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Design, synthesis and evaluation of dual pharmacology β_2-adrenoceptor agonists and PDE4 inhibitors

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ABSTRACT

A novel series of formoterol–phthalazinone hybrids were synthesised and evaluated as dual pharmacology β_2-adrenoceptor agonists and PDE4 inhibitors. Most of the hybrids displayed high β_2-adrenoceptor agonist and moderate PDE4 inhibitory activities. The most potent compound, (*R*,*R*)-**11c**, exhibited agonist (EC_{50} = 1.05 nM, pEC_{50} = 9.0) and potent PDE4B2 inhibitory activities (IC_{50} = 0.092 μM).

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Chronic obstructive pulmonary disease (COPD) is a chronic respiratory disease that affects millions of people worldwide. COPD is characterised by limited airflow to and from the lungs, which is not fully reversible.[1] Although the aetiology of COPD remains to be fully understood, smooth muscle dysfunction and chronic inflammation are known to play important roles in the pathophysiology of the disease. Smooth-muscle dysfunction results in exaggerated bronchoconstriction, bronchial hyperresponsiveness, excessive proliferation (hyperplasia), and excessive growth (hypertrophy) of the airway smooth-muscle cells[2] and release of proinflammatory mediators.[3]

β_2-Adrenoceptor agonists induce bronchodilatory effects mediated by the relaxation of airway smooth muscles by increasing cAMP. Thus, inhaled β_2 adrenoceptor agonists are widely used to treat asthma and COPD, providing symptomatic relief by inducing bronchodilation via the relaxation of airway smooth muscle.[4] Currently, salbutamol (a typically short-acting agonist with a rapid onset of action), salmeterol and formoterol (the two most prescribed inhaled long-acting β_2-agonists) are clinically used as β_2-agonists. In addition, a once-daily β_2-agonist indacaterol has been approved in the USA and Europe for the treatment of COPD.

In recent years, PDE4 has been examined as a suitable target for anti-inflammatory therapy to treat respiratory diseases.[5,6] PDE4 inhibitors have been reported to downregulate inflammatory cell activity in vitro[7,8] and exhibit anti-inflammatory and bronchodilatory activity in animal models.[9] These therapeutic effects could be used in the development of new agents, such as steroid-sparing compounds, to treat diseases associated with chronic airway inflammation, particularly in the management of asthma and COPD.[10]

The multifaceted conditions of some diseases have led to the development of multifunctional drugs. These drugs possess two or more complementary biological activities and may represent an important advancement in the treatment of diseases.[11–13] Using a multivalent approach to drug discovery, Hughes et al. designed and synthesised dual pharmacology molecules that function as bronchodilators (Fig. 1, **1**) and target both the M3 muscarinic acetylcholine and β_2-adrenergic receptors.[11] Jones et al. designed and developed of dual pharmacology β_2 agonists–M3 antagonists (Fig. 1, **2**) for the treatment of COPD.[14]

Recently, we used a multivalent approach to synthesise a class of dual pharmacology bronchodilators (Fig. 1, **3**) that target both the β_2-adrenoceptor and PDE4.[15] Here, we present the synthesis and evaluation of a new series of hybrids that in one molecule combine both formoterol, which is one of the two most prescribed inhaled long-acting β_2-agonists, and the PDE4 inhibitor phthalazinone (Fig. 2).

The synthetic route of dual β_2-agonists and PDE4 inhibitors (**11a**–**11c**) is shown in Scheme 1. 1,2-Dimethoxybenzene was reacted with 1,2-cyclohexanedicarboxylic anhydride to afford a ketone acid **4**, which was subsequently reacted with hydrazine to

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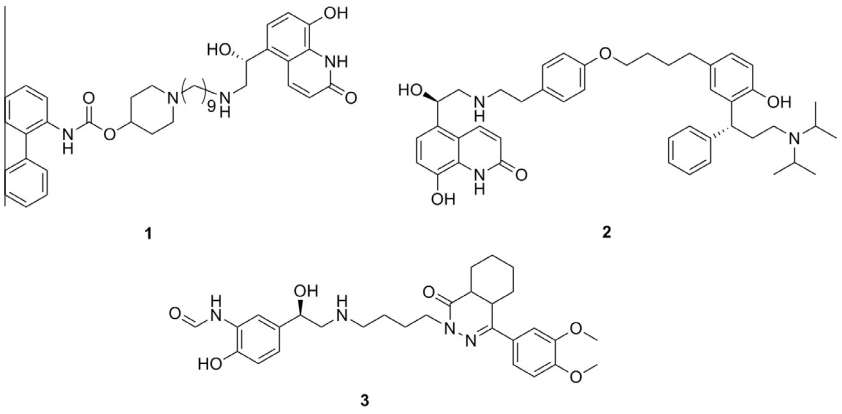


Figure 1. Structures of reported dual pharmacology bronchodilators for the treatment of asthma or COPD.

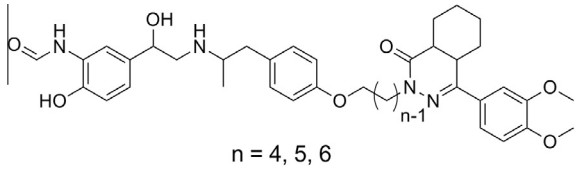


Figure 2. The synthesised β_2-adrenoceptor agonists-PDE4 inhibitors.

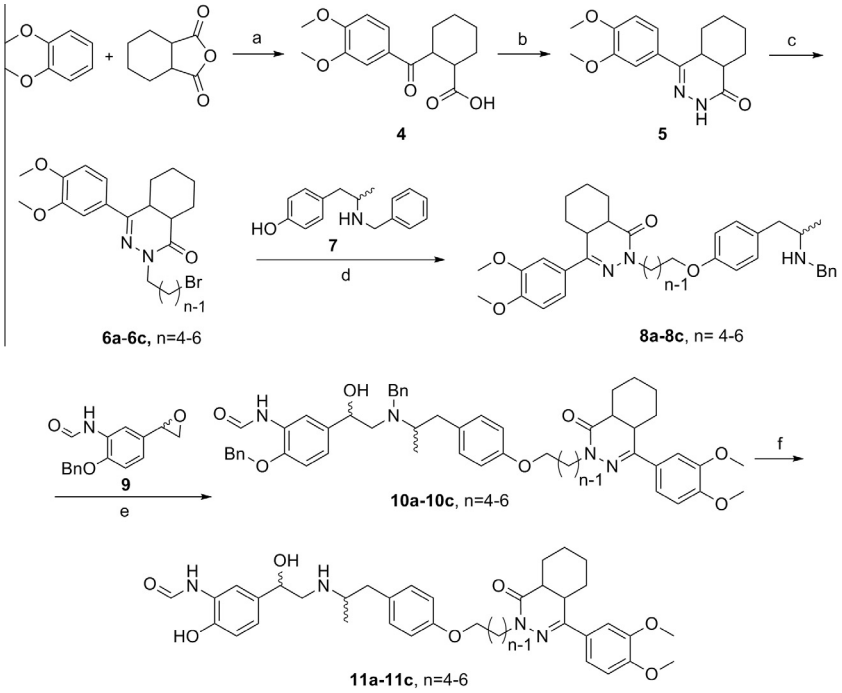
yield the known PDE4 inhibitor 4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydro-2*H*-phthalazin-1-one (**5**).[16,17] *N*-Alkyl phthalazinones **6a–6c** were obtained by treating compound **5** with dibromoalkanes and NaH in DMF. The intermediates were then reacted with 4-(2-(benzylamino)propyl)phenol (**7**) to provide compounds **8a–8c**. Finally, the target compounds (**11a–11c**) were obtained by coupling epoxide **9** with compounds **8a–8c** followed by deprotection via hydrogenation in the presence of Pd/C.

The racemic secondly amine **7** was synthesised in a one-pot reaction reductive hydrogenation and subsequent O-demethylation reaction in an overall yield of 72% (Scheme 2).

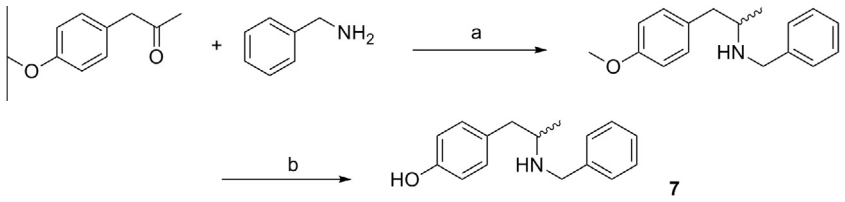
Epoxide intermediate **9**, the intermediate containing the pharmacophore of the β_2-adrenoceptor agonist, was prepared using commercially available 4-hydroxy-3-nitro-acetophenone in several steps according to previously reported procedures (Scheme 3).[18]

In order to investigate the relationship between the chiral center of the compounds and the activity of the β_2-adrenoceptor, an enantiomer of **11c**, (*R*,*R*)-**11c**, was synthesized using enantiopure secondly amine (*R*)-**7** and enantiopure epoxide (*R*)-**9** as the intermediates according to the synthetic route shown in Scheme 1. (*R*)-**7** and (*R*)-**9** were prepared according to procedures reported previously.[18]

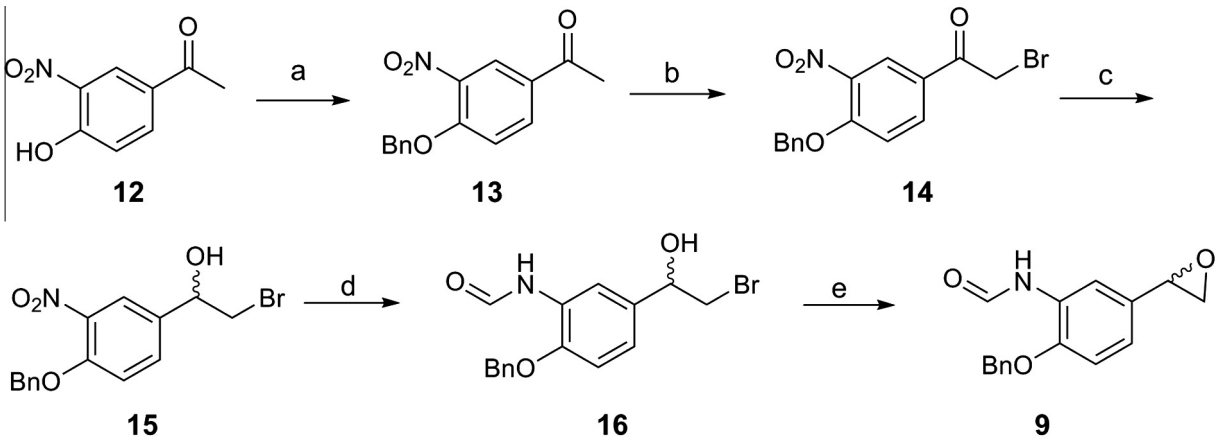
For studying the in vitro β_2-adrenoceptor agonist activity of target compounds, The effects of **11a–11c** and (*R*,*R*)-**11c** on the tracheal rings of guinea pigs were assessed using (*R*,*R*)-formoterol according to a previously described protocol.[19,20] The concentration–response curves are showen in Figure 3 and the EC_{50} and pEC_{50} values are summarized in Table 1. The maximum relaxant effect exhibited by Isoproterenol was considered to be 100%. The EC_{50} values were estimated from the concentration–response



Scheme 1. Synthetic scheme for synthesis of dual β_2-adrenoceptor agonists-PDE4 inhibitors. Reagents and conditions: (a) $AlCl_3$, CH_2CH_2, reflux, 76%; (b) NH_2NH_2, EtOH, reflux, 32%; (c) $Br(CH_2)_nBr$, NaH, DMF, rt, 80–88%; (d) **7**, K_2CO_3, DMF, 60 °C, 60–65%; (e) **9**, neat, 120 °C, 55–61%; (f) 10% Pd/C, H_2, MeOH, rt, 58–65%.

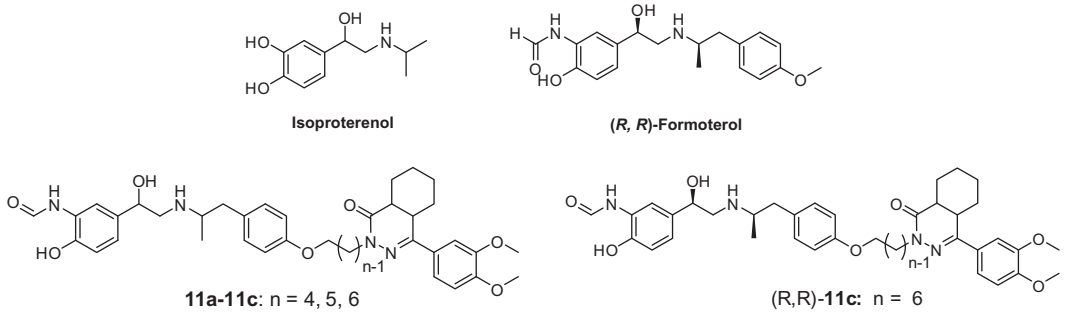


Scheme 2. Recemic synthesis of secondary amine **7**. Reagents and conditions: (a) 5% Pt/C, H_2, MeOH, 60 °C, 95%; (b) BBr_3, CH_2Cl_2, 10 °C, 76%.



Scheme 3. Recemic synthesis of epoxide intermediate **9**. Reagents and conditions: (a) (chloromethyl)benzene, K_2CO_3, actone–H_2O, 60 °C, 90.5%; (b) bromine, acetic acid, 20 °C, 85%; (c) NaBH4, THF, 0 °C to rt, 91%; (d) Pt/C, Me_2S, H_2; formaldehyde, acetic anhydride, 0 °C to rt, 83%; (e) K_2CO_3, THF/MeOH, rt, 98%.

Table 1
EC_{50} for β_2-adrenergic agonist compounds in the function ex vivo guinea pig trachea bioassay



Compounds	*n*	EC_{50}[a] (nM)	pEC_{50}
Isoproterenol	—	31.64 ± 0.50	7.5 (7.8[b])
(*R*,*R*)-Formoterol	—	0.13 ± 0.01	9.9 (9.5[c])
11a	4	10.32 ± 0.22	8.0
11b	5	12.59 ± 0.17	7.9
11c	6	5.02 ± 0.03	8.3
(*R*,*R*)-**11c**	6	1.05 ± 0.01	9.0

[a] Data are of average of three determinations ± SEM.
[b] Ref. 19.
[c] Ref. 21.

curves by means of nonlinear regression analysis with the experimental data.

The results shown in Figure 3 indicate that all of the hybrids exhibited relaxant effects on tracheal rings precontracted with histamine. All of the compounds induced concentration-dependent relaxation, and the maximum response (E_{max}) to each compound was similar to that evoked by the reference compound isoproterenol. Among the three target compounds (**11a**–**11c**), compound **11c**, which contains a 6-carbon linker between the phthalazinone and formoterol moieties, displayed the most potent β_2-adrenoceptor agonist activity (Table 1) with EC_{50} and pEC_{50} value (related to pK_d) of 5.02 nM and 8.3, respectively. This value is higher than that of isoproterenol (EC_{50} = 31.64 nM, pEC_{50} = 7.5). (*R*,*R*)-**11c** was also examined as a β_2-adrenoceptor agonist using an in vitro evaluation, and the results showed that (*R*,*R*)-**11c** (EC_{50} = 1.05 nM, pEC_{50} = 9.0) exhibited about a fivefold increase in potency compared to its racemic form (**11c**, EC_{50} = 5.02 nM, pEC_{50} = 8.3). However, (*R*,*R*)-**11c** was less potent than that of (*R*,*R*)-formoterol (EC_{50} = 0.13 nM, pEC_{50} = 9.9).

When isolated guinea pig tracheal rings were precontracted with histamine and incubated with the well-known β_2-AR selective inverse agonist ICI-118551 (1×10^{-9} to 1×10^{-7} M), the dose–re-

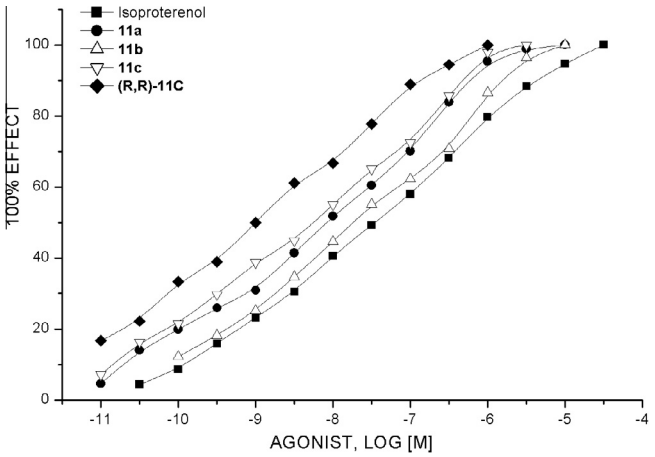


Figure 3. Effects of agonists and isoproterenol on isolated guinea pig tracheal rings precontracted with 30 μM of histamine. The maximum relaxant effect exhibited by Isoproterenol was considered to be 100%. Each data point represents the mean effect ($n = 3$).

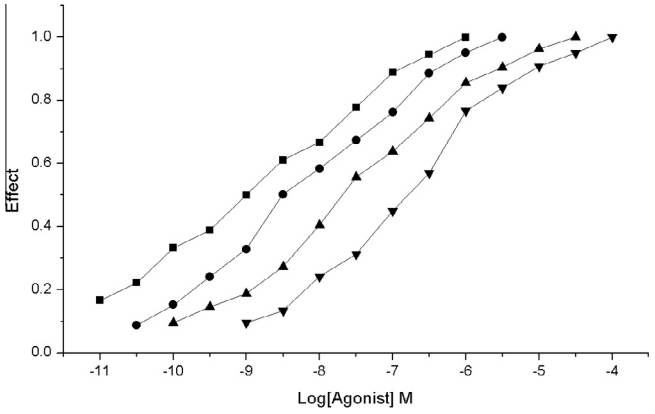


Figure 4. The antagonistic action for ICI-118551 on guinea pig tracheal muscle precontracted with histamine and used (*R*,*R*)-**11c** as agonist. ■ = (*R*,*R*)-**11c** ($1 \times 10^{-11} \sim 1 \times 10^{-6}$ M) in absence of ICI-118551; ● = (*R*,*R*)-**11c** ($1 \times 10^{-10.5} \sim 1 \times 10^{-6}$ M) in the presence of ICI-118551 (1×10^{-9} M); ▲ = (*R*,*R*)-**11c** ($1 \times 10^{-10} \sim 1 \times 10^{-5}$ M) in the presence of ICI-118551(1×10^{-8} M); ▼ = (*R*,*R*)-**11c** ($1 \times 10^{-9} \sim 1 \times 10^{-4}$ M) in the presence of ICI-118551(1×10^{-7} M).

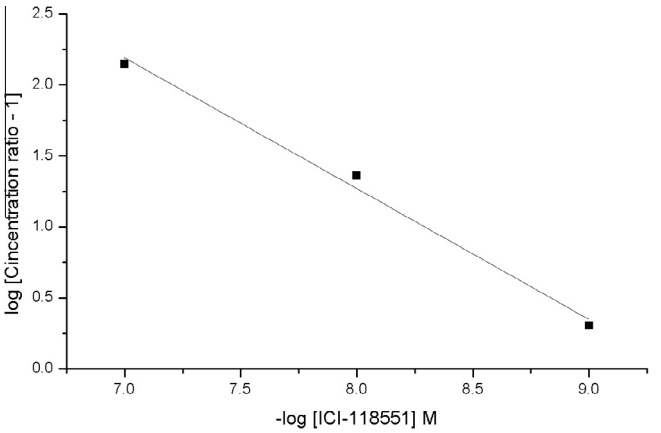


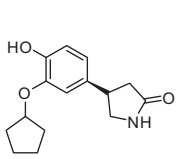
Figure 5. Schild plot for the antagonism of ICI-118551 versus (*R*,*R*)-**11c** on isolated guinea pig trachea strips precontracted with histamine. The pA2 values were calculated from the formulation pA2 = Log (CR-1)—Log [B]. Values for concentration ratio (CR) were obtained from EC_{50} values in the presence or absence of ICI-118551.

sponse curve for compound (*R*,*R*)-**11c** shifted to the right (Fig. 4), indicating antagonist effects and the pharmacology of compound (*R*,*R*)-**11c** is partly driven as β_2-adrenoceptor agonist. The result of schild plot (Fig. 5) showed that the pA_2 value for the antagonist activity of ICI-118551 in the presence of (*R*,*R*)-**11c** was 9.36.

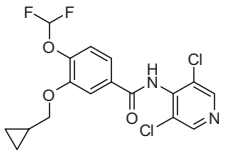
It is well known that the PDE4 family consist of four isoforms: PDE4A, 4B, 4C and 4D. Knockout studies have revealed that PDE4B could suppress TNF-a production, which suggested that PDE4B inhibitors are expected to be useful anti-inflammatory agents.[22] PDE4B is a hydrolytic enzyme responsible for the degradation of the second messenger cAMP. As an increase in intracellular levels of cAMP can reduce the activation of a wide range of inflammatory, increasing the cAMP concentration by inhibiting PDE4B activity is thought to be one of the approach to treatment of COPD.[23] Considering PDE4B2 is the predominant PDE4B species and study[24] certified it undergoes differential regulation of gene expression in human monocytes and neutrophils, we choose PDE4B2 isoform as assay enzyme.

Compounds **11a–11c** were also tested for inhibition of cAMP hydrolysis by recombinant human PDE4B2 in vitro using a colourimetric assay from Biomol (Enzo Life Science) and the protocol described by the manufacturer. The known PDE4 inhibitors

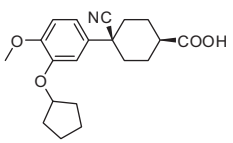
Table 2
Inhibition of cAMP hydrolysis by recombinant human PDE4B2 in the presence of **11** and new compounds[a]



(R)-Rolipram



Roflumilast



Cilomilast

Compounds	*n*	PDE4B2 inhibition IC_{50} (μM)
(*R*)-Rolipram	—	0.500 ± 0.028
5	—	0.520 ± 0.042
11a	4	0.117 ± 0.008
11b	5	0.118 ± 0.015
11c	6	0.104 ± 0.010
(*R*,*R*)-**11c**	6	0.092 ± 0.009
Cilomilast	—	0.120 (0.095[b])
Roflumilast	—	0.001 (0.0008[c])

[a] Data are of average of three determinations ± SEM.
[b] Ref. 25.
[c] Ref. 26.

Table 3
Values (MW, HBD, HBA, PSA, and Log*P*)[a] of **11a**, **11b**, **11c**

	11a	11b	11c
MW	672	686	700
HBD	4	4	4
HBA	9	9	9
PSA	141.95	141.95	141.95
Log*P*	4.89	5.33	5.78
AClog*P*	4.53	4.99	5.46
*A*log*P*	5.08	5.53	5.99

[a] *Abbreviations:* MW, molecular weight; HBD, hydrogen-bond donor atoms; HBA, hydrogen-bond acceptor atoms; PSA, polar surface area; log*P*, AClog*P*, and *A*Log*P*, calculated logarithm of the octanol–water partition coefficient. MW, HBD, HBA, NROT, PSA, and Log*P* were calculated online by Marvin software.[27] AClog*P* and *A*Log*P* were calculated online by the ALOGPS 2.1 program.[28]

compound **5** and rolipram were used as standards (Table 2). The results in Table 2 indicate that all target compounds provided good PDE4B2 inhibitory activity (Table 2, compounds **11a**–**11c** exhibited IC_{50} values of 0.117, 0.118, and 0.104 μM, respectively). The higher potency of compounds **11a**–**11c** compared to that of the lead compound **5** suggests that *N*-substitution is favourable for the inhibition of human PDE4B2. The stereoisomer (*R*,*R*)-**11c** (IC_{50} = 0.092 μM) showed a similar inhibitory activity against PDE4B2 with **11c** (IC_{50} = 0.104 μM).

In order to evaluate the Drug-Like properties of target compounds, the important pharmacological parameters of **11a**–**11c** were calculated online by the computer programs Marvin[27] and ALOGPS 2.1.[28] The values were presented in Table 3.

In summary, we have developed a series of dual pharmacology molecules that behave as both β_2-adrenoceptor agonists and PDE4 inhibitors. These compounds displayed moderate to high β_2-adrenoceptor agonist activities on isolated guinea pig tracheal rings precontracted with histamine. Among them, compound (*R*,*R*)-**11c** exhibited the most potent agonist activity with a pEC_{50} value of approximately 9.0. Moreover, compound (*R*,*R*)-**11c** displayed strong PDE4B2 inhibitory activity with an IC_{50} of 0.092 μM. Further studies to investigate the binding affinity of compound (*R*,*R*)-**11c** to the human β_2-adrenoceptor and the efficacy in an animal model of COPD are in progress.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.11.028.

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