Arch Pharm Res Vol 22, No 2, 202-207, 1999

Synthesis and Biological Studies of Catechol Ether Type Derivatives as Potential Phosphodiesterase (PDE) IV Inhibitors

Chung K. Rhee[1], Jong Hoon Kim[1], Byung-Chul Suh[1], Myung Xik Xiang[1], Yong Sik Youn[1], Won Young Bang[2], Euikyung Kim[2], Jae Kyu Shin[2] and Younha Lee[2]

[1]Department of Medicinal Chemistry, [2]Department of Pharmacology, R&D Center, Cheil Jedang Corp., 522-1 Dok-pyong-Ri, Majang-Myon, Ichon-Si, Kyonggi-Do 467-810, Korea

(Received November 2, 1998)

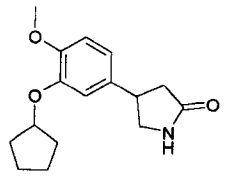
New series of catechol ether type derivatives **5**, **6** have been synthesized and applied to biological tests. Even though it is a preliminary data, some of our target molecules show the promising result against PDE IV inhibition. SAR and biological studies with synthetic compounds will be discussed in detail.

Key words : Phosphodiesterase (PDE), Anti-asthma therapy, Structure-activity relationship (SAR), Catechol ether type PDE IV inhibitors

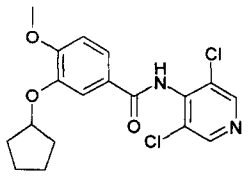
INTRODUCTION

Cyclic nucleotides such as cyclic adenosine 5'-monophosphate (cAMP) and cyclic guanosine 5'-monophosphate (cGMP) are important cellular secondary messengers and linked to a variety of biological responses in cells responding to extracellular stimuli (Erneux *et al.*, 1985; Cortijo *et al.*, 1993). Phosphodiesterases (PDEs) inactivate these cyclic nucleotides by catalyzing hydrolysis of the 3'-phosphoester bond to form the corresponding inactive 5'-nucleotide products. To date, at least seven families of PDE isozymes are identified on the basis of their substrate selectivity and sensitivity to isozyme-selective inhibitors (Nicholson *et al.*, 1991; Weishaar *et al.*, 1985). PDE type IV specifically hydrolyzes cAMP to 5'-AMP (Beavo *et al.*, 1994; Nicholson *et al.*, 1991). Meanwhile, this isozyme is insensitive to cGMP and calcium. There is a significant evidence that cAMP mediates both airway smooth muscle relaxation and down-regulation of immune cell and inflammatory cell activities (Kammer, 1988; Nicholson *et al.*, 1991; Torphy and Undem, 1991). PDE IV is a major cAMP-specific PDE isozyme present in the inflammatory cells, which are suggested to associate with asthma (Weishaar *et al.*, 1985; Beavo and Reifsnyder,, 1990). Hence, for the last decades, the discovery of phosphodiesterase (PDE) IV inhibitors for the treatment of asthma and inflammation has been the focus of a great deal of interest (Weishaar *et al.*, 1985; Beavo and Reifsnyder, 1990).

In recent years, selective and potent PDE IV inhibitors such as rolipram (Marivet *et al.*, 1989), RP-73401 (Ashton *et al.*, 1994), CDP-840 (Lynch *et al.*, 1997), denbufylline (Nicholson *et al.*, 1991), CP-80,633 (Cohan *et al.*, 1995) and SB 207,499 (Christensen *et al.*, 1998) have been available. However, many potent PDE IV inhibitors are suffering from unwanted side effects such as nausea and emesis (Schmiechen *et al.*, 1990; Barnette *et al.*, 1995). As a part of our ongoing effort to develop more potent and selective PDE IV inhibitors with reduced side effects, we have designed and synthesized new compounds **5**, **6** and examined SAR using their PDE IV inhibitory activities.



Rolipram



RP-73401

MATERIALS AND METHODS

All solvents were distilled prior to use. Unless noted, other commercially available reagents were used as received. Unless noted, reactions were carried out under an atmosphere of argon, and reaction temperatures were referred to the bath. Acid chloride **4** was prepared by the following procedures of RP-73401 (Ashton *et al.*, 1994). Yields of analogues **5**, **6** were not optimized.

Correspondence to: Chung K. Rhee, Department of Medicinal Chemistry, R&D Center, Cheil Jedang Corp., 522-1 Dokpyong-Ri, Majang-Myon, Ichon-Si, Kyonggi-Do, 467-810, Korea
E-mail: ckrhee@www.cheiljedang.com

General procedures for benzamide compounds 5

Acid chloride **4** (1.5 g, 5.9 mmol) was added to a solution of amide compound (4.2 mmol) in dry pyridine (20 mL) and the reaction mixture was heated at 60°C overnight. After cooling to room temperature, the reaction mixture was quenched with water (20 mL) and extracted with $CHCl_3$ (2×50 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was recrystallized from organic solvent to give **5** as a solid.

3-(3-Cyclopentyloxy-4-methoxyphenylcarboxamido)-2-thiophencarboxamide (5A)

The title compound (1.14 g, 75%) was synthesized with acid chloride **4** and 3-aminothiophen-2-carboxamide (597 mg). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 12.42 (1H, brs), 8.11 (1H, d, J=5.5 Hz), 7.84 (1H, d, J=5.5 Hz), 7.51 (1H, dd, J=8.5, 2 Hz), 7.45 (1H, d, J=2 Hz), 7.14 (1H, d, J=8.5 Hz), 4.99-4.83 (1H, m), 3.84 (3H, s), 2.13-1.64 (8H, m).

Methyl 3-(3-cyclopentyloxy-4-methoxyphenylcarboxamido)-2-thiophencarboxylate (5B)

The title compound (1.39 g, 88%) was synthesized with acid chloride **4** and methyl 3-amino-2-thiophencarboxylate (660 mg). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 11.24 (1H, br s), 8.33 (1H, d, J=5.5 Hz), 7.64 ((1H, d, J=2 Hz), 7.61 (1H, dd, J=8.5, 2 Hz), 7.57 (1H, d, J=5.5 Hz), 6.99 (1H, d, J=8.5 Hz), 5.11-4.90 (1H, m), 3.97 (3H, s), 3.96 (3H, s), 2.22-1.67 (8H, m).

4-(3-Cyclopentyloxy-4-methoxyphenylcarboxamido)-1-*N*-imidazole-5-carboxamide (5C)

The title compound (950 mg, 66%) was synthesized from acid chloride **4** and 4-amino-5-imidazole-carboxamide hydrochloride (683 mg). ^{1}H NMR (400 MHz, DMSO-d_6, δ) 10.96 (1H, brs), 7.52 (1H, d, J=8.5 Hz), 7.43 (1H, brs), 7.52-7.41 (1H, m), 7.14 (1H, d, J=8.5 Hz), 4.91-4.80 (1H, m), 3.88 (3H, s), 2.03-1.63 (8H, m).

3-(3-Cyclopentyloxy-4-methoxyphenylcarboxamido)-1*H*-4-pyrazolecarboxamide (5D)

The title compound (930 mg, 64%) was synthesized from acid chloride **4** and 3-aminopyrazole-4-carboxamide hemisulphate (736 mg). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 13.32 (1H, brs), 11.41 (1H, brs), 8.01 (1H, brs), 7.82 (1H, brs), 7.53 (1H, d, J=8.5 Hz), 7.41 (1H, brs), 7.15 (1H, d, J=8.5 Hz), 4.85-4.70 (1H, m), 3.80 (3H, s), 2.01-1.53 (8H, m).

5-(3-Cyclopentyloxy-4-methoxyphenylcarboxamido)-1*H*-1,2,3-triazole-4-carboxamide (5E)

The title compound (900 mg, 62%) was synthesized from acid chloride **4** and 4-amino-1,2,3-trizole-5-carboxamide (588 mg). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 10.87 (1H, brs), 8.10 (1H, brs), 7.64 (1H, brs), 7.50 (1H, dd, J=8.5, 2 Hz), 7.43 (1H, d, J=2 Hz), 7.10 (1H, d, J=8.5 Hz), 4.93-4.84 (1H, m), 3.82 (3H, s), 2.11-1.66 (8H, m).

5-(3-Cyclopentyloxy-4-methoxyphenylcarboxamido)-1-benzyl-1,2,3-triazole-4-carboxamide (5F)

The title compound (1.16 g, 62%) was synthesized from acid chloride **4** and 4-amino-1,2,3-trizole-5-carboxamide (974 mg). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 10.87 (1H, brs), 8.10 (1H, brs), 7.64 (1H, brs), 7.50 (1H, dd, J=8.5, 2 Hz), 7.43 (1H, d, J=2 Hz), 7.10 (1H, d, J=8.5 Hz), 4.93-4.84 (1H, m), 3.82 (3H, s), 2.11-1.66 (8H, m).

2-(3-Cyclopentyloxy-4-methoxyphenylcarboxamido) benzamide (5G)

The title compound (1.26 g, 85%) was synthesized from acid chloride **4** and anthranilamide (572 mg). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 12.16 (1H, brs), 8.81 (1H, d, J=7 Hz), 7.63 (4H, m), 7.12 (1H, dd, J=8.5, 2 Hz), 7.02 (1H, d, J=8.5 Hz), 4.88-4.77 (1H, m), 3.90 (3H, s), 2.01-1.62 (8H, m).

General procedure for cyclized compounds 6

A solution of Benzamide (2.5 mmol) in 1N-NaOH (25 mL) and EtOH (5 mL) was heated under reflux for 4 h. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure. The resulting residue was acidified with 1N-HCl, filtered, then washed with water to yield **6** as a white solid.

2-(3-Cyclopentyloxy-4-methoxyphenyl)-3,4-dihydrothieno[3,2-d]pyrimidin-4-one (6A)

The title compound (710 mg, 83%) was synthesized with **5A** (901 mg). ^{1}H NMR (400 MHz, DMSO-d_6, δ) 12.56 (1H, brs), 8.19 (1H, d, J=5 Hz), 7.81 (1H, dd, J=8.5,2 Hz), 7.75 (1H, d, J=2 Hz), 7.44 (1H, d, J=5 Hz), 7.10 (1H, d, J=8.5 Hz), 5.03-4.82 (1H, m), 3.83 (3H, s), 2.01-1.49 (8H, m).

2-(3-Cyclopentyloxy-4-methoxyphenyl)-6,7-dihydro-1*H*-6-purinone (6C)

The title compound (572 mg, 70%) was synthesized with **5C** (860 mg). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 12.60 (1H, brs), 8.61 (1H, s), 7.78 (1H, dd, J=8.5,2 Hz), 7.72 (1H, d, J=2 Hz), 7.11 (1H, d, J=8.5 Hz), 5.12-4.94 (1H, m), 3.84 (3H, s), 2.10-1.53 (8H, m).

5-(3-Cyclopentyloxy-4-methoxyphenyl)-6,7-dihydro-3*H*-[1,2,3]triazolo[5,4-d]pyridine-7-one (6E)

The title compound (556 mg, 68%) was synthesized

with **5E** (863 mg). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 10.38 (1H, brs), 7.40 (1H, dd, J=8.5 Hz), 7.15-7.05 (2H, m), 6.82 (1H, brs), 4.88-4.75 (11H, m), 3.79 (3H, s), 2.09-1.62 (8H, m).

2-(3-Cyclopentyloxy-4-methoxyphenyl)-3,4-dihydro-4-quinazoline (6G)

The title compound (673 mg, 80%) was synthesized with **5G** (886 mg). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 12.45 (1H, brs), 8.13 (1H, d, J=7 Hz), 8.03-7.85 (3H, m), 7.70 (1H, d, J=8.5 Hz), 5.00-4.92 (1H, m), 3.93-3.82 (1H, m), 2.16-1.58 (8H, m).

Molecular modeling

3D pharmacophore model was generated with RP-73401 series compounds (Ashton *et al.*, 1994) using Catalyst (version 3.0) from MSI. The conformers of each compound were generated using the poling algorithm within Catalyst, then analyzed to obtain 3D pharmacophore model. The best conformation of the synthesized compound, which was best fit to 3D pharmacophore model, was compared to the best conformation of RP-73401. It was carried out using Insight II (version 95) from MSI. The modeling studies have been performed using a Silicon Graphics Indigo operating under IRIX 5.3.

PDE IV enzyme purification

Tissue preparation: Male Sprague Dawley rat was sacrificed and the isolated liver was homogenized in 4x (v/w) ice-cold homogenization buffer (50 mM Tris-HCl pH 7.5, 0.25 M sucrose, 5 mM benzamidine, 0.2 mM PMSF, 20 μM leupeptin, 0.1 mM EGTA, 0.1 mM dithiothreitol) using Polytron. The homogenate was filtered through two layers of nylon mesh and centrifuged at 700 g for 10 min. The supernatant was spun at 165,000 g for 60 min and the supernatant was further fractionated for PDE activity using Mono-Q columns.

Mono-Q ion exchange chromatography: The final 165,000 g supernatant (10 mL) was loaded at 1 mL/min flow rate onto a Mono-Q column (5 mm$\times$50 mm) pre-equilibrated by sequential washing with 5 mL of Buffer A (50 mM Tris-HCl, pH 7.5), 10 mL of Buffer B (0.5 M NaCl), then 5 mL of Buffer A. PDE fractions were eluted at 1 mL/min flow rate using linear gradient salt (0~0.5 M NaCl) and the PDE activity of fractions were determined for pooling and saved in liquid nitrogen until use in PDE enzyme assay.

PDE IV enzyme assay

PDE IV enzyme assay was performed in 0.5 mL of final volume, with pre-assay additions made on ice. PDE IV substrate, [^{3}H]cAMP was prepared (ca. 28 Ci/mmol; ca. 100,000 cpm/100 μL) in freshly prepared assay buffer (40 mM Tris pH 8.0, 10 mM $MgCl_2$, 0.125 mg/mL BSA, 3.75 mM 2-mercaptoethanol) and 200 μL of the substrate was added to a reaction tube with 100 μL of testing compound and 100 μL of purified PDE IV enzyme and additional assay buffer to make 0.5 mL final volume. Reaction tubes were incubated at 30°C for 20 min, then the reaction was terminated by placing the tube in boiling water for 45~60 seconds before cooling on ice. The reaction was further treated with 100 μL (1 mg/mL) of snake venom (*Ophiophagus hannah*) from Sigma Chemical Co. at 30°C for 10 min, then reaction product, [^{3}H]adenosine was separated from unmetabolized substrates by eluting (0.1 N-NaOH) the sample in ion-exchange column chromatography with AG1-X2 resin (Bio-Rad). The radioactivity of the elute was measured using scintillation counting.

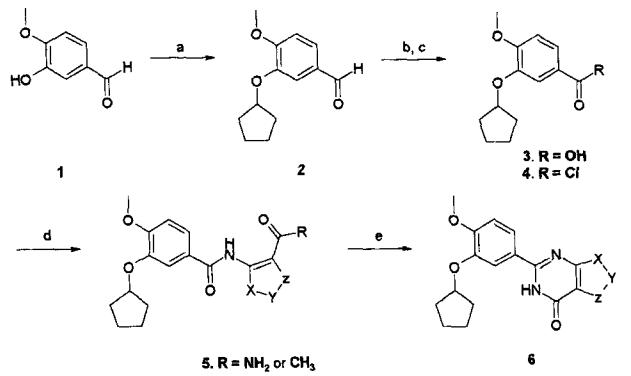
Trachea relaxation

Guinea pig trachea was isolated from non-sensitized animals, then suspended in 20 mL of Krebs-Henseleit solution in organ bath and muscle contraction was measured using isometric transducer (Grass FT03). The tissue was allowed to equilibrate by intermittent washing with fresh buffer for a few hours, then challenged with histamine (10^{-5} M) for contraction and the muscle relaxant effect of testing compounds was measured with the serial addition of increasing concentrations of the compounds. At the end of the experiment, isoproterenol (10^{-6} M) was added to elicit maximum relaxation. Results were calculated as a percentage of the maximum and determined EC_{50}.

RESULTS AND DISCUSSION

Rolipram and xanthine type compounds have been used as prototype compounds for the design of the target molecules. Rolipram has an electron rich and polarizable group, such as amide carbonyl group, and an adjacent aromatic group (Marivet *et al.*, 1989). The combination of rolipram and xanthine derivatives produced a new type of compound **6** in which these functional groups are contained.

As shown in Scheme **1**, the benzamide molecule **5** was prepared from isovanillin (**1**) by known procedures (Ashton *et al.*, 1994). Alkylation of isovanillin (**1**) and cyclopentyl bromide with K_2CO_3 and KI in DMF gave aldehyde **2** as a yellow oil in 81% yield. Oxidation of aldehyde **2** with $NaClO_2$ and H_2NSO_2H in AcOH proceeded smoothly to yield acid **3** as a white solid in 92% yield. Treatment of acid **3** with $SOCl_2$ in toluene afforded acid chloride **4** as a light yellow oil in 98% yield. Benzamide derivatives **5** were obtained by the reaction of acid chloride **4** with various amino



Scheme 1. Synthesis of benzamide derivatives. a) Bromocyclopentane, NaH, DMF, 60°C, (81%), b) $NaClO_2$, H_2NSO_3H, AcOH, (92%), c) $SOCl_2$, toluene, (98%), d) RNH_2, Pyridine, (60%~90%) and e) 1N NaOH, EtOH (60%~85%).

amide derivatives in 60~90% yields. These compound **5** have a similar structure to that of RP-73401. Amidoamide compound **5** was treated with NaOH/EtOH to give a cyclized analog **6** in 60~85% yields.

Our compounds were evaluated for PDE IV inhibitory activities against rat liver PDE IV and their structure-activity relationships were examined. The preliminary results of biological tests were summarized in Table I. Analog **5A** was identified to be the most active in inhibiting PDE IV among analogs **5** and **6** but less active than RP-73401. Trachea relaxation studies using isolated Guinea-pig trachea showed that analogs **5A** and **6C** were 50 times less active than RP-73401 but 15 times more active than theophylline. Analog **5A** was twice more potent than ester analog **5B** in inhibiting PDE IV. Analogs **5C-F** and **6** were observed to have not significant inhibitory activities. Analog **6C** showed IC_{50} values of 2.8 μM in PDE IV assay. In addition, synthesized analog **5G** was much less potent than RP-73401.

Previous studies with RP-73401 indicated that dichloro substituted pyridine moiety of RP-73401 might be crucial (Ashton *et al.*, 1994; Smellie *et al.*, 1995). It was well known that methoxy and cyclopentoxy moieties from benzene ring of PDE IV inhibitors such as rolipram might be important to enhance PDE IV inhibitory activity. 3D pharmacophore model also showed that the substituted dichloro moiety and the nitrogen atom in pyridine ring of RP-73401 were crucial to increase the potency (Fig. 1). Since methoxy and cyclopentoxy moieties from benzene ring were contained in our analogs, it might be necessary to examine the best fitting conformation of each compound to 3D pharmacophore model. Mimic of the nitrogen atom of pyridine ring in RP-73401 might be interesting as well. The nitrogen atom was supposed to act as hydrogen bonding acceptor. Analog **5A** showed a good overlay in overall 3D geometry with RP-73401 (Fig. 2). In addition, sulfur atom of **5A** was superimposed closely on nitrogen atom of pyridine ring in RP-73401. However, analog **5A** does not mimic

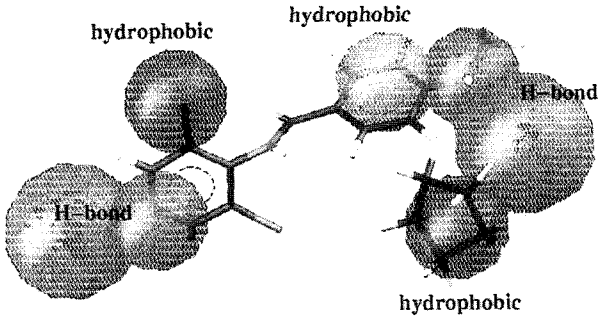


Fig. 1. Best conformation of RP-73401 fit to Catalyst generated 3D pharmacophore model. 3D pharmacophore model consists of three hydrophobic sites and two hydrogen bonding acceptor sites.

Table I. Biological activities of analogs **5** and **6**

Compound	R	X, Y, Z	PDE IV Inhibition (IC_{50}=μM)	Trachea Relaxation (EC_{50}=μM)
5A	NH_2	X=CH, Y=CH, Z=S	0.8±0.1	3.07±0.43
5B	CH_3	X=CH, Y=CH, Z=S	1.5±0.2	n.d.
5C	NH_2	X=NH, Y=CH, Z=N	>10	n.d.
5D	NH_2	X=NH, Y=N, Z=CH	6.0±0.8	n.d.
5E	NH_2	X=NH, Y=N, Z=N	>10	n.d.
5F	NH_2	X=NBn, Y=N, Z=N	>10	n.d.
5G	NH_2	X=CHCH, Y=CH, Z=CH	3.1±0.5	5.44±0.74
6A		X=CH, Y=CH, Z=S	>10	n.d.
6C		X=NH, Y=CH, Z=N	2.8±0.4	3.12±0.50
6E		X=NH, Y=N, Z=N	>10	n.d.
6G		X=CHCH, Y=CH, Z=CH	>10	n.d.
	Rolipram		0.3±0.04	n.d.
	RP-73401		0.001±0.000	0.062±0.013
	Theophylline		660±76	44.1±7.3

n.d.: not determined.

Fig. 2. The overlay of RP-73401 and analog 5A

Fig. 3. The overlay of RP-73401 and analog 6C

substituted dichloro moieties of pyridine ring in RP-73401 and, therefore, the activity of analog **5A** might not be improved to the level of RP-73401. Meanwhile, since the carbonyl oxygen of guanine ring in analog **6C** was not superimposed on the nitrogen in RP-73401 and overall overlay of analog **6C** with RP-73401 was not good, therefore, **6C** might be less active than the reference compound (Fig. 3).

In conclusion, the preliminary biological results showed that most analogs have much weaker PDE inhibitory activities compared with RP-73401, some of analogs such as **5A** and **6C** were observed to have promising PDE IV inhibitory activities. Smooth muscle relaxation studies using isolated Guinea pig trachea indicated that analogs **5A** and **6C** were 50 times less active than RP-73401 but 15 times more active than theophylline. Meanwhile, bioavailability of analog **5A** was 27%, while rolipram was 35% and RP-73401 was observed to be very low. Employment of these novel pyrimidine system for further potentially bioactive compounds is envisaged and will be reported in near future.

REFERENCES CITED

Ashton, M. J., Cook, D. C., Fenton, G., Karlsson, J. A., Palfreyman, M. N., Raeburn, D., Ratcliffe, A. J., Souness, J. E., Thurairatnam, S. and Vicker, N., Selective type IV phosphodiesterase inhibitors as antiasthmatic agents. The syntheses and biological activities of 3-(cyclopentyloxy)-4-methoxybenzamides and analogues. *J. Med. Chem.*, 37, 1696-1703 (1994).

Barnette, M. S., Grous, M., Cieslinski, L. B., Burman, M., Christensen, S. B. and Torphy, T. J., Inhibitors of phosphodiesterase IV (PDE IV) increase acid secretion in rabbit isolated gastric glands: correlation between function and interaction with a high-affinity rolipram binding site. *J. Pharmacol. Exp. Ther.*, 273, 1396-1402 (1995).

Beavo, J. A., Conti, M. and Heaslip, R. J., Multiple cyclic nucleotide phosphodiesterases. *Mol. Pharmacol.*, 46, 399-405 (1994).

Beavo, J. A. and Reifsnyder, D. H., Primary sequence of cyclic nucleotide phosphodiesterase isozymes and the design of selective inhibitors. *Trends Pharmacol. Sci.*, 11, 150-155 (1990).

Christensen, S. B., Guider, A., Forster, C. J., Gleason, J. G., Bender, P. E., Karpinski, J. M., DeWolf, W. E. Jr., 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., Ryan, M. D., Eggleston, D. S., Haltiwanger, R. C. and Torphy, T. J., 1,4-Cyclohexanecarboxylates: potent and selective inhibitors of phosphodiesterase 4 for the treatment of asthma. *J. Med. Chem.*, 41, 821-835 (1998).

Cohan, V. L., Showell, H. J., Pettipher, E. R., Fisher, D. A., Pazzoles, C. J., Watson, J. W., Turner, C. R. and Cheng, J. B., *In vitro* pharmacology of the novel type IV phosphodiesterase (PDE_{IV}) inhibitor, CP-80,633. *J. Allergy Clin. Immunol.*, 95, 350 (1995) [Abstract].

Cortijo, J., Bou, J., Beleta, J., cardelus, I., Llenas, J., Motciool, E. and Gristwood, R. W., Investigation into the role of phosphodiesterase IV in bronchorelaxation, including studies with human bronchus. *Br. J. Pharmacol.*, 108, 562-56 (1993).

Erneux, C., Van Sande, J., Milot, F., Cochaux, P., Decosster, C. and Dumont, J. E., A mechanism in the control of intracellular cAMP level: the activation of a calmodulin-sensitive phosphodiesterase by a rise of intracellular free calcium. *Mol. Cell. Endocrinol.*, 43, 123-134 (1985).

Holbrook, M., Gozzard, N., James, T., Higgs, G. and Hughes, B., Inhibition of bronchospasm and ozone-induced airway hyperresponsiveness in the guinea-pig by CDP840, a novel phosphodiesterase type 4 inhibitor. *Br. J. Pharmacol.*, 118, 1192-1200 (1996).

Kammer, G. M. The adenylate cyclase-cAMP-protein kinase pathway and regulation of immune response. *Immunol. Today* 9, 222-229 (1988).

Lynch, J. E., Choi, W. -B., Churchill, H. R. O., Volante, R. P., Reamer, R. A. and Ball, R. G., Asymmetric synthesis of CD840 by Jacobsen Epoxidation. An usual Syn selective reduction of an

epoxide. *J. Org. Chem.*, 62, 9223-9228 (1997).

Marivet, M. C., Bourguignon, J. -J., Lugnier, C., Mann, A., Stoclet, J. -C. and Wermuth, C., Inhibition of cyclic adenosine-3',5'-monophosphate phosphodiesterase from vascular smooth muscle by rolipram analogues. *J. Med. Chem.*, 32, 1450-1457 (1989).

Nicholson, C., Challis, R. and Shakio, M., Differential modulation of tissue function and therapeutic potential of selective inhibitors of cyclic nucleotide phosphodiesterase isoenzymes. *Trends Pharmacol. Sci.*, 12, 19-27 (1991).

Schmiechen, R., Schneider, H. H. and Watchtel, H., Close correlation between behavioral response and binding *in vivo* for inhibitors of the rolipram-sensitive phosphodiesterase. *Psychopharmacology*, 102, 17-20 (1990).

Torphy, T. J. and Undem, B. J., Phosphodiesterase inhibitors: New opportunities for the treatment of asthma. *Thorax* 46, 512-523 (1991).

Smellie, A., Teig, S. L. and Towbin, P., "Poling: Promoting Conformational Coverage". *J. Comp. Chem.*, 16, 171-187 (1995).

Weishaar, R. E., Cain, M. H. and Bristol, J. A., A new generation of phosphodiesterase inhibitors: multiple molecular forms of phosphodiesterase and the potential for drug selectivity. *J. Med. Chem.*, 28, 537-545 (1985).