Bioorganic & Medicinal Chemistry Letters 23 (2013) 1548–1552

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Hybrids consisting of the pharmacophores of salmeterol and roflumilast or phthalazinone: Dual β_2-adrenoceptor agonists-PDE4 inhibitors for the treatment of COPD

Anqiu Liu [a], Ling Huang [a], Zhiren Wang [a], Zonghua Luo [a], Fei Mao [a], Wenjun Shan [a], Jiaxing Xie [b], Kefang Lai [b,*], Xingshu 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 11 September 2012
Revised 1 November 2012
Accepted 15 November 2012
Available online 29 November 2012

Keywords:
COPD
Bronchodilator
β_2-Adrenoceptor agonist
PDE4 inhibitor

ABSTRACT

A novel class of dual pharmacology bronchodilators targeting both β_2-adrenoceptor and PDE4 was designed and synthesised by combining the pharmacophores of salmeterol and roflumilast or phthalazinone. All the compounds exhibited better β_2-adrenoceptor agonist activities (pEC_{50} = 8.47–9.20) than the reference compound salmeterol (pEC_{50} = 8.3) and good inhibitory activity on PDE4B2 (IC_{50} = 0.235–1.093 μM).

© 2012 Elsevier Ltd. All rights reserved.

Chronic obstructive pulmonary disease (COPD) is characterised by limited expiratory airflow, which is the result of several types of anatomical lesions, including loss of lung elastic recoil, fibrosis, and narrowing of small airways.[1] It is the fourth leading cause of mortality worldwide; over 600 million individuals worldwide have COPD, and nearly 3 million die from this disorder annually.[2] Although the exact pathogenesis of COPD is unknown, smooth muscle dysfunction and chronic inflammation are known to play important roles in the disease. Consequently, both anti-inflammatory and bronchodilator medicines are used extensively for the treatment of this disorder.

Inflammation in COPD is not present only in the lungs but is instead a significant systemic inflammation.[3] It is believed that lung inflammation spreads to the systemic circulatory system through the rich lung capillary network causing systemic inflammation. COPD is postulated to be involved in the pathogenesis of systemic inflammation.[3,4] It is well known that cyclic adenosine monophosphate (cAMP) blocks proliferation and chemotaxis of inflammatory cells, inhibits proinflammatory cell activity, and suppresses the release of inflammatory and cytotoxic mediators in the lungs.[5] Phosphodiesterases can hydrolyse cAMP to 5′-adenosine monophosphate, rendering it inactive. Among the eleven members of the phosphodiesterase enzyme family, phosphodiesterase type 4 (PDE4)[6,7] is particularly abundant in inflammatory, immune, and airway smooth muscle cells.[8] The inflammatory response is highly sensitive to levels of cAMP. PDE4 inhibitors can block cAMP hydrolysis and provide anti-inflammatory activity in the airways by inhibiting the activity of PDE4. In recent years, PDE4 inhibitors have been evaluated as promising therapies for the treatment of inflammatory pulmonary disorders, including asthma and COPD.[9] Roflumilast, a PDE4 inhibitor, has been approved by the FDA as a long-term maintenance therapy for COPD.[10]

β_2-Adrenoceptor agonists are well established in the treatment of both asthma and COPD due to their rapidly evoking bronchodilatation effects.[11] The mechanism of action of this class of compounds is believed to involve the stimulation of adenylyl cyclase and subsequent activation of cAMP/cAMP-dependent protein kinase cascade.[12,13] Current β_2-receptor agonists include short-acting (e.g., salbutamol and fenoterol) and long-acting (e.g., salmeterol, formoterol and indacaterol) species.[14] Among them, salmeterol and formoterol are the two most prescribed long-acting β_2-receptor agonists, which provide longer lasting bronchodilation, improve quality of life and are associated with a lower frequency of exacerbation compared with short-acting β_2 agonists.

The multifaceted symptoms associated with some diseases have encouraged active research in the development of multifunctional drugs. For the treatment of COPD and asthma, successful use of combination products such as Combivent® (salmeterol and fluticasone propionate) and Symbicort® (formoterol and budesonide),

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

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.11.058

as well as combination studies with tiotropium and formoterol, have confirmed the complementary effects of simultaneously targeting multiple mechanisms with two drugs, which can provide greater improvement in lung function compared to single agent bronchodilators.[15]

In previous work, we have developed of a multivalent approach to synthesise a class of dual-pharmacology bronchodilators that target both the β_2-adrenoceptor and PDE4.[16] Here, we report the synthesis of a new series of hybrids that combine both salmeterol and the PDE4 inhibitors roflumilast or phthalazinone (a potent PDE4 inhibitor),[17,18] which are expected to provide both β_2-adrenoceptor agonism activity and PDE4 inhibition activity (Fig. 1).

The syntheses of the dual β_2-agonists and PDE4 inhibitors (**11a–d** and **17a–d**) are shown in Schemes 1–3. First, compound **3** was prepared in several steps according to procedures reported previously.[19] The reduction of **3** with sodium borohydride provided alcohol **4**, which was then protected with TBDMSCl to give compound **5**, the synthetic intermediates containing the pharmacophore of the β_2-adrenoceptor agonist.

The synthetic intermediate containing the pharmacophore of the PDE4 inhibitor roflumilast **9a–d** was was shown in Scheme 2. First, the reaction of isoindoline-1,3-dione with dibromides gave **6a–d**, which were reacted with 3-hydroxy-4-methoxybenzaldehyde to provide intermediates **7a–d**. The oxidation of compounds **7a–d** with $NaClO_2$ in the presence of H_2O_2 gave intermediates **8a–d**, which reacted in succession with $SOCl_2$, 4-amino-3,5-dichloropyridine and H_2NNH_2 to provide **9a–d**, the synthetic intermediates containing the pharmacophore of the PDE4 inhibitor roflumilast. Intermediates **10a–d** were prepared by the coupling of **5** and **9** in the presence of triethylamine**.** Finally, target compounds **11a–d** were obtained by the deprotection of **10a–d** with the NH_4F–EtOH–H_2O system followed by reaction with hydrogen chloride in alcohol.

Target compounds **17a–d** were synthesised according the route shown in Scheme 3. First, compound **15a–d** was prepared from 1,2-dimethoxybenzene and *cis*-1,2-cyclohexanedicarboxylic anhydride according the literature.[16] Compounds **15a–d** were reacted with intermediate **3** to provide **16a–d**. Finally, the dual β_2-agonists and PDE4 inhibitors **17a–d** were obtained by deprotection of compounds **16a–d** in the presence of Pd/C under hydrogen followed by reaction with $NaBH_4$ then treated with hydrochloric acid.

The β_2-adrenoceptor agonist in vitro assays were carried out using a protocol described previously.[20] Isoprenaline and salmeterol, the known β_2-adrenoceptor agonists, were used as reference compounds. The effect of the synthesised compounds on the tracheal rings of guinea pigs pre-contracted by histamine (Fig. 2) indicated that all the tested compounds produced a relaxant effect and induced a concentration-dependent relaxation. The maximum response (E_{max}) to each was similar to that evoked by the reference compound isoprenaline. The results in Table 1 indicate that all eight synthesised compounds exhibited more potent β_2-adrenoceptor agonist activity (up to a 9.20 pEC_{50} value, related to pK_d) than the reference compounds isoprenaline ($pEC_{50} = 7.5$) and salmeterol ($pEC_{50} = 8.3$). Among them, the series (**17a–17d**) connected by a 2–6 carbon linker between the PDE4 inhibitor phthalazinone and the β_2-adrenoceptor agonist moiety salmeterol exhibited better activities (with the pEC_{50} values of 9.08–9.20) than **11a–11d** (with the pEC_{50} values of 8.47–8.71), the hybrids consisting of salmeterol and a roflumilast moiety. However, it seems that the length of the linkers have little effect on the activities of both series of compounds.

Compounds **11a–11d** and **17a–17d** were also evaluated for inhibition of cAMP hydrolysis by recombinant human PDE4B2 in vitro by a colorimetric assay method from Biomol (Enzo Life Science, Inc., NY, USA) following the protocol described by the manufacturer and using rolipram and roflumilast as reference PDE4 inhibitors.[22] The results in Table 2 show that most of the target compounds provided good PDE4B2 inhibitory activity (Compounds **11a**, **11b**, **11c** and **11d** gave IC_{50} values of 1.093, 0.601, 0.394 and 0.235 μM; compounds **17a**, **17b**, **17c** and **17d** gave IC_{50}

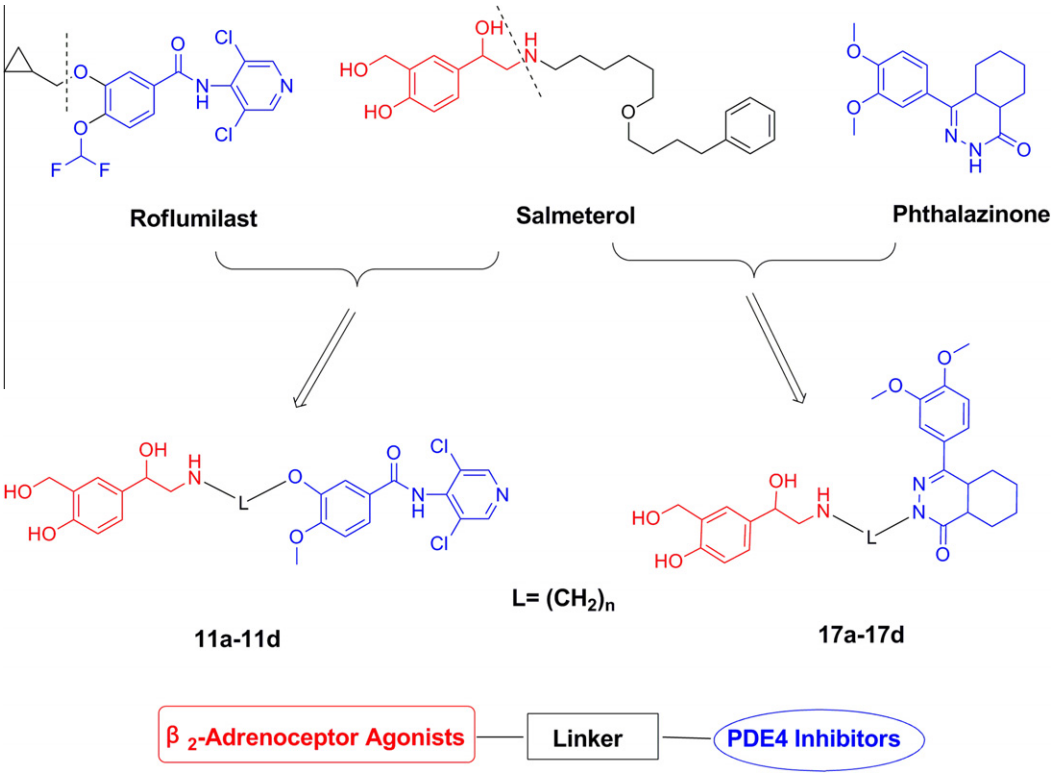
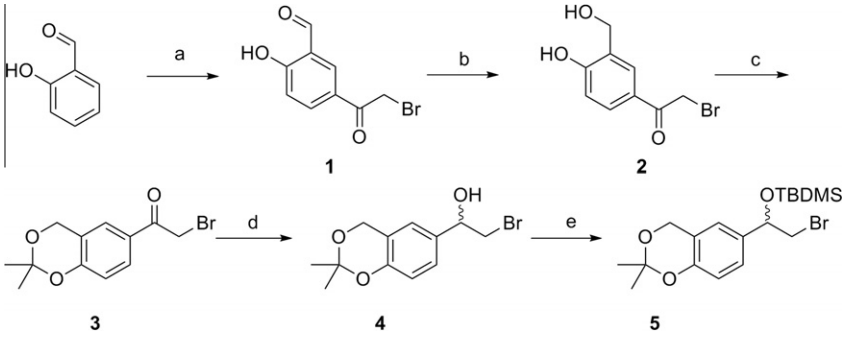
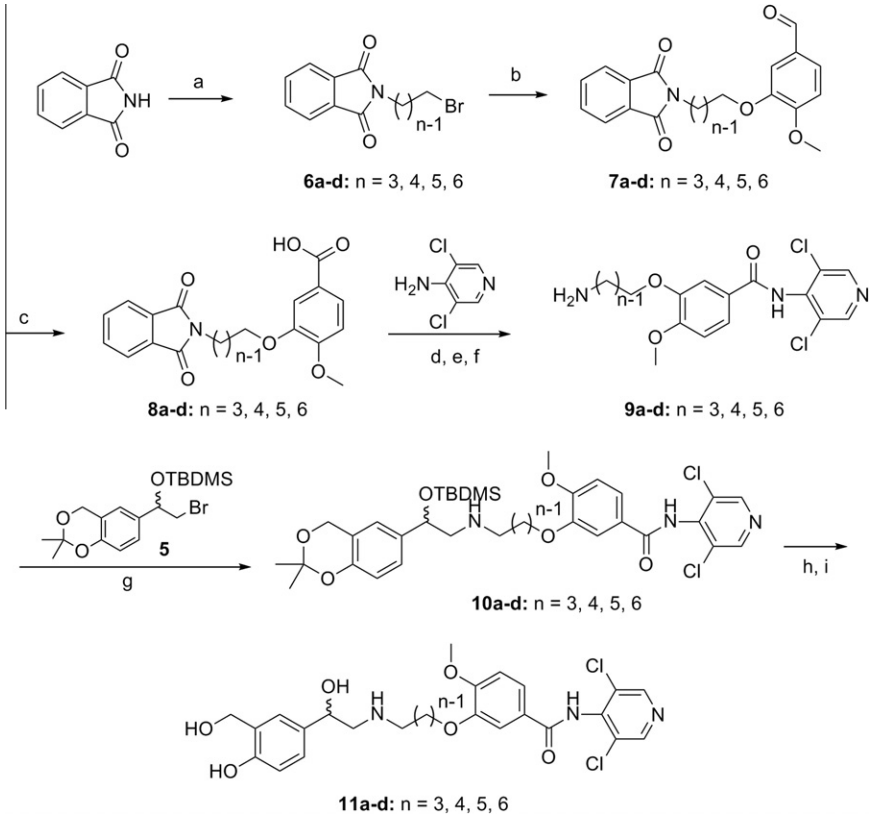


Figure 1. Design strategy for compounds **11a–11d**, **17a–11d**.



Scheme 1. Reagents and conditions: (a) $AlCl_3$, $BrCH_2COBr$, CH_2Cl_2, reflux, 15 h, 80%; (b) $NaBH_4$, acetic acid, 10 °C, 1 h, 85%; (c) 2,2-dimethoxypropane, CH_2Cl_2, rt, 10 h, 82%; (d) $NaBH_4$, methanol, 0 °C to rt, 1 h, 95%; (e) TBDMSCl, imidazole, DMF, rt, 16 h, 57%.

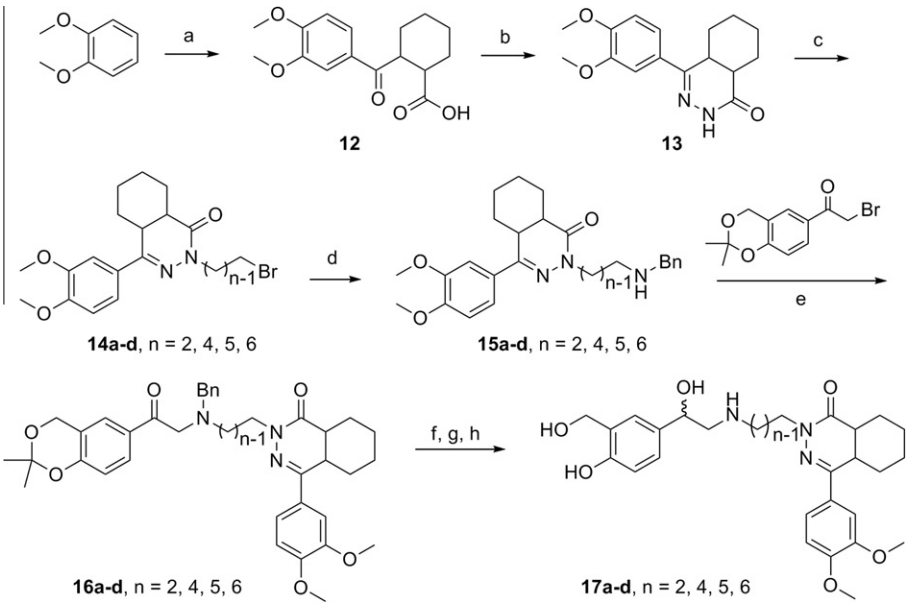


Scheme 2. Reagents and conditions: (a) K_2CO_3, DMF, $Br(CH_2)_nBr$, rt, 16 h, 78–85%; (b) K_2CO_3, KI, DMF, isovanillin, 65 °C, 18 h, 73–81%; (c) $NaClO_2$, H_2O_2, acetonitrile, rt, 24 h, 85–91%; (d) $SOCl_2$, reflux, 6 h; (e) NaH, 4-amino-3,5-dichloropyridine, DMF, 20 °C then rt, 1 h; (f) $H_2NNH_2{\cdot}H_2O$, EtOH, reflux, 2 h, 17–20% over three steps; (g) DMF, Et_3N, 65 °C, 24 h, 36–41%;(h) NH_4F, EtOH, H_2O, 45 °C, 48 h; (i) 1 M HCl, EtOH, rt, 12 h, 61–67% over two steps.

values of 0.282, 0.278, 0.271 and 0.263 μM, respectively). Preliminary structure–activity relationship analysis revealed that the inhibitory potency of the two series is closely related to the length of the alkyl chain, but the salmeterol–roflumilast series is more obvious. Compound **11d**, with six methylene groups between the β_2-adrenoceptor agonist moiety and PDE4B2 roflumilast moiety provided the best inhibitory potency (IC_{50} = 0.235 μM) of all the tested compounds, while compound **11d**, with three methylene groups between the two pharmacophores, gave the weakest inhibitory potency (IC_{50} = 1.093 μM). Although the trend in inhibitory potency is similar to that of compounds **11a**–**11d**, compounds **17a**–**17d** show less variation in potency when varying the linker from two methylene groups to six methylene groups (**17a**, two methylene groups, IC_{50} = 0.282 μM; **17d**, six methylene groups, IC_{50} = 0.263 μM).

It is worthy to note that there is still a far distance to ideal balance of two activities potencies in a single molecule (The pIC_{50} = 6.58 for PDE4, while the pEC_{50} = 9.20 for β_2-adrenoceptor agonists **17d**). The crystal structure of the complex of roflumilast and PDE4 showed the cyclopropyl pharmacophore of roflumilast has a good interaction with the hydrophobic groove of PDE4. The loss of activity for the PDE4 pharmacophore in the hybrids should be attributed to the bulky and polarity effect of the molecule which cyclopropyl moiety of roflumilast was replaced by β_2-adrenoceptor agonists. Therefore, to optimize the structures of the PDE4 inhibitor moiety for increasing the inhibitory potency of the PDE4 would be the emphases in future work.

In a summary, we have designed, synthesised and evaluated a series of dual functional molecules that possess the pharmacophores of long-acting β_2-receptor agonist salmeterol and PDE4



Scheme 3. Reagents and condition: (a)*cis*-1,2-cyclohexanedicarboxylic anhydride, $AlCl_3$, CH_2Cl_2, reflux, 20 h, 82%; (b) H_2NNH_2, EtOH, reflux, 3 h, 93%; (c) $Br(CH_2)_nBr$, NaH, DMF, 0 °C to rt, 1 h, 78–84%; (d) benzylamine, rt, 10 h, 66–77%; (e) CH_2Cl_2, Et_3N, rt,12 h, 65–71%; (f) Pd/C, H_2, 500 psi, 24–30 h; (g) $NaBH_4$, methanol, 0 °C to rt, 1 h; (h)1 M HCl, EtOH, rt, 12 h, 66–72% over three steps.

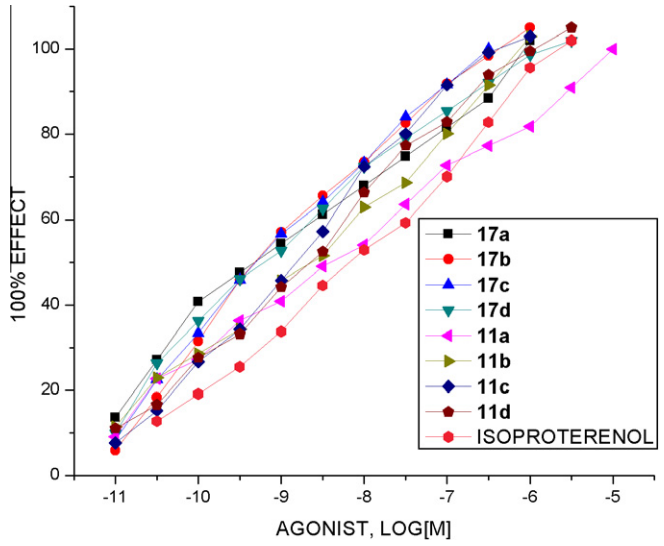


Figure 2. Effect of agonists and isoproterenol on isolated guinea pig tracheal rings pre-contracted by histamine at 30 μM. The maximum relaxant effect by isoproterenol was considered 100%. Each point represents the mean effect ($n = 3$).

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

Compound	*n*	pEC_{50}[b]	E_{max} (%)
Isoprenaline	—	7.54	100
11a	3	8.47	100
11b	4	8.55	103
11c	5	8.71	103
11d	6	8.50	105
17a	2	9.19	102
17b	4	9.17	100
17c	5	9.08	100
17d	6	9.20	102
Salmeterol	—	8.3[c]	—

[a] Effect of isoproterenol and new β_2-adrenoceptor agonists on isolated guinea pig tracheal rings pre-contracted 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 **13** and new compounds[a]

Compounds	*n*	PDE4B2 inhibition IC_{50} (μM)
(*R*)-Rolipram	—	0.500 ± 0.032
Phthalazinone (**13**)	—	0.520 ± 0.048
11a	3	1.093 ± 0.020
11b	4	0.601 ± 0.011
11c	5	0.394 ± 0.024
11d	6	0.235 ± 0.010
17a	2	0.282 ± 0.08
17b	4	0.278 ± 0.012
17c	5	0.271 ± 0.09
17d	6	0.263 ± 0.07
Roflumilast	—	0.001 (0.0008[b])

[a] Data are of average of three determinations ± SEM.
[b] Ref. 23.

inhibitors roflumilast or phthalazinone. All the compounds exhibited better β_2-adrenoceptor agonist activities on isolated guinea pig tracheal rings pre-contracted by histamine than the reference compounds isoprenaline and salmeterol. Meanwhile, most of the hybrids are more potent than (*R*)-Rolipram, the first generation PDE4 inhibitor, It is worthy to note that compound **17d**, with six methylene groups between the β_2-adrenoceptor agonist moiety and PDE4B2 phthalazinone moiety, showed high inhibitory activity toward PDE4B2 (with an IC_{50} value 0.263 μM) and exhibited the most potent agonist activity. As a preliminary study, we use alkyl linkers of varying lengths to join the two pharmacophores, which lead to a flat SAR. Further investigations including the linkers modification, and into inhaled COPD candidates (considering the route of most drugs for the treatment of COPD are inhaled approach) based on these results are in progress.

Acknowledgments

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 http://dx.doi.org/10.1016/j.bmcl.2012.11.058.

References and notes

1. Saetta, M.; Turato, G.; Maestrelli, P.; Mapp, C. E.; Fabbri, L. M. *Am. J. Respir. Crit. Care Med.* **2001**, *163*, 1304.
2. Mui, T. S. Y.; Man, S. F. P.; Sin, D. D. *Expert Rev. Clin. Immunol.* **2008**, *4*, 365.
3. Gan, W. Q.; Man, S. F. P.; Senthilselvan, A.; Sin, D. D. *Thorax* **2004**, *59*, 574.
4. Broekhuizen, R.; Grimble, R. F.; Howell, W. M.; Shale, D. J.; Creutzberg, E. C.; Wouters, E. F.; Schols, A. N. *Am. J. Clin. Nutr.* **2005**, *1059*, 82.
5. Schalkwyk, E. V.; Strydom, K.; Williams, Z.; Venter, L.; Leichtl, S.; Christine, S. W.; DirK, B.; Philip, G. B. *J. Allergy Clin. Immunol.* **2005**, *116*, 292.
6. Bender, A. T.; Beavo, J. A. *Pharmacol. Rev.* **2006**, *58*, 488.
7. Lerner, A.; Epstein, P. M. *Biochem. J.* **2006**, *393*, 21.
8. Gratteri, P.; Bonaccini, G.; Melani, F. *J. Med. Chem.* **2005**, *48*, 1657.
9. Vignola, A. M. *Resp. Med.* **2004**, *98*, 495.
10. Pinner, N. A.; Hamilton, L. A.; Hughes, A. *Clin. Ther.* **2012**, *34*, 56.
11. Waldeck, B. *Eur. J. Pharmacol.* **2002**, *445*, 1.
12. Giembycz, M. A.; Newton, R. *Eur. Respir. J.* **2006**, *27*, 1286.
13. Lotvall, J. *Respir. Med.* **2000**, *94*, S6.
14. Hughes, A. D.; Chin, K. H.; Dunham, S. L.; Jasper, J. R.; King, K. E. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1354.
15. van Noord, J. A.; Aumann, J. L.; Janssens, E.; Smeets, J. J.; Zaagsma, J.; Mueller, A.; Cornelissen, P. J. G. *Respir. Med.* **2010**, *104*, 995.
16. Shan, W. J.; Huang, L.; Zhou, Q.; Jiang, H. L.; Luo, Z. H.; Lai, K. f.; Li, X. S. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1523.
17. VanderMey, 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.
18. VanderMey, M.; Boss, H.; Couwenberg, D.; Hatzelmann, A.; Sterk, G. J.; Goubitz, K.; Schenk, H.; Timmerman, H. *J. Med. Chem.* **2002**, *45*, 2526.
19. Liu, J. T.; Zhou, D.; Jia, X.; Huang, L.; Li, X. S.; Chan; Albert, X. S. *Tetrahedron: Asymmetry* **1824**, *2008*, 19.
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. *Cyclic nucleotide phosphodiesterase assay kit*: AK-800.
23. Hatzelmann, A.; Schudt, C. *J. Pharmacol. Exp. Ther.* **2001**, *297*, 267.