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Phthalazine PDE4 Inhibitors. Part 3: The Synthesis and In Vitro Evaluation of Derivatives with a Hydrogen Bond Acceptor

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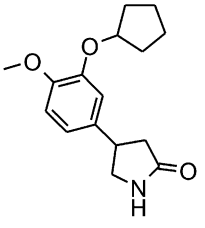
Abstract—This communication describes the synthesis and in vitro evaluation of a novel and potent series of phthalazine phosphodiesterase type (IV) (PDE4) inhibitors. The interaction with two distinct polar binding sites allowed us to eliminate the cyclopentyloxy substitution from rolipram-like analogues. © 2001 Elsevier Science Ltd. All rights reserved.

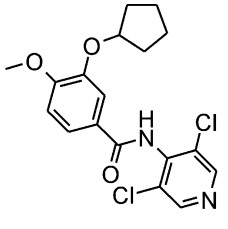
Phosphodiesterase type IV (PDE4) is a cAMP-specific phosphodiesterase highly expressed in inflammatory cells and in airway smooth muscle.[1] The observation that an elevation of cAMP in these cells can suppress inflammatory effects and can induce muscle relaxation has stimulated great interest in developing selective PDE4 inhibitors as therapeutic agents for asthma and other inflammatory diseases.[2]

With the three-dimensional structure of the active site only recently described,[3] PDE4 inhibitors have been designed mostly starting from the archetypal inhibitor rolipram (**1**) by replacing the pyrrolidone of **1** with other functionality.[4] Among the most potent analogues RP 73401 (**2**)[5] and GW 3600 (**3**)[6] present interesting and distinct pharmacophores for the pyrrolidone ring. It has been proposed that the acetyl carbonyl of **3** replaces the lactam carbonyl in rolipram (**1**)[6] and we wondered if the carbamic group could mimic the pyridine ring of **2** at a distinct binding site.

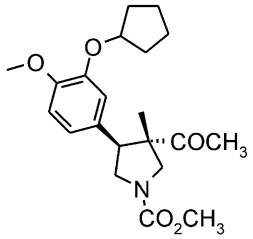
In order to explore this hypothesis we decided to add a binding moiety in our recently described class of phthalazine PDE4 inhibitors represented by **4**[7] and **5**[8] by means of a carbonyl function or a suitable hydrogen bond acceptor on the pyrazine ring as depicted in derivatives **6**.



Rolipram (**1**)



RP 73401 (**2**)



GW 3600 (**3**)

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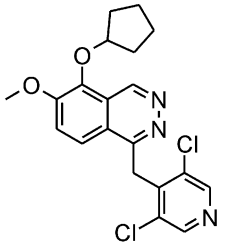

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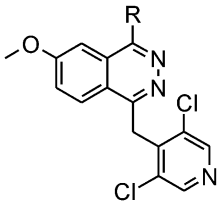

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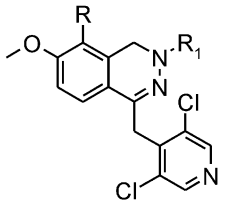


6

In fact, molecular modelling simulation of the overlay of **2**, **3** and **6a** (see Table 1) showed that proper substitution in position 3 of the phthalazine nucleus could fit with the carbonyl group of **3** (Fig. 1).[9]

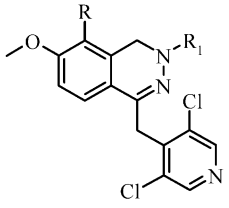
In implementing this modification, we hoped to increase enzyme inhibitory potency in order to eliminate the cyclopentyloxy group from the aromatic ring. Such substitution is commonly observed in rolipram-like PDE4 inhibitors[10] and its lipophilicity could contribute to the poor pharmacokinetic and metabolic profile often seen in this class of molecules.[11]

Chemistry

The synthesis of the requisite phthalazines is illustrated in Schemes 1 and 2. Catalytic hydrogenation of **4** (Scheme 1) gave dihydro derivative **7** chemo-selectively. Compound **7** slowly decomposes on standing, and was directly acylated with the proper reagent affording the target products **6a**,**b**.

Scheme 2 describes the strategy adopted for the synthesis of 5-unsubstituted phthalazine **6c**–**p**. *Ortho*-deprotonation of known protected acid **8**[12] and subsequent

Table 1.



General Structure

Compd	R	R_1	PDE4 IC_{50} (nM)	Rolipram binding K_i (nM)	RBS/PDE4 ratio
1 (Rolipram)			1680	1.6	0.001
2 (RP 73041)			1	1.5	1.5
Ariflo			73	38	0.5
4			53	149	2.8
6a		$COCH_3$	30	128	4.3
6b		SO_2CH_3	2	5	2.5
6c	H	H	14% (10^{-7} M)	13% (10^{-6} M)	
6d	H	COH	45% (10^{-7} M)	138	
6e	H	$COCH_3$	51	397	7.8
6f	H	$COCH_2CH_3$	28	237	8.5
6g	H	$COCH(CH_3)_2$	25	498	19.9
6h	H	COPh	30	17% (10^{-7} M)	>20
6i	H	$COCH_2Ph$	12	36% (10^{-7} M)	>20
6j	H	SO_2CH_3	21	60	2.9
6k	H	$COCOOCH_2CH_3$	113	212	1.9
6l	H	$COOCH_3$	72	199	2.8
6m	H	$CONH_2$	36	63	1.7
6n	H	$CONHCH_3$	131	235	1.8
6o	H	$CON(CH_3)_2$	35% (10^{-7} M)	473	
6p	H	CONHOH	85	208	2.4
13			0% (10^{-7} M)	42% (10^{-5} M)	

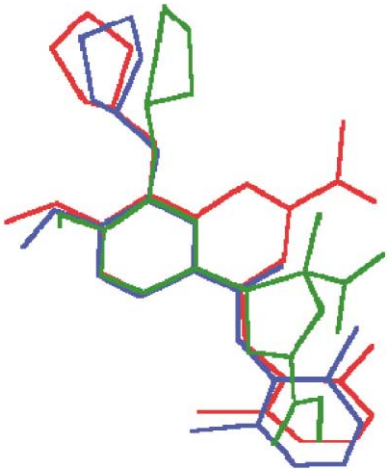


Figure 1. Overlay of **2** (blue), **3** (green), and **6a** (red). The overlay is based on RMS fitting of the respective catechol oxygens and the pyridine nitrogens of **2** and **6a** with the carbamic group of **3**.

formylation produced **9** in high yield. Acid hydrolysis forming **10** followed by hydrazine cyclisation provided phthalazinone **11** uneventfully. $POCl_3$ chlorination gave **12**, which upon treatment with the sodium salt of 3,5-dichloro-4-methylpyridine furnished intermediate **13**. Optimised condition for the catalytic hydrogenation resulted in stable **6c**, followed by treatment with proper reagents to access desired compounds **6d–p**.

Biological Results and Discussion

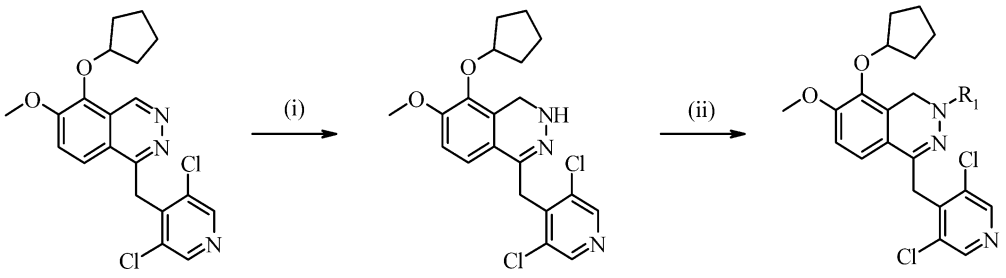
Table 1 summarises the in vitro activity of phthalazines with respect to human neutrophil PDE4 inhibition (IC_{50}, nM)[13] and association the high affinity rolipram binding site (K_i, nM).[14] Activity of the three standards was determined in-house using these procedures. Ariflo was added in comparison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

Disappointingly, only a slight variation in PDE4 catalytic activity was observed passing from **4** to **6a** but changing from the acetyl to the methanesulphonyl substitution (**6b**) the expected activity improvement was obtained. This result confirms the existence of two distinct binding sites in PDE4 enzyme that accommodate the pyridine ring and a polar substituent. In the rigid phthalazine scaffold, the tetrahedral sulphonyl group is probably better orientated to accept a hydrogen bond.

With these results in hand, we moved our attention to the 5-unsubstituted phthalazine derivatives. Compound **13** and dihydro derivative **6c** were devoid of activity confirming the importance that the cyclopentyloxy substituent plays in the PDE4 inhibition. We were pleased to verify that introducing a polar π-bond such as in **6e** and **6j**, potent PDE4 inhibitory activity was restored even in absence of the catechol cyclopentyl substitution. It is worth noting the reduced difference in activity between **6j** and **6e** compared to **6b** and **6a**. Probably the absence of the sterically demanding cyclopentyloxy in **6e** makes it possible to better accommodate within the binding pocket.

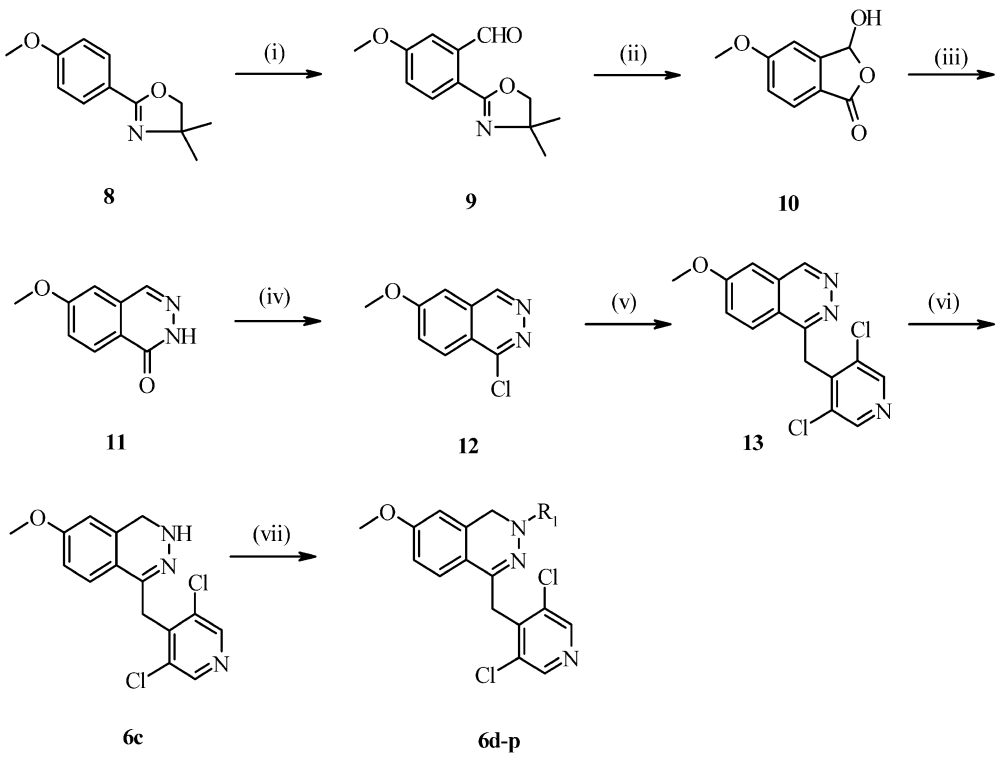
Following these results, a brief SAR study of substitution at R_1 was performed varying the acetyl moiety. Increasing the size and lipophilicity of the substituent, from formyl to phenylacetyl, **6d–i** gave gradual improvement in PDE4 inhibitory activity. A similar trend was observed for the selectivity for the catalytic binding site over the rolipram-binding site (RBS/PDE4 ratio). Such selective binding is claimed to be a potential property for overcoming the side effects often seen with potent PDE4 inhibitors.[16] Derivatives **6k–p** showed that other acyl substituents were allowed, with a drop of PDE4 inhibition increasing the substitution in the urea series (**6m–o**). No improvement of PDE inhibitory activity was produced by hydroxamic derivative **6p** in contrast with what has been reported in other series of rolipram analogues.[17]

In conclusion, the synthesis and the in vitro evaluation of a novel series of potent phthalazine PDE4 inhibitors has been reported, demonstrating that two distinct binding pockets exist in the PDE4 enzyme that accommodate the pyridine ring and a suitable hydrogen bond donor. The interaction with these two binding sites allowed us to eliminate the catechol substitution usually observed in rolipram analogues. Moreover, the new binding site can tolerate large lipophilic substituents, which leads to a great improvement in the RBS/PDE4 ratio. Studies that further characterise this class of molecules are in progress and the obtained result will be reported in due course.



4 **7** **6a, b**

Scheme 1. Reagents and conditions: (i) 4 atm H_2, PtO_2, THF, rt, 24 h, 75%; (ii) R_1Cl, Et_3N, CH_2Cl_2, rt, 1 h, 60–80%.



Scheme 2. Reagents and conditions: (i) BuLi, Et_2O, 0 °C, 4 h then DMF, rt, 8 h, 84%; (ii) 50% aq H_2SO_4, EtOH, reflux, 20 h, 90%; (iii) hydrazine hydrate, acetic acid, 80 °C, 4 h, 82%; (iv) $POCl_3$, CH_3CN, reflux, 3 h, 98%; (v) 3,5-dichloro-4-methylpyridine, NaH, DMF, rt, 20 h, 80%; (vi) 2 atm H_2, cat PtO_2, THF, rt, 22 h, 99%; (vii) for **6d**: formylimidazole, THF, rt, 24 h, 93%. For **6e–k**: R_1Cl, Et_3N, THF, rt, 3 h, 66–86%. For **6l–p** ($R_1 = R_2CO$): 1,1′-carbonyldiimidazole, THF, rt, 1 h then R_2H, rt, 3 h, 25–86%.

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