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First dual M_3 antagonists-PDE4 inhibitors: Synthesis and SAR of 4,6-diaminopyrimidine derivatives

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Abstract—SAR around 4,6-diaminopyrimidine derivatives allowed the discovery of the first potent dual M_3 antagonists and PDE4 inhibitors. Various chemical modulations around that scaffold led to the discovery of ucb-101333-3 which is characterized by the most interesting profile on both targets.

COPD (chronic obstructive pulmonary disease) is a chronic, progressive, and poorly reversible condition characterized by impaired expiratory outflow and abnormal inflammatory response of the lungs to noxious particles and gases. Chronic bronchitis, resulting in small airway obstruction and mucus plugging, and emphysema, responsible for parenchyma destruction, are the two main components of this disease state, the top three symptoms being coughing, wheezing and, above all, shortness of breath.[1–6]

COPD is one of the most common chronic diseases worldwide. It affects 4–6% of people older than 45 and is predicted to be the third leading cause of death by 2020. Smoking tobacco is by far the most important risk factor for the development and progression of the pathological condition.[1–6]

There are currently no drug therapies capable of slowing down the progression of disease. Patients are commonly treated with drugs developed for asthma but it proves to be quite ineffective probably because the pathophysiologies of asthma and COPD differ markedly.[2,7] The primary inflammatory cells associated with asthma are $CD4^+$ T-cells, mast cells and eosinophils, while those associated with COPD are macrophages, $CD8^+$ T-cells, and neutrophils.[1,2]

Therefore, there is an urgent need to develop new treatment approaches.[3–6]

Bronchodilator drugs are the current mainstay of treatment for symptoms' relief.[8,9] Anticholinergic bronchodilators, particularly selective muscarinic M_3 antagonists, are currently the preferred choice for the symptomatic management of COPD as they are more effective and have fewer side-effects than β_2-adrenoceptor agonists.[8] Tiotropium bromide (Spiriva®), a new anticholinergic with a very long duration of action, has been recently registered and proved to be suitable for once-daily administration as a dry powder (see Fig. 1).[10,11]

However, although bronchodilators are quite effective to improve symptoms, they do not address the underlying chronic inflammation or the changes in airway structure. Contrary to asthma, standard treatment with corticosteroids as anti-inflammatory agents has demonstrated limited efficacy. However, among the new anti-inflammatory agents currently being developed, PDE4 inhibitors proved to be very effective in attenuating the responses of various inflammatory cells through their ability to elevate cAMP levels. They are known

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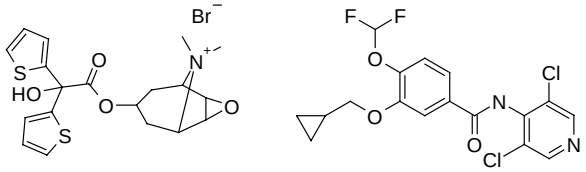


Tiotropium Bromide (Spiriva®) Roflumilast (Daxas®)

Figure 1. Selected M_3 antagonist and PDE4 inhibitor targeting COPD.

to modulate activity, migration, and apoptosis of neutrophils by inhibiting the production and release of chemokines, superoxide free radicals, leukotrienes, and proteolytic and toxic granular enzymes.[12–14]

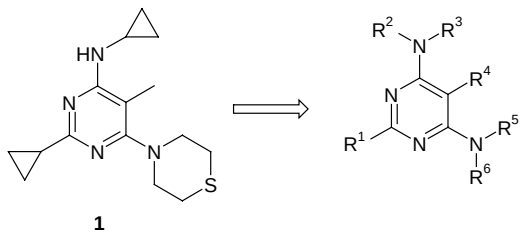
The tendency of first generation PDE4 inhibitors (e.g., rolipram) to cause side effects such as nausea, emesis, and headache has limited their beneficial effect in clinical studies. Nevertheless, new generation compounds[13,14] such as Roflumilast (Daxas®)[15,16] are currently in Phase III for the treatment of COPD and have been shown to significantly improve lung function and symptoms of patients (see Fig. 1).

Thus, the combination of selective muscarinic M_3 antagonism with selective PDE4 inhibition may lead to a new class of drugs combining both bronchodilating and anti-inflammatory properties.

In this paper, we present the discovery of a family of dual M_3 antagonists and PDE4 inhibitors as potential new drugs for COPD treatment. We focused on the structural optimization of cyclopropyl-(2-cyclopropyl-5-methyl-6-thiomorpholin-4-yl-pyrimidin-4-yl)-amine **1** (Fig. 2), one of the first chemical leads discovered by screening from our in-house library. Full details of SAR studies are given.[17]

The general method of synthesis of compounds listed in Tables 1–4 is outlined in Scheme 1.

Briefly, condensation of 2-substituted dialkylmalonates with various amidines in the presence of sodium ethoxide affords 4,6-dihydroxypyrimidines **I** [18] which were then chlorinated with phosphorus oxychloride in the presence of *N*,*N*-diethylaniline.[19] The resulting 4,6-dichloropyrimidines **II** are subsequently substituted with various primary or secondary amines to give the desired compounds **IV**.



1

Figure 2.

Compounds bearing a nitro substituent in R^4 have been prepared by nitration of the corresponding 4,6-dihydroxypyrimidine **I-a** using glacial acetic acid and nitric acid.[20] Bromination of compound **IV-a** with *N*-bromosuccinimide afforded **IV-b**.[21]

The reduction of **IV-c** with sodium dithionite in the presence of ammonia afforded **IV-d**.[22]

The synthesis of compounds **VII** listed in Table 4 is outlined in Scheme 2.

Condensation of amidines with 2-{3-[(*tert*-butoxy-hydroxy-methyl)-amino]-alkyl}-diethylmalonates in the presence of base afforded the corresponding 4,6-dihydroxypyrimidines. Their reaction with phosphorus oxychloride in the presence of *N*,*N*-diethylaniline afforded the deprotected dichloropyrimidines, which were then heated at high temperature to give the cyclized compounds **VI**. Subsequent substitution with secondary amines afforded the desired compounds **VII**.

A series of 4,6-diaminopyrimidines were synthesized and evaluated for their ability to inhibit phosphodiesterase type 4 (PDE4), prepared from U937 cells,[23] and to bind to the M_3 receptor.[24]

The first modification envisaged was the replacement of the thiomorpholine moiety, which was suspected to be rapidly oxidized. A variety of compounds bearing various secondary and tertiary amines have been prepared and tested. As mentioned in Table 1, the replacement of thiomorpholine by amines bearing hydrophilic groups (thiomorpholine-1,1-dioxide, morpholine, 4-hydroxypiperidine, and piperidin-4-one) resulted in a dramatic loss of M_3 affinity, PDE4 inhibiting activity is also decreasing but to a lesser extent.

Various physicochemical parameters were measured for compounds listed in Table 1 and were correlated to M_3 affinity. A positive correlation was found between the experimental lipophilicity parameter K′IAMex[25] and the M_3 affinity for the tertiary amine substituents which adequately fill the space of a hypothetical hydrophobic pocket within the receptor. The introduction of larger lipophilic groups has however an unfavorable impact on affinity. Equation 1, obtained by multiple regression analysis (MRA) employing diverse common physicochemical descriptors (π, σ_m, *F*, *R*, MR, and Verloop…), indeed shows a dependency of M_3 affinity towards both lipophilicity (K′IAMex) and steric bulk (Verloop parameters L, B3, and B4).

$$pK_i = 0.83 \log K'IAMex - 0.61L + 0.73B3 - 1.02B4 + 6.84$$
$$n = 18 \quad r^2 = 0.81.$$

In most cases, no dramatic change of PDE4 inhibiting activity was observed following those substitutions but it nevertheless appears that the optimal ring size is 6 (piperidines) or 7 (azepane), pyrrolidine or azocane being less potent.

Table 1. Modulation of NR^5R^6

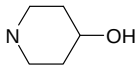
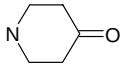
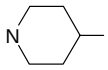
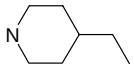
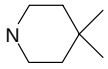
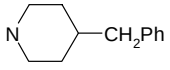
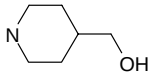
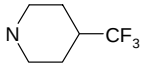
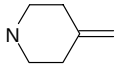
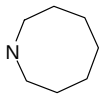
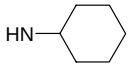
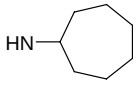
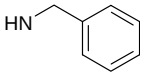
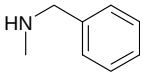
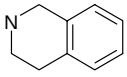
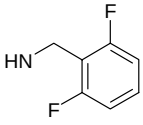
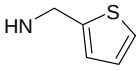
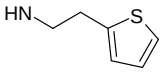
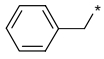
Compound	NR^5R^6=	PDE4 IC_{50} (μM)	M_3K_i (nM)	log K′IAMex
1		0.32	79	2.14
2		0.79	#	1.45
3		4.0	501	1.20
4		3.2	3200	1.35
5		0.50	13	2.50
6		0.63	16	2.80
7		0.63	50	3.21
8		0.79	32	2.37
9		0.32	25	3.02
10		0.79	158	3.66
11		2.0	790	3.89
12		0.63	501	1.57
13		2.0	100	2.94
14		0.40	50	2.50
15		0.40	79	2.70
16		1.6	100	2.36
17		0.63	3.2	2.89
18		1.6	16	3.05
19		2.5	316	2.71
20		2.0	398	2.98

Table 1 (*continued*)

Compound	NR^5R^6=	PDE4 IC_{50} (μM)	M_3K_i (nM)	log K′IAMex
21		0.5	6.3	3.11
22		4.0	200	2.36
23		0.40	251	2.70
24		0.50	316	2.97
25		2.0	63	2.38
26		6.3	126	2.21
27		2.5	79	2.57

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

Table 2. Modulations of R^1

Compound	R^1=	PDE4 IC_{50} (μM)	M_3K_i (nM)
17		0.63	3.2
28		2.0	40
29		1.26	79
30		2.5	25
31		2.0	126
32		1.0	501
33[26]		3.2	32
34[26]		2.5	398

Cycloalkylamino substituents as well as several benzylamines have also been synthesized and tested (Table 1). Lower M_3 affinities are obtained in most cases except for **25** and **27**.

Table 3. Modulations of R^4

Compound	R^4=	NR^5R^6=	PDE4 IC_{50} (μM)	M_3K_i (nM)	pK_a
1	CH_3		0.32	79	6.99
17	CH_3		0.63	3.2	8.13
35	H		0.40	63	8.03
36	F		0.63	251	6.44
37	Br		0.63	316	6.21
38	NO_2		1.26	2512	4.64
39	NH_2		0.79	79	7.15
40	OCH_3		2.5	398	6.47
41	F		0.25	#	5.27
42	CH_2CH_3		1.6	100	6.78

Table 4. Modulations of NR^2R^3

Compound	NR^2R^3=	R^4=	PDE4 IC_{50} (μM)	M_3K_i (nM)
17		Me	0.63	3.2
43		Me	2.0	7.9
44		Me	6.3	10
45	 (+,-)	Me	7.9	7.9
46			0.5	50
47			1.3	10

However, these examples emphasize the importance of tertiary amines for PDE4 inhibiting activity. For example, *N*-methyl-benzylamine and 1,2,3,4-tetrahydroisoquinoline substituents are about 10-fold more potent than benzylamine itself (compare compounds **22**, **23**, and **24** in Table 1).

After this first series of substitutions, the compound **17**, bearing an azepanyl moiety, has been found to be characterized by the most interesting balance between M_3 affinity (3.2 nM), and PDE4 inhibiting activity (630 nM) and has therefore been selected for further optimization.

All tentatives to replace the cyclopropyl ring (R^1) resulted in a loss of M_3 affinity as well as PDE4 inhibiting activity, as reported in Table 2.

These results clearly show the importance of steric factors in that area. Increased ring size as well as larger alkyl or benzyl groups is detrimental to both activities.

The nature of the substituent in 5-position (R^4) is also highly important for the basicity and therefore for the affinity for the M_3-R as exemplified in Table 3. It has however nearly no impact on PDE4 inhibiting activity.

For example, electronegative substituents such as halogens (p$K_a \sim 6.5$)[27] or nitro (p$K_a \sim 4.6$) led to a 25- to 800-fold decrease in M_3 affinity. The affinity is maintained if the methyl group is replaced by hydrogen or a small alkyl group (ethyl).

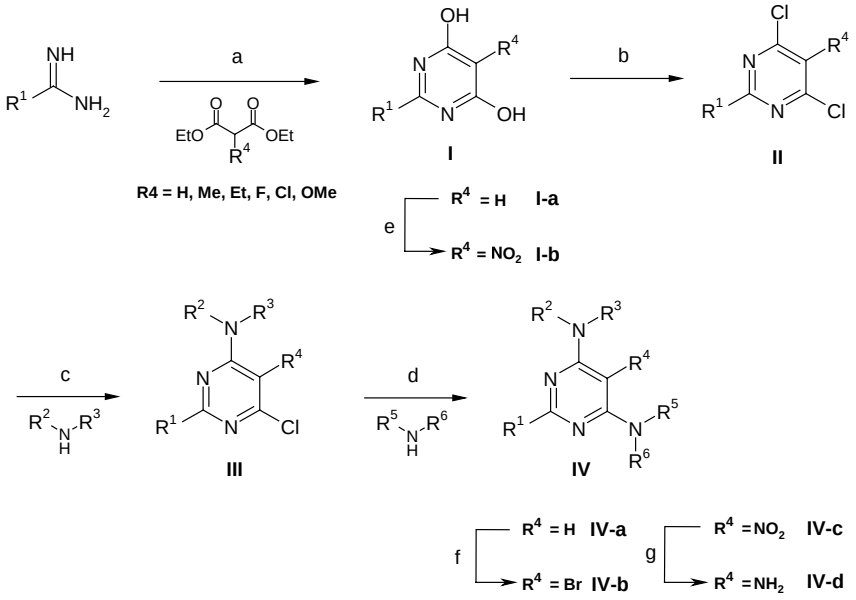
M_3 affinity is however four times lower in the particular case of the methoxy group (**40**) compared to the ethyl group (**42**). For compound **40**, the counter-intuitive decrease in basicity is explained by an intramolecular H-bond between the oxygen and the cyclopropylamino moiety. This assumption has been confirmed by a X-ray structure of the compound **40**.[28]

PDE4 inhibiting activity proved to be much more sensitive toward the nature of the substituent in 4-position (NR2R3) as shown in Table 4.

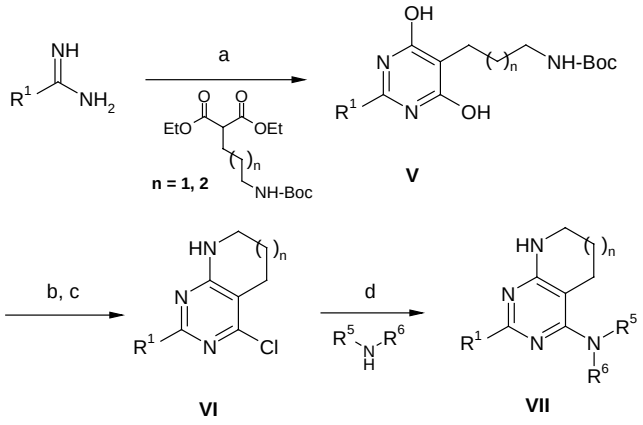
For example, removal, substitution in various positions or replacement of the cyclopropane by larger rings led to an important loss of activity, therefore highlighting the importance of steric factors in that area. Affinities for the M_3-R are, in most cases, insensitive to these modifications.

Bicyclic derivatives **46** and **47** in which R^3 and R^4 are linked together to form 5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidine or 6,7,8,9-tetrahydro-5*H*-pyrimido[4,5-*b*]azepine derivatives have also been synthesized according to Scheme 2. These are characterized by an interesting profile on both targets but suffer from lower selectivities than open derivatives toward other receptors.

Compound **17** (ucb-101333-3) was therefore selected as the most promising candidate to be further studied in several assays. Its in vitro profile as well as several physicochemical data is detailed in Table 5. It is characterized by a high selectivity for PDE4 versus other PDE enzymes and towards the high affinity rolipram binding site (HARBS). The anti-inflammatory activity of **17** has also been confirmed in several cell-based assays (TNF-α or elastase release from polymorphonuclear



Scheme 1. Synthesis of compounds **IV**. Reagents and conditions: (a) NaOEt, EtOH, 60 °C; (b) $POCl_3$, *N*,*N*-diethylaniline, 100 °C; (c) neat, 45 °C; (d) neat, 100–120 °C; (e) HOAc, HNO_3, 0 °C then 30 °C; (f) NBS, $CHCl_3$, 60 °C; (g) $Na_2S_2O_4$, NH_4OH, dioxane/H_2O, rt.



Scheme 2. Synthesis of compounds **VII**. Reagents and conditions: (a) NaOEt, EtOH, 60 °C; (b) $POCl_3$, *N*,*N*-diethylaniline, 100 °C; (c) 1-methoxy-2-propanol, 140 °C (d) neat, 100–120 °C.

neutrophils). An interesting selectivity for the M_3 receptor toward the other muscarinic receptor subtypes (M_2 in particular) is also noticed and has been confirmed using the isolated guinea pig trachea or left atrium both stimulated by carbachol as functionally integrated M_3 and M_2 assays, respectively. ucb-101333-3 has also been screened against more than 40 different targets and no significant interactions were observed at 10 μM.

However, before pursuing the complete characterization of this compound, a salt selection was performed in order to avoid potential formulation issues. A nice, nonhygroscopic powder has been obtained by making a salt with fumaric acid. The stoichiometry (sesquifumarate) as well as the protonated center on the aromatic nitrogen in 3-position have been unambiguously assigned through X-ray diffraction. The crystallographic data are available in Cambridge Structural Database.[29]

Table 5. ucb-101333-3′s in vitro profile

	Ucb-101333-3 (**17**)
Muscarinic profile	
pK_i M_3	8.5
pK_i M_1	7.7
pK_i M_2	7.2
pK_i M_4	8.0
pK_i M_5	7.2
pA_2 M_3[d]	7.9
pA_2 M_2[e]	7.0
Physicochemical profile	
Log *D* (pH 7.4)	2.90
Log K′IAMex	2.75
S (pH 1, 4, 7.4)	7, 4, 0.01 mg/ml
PDE profile	
pIC_{50} PDE4 (U937 cells)	6.1
pK_i HARBS	5.3
PDE1 (bovine brain)	2% inhibition[a]
PDE2 (human platelets)	7% inhibition[a]
PDE3 (human platelets)	4% inhibition[a]
PDE5 (human platelets)	6% inhibition[a]
PDE6 (bovine retina)	26% inhibition[b]
PDE7 (HuT-78 cells)	31% inhibition[c]
Inhibition of LPS-induced TNF-α release from human PMN (pIC_{50})	5.9
Inhibition of fMLP-induced elastase release from human PMN (pIC_{50})	6.1
pD_2 PDE4[f]	6.1

[a] Inhibition at 10 μM.
[b] Inhibition at 1 μM.
[c] Inhibition at 100 μM.
[d] On guinea pig trachea contracted with carbachol.
[e] On guinea pig paced left atrium stimulated with carbachol.
[f] On guinea pig trachea precontracted with LTD_4.[30]

As a conclusion, we have identified, for the first time, a new family of potent dual M_3 antagonists and PDE4 inhibitors. Chemical modulations and SAR study

around the 4,6-diaminopyrimidine scaffold allowed us to identify ucb-101333-3 which has been selected for extensive DMPK and pharmacological evaluation on the basis of its excellent affinity/activity and selectivity profiles as well as adequate physicochemical properties. Those results as well as its characterization in relevant animal models of COPD will be reported in due course.

Acknowledgments

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23. Inhibition of PDE4 enzyme activity was measured in the supernatant from U937 cells prestimulated with salbutamol and rolipram to upregulate PDE4 expression. Enzyme activity was measured using [^{3}H]cAMP as a substrate.
24. 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.
25. Capacity factors (k′IAM) were established on a IAM.PC.DD 2 Drug-Discovery HPLC column 30×4.6 mm (Regis Tech Inc., Morton Grove, IL, USA). The mobile phase consisted of different mixtures of phosphate-buffered saline (pH 7.4) and acetonitrile as co-solvent. The published data are the extrapolated values at 0% in CH_3CN. See also: Ong, S.; Liu, H.; Pidgeon, C. *J. Chromatogr. A* **1996**, *728*, 113.
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27. 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.
28. CCDC 290973 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif.
29. CCDC 290974 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif.
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