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DISCOVERY OF MICROMOLAR PDE IV INHIBITORS THAT EXHIBIT MUCH REDUCED AFFINITY FOR THE [^{3}H]ROLIPRAM BINDING SITE: 3-NORBORNYLOXY-4-METHOXYPHENYLMETHYLENE OXINDOLES.

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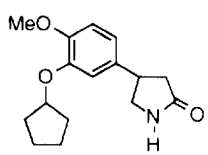
Abstract: The synthesis and the *in vitro* properties of a novel series of potent and selective phosphodiesterase type IV (PDE IV) inhibitors is described. Despite bearing structural similarity to rolipram, several of these compounds have much reduced affinity for the [^{3}H]rolipram binding site.

The inhibition of phosphodiesterases for the treatment of asthma is a well recognized mechanistic approach, and indeed, theophylline, a non-specific PDE inhibitor, has long been a major drug of choice for this disease.[1] Over the last decade, the isolation of at least five subtypes of PDE, classified on the basis of substrate specificity, and their differential expression in various cell types has provided the opportunity to develop more selective agents.[2] In particular, the inhibition of type IV (PDE IV) has emerged as an exciting new area of research for the treatment of asthma which is anticipated to provide the desired antiinflammatory activity without the cardiovascular side effects associated with the inhibition of PDE III.[3,4] Much of the work in the PDE IV area,[5] including our own CP-80,633 **2**,[6] has used rolipram **1** as a starting point for SAR development. Rolipram is a micromolar PDE IV inhibitor (IC_{50} = 3.45 ± 0.91 μM, n = 7) against human PDE IV isolated from human lung tissue and binds to a high-affinity site in mouse brain homogenates (IC_{50} = 0.004 ± 0.002 μM, n= 73).[7] This binding site is known as the [^{3}H]rolipram binding site and has been shown to be associated with the same protein as the PDE IV catalytic site.[8] However, except for a limited number of PDE inhibitors, the SARs for these two sites do not appear to be correlated and the functional relationship between these two is not fully understood.[3b] Nevertheless, rolipram is roughly 1000X more potent at binding to its high affinity binding site than it is at binding to the PDE IV catalytic site. Although the physiological significance of this [^{3}H]rolipram binding site is not clearly understood, it has been associated with emesis and nausea which is commonly associated with PDE IV inhibitors.[9]



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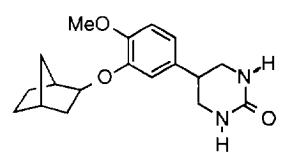

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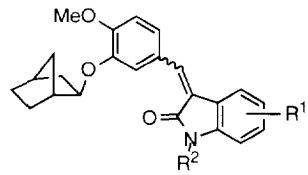
The known PDE IV inhibitors are either combined PDE III/PDE IV inhibitors,[10] or have high affinity for the [^{3}H]rolipram binding site,[3b] often with 100 to 1000 fold greater affinity than that

exhibited for the PDE IV catalytic site. Since we believed that a selective PDE IV inhibitor would indeed offer improved therapy with reduced side effects, we accordingly undertook a synthetic program to identify compounds that would have potent PDE IV inhibitory activity but that would not exhibit affinity for the [^{3}H]rolipram binding site,[11] nor exhibit PDE III inhibitory activity. We found by changing the pyrrolidone ring to an oxindole ring and then modifying the substituents on the oxindole, we were able to achieve the desired pharmacological profile.



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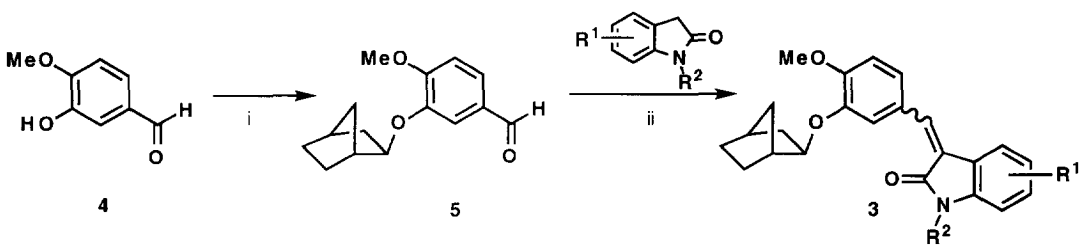
The general scheme for making these compounds is illustrated in Scheme I. Treatment of isovanillin with *endo*-2-norborneol under Mitsunobu conditions provided 4-methoxy-3-norbornyloxy-benzaldehyde **5**[12] in 44 % yield. This material was then subjected to an aldol reaction followed by *in situ* dehydration (pyrrolidine/methanol at room temperature) with a variety of substituted oxindoles. The products were then separated into their *E* and *Z* isomers by column chromatography to yield the geometrically pure title compounds **3**.

Scheme I



i. endo-2-norborneol, triphenylphosphine, DEAD, THF, reflux 48 h, 44%; ii. methanol, pyrrolidine.

Since our goal was to develop a potent and selective PDE IV inhibitor, the *in vitro* profile that we sought consisted of an IC_{50} less than 5.0 μM in the PDE IV binding assay,[13] an IC_{50} greater than 100 μM in the PDE III binding assay, and an IC_{50} greater than 50 μM in the [^{3}H]rolipram binding assay.[14]

Our first compound in this structural class, **6** (Table I), was a very exciting lead. Although it did not meet the selection criteria, it was the first time that we had observed a PDE IV inhibitor with the selectivity in the direction that we were seeking (ten fold greater for PDE IV over rolipram binding). It is interesting to note that the regioisomer, **7**, did not demonstrate this selectivity.[15]

The chemistry follow-up plan involved the synthesis of a variety of analogs, varying the substitution at all the positions around the phenyl ring of the oxindole functionality; varying the substitution on the nitrogen of the oxindole ring; as well as heterocyclic replacement of the phenyl

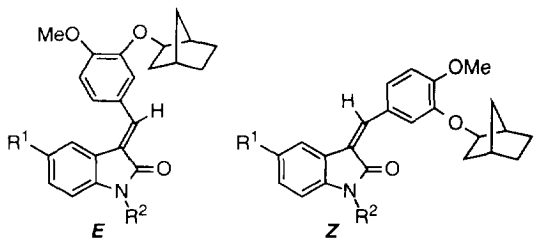


Table I. Pharmacological Profile of Unsubstituted Catechol Oxindoles[a]

Cpd	R^1	R^2	Stchm	PDE IV IC_{50} (μM)	[^{3}H] Rolipram IC_{50} (μM)	PDE III IC_{50} (μM)
6	H	H	E	0.48	4.6	42% @ 100
7	H	H	Z	3.7	3.2	100

[a] All values are individual determinations. See footnotes for variability of reference compounds for each assay.

ring of the oxindole ring. It soon became clear from the extensive SAR in this series that the PDE IV inhibitory activity and [^{3}H]rolipram binding do not correlate, even within this class of relatively closely related structures.[16,17] This lack of correlation had been noted earlier[3b] and implies that the architecture of the [^{3}H]rolipram binding site is not identical to the enzyme inhibitory site of the PDE IV enzyme. The ultimate results of this SAR development were the discoveries that (1) electron withdrawing groups in the 5 position (R^1) confer the desired pharmacological profile, (2) that the Z configuration is in general preferred, and (3) that nothing larger than a methyl group is tolerated on the oxindole nitrogen. Representative compounds from this series which best meet the targetted criteria are illustrated in Table II, along with rolipram **1** for comparison.[18]

Table II. Pharmacological Profile of Substituted Catechol Oxindoles[a]

Cpd	R^1	R^2	Stchm	PDE IV IC_{50} (μM)	[^{3}H] Rolipram IC_{50} (μM)	PDE III IC_{50} (μM)
8	Br	H	Z	1.7	50	17% @ 100
9	NO_2	H	Z	1.2 ± 0.5 (3)	62	18% @ 100
10	Cl	Me	Z	3.4 ± 2.4 (3)	58	-9% @ 100
11	Br	Me	Z	3.6	57	-7% @ 100
12	NO_2	Me	E	7.1	66	25% @ 100
1	na[b]	na	na	3.5 ± 0.9 (7)	0.004 + 0.002 (73)	23 % @ 100

[a] All values are individual determinations, unless otherwise indicated. See footnotes for variability of reference compounds for each assay. [b] Not applicable.

In summary, it is clear that these oxindoles are invaluable tools to chemically explore not only the active site of the PDE IV enzyme and the [^{3}H]rolipram binding site and the interrelationship

between the two, but also to evaluate what significance these unique pharmacological profiles have on the efficacy and side effect profile of drugs for the treatment of asthma.

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13. The method of determining PDE activity was similar to the procedure reported by Reeves, M. L.; Leigh, B. K.; England, P. J. (*Biochem. J.* 1987, *241*, 535) using human lung PDE IV. Data on reference standards, CP-76,593 (the racemate of CP-80,633 **2**) and milrinone (Harrison, S. A.; *Mol. Pharmacol.* **1986**, *29*, 506), illustrate the confidence limits for the PDE IV and PDE III screens, respectively. For the PDE IV assay, CP-76,593 gave an IC_{50} of 1.5 ± 1.2 μM (n = 150). For the guinea pig PDE III assay, milrinone gave an IC_{50} of 8.5 ± 2.5 μM (n = 3). For the human PDE III assay, milrinone gave an IC_{50} of 3.3 ± 1.3 μM (n = 41). Statistics were done with a Student's *t* test.
14. [^{3}H]Rolipram Binding Assay. Fresh mouse brains were homogenized in 20 volumes of ice-cold 50 mM Tris•HCl (pH = 8.0) buffer containing 5 mM $MgCl_2$ in a Polytron PT-10 homogenizer (Brinkman Instruments). The resulting homogenate was centrifuged at 30,000 x g for 20 min at 4 °C. The pellet was washed by resuspension in 20 volumes of fresh buffer and recovered by centrifugation as before. The final pellet was suspended in Tris buffer (0.5 mg protein/ml) for binding experiments. Incubation mixtures in duplicates consisted of 0.1 ml (±)-[^{3}H]rolipram (2-8 nM final), 0.01 mL inhibitor and 0.9 mL membrane preparation. Rolipram (10 μM) was used for non-specific binding. After 60 min incubation at 4 °C, the contents of the incubation tubes were filtered through a Whatman GF/C glass filter paper. The membranes were washed 3 times with 3 mL ice-cold buffer, and radioactivity on the separated filter disks was determined in a liquid scintillation counter. Data on the reference standard, rolipram, illustrate the confidence limits for the [^{3}H]rolipram binding assay. For this screen, rolipram gave an IC_{50} of 0.004 ± 0.002 μM (n = 73). Statistics were done with a Student's *t* test.
15. There is no E/Z isomerization in vitro.
16. In contrast, see reference 12.
17. For instance, the correlation coefficient value of PDE IV vs. [^{3}H]rolipram for one group of analogs is 0.121.
18. Full SAR papers on these oxindoles will be reported shortly.

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