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Design, synthesis, and evaluation of 2-aryl-7-(3′,4′-dialkoxyphenyl)-pyrazolo [1,5-*a*]pyrimidines as novel PDE-4 inhibitors

Ikyon Kim [a], Jong Hwan Song [a], Chang Min Park [a], Joon Won Jeong [a], Hyung Rae Kim [a], Jin Ryul Ha [a], Zaesung No [b], Young-Lan Hyun [c], Young Sik Cho [d], Nam Sook Kang [e,*], Dong Ju Jeon [a,*]

[a] *Medicinal Chemistry Research Center, Korea Research Institute of Chemical Technology, Daejeon 305-600, Republic of Korea*
[b] *Institut Pasteur Korea, Sampyeong-dong 696, Bundang-gu, Seongnam-si, Gyeonggi-do, Republic of Korea*
[c] *Crystalgenomics, Inc. Seoul 138-736, Republic of Korea*
[d] *Pharmacology Research Center, Korea Research Institute of Chemical Technology, Daejeon 305-600, Republic of Korea*
[e] *Drug Discovery Platform Technology Team, Korea Research Institute of Chemical Technology, Daejeon 305-600, Republic of Korea*

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ABSTRACT

Described herein is design, synthesis, and biological evaluation of novel series of 2-aryl-7-(3′,4′-dialkoxyphenyl)-pyrazolo[1,5-*a*]pyrimidines acting as inhibitors of type 4 phosphodiesterase (PDE4) which is known as a good target for the treatment of asthma and COPD. For this purpose, structure optimization was conducted with the aid of structure-based drug design using the known X-ray crystallography. Also, biological effects of these compounds on the target enzyme were evaluated by using in vitro assays, leading to the potent and selective PDE-4 inhibitor (IC_{50} < 10 nM).

Phosphodiesterases (PDEs), a super family of 11 isozymes, are responsible for the hydrolysis of cAMP and c-GMP.[1] Cyclic nucleotides are important intracellular secondary messengers in cell function, relaying the signals from hormones at specific cell-surface receptors. An increase of cAMP due to the stimulation of adenylyl cyclase or the inhibition of PDEs affects the activity of immune system and inflammatory cells.[2] Thus, PDE4, a cAMP specific PDE, received much attention as a target for the treatment of the diseases such as asthma and Chronic Obstructive Pulmonary Disease (COPD).[3]

Since the first report of rolipram as a selective inhibitor of PDE4,[4] a number of compounds have been studied to increase the activity and to reduce the side effects such as nausea, vomiting, psychotropic activity, and increased gastric secretion (Fig. 1).[5]

In this communication, we wish to report the potent and selective PDE4D inhibitors. First of all, using the crystal structures of the ligand-bound PDE4D (PDB entry; 1XOQ),[6,7] we hoped to design molecules to bind the S1 and S3 sites while not to directly bind the metal binding S2 site as shown in Figure 2. Initially, while con-

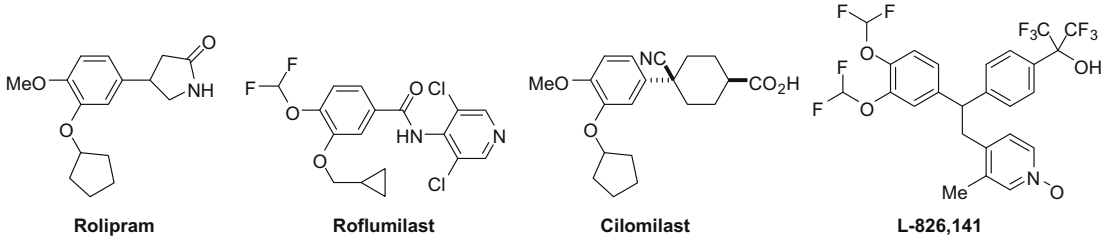

Figure 1. Structures of catechol-type PDE4 inhibitors.

* Corresponding authors. Tel.: +82 42 860 7452; fax: +82 42 860 7456 (N.S.K).
E-mail address: nskang@krict.re.kr (N. Sook Kang).

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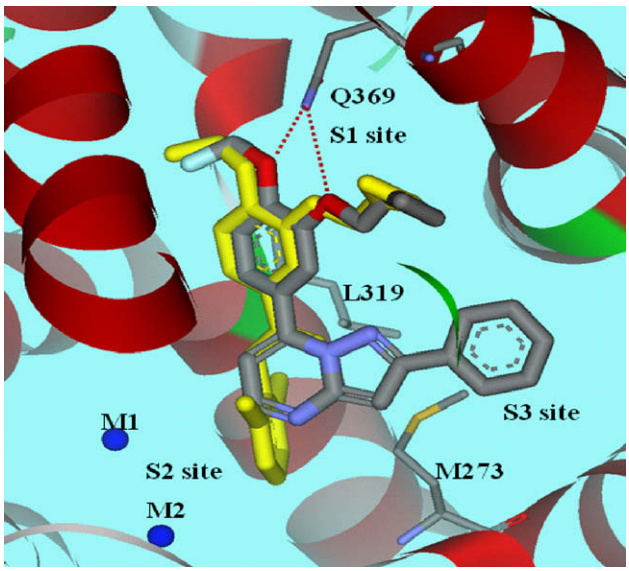


Figure 2. The crystallographic structure of roflumilast (PDB entry; 1XOQ, yellow) and docking structure of our compound (gray).

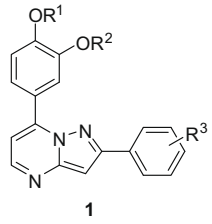


Figure 3. New scaffold proposed by SBDD.

serving the S1 site of roflumilast, we tried to introduce new linker fragment positioning at side chains of L319 and M273 to bridge S1 and S3 sites from our in-house fragment library, consisting of hetero-cyclic compounds. Through the virtual fragment exploration using docking method for potent PDE4 inhibitors, 2,7-diphenylpyrazolo[1,5-*a*]pyrimidine scaffold **1** as the bridging moiety between S1 and S3 sites was selected (Fig. 3). For docking by LigandFit[8] interfaced with Accelrys Discovery Studio2.0, NH_2 group of side chain in Q369 residue was defined as the hydrogen donor interaction site and the default parameters used. The predicted docking mode for roflumilast was well generated showing RMSD value, 0.33 Å, compared the crystallographic structure. Through the docking study for roflumilast and scaffold **1** ($R^1 = CHF_2$, R^2 = cyclopropylmethyl), Dock Score were 63.648 and 64.845 kcal/mol, respectively.

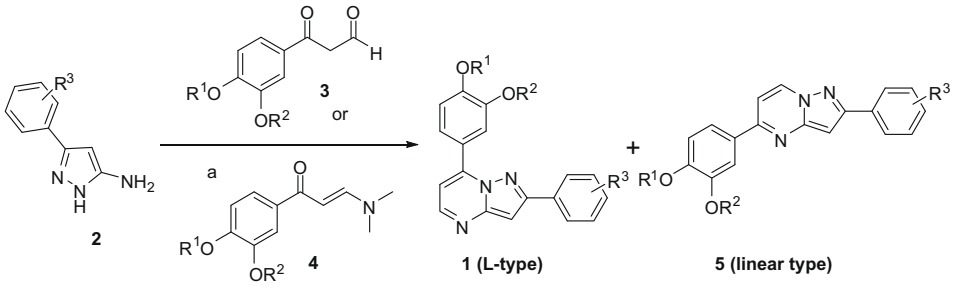
As shown in Scheme 1, these pyrazolo[1,5-*a*]pyrimidines[8] were envisaged to derive from condensation of β-ketoaldehydes **3** or β-enaminoketones **4** with aminopyrazoles **2**. Two types of products (L-type and linear type) were anticipated depending on the mode of cyclization.

The key intermediate, aminopyrazoles **2** were prepared in two steps (Scheme 2). Thus, reaction of ethyl benzoate with the anion of acetonitrile gave cyanoacetophenones **6**, which were converted to aminopyrazoles **2** upon exposure to hydrazine hydrate in 60–80% yields.[9] The requisite arylacetoaldehydes were synthesized from the reaction of enolates derived from acetophenones with ethyl formate in moderate yields,[10] whereas β-enaminoketones **4** were easily prepared by treatment of acetophenones with DMF-DMA (Scheme 2).[11]

Indeed, 5-aminopyrazoles **2** were coupled with arylacetoaldehydes **3** in acetic acid at room temperature to give pyrazolopyrimidines, **1** and **5**. Alternatively, **1** and **5** were obtained by condensation of **2** with **4**. Interestingly, whereas dehydrative cyclization of aminopyrazoles **2** with arylacetoaldehydes **3** gave a regioisomeric mixture of pyrazolo[1,5-*a*]pyrimidines, **1** and **5** in an approximately 1:1 ratio, **1**[12] was obtained as a major isomer by employing β-enaminoketone **4** as a coupling partner.[13] Regioisomer **5** was observed in a small portion in the latter case, and could be separated by silica gel column chromatography. As shown in Figure 4, structure assignment of **1n** was unambiguously confirmed by X-ray crystallographic analysis.[14] This structure takes an L-shape and three aromatic moieties are nearly on the same plane.

These derivatives were assayed against purified human PDE4D and their inhibitory effects are shown in Table 1. For **1a–1j** where R^1 is methyl and R^2 is cyclopentyl, it was found that the compounds bearing *meta*-substituted phenyl group exhibited more potent inhibitory activity than the compounds having *ortho*- or *para*-substituted phenyl group. To validate their activity, the level of cAMP was measured using U937 cell at 20 μM. Disappointingly, however, the levels of cAMP were lower compared with their PDE4D inhibition activity.

We further evaluated PDE-4 enzyme inhibition of a series of 2-phenyl-7-(3′-cyclopropylmethoxy-4′-difluoromethoxyphenyl)pyrazolo[1,5-*a*]pyrimidines carrying *meta*-substituted phenyl groups (compounds **1k–1p** in Table 1).[15] At first, we checked the level of cAMP using U937 cell at 10 μM for compounds **1k** and **1m**, which indicated that the derivatives of 2-phenyl-7-(3′-cyclopropylmethoxy-4′-difluoromethoxyphenyl)pyrazolo[1,5-]pyrimidines increase the level of cAMP. With these results in hand, a wide variety of derivatives were synthesized and tested for their in vitro activities.[16] Although relatively bulky groups containing polar moiety are required for higher activity, introduction of diverse functional groups for R has kept the inhibitory activity against PDE4D. These results can be rationalized by our hypothesis that R group occupying S3 binding site is exposed to outward of PDE4D binding pocket



Scheme 1. Reagents and conditions: (a) AcOH, rt, 12 h, 60–90%.