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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

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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-

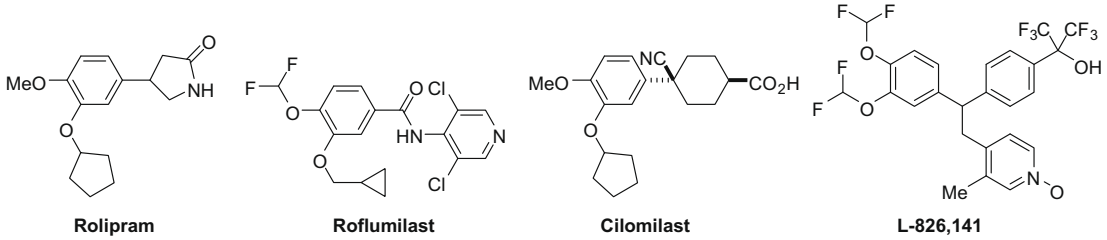

Figure 1. Structures of catechol-type PDE4 inhibitors.

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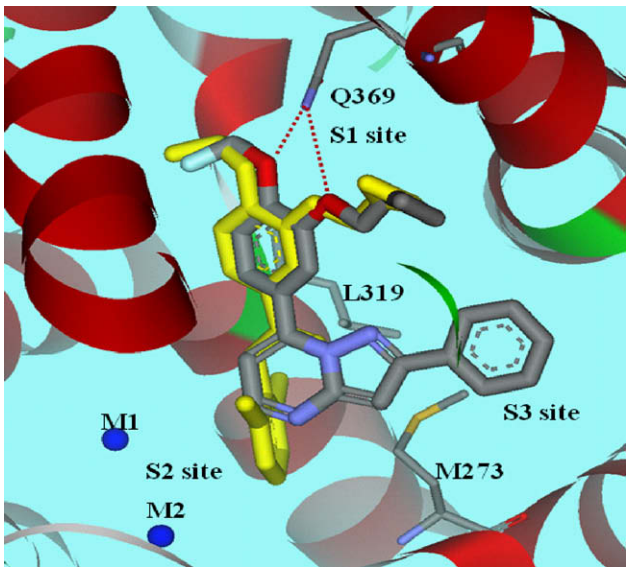


Figure 2. The crystallographic structure of roflumilast (PDB entry; 1XOQ, yellow) and docking structure of our compound (gray).

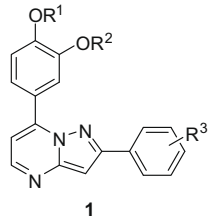


Figure 3. New scaffold proposed by SBDD.

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.

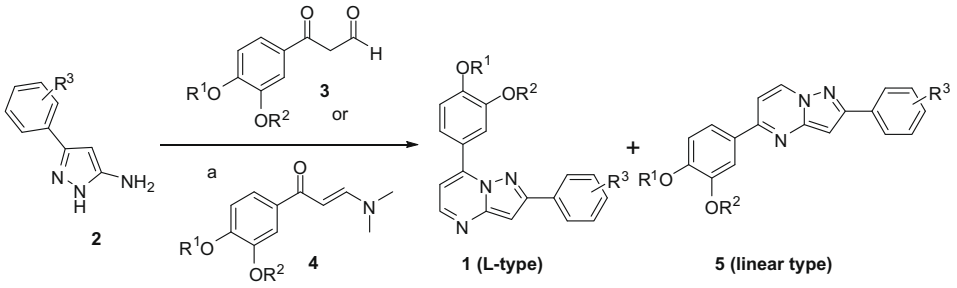
As shown in Scheme 1, these pyrazolo[1,5-*a*]pyrimidines[8] were envisaged to derive from condensation of β-ketoaldehydes **3** or β-enaminoketones **4** with aminopyrazoles **2**. Two types of products (L-type and linear type) were anticipated depending on the mode of cyclization.

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]

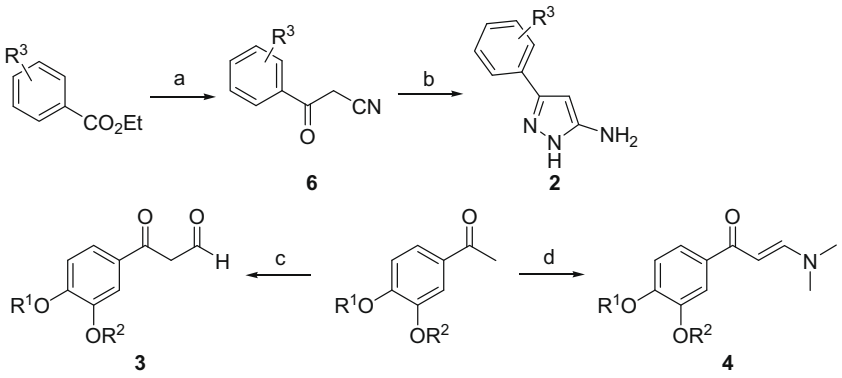
Indeed, 5-aminopyrazoles **2** were coupled with arylacetoaldehydes **3** in acetic acid at room temperature to give pyrazolopyrimidines, **1** and **5**. Alternatively, **1** and **5** were obtained by condensation of **2** with **4**. Interestingly, whereas dehydrative cyclization of aminopyrazoles **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%.



Scheme 2. Reagents and conditions: (a) CH_3CN, NaH, THF, rt, 40–80%; (b) NH_2NH_2, EtOH, reflux, 60–80%; (c) ethyl formate, NaH, THF, rt, 12 h, 60–90%; (d) DMF-dimethylacetal, toluene, reflux, 60–90%.

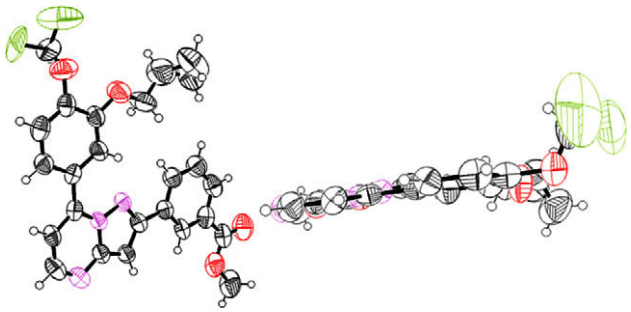


Figure 4. Crystal structure of **1n.**

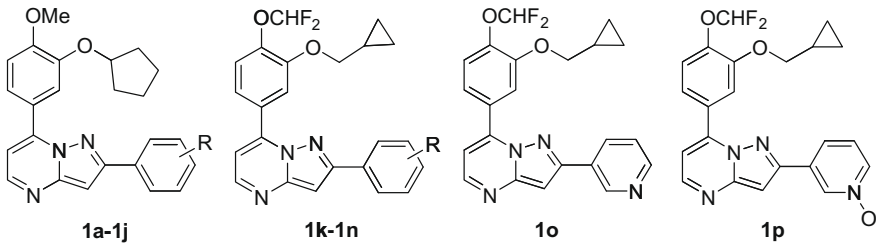
which directly contacts with solvent molecules as anticipated through modeling study.

Finally, we introduced pyridine moiety instead of benzene ring (compounds **1o** and **1p** in Table 1), which resulted in maintaining

Table 2
PDE isozyme selectivity profile of **1l**, **1o**, and **1p**

Compd	IC_{50}(μM)			
	PDE-4	PDE-3	PDE-5	PDE-7
1l	0.006	2.0	>5	>5
1o	0.009	>5	3.5	3.0
1p	0.026	>5	>5	>5

Table 1



Compd	R	IC_{50} (nM)	cAMP
1a	H	70	NA[a]
1b	2-Br	140	NA
1c	2-OMe	70	NA
1d	4-Cl	90	NA
1e	4-Br	130	NA
1f	4-OMe	60	NA
1g	3-Cl	20	19.7[b]
1h	3-Br	30	11.4[b]
1i	3-OMe	40	20.1[b]
1j	2,5-Cl_2	30	10.4[b]
1k	3-Br	13	57.1[c]
1l	3-I	6	19.8[c]
1m	3-OMe	30	44.4[c]
1n	3-COOMe	37	NA
1o	Pyridine	27	66.9
1p	N-oxide	42	1.61 μM[d]

[a] NA: not available.
[b] % cAMP level relative to control at 20 μM in U937 cells.
[c] % cAMP level relative to control at 10 μM in U937 cells.
[d] EC_{50} of **1p** for cAMP accumulation in U937 cells.

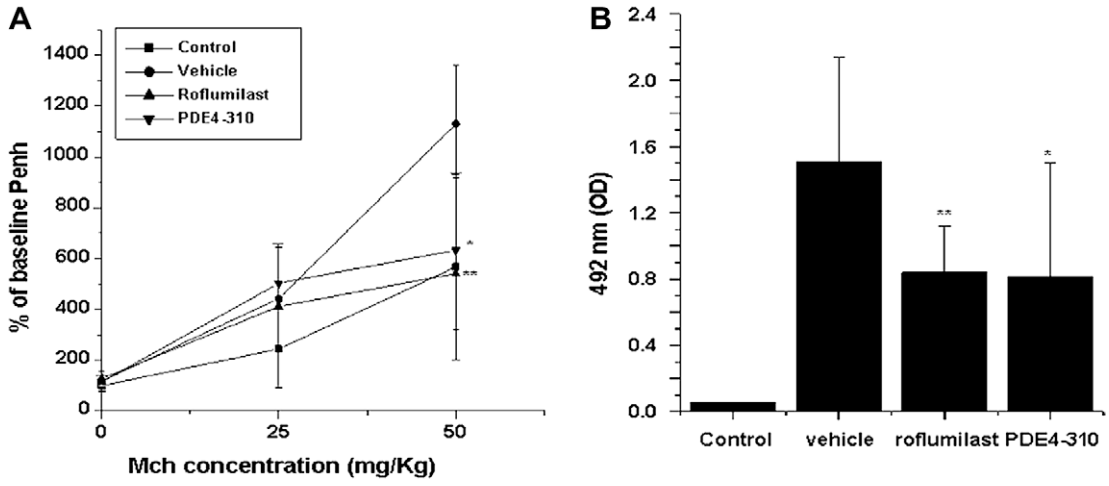


Figure 5. The Inhibitory effects of compound **1p** on OVA-induced airway hyperresponsiveness and eosinophil peroxidase activity. AHR was measured in mice 48 h after OVA inhalation (A). Results are mean ± SD, $n = 7–10$ per group. $^{*}p < 0.05$ and $^{**}p < 0.01$ versus. vehicle control (0.5% CMC). Also, EPO activity was measured in BALF 62 h after OVA inhalation. Results are mean ±SD, $n = 9$ to 10 per group. $^{*}p < 0.05$ and $^{**}p < 0.001$ versus. vehicle control.

the in vitro activity for PDE4D. The treatment of cells with compound **1p** led to the considerable accumulation of cAMP with EC_{50} of 1.61 μM. Also, our scaffold exhibited isozyme selectivity as shown in Table 2.

From in vitro profiles of compound **1p**, its in vivo efficacy was further evaluated in ovalbumin-induced asthma animal model. Penh values to 25 and 50 mg/mg in vehicle group were about two fold higher than those in controls, but as a reference, administration of 30 mg/kg (mpk) roflumilast suppressed completely the responsiveness to Mch. In mice treated with compound **1p** (100 mpk), a significant decrease in airway responsiveness to 50 mg/ml Mch was observed (Fig. 5A). Moreover, to examine the preventive effect of **1p** on pulmonary inflammation, eosinophil peroxidase (EPO) activity in bronchoalveolar lavage fluid (BALF) was evaluated as an indicator of infiltrated eosinophils. As shown in Figure 5B, EPO activity was highly elevated in vehicle group compared with control while either **1p** or roflumilast treatment diminished the EPO activity as low as about 50% of vehicle group. Based on these results, it is proposed that compound **1p** has a protective effect on OVA-induced AHR and its beneficial effect results partly from the suppression of eosinophil infiltration.

In conclusion, we have identified a novel series of 2-aryl-7-(3′,4′-dialkoxyphenyl)-pyrazolo[1,5-*a*]pyrimidines which show good PDE4 inhibitory activities. Further evaluation of selectivity to other PDEs and anti-inflammatory activity in the animal model are now underway and will be reported in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.12.070.

References and notes

1. Beavo, J. A.; Reifsnyder, D. H. *Trends Pharmacol. Sci.* **1990**, *11*, 150.
2. Verghese, M. W.; Snyderman, R. In *Human Monocytes*; Zembala, M., Asherson, G. L., Eds.; Academic: London, 1989; p 101.
3. (a) Giembycz, M. A.; Dent, G. *Clin. Exp. Allergy* **1992**, *22*, 337; (b) Torphy, T. J.; Livi, G. P.; Christensen, S. B. *Drug News Perspect.* **1993**, *6*, 203; (c) Christensen, S. B.; Torphy, T. J. *Annu. Rep. Med. Chem* **1994**, *29*, 185.
4. Schwabe, U.; Miyake, M.; Ohga, Y.; Daly, J. *Mol. Pharmacol.* **1976**, *12*, 900.
5. (a) Zeller, E.; Stief, H. J.; Pflug, B.; Sastre, Y.; Hernandez, M. *Pharmacopsychiatry* **1988**, *59*, 358; (b) Masamune, H.; Cheng, J. B.; Cooper, K.; Eggler, J. F.; Marfat, A.; Marshall, S. C.; Shirley, J. T.; Tickner, J. E.; Umland, J. P.; Vazquez, E. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1965; (c) Cheng, J. B.; Cooper, K.; Duplantier, A. J.; Eggler, J. F.; Kraus, K. G.; Marshall, S. C.; Marfat, A.; Masamune, H.; Shirley, J. T.; Tickner, J. E.; Umland, J. P. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1969; (d) 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; (e) Chambers, R. J.; Marfat, A.; Cheng, J. B.; Cohan, V. L.; Damon, D. B.; Duplantier, A. J.; Hibbs, T. A.; Jenkinson, T. H.; Johnson, K. L.; Kraus, K. G.; Pettipher, E. R.; Salter, E. D.; Shirley, J. T.; Umland, J. P. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 739; (f) Piaz, V. D.; Giovannoni, M. P.; Castellana, C.; Palacios, J. M.; Beleta, J.; Doménech, T.; Segarra, V. *J. Med. Chem.* **1997**, *40*, 1417; (g) Hulme, C.; Poli, G. B.; Huang, F.-C.; Souness, J. E.; Djuric, S. W. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 175; (h) Friesen, R. W.; Ducharme, Y.; Ball, R. G.; Blouin, M.; Boulet, L.; Côté, B.; Frenette, R.; Girard, M.; Guay, D.; Huang, Z.; Jones, T. R.; Laliberté, F.; Lynch, J. J.; Mancini, J.; Martins, E.; Masson, P.; Muise, E.; Pon, D. J.; Siegel, P. K. S.; Styhler, A.; Tsou, N. N.; Turner, M. J.; Young, R. N.; Girard, Y. *J. Med. Chem.* **2003**, *46*, 2413.
6. Huai, Q.; Wang, H.; Sun, Y.; Kim, H. Y.; Liu, Y.; Ke, H. *Structure* **2003**, *11*, 865.
7. Card, G. L.; England, B. P.; Suzuki, Y.; Fong, D.; Powell, B.; Lee, B.; Luu, C.; Tabrizizad, M.; Gillette, S.; Ibrahim, P. N.; Artis, D. R.; Bollag, G.; Milburn, M. V.; Kim, S. H.; Schlessinger, J.; Zhang, K. Y. *Structure* **2004**, *12*, 2233.
8. For selected synthesis and biological evaluation of pyrazolopyrimidines, see: (a) Hammouda, H. A.; El-Barbary, A. A.; Sharaf, M. A. F. *J. Heterocycl. Chem.* **1984**, *21*, 945; (b) Bruni, F.; Selleri, S.; Costanzo, A.; Guerrini, G.; Casilli, M. L.; Giusti, L. *J. Heterocycl. Chem.* **1995**, *32*, 291; (c) Almansa, C.; de Arriba, A. F.; Cavalcanti, F. L.; Gomez, L. A.; Miralles, A.; Merlos, M.; Garcia-Rafanell, J.; Forn, J. *J. Med. Chem.* **2001**, *44*, 350; (d) Elmaati, T. M. A.; Said, S. B.; Elenein, N. S. A.; Khodeir, N. M.; Sofan, M. M. *J. Heterocycl. Chem.* **2003**, *40*, 481; (e) Danagulyan, G. G.; Mkrtchyan, A. D.; Panosyan, G. A. *Chem. Heterocycl. Compd.* **2005**, *41*, 485; (f) Selleri, S.; Gratteri, P.; Costagli, C.; Bonaccini, C.; Costanzo, A.; Melani, F.; Guerrini, G.; Ciciani, G.; Costa, B.; Spinetti, F.; Martini, C.; Bruni, F. *Bioorg. Med. Chem.* **2005**, *13*, 4821; (g) Zhou, H.-B.; Sheng, S.; Compton, D. R.; Kim, Y.; Joachimiak, A.; Sharma, S.; Carlson, K. E.; Katzenellenbogen, B. S.; Nettles, K. W.; Greene, G. L.; Katzenellenbogen, J. A. *J. Med. Chem.* **2007**, *50*, 399; (h) Powell, D.; Gopalsamy, A.; Wang, Y. D.; Zhang, N.; Miranda, M.; McGinnis, J. P.; Rabindran, S. K. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1641; (i) Shaaban, M. R.; Saleh, T. S.; Farag, A. M. *Heterocycles* **2007**, *71*, 1765; (j) Fookes, C. J. R.; Pham, T. Q.; Mattner, F.; Greguric, I.; Loc'h, C.; Liu, X.; Berghofer, P.; Shepherd, R.; Gregoire, M.-C.; Katsifis, A. *J. Med. Chem.* **2008**, *51*, 3700; (k) Farag, A. M.; Mayhoub, A. S.; Barakat, S. E.; Bayomi, A. H. *Bioorg. Med. Chem.* **2008**, *16*, 4569.
9. (a) El Agamey, A. G. A.; El-Sakka, I.; El-Shahat, Z.; Elnagdi, M. H. *Arch. Pharm.* **1984**, *317*, 289; (b) Ivanov, C. *Chem. Ber.* **1954**, *87*, 1600.
10. (a) Sezer, O.; Anac, O. *Helv. Chim. Acta* **1994**, *77*, 2323; (b) Wright, S. W.; Harris, R. R.; Kerr, J. S.; Green, A. M.; Pinto, D. J.; Bruin, E. M.; Collins, R. J.; Dorow, R. L.; Mantegna, L. R.; Sherk, S. R.; Covington, M. B.; Nurnberg, S. A.; Welch, P. K.; Nelson, M. J.; Magolda, R. L. *J. Med. Chem.* **1992**, *35*, 4061.
11. (a) Mustazza, C.; Maria, R.; Del, G.; Borioni, A.; Gatta, F. *J. Heterocycl. Chem.* **2001**, *38*, 1119; (b) Westaway, S. M.; Thompson, M.; Rami, H. K.; Stemp, G.; Trouw, L. S.; Mitchell, D. J.; Seal, J. T.; Medhurst, S. J.; Lappin, S. C.; Biggs, J.; Wright, J.; Arpino, S.; Jerman, J. C.; Cryan, J. E.; Holland, V.; Winborn, K. Y.;

Coleman, T.; Stevens, A. J.; Davis, J. B.; Gunthorpe, M. J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5609.
12. For NMR data of **1**, see the Supplementary data.
13. In addition, these β-ketoaldehydes are usually difficult to handle compared with β-enaminoketones.
14. CCDC 741294 contains the supplementary crystallographic data for compound **1n**. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via ww.ccdc.cam.ac.uk/data_request/cif.
15. Compound **1p** was prepared by *m*CPBA oxidation of compound **1o**.
16. For the full in vitro data, see the Supplementary data.