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The Selective Inhibition of Phosphodiesterase IV by Benzopyran Derivatives of Rolipram

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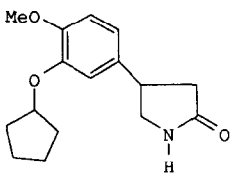
Abstract: A series of benzopyran derivatives of rolipram has been prepared, one of which, **8a**, proved to be a potent inhibitor of the PDE IV isoenzyme. The enantiomers of **8a** were separated and activity shown to reside mainly in the (+) enantiomer. These novel compounds display much reduced activity on the high affinity form of PDE IV relative to rolipram.

Inactivation of cyclic nucleotides (cAMP and cGMP) is known to be catalysed by at least five major classes of phosphodiesterase isoenzymes (PDE I-V) which have been characterised on the basis of their substrate specificity, sensitivity to selective inhibitors and enzyme kinetics.[1] While the role of each of these isoenzymes in specific tissues or cells is often complex, the inhibition of PDE IV in particular constitutes an attractive therapeutic target since studies in human tissues demonstrate that this is the predominant functional isoenzyme in many inflammatory cells.[2,3] Inhibitors of PDE IV may be of particular value in the treatment of diseases such as asthma, in which there is increasing awareness of an underlying inflammatory involvement, and for which theophylline, a non-specific PDE inhibitor, has found extensive use.[4] Theophylline, however, suffers from dose-limiting side-effects, some of which may inevitably arise as a result of its poor PDE isoenzyme specificity.[5]



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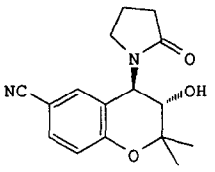

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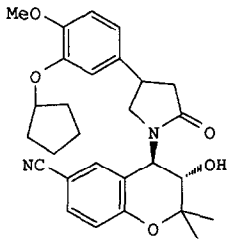

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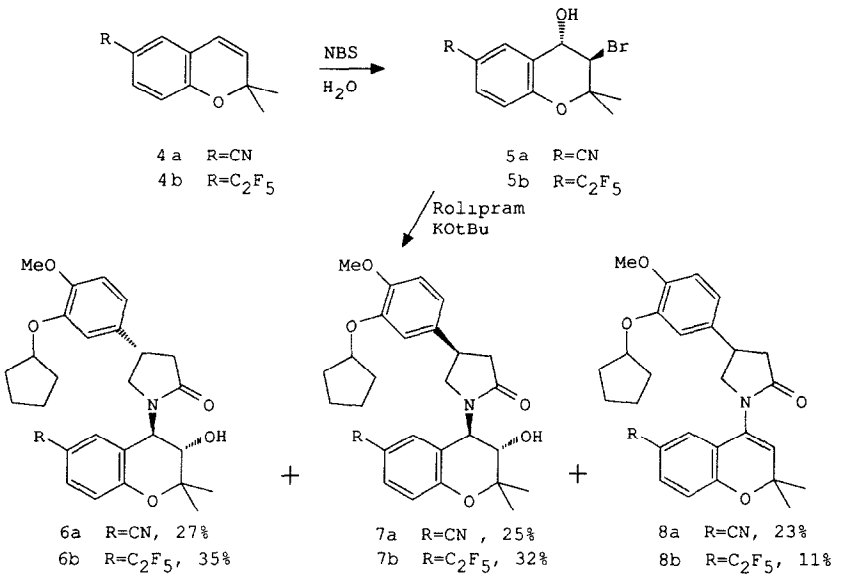
A number of selective inhibitors of PDE IV have been reported[6] but rolipram **1** was chosen as a starting point for subsequent modification since it offered the opportunity for synthetic flexibility. Moreover, from the known rolipram SAR[7] it was anticipated that molecules embodying enhanced bronchodilator characteristics could be devised by constructing molecular hybrids with the potassium channel activator cromakalim **2**.[8] Thus, compounds such as **3** satisfy not only the SAR requirements of rolipram for PDE IV inhibitory activity, but also the requirements of the benzopyran-based potassium channel activators for bronchodilator activity.[7,8]

The synthesis of the target compounds **3** from the key intermediate benzopyrans **4** is illustrated in Scheme 1. In the case of the 6-cyano analogue, 6-cyanobenzopyran was first converted into the

corresponding bromohydrin **5a** by reaction with moist N-bromosuccinimide[9] which on subsequent treatment with racemic rolipram in the presence of potassium t-butoxide yielded a mixture of three compounds[10] which were readily separated by chromatography. Of these, two were identified as the diastereoisomers **6a** and **7a**, epimeric at the aryl substituent on the pyrrolidinone ring, for which the relative configuration was established by detailed nOe experiments, and the third was the anhydro product **8a**. In the case of **6a**, the presence of a mutual nOe between H5 and H5'b and one between H5'a and H3 was indicative of a preferred conformation having the pyrrolidinone ring orthogonal to the benzopyran moiety and the carbonyl group *syn* to H4. Such a conformation is consistent with that previously found for cromakalim and related derivatives.[11,12] Mutual nOe's were also noted between H5 and H11' and between H5 and H7', indicating a close spatial relationship of the two aromatic rings and, by implication, providing evidence for the relative configuration at C-4 and C-4' as shown (Figure 1).



Scheme 1

A similar analysis of the nOe data from **7a** indicated a close spatial proximity for H3 and H5', but in this instance it was not possible to distinguish between H5'a and H5'b due to overlapping chemical shifts. The absence of a nOe between H4 and H5', suggested that the preferred conformation again positioned the pyrrolidinone ring orthogonal to the benzopyran moiety with the carbonyl group *syn* to H4. The mutual nOe between H5 and H4' dictated that the relative stereochemistry at C-4 and C-4' was that shown in Figure 1.

Like rolipram, the anhydro compound **8a** was a potent inhibitor of guinea-pig cardiac ventricle PDE IV with an IC_{50} = 1 μM (Table 1). Although considerably less potent, the benzopyranol **7a** also showed significant inhibition of PDE IV, but the diastereoisomer **6a**, by contrast, was devoid of activity at concentrations < 100 μM. No significant inhibition of PDE I, II, III or Va isoenzymes was noted with any of the compounds. Rolipram has also been shown to bind to specific high affinity binding sites in the brain, although the relationship of this binding site to the catalytic site is not fully understood.[13] Studies with human recombinant PDE IV, however, suggest that both the binding and catalytic sites are properties of the same protein.[14] We have consequently evaluated compounds for their ability to displace [^{3}H]-

rolipram from its high affinity binding site in rat cortex and shown that unlike rolipram itself, compound **8a**, which is of equivalent potency as an inhibitor of PDE IV, is some thirtyfive times less potent at the high affinity binding site (Table 1). Similarly, neither **6a** nor **7a** showed significant inhibition of rolipram binding at concentrations < 10 μM. While the relevance of the high affinity binding site has still to be established, the different spectrum observed with compound **8a** prompted its enantiomeric separation by chiral hplc.[15] The (+)-enantiomer **9** was subsequently shown to be approximately sixfold more potent than the (-)-enantiomer **10** as an inhibitor of PDE IV, but to be little different in its affinity for the rolipram binding site (Table 1).

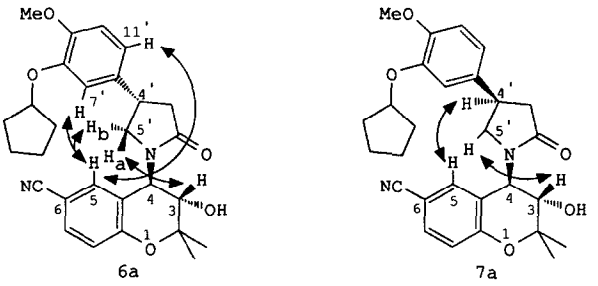


Figure 1 nOe Interactions Observed in Compounds **6a** and **7a**

Potassium channel activators have been shown to evoke potent relaxation of spontaneously induced tone in guinea-pig isolated trachealis and this model provides a useful primary screen for such activity.[8] None of the compounds **6a**, **7a** and **8a** showed any useful relaxant activity when evaluated in this model, however, suggesting an inability to effect potassium channel opening at concentrations < 20 μM. Since the 6-pentafluoroethyl group has been shown to enhance both the potency of potassium channel activators and their selectivity towards airway smooth muscle[16] the pentafluoroethyl analogues **6b**, **7b** and **8b** were prepared from 6-(pentafluoroethyl)benzopyran **4b** following a similar sequence of reactions to those described above (Scheme 1). In this instance, however, slightly improved yields of the two diastereoisomeric products **6b** and **7b** were isolated at the expense of the dehydrated product **8b**, and structural assignments were made by comparison of their NMR spectra with those of the respective 6-cyano compounds. Somewhat surprisingly, none of the pentafluorinated compounds **6b**, **7b** and **8b** elicited inhibitory activity on PDE IV at the concentrations tested, the reasons for this loss of activity are not understood. Moreover, none of these compounds evoked a significant relaxation of spontaneous tone in guinea-pig trachealis, indicating that they lacked the ability to open potassium channels.

Table 1

	PDE IV Inhibition IC_{50} μM	Rolipram Binding IC_{50} μM
Rolipram **1**	1 ± 0.2	0.007
6a	>100	>10
7a	18 ± 7	>10
8a	1 ± 0.1	0.25
9	2 ± 0.8	0.58
10	12 ± 5	0.46

Since the NMR experiments described above confirmed the optimal spatial relationship of the pyrrolidinyl function to that of the benzopyran nucleus for potassium channel activating activity[11,12], it seems improbable that inactivity arises through an inappropriate conformational disposition of these two features in compounds **6a**, **7a**, **6b** and **7b**, although it is conceivable that this may be the case with the anhydro derivatives **8a** and **8b**. Although not proven, it is more likely that the large C-4' aryl substituents present in compounds **6**, **7** and **8** cannot be accommodated within the active site at which the potassium channel activators interact.

In conclusion we have found that the bulky N-(6-cyanobenzopyran-4-yl) substitution (**8a**) does not interfere with the ability of rolipram to inhibit PDE IV and that the predominant activity resides in the (+)-enantiomer **9**. Both **8a** and **9** show a reduced affinity for the high affinity rolipram binding site in rat brain tissue relative to rolipram itself, the implications of which remain to be determined.

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