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# Phthalazine PDE4 Inhibitors. Part 2: The Synthesis and Biological Evaluation of 6-Methoxy-1,4-disubstituted Derivatives

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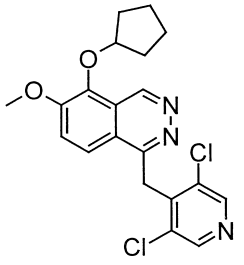
**Abstract**—This communication describes the synthesis and in vitro evaluation of a novel and potent series of phosphodiesterase type IV (PDE4) inhibitors. The compounds described present substituents in position 4 of the phthalazine ring to replace the commonly observed cyclopentyloxy moiety of rolipram analogues. Preliminary evidences of reduced side effects compared to standards and improved pharmacokinetic properties for selected derivatives are also reported. 

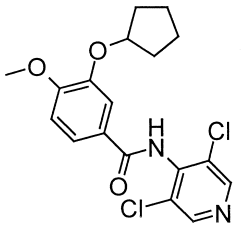
Phosphodiesterase type IV (PDE4) metabolises cyclic adenosine monophosphate in pulmonary inflammatory and immune cells, and in pulmonary smooth muscle cells, and thereby promotes bronchoconstriction and airway inflammation.[1] Selective PDE4 inhibitors would, therefore, be expected to produce both bronchodilatatory and anti-inflammatory effects in patients with asthma.[2]

In a previous paper we have described a novel series of phthalazine PDE4 inhibitors represented by **1**,[3] which is a conformationally constrained analogue of RP 73401 (**2**), one of the most potent PDE4 inhibitors described so far.[4]

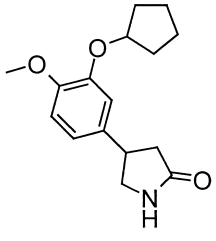
Improved selectivity for the catalytic binding site over the rolipram binding site, and preliminary evidences of reduced side effects of **1** compared to **2**, rolipram (**3**), and Ariflo (**4**) suggested an active role of the added pyridazine nucleus to the observed activity.



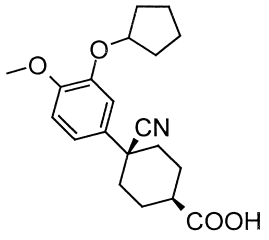
**1**



RP 73401 (**2**)



Rolipram (**3**)



Ariflo (**4**)

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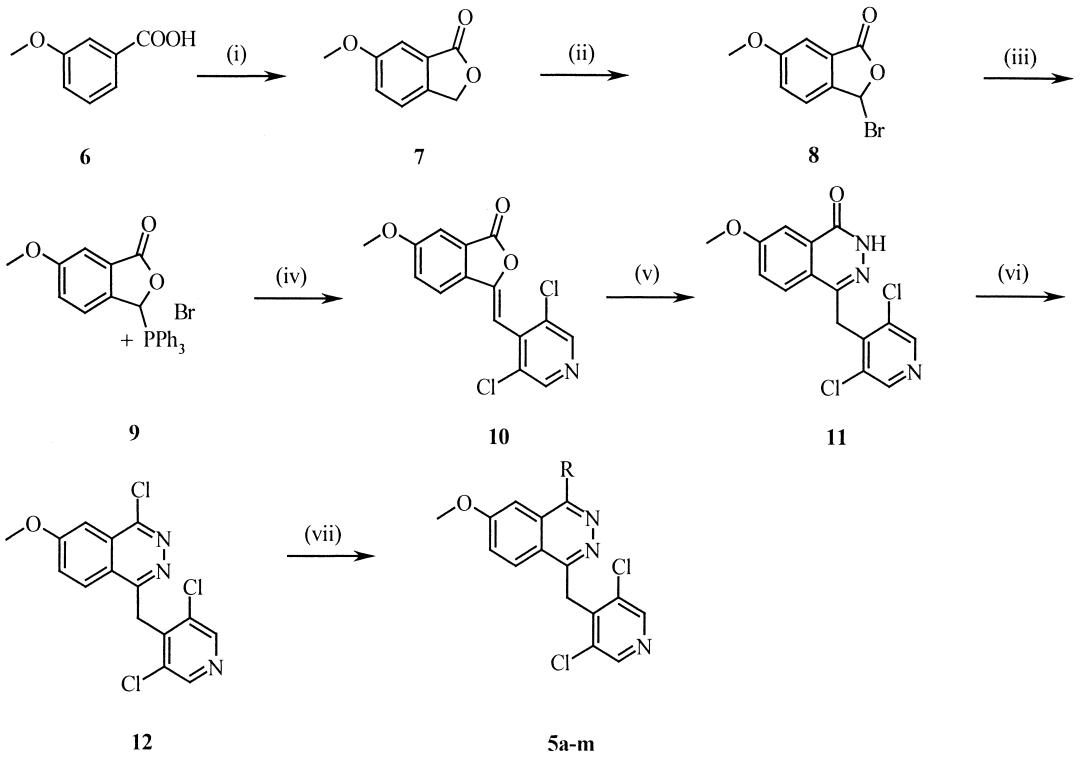
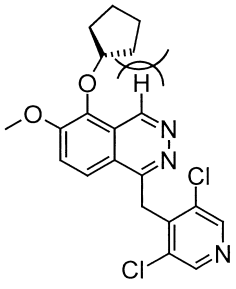



**Scheme 1.** Reagents and conditions: (i) 37% aq HCHO (1.3 equiv), 37% HCl (1.5 equiv), acetic acid, 90 °C, 14 h, 46%; (ii) NBS, cat AIBN, chlorobenzene, 85 °C, 0.7 h, 98%; (iii) triphenylphosphine, THF, reflux, 7 h, 86%; (iv) 3,5-dichloro-4-pyridinecarboxaldehyde, $Et_3N$, THF, 1 h rt then 2 h reflux, 95%; (v) hydrazine hydrate, MeOH, acetic acid, reflux, 2 h, 99%; (vi) $POCl_3$, reflux, 4 h, 94%; (vii) for **5a**: 2 atm $H_2$, 10% Pd/C, 32% NaOH (1.5 eq), DMF, rt, 2 h, 52%. For **5b–f**: RH, DMF, 100 °C, 20 h, 34–79%. For **5g–j**: RH, NaH, DMF, 100 °C, 20 h, 49–77%. For **5k**: phenyl lithium, $ZnCl_2$, THF, rt, 1 h, then **12**, cat $PdAcO_2/2PPh_3$, reflux, 24 h, 55%. For **5l**: phenylacetylene, piperidine, cat $PdCl_2/2PPh_3/CuI$, DMF, rt, 20 h, 52%. For **5m**: 2-methyl-3-butyn-2-ol, $K_2CO_3$, cat $PdCl_2/2PPh_3/CuI$, DMF, rt, 16 h, then cat NaH, toluene, reflux, 4 h, 61%.

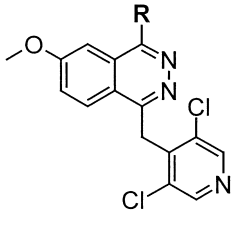
Potent PDE4 inhibition was obtained for this class of compounds indicating that a planar dihedral angle between the phenyl ring and the linker region of rolipram-like analogues is allowed. However the derivative **1** showed a decreased potency compared to open amide **2**.

A possible reason for this behaviour could derive from an unfavourable interaction between the cyclopentyloxy and the *peri*-hydrogen in position 4 of the phthalazine nucleus. In fact, studies carried out with rolipram analogues having rigid heterocycles as surrogates for the catechol substitution[5] showed that the bulky alkoxy group must adopt an orientation toward the region of space occupied by the pyrazine ring in **1** to explicate optimal PDE4 inhibition activity.

On the other hand, the presence of the added ring gave us the possibility to evaluate if proper substituents in position 4 of the phthalazine nucleus, as depicted in derivatives **5**, could replace the cyclopentyloxy moiety at its important enzyme recognition site. In implementing this modification we hoped to improve the pharmacokinetic profile of this class of molecules while retaining the desired safety profile of **1**.



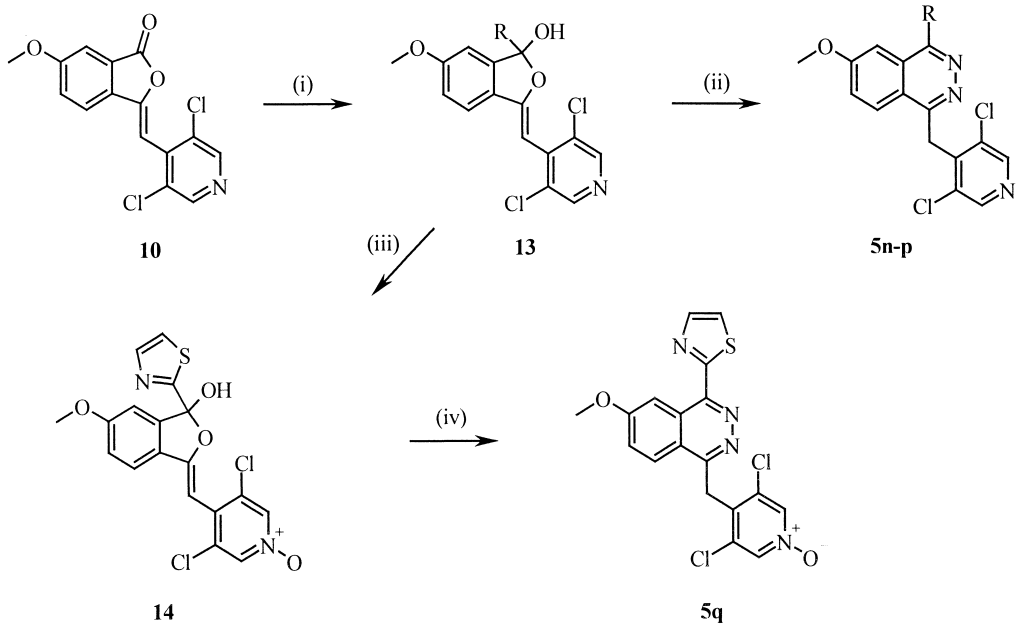
**1**



**5**

## Chemistry

The synthesis of the requisite phthalazines is illustrated in Schemes 1 and 2. Chloroformylation of 3-methoxybenzoic acid **6** (Scheme 1) and subsequently radical bromination of **7** produced **8** in good yield. Treatment with triphenylphosphine to give **9** and Wittig olefination with 3,5-dichloro-4-pyridinecarboxaldehyde[6] afforded compound **10** as a 7:3 mixture of diastereoisomers. Hydrazine cyclization forming phthalazine **11** followed by $POCl_3$ chlorination gave **12**, which upon treatment with the proper reagents provided the target compounds **5a–m**.




**Scheme 2.** Reagents and conditions: (i) RLi or RMgX, $Et_2O$, −78 °C, 0.75 h, 38–65%; (ii) hydrazine hydrate, MeOH, acetic acid, reflux, 2 h, 85–98%; (iii) 55% *m*-chloroperbenzoic acid, $CHCl_3$, reflux, 18 h, 55%; (iv) hydrazine hydrate, MeOH, acetic acid, reflux, 18 h, 68%.

In Scheme 2 is described the strategy adopted for the synthesis of phthalazine **5n–q**. Low temperature alkylation with selected lithium derivatives or Grignard reagents of intermediate **10** to give **13** and subsequent treatment with hydrazine furnished derivatives **5n–p** uneventfully. Finally **13** (R = thiazolyl) was selectively oxidised to the corresponding *N*-oxide **14** using *m*-CPBA and transformed into **5q** by means of usual hydrazine cyclisation.

## Biological Results and Discussion

Table 1 summarises the in vitro activity of phthalazines with respect to human neutrophil PDE4 inhibition ($IC_{50}$, nM),[7] association with the high affinity rolipram binding site ($K_i$, nM)[8] and human monocytes $TNF_\alpha$ synthesis inhibition ($IC_{50}$, nM).[9] Activity of the three standards was determined in-house using these procedures.

As expected, phthalazine **5a**, which is unsubstituted in positions 4 and 5, was devoid of PDE4 inhibitory activity confirming the importance that a substituent in that space region plays in the binding interaction. Introducing a substituent in position 4 a great activity enhancement was observed for several derivatives. Whether the added substitution improves the affinity for the PDE4 enzyme by mimicking the commonly observed cyclopentyloxy moiety of rolipram-like inhibitors or it interacts with a hitherto unexplored binding site remains to be clarified.

The inhibition of PDE4 catalytic activity proved to be quite sensitive to substituent modification in position 4 of the phthalazine ring rendering a structure–activity relationship difficult to rationalise. However, potent inhibition for PDE4 has been obtained for derivatives that present substituents with diverse physico-chemical characteristics giving us the possibility to modulate their pharmacokinetic profile. The introduction of a polar tertiary amine gave potent derivatives **5d** and **5f**, which were soluble in water as hydrochloride salts. Phenyl substituted **5k** and its heterocyclic analogues **5i** and **5p** showed both potent PDE4 activity and whole cell activity.

For most compounds, improved $TNF_\alpha$ inhibition compared to PDE4 inhibition was obtained by increasing the polarity of the substituent in position 4 (for instance **5f** versus **5k**). This is probably due to an augmented capability for more polar derivatives to penetrate into the cells, even if a different affinity toward PDE4 subtypes can not be ruled out.[1] It is worth noting the PDE4 inhibitory potency of pyridine *N*-oxide **5q** in accordance with what has been reported in literature.[4,10]

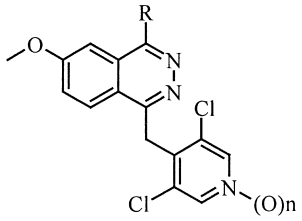
For all active derivatives an excellent selectivity for the catalytic binding site over the rolipram binding site compared to standards was retained with the bulky lipophilic **5l** as the most selective in this series. Such selective binding is claimed to be a potential property for overcoming the side effects often seen with potent PDE4 inhibitors.[9]

Preliminary studies to evaluate activity, potential side effects and pharmacokinetic properties of this novel series were performed comparing phthalazines **5k** and **5i** to standard. Ariflo (**4**) was chosen because it has been recently described as a second-generation inhibitor of PDE4 with a good bioavailability and a decreased potential for side effects.[11] Their in vitro metabolic stability,[12] oral bioavailability in the rat,[13] inhibition of eosinophil infiltration in the guinea pig,[14] ability to increase acid secretion in isolated whole rat stomach[15] and to induce emesis in the dog[16] are reported in Table 2.

Both the in vitro and the in vivo assays suggested an improved therapeutic potential for **5k** and **5i**. Stability in rat hepatocytes and promising bioavailability in the rat were encouraging for obtaining oral activity. Moreover **5k** and **5i** confirmed their activity in a guinea pig model of late-phase response to antigen. We were also very pleased to verify that, at the maximum dose tested, no sign of emesis was detected. Studies are in progress to verify if this favourable behaviour of **5k** and **5i** is shared among this series.

In conclusion, the synthesis and biological evaluation of a novel series of potent phthalazine PDE4 inhibitors has been reported, demonstrating that it is possible to

**Table 1.**



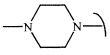
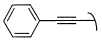

| Compound | R | n | PDE4 $IC_{50}$ (nM) | Rolipram binding $K_i$ (nM) | $TNF_\alpha$ $IC_{50}$ (nM) |
|---|---|---|---|---|---|
| **3** (Rolipram) | | | 1680 | 1.6 | 225 |
| **2** (RP 73041) | | | 1 | 1.5 | 1.3 |
| **4** (Ariflo) | | | 73 | 38 | 158 |
| **1** | | | 53 | 149 | 254 |
| **5a** | H | 0 | 0% ($10^{-7}$ M) | 42% ($10^{-5}$ M) | — |
| **5b** | *n*PrNH | 0 | 19% ($10^{-7}$ M) | — | — |
| **5c** | $(CH_3)_2N$ | 0 | 39% ($10^{-7}$ M) | — | — |
| **5d** |  | 0 | 132 | 452 | 62 |
| **5e** |  | 0 | 16% ($10^{-7}$ M) | — | — |
| **5f** |  | 0 | 38 | 89 | 9 |
| **5g** |  | 0 | 6% ($10^{-7}$ M) | — | — |
| **5h** |  | 0 | 17% ($10^{-7}$ M) | — | — |
| **5i** |  | 0 | 241 | 383 | 72 |
| **5j** |  | 0 | 132 | 436 | 166 |
| **5k** | Ph | 0 | 37 | 271 | 46 |
| **5l** |  | 0 | 48 | 21% ($10^{-6}$ M) | 273 |
| **5m** |  | 0 | 93 | 204 | 87 |
| **5n** | $C_2H_5$ | 0 | 23% ($10^{-7}$ M) | — | — |
| **5o** | $Ph(CH_2)_4$ | 0 | 19% ($10^{-7}$ M) | — | — |
| **5p** |  | 0 | 14 | 34 | 8 |
| **5q** |  | 1 | 4 | 17 | 3 |

**Table 2.**

| Compound | Met. stab. $Cl._{int}$ ($\mu L \times min^{-1} \times 10^6 cells^{-1}$) | Bioav. (rat) F% | Eosinophilia (guinea pig) % inhibition at 30 μmol/kg ip | Acid secretion $IC_{50}$ (μM) | Emesis (dog model) $ED_{50}$ (μmol/kg iv) |
|---|---|---|---|---|---|
| **4** (Ariflo) | 9.2 | 35 | 17 | 1 | 10 |
| **5k** | 5.8 | 27 | 34 | 4 | >30 (0/8) |
| **5i** | 1.5 | 27 | 37 | >30 (33%) | >30 (0/8) |

replace the cyclopentyloxy moiety of rolipram with a proper substituent in position 4 of the phthalazine ring. Further 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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12. Metabolic stability method: Fresh rat hepatocytes were obtained by in situ perfusion of the liver. The cellular vitality was >80%. Hepatocytes were incubated at 37 °C for up to 120 min in either Williams' E medium or Hanks' balanced salt solution at a concentration of $3–4\times10^6$ cells/mL of suspension. Compounds were dissolved in DMSO and used at different concentrations 1–100 μM (final concentration of DMSO = 1%). After liquid–liquid extraction or deproteinisation samples were submitted to HPLC/UV analysis. The metabolic stability of each compound was expressed as $Cl_{int}$ calculated as $V_{max}/K_m$.
13. In vitro dosing was performed at 3 μmol/kg as a sol in PEG200/DMSO 85/15. Oral dosing was performed at 10 μmol/kg in the same medium.
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16. Emesis method: Male and female beagle dogs (10–12 kg) were used. Test 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 treatment. The dose that induced emesis in at least 4 out of 8 animals has been determined.