Bioorganic & Medicinal Chemistry Letters 22 (2012) 1523–1526

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Dual β_2-adrenoceptor agonists-PDE4 inhibitors for the treatment of asthma and COPD

Wen-Jun Shan [a], Ling Huang [a], Qi Zhou [a], Huai-Lei Jiang [a], Zong-Hua Luo [a], Ke-fang Lai [b,*], Xing-Shu Li [a,*]

[a] Institute of Drug Synthesis and Pharmaceutical Process, School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510006, China
[b] State Key Laboratory of Respiratory Diseases, Guangzhou Medical College, Guangzhou 510120, China

ARTICLE INFO

Article history:
Received 5 October 2011
Revised 26 December 2011
Accepted 6 January 2012
Available online 14 January 2012

Keywords:
Asthma and COPD
Bronchodilator
β_2-Adrenoceptor agonist
PDE4 inhibitor

ABSTRACT

We designed and synthesized a novel class of dual pharmacology bronchodilators targeting both β_2-adrenoceptor and PDE4 by applying a multivalent approach. The most potent dual pharmacology molecule, compound **29**, possessed good inhibitory activity on PDE4B2 (IC_{50} = 0.278 μM, which was more potent than phthalazinone, IC_{50} = 0.520 μM) and possessed excellent relaxant effects on tracheal rings precontracted by histamine (pEC_{50} = 9.3).

Asthma and chronic obstructive pulmonary disease (COPD) are both prevalent and chronic respiratory diseases that affect millions of people worldwide.[1] Although the etiology of asthma and COPD are not completely understood, smooth muscle dysfunction and chronic inflammation are known to play important roles in the pathophysiology of these diseases. Thus, both anti-inflammatory and bronchodilator medicines are used extensively in this context.

The β_2-adrenoceptor agonists (β_2-agonists) are the most effective bronchodilators, offering proven benefits in reducing the burden of asthma and COPD.[2,3] Activation of the β_2-adrenoceptor results in the activation of adenylyl cyclase (AC) via a stimulatory G-protein (Gs) and an increase in the cAMP concentration,[4] which is a key regulator of numerous signalling cascades.[5] The currently used β_2-agonists are illustrated in Figure 1. Among them, salbutamol (Fig. 1, **1**) is a typically short-acting agonist with a rapid onset of action. Salmeterol (Fig. 1, **2**) and formoterol (Fig. 1, **3**) are the two most prescribed representatives of inhaled long-acting β_2-agonists. Recently, indacaterol (Fig. 1, **4**) has been approved in the USA and Europe as a once-daily β_2-agonist for the treatment of COPD.[6]

The phosphodiesterase (PDE) enzymes, important regulators of intracellular cyclic nucleotide signalling,[7,5] catalyze the hydrolysis of cAMP and cGMP to the corresponding nucleoside 5′-monophosphates. PDE4, one of the eleven PDE enzyme families,[8,9] is particularly abundant in inflammatory-, immune- and airway smooth muscle cells.[10] PDE4 inhibitors have been evaluated as promising therapies for the treatment of inflammatory pulmonary disorders, including asthma and COPD.[11] The PDE4 family consist of four isoforms: PDE4A, 4B, 4C and 4D. Knockout studies have revealed that PDE4B could suppress TNF-a production.[12] In this regard, PDE4B inhibitors are expected to be useful anti-inflammatory agents. There are a number of PDE4 inhibitors, including rolipram (Fig. 2, **5**), rofiumilast (Fig. 2, **6**), cilomilast (Fig. 2, **7**) and AWD12-281(Fig. 2, **8**), that have been investigated in clinical trials.[13] More recently, rofiumilast has been approved by the FDA for the treatment of COPD. This approval has stimulated the development of PDE4 inhibitors greatly.

The multifaceted condition of some diseases has encouraged active research in the development of multifunctional drugs. These drugs possess two or more complementary biological activities and they may represent an important advance in the treatment of the diseases.[14–16] Inspired by the development of multifunctional drugs, we wished to design a new kind of inhibitor for the treatment of both asthma and COPD, which could target the β_2-adrenoceptor and PDE4. In this Letter, we describe the preliminary study toward the synthesis and evaluation of novel hybrids that possess dual activity as β_2-agonists and PDE4B inhibitors.

The synthetic route of dual β_2-agonists and PDE4 inhibitors (**28–31**) is shown in Schemes 1–4. First, epoxide **13**, the synthetic intermediate containing the pharmacophore of the β_2-adrenoceptor agonist, was synthesized from the commercially available 4-hydroxy-3-nitro-acetophenone by several steps according to procedures reported previously.[17] Then, intermediates **20–23**, which possess the pharmacophore of the PDE4 inhibitor, were synthesized via the route outlined in Scheme 2. The reaction of 1,2-dimethoxybenzene with 1,2-cyclohexanedicarboxylic anhydride provided compound **14**. Compound **14** was then reacted with hydrazine to

* Corresponding authors. Tel./fax: +86 20 3994 3050.
E-mail address: lixs@mail.sysu.edu.cn (X.-S. Li).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2012.01.013

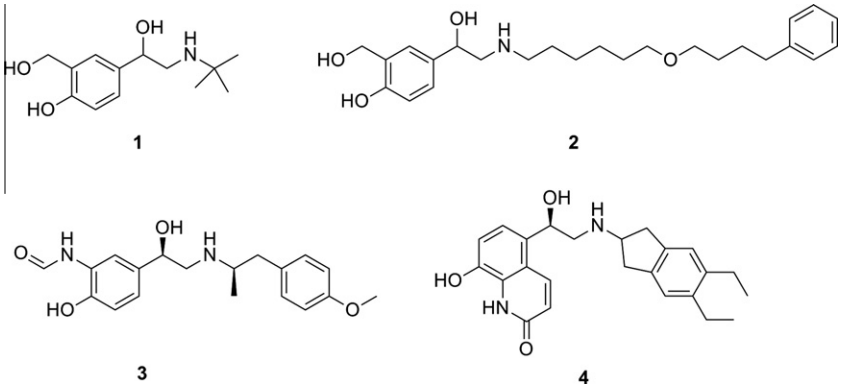


Figure 1. Structure of salbutamol (**1**), salmeterol (**2**), formoterol (**3**), indacaterol (**4**).

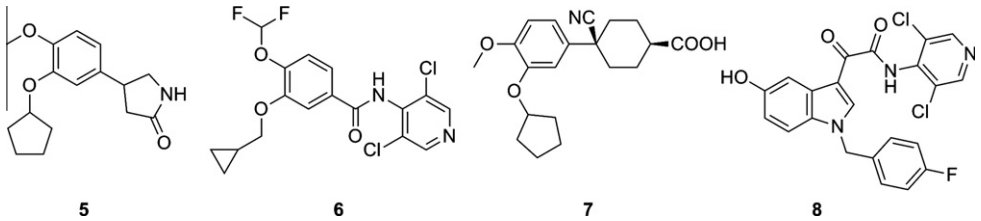
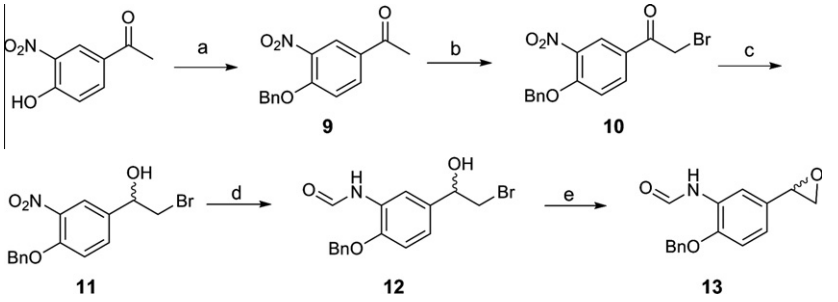
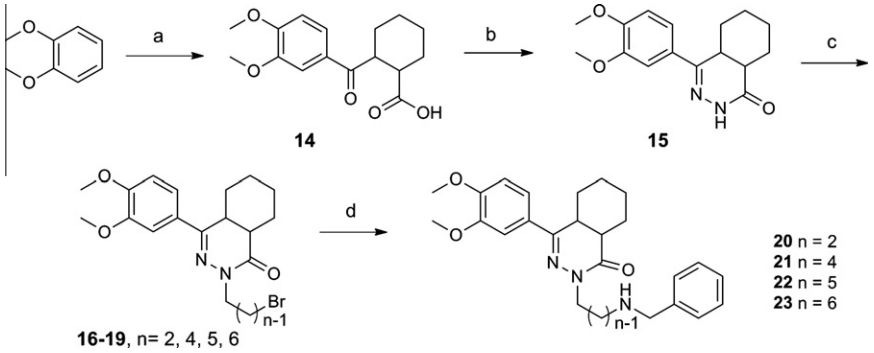


Figure 2. Structure of rolipram (**5**), roflumilast (**6**), cilomilast (**7**) and AWD12-281 (**8**).

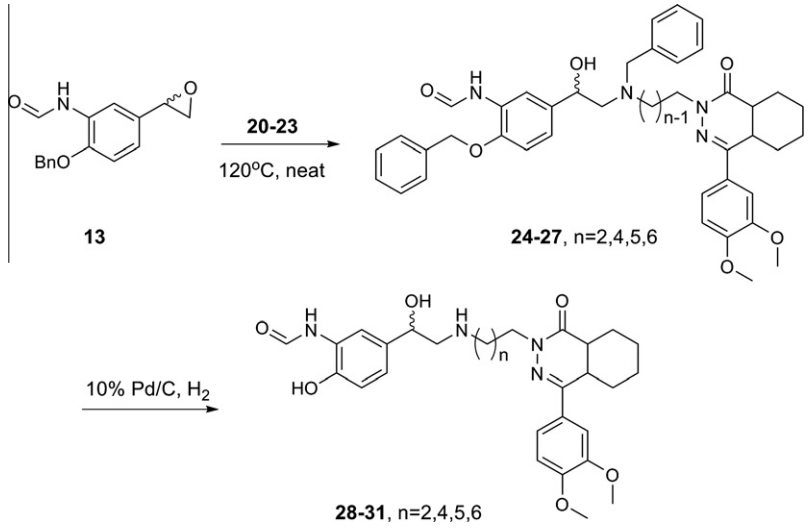


Scheme 1. Reagents and conditions: (a) (chloromethyl)benzene, K_2CO_3; (b) bromine, acetic acid; (c) $NaBH_4$, THF; (d) Pt/C, Me_2S, H_2; formaldehyde, acetic anhydride; (e) K_2CO_3, THF/MeOH.

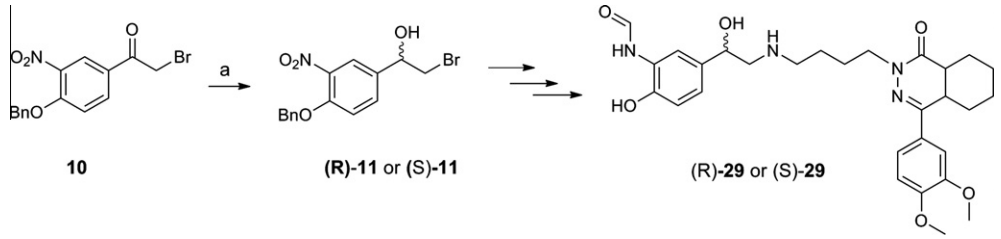


Scheme 2. Reagents and condition: (a) 1,2-cyclohexanedicarboxylic anhydride, $AlCl_3$, CH_2Cl_2; (b) H_2NNH_2, EtOH, reflux; (c) $Br(CH_2)_nBr$, NaH, DMF; (d) benzylamine.

afford 4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydro-2*H*-phthalazin-1-one (**15**), a known PDE4 inhibitor synthesized by Van der Mey et. al.[18,19] *N*-Alkyl phthalazinones **16–19** were obtained by treating compound **15** with NaH and dibromoalkanes in DMF. The reaction of **16–19** with benzylamine provided compounds **20**–**23**, respectively. Finally, the target compounds were synthesized by the coupling of epoxide **13** with compounds **20–23**, followed by the deprotection via hydrogenation in the presence of Pd/C. In order to investigate the relationship between the chiral center of the synthesized compounds and the activity of the β_2-adrenoceptor, each



Scheme 3. Reagents and condition: (a) **20**–**23**, 120 °C, neat; (b) 10% Pd/C, H_2.



Scheme 4. Synthetic route of (*R*)-**29** or (*S*)-**29**. Reagents and condition: (a) Ru-(*R*, *R*)-TsDPEN or Ru-(*S*, *S*)-TsDPEN, HCOONa, H_2O.

enantiomer of **29**, (*R*)-**29** and (*S*)-**29** (Scheme 4) was synthesized by the reaction of a chiral epoxide (prepared from the alcohol **11**) and intermediate **21**.

To study the in vitro β_2-adrenoceptor agonist and PDE4 inhibitory activities, in vitro assays were carried out. First, the effect on the tracheal rings of guinea pigs were assessed with a protocol described previously.[20] The results detailed in Figure 3 indicated that all the compounds examined produced a relaxant effect on the precontracted tracheal rings by histamine. All the compounds induced a concentration-dependent relaxation, and the maximum response (E_{max}) to each was similar to that evoked by the reference compound isoprenaline. Among the four target compounds (**28–31**), compound **29**, connected by a 4-carbon linker between the PDE4 inhibitor and the β_2-adrenoceptor agonist moiety, exhibited the most potent β_2-adrenoceptor agonist activity (Table 1), with the pEC_{50} value (related to pK_d) of 9.3. This value is higher than that of isoprenaline (7.5). (*R*)-**29** and (*S*)-**29** were also examined as β_2-adrenoceptor agonists by an in vitro evaluation, and the results showed that (*R*)-**29** had about a fivefold potency increase over its racemic form, and about three orders of magnitude higher potency than its enantiomer, (*S*)-**29**.

When isolated guinea pig tracheal rings were precontracted with histamine, the dose–response curve of (*R*)-**29** was shifted to the right when using the well-known β_2-AR selective inverse agonist, ICI-118551[22] (1×10^{-9}–1×10^{-7} M), showing competitive

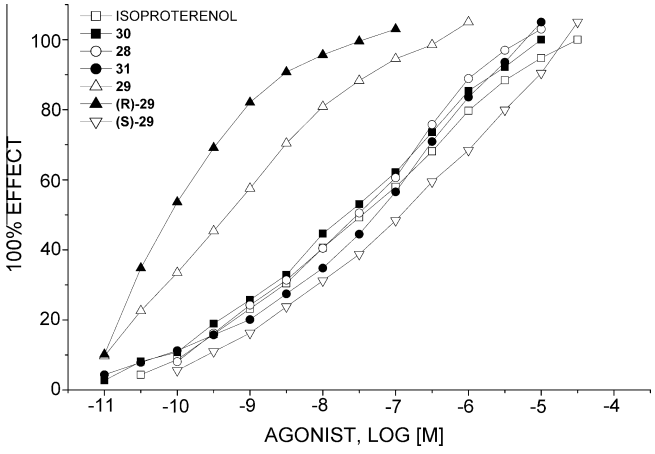


Figure 3. Effect of agonists and isoproterenol on isolated guinea pig tracheal rings precontracted by histamine at 30 μM. The maximum relaxant effect by isoproterenol was considered 100%. Each point represents the mean effect ($n = 3$).

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

Compound	*n*	pEC_{50}[b]	E_{max} (%)
Isoprenaline	—	7.5	100
28	2	7.7	103
29	4	9.3	105
30	5	7.7	100
31	6	7.3	105
(R)-**29**	4	10.0	103
(S)-**29**	4	6.9	105
Salmeterol	—	8.3[c]	—
(*R*, *R*)-3.Tartrate	—	9.9	100

[a] Effect of isoproterenol and new β_2-adrenoceptor agonists on isolated guinea pig teacheal rings precontracted by histamine at 30 μM. The maximum relaxant effect by isoproterenol was considered 100%.
[b] pEC_{50} values are the mean of three experiments.
[c] Ref. 21

Table 2
Inhibition of cAMP hydrolysis by recombinant human PDE4B2 in the presence of **15** and new compounds[a]

Compounds	*n*	PDE4B2 inhibition IC_{50} (μM)
(R)-Rolipram	–	0.500 ± 0.028
15	–	0.520 ± 0.042
28	2	0.280 ± 0.021
29	4	0.278 ± 0.013
(R)-**29**	4	0.265± 0.009
(S)-**29**	4	0.284 ± 0.016
30	5	0.257 ± 0.020
31	6	0.251 ± 0.015
Cilomilast	–	0.120 (0.095[b])
Rofiumilast	–	0.001 (0.0008[c])

[a] Data are of average of three determinations ± SEM.
[b] Ref. 24
[c] Ref. 25

antagonist effects. The pA_2 value for the antagonist activity of ICI-118551 in the presence of (*R*)-**29** was 9.15.

Compound **15** and rolipram, the known PDE4 inhibitors, are used as standard (Table 2). Compounds **28–31** were also tested for inhibition of cAMP hydrolysis by recombinant human PDE4B2 in vitro, using a colorimetric assay method from Biomol (Enzo Life Science), following the protocol described by the manufacturer.[23] The results outlined in Table 2 indicate that all target compounds provided excellent PDE4B2 inhibitory activity (Table 2, compounds **15**, **28**, **29**, **30**, and **31** gave the IC_{50} value of 0.520, 0.280, 0.278, 0.257 and 0.251 μM, respectively). A simple structure–activity relationship analysis showed that the PDE4B2 inhibitory potency is closely related to the length of the alkylene chain. Compound **31**, with six methylene groups between the β_2-adrenoceptor agonist moiety and phthalazinone, provided the greatest inhibitory potency in the series. The higher potency of compounds **28–31** compared with lead compound **15** suggests that N-substitution is favorable for the inhibition of human PDE4B2. As reported in literature,[26] cis-**15** is more potent than its trans-isomer for the inhibition of PDE4. For searching more potent agents for treatment asthma and COPD, the study of the different diastereomers of these dual β_2-adrenoceptor agonists-PDE4 inhibitors is in progress in our group.

In conclusion, we have presented the design, synthesis and evaluation of a series of dual functional molecules that behave as β_2-adrenoceptor agonists and PDE4 inhibitors for the first time. The compounds displayed moderate to high β_2-adrenoceptor agonist activities on isolated guinea pig tracheal rings precontracted by histamine. Among them, compound (*R*)-**29** exhibited the most potent agonist activity, with a pEC_{50} value of up to 10.0. Moreover, the results showed that N-substitution of the phthalazinone resulted in a significant increase in the PDE4B2 inhibitory potency.

Acknowledgment

We thank the Natural Science Foundation of China (20972198) and State Key Laboratory of Respiratory Diseases (2007DA-80154F1110) for financial support of this study.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2012.01.013.

References and notes

1. Barnes, P. J. *Nat. Rev. Immunol.* **2008**, *8*, 183.
2. Barnes, P. J. *J. Allergy Clin. Immunol.* **1998**, *102*, 531.
3. Boushey, H. A. *J. Allergy Clin. Immunol.* **1998**, *102*, 5.
4. Kotlikof, M. I. *Ann. Rev. Physiol.* **1996**, *58*, 115.
5. Bender, A. T.; Beavo, J. A. *Pharmacol. Rev.* **2006**, *58*, 488.
6. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm261649.htm.
7. Lugnier, C. *Pharmacol. Ther.* **2006**, *109*, 366.
8. Halene, T. B.; Siegel, S. J. *Drug Discovery Today* **2007**, *12*, 870.
9. Houslay, M. D.; Shafer, P.; Zhang, K. Y. *Drug Discovery Today* **2005**, *10*, 1503.
10. Yuasa, K.; Kanoh, Y.; Okumura, K.; Omori, K. *Eur. J. Biochem.* **2001**, *268*, 168.
11. Fan, Chung. K. *Eur. J. Pharmacol.* **2006**, *533*, 110.
12. Jin, S.-L. C.; Conti, M. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 7628.
13. Keller, T. H.; Bray-French, K.; Demnitz, F. W. J.; Müller, T.; Pombo-Villar, E.; Walker, C. *Chem. Pharm. Bull.* **2001**, *49*, 1009.
14. Provins, L.; Christophe, B.; Danhaive, P.; Dulieu, J.; Durieu, V.; Gillard, M.; Lebon, F.; Lengelé, S.; Quéré, L.; van Keulen, B. *Bioorg. Med. Chem. Lett.* **1834**, *2006*, 16.
15. Cashman, J. R.; Voelker, T.; Zhang, H.-T.; O'Donnell, J. M. *J. Med. Chem.* **2009**, *52*, 1530.
16. Hughes, A. D.; Chin, K. H.; Dunham, S. L.; Jasper, J. R.; King, K. E.; Lee, T. W.; Mammen, M.; Martin, J.; Steinfeld, T. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1354.
17. Huang, L.; Liu, J.; Shan, W.; Liu, B.; Shi, A.; Li, X. *Chirality* **2010**, *22*, 206.
18. Van der Mey, M.; Hatzelmann, A.; Van Klink, G. P.; Van der Laan, I. J.; Sterk, G. J.; Thibaut, U.; Ulrich, W. R.; Timmerman, H. *J. Med. Chem.* **2001**, *44*, 2523.
19. Van der Mey, M.; Boss, H.; Couwenberg, D.; Hatzelmann, A.; Sterk, G. J.; Goubitz, K.; Schenk, H.; Timmerman, H. *J. Med. Chem.* **2002**, *45*, 2526.
20. Soriano-Ursúa, M. A.; Correa-Basurto, J.; Valencia-Hernández, I.; Amezcua-Gutiérrez, M. A.; Padilla-Martínez, I. I.; Trujillo-Ferrara, J. G. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5623.
21. Procopiou, P. A.; Barrett, V. J.; Bevan, N. J.; Biggadike, K.; Butchers, P. R.; Coe, D. M.; Conroy, R.; Edney, D. D.; Field, R. N.; Ford, A. J.; Guntrip, S. B.; Looker, B. E.; McLay, I. M.; Monteith, M. J.; Morrison, V. S.; Mutch, P. J.; Richards, S. A.; Sasse, R.; Smith, C. E. *J. Med. Chem.* **2009**, *52*, 2280.
22. Bilski, A. J.; Halliday, S. E.; Fitzgerald, J. D.; Wale, J. L. *J. Cardiovasc. Pharmacol.* **1983**, *5*, 430.
23. Cyclic nucleotide phosphodiesterase assay kit: AK-800.
24. Christensen, S. B.; Guider, A.; Forster, C. J.; Gleason, J. G.; Bender, P. E.; Karpinski, J. M.; DeWolf, W. E.; Barnette, M. S.; Underwood, D. C.; Griswold, D. E.; Cieslinski, L. B.; Burman, M.; Bochnowicz, S.; Osborn, R. R.; Manning, C. D.; Grous, M.; Hillegas, L. M.; Bartus, J. O. L.; Ryan, M. D.; Eggleston, D. S.; Haltiwanger, R. C.; Torphy, T. J. *J. Med. Chem.* **1998**, *41*, 821.
25. Hatzelmann, A.; Schudt, C. *J. Pharmacol. Exp. Ther.* **2001**, *297*, 267.
26. Van der Mey, M.; Hatzelmann, A.; Van der Laan, I. J.; Sterk, G. J.; Thibaut, U.; Timmerman, H. *J. Med. Chem.* **2001**, *44*, 2511.