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The Synthesis and Biological Evaluation of a Novel Series of Phthalazine PDE4 Inhibitors I

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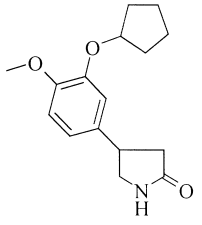
Abstract—This communication describes the synthesis and in vitro evaluation of a novel and potent series of phosphodiesterase type IV (PDE4) inhibitors. The compounds described represent conformationally constrained analogues of RP 73401, Piclamilast. Preliminary evidences of reduced side effects of **11** compared to standards are also reported.

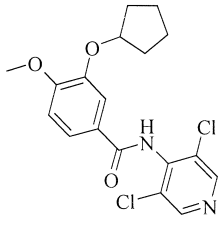
PDE4 is a cAMP-specific phosphodiesterase highly expressed in inflammatory cells and in airway smooth muscle. Inhibition of PDE4 results in an elevation of cAMP in these cells, which mediates anti-inflammatory effects and muscle relaxation in a variety of animal models.[1] The potential use of PDE4 inhibitors as anti-inflammatory agents for the treatment of asthma and other inflammatory disorder has generated great interest in this area.[2]

The archetypal PDE4 inhibitor rolipram (**1**) has been the starting point for the majority of medicinal chemistry efforts[2] and from which derives the commonly observed 4-methoxy-3-cyclopentyloxy substitution on the aromatic ring.[3]

Initially, most of the research in this area dealt with the replacement of the pyrrolidinone of **1** with other functionality[4] and from this work RP 73401 (**2**)[5] emerged as one of the most potent PDE4 inhibitors.



Rolipram (**1**)



RP 73401 (**2**)

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Subsequently, many efforts have focused on finding suitable isosteres for the 3,4-dialkoxyphenyl moiety of **1** (or its analogues).[6–8]

Until now, no studies have been carried out to evaluate the possible conformations that the phenyl ring can adopt during the interaction with the enzyme. In order to gather information on this aspect, we designed rigid analogues of **2** where the carbonyl function is replaced by a π-bond of an aromatic ring.

In implementing this modification, we also hoped to retain the necessary recognition elements for enzyme inhibition while reducing the unwanted side effects, such as emesis[9] and gastrointestinal acid secretion.[10] The phthalazine nucleus was chosen in order to increase the polarity of **2**.

Chemistry

The synthesis of the requisite phthalazines is illustrated in Schemes 1–3. Oxidation of aldehyde **3** (Scheme 1) to **4**, followed by amidation afforded intermediate **5**. Low temperature deprotonation and formylation gave derivative **6**.

Acid hydrolysis of both the benzyl ether and amide bond produced **7** in high yield. Hydrazine cyclization forming **8** followed by *O*-alkylation with selected mesylates afforded compounds **9**. $POCl_3$ chlorination gave **10**, which upon treatment with the sodium salt of 3,5-dichloro-4-methylpyridine or 4-amino-3,5-dichloropyridine provided the target compounds **11**–**15**. Selective

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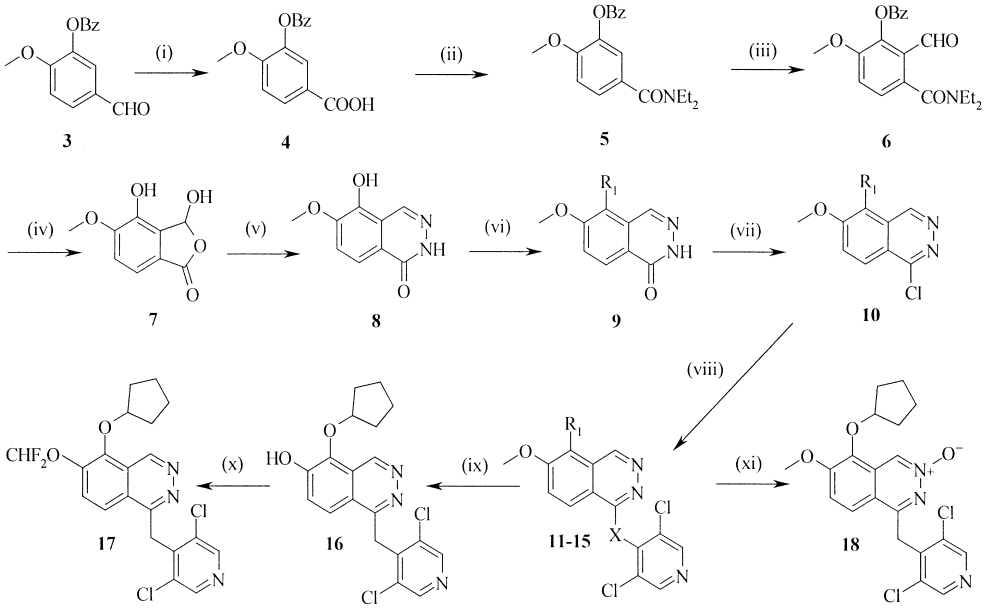
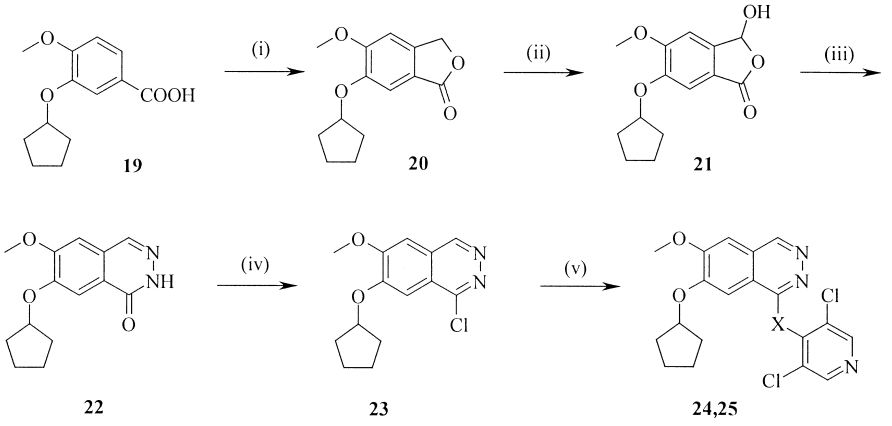


Scheme 1. Reagents and conditions: (i) $Bu_4N^+KMnO_4^-$, pyridine, rt, 3 h, 88%; (ii) $SOCl_2$, reflux, 2 h; then Et_2NH, CH_2Cl_2, 10 °C, 1 h, 94%; (iii) *s*-BuLi, TMEDA, DMF, THF, −78 °C, 4 h, 32%; (iv) 10% HCl in acetic acid, reflux, 18 h, 100%; (v) hydrazine hydrate, EtOH, 60 °C, 5 min, 72%; (vi) $R^1O_2SCH_3$, Na_2CO_3, cat KI, DMF, 90 °C, 16 h, 66–88%; (vii) $POCl_3$, reflux, 1 h, 95–98%; (viii) 3,5-dichloro-4-methylpyridine or 4-amino-3,5-dichloropyridine, NaH, DMF, rt, 1.5 h, 22–60%; (ix) **11**, sodium *p*-thiocresolate, DMF, 90 °C, 2 h, 40%; (x) $CHClF_2$, K_2CO_3, cat KI, DMF, 75 °C, 4.5 h, 21%; (xi) **11**, 55% *m*-chloroperbenzoic acid, CH_2Cl_2, rt, 1 h, 64%.



Scheme 2. Reagents and conditions: (i) 32% aq HCHO (1.2 equiv), 20% HCl, 60 °C, 48 h, 60%; (ii) NBS, cat benzoyl peroxide, CCl_4, reflux, 2 h, then 5% HCl, reflux, 4 h, 60%; (iii) hydrazine hydrate, EtOH, 60 °C, 5 min, 93%; (iv) $POCl_3$, reflux, 1 h, 98%; (v) 3,5-dichloro-4-methylpyridine or 4-amino-3,5-dichloropyridine, NaH, DMF, rt, 1.5 h; (**24**: 59%; **25**: 22%).

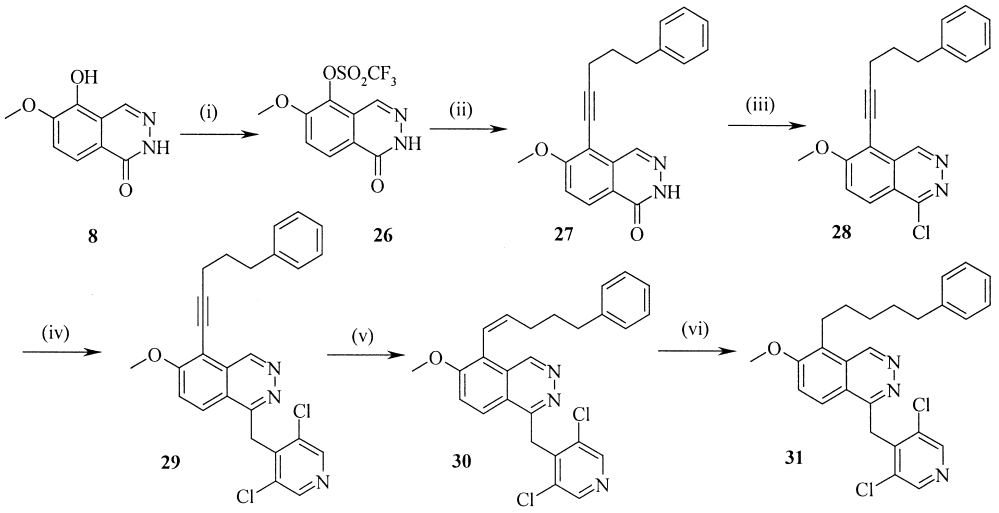
demethylation of **11** to give **16** and difluorocarbene insertion resulted in the methoxy bioisostere derivative **17**. Regiochemical oxidation of **11** to its corresponding *N*-oxide **18** proceeded smoothly with *m*-CPBA.[11]

In Scheme 2 is described the strategy adopted for the synthesis of phthalazines bearing the cyclopentyloxy substitution in position 7. Chloroformylation of known acid **19**[5] and subsequent radical oxidation of **20** produced **21** uneventfully. Following the same pathway utilized for the 5,6-disubstituted phthalazines, the desired derivatives **24** and **25** were obtained.

Intermediate **8** (Scheme 3) formed triflate **26** that was sufficiently reactive to give Pd^0 cross-coupling with alkynyl derivatives. This behaviour gave us the possibility to prepare compounds such as **27**. Usual transformation to chloride **28** and subsequent alkylation provided **29** as described above. Sequential catalytic hydrogenation afforded **30** and **31**, respectively.

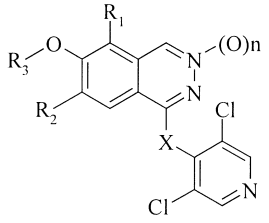
Biological Results and Discussion

Table 1 summarizes the in vitro activity of phthalazines with respect to human neutrophil PDE4 inhibition (IC_{50}, nM),[12] association with the high affinity rolipram binding site (K_i, nM)[13] and human monocytes TNF_α synthesis inhibition (IC_{50}, nM).[14] Activity of the three standards was determined in-house using these procedures. SB 207499 (Ariflo) was added for comparison because it has been recently described as a second-generation inhibitor of PDE4 with a decreased potential for side effects.[15]



Scheme 3. Reagents and conditions: (i) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, −5 °C, 1 h, 73%; (ii) pent-4-ynylbenzene, 5% bis(triphenylphosphine)$PdCl_2$, Et_3N, DMF, 90 °C, 3 h, 65%; (iii) $POCl_3$, 80 °C, 0.5 h, 100%; (iv) 3,5-dichloro-4-methylpyridine, NaH, DMF, rt, 3 h, 51%; (v) H_2, 10% Pd/C, THF, rt, 1 h, 43%; (vi) 4 atm H_2, 10% Pd/C, THF, rt, 48 h, 17%.

Table 1.



General Structure

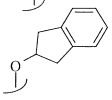
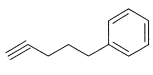
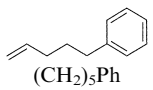
Compound	X	R_1	R_2	R_3	n	PDE4 IC_{50} (nM)	Rolipram binding K_i (nM)	TNF_α IC_{50} (nM)
1 (Rolipram)						1680	1.6	225
2 (RP 73041)						1	1.5	1.3
SB 207499 (Ariflo)						73	38	158
11	CH_2		—	CH_3	0	53	149	254
12	NH		—	CH_3	0	59	95	187
13	CH_2		—	CH_3	0	30	16	252
14	CH_2	$O(CH_2)_5Ph$	—	CH_3	0	57	13	83
15	CH_2		—	CH_3	0	153	286	—
17	CH_2		—	CHF_2	0	39	46	167
18	CH_2		—	CH_3	1	42	121	139
24	CH_2	—		CH_3	0	186	—	—
25	NH	—		CH_3	0	7% (10^{-7} M)	—	—
29	CH_2		—	CH_3	0	10	71	30
30	CH_2		—	CH_3	0	19	164	52
31	CH_2	$(CH_2)_5Ph$	—	CH_3	0	30	320	103

Table 2

Compound	Acid secretion IC_{50} (μM)	Emesis (dog model) ED_{50} (μmol/kg iv)
1 Rolipram	0.04	0.3
2 (RP 73041)	0.07	1
SB 207499 (Ariflo)	1	10
11	7	>10 (0/8)

Potent inhibition for PDE4 has been obtained for most of the phthalazines synthesized and these data well correlate with TNF_{α} inhibition observed. These activities strongly indicate that a planar dihedral angle between the phenyl ring and the linker region of rolipram-like PDE4 inhibitors is allowed. Of the two possible planar conformations, that represented by phthalazines **11** and **12** (cyclopentyloxy substitution in position 5) is clearly preferred compared with the substitution in position 7 (compound **24** and **25**). The replacement of the nitrogen with a methylene linker between the phthalazine nucleus and the pyridine (**11** vs **12**) does not affect or improves (**24** vs **25**) the activity, suggesting only a spacer role for this part of PDE4 inhibitors, at least in this series.

In general, the role of the alkoxy substituents confirmed the known structure–activity relationship of rolipram-derived PDE4 inhibitors.[3,16] It is interesting to note the increased affinity for the rolipram-binding site shown by the bulky, more lipophilic derivatives **13** and **14** compared to **11**. On the contrary, it is worth noting the excellent selectivity of compounds **29**–**31**, with the alkyl substituents directly on the aromatic ring, for the catalytic binding site over the rolipram-binding site. Such selective binding is a potential property for overcoming the side effects often seen with potent PDE4 inhibitors.[14]

Preliminary studies to evaluate the potential side effects of this novel series were performed comparing phthalazine **11** to standards. Their ability to increase acid secretion in isolated whole rat stomach[17] and to induce emesis in dog[18] is reported in Table 2.

Both the in vitro and the in vivo assays suggested an improved therapeutic potential for **11**. We were very pleased to verify that, at the maximum solubility obtainable for **11** in the vehicle, no sign of emesis was detected. Studies are in progress to verify if this favourable behaviour of **11** is the consequence of the improved selectivity for the catalytic over the high affinity rolipram-binding site or is due to a preferential affinity toward the PDE4 subtypes.[1]

In conclusion, the synthesis and in vitro evaluation of a novel series of potent PDE4 inhibitors has been reported, demonstrating that the phthalazine nucleus is an effective, polar scaffold to design rolipram-like PDE4 inhibitors. Preliminary results on efficacy and safety of this class are being produced and the progress in this area will be reported in the near future.

References and Notes

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18. Emesis method: Male and female beagle dogs (10–12 kg) were used. Tests compounds were dissolved in PEG200/DMSO 85/15 and administered by iv injection (3 mL) with an ascending dose regiment, waiting 1 h between two successive treatments. The dose that induced emesis in at least four out eight animals has been determined.