Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 2355–2358

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Improvement of Therapeutic Index of Phosphodiesterase Type IV Inhibitors as Anti-Asthmatics

Euikyung Kim,[a,c,*] Hyung-Ok Chun,[a] Sung-Hak Jung,[a] Jong Hoon Kim,[b] Jae-Mok Lee,[b] Byung-Chul Suh,[b] Myung Xik Xiang[b,d] and Chung K. Rhee[b,d,*]

[a]*Pharmacology and Analytical Chemistry Research, 319 Hyundai I Valley, 223-12 Sandaewon-dong, Choongwon-gu, Sungnam-si, Kyonggi-do, 462-120, Republic of Korea*

[b]*Division of New Drug Discovery Project, Institute of Science & Technology, CJ Co., 522-1 Dokpyong-ri, Majang-myon, Ichon-si, Kyonggi-do, 467-810, Republic of Korea*

[c]*Department of Life Science, Division of Molecular and Life Science, Pohang University of Science & Technology, Pohang, Kyungbuk 790-784, Republic of Korea*

[d]*R & D Center, Leadgene Co., 319 Hyundai I Valley, 223-12 Sandaewon-dong, Choongwon-gu, Sungnam-si, Kyonggi-do, 462-120, Republic of Korea*

Received 28 March 2003; revised 15 April 2003; accepted 17 April 2003

Abstract—A new series of catechol hydrazines was synthesized and their structure–activity relationship (SAR) was analyzed for developing an effective phosphodiesterase 4 (PDE4) inhibitor as an anti-asthmatic drug candidate. Among the (E)-Analogues tested using in vitro assays, **5CC** showed a strong PDE4 inhibitory activity and a significantly improved rolipram binding profile compared with rolipram, a prototype PDE4 inhibitor. Moreover, from in-vivo asthma model, we observed that (E)-Analogue **5CC** had a good efficacy against guinea-pig respiratory tract inflammation and bronchoconstriction, along with a remarkably reduced emetic side effect, compared with rolipram. Conclusively, (E)-Analogue **5CC** seems to be a promising candidate for the development of anti-asthmatic PDE4 inhibitors.

Asthma is an airway inflammatory disease with a symptomatic bronchial muscle contraction, which often causes respiratory arrest in the asthmatic subjects. Cyclic AMP (cAMP) is a well-known key modulator of respiratory tract muscle contraction and inflammation.[1] From this reason, cAMP-hydrolyzing phosphodiesterase (PDE) has became one of the hottest targets for the development of new anti-asthmatic drugs over the last several years. Up to now, eleven families of PDE isozymes have been identified and classified according to their substrate selectivity and sensitivity to specific inhibitors.[2] Among these, PDE4 is a major cAMP-hydrolyzing enzyme found in airway immune and inflammatory cells of asthma. Within each phosphodiesterase family, multiple variants can be generated by alternative splicing among the 5′-end exons and/or the use of different transcription initiation sites.[3] PDE4 has four variants(subtypes A, B, C, and D), each of which is derived from a distinct gene.[4–9] Among them, PDE4B is the most predominant PDE isozyme found in inflammatory cells including human monocytes and neutrophils.[10–14] In addition to anti-inflammatory activity, cAMP has another important biological function that induce relaxation of airway smooth muscle,[15] which can relieve asthmatic patients from respiratory arrest. These results suggest that PDE4 can be an attractive therapeutic target against chronic respiratory diseases, such as asthma and COPD (chronic obstructive pulmonary disease). From the works of other investigators, several potent PDE4 inhibitors have been developed such as rolipram,[16] RP-73401,[17] CDP-840,[18] and SB-207499.[19]

However, PDE4 inhibitors are well known to have intrinsic adverse side effect such as emesis, which has a strong correlation with the binding affinity of a compound to the rolipram binding site in brain.[20,21] As a part of our ongoing effort to develop more potent and selective PDE IV inhibitors with reduced side effects,[22] we have designed and synthesized a new series of catechol hydrazines, and evaluated their therapeutic poten-

*Corresponding author. Tel.: +82-54-279-2973; fax: +82-54-279-2199; e-mail: ekim@postech.ac.kr (E. Kim). Tel.: +82-31-737-9641; fax: +82-31-737-9645; e-mail: ckrhee@leadgene.com (C.K. Rhee).

0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00405-0

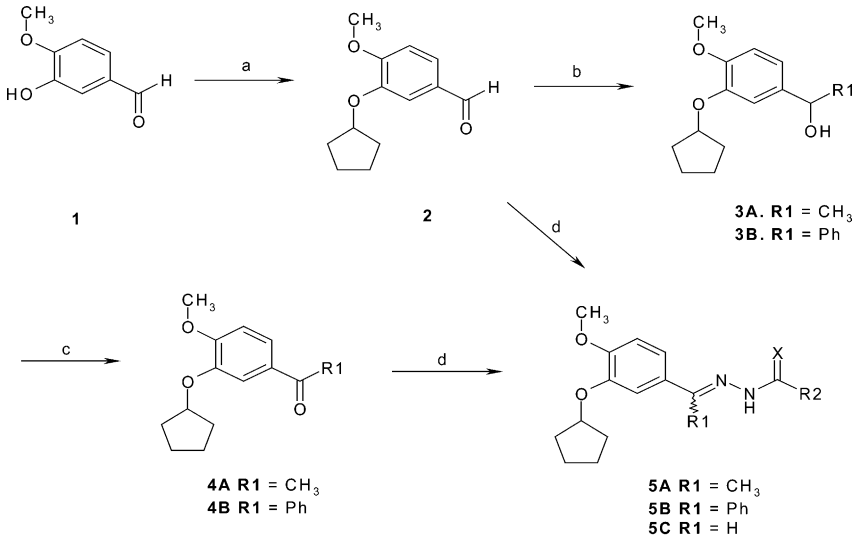
tial against asthma.[23] SAR studies using molecular modeling with those reference compounds such as rolipram and SB-207499 allowed us to design new molecules for PDE IV inhibitors. Here we wish to report the synthesis of target compound **5** and their biological activity.

As outlined in the Scheme 1, the target molecules **5** were easily prepared from isovanillin **1** by known procedures.[18] Alkylation of isovanillin **1** and cyclopentyl bromide with K_2CO_3 and KI in DMF gave rise to aldehyde **2** as yellow oil in 81% yield. Aldehyde **2** was alkylated with CH_3Li or PhLi to produce alcohol **3A**, **3B**, respectively. Oxidation of alcohol **3A**, **3B** with PDC was carried out to yield ketone **4A**, **4B**, respectively. Treatment of ketone **4A** or aldehyde **2** with hydrazine derivatives gave a mixture of (*Z*)- and (*E*)-isomers **5A** (1:10), **5C** (1:20), respectively. However, a mixture of (*Z*)- and (*E*)-isomers **5B** (1:1–1.5) was observed in the reaction of ketone **4B** with hydrazine derivatives. The structure of (*Z*)- and (*E*)-isomers **5** was assigned by ^1H proton NMR. The signal of the methoxy groups of (*Z*)-isomers **5** is shifted downfield (3.83 ppm) compare to that of (*E*)-isomers (3.77 ppm). (*E*)-isomers **5** were obtained pure by recrystalization and then applied to biological tests.[23]

As predicted from molecular modeling studies, (*E*)-isomers of the analogues were about 3–4 times more potent than (*Z*)-isomers (data not shown). In the present study, we focused on SAR of (*E*)-isomers and the results of their SAR studies are summarized in Table 1. Synthesized compounds were evaluated for PDE4 inhibitory activity against purified rat liver PDE4 enzyme as well as binding affinity to the high affinity-rolipram binding site using crude rat brain homogenate. The results suggest the size of R1 on (*E*)-analogues plays a very interesting role on the biological activities of this series of compounds. Either a larger R1 (phenyl in analogues **5B**) or a smaller R1 (hydrogen in analogues **5C**) gave a significantly higher potency in PDE4 inhibition comparing with an intermediate size R1 (methyl in analogues **5A**). Furthermore, amide substituted R2 analogues were observed to be 2 to 3 times more potent than carbonyl substituted R2 analogues (Table 1). Among them, analogues **5BC** and **5CC** were the most potent in the series and have similar affinity to rolipram. In terms of a rolipram binding, a larger R1 (analogues **5B**) significantly reduced the affinity of analogues to the rolipram binding site in brain comparing with the intermediate size R1 (analogues **5A**) or the smaller R1 (analogues **5C**). The results suggest larger R1 (phenyl in analogues **5B**) is important for reducing the affinity of the analogues to high affinity rolipram binding site in brain. As shown in Table 1, B/A ratio, which represents selectivity against high affinity rolipram binding versus low affinity PDE4 inhibition, imply that analogues **5BC** and **5CC** are substantially less likely to induce emesis comparing with rolipram, a prototype reference compound.

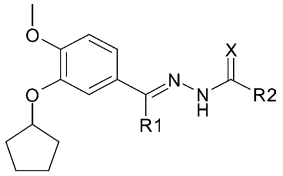
In the trachea relaxation study using isolated guinea-pig trachea, analogue **5CC** shows a potent trachea muscle relaxant activity, but analogue **5BC** does not (data not shown). Therefore, the further in vivo studies were focused on analogue **5CC** regarding the antiasthmatic activity and the emetic side effects. The results of in vivo studies were summarized in Table 2. The data show that analogue **5CC** has a significantly less emetic side effect than rolipram, while retaining similar potencies in brochodilatory and anti-inflammatory activities. These in vivo results are consistent with those of in vitro experiments (PDE IV enzyme inhibition and [^3H] rolipram binding in Table 1).

Selective PDE4 inhibitors have been previously observed to have antiasthmatic activities from various kinds of in vitro and in vivo models. The therapeutic potential of these agents against asthma has been documented by their dual pharmacological activities, which



Scheme 1. Synthesis of catechol hydrazine derivatives **5.** Reactions and conditions: (a) Bromocyclopentane, NaH, DMF, 60°C, (81%); (b) CH_3Li, THF for **3**. (95%), PhLi, THF for **4**, (90%); (c) PDC, CH_2Cl_2, Molecular sieve 4A°, (95%); (d) hydrazine derivatives, MeOH, reflux. (85-95%). * unoptimized yields.

Table 1. Biological activities of (E)-analogues 5



(E)-Analogues 5

Compd	R1	X	R2	PDE4 Inhibition (A) ($IC_{50=\mu}$ M)	[^{3}H]Rolipram binding (B) ($IC_{50=\mu}$M)	B/A[a]
5AA	CH_3	O	CH_3		0.092	<0.009
5AB	CH_3	O	CH_2CH_3	>10	0.042	<0.004
5AC	CH_3	O	NH_2	>10	0.146	<0.015
5AD	CH_3	S	NH_2	4.51	0.336	0.08
5AE	CH_3	NH	NH_2	>10	3.455	<0.35
5BA	Phenyl	O	CH_3	3.72	2.02	0.54
5BB	Phenyl	O	CH_2CH_3	4.90	>5	1.02
5BC	Phenyl	O	NH_2	0.58	1.197	2.06
5BD	Phenyl	S	NH_2	1.60	2.31	1.44
5BE	Phenyl	NH	NH_2	1.56	1.559	1.0
5CA	H	O	CH_3	1.75	0.019	0.01
5CB	H	O	CH_2CH_3	n.d.[b]	2.73	—
5CC	H	O	NH_2	0.60	0.069	0.12
5CD	H	S	NH_2	1.42	0.046	0.03
5CE	H	NH	NH_2	>10	>5	—
	Rolipram			0.31	0.0023	0.007

[a]Selectivity, (the value for [^{3}H]rolipram binding/the value for PDE4 inhibition).
[b]Not determined.

Table 2. Comparison of biological activities between 3C and rolipram

Compd	Bronchoconstriction[a] (% inh at 1.0 mg/kg, iv)	BAL[b] (% inh at 30 mg/kg, po)	Ferret emesis[c]
5CC	90.3	63	0/5 at 10 mg/kg, po
Rolipram	95.4	62	2/5 at 0.03 mg/kg, po

[a]Antigen-induced bronchoconstriction in passively-sensitized guinea pigs (iv, testing compounds were administered 10 min before OVA challenge).
[b]Antigen-induced eosinophil infiltration of BAL (Broncho alveolar lavage) in passively-sensitized guinea pigs (po, testing compounds were administered 90 min before OVA challenge).
[c]Number of animals exhibiting emesis or prodromal syndrome/total animals tested.

are bronchodilatory action and anti-inflammatory action. However, most PDE4 inhibitory compounds have characteristic side effects, including nausea, vomiting and gastric acid secretion, which become a bottleneck of these agents for being a drug candidate. Therefore, it has become inevitable for the drug development to discover a compound that has a prominent anti-inflammatory effect but less side effect. The side effects of PDE4 inhibitory compounds appear to be related to the inhibition of HPDE4 (which can be indirectly assessed by the affinity of a compound to the brain rolipram binding site), a distinct conformer of the enzyme that is enriched in the central nervous system. Therapeutic profile (retaining antiasthmatic activities against minimum emetic side effect) of analogue **5CC** showed the greatest improvement of the series of phosphodiesterase 4 inhibitors tested in this study. We expect that analogue **5CC** may give some information for the development of PDE4 inhibitors as antiasmatic drugs with an improved therapeutic profile.

References and Notes

1. Le Jeune, I. R.; Shepherd, M.; Van Heeke, G.; Houslay, M. D.; Hall, I. P. *J. Biol. Chem.* **2002**, *277*, 35980.
2. Essayan, D. M. *J. Allergy Clin. Immunol.* **2001**, *108*, 671.
3. Bolger, G. B. *Cell. Signal* **1994**, *8*, 851.
4. Baecker, P. A.; Obernolt, R.; Bach, C.; Yee, C.; Shelton, E. R. *Gene* **1994**, *138*, 253.
5. Bolger, G.; Michaeli, T.; Martins, T.; St John, T.; Steiner, B.; Rodgers, L.; Riggs, M.; Wichael, M.; Ferguson, K. *Mol. Cell. Biol.* **1993**, *13*, 6558.
6. Engels, P.; Sullivan, M.; Muller, T.; Lubbert, H. *FEBS Lett.* **1995**, *358*, 305.
7. McLaughlin, M. M.; Cieslinski, L. B.; Burman, M.; Torphy, T. J.; Livi, G. P. *J. Biol. Chem.* **1993**, *268*, 6470.
8. Livi, G. P.; Kmetz, P.; Mchale, M. M.; Cieslinski, L. B.; Sathe, G. M.; Taylor, D. P.; Davis, R. L.; Torphy, T. J.; Balcarek, J. M. *Mol. Cell. Biol.* **1990**, *10*, 2678.
9. Obernolte, R.; Bhakta, S.; Alvarez, R.; Bach, C.; Zuppan, P.; Mulkins, M.; Jarnagin, K.; Shelton, E. R. *Gene* **1993**, *129*, 239.
10. Dent, G.; Magnussen, H.; Rabe, K. F. *Lung* **1994**, *172*, 129.
11. Muller, T.; Engles, P.; Fozard, J. R. *Trends Pharmacol. Sci.* **1995**, *17*, 294.
12. Nicholson, C. D.; Shahid, M. *Pulm. Pharmacol.* **1994**, *7*, 1.
13. Palfreyman, M. N.; Souness, J. E. *Prog. Med. Chem.* **1996**, *33*, 1.
14. Torphy, T. J. *Am. J. Respir. Crit. Care. Med.* **1998**, *157*, 351.

15. Cortijo, J.; Bou, J.; Beleta, J.; Cardelus, I.; Llenas, J.; Morcillo, E.; Gristwood, R. W. *Br. J. Pharmacol.* **1993**, *108*, 562.
16. Marivet, M. C.; Bourguignon, J. J.; Lugnier, C.; Mann, A.; Stoclet, J. C.; Wermuth, C. G. *J. Med. Chem.* **1989**, *32*, 1450.
17. Ashton, M. J.; Cook, D. C.; Fenton, G.; Karlsson, J. A.; Palfreyman, M. N.; Raeburn, D.; Ratcliffe, A. J.; Souness, J. E.; Thurairatnam, S.; Vicker, N. *J. Med. Chem.* **1994**, *37*, 1696.
18. Hughes, B.; Howat, D.; Lisle, H.; Holbrook, M.; James, T.; Gozzard, N.; Blease, K.; Hughes, P.; Kingaby, R.; Warrellow, G.; Alexander, R.; Head, J.; Boyd, E.; Eaton, M.; Perry, M.; Wales, M.; Smith, B.; Owens, R.; Catterall, C.; Lumb, S.; Russell, A.; Allen, R.; Merriman, M.; Bloxham, D.; Higgs, G. *Br. J. Pharmacol.* **1996**, *118*, 1183.
19. Barnette, M. S.; Christensen, S. B.; Essayan, D. M.; Grous, M.; Prabhakar, U.; Rush, J. A.; Kagey-Sobotka, A.; Torphy, T. J. *J. Pharmacol. Exp. Ther.* **1998**, *284*, 420.
20. Barnette, M. S.; Grous, M.; Cieslinski, L. B.; Burman, M.; Christensen, S. B.; Torphy, T. J. *J. Pharmacol. Exp. Ther.* **1995**, *273*, 1396.
21. Duplantier, A. J.; Biggers, M. S.; Chambers, R. J.; Cheng, J. B.; Cooper, K.; Damon, D. B.; Eggler, J. F.; Kraus, K. G.; Marfat, A.; Masamune, H.; Pillar, J. S.; Shirley, J. T.; Umland, J. P.; Watson, J. W. *J. Med. Chem.* **1996**, *39*, 120.
22. Rhee, C. K.; Kim, J. H.; Suh, B. C.; Xiang, M. X.; Youn, Y. S.; Bang, W. Y.; Kim, E.; Shin, J. K.; Lee, Y. *Arch. Pharm. Res.* **1999**, *22*, 202.

23. *Animals and drugs*. All the experimental animals used in this study were housed until use in cages containing a layer of woodshavings, under conditions of constant ambient temperature (21 ± 1C), constant humidity and normal light/dark cycle. All the testing compounds of the present study except positive control, rolipram (Tocris Cookson Ltd.), were synthesized in Division of New Drug Discovery Project, R&D center of Biosciences at Cheil Jedang Co.

In vitro experiments. (1) As an efficacy test, phosphodiesterase 4 (PDE4) enzyme assay was measured as previously described.[22] (2) For the assessment of emetic side effect of PDE4 inhibitors, rolipram binding assay was performed. Briefly, rats (130–170 g) were sacrificed and hypothalamic region of the brain was dissected out for the preparation of rat brain homogenate. Brain tissue preparation was performed on ice or in 4 °C cold room unless otherwise indicated. The brain tissue was homogenized in 10× (v/w) of homogenization buffer (50 mM Tris–HCl, 1.2 mM $MgCl_2$, pH 7.5) using glass homogenizer. Crude homogenate was centrifuged at 30,000 *g* for 20 min and the pellet was washed in 10 volumes of fresh homogenization buffer. The final pellet was resuspended in fresh homogenization buffer and stored in liquid nitrogen until use. Rolipram binding assay was performed in a total of 0.5 mL reaction volume. In assay buffer of 50 mM Tris–HCl (pH 7.5), 5 mM $MgCl_2$, 50 μM 5′-AMP, 2 nM [^{3}H]rolipram (86 Ci/mmol), various concentrations of testing compound was added and the reaction was started by the addition of brain homogenate (receptor). After the incubation for 1 h at 30 °C, the reaction was stopped by vacuum filtration with ice-cold harvesting buffer (50 mM Tris–HCl, pH 7.5) through GF/B filters that have been pre-soaked in 0.3% polyethylenimine. Nonspecific binding was determined in the presence of 1 μM unlabeled rolipram in the reaction. The binding was calculated by measuring the radioactivity of the filter.

In vivo experimental procedure. All the in vivo experiments, except ferret emesis study, were performed using guinea pig (about 250 g body weight). (1) Antigen-induced bronchoconstriction: Guinea pig was passively immunized at day 0 with anti-OVA guinea-pig serum and tracheotomy was performed at day 1 for testing antiasthmatic effect of a compound in antigen-induced bronchoconstriction. A polyethylene cannule was inserted into trachea for artificial ventilation with room air (10 mL/kg, 50 breaths/min) and pressure transducer was attached to measure intra-tracheal pressure. Left jugular vein was cannulated to allow drug administration. Ten minutes after the administration of a testing compound (1.0 mg/kg, iv), airway tract contraction was induced by the administration of OVA (0.3 mg/kg, iv) through the jugular vein to the guinea pig. The pulmonary dilatory effect of a testing compound was determined by measuring the level of intra-tracheal pressure for 10 min after the OVA administration. (2) Broncho Alveolar Lavage (BAL): Guinea pig was passively immunized at day 0 with anti-OVA guinea-pig serum. Twenty-four h later, the guinea pig was orally administered with a testing compound (30 mg/kg) and 90 min later challenged with OVA (0.2%) by nebulization for 10 min. After another twenty four hours later, broncho-alveolar lavage (BAL) was obtained from the respiratory tract of guinea pigs and differential white blood cell count was determined to evaluate degree of inflammation induced by antigen challenge. (3) Ferret emesis study: Ferrets were fasted for the overnight, then administered orally with testing compounds (suspended in propylene glycol) at concentrations as indicated in the result. The emetic response and prodromal syndrome were continuously monitored for up to 3 h after the administration of each testing compound. Rolipram was used as a positive control.