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Dual M_3 antagonists-PDE4 inhibitors. Part 2: Synthesis and SAR of 3-substituted azetidinyl derivatives

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Abstract—Introduction of 3-substituted azetidinyl substituents onto the 4,6-diaminopyrimidine scaffold allowed the improvement of PDE4 inhibiting activities. Preliminary in vivo activity in pulmonary inflammation models is reported.

In a previous paper,[1] we have reported our work towards the discovery of potent dual M_3 antagonists and PDE4 inhibitors as a new class of drugs combining both bronchodilating and anti-inflammatory properties as potential treatment of COPD (Chronic Obstructive Pulmonary Disease).

SAR around the 4,6-diaminopyrimidine scaffold led us to the identification of ucb-101333-3, characterized by a very interesting profile on both targets and selected for further extensive evaluation (Fig. 1).

Figure 1. 4,6-Diaminopyrimidines as dual M_3 antagonists/PDE4 inhibitors.

These compounds were nevertheless characterized by still moderate PDE4 inhibiting activities, mostly in the micromolar range. In order to improve them, we have decided to pursue the lead optimization process focusing on the modulation of the substituent in 4-position.

As reported before,[1] PDE4 inhibiting activity proved to be extremely sensitive towards the nature of this substituent since the removal, the substitution or the replacement of the cyclopropane by larger rings led to an important loss of activity, therefore highlighting the importance of steric factors in that area.

In order to roughly evaluate the maximal tolerated size, we have studied a series of pyrimidines bearing cyclic amines of growing size (Fig. 2), other substituents being kept constant to allow comparisons with ucb-101333-3.

As expected, it appears that compounds **3** and **4** bearing a 6- or a 7-membered ring were characterized by a very low PDE4 inhibiting activity. A pyrrolidine moiety is nevertheless tolerated in that position but with an important loss of potency in comparison to the cyclopropylamine. This compound (**2**) is however characterized by the best M_3 affinity observed in the series.

Surprisingly, the introduction of an azetidine substituent resulted in slightly better PDE4 inhibiting activity than

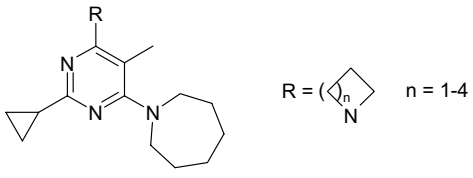

Figure 2. Modulation of 4-position by various cyclic amines.

Keywords: Muscarinic receptor(s); Phosphodiesterase; Pyrimidine; Azetidine; COPD.
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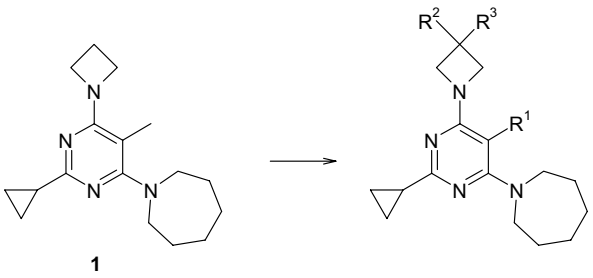


Figure 3.

ucb-101333-3, whereas M_3 affinity was slightly decreased but still acceptable. This important result led us to start an in-depth exploration of the azetidine moiety and, in particular, of the substitution in 3-position (Fig. 3).

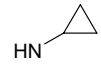
The general method of synthesis of compounds listed in Tables 1 and 2 is outlined in Scheme 1. Briefly, 4,6-dichloropyrimidine derivatives **I**[1] are first substituted with various cyclic amines. The reaction is performed in the presence of potassium carbonate in the only case of azetidine derivatives, used as hydrochloride salts. The resulting 6-chloropyrimidines **II** or **IV** are then substituted by azepane to afford the desired compounds **III** or **V**.

Compound **III-e** bearing an azetidin-3-one moiety has been prepared by oxidation of the corresponding 3-azetidinol **III-a** by the pyridine–sulfur trioxide complex.

Substitution of the mesylate **III-b** with sodium cyanide or sodium bromide afforded **III-c** and **III-d**, respectively.

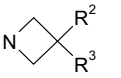
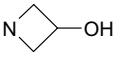
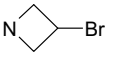
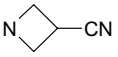
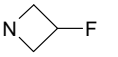
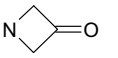
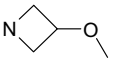
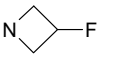
It has to be noticed that the compounds bearing a methyl group in 5-position are characterized by some instability in highly acidic media (pH 1) giving rise to the formation of 6-azepan-1-yl-2-cyclopropyl-5-methyl-pyrimidin-4-ol. Unsubstituted analogues proved to be stable in the same conditions.

Table 1. Modulations of R

Compound	R	PDE4 IC_{50} (μM)	M_3 K_i (nM)
ucb-101333-3		0.63	3.2
1		0.32	16
2		3.2	1.3
3		#	79
4		#	40

Results are expressed as IC_{50} (μM), K_i (nM) or # (when <50% inhibition of radioligand specific binding by 10 μM of compound).

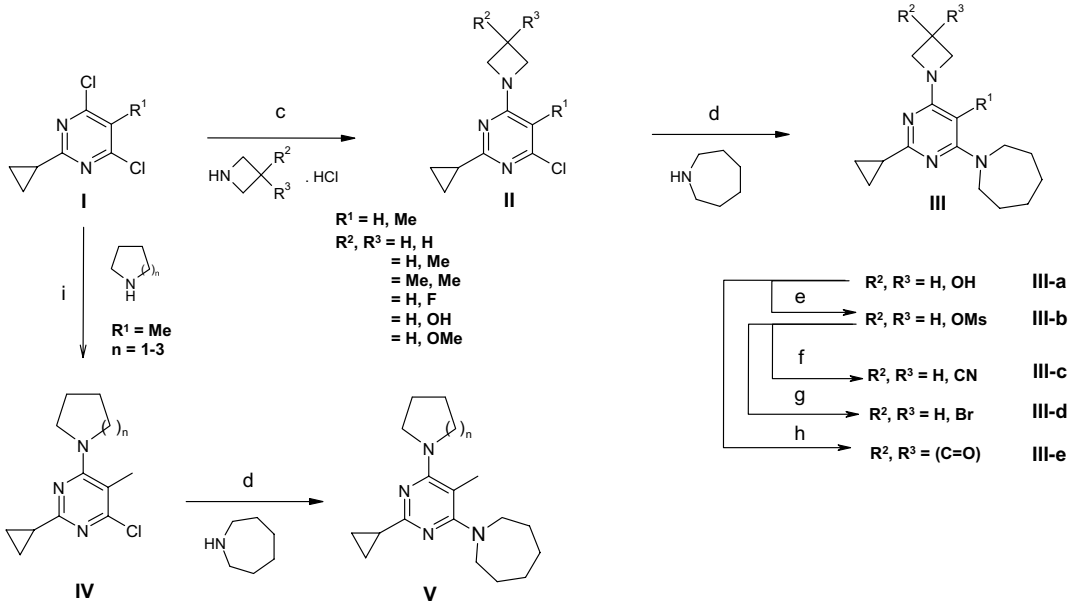
Table 2. Modulation of R^2 and R^3

Compound		R^1	PDE4 IC_{50} (μM)	M_3 K_i (nM)	pK_a
1		Me	0.32	16	7.1
5		Me	0.79	20	8.0
6		Me	5.0	32	7.0
7		Me	0.63	158	7.0
8		Me	2.0	200	6.1
9		Me	1.0	794	6.4
10		Me	0.079	200	6.6
11		Me	0.50	631	6.4
12		Me	4.0	40	6.8
13		H	0.79	79	7.4
14		H	2.0	13	7.5
15		H	0.20	501	6.9

A series of 4,6-diaminopyrimidines were therefore synthesized and evaluated for their ability to inhibit phosphodiesterase type 4 (PDE4), prepared from U937 cells,[2] and to bind to the M_3 receptor.[3] The results are displayed in Table 2.

The introduction of one methyl group in 3-position resulted in a slight decrease of PDE4 inhibiting activity, whereas M_3 affinity is kept constant. As already mentioned, this observation may be correlated with the sensitivity of PDE4 activity towards steric crowding in that area. Not surprisingly, the *gem*-dimethylated analogue **6** also displayed a much lower activity as well as the 3-methoxy derivative (**12**).

However, compounds bearing more polar substituents such as hydroxy (**7**), cyano (**9**) or carbonyl (**11**) were characterized by a more important loss of M_3 affinity probably due to lower basicities (see pK_a values in Table 2),[4] particularly in the case of **9** and **11**.



Scheme 1. Synthesis of compounds. Reagents and conditions: (a) NaOEt, EtOH, 60 °C; (b) $POCl_3$, *N*,*N*-diethylaniline, 100 °C; (c) K_2CO_3, CH_2Cl_2, 45 °C; (d) heat, 100–120 °C; (e) CH_3SO_2Cl, NEt_3, CH_2Cl_2, 0 °C to rt; (f) NaCN, DMF, 120°C; (g) NaBr, DMF, 100 °C; (h) Pyr·SO_3, NEt_3, DMSO, rt; (i) heat, 50 °C.

No dramatic changes in PDE4 inhibiting activity were nevertheless observed following those substitutions.

3-Halogeno-(bromo-**8** and fluoro-**10**) azetidine derivatives have also been synthesized and tested.

Lower M_3 affinities were observed in both cases mainly due to expected decreased basicities in comparison to unsubstituted analogues.

Interestingly, compound **10** bearing a fluorine atom displayed the highest PDE4 inhibiting activity ever seen in the pyrimidine series. The azetidine moiety seems to fit in a narrow hydrophobic channel (a pyrrolidine or a piperidine is therefore too large). Long, narrow and hydrophobic substituents are therefore required, the 3-fluoroazetidine being the optimal one.

The nature of the substituent in 5-position of the pyrimidine has also been rapidly studied. It appears that the removal of the methyl group systematically led to a slight decrease of both PDE4 activity and M_3 affinity, as expected from previous results.[1]

On the basis of those results, compounds **1** and **10** have been selected for further characterization in several in vitro assays and compared with ucb-101333-3.

Their in vitro profile is detailed in Table 3. It appears that the significantly higher PDE4 inhibiting activity of compounds **1** and **10** has also been confirmed in cell-based assays such as the fMLP-induced elastase release from polymorphonuclear neutrophils. Furthermore, a high level of selectivity towards more than 40 other targets (GPCRs, other PDE isoenzymes, ion channels...) is observed, as in the case of ucb-101333-3.

Table 3. In vitro profile

Profile	ucb-101333-3	**1**	**10**
pK_i M_3	8.5	7.8	6.8
pA_2 M_3[a]	7.9	6.0	nd
pIC_{50} PDE4	6.1	6.6	7.3
pD_2 PDE4[b]	6.1	nd	7.4
pK_i HARBS	5.3	5.3	6.0
Inh. of fMLP-induced elastase release from human PMN (IC_{50}, μM)	0.79	0.19	0.38

[a] On guinea pig trachea contracted with carbachol.
[b] On guinea pig trachea precontracted with LTD4.[5]

A preliminary characterization of some of these compounds in several in vivo models of pulmonary inflammation has been performed. We have surprisingly shown that ucb-101333-3, despite its quite modest PDE4 inhibiting activity (IC_{50} = 0.63 μM) in comparison to compounds **1** and **10**, displays the most favourable anti-inflammatory properties, including in the disease relevant cigarette smoke-induced pulmonary cells, recruitment model in mice.[6,7] Indeed, it dose-dependently inhibits significantly polymorphonuclear neutrophils (Fig. 4), total BALF cells, and mKC (data not shown) when given by nose-only aerosol at concentrations (ED_{50} = 10 mg/ml, 15 min nebulization) comparable to the ones reported for cilomilast and roflumilast.[7]

ucb-101333-3 has also been tested on emphysema development and MMP's activity in a model of cadmium-induced emphysema in rats and displays protective effects on macrophages and neutrophils but also on emphysema development.[8,9]

As a conclusion, chemical modulations around the azetidine substituent allowed us to identify new compounds

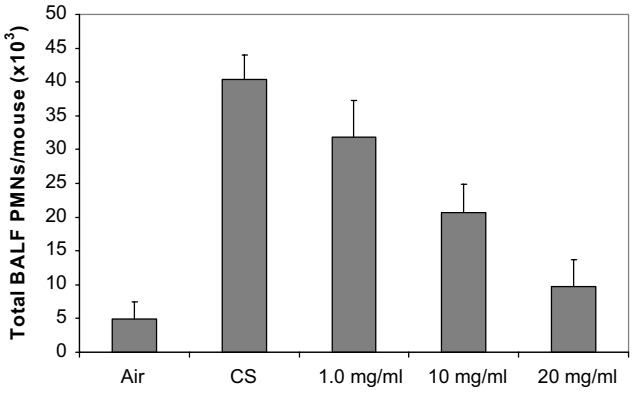


Figure 4. Effect of ucb-101333-3 on cigarette smoke-induced PMN's recruitment in mice. CS, cigarette smoke.

as potent dual M_3 antagonists and PDE4 inhibitors. These compounds display significantly higher PDE4 inhibiting activities than the corresponding cyclopropylamino derivatives (e.g., ucb-101333-3) but slightly lower M_3 affinities. They have therefore given us an access to a larger set of compounds, with a wider range of in vitro activities. Preliminary in vivo profiling has nevertheless demonstrated that ucb-101333-3 was the most potent compound in a range of pulmonary inflammation models.

Acknowledgements

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References and notes

1. Provins, L.; Christophe, B.; Danhaive, P.; Dulieu, J.; Durieu, V.; Gillard, M.; Lebon, F.; Lengelé, S.; Quéré, L.; van Keulen, B. J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1834.
2. Inhibition of PDE4 enzyme activity was measured in supernatant from U937 cells prestimulated with salbutamol and rolipram to upregulate PDE4 expression. Enzyme activity was measured using [^{3}H]cAMP as a substrate. Data shown in tables are average results of two independent experiments.
3. Affinity for M_3 muscarinic receptor was determined by competition experiments with [^{3}H]N-methylscopolamine performed in CHO cell membranes expressing recombinant human receptors. Data shown in tables are average results of 2 independent experiments.
4. All titrations were performed using a GlpKa™ (Sirius, Forest Row, UK) and done in aqueous solution or methanol–water mixture with 0.15 M KCl at 25 °C using standardized 0.5 M HCl titrant.
5. Christophe, B.; Lelubre, F.; Provins, L.; Peck, M.; Massingham, R. *The Pharmacologist* **2002**, *44*, A227.
6. BALB/c female mice (8–10 weeks old, Charles River Laboratories) were placed into a plexiglass chamber. Smoke was generated from research grade cigarettes (1R3, University of Kentucky) using a Harvard rodent ventilator (35 strokes/min, 2.8 ml/stroke, approximate burn time 4 min). A compressed air source was used to dilute the concentration of smoke within the exposure chamber. Animals were exposed to a total of 5 cigarettes approximately every 15 min. Control animals were exposed to compressed air for the appropriate exposure time. Animals were exposed to an aerosol of test compound or vehicle. Mice were exposed to two 15 min nose-only aerosols generated using a BioAerosol Nebulizing Generator (CH Technologies). Mice were euthanized with sodium pentobarbital 48 h after smoke exposure. Lungs were lavaged with two 0.5 ml aliquots of HBSS (w/o Ca^{++}, Mg^{++}). Approximate recovery was 80%. BALF was centrifuged and supernatant aliquots stored at −80 °C until cytokine assay. Red cells were lysed with 100 μl dH_2O and osmolarity restored with 100 μl 2× HBSS (−/−). The cells were centrifuged again and the pellet was resuspended in 150 μl 0.1% BSA/HBSS. Total cells recovered are determined using a Coulter counter. Cytospin slides were prepared for differential cell count determination. Slides were stained with 3-step stain for differential counting, and 300 cells were counted per slide. Supernatant KC was analyzed by ELISA (R&D Systems) following manufacturer's instructions. The compounds were prepared in saline as a vehicle.
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9. Kirschvink, N.; Fiévez, N.; Marlin, D.; Smith, N.; Provins, L.; Gustin, P. *Eur. Respir. J.* **2005**, *26*(Suppl. 49), 104s.