.2, 148.0, 147.7, 132.0, 131.6, 128.6, 128.2, 127.8, 126.6, 125.7, 124.8, 124.1, 113.0, 112.6, 112.3, 112.1, 111.6, 111.5, 111.0, 110.5, 101.4, 101.0, 57.7, 56.5, 56.3, 56.1, 51.5, 41.4, 34.5, 33.9, 32.4, 30.1, 29.6, 28.2. ESI-MS m/z 381.0 $[M+H]^+$. HR-MS: (ESI, *m*/*z*) calcd for $C_{22}H_{25}N_2O_4^+$ $[M+H]^+$ 381.1809, found 381.1817.

(S)-6,7-dimethoxy-1-((5-methoxy-2-methyl-1H-indol-3-yl)methyl)-3,4-dihydroisoquinoline-2(1H)-carbaldehyde (40). White solid. This compound was prepared using a similar method of compound **1**, by replacing **6a** with **17g**. HPLC purity: 97.24 %. m.p.: 106-108 ^{o}C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.23, 7.54 (2 × s, 1H), 8.00, 7.83 (2 × s, 1H), 7.20, 7.14 (2 × d, *J* = 8.7 Hz, 1H), 7.00, 6.98 (2 × d, *J* = 2.5 Hz, 1H), 6.83, 6.76 (2 × dd, *J* = 8.7, 2.5 Hz, 1H), 6.67, 6.57 (2 × s, 1H), 6.66, 6.14 (s, 1H), 5.55 – 5.53, 4.69 – 4.66 (2 × m, 1H), 4.47 – 4.44, 3.65 – 3.51 (2 × m, 1H), 3.90 - 3.47 (3 × 2 × s, 9H), 3.32 – 3.01 (m, 3H), 2.94 – 2.83 (2 × m, 1H), 2.81 – 2.64 (2 × m, 1H), 2.19, 2.07 (2 × s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.5, 161.0, 154.0. 153.9, 148.1, 147.7, 147.5, 147.0, 134.1, 133.3, 130.6, 130.0, 129.4, 128.4, 127.6, 127.5, 126.1, 125.1, 111.5, 111.2, 111.1, 111.0, 110.6, 110.0, 109.9, 107.7, 107.0, 100.6, 100.4, 56.9, 56.0, 55.9, 55.8, 55.7, 55.6, 55.4, 51.1, 41.1, 34.3, 32.5, 31.0, 28.9, 27.8, 11.6, 11.5. ESI-MS m/z 395.0 $[M+H]^+$. HR-MS: (ESI, *m*/*z*) calcd for $C_{23}H_{27}N_2O_4^+$ $[M+H]^+$ 395.1965, found 395.1972.

6,7-dimethoxy-1-((1-methyl-1H-indol-3-yl)methyl)-3,4-dihydroisoquinoline-2(1H)-carbaldehyde (41). White solid. This compound was prepared using a similar method of compound **2**, by replacing **5a** with **16b**. HPLC purity: 99.66 %. m.p.: 120-122 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.08, 7.49 (2 × s, 1H), 7.71, 7.61 (2 × d, *J* = 7.8 Hz, 1H), 7.39, 7.37 (2 × d, *J* = 8.6 Hz, 1H), 7.26 – 6.97 (m, 3H), 6.93, 6.67 (2 × s, 1H), 6.73, 6.45 (2 × s, 1H), 5.44, 4.82 (2 × dd, *J* = 10.5, 4.2 Hz, 1H), 4.32 – 4.14, 3.72 – 3.67 (2 × m, 1H), 3.74, 3.73 (2 × s, 6H), 3.70, 3.48 (2 × s, 3H), 3.46 –

3.38, 3.27 – 3.01 (2 × m, 3H), 2.88 – 2.60 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.7, 161.6, 148.4, 147.9, 147.7, 147.3, 137.3, 137.0, 128.3, 128.0, 127.9, 127.4, 126.2, 125.2, 122.0, 121.7, 119.4, 119.2, 118.3, 111.7, 111.3, 110.7, 110.6, 110.1, 110.0, 109.8, 109.1, 57.7, 56.2, 56.1, 56.0, 55.6, 51.4, 40.9, 34.0, 33.4, 32.9, 32. 8, 31.9, 29.4, 27.9. ESI-MS m/z 365.1 $[M+H]^+$. HR-MS: (ESI, *m*/*z*) calcd for $C_{22}H_{25}N_2O_3^+$ $[M+H]^+$ 365.1860, found 365.1868.

(S)-6,7-dimethoxy-1-((5-phenyl-1H-indol-3-yl)methyl)-3,4-dihydroisoquinoline-2(1H)-carbaldehyde (42). White solid. This compound was prepared using a similar method of compound **1**, by replacing **6a** with **20a**. HPLC purity: 97.28 %. m.p.: 222-223 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.98, 10.90 (2 × s, 1H), 8.11, 7.84 (2 × s, 1H), 7.76 – 7.57 (m, 3H), 7.49 – 7.25 (m, 5H), 7.21, 7.06 (2 × d, *J* = 2.3 Hz, 1H), 6.95, 6.67 (2 × s, 1H), 6.74, 6.58 (2 × s, 1H), 5.47, 4.90 (2 × dd, *J* = 10.1, 4.4 Hz, 1H), 4.24 – 4.10, 3.70 – 3.63 (2 × m, 1H), 3.73 (s, 3H), 3.69, 3.53 (2 × s, 3H), 3.32 – 3.08 (m, 3H), 2.83 – 2.58 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.7, 161.6, 148.5, 148.1, 147.8, 147.6, 142.7, 142.5, 136.1, 135.7, 133.7, 133.2, 128.9, 128.8, 128.6, 128.0, 127.6, 127.5, 127.4, 126.6, 126.4, 126.3, 125.5, 124.4, 123.9, 122.5, 122.0, 117.7, 117.0, 112.6, 112.0, 111.9, 111.8, 111.4, 111.3, 110.8, 110.3, 57.6, 56.3, 56.1, 56.0, 55.9, 51.5, 41.1, 34.2, 33.6, 32.0, 29.3, 27.9. ESI-MS m/z 427.0 $[M+H]^+$. HR-MS: (ESI, *m*/z) calcd for $C_{27}H_{27}ClN_2O_3^+$ $[M+H]^+$ 427.2016, found 427.2022.

(S)-1-((4-chloro-1H-indol-3-yl)methyl)-7-ethoxy-6-methoxy-3,4-dihydroisoquinoline-2(1H)-carbaldehyde (43). White solid. This compound was prepared using a similar method of compound **33**, by replacing **1a** with **1b**. HPLC purity: 99.47 %. m.p.: 180-181 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62, 8.48 (2 × s, 1H), 8.09, 7.41 (2 × s, 1H), 7.26 – 7.24 (m, 1H), 7.23 – 7.06 (m, 1H), 7.15 – 7.00 (m, 2H), 6.92, 6.59 (2 × s, 1H), 6.64, 6.57 (2 × s, 1H), 5.77 – 5.73, 4.89 – 4.85 (2 × m, 1H), 4.58 – 4.53, 3.49 – 3.46 (2 × m, 1H), 4.08 (q, *J* = 7.0 Hz, 2H), 3.87, 3.85 (2 × s, 3H), 3.78 (dd, *J* = 14.5, 3.2 Hz, 1H), 3.21 – 3.14 (2 × m, 1H), 3.09 – 3.03 (2 × m, 1H), 2.98 – 2.84 (2 × m, 1H), 2.78 – 2.74 (2 × m, 1H), 1.48, 1.38 (2 × t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.2, 148.8, 147.3, 138.5, 128.2, 126.2, 126.1, 126.0, 123.8, 123.3, 121.2, 112.1, 112.0, 111.7, 111.0, 64.8, 58.7, 56.4, 34.3, 34.1, 28.3, 15.12. ESI-MS m/z

399.0 $[M+H]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{22}H_{23}ClN_2O_3^+$ $[M+H]^+$ 399.1470, found 399.1476.

1-((6-chloro-1H-indol-3-yl)methyl)-7-ethoxy-6-methoxy-3,4-dihydroisoquinoline-2(1H)-carbaldehyde (44). White solid. This compound was prepared using a similar method of compound **34**, by replacing **1a** with **1b**. HPLC purity: 99.09 %. m.p.: 118-120 ^oC. ^1H NMR (500 MHz, DMSO-d_6) δ 11.02, 10.09 (2 × s, 1H), 8.04, 7.45 (2 × s, 1H), 7.69, 7.56 (2 × d, *J* = 8.4 Hz, 1H), 7.39, 7.36 (2 × s, 1H), 7.19, 7.04 (2 × s, 1H), 6.98, 6.97 (2 × d, *J* = 8.4 Hz, 1H), 6.93, 6.67 (2 × s, 1H), 6.73, 6.53 (2 × s, 1H), 5.42 – 5.39, 4.79 – 4.76 (2 × m, 1H), 4.21 – 4.08, 3.41 – 3.36 (2 × m, 1H), 4.01 – 3.95, 3.78 – 3.74 (2 × m, 2H), 3.74, 3.71 (2 × s, 3H), 3.25 – 3.02 (m, 3H), 2.80 – 2.60 (2 × m, 2H), 1.32, 1.23 (2 × t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.4, 160.6, 147.9, 147.7, 146.3, 146.1, 136.5, 136.4, 128.4, 127.7, 126.4, 125.9, 125.7, 125.6, 125.4, 124.9, 120.1, 119.9, 118.7, 118.6, 112.0, 111.9, 111.0, 110.9, 110.8, 63.9, 63.5, 56.4, 55.4, 55.3, 50.2, 33.0, 32.1, 31.1, 28.7, 27.2, 14.8, 14.7. ESI-MS *m/z* 399.1 $[M+H]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{22}H_{24}ClN_2O_3^+$ $[M+H]^+$ 399.1834, found 399.1848.

(S)-1-((6-chloro-1H-indol-3-yl)methyl)-7-ethoxy-6-methoxy-3,4-dihydroisoquinoline-2(1H)-carbaldehyde (45). White solid. This compound was prepared using a similar method of compound **35**, by replacing **1a** with **1b**. HPLC purity: 99.69 %. m.p.: 98-100 ^oC. ^1H NMR (500 MHz, $CDCl_3$) δ 8.54, 8.32 (2 × s, 1H), 8.14, 7.55 (2 × s, 1H), 7.48, 7.42 (2 × d, *J* = 8.4 Hz, 1H), 7.34, 7.31 (2 × d, *J* = 1.8 Hz, 1H), 7.12, 7.04 (2 × dd, *J* = 8.5, 1.8 Hz, 1H), 6.91, 6.86 (s, 1H), 6.70, 6.55 (2 × s, 1H), 6.64, 6.37 (2 × s, 1H), 5.62 – 5.60, 4.67 – 4.65 (2 × m, 1H), 4.49 – 4.46, 3.56 – 3.53 (2 × m, 1H), 4.09 – 4.04, 3.84 – 3.73 (2 × m, 2H), 3.87, 3.84 (2 × s, 3H), 3.37 – 3.13 (m, 3H), 2.92 – 2.80 (2 × m, 1H), 2.75 – 2.62 (2 × m, 1H), 1.48, 1.34 (2 × t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.7, 161.6, 148.9, 148.4, 147.1, 146.9, 136.9, 136.5, 128.3, 128.0, 127.7, 126.6, 126.3, 125.6, 125.4, 124.4, 123.8, 120.6, 120.4, 120.0, 119.1, 112.2, 112.0, 111.8, 111.7, 111.6, 111.0, 65.0, 64.4, 57.6, 56.1, 56.0, 51.4, 41.0, 34.1, 33.3, 32.0, 29.3, 29.0, 27.9, 15.0, 14.8. ESI-MS *m/z* 399.1 $[M+H]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{22}H_{24}ClN_2O_3^+$ $[M+H]^+$ 399.1470, found 399.1476.

(S)-1-((7-chloro-1H-indol-3-yl)methyl)-7-ethoxy-6-methoxy-3,4-dihydroisoquinoline-2(1H)-carbaldehyde (46). White solid. This compound was prepared using a similar method of compound **36**, by replacing **1a** with **1b**. HPLC purity: 99.69 %. m.p.: 107-109 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.29, 11.19 (s, 1H), 8.04, 7.46 (2 × s, 1H), 7.68, 7.56 (2 × d, J = 7.9 Hz, 1H), 7.26 – 6.95 (m, 3H), 6.93, 6.67 (2 × s, 1H), 6.73, 6.54 (2 × s, 1H), 5.44 – 5.41, 4.82 – 4.79 (2 × m, 1H), 4.17 – 4.12, 3.70 – 3.66 (2 × m, 1H), 4.03 – 3.75 (m, 2H), 3.74, 3.71 (2 × s, 3H), 3.27 – 3.07 (m, 3H), 2.74 - 2.66 (m, 2H), 1.31, 1.24 (2 × t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.6, 160.8, 148.1, 147.9, 146.5, 146.2, 133.0, 132.9, 129.8, 129.3, 128.5, 127.8, 126.0, 125.7, 125.6, 125.3, 120.6, 120.5, 119.6, 119.5, 118.0, 117.8, 115.9, 112.3, 112.1, 64.0, 63.7, 56.6, 55.6, 55.5, 50.2, 33.1, 32.4, 31.3, 29.2, 28.8, 27.3, 26.7, 22.2, 14.9, 14.8. ESI-MS m/z 399.1 $[M+H]^+$. HR-MS: (ESI, *m*/*z*) calcd for $C_{22}H_{23}ClN_2O_3^+$ $[M+H]^+$ 399.1470, found 399.1476.

(S)-1-((5-bromo-1H-indol-3-yl)methyl)-7-ethoxy-6-methoxy-3,4-dihydroisoquinoline-2(1H)-carbaldehyde (47). White solid. This compound was prepared using a similar method of compound **37**, by replacing **1a** with **1b**. HPLC purity: 99.69 %. m.p.: 105-106 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.17, 11.09 (2 × s, 1H), 8.05, 7.49 (2 × s, 1H), 7.87, 7.68 (2 × d, J = 1.9 Hz, 1H), 7.33 - 7.28 (m, 1H), 7.20 – 7.07 (m, 2H), 6.98, 6.67 (2 × s, 1H), 6.73, 6.58 (2 × s, 1H), 5.30 – 5.37, 4.81 – 4.78 (2 × m, 1H), 4.17 – 4.11, 3.71 – 3.65 (2 × m, 1H), 4.04 – 3.75 (m, 2H), 3.73, 3.71 (2 × s, 3H), 3.22 – 3.05 (m, 3H), 2.78 – 2.61 (m, 2H), 1.32, 1.24 (2 × t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.6, 160.8, 148.1, 148.0, 146.5, 146.3, 135.0, 134.9, 129.5, 129.1, 128.6, 127.9, 126.3, 125.8, 125.7, 123.5, 123.4, 121.1, 113.5, 113.4, 112.4, 112.3, 112.2, 111.4, 111.2, 110.7, 110.6, 64.1, 63.8, 56.4, 55.6, 55.5, 50.3, 33.2, 32.2, 31.3, 28.8, 27.3, 14.9, 14.8. ESI-MS m/z 442.9 $[M+H]^+$. HR-MS: (ESI, *m*/*z*) calcd for $C_{22}H_{24}BrN_2O_3^+$ $[M+H]^+$ 443.0965, found 443.0970.

(S)-7-ethoxy-6-methoxy-1-((5-methyl-1H-indol-3-yl)methyl)-3,4-dihydroisoquinoline-2(1H)-carbaldehyde (48). White solid. This compound was prepared using a similar method of compound **38**, by replacing **1a** with **1b**. HPLC purity: 99.51 %. m.p.: 88-90 ^{o}C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22, 7.58 (2 × br, 1H), 8.15, 7.37 (2 ×

s, 1H), 7.36, 7.30 (2 × s, 1H), 7.27, 7.21 (2 × d, *J* = 8.3 Hz, 1H), 7.05, 6.99 (2 × d, *J* = 8.4 Hz, 1H), 6.88, 6.84 (2 × s, 1H), 6.72, 6.55 (2 × s, 1H), 6.64, 6.39 (2 × s, 1H), 5.66 – 5.62, 4.72 – 4.65 (2 × m, 1H), 4.53 – 4.45, 3.57 – 3.50 (2 × m, 1H), 4.12 – 4.02, 3.82 – 3.67 (2 × m, 2H), 3.87, 3.84 (2 × s, 3H), 3.40– 3.08 (m, 3H), 2.96 – 2.86 (2 × m, 1H), 2.78 – 2.59 (2 × m, 1H), 2.50, 2.42 (2 × s, 3H), 1.48, 1.33 (2 × t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.8, 161.6, 148.8, 148.3, 147.0, 146.8, 134.9, 129.0, 128.8, 128.1, 128.0, 127.2, 126.4, 125.4, 124.1, 123.9, 123.7, 123.3, 118.8, 117.9, 112.3, 112.1, 112.0, 111.6, 111.4, 110.9, 110.7, 65.0, 64.4, 57.5, 56.1, 56.1, 51.3, 41.0, 34.1, 33.5, 31.9, 29.3, 28.0, 21.8, 21.7, 15.0, 14.8. ESI-MS *m/z* 401.0 $[M+Na]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{23}H_{27}N_2O_3^+$ $[M+H]^+$ 379.2016, found 379.2015.

(S)-7-ethoxy-6-methoxy-1-((5-methoxy-1H-indol-3-yl)methyl)-3,4-dihydroisoquinoline-2(1H)-carbaldehyde (49). White solid. This compound was prepared using a similar method of compound **39**, by replacing **1a** with **1b**. HPLC purity: 97.82 %. m.p.: 93-95 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.62 (s, 1H), 7.27, 7.21 (2 × d, *J* = 8.8 Hz, 1H), 7.00, 6.98 (2 × d, *J* = 2.4 Hz, 1H), 6.91, 6.86 (2 × s, 1H), 6.88, 6.82 (2 × dd, *J* = 8.8, 2.4 Hz, 1H), 6.69, 6.54 (2 × s, 1H), 6.63, 6.39 (2 × s, 1H), 5.64 – 5.61, 4.70 - 4.61 (2 × m, 1H), 4.49 – 4.43, 3.57 – 3.51 (2 × m, 1H), 4.08 – 4.02, 3.78 – 3.72 (2 ×m, 2H), 3.87, 3.83 (2 × s, 3H), 3.87, 3.39 (2 × s, 3H), 3.37 – 3.08 (m, 3H), 2.95 – 2.77 (2 × m, 1H), 2.78 – 2.60 (2 × m, 1H), 1.46, 1.33 (2 × t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.0, 161.9, 154.6, 154.5, 149.10, 148.5, 147.3, 147.1, 132.1, 131.6, 128.6, 128.2, 127.8, 126.7, 125.7, 124.8, 124.1, 112.9, 112.6, 112.4, 112.3, 112.2, 112.1, 111.8, 111.4, 101.3, 101.0, 65.2, 64.6, 57.7, 56.5, 56.4, 56.3, 56.1, 51.5, 41.4, 34.5, 33.9, 32.4, 29.6, 28.2, 15.2, 15.1. ESI-MS *m/z* 395.2 $[M+H]^+$. HR-MS: (ESI, *m/z*) calcd for $C_{23}H_{27}N_2O_4^+$ $[M+H]^+$ 395.1965, found 395.1975.

(S)-7-ethoxy-6-methoxy-1-((5-methoxy-2-methyl-1H-indol-3-yl)methyl)-3,4-dihydroisoquinoline-2(1H)-carbaldehyde (50). White solid. This compound was prepared using a similar method of compound **40**, by replacing **1a** with **1b**. HPLC purity: 97.67 %. m.p.: 85-87 ^{o}C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.57, 10.53 (2 × s,

1H), 8.12, 7.43 (s, 1H), 7.12, 7.09 (2 × d, *J* = 8.6 Hz, 1H), 6.97, 6.83 (2 × d, *J* = 2.5 Hz, 1H), 6.82, 6.66 (2 × s, 1H), 6.74, 6.26 (2 × dd, *J* = 8.7, 2.4 Hz, 1H), 6.63, 6.58 (2 × dd, *J* = 8.6, 2.4 Hz, 1H), 5.30, 4.69 (2 × dd, *J* = 9.5, 4.5 Hz, 1H), 4.15 – 4.10, 3.61 – 3.55 (2 × m, 1H), 4.00 - 3.93 (2 × m, 2H), 3.76, 3.70 (2 × s, 3H), 3.74, 3.66 (2 × s, 3H), 3.37, 3.16 (2 × ddd, *J* = 12.8, 10.7, 5.0 Hz, 1H), 3.12 – 2.92 (m, 2H), 2.77 – 2.57 (m, 2H), 2.15, 2.02 (2 × s, 3H), 1.31, 1.18 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.6, 160.6, 153.2, 153.0, 148.1, 147.8, 146.5, 146.1, 134.3, 130.4, 130.3, 129.2, 128.7, 128.3, 127.6, 126.1, 125.8, 112.4, 112.2, 112.0, 111.0, 110.9, 109.7, 109.5, 106.6, 106.4, 100.5, 100.2, 64.0, 63.5, 56.2, 55.6, 55.5, 55.4, 55.20, 50.3, 33.8, 31.9, 30.7, 28.7, 27.5, 14.9, 14.8, 11.5. ESI-MS m/z 409.0 $[M+H]^+$. HR-MS: (ESI, *m*/*z*) calcd for $C_{24}H_{29}N_2O_4^+$ $[M+H]^+$ 409.2122, found 409.2130.

(S)-7-ethoxy-6-methoxy-1-((1-methyl-1H-indol-3-yl)methyl)-3,4-dihydroisoquinoline-2(1H)-carbaldehyde (51). White solid. This compound was prepared using a similar method of compound **22**, by replacing **2** with **13h**. HPLC purity: 99.86 %. m.p.: 66-68 ^{o}C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.08, 7.51 (2 × s, 1H), 7.69, 7.60 (2 × d, *J* = 7.9 Hz, 1H), 7.38 (2 × m, 1H), 7.20 – 6.96 (m, 3H), 6.90, 6.67 (2 × s, 1H), 6.73, 6.39 (2 × s, 1H), 5.44 - 5.41, 4.81 – 4.79 (2 × m, 1H), 4.19 – 4.15, 3.46 – 3.38 (2 × m, 1H), 3.99 – 3.95, 3.70 – 3.63 (2 × m, 2H), 3.73, 3.70 (2 × 2 × s, 6H), 3.27 – 3.06 (m, 3H), 2.75 – 2.65 (2 × m, 2H), 1.31, 1.19 (2 × t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.7, 160.9, 148.2, 148.0, 146.6, 146.25, 137.0, 136.9, 129.0, 128.8, 128.5, 128.2, 128.0, 127.8, 125.9, 125.7, 121.4, 121.3, 119.2, 119.1, 118.8, 118.7, 112.4, 112.3, 110.4, 110.2, 109.9, 109.7, 64.2, 63.7, 56.8, 55.8, 55.7, 50.4, 33.3, 32.6, 32.5, 31.6, 29.0, 27.5, 15.1, 14.1. ESI-MS m/z 379.1 $[M+H]^+$. HR-MS: (ESI, *m*/*z*) calcd for $C_{23}H_{27}N_2O_3^+$ $[M+H]^+$ 379.2016, found 379.2016.

(S)-7-ethoxy-6-methoxy-1-((5-phenyl-1H-indol-3-yl)methyl)-3,4-dihydroisoquinoline-2(1H)-carbaldehyde (52). White solid. This compound was prepared using a similar method of compound **42**, by replacing **1a** with **1b**. HPLC purity: 99.23 %. m.p.: 111-113 ^{o}C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.37, 8.20 (2 × s, 1H), 8.17, 7.74 (2 × s, 1H), 7.66 - 7.27 (m, 8H), 6.98, 6.97 (2 × d, *J* = 2.3 Hz, 1H), 6.73, 6.54 (2 × s, 1H), 6.64, 6.50 (2 × s, 1H), 5.69 – 5.66, 4.76- 4.69 (2 × m, 1H), 4.53 – 4.45, 3.56 – 3.50 (2

× m, 1H), 4.09 – 4.01, 3.85 – 3.75 (2 × m, 2H), 3.86, 3.80 (2 × s, 3H), 3.39 – 3.14 (m, 3H), 2.96 – 2.77 (2 × m, 1H), 2.79 – 2.60 (2 × m, 1H), 1.46, 1.33 (2 × t, *J* = 7.0 Hz, 3H).^{13}C NMR (126 MHz, $CDCl_3$) δ 161.8, 148.8, 148.4, 147.1, 142.7, 142.5, 136.1, 135.7, 133.6, 133.1, 128.4, 128.8, 127.9, 127.6, 127.5, 127.3, 126.6, 126.4, 126.3, 125.5, 124.5, 124.0, 122.4, 121.8, 117.6, 116.8, 112.4, 112.2, 112.0, 111.8, 111.6, 111.4, 65.0, 64.4, 57.7, 56.1, 56.0, 51.6, 41.2, 34.3, 33.6, 31.9, 29.3, 27.9, 15.0, 14.8. ESI-MS m/z 441.0 $[M+H]^+$. HR-MS: (ESI, *m*/*z*) calcd for $C_{28}H_{29}N_2O_3^+$ $[M+H]^+$ 441.2173, found 441.2185.

4.2. Pharmacology

4.2.1. Protein purification and crystallization. The recombinant human PDE4D catalytic domain (86-413) was expressed in *E.coli* BL21 codon PLUS (DE3). Briefly, complementary DNA was subcloned into pET15b with an N-terminal 6x His tag. Protein was expressed in *E.coli* for 16 h at 18 °C after adding isopropyl-β-d-thiogalactoside at a final concentration of 100 μM. Cells were collected and disrupted with the buffer containing 50 mM NaH_2PO_4 pH 7.0, 200 mM NaCl, 1 mM TCEP, 10 mM imidazole. Cell lysate was applied ultracentrifugation at 15000 rpm for 40 min. The supernatant was collected and incubated with nickel affinity resin (Ni-NTA, GE Healthcare) at 4 °C for 30 min. The mixed solution was passed through a Ni-NTA column (GE Healthcare), then washed and eluted with the lysis buffer containing 50 mM and 250 mM imidazole, respectively. Protein was further purified by the anion exchange chromatography (Q-Sepharose, GE Healthcare) and size-exclusion chromatography (Superdex200, GE Healthcare). Finally, the recombinant PDE4D catalytic domain was concentrated to 10-12 mg/mL for crystallization with the buffer containing 18% PEG 3350 (w/v), 0.1 M HEPES (pH 7.0), 0.2 M $MgCl_2$, 10% isopropanol (v/v), 30% ethylene glycol (v/v) at 4 °C using hanging drop vapor-diffusion method. Crystals were appeared within 2 days and subsequently soaked into crystallization buffer with 5-10 mM compounds. Using commercial perfluoropolyether cryo oil (PFO) as croprotectant, crystals were flash-frozen into liquid nitrogen.

4.2.2. X-ray structure determination. Diffraction data for PDE4D-**36** and PDE4D-**apremilast** complex were collected at beamline BL17U1 and BL18U1 at the Shanghai Synchrotron Radiation Facility (SSRF) [32]. Reflections were processed using HKL2000/3000, the structure was solved by molecular replacement using Phaser in CCP4 suit with a search model of previously solved PDE4D apo structure with all water molecules removed [33, 34]. Model building and subsequent several rounds of refinement were processed using COOT and PHENIX [35, 36]. Data collection and refinement statistics are shown in Table S1.

4.2.3. PDEs enzyme inhibition assay. The catalytic activity of PDE4D and other PDEs were monitored by measuring the hydrolysis of [^{3}H]-cAMP or [^{3}H]-cGMP into [^{3}H]-AMP or [^{3}H]-GMP using a phosphodiesterase scintillation proximity assay (SPA) [24]. Following assay buffer condition: 50 mM Tris pH 7.5, 8.3 mM $MgCl_2$, 1.7 mM EGTA, protein and compounds were diluted to a concentration of 2 nM and 50 μM to 1 nM, respectively. The reaction was performed by adding 80 μL of protein solution, 10 μL of test compound and 10 μL of [^{3}H]-cAMP or [^{3}H]-cGMP (0.5 μCi/mL) in a "low binding" plate and then incubated at 30 °C for 30 min. Then 25 μL of phosphodiesterase SPA beads (RPNQ0150, PerkinElmer Inc.) was added to quench the reaction. Settle all the plated for 20 min at room temperature before being counted with MicroBeta^2 counter (PerkinElmer Inc.). At least three independent experiments were performed for the determination of IC_{50} values which were shown as mean ± SD. Experimental data were analyzed using GraphPad Prism software, version 8.0 (GraphPad Inc.).

4.2.4. LPS-induced TNF-α secretion in RAW264.7 cells. Murine RAW264.7 cells were obtained from ATCC (Manassas, VA, USA) and maintained in Dulbecco's Modified Eagle's Media (DMEM, Gibco, Grand Island, NY, USA) in the presence of 10% Fetal Bovine Serum (FBS, Hyclone, South Logan, UT, USA), 100 U/ml penicillin (Sigma-Aldrich, St. Louis, MO, USA) and 100 μg/ml streptomycin (Sigma-Aldrich). To evaluate the anti-inflammatory activity of tested compounds, RAW264.7 cells were incubated with the compounds at the indicated concentrations (containing 0.125% DMSO) in the presence or absence of lipopolysaccharide (LPS,

Escherichia coli O55:B5, Sigma-Aldrich) for 24 h in a humidified incubator of 5% CO_2. The supernatants were collected for TNF-α determination by using the mouse TNF-α ELISA kits (BD Biosciences, San Diego, CA, USA). Meanwhile, 20 μl of CCK-8 reagents (Dojindo, Kumamoto, Japan) were added and the OD values were measured after 1 h incubation at 450 nm (650 nm calibration) by a microplate reader (Molecular Devices, Sunnyvale, CA, USA) to estimate the cytotoxicity of the tested compounds. The CC_{50} and IC_{50} values were calculated using the log (inhibitor) versus normalized response nonlinear fit (Graph Pad Prism 6.0, La Jolla, CA, USA).

4.2.5. Human PBMC inhibitory activity assay. HPBMCs were purchased from SAILY BIO (Shanghai, China). Inhibition of TNF-α secretion of tested compounds were measured in LPS-stimulated human PBMCs (hPBMC) as previously reported [24]. HPBMCs were purchased from SAILY BIO (Shanghai, China). Cells were cultured in RPMI-1640 medium (Gibco, Grand Island, NY, USA) supplemented with 10% FBS (Gibco), 100 U/mL penicillin, 2 mM L-glutamine, and 100 μg/mL streptomycin. Then added compounds to cultured hPBMCs, and 1 h later, LPS was added to stimulate the cell with final DMSO concentration of 0.1% for 18-20 h at 37 °C with 5% CO_2. Supernatants were harvested and the total TNF-α were determined by the TNF-α ELISA Kit (Abcam, Cambridge, UK) using a Bio-Tek Synergy4 plate reader. At least three independent experiments were performed for the determination of IC_{50} values of tested compounds which were shown as mean ± SD. All experimental data were analyzed by using GraphPad Prism software, version 8.0 (GraphPad Inc.).

4.2.6. Caco-2 permeability assay. Permeability of compounds **1** and **36** was measured using the procedure reported previously [37].

4.2.7. Imiquimod-induced murine psoriatic skin inflammation. Inbred 6-8-week-old female BALB/c mice (IACUC: 2019-10-TW-33) were obtained from Shanghai Laboratory Animal Center of the Chinese Academy of Sciences. All mice were allowed to acclimatize in the specific pathogen-free (SPF) conditions for 1 week before any experiments were started. Mice were raised in a 12 h light/dark cycle with humidity (55 ± 5%) and temperature (22 ± 1 °C). The animal experiments were

performed according to the National Institutes of Health Guides for the Care and Use of Laboratory Animals and were approved by the Bioethics Committee of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

To induce the experimental psoriatic skin inflammation, mice were randomly divided into 5 groups (n = 5): Normal, Vehicle (Imiquimod (IMQ, Sichuan Mingxin Pharmaceutical, Sichuan, China)-applied only), Calcipotriol treatment (IMQ with Calcipotriol ointment, Huabang Pharmaceutical, Chongqing, China), 4% Compound **36** ointment (IMQ with 4% Compound **36** ointment), and 2% Compound **36** ointment (IMQ with 2% Compound **36** ointment). The psoriatic skin inflammation was established by topically applying 62.5 mg of IMQ cream on the shaved back skins. Calcipotriol and compound **36** ointment were topically applied at the dose of 62.5 mg per day. During the treatment for 7 days, murine body weights were recorded daily and to score the severity of skin inflammation, the clinical Psoriasis Area and Severity Index (PASI) was scored according to the previous description [24].

4.2.8. Transdermal absorption characteristics in mice. The ointment of compound **36** were topically applied (62.5 mg/kg) for 7 days. Then the skin tissue of each mouse was collected and the skin suspensions were prepared. After ultrasonic for 10 min and vortex for 1 min, the suspensions were separated by centrifugation at 15000 rpm for 5 min. The mixture of supernatant with water (v: v = 1:1) was used for analysis. The concentration of **36** in skin tissue was measured using LC-MS/MS (Waters ACQUITY I-Class System/ Waters ACQUITY XEVO TQ-S(ESI)).

4.2.9. Statistical analysis. All data were shown as Mean ± SEM. Statistical differences were determined using one-way analysis of variance (ANOVA) with Dunnet's multiple comparisons test using GraphPad software 6.0. *P*-values less than 0.05 were considered significant.

■ Associated content

Supporting Information

Supplementary tables, synthetic methods of key intermediates, NMR and HR-MS spectra (PDF)

Accession Codes

The atomic coordinates and structure factors have been deposited into the Protein Data Bank with accession codes 7CBJ (PDE4D-**36**), 7CBQ (PDE4D-**Apremilast**). The atomic coordinates and experimental data will be released upon article publication.

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Notes

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■ Abbreviations

PDE4, phosphodiesterase 4; cAMP, cyclic adenosine monophosphate; BBR, Berberine; SPA, scintillation proximity assay; LPS, lipopolysaccharide; hPBMC, human peripheral blood mononuclear cells; IMQ, imiquimod.

■ References

[1] W.-H. Boehncke, M.P. Schön, Psoriasis, The Lancet, 386 (2015) 983-994.

[2] J.E. Hawkes, T.C. Chan, J.G. Krueger, Psoriasis pathogenesis and the development of novel targeted immune therapies, J. Allergy. Clin. Immunol., 140 (2017) 645-653.

[3] E. Ogawa, Y. Sato, A. Minagawa, R. Okuyama, Pathogenesis of psoriasis and development of treatment, J. Dermatol., 45 (2018) 264-272.

[4] A. Ventura, M. Mazzeo, R. Gaziano, M. Galluzzo, L. Bianchi, E. Campione, New insight into the pathogenesis of nail psoriasis and overview of treatment strategies, Drug. Des. Devel. Ther., 11 (2017) 2527-2535.

[5] S.B. Kaushik, M.G. Lebwohl, Psoriasis: Which therapy for which patient: Psoriasis comorbidities and preferred systemic agents, J. Am. Acad. Dermatol., 80 (2019) 27-40.

[6] C.T. Ritchlin, A. Kavanaugh, D.D. Gladman, P.J. Mease, P. Helliwell, W.H. Boehncke, K. de Vlam, D. Fiorentino, O. Fitzgerald, A.B. Gottlieb, N.J. McHugh, P. Nash, A.A. Qureshi, E.R. Soriano, W.J. Taylor, R. Group for, P. Assessment of, A. Psoriatic, Treatment recommendations for psoriatic arthritis, Ann. Rheum. Dis., 68 (2009) 1387-1394.

[7] Z.K. Jabbar-Lopez, Z.Z.N. Yiu, V. Ward, L.S. Exton, M.F. Mohd Mustapa, E. Samarasekera, A.D. Burden, R. Murphy, C.M. Owen, R. Parslew, V. Venning, R.B. Warren, C.H. Smith, Quantitative Evaluation of Biologic Therapy Options for

Psoriasis: A Systematic Review and Network Meta-Analysis, J. Invest. Dermatol., 137 (2017) 1646-1654.

[8] P. Li, Y. Zheng, X. Chen, Drugs for Autoimmune Inflammatory Diseases: From Small Molecule Compounds to Anti-TNF Biologics, Front. Pharmacol., 8 (2017) 460.

[9] T. Torres, P. Filipe, Small Molecules in the Treatment of Psoriasis, Drug. Dev. Res., 76 (2015) 215-227.

[10] P.A. Harris, S.B. Berger, J.U. Jeong, R. Nagilla, D. Bandyopadhyay, N. Campobasso, C.A. Capriotti, J.A. Cox, L. Dare, X. Dong, P.M. Eidam, J.N. Finger, S.J. Hoffman, J. Kang, V. Kasparcova, B.W. King, R. Lehr, Y. Lan, L.K. Leister, J.D. Lich, T.T. MacDonald, N.A. Miller, M.T. Ouellette, C.S. Pao, A. Rahman, M.A. Reilly, A.R. Rendina, E.J. Rivera, M.C. Schaeffer, C.A. Sehon, R.R. Singhaus, H.H. Sun, B.A. Swift, R.D. Totoritis, A. Vossenkamper, P. Ward, D.D. Wisnoski, D. Zhang, R.W. Marquis, P.J. Gough, J. Bertin, Discovery of a First-in-Class Receptor Interacting Protein 1 (RIP1) Kinase Specific Clinical Candidate (GSK2982772) for the Treatment of Inflammatory Diseases, J. Med. Chem., 60 (2017) 1247-1261.

[11] A. Martinez, C. Gil, cAMP-specific phosphodiesterase inhibitors: promising drugs for inflammatory and neurological diseases, Expert. Opin. Ther. Pat., 24 (2014) 1311-1321.

[12] J.G. Sun, Y.M. Deng, X. Wu, H.F. Tang, J.F. Deng, J.Q. Chen, S.Y. Yang, Q.M. Xie, Inhibition of phosphodiesterase activity, airway inflammation and hyperresponsiveness by PDE4 inhibitor and glucocorticoid in a murine model of allergic asthma, Life. Sci., 79 (2006) 2077-2085.

[13] A. Gavalda, R.S. Roberts, Phosphodiesterase-4 inhibitors: a review of current developments (2010 - 2012), Expert. Opin. Ther. Pat., 23 (2013) 997-1016.

[14] H. Li, J. Zuo, W. Tang, Phosphodiesterase-4 Inhibitors for the Treatment of Inflammatory Diseases, Front. Pharmacol., 9 (2018) 1048.

[15] H. Li, C. Fan, C. Feng, Y. Wu, H. Lu, P. He, X. Yang, F. Zhu, Q. Qi, Y. Gao, J. Zuo, W. Tang, Inhibition of phosphodiesterase-4 attenuates murine ulcerative colitis through interference with mucosal immunity, Br. J. Pharmacol., 176 (2019) 2209-2226.

[16] K.Y. Zhang, P.N. Ibrahim, S. Gillette, G. Bollag, Phosphodiesterase-4 as a potential drug target, Expert. Opin. Ther. Targets., 9 (2005) 1283-1305.

[17] D.H. Maurice, H. Ke, F. Ahmad, Y. Wang, J. Chung, V.C. Manganiello, Advances in targeting cyclic nucleotide phosphodiesterases, Nat. Rev. Drug. Discov., 13 (2014) 290-314.

[18] H.W. Man, P. Schafer, L.M. Wong, R.T. Patterson, L.G. Corral, H. Raymon, K. Blease, J. Leisten, M.A. Shirley, Y. Tang, D.M. Babusis, R. Chen, D. Stirling, G.W. Muller, Discovery of (S)-N-[2-[1-(3-ethoxy-4-methoxyphenyl)-2-methanesulfonylethyl]-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl] acetamide (apremilast), a potent and orally active phosphodiesterase 4 and tumor necrosis factor-alpha inhibitor, J. Med. Chem., 52 (2009) 1522-1524.

[19] A.C. Palfreeman, K.E. McNamee, F.E. McCann, New developments in the management of psoriasis and psoriatic arthritis: a focus on apremilast, Drug. Des. Devel. Ther., 7 (2013) 201-210.

[20] P. Gisondi, G. Girolomoni, Apremilast in the therapy of moderate-to-severe chronic plaque psoriasis, Drug. Des. Devel. Ther., 10 (2016) 1763-1770.

[21] P.R. Vuddanda, S. Chakraborty, S. Singh, Berberine: a potential phytochemical with multispectrum therapeutic activities, Expert. Opin. Investig. Drugs., 19 (2010) 1297-1307.

[22] K. Zou, Z. Li, Y. Zhang, H.Y. Zhang, B. Li, W.L. Zhu, J.Y. Shi, Q. Jia, Y.M. Li, Advances in the study of berberine and its derivatives: a focus on anti-inflammatory and anti-tumor effects in the digestive system, Acta. Pharmacol. Sin., 38 (2017) 157-167.

[23] X. Wang, S. Feng, N. Ding, Y. He, C. Li, M. Li, X. Ding, H. Ding, J. Li, J. Wu, Y. Li, Anti-Inflammatory Effects of Berberine Hydrochloride in an LPS-Induced Murine Model of Mastitis, Evid. Based. Complement. Alternat. Med., 2018 (2018) 5164314.

[24] X. Zhang, G. Dong, H. Li, W. Chen, J. Li, C. Feng, Z. Gu, F. Zhu, R. Zhang, M. Li, W. Tang, H. Liu, Y. Xu, Structure-Aided Identification and Optimization of Tetrahydro-isoquinolines as Novel PDE4 Inhibitors Leading to Discovery of an

Effective Antipsoriasis Agent, J. Med. Chem., 62 (2019) 5579-5593.

[25] H. Li, J. Li, X. Zhang, C. Feng, C. Fan, X. Yang, R. Zhang, F. Zhu, Y. Zhou, Y. Xu, H. Liu, W. Tang, DC591017, a phosphodiesterase-4 (PDE4) inhibitor with robust anti-inflammation through regulating PKA-CREB signaling, Biochem. Pharmacol., 177 (2020) 113958.

[26] C. Jansen, A.J. Kooistra, G.K. Kanev, R. Leurs, I.J. de Esch, C. de Graaf, PDEStrIAn: A Phosphodiesterase Structure and Ligand Interaction Annotated Database As a Tool for Structure-Based Drug Design, J. Med. Chem., 59 (2016) 7029-7065.

[27] J.D. Moseley, P.M. Murray, E.R. Turp, S.N.G. Tyler, R.T. Burn, A mild robust generic protocol for the Suzuki reaction using an air stable catalyst, Tetrahedron, 68 (2012) 6010-6017.

[28] S. Mang, H. Bucher, P. Nickolaus, Development of a Scintillation Proximity Assay (SPA) Based, High Throughput Screening Feasible Method for the Identification of PDE12 Activity Modulators, Curr. Drug. Discov. Technol., 13 (2016) 144-151.

[29] L.A. O'Neill, Targeting signal transduction as a strategy to treat inflammatory diseases, Nat. Rev. Drug. Discov., 5 (2006) 549-563.

[30] B. Flutter, F.O. Nestle, TLRs to cytokines: mechanistic insights from the imiquimod mouse model of psoriasis, Eur. J. Immunol., 43 (2013) 3138-3146.

[31] M. Gilliet, C. Conrad, M. Geiges, A. Cozzio, W. Thurlimann, G. Burg, F.O. Nestle, R. Dummer, Psoriasis triggered by toll-like receptor 7 agonist imiquimod in the presence of dermal plasmacytoid dendritic cell precursors, Arch. Dermatol., 140 (2004) 1490-1495.

[32] Q.-S. Wang, K.-H. Zhang, Y. Cui, Z.-J. Wang, Q.-Y. Pan, K. Liu, B. Sun, H. Zhou, M.-J. Li, Q. Xu, C.-Y. Xu, F. Yu, J.-H. He, Upgrade of macromolecular crystallography beamline BL17U1 at SSRF, Nuclear Science and Techniques, 29 (2018).

[33] Collaborative, The CCP4 suite: programs for protein crystallography, Acta. Crystallographica. Section. D., 50 (1994) 760-763.

[34] K.Y. Zhang, G.L. Card, Y. Suzuki, D.R. Artis, D. Fong, S. Gillette, D. Hsieh, J. Neiman, B.L. West, C. Zhang, M.V. Milburn, S.H. Kim, J. Schlessinger, G. Bollag, A glutamine switch mechanism for nucleotide selectivity by phosphodiesterases, Mol. Cell., 15 (2004) 279-286.

[35] P.D. Adams, R.W. Grosse-Kunstleve, L.-W. Hung, T.R. Ioerger, A.J. McCoy, N.W. Moriarty, R.J. Read, J.C. Sacchettini, N.K. Sauter, T.C. Terwilliger, PHENIX: building new software for automated crystallographic structure determination, Acta. Crystallographica. Section. D., 58 (2002) 1948-1954.

[36] P. Emsley, K. Cowtan, Coot: model-building tools for molecular graphics, Acta. Crystallographica. Section. D., 60 (2004) 2126-2132.

[37] S. Skolnik, X. Lin, J. Wang, X.H. Chen, T. He, B. Zhang, Towards prediction of in vivo intestinal absorption using a 96-well Caco-2 assay, J. Pharm. Sci., 99 (2010) 3246-3265.

Highlights:

- 51 tetrahydroisoquinolines derivatives as novel PDE4 inhibitors were designed, synthesized, and evaluated.
- The structure-activity relationship (SAR) of this novel series of compounds was demonstrated.
- The optimum compound **36** exhibited high safety, potency (PDE4D IC_{50} = 0.36 μM, TNF-α IC_{50} = 6.76 μM), permeability and selectivity *in vitro*.
- The crystal structure of PDE4D in complex with compound **36** was solved.
- Compound **36** exhibited superior therapeutic efficacy compared with calcipotriol against the IMQ-induced murine psoriasis-like skin inflammation.

The authors declare no competing financial interest.