Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 3229–3234

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

N-ARYLROLIPRAM DERIVATIVES AS POTENT AND SELECTIVE PDE4 INHIBITORS

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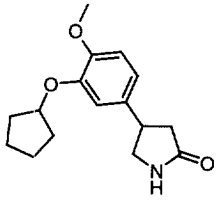
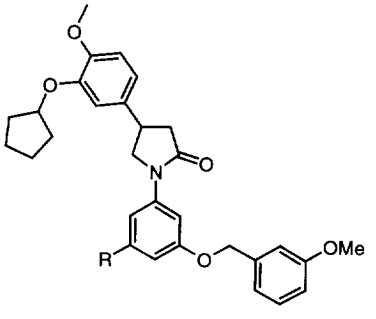
Received 12 August 1998; accepted 6 October 1998

Abstract: Derivatization of rolipram led to the identification of 3-[4-(3-cyclopentyloxy-4-methoxyphenyl)-2-oxo-pyrrolidin-1-yl]-5-(3-methoxybenzyloxy)-benzoic acid *N',N'*-dimethylhydrazide (**4**), a potent and selective inhibitor of PDE4, which inhibits the activation of human leukocytes with pIC_{50} values in the range of 7.3 - 7.8, and blocks antigen induced eosinophilia in Brown Norway rats at a dose of 1 mg/kg (i.t.).

Modulation of cyclic adenosine-3,5-monophosphate (cAMP) has emerged as one of the most promising approaches in the search for new anti-asthma drugs.[2] Cellular levels of this important second messenger are regulated by phosphodiesterase enzymes which hydrolyze cAMP to inactive metabolites. While seven subtypes of the PDE family are known, recent observations have shown that phosphodiesterase 4 (PDE4) is the principle regulator of cAMP in inflammatory cells.[3] Furthermore, several research groups have reported evidence that selective PDE4 inhibitors suppress inflammatory mediator release from activated leukocytes and inhibit the recruitment of inflammatory cells into the airways of animals.[2,4]



Rolipram **1**



R = H **2**
R = COOH **3**
R = $CONHNMe_2$ **4**

Rolipram (**1**), the prototypical, selective PDE4 inhibitor has been used by many research groups as a starting point in their search for proprietary drug candidates.[5-8] Although this has lead to the identification of several

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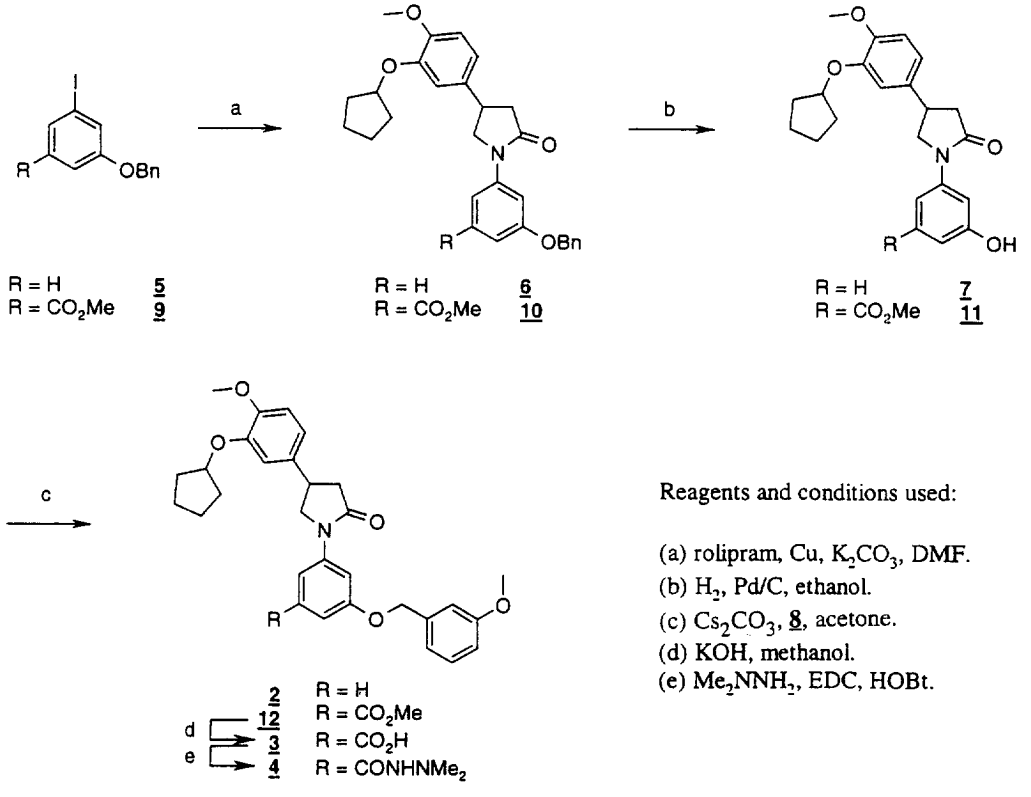
0960-894X/98/$ - see front matter
PII: S0960-894X(98)00583-6

development compounds, the search for anti-inflammatory agents which can replace inhaled corticosteroids as first line anti-asthma treatment is continuing.[9]

Our effort has been aimed at finding rolipram derivatives, suitable for inhalation, which show improved PDE4 potency while maintaining selectivity versus PDE3. In theory this should allow for anti-inflammatory action in the lung, while at the same time reducing the chance for side effects by limiting systemic exposure. In contrast to other research groups which have mainly focused on modifications at either the aromatic catechol ether or the pyrrolidone ring of rolipram,[5-8] we have concentrated our chemistry program on *N*-phenyl-rolipram derivatives. In this communication we describe the synthesis and biological profile of the racemic compounds **2**, **3** and **4** which are highly potent and selective PDE4 inhibitors and show very promising activity in an animal model of airway inflammation.

Compound **2** was prepared conveniently in three steps from 3-benzyloxy-iodobenzene (**5**). Copper catalyzed coupling[10] of **5** with rolipram gave **6** (81%), which was converted to the target molecule by hydrogenolysis of the benzyl ether protecting group, followed by alkylation of the resulting phenol **7** with 3-methoxybenzylchloride (**8**) in the presence of cesium carbonate (Scheme 1).



Scheme 1

4-(3-cyclopentyloxy-4-methoxy-phenyl)-1-[3-(3-methoxy-benzyloxy)-phenyl]-pyrrolidin-2-one (**2**) proved to be an excellent inhibitor of the PDE4 isozymes,[11] being about two orders of magnitudes more potent than rolipram (Table 1). Like rolipram, **2** exhibits little selectivity between the isozymes, being equipotent (within the error of the experiments) on PDE4A, B and D. Despite the increase in potency on the PDE4 isozymes, **2** remained virtually inactive as an inhibitor of PDE3.

While compound **2** was a very promising lead compound, it exhibited variable activity in secondary, cellular assays and could not be administered to animals due to its highly hydrophobic nature.[12] In order to improve the physical properties of the *N*-phenyl roliprams we introduced hydrophilic substituents in the 5-position of the central phenyl ring in **2**. Structure activity studies had indicated that this position could accommodate a wide variety of substituents without deleterious effects on PDE4 potency.

The synthesis of **3** and **4** is shown in Scheme 1. Methyl 5-hydroxy-3-iodobenzoate[13] was benzylated to give **9** and then coupled in the usual way with rolipram. Removal of the benzyl protecting group of **10**, reaction of 3-methoxybenzylchloride with the resulting phenol **11** (51% for two steps) and hydrolysis of the methyl ester gave 3-[4-(3-cyclopentyloxy-4-methoxyphenyl)-2-oxo-pyrrolidin-1-yl]-5-(3-methoxybenzyloxy)-benzoic acid (**3**). As our hypothesis had suggested, the newly introduced carboxyl group was well tolerated (Table 1), and as expected the compound showed improved physicochemical characteristics, being appreciably soluble in bicarbonate solution as well as in aqueous ethanol.[14] Further derivatization culminated in the synthesis of compound **4** (Scheme 1), a highly potent inhibitor with nanomolar activity on all PDE4 isozymes and over 10000 fold selectivity versus PDE3 (Table 1).

Table 1. Inhibition of PDE4 isozymes and PDE3[a,15]

Compound	PDE4A	PDE4B	PDE4D	PDE3	rolipram binding[16]
rolipram (**1**)	5.9 (± 0.2)	5.7 (± 0.3)	6.4 (± 0.1)	< 4	8.4 (± 0.2)
2	8.2 (± 0.4)	8.6 (± 0.2)	8.1 (± 0.2)	< 4	8.1 (± 0.3)
3	7.9 (± 0.1)	7.7 (± 0.2)	7.9 (± 0.2)	< 4	7.5 (± 0.3)
4	8.6 (± 0.6)	8.3 (± 0.2)	8.8 (± 0.6)	< 4.	8.3 (± 0.2)

[a] Data indicated as pIC_{50} values (± SEM) ; n = 2-6.

PDE4 enzymes seem to exist in two different states, distinguishable by either low or high affinity for rolipram.[17] While the precise biological role of the different forms of the PDE4 enzymes is unclear, there have been suggestions that the activity of inhibitors at the high affinity site may correlate with their potential to

exhibit side effects.[18] Compared to rolipram, compounds **2**, **3** and **4** have improved potency as inhibitors of the isozymes, while the activity at the high affinity binding site remains the same.

With two promising candidate compounds in hand, we proceeded to evaluate their anti-asthma potential in a number of cellular assays. There is convincing evidence that selected T cell populations play a key role in the orchestration of the asthmatic inflammation by the production of a characteristic cytokine pattern in response to inhaled allergens and other stimuli.[19] IL-4 and IL-5 produced by these allergen specific TH2 cells are intimately involved in the regulation of IgE production as well as in the development, activation and selective accumulation of eosinophils, two characteristic features of allergic asthma. Besides TH2 cells, eosinophils themselves are major pro-inflammatory cells in asthma, producing reactive oxygen species and releasing cationic proteins which are thought to be prime contributors to epithelial cell damage and increased hyperreactivity of asthmatic airways.

Based on this knowledge we chose representative assay systems, which would allow us to study the effect of our *N*-phenyl rolipram derivatives on primary human cells. In an attempt to mimic allergen induced T cell activation events, human peripheral blood mononuclear cells (HPBMC) were stimulated with anti-CD3 antibodies, known to induce T-cell proliferation as well as cytokine release. Both compounds **3** and **4** potently inhibited proliferation and the release of a characteristic TH2 (IL-4) as well as TH1 cell cytokine (IFN-γ) from this key effector cell (Table 2). Moreover, stimulation of HPBMC with lipopolysaccharide (LPS) leads to production of TNF-α from monocytes/macrophages, which was also suppressed by our new rolipram analogues (Table 2). Finally both of our drug candidates showed an improved profile compared to rolipram in inhibiting fMLP (N-formyl-Met-Leu-Pro-OH) induced oxidative burst from human eosinophils (Table 2).

Table 2. Inhibition of human leukocyte activation[a]

Compound	human peripheral blood mononuclear cells				human eosinophils
	anti-CD3 induced T-cell proliferation[20]	anti-CD3 induced IL-4 release[20]	anti-CD3 induced IFN-γ release[20]	LPS induced TNF-α release[21]	fMLP induced oxidative burst[22]
rolipram	5.7 (± 0.2)	6.3 (± 0.5)	5.6 (± 0.6)	7.1 (± 0.2)	6.1 (± 0.2)
3	7.2 (± 0.2)	6.6 (± 0.4)	7.5 (± 0.1)	7.4 (± 0.4)	6.6 (± 0.2)
4	7.3 (± 0.2)	7.6 (± 0.4)	7.8 (± 0.3)	7.7 (± 0.2)	7.3 (± 0.3)

[a] Data indicated as pIC_{50} values (± SEM) ; n = 2-7.

Especially compound **4** is a remarkably potent and balanced inhibitor, being equipotent in modulating the proliferation of T cells, blocking the release of inflammatory cytokines from T cells and monocytes as well as

inhibiting the oxidative burst from eosinophils, with pIC_{50} values ranging from 7.3 to 7.8 (Table 2).

To analyze the potential anti-asthma activity of our *N*-phenyl-rolipram derivatives *in vivo*, we tested the compounds in an animal model of antigen-induced pulmonary eosinophilia.[23] For this purpose, ovalbumin (OA) sensitized brown Norway rats were challenged with aerosolized antigen (OA), which resulted in a selective accumulation of eosinophils as well as the release of increased levels of the eosinophil granule derived eosinophil peroxidase (EPO) in bronchoalveolar lavage (BAL) samples obtained from these animals (Fig.1). Both of our candidate compounds, when administered 1h before and 24h after challenge by the intratracheal route as suspensions in saline, significantly inhibited both measures of airway inflammation. Figure 1 shows the effect of **3** and **4** on antigen induced eosinophil recruitment and activation at a dose of 3.2 and 1 mg/kg respectively.

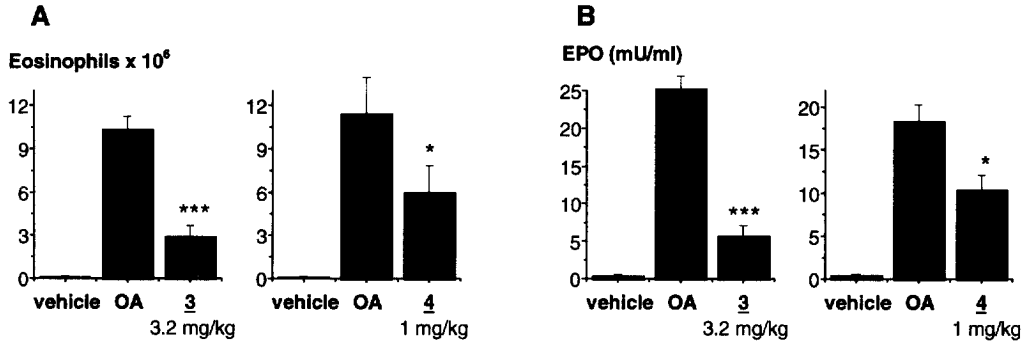


Figure 1. Effect **3** (3.2 mg/kg) and **4** (1 mg/kg) on ovalbumin induced eosinophil accumulation (A) and eosinophil activation (B) in actively sensitized brown Norway rats. * $p<0.05$, ***<0.001 indicates significant difference by comparison with vehicle and ovalbumin (OA) treated animals, n = 5.

In conclusion, we have identified a new class of rolipram derivatives which are highly potent inhibitors of human leukocyte activation and show interesting activity in animal models of airway inflammation. We are currently synthesizing several compounds of this class in enantiomerically pure form with the aim of further studying their potential as anti-asthma drugs .

References and Notes

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14. 3 mg **3** could be dissolved in 1 mL 1M $NaHCO_3$ solution and then diluted with buffer as required. 2 mg **4** could be dissolved in 0.2 mL of ethanol and then diluted as required with buffer.
15. PDE4 activity was assessed as previously described.[24] With the exception of PDE4B[25] (rat; expressed in *S. cerevisae*), isozyme preparations were from human sources: PDE4A,[26] PDE4D[27] expressed in *S. cerevisae*.
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20. Mononuclear cells (MNC) were isolated from blood of normal individuals by Ficoll-hypaque gradient centrifugation. Cell density was adjusted to $1x10^6$ cell/mL. 100 µL of the MNC suspension was placed in 96 well culture plates and 50 µL of either medium or compounds were added. After a 10 min preincubation, cells were stimulated with 50 µL of anti-CD3 monoclonal antibodies (OKT-3, 100 ng/mL), incubated for either 24 h (cytokine production) or 48 h (proliferation assay) at 37°C in a humidified incubator with 5% CO_2. To determine the proliferative response of anti-CD3 stimulated MNCs, [^{3}H]-thymidine was added to the culture plates for the last 6 h of the incubation period and incorporated radioactivity was measured in an automated liquid scintillation counter. Cytokines were measured in supernatants harvested after 24 h of incubation. IL-4 and IFNγ were measured by sandwich ELISA using two monoclonal antibodies recognizing different epitopes of the specific cytokine. Antibodies used for measuring IL-4 and IFN-γ were purchased from Mabtech, Stockholm, Sweden. The sensitivity of the cytokine ELISAs was around 10 pg/mL.
21. Cell were stimulated with LPS (1µg/mL) and IFNγ (10 ng/mL) and supernatants were harvested after 24h of incubation at 37°C in a humidified incubator with 5% CO_2. Concentration of TNFα in these supernatants was measured by sandwich ELISA using two monoclonal antibodies recognizing different epitopes of the specific cytokine (mAb357/101-4 and biotinylated 2-179/E11).
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