Synthesis and Structure–Activity Relationship of *N*-Arylrolipram Derivatives as Inhibitors of PDE4 Isozymes

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Structure activity studies of *N*-phenylrolipram derivatives have led to the identification of highly potent PDE4 inhibitors. The potential of these inhibitors for cellular activity was routinely assessed in an assay of fMLP induced oxidative burst in human eosinophils. Since first generation PDE4 inhibitors have been plagued with a number of unwanted side effects, parallel structure activity studies for competition with the [^{3}H]-rolipram binding site in rat brain were performed. In this fashion 5-[4-(3-cyclopentyloxy-4-methoxyphenyl)-2-oxo-pyrrolidin-1-yl]-3-(3-methoxybenzyloxy)benzoic acid *N′*,*N′*-dimethylhydrazide (22) was identified as a potent inhibitor of PDE4 which exhibits >1000 fold selectivity *versus* PDE3, and is a nanomolar inhibitor in all the cellular assays tested. Studies on the stereoselectivity of PDE4 inhibition of this class of rolipram based compounds revealed, that for example (*S*)-11 is a more potent inhibitor than (*R*)-11. This effect can also be observed in primary human cells where the (*S*)-enantiomer is about 10 fold more potent than the corresponding (*R*)-enantiomer.

Key words Rolipram; PDE inhibition; *N*-arylrolipram derivative; rolipram binding

In past years we have seen a major shift in the perception of asthma as a complex disease characterized by a combination of reversible obstruction, hyperreactivity and chronic inflammation of the airways rather than simply in terms of disordered smooth muscle function.[2)] While all three of these pathological features of asthma are important, it is now widely accepted that controlling inflammation is the most promising approach for the treatment of chronic disease.[2)] Accordingly, treatment regimens have been adjusted to include inhaled steroids as first line therapy even for mild disease.[3)] However, despite our improved understanding of the underlying cellular events responsible for the progression of asthma and the widespread use of anti-inflammatory therapy, morbidity and mortality due to asthma have continued to increase.[4)] As a consequence, alternative strategies to inhibit the chronic inflammation in asthmatic airways have been investigated with the aim of supplementing or replacing corticosteroids.

Modulation of cyclic adenosine-3,5-monophosphate (c-AMP) has emerged as one of the most promising approaches in the search for new anti-asthma drugs.[5)] Cellular levels of this important second messenger are regulated by phosphodiesterase enzymes (PDE) which hydrolyze c-AMP to inactive metabolites. While seven subtypes of the PDE family are known, recent observations have shown that phosophodiesterase-4 (PDE4) is the principle regulator of c-AMP in inflammatory cells.[6)] 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.[5,7)]

Due to the presence of the enzymes in immune and inflammatory cells and the biological role of c-AMP in these cells, PDE4 inhibitors have broad anti-inflammatory effects *in vitro* and *in vivo*,[5,7)] however their wide distribution in the body has also raised concerns about dose limiting side effects.[8)] Clinical studies with rolipram and studies on animals with a number of selective PDE4 inhibitors have shown that the most likely side effects are nausea and vomiting and increased gastric acid secretion.[8)] Accordingly the focus of current research effort is aimed at identifying PDE4 inhibitors with improved side effect profile compared to first generation compounds like rolipram, denbufyllin or even theophyllin which has been claimed to owe its clinical efficacy to PDE4 inhibition.[9)]

Rolipram (**1**), the prototypical, selective PDE4 inhibitor has been used by many research groups as a starting point in their search for potent and selective PDE4 enzyme inhibitors.[10—14)] This has led to the identification of a number of clinical candidates of which RP-73401 (**2**) and SB-207499 (**3**) are the best known representatives. While **3** is currently in phase III clinical studies in both asthma and COPD,[15,16)] the development of compound (**2**) in the asthma indication seems to have been abandoned.

We report herein the structure activity of a new class of rolipram derived PDE4 inhibitors, which were designed to supplement or, ideally, replace corticosteroids as local anti-inflammatory drugs. As a consequence they were designed for delivery by inhalation. In theory this should allow for potent anti-inflammatory action in the lung, while at the same time reducing the chance for side effects.

Chemistry

N-Phenyl substitution of rolipram was conveniently achieved using copper catalyzed coupling with aryl halides (Table 1). This method pioneered by Yamamoto[17)] proved to be very versatile for the synthesis of a wide range of *N*-arylrolipram derivatives in moderate to high yield.[18)] In general aryl iodides were the preferred halides in these coupling reactions, whereas bromides only gave satisfactory results in the case of bromobenzene or when electron withdrawing groups were present (*e.g.* synthesis of **9**).[18)] The amount of copper powder used to facilitate the reaction was adjusted according to the reactivity and the availability of the aryl halide. For example the readily available bromobenzene was used in a large excess (6 eq) without any solvent in the pres-

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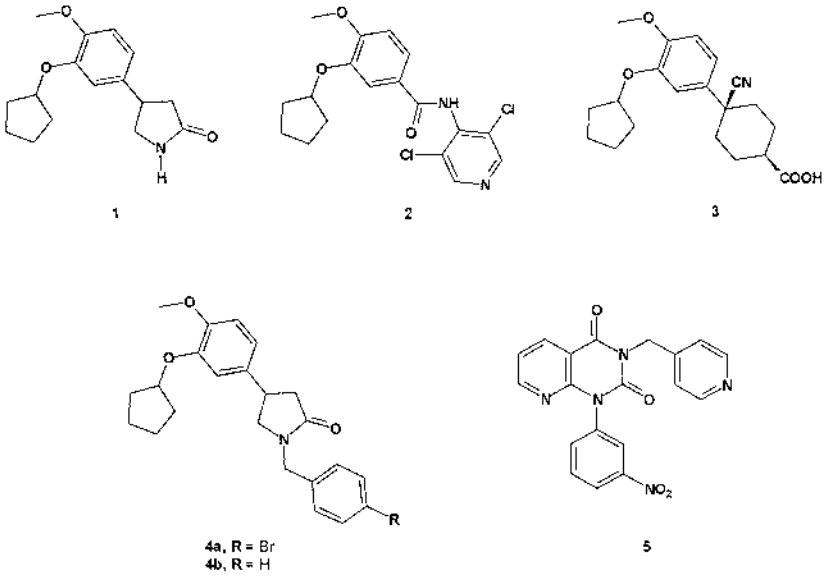
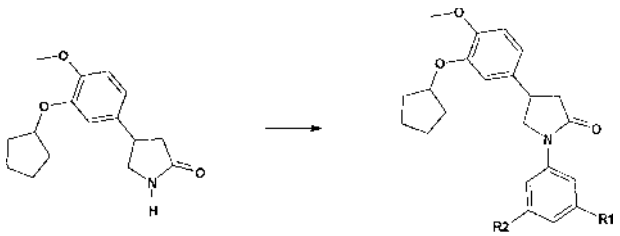


Table 1. Copper Mediated Coupling of Rolipram with Arylhalides



Aryl halide	Method[a]/time (h)/temp (°C)	R1	R2	Product (yield %)
Bromobenzene	A/3.5/150	H	H	**6** (88)
3,5-Dimethoxy-iodobenzene	B/4.5/reflux	OMe	OMe	**8** (61)
3-Nitro-bromobenzene	B/20/150	NO_2	H	**9** (73)
3-Benzyloxy-iodobenzene	B/22/150	OBn	H	**10** (50)
3-(3-Methoxybenzyloxy)iodobenzene	C/4/140	$OCH_2C_6H_4$–*m*-OMe	H	**11** (81)
Methyl 5-benzyloxy-3-iodobenzoate (**13**)	C/21/140	OBn	CO_2Me	**12** (59)

a) For synthetic Methods A—C, see general procedure in the experimental section.

ence of 1.85 eq of copper powder (Method A), while the more precious iodide (**13**) was reacted with rolipram in equimolar amounts in the presence of a large excess of copper powder (Method C).

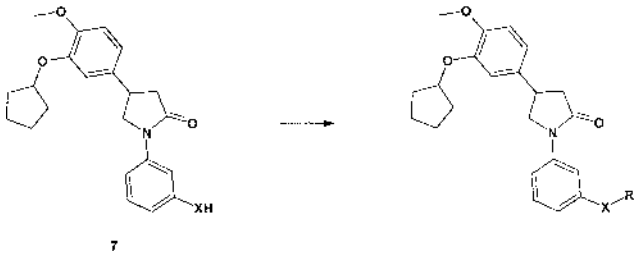
The phenol (**7**, see Chart 1) was used as a convenient starting point for the synthesis of more complex PDE4 inhibitors (Table 2). The esters (**14**) and (**15**) were prepared in good yield by reacting **7** with the corresponding acyl halides in pyridine. The ether (**17**) was synthesized by alkylation of the sodium salt of **7** with 4-methoxybenzyl chloride in DMF. One of the key compounds in this series 4-(3-cyclopentyloxy-4-methoxyphenyl)-1-[3-(3-methoxybenzyloxy)phenyl]-pyrrolidin-2-one (**11**) could be prepared either by direct copper catalyzed coupling of 3-(3-methoxybenzyloxy)iodobenzene with rolipram (Table 1), or alternatively by reaction of **7** with 3-methoxybenzyl chloride in the presence of cesium carbonate (Table 2). Finally, hydrogenation of the nitro substituted derivative (**9**) with palladium on charcoal, followed by direct acylation of the resulting aniline with 4-methoxybenzoyl chloride yielded benzamide (**16**).

Chart 1 describes the synthesis of the 3,5-disubstituted *N*-phenyl rolipram derivatives. Methyl 5-hydroxy-3-iodobenzoate[19] was benzylated to give **13**, which was then coupled in the usual way with rolipram (Table 1). The resulting ester (**12**) was the key intermediate for both the 3-benzyloxyphenyl- and the 3-(3-methoxybenzyloxy)phenyl derivatives. Hydrolysis of **12** produced the acid (**18**). Alternatively, removal of the benzyl protecting group of **12**, and reaction of 3-methoxybenzyl chloride with the resulting phenol (**19**) produced **20**, which was hydrolyzed to give 3-[4-(3-cyclopentyloxy-4-methoxyphenyl)-2-oxo-pyrrolidin-1-yl]-5-(3-methoxybenzyloxy)benzoic acid (**21**). Reaction of **21** with *N*,*N*-dimethyl hydrazine finally produced the potent PDE4 inhibitor (**22**).

Results and Discussion

Reference Compounds In order to compare our PDE4 isozymes with material used by other research groups, we prepared the reference inhibitors (**2**,[20] **3**,[16] **5**[21]) using literature procedures. Table 3 summarizes the results obtained for these three compounds and our lead structure rolipram. While the results for compounds (**2**) and (**5**) are basically

Table 2. Synthesis of Compounds **11**, **14** to **17**[a)]



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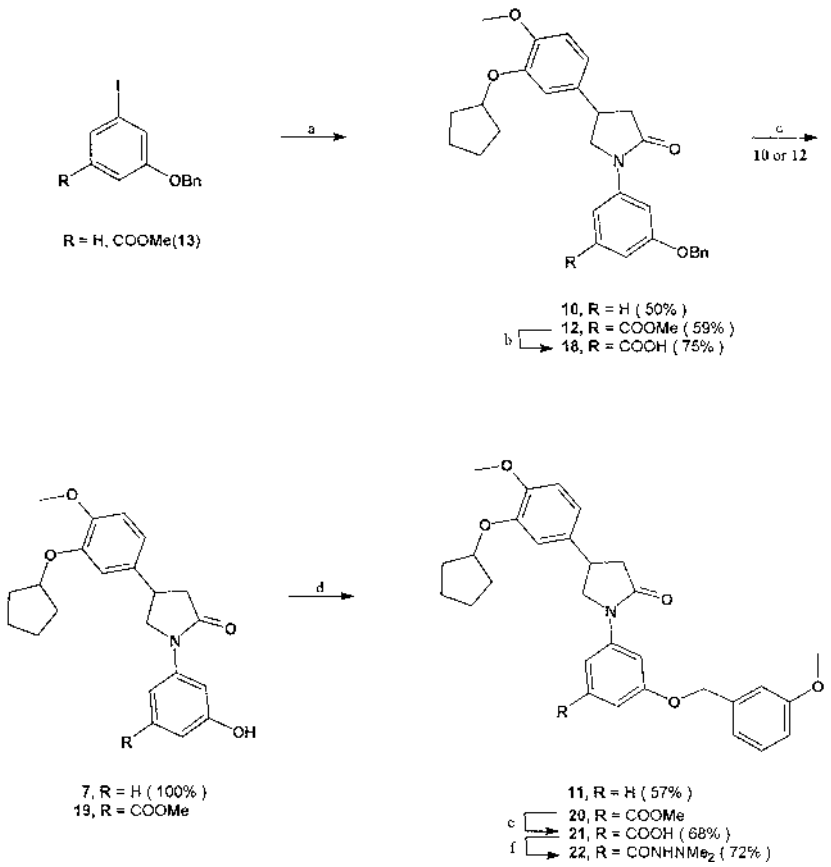
Halide	X	Reagents	R	Product (yield %)
m-(MeO)$C_6H_4CH_2Cl$	O	Cs_2CO_3	$CH_2C_6H_4$–*m*-OMe	**11** (57)
C_6H_5COCl	O	Pyridine	COC_6H_5	**14** (72)
p-(MeO)C_6H_4COCl	O	NaH/DMF	COC_6H_4–*p*-OMe	**15** (73)
p-(MeO)C_6H_4COCl	NH	Pyridine	COC_6H_4–*p*-OMe	**16** (99)
p-(MeO)$C_6H_4CH_2Cl$	O	NaH/DMF	$CH_2C_6H_4$–*p*-OMe	**17** (78)

a) For detailed procedures see experimental part.



Reagents and conditions: a: rolipram, Cu, K_2CO_3, DMF; b: aq. NaOH, methanol; c: H_2, Pd/C, methanol; d: Cs_2CO_3, *m*-(MeO)$C_6H_4CH_2Cl$, acetone; e: aq KOH, ethanol; f: Me_2NNH_2, EDC, HOBt, DMF.

Chart 1

identical to literature values,[15)] SB-207499 (**3**) in our hands is slightly less potent than reported.[15,16)] Gratifyingly however, we were able to confirm the selectivity of **3** for PDE-4D.

Design and Structure–Activity Relationship on the PDE4 Isozymes In contrast to other research groups which have mainly focused on modifications at the aromatic catechol ether or the replacement of the pyrrolidone ring of rolipram,[10—16,20)] we sought to find potent and selective PDE4 inhibitors through substitution at the pyrrolidone nitrogen. Indications that this strategy might be successful had been provided by Christensen[22a)] and by Bourguignon,[22b)] who had shown that *N*-substitution of rolipram could lead to improved PDE4 potency, when *N*-(*p*-bromobenzyl)rolipram (**4a**) was synthesized for structural studies.[22a)]

Our studies started with the synthesis of compounds (**6**) and (**4b**) (a close analogue of **4a**). These initial targets were chosen based on the working hypothesis that *N*-phenyl roliprams would show comparable activity to known *N*-ben-

Table 3. Inhibition of PDE4 Isoenzymes by Reference Compounds[a)]

Compound	PDE4A	PDE4B	PDE4C	PDE4D
RP-73401 (**2**)	3.3 (±1.1)	2.6 (±1.3)	11 (±1.4)	1.6 (±0.4)
SB-207499 (**3**)	410 (±90)	310 (±110)	840 (±180)	79 (±28)
RS-25344 (**5**)	40 (±9)	34 (±5)	203 (±9)	1.8 (±0.2)

a) Data indicated as mean IC_{50} (±S.E.M.) values in nM (n=2—5).

Table 4. Inhibition of PDE4 Isoenzymes by *N*-Arylrolipram Derivatives[a)]

	PDE4A	PDE4B	PDE4C	PDE4D	PDE3
1	1380 (±370)	1340 (±300)	7940 (±240)	400 (±80)	>100000
4b	520 (±60)	370 (±60)	2480 (±750)	320 (±120)	>100000
6	440 (±110)	430 (±21)	1650 (±50)	410 (±17)	>100000
7	280 (±8)	160 (±85)	2430 (±140)	51 (±23)	>100000
8	100 (±5)	69 (±9)	330 (±110)	72 (±30)	n.d.
14	20 (±8)	8 (±2)	59 (±12)	13 (±6)	>100000
10	128 (±41)	25 (±2)	440 (±140)	81 (±31)	n.d.
12	115 (±14)	66 (±16)	1270 (±280)	81 (±7)	>10000
18	24 (±2)	44 (±9)	280 (±80)	30 (±2)	>10000
15	8 (±5)	3 (±1)	81 (±27)	8 (±1)	>10000
16	59 (±17)	26 (±10)	143 (±14)	40 (±5)	>10000
17	40 (±10)	32 (±14)	980 (±22)	16 (±7)	>10000
11	9 (±2)	3 (±1)	320 (±22)	10 (±2)	>10000
21	13 (±1)	20 (±7)	128 (±16)	13 (±2)	>10000
22	2.5 (±0.5)	5 (±1)	16 (±6)	2 (±0.5)	>10000

a) Data indicated as mean IC_{50} (±S.E.M.) values in nM (n=2—6). n.d.=not done.

zyl roliprams, and could therefore serve us as a starting point in our search for potent and selective PDE4 inhibitors. As we had hoped, the simple *N*-phenyl substituted rolipram (**6**) showed improved potency compared to the lead compound (**1**) and was similar in activity on the PDE4 isozymes to the *N*-benzyl compound (**4b**) (Table 4). Since the ready availability of a wide variety of phenyl halides promised to give us a synthetically very simple and direct access to a new series of proprietary inhibitors, we continued to pursue derivatives of **6**. Simple introduction into the phenyl ring of nitro, amino (data not shown) or phenolic groups (*e.g.* **7**) had very little effect on the potency of the compounds compared with **6**. The first breakthrough occurred with benzoate (**14**), a compound which is easily accessible in two steps from rolipram, and showed over 50 times improved potency compared to our lead compound (**1**).

Not surprisingly **14** was equipotent (within the error of the experiment) on the three important isozymes[23)] PDE4A, PDE4B and PDE4D, while PDE4C was inhibited to a slightly lesser extent. This pattern of activity is common for rolipram based inhibitors,[15)] and indeed all of the inhibitors synthesized in this study and summarized in Table 4 are between 5 and 50 times less potent on PDE4C as compared to the other isozymes.

Substitution of the benzoate (**14**) with a *p*-methoxy group (**15**) lead to a further improvement in activity, while the corresponding benzamide (**16**) was considerably less active. It was clear however, that despite their potency, the benzoates (**14**, **15**) could not be taken as serious drug candidates, since they were expected to be easily hydrolized *in vivo*. As a consequence the corresponding ethers were investigated. The simple 3-benzyloxy ether (**10**) was about 5 fold less active than the corresponding benzoate (**14**), but the result was thought to be encouraging enough to warrant further investigation. As in the benzoate series the introduction of a *p*-methoxy group (**17**) lead to an improvement in the activity on all isozymes, however the most potent compound was found to be the 3-(3-methoxybenzyloxy)phenyl substituted rolipram (**11**).

While **11** was an excellent PDE4 inhibitor, it proved to be very hydrophobic (clog P=5.2), which caused problems in secondary assays and *in vivo* models.[24)] Since our compounds were designed for administration by inhalation, molecules with high log P were not thought to be inherently flawed. Indeed, experience in the field of inhaled β-agonists suggests that strongly hydrophobic compounds may be preferred in terms of duration of action. However the difficulty of dissolving **11** in solvents suitable for *in vivo* studies was assumed to be responsible for its lack of activity in the Brown Norway rat model of airways inflammation.[24)]

In order to improve the physical properties of these new rolipram derivatives we introduced hydrophilic substituents in the 5-position of the *N*-phenyl ring in **11**. Previous studies had indicated that this position may be able to accommodate another substituent without deleterious effects on PDE4 potency (see for example compound **8**). Our hypothesis proved to be correct and the newly introduced polar groups were well tolerated. The first compound synthesized in this series, methyl 3-benzyloxy-5-[4-(3-cyclopentyloxy-4-methoxyphenyl)-2-oxo-pyrrolidin-1-yl]benzoate (**12**), was equipotent with the *N*-phenyl-monosubstituted analogue (**10**). Hydrolysis of **12** lead to the carboxylic acid (**18**) which was an even more potent PDE4 inhibitor and showed much improved physicochemical characteristics.[24,25)] Replacement

of the benzyl group in **18** with a 3-methoxybenzyl group (**21**) led to a further increase in PDE4 potency as had been observed in the previous series. Further extension of this series led finally to the discovery of **22**, a low nanomolar inhibitor of all PDE4 isozymes, which is more than two orders of magnitude more potent than our lead compound rolipram.

Rolipram Binding PDE4 enzymes exist in two different states, distinguishable by either low or high affinity for (*R*)-rolipram.[26] While the presence of the two forms of PDE4 has been demonstrated both in recombinant enzymes and cellular systems,[5,26] it is still unclear what mechanism regulates the distribution of the two states of PDE4. By studying the side effect profile of PDE4 inhibitors in animal models and the resulting correlations of this pharmacological effect with the affinity of compounds for the high and low affinity forms of PDE4 enzymes, Torphy and coworkers[8,16] have developed a hypothesis which states that inhibitors with reduced ability to compete for the high affinity [^{3}H]-rolipram binding site (h-PDE4), while maintaining potent activity in inhibiting PDE4 catalytic activity (l-PDE4), exhibit an improved therapeutic index compared to first generation PDE4 inhibitors. Since SB-207499 (**3**), a second generation PDE4 inhibitor with a significantly improved side effect profile,[15] was designed on this premise, it was appropriate to test our PDE4 inhibitors for their h-PDE4 activity.

Competition of rolipram derivatives (**6**—**22**) for the [^{3}H]-rolipram binding site in rat brain membranes, (an assay which has commonly been used to determine h-PDE4 activity) was measured (Table 5) and compared with the standard compounds (**1**) and (**3**). A closer inspection of these values suggests that the structure activity of our compounds is very similar in both the PDE4 enzymatic assays as well as in the assay which measures h-PDE4 activity. Figure 1 shows a graph plotting the potency of the compounds at the two binding sites. To illustrate our findings, we have arbitrarily chosen PDE4D activity for this correlation, however since none of our compounds shows significant isozyme selectivity, the results are similar for PDE4A and PDE4B (graph not shown). The correlation between the potencies of our compounds (**6**—**22**) at the two binding sites is surprisingly good, giving a regression coefficient of 0.88 (S.D. 0.26; $p \leqq 0.001$). As can be seen from Fig. 1, rolipram is the only compound which clearly stands out, while SB-207499 (**3**) is indistiguishable in its behaviour from the rolipram derivatives (**6**—**22**). The data suggest that all of our compounds have a significantly improved l-PDE4/h-PDE4 ratio compared to rolipram and should therefore have a side effect profile similar to SB-207499.

Cellular Assays Since PDE4 inhibitors work at an intracellular level, the panel of compounds was routinely tested in an assay of fMLP induced oxidative burst in human eosinophils (HUEOS) (Table 5). This assay served two functions, namely it allowed us to gauge the potential of the rolipram derivatives to penetrate cellular membranes, and secondly it allowed us to study the effect of the inhibitors in one of the most important pro-inflammatory cells in asthma. Reactive oxygen species and cationic proteins, which are released upon eosinophil activation, are thought to be prime contributors to epithelial cell damage and increased hyperreactivity of asthmatic airways.

There have been suggestions in the literature that for PDE4 inhibitors the inhibition of guinea pig eosinophil oxidative burst correlates with PDE4 catalytic activity.[27] In our data set the activity of the inhibitors on PDE4D shows no significant correlation with their activity to inhibit the production of reactive oxygen species from human eosinophils ($R=0.45$; S.D.$=0.56$; $p=0.09$). Nevertheless the HUEOS assay proved to be very useful as a secondary assay, giving us a rapid functional readout of PDE4 inhibition, which allowed us to eliminate compounds with poor cellular activity at an early stage. Gratifyingly, both of our most promising inhibitors (**21**, **22**) potently inhibited the activation of this key effector cell in asthma.

There is convincing evidence that selected T cell populations play a key role in the orchestration of the inflammatory response to inhaled allergens and other stimuli in asthma by the production of a characteristic cytokine pattern.[28] 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.

In an attempt to mimic allergen induced T cell activation

Table 5. HUEOS[a] and High Affinity Rolipram Binding Data[b]

Compound	HUEOS	Rolipram binding
1	88 (±20)	4.0 (±1.5)
2	n.d.	0.8 (±0.3)
3	n.d.	40 (±13)
6	6200 (±310)	400 (±55)
7	640 (±140)	97 (±66)
14	370 (±150)	n.d.
10	370 (±110)	n.d.
12	410 (±82)	180 (±20)
18	170 (±40)	17.4 (±0.8)
15	170 (±45)	45 (±5)
16	210 (±86)	23 (±4)
17	1700 (±300)	n.d.
11	61 (±18)	8 (±1)
21	38 (±8)	32 (±4)
22	20 (±4)	5 (±2)

a) Human eosinophil oxidative burst. *b*) Data indicated as mean IC_{50} (±S.E.M.) values in nM ($n=2$—3).

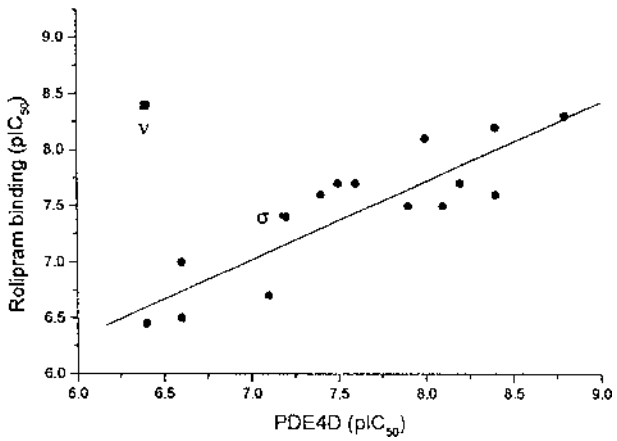


Fig. 1. Correlation of pIC_{50} for PDE4D Activity *versus* pIC_{50} for Inhibition of [^{3}H]-Rolipram Binding to Rat Brain Membranes for Compounds **6**—**22**

The standard compounds rolipram (ν) and SB-207499 (σ) are shown for comparison. The regression coefficient for compounds **6**—**22** is $R=0.88$ (S.D. 0.26; $p \leqq 0.001$).

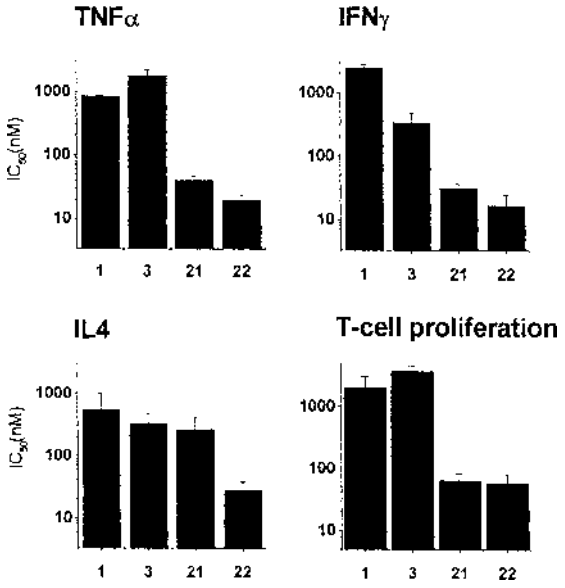


Fig. 2. Effect of Compounds **1**, **3**, **21** and **22** on LPS Induced TNF-α Production as Well as Anti-CD3 Induced IL-4, IFN-γ Production and Anti-CD3 Stimulated T-Cell Proliferation from Human Peripheral Blood Mononuclear Cells

For details see experimental section. Data shown as IC_{50}s (n=2—6).

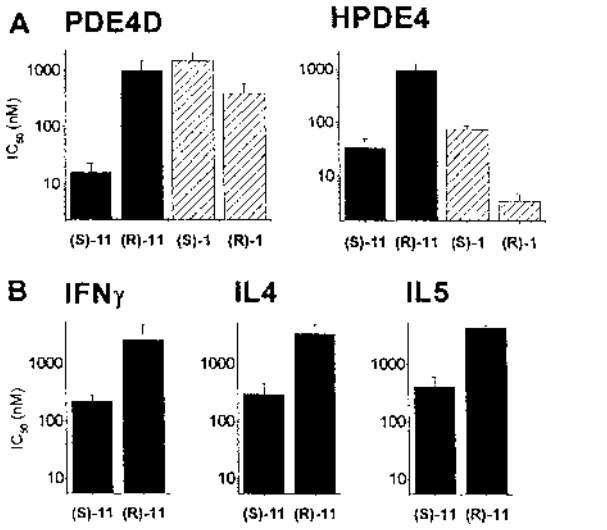


Fig. 3. (A) Stereoselectivity of Inhibition of PDE4D Enzymatic Activity and High Affinity [^{3}H]-Rolipram Binding (h-PDE4) of the (*R*)- and (*S*)-Enantiomers of Rolipram (**1**) and Compound **11**
(B) Stereoselectivity of Anti-CD3 Induced IFN-γ, IL-4 and IL-5 Production by (*R*)- and (*S*)-**11**

The assays were performed with human peripheral mononuclear cells. Data shown as mean IC_{50} (±S.E.M.) values (n=2—4).

events, human peripheral blood mononuclear cells (HPBMC) were stimulated with anti-CD3 antibodies, and the effect of selected inhibitors on cytokine release measured. Figure 2 shows the results of compounds (**21**) and (**22**) on IL-4 and IFN-γ release. While both rolipram derivatives are equipotent in inhibiting the TH1 cytokine IFN-γ, **22** is clearly superior in suppressing the TH2 cytokine IL-4, which has been implicated as one of the key mediators in the induction and maintenance of asthma. Compared to our lead compound rolipram but also the second generation PDE4 inhibitor SB-207499 (**3**), **22** shows a clearly improved profile in this key assay.

Finally we tested the activity of our most promising compounds on the production of TNF-α, a pro-inflammatory cytokine primarily produced by macrophages and monocytes, which has been shown to play an important role in the late response to allergen challenge.[29] In the event, stimulation of HPBMC with lipopolysaccharide leads to production of TNF-α, which was nicely suppressed by our new rolipram analogues (Fig. 2). Again compound **22** proved to be about 50 times more potent than either **1** or **3**.

Stereoselective Inhibition of PDE4 For convenience, all of our initial work was performed with racemic inhibitors. We have however recently developed a convenient, large scale synthesis of (*R*)- and (*S*)-rolipram[30] which provided access to enatiomerically pure inhibitors. While **22** is one of our most interesting compounds, we currently cannot disclose data on the enantiomers of this compound. Due to the interesting findings about the inhibition stereoselectivity, we have included data on (*R*)- and (*S*)-**11**, two compounds which have been released for publication, in contrast to other PDE4 inhibitors of this series. Both enantiomers of rolipram could be coupled with iodo-3-(3-methoxybenzyloxy)benzene under the usual conditions to give (*R*)-**11** and (*S*)-**11** in greater than 99% ee as determined by chiral HPLC. The stereochemistry of (*R*)-**11** and (*S*)-**11** was assigned based on the known chirality of the two enantiomers of rolipram.

In our hands,[30] both enantiomers of rolipram are micromolar inhibitors of the PDE4 isozymes, the (*R*)-enantiomer being the more potent inhibitor on all four isozymes, exhibiting eudismic ratios[31] between 3 and 5. This finding is in overall agreement with the existing literature. While it has been shown that the eudismic ratio can vary depending on the source of the PDE enzyme, there is general agreement that (*R*)-(−)-rolipram is the more potent of the two enantiomers.[22,33]

As representative examples, Fig. 3 shows a comparison of the IC_{50}'s for PDE4D inhibition of the (*R*)- and (*S*)-enantiomers of compounds (**1**) (rolipram) and (**11**). Surprisingly (*S*)-**11** is approximately 30 fold more potent as an inhibitor of the PDE4D isozyme than its corresponding (*R*)-enantiomer. The results for the other isozymes are not shown, since again there is no significant difference between the potency and the potency ratio of the two enantiomers on the different isozymes. In agreement with our findings with the racemic inhibitors, h-PDE4 activity closely paralelled the catalytic activity in (*R*)-**11** and (*S*)-**11** (Fig. 3). As a comparison Fig. 3 also shows the previously published[30] h-PDE4 values for the enantimers of rolipram.

As expected this stereoselectivity of PDE4 inhibition can also be observed in cellular assays (Fig. 3). Both of the T-cell derived cytokines IFN-γ and IL-4 are preferentially inhibited by (*S*)-**11** (Fig. 3), even though the stereoselectivity is slightly lower (eudismic ratio=10). We currently have no explanation for the differing stereoselectivities between (*R*)- and (*S*)-rolipram on the one hand, and (*R*)- and (*S*)-**11** on the other. The recent X-ray crystal structure of PDE4D could be used to gain a better understanding of this phenomenon, however with uncertainty over how **11** is oriented in the active site, any explanantion about the source of this selectivity is speculative at this moment.

Conclusion

In the current study we have reported the structure–activity relationship of a new series of rolipram based PDE4 inhibitors. The compounds were evaluated for inhibitory activity on all four PDE4 isozymes and for selectivity against PDE3. At the same time the compounds were tested for cellular activity in a human eosinophil oxidative burst assay and their potential to bind to the [^{3}H]-rolipram binding site in rat brain was assessed. This investigation lead to the identification of a highly potent PDE4 inhibitor (**22**), which showed greater than 1000 fold selectivity *versus* PDE3 and was an excellent inhibitor of eosinophil oxidative burst as well as TNF-α, IL-4 and IFN-γ production from human peripheral blood mononuclear cells. Correlations of PDE4D inhibition potency with rolipram binding data indicate that **22** has a similar side effect profile as the currently leading PDE4 inhibitor SB-207499, while being considerable more potent as a PDE4 inhibitor *in vitro* as well as in all the cellular assays examined. These new potent rolipram derivatives exhibit stereospecific inhibition of the PDE4 isozymes, however in contrast to rolipram the (*S*)-enantiomer is the more potent inhibitor. This effect can also be observed in primary human cells where the (*S*)-enantiomer is about 10 fold more potent than the corresponding (*R*)-enantiomer.

Experimental

General Melting points are corrected. ^{1}H-NMR (360 MHz) spectra were recorded on a Bruker AM-360 instrument, using residual solvent protons as references. Infrared spectra were recorded on a Bruker IFS 66 apparatus and mass spectra on a VG 70-SE instrument. Chromatography was carried out using silica gel (Merck, Kieselgel 60 F_{254}, 230—400 mesh) and analytical TLC was performed using precoated Kieselgel 60 F_{254} glass plates. AR grade solvents (Fluka) and commercial reagents (Fluka, Aldrich) were used without further purification in all cases. Brine refers to a saturated aqueous NaCl solution and concentration implies the use of a rotary evaporator at water aspirator pressure.

Inhibition of Human PDE3 PDE3 was prepared from human platelets by ultrasonic homogenization. Platelets were washed once with PBS, suspended in 10 ml of buffer H (sucrose 0.25 M, EDTA 1 mM, tris–HCl 10 mM, dithiothreitol 1 mM, pH 7.4) containing the following protease inhibitor solutions: 5 μl/ml of phenylmethyl-sulphonylfluoride (7 mg/ml in 2-propanol), 1 μg/ml leupeptin and pepstatin A (1 mg/ml each in ethanol). After sonication (15 s at 4 °C, Branson probe sonicator), homogenates were centrifuged at 2200×***g***. The pellet was resuspended in the same volume of buffer H and the sonication repeated. Pooled supernates were stored at −20 °C. Activity was assayed as described.[34]

Inhibition of Human cAMP-Specific Phosphodiesterase (PDE4) Isoenzymes PDE4 activity was assessed as previously described.[34] With the exception of PDE4B[35] (rat; expressed in *S. cerevisae*), all isoenzyme preparations were from human sources: PDE4A,[36] PDE4C,[34] PDE4D[37] expressed in *S. cerevisae*.

Anti-CD3 Induced Proliferation and Cytokine Production Mononuclear cells (MNC) were isolated from blood of normal individuals by Ficoll-hypaque gradient centrifugation (20 min at 800×***g***). The interphase was collected, washed twice in PBS and resuspended in RPMI1640 supplemented with 10% FCS. Cell density was adjusted to 1×10^6 cell/ml. One hundred microliters of the MNC suspension was placed in 96 well culture plates and 50 μl of either medium or compounds in the indicated concentrations 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 IFN-γ 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. In all cases, binding of the second antibody was analyzed by stepwise incubation with streptavidin–alkaline phosphatase conjugate (Mabtech, Stockholm, Sweden) and 4-nitrophenylphosphate disodium salts (Sigma Chemical Co.). Optical density was measured at 405 nm and cytokine concentrations were calculated based on the results from serial dilutions of standard recombinant mouse IL-4 and IFN-γ, respectively. The sensitivity of the cytokine ELISAs was around 10 pg/ml.

Induction and Measurement of TNFα Production Mononuclear cells were isolated as above and resuspended in RPMI1640 supplemented with 10% FCS. Cell density was adjusted to 1×10^6 cell/ml. Cells were stimulated with LPS (1 μg/ml) and IFN-γ (10 ng/ml) and supernatants were harvested after 24 h of incubation at 37 °C in a humidified incubator with 5% CO_2. Concentration of TNF-α 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). Optical density was measured at 405 nm and cytokine concentration was calculated based on the results from serial dilutions of standard recombinant human TNF-α.

Rolipram Receptor-Binding Assay Method Rat brains were suspended in 10 ml/g of ice-cold buffer (tris 20 mM, $MgCl_2$ 1 mM, dithiothreitol 0.1 mM, pH 7.5), homogenized using 2 bursts (30 s each) of a Polytron homogenizer (Kinematica, Switzerland), centrifuged (600×***g***, 10 min, 4 °C). The supernatants were pooled, and centrifuged (24000×***g***, 10 min, 4 °C). The pellets were resuspended in 5 ml/g of buffer. Protein content was determined using the method of Bradford,[38] according to the instructions of the manufacturer (BioRad). The assay was carried out as described by Schneider *et al*.[39]

Oxidative Burst from Human Eosinophils Blood was obtained from normal individuals. Granulocytes were separated from mononuclear cells by Ficoll hypaque gradient centrifugation. Erythrocytes were lysed by two cycles of hypotonic lysis and the remaining granulocytes were incubated with anti-CD16 coated immunomagnetic particles (Miltenyi Biotec, Bergisch Gladbach, Germany). Magnetically labeled neutrophils were then depleted by passing the granluocytes through a MACS (magnetic cell separation) column which resulted in a more than 98% pure eosinophil preparation. These purified human eosinophils were diluted in HBSS and pipetted into 96 well microtitreplates (MTP) at 10^4 cells/well. Each well contained a 200 μl sample comprising: 100 μl eosinophil suspension, 50 μl HBSS, 10 μl lucigenin, 20 μl activation stimulus, 20 μl compound of interest. The samples were incubated with compound or vehicle for between 10 and 30 min prior to addition of fMLP (0.01—10 μM). MTPs were agitated (Titertek MTP mixer) to facilitate mixing of the cells and medium, and the MTP placed into a Hamamatsu luminometer. Total chemiluminescence and the temporal profile of each well was measured simultaneously over 20 min and the results expressed as a percentage of fMLP-induced chemoluminescence in the absence of compound. Results were fitted to the Hill equation and IC_{50} values calculated. All compounds were dissolved in dimethyl sulphoxide and thereafter diluted in buffer.

General Procedure for the Copper Catalyzed Coupling of Rolipram with Aryl Halides Method A: A mixture of rolipram (**1**), the appropriate aryl halide (2—6 eq), Cu powder (1.85 eq), K_2CO_3 (1.5 eq) and KI (1.5 eq) was heated without solvent under an Argon atmosphere at the time and temperature indicated in Table 1.

Method B: A mixture of **1**, the appropriate aryl halide (1.5 eq), Cu powder (0.16 eq) and K_2CO_3 (1.4 eq) in dry DMF was heated under an Argon atmosphere at the time and temperature indicated in Table 1.

Method C: A mixture of **1**, the appropriate aryl halide (1.0 eq), Cu powder (22 eq) and K_2CO_3 (1.5 eq) in dry DMF was heated under an Argon atmosphere at the time and temperature indicated in Table 1.

Work up: The reaction mixture was filtered through a bed of hyflo and concentrated *in vacuo* (Methods B and C) or treated with EtOAc whilst still warm, worked-through with a spatula or glass rod and then filtered through hyflo (Method A). The filtrate was then diluted with EtOAc, washed with water and brine, dried (Na_2SO_4), filtered and concentrated. The crude products were purified by flash chromatography and/or recrystallization from an appropriate solvent.

4-(3-Cyclopentyloxy-4-methoxyphenyl)-1-phenyl-pyrrolidin-2-one (6) mp 124—126 °C; ^{1}H-NMR ($CDCl_3$) δ: 7.68 (2H, d, J=7 Hz), 7.37 (2H, dd, J=7, 7 Hz), 7.12 (1H, dd, J=7, 7 Hz), 6.99 (1H, d, J=2 Hz), 6.89 (1H, d, J=8 Hz), 6.87 (1H, dd, J=8, 2 Hz), 4.80 (1H, m), 4.15 (1H, dd, J=9, 6 Hz), 3.81 (1H, dd, J=9, 9 Hz), 3.71 (3H, s), 3.62 (1H, dddd, J=9, 9, 9, 6 Hz), 2.83 (1H, dd, J=16, 9 Hz), 2.70 (1H, dd, J=16, 9 Hz), 1.95—1.47 (8H, m). IR (KBr) cm^{-1}: 1685. MS *m*/*z*: 351 (M^+). *Anal.* Calcd for $C_{22}H_{25}NO_3$: C, 75.19; H, 7.17; N, 3.99. Found: C, 74.90; H, 7.00; N, 4.00.

4-(3-Cyclopentyloxy-4-methoxyphenyl)-1-(3,5-dimethoxyphenyl)-pyrrolidin-2-one (8) mp 52—54 °C. ^{1}H-NMR ($CDCl_3$) δ: 6.99 (1H, d, J=1 Hz), 6.95—6.82 (4H, m), 6.32 (1H, br s), 4.80 (1H, m), 4.14 (1H, br dd, J=9, 9 Hz), 3.75 (1H, m), 3.74 (6H, s), 3.72 (3H, s), 3.60 (1H, m), 2.83 (1H, dd, J=16, 9 Hz), 2.70 (1H, dd, J=16, 9 Hz), 1.95—1.47 (8H, m). IR (KBr) cm^{-1}: 1692. MS m/z: 411 (M^+). *Anal.* Calcd for $C_{24}H_{29}NO_5$: C, 70.05; H, 7.10; N, 3.40. Found: C, 69.70; H, 7.40; N, 3.60.

4-(3-Cyclopentyloxy-4-methoxyphenyl)-1-(3-nitrophenyl)-pyrrolidin-2-one (9) mp 99—101 °C. ^{1}H-NMR ($CDCl_3$) δ: 8.36 (1H, dd, J=1, 1 Hz), 8.21 (1H, ddd, J=7, 1, 1 Hz), 8.00 (1H, ddd, J=7, 1, 1 Hz), 7.55 (1H, dd, J=7, 7 Hz), 6.88—6.79 (3H, m), 4.78 (1H, m), 4.23 (1H, dd, J=9, 7 Hz), 3.90 (1H, dd, J=9, 7 Hz), 3.85 (3H, s), 3.69 (1H, dddd, J=9, 9, 9, 9 Hz), 3.05 (1H, dd, J=16, 9 Hz), 2.83 (1H, dd, J=16, 9 Hz), 1.98—1.50 (8H, m). IR (KBr) cm^{-1}: 1698. MS m/z: 396 (M^+).

1-(3-Benzyloxyphenyl)-4-(3-cyclopentyloxy-4-methoxyphenyl)-pyrrolidin-2-one (10) mp 103—105 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.48—7.23 (7H, m), 7.16 (1H, dd, J=7, 2 Hz), 6.88—6.75 (4H, m), 5.09 (2H, s), 4.77 (1H, m), 4.15 (1H, dd, J=9, 7 Hz), 3.85 (3H, s), 3.82 (1H, dd, J=9, 7 Hz), 3.61 (1H, dddd, J=9, 9, 9, 9 Hz), 2.99 (1H, dd, J=16, 9 Hz), 2.76 (1H, dd, J=16, 9 Hz), 1.98—1.51 (8H, m). IR (KBr) cm^{-1}: 1686. MS m/z: 458 (MH^+). *Anal.* Calcd for $C_{29}H_{31}NO_4$: C, 76.12; H, 6.83; N, 3.06. Found: C, 76.10; H, 6.90; N, 3.30.

4-(3-Cyclopentyloxy-4-methoxyphenyl)-1-[3-(3-methoxybenzyloxy)-phenyl]-pyrrolidin-2-one (11) mp 73—74 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.43 (1H, dd, J=1, 1 Hz), 7.32—7.25 (2H, m), 7.16 (1H, m), 7.01 (2H, m), 6.89—6.75 (5H, m), 5.05 (2H, s), 4.77 (1H, m), 4.15 (1H, dd, J=9, 7 Hz), 3.85 (3H, s), 3.83 (1H, dd, J=9, 7 Hz), 3.83 (3H, s), 3.61 (1H, dddd, J=9, 9, 9, 9 Hz), 3.00 (1H, dd, J=16, 9 Hz), 2.77 (1H, dd, J=16, 9 Hz), 1.95—1.50 (8H, m). IR (KBr) cm^{-1}: 1687. MS m/z: 487 (M^+). *Anal.* Calcd for $C_{30}H_{33}NO_5$: C, 73.89; H, 6.77; N, 2.87. Found: C, 73.64; H, 6.74; N, 3.04.

Alternative Preparation of (11) A mixture of the phenol (**7**, *vide infra*) (200 mg, 0.545 mmol), *m*-methoxybenzylchloride (116 mg, 0.746 mmol, 1.3 eq) and Cs_2CO_3 (266 mg, 0.817 mmol, 1.5 eq) in isobutylmethyl ketone (12 ml) was stirred at room temperature for 2 h, at 60 °C for 1.5 h and at 80 °C for 15 h. The resulting light-gray suspension was filtered through hyflo and the filtrate concentrated *in vacuo.* The residue was taken up in EtOAc and washed with 2 N NaOH and brine. Drying (Na_2SO_4), filtration and concentration provided a yellow oil (302 mg) which was chromatographed on silica (eluent: hexane/EtOAc, 3/1) to give a clear, colorless oil, crystallization of which from *i*-PrOH furnished the ether (**11**) as white blocks (150 mg, 57%).

***R*-(+)-4-(3-Cyclopentyloxy-4-methoxyphenyl)-1-[3-(3-methoxybenzyloxy)-phenyl]-pyrrolidin-2-one ((*R*)-(+)-11)** For spectral data see above for compound **11**: mp 61—62 °C (*i*-Pr_2O/hexane/pentane). $[\alpha]_D^{20}$ +7.1° (c=0.31, MeOH). CD Spectrum.[30]

***S*-(−)-4-(3-Cyclopentyloxy-4-methoxyphenyl)-1-[3-(3-methoxybenzyloxy)-phenyl]-pyrrolidin-2-one ((*S*)-(−)-11)** For spectral data see above for compound **11**: mp 57—59 °C (*i*-Pr_2O/hexane/pentane). $[\alpha]_D^{20}$ −6.2° (c=0.50, MeOH). CD Spectrum.[30]

Methyl 3-Benzyloxy-5-[4-(3-cyclopentyloxy-4-methoxyphenyl)-2-oxo-pyrrolidin-1-yl]-benzoate (12) Amorphous solid. ^{1}H-NMR ($CDCl_3$) δ: 7.87 (1H, m), 7.65 (1H, m), 7.50—7.30 (6H, m), 6.90—6.70 (3H, m), 5.13 (2H, s), 4.77 (1H, m), 4.20 (1H, dd, J=10, 8 Hz), 3.90 (3H, s), 3.88 (1H, dd, J=10, 8 Hz), 3.82 (3H, s), 3.64 (1H, m), 2.99 (1H, dd, J=16, 9 Hz), 2.79 (1H, dd, J=16, 9 Hz), 2.00—1.50 (8H, m). IR (KBr) cm^{-1}: 1704. MS m/z: 516 (MH^+). *Anal.* Calcd for $C_{31}H_{33}NO_6$: C, 72.21; H, 6.45; N, 2.72. Found: C, 72.33; H, 6.61; N, 2.75.

4-(3-Cyclopentyloxy-4-methoxyphenyl)-1-(3-hydroxyphenyl)-pyrrolidin-2-one (7) A solution of the benzyl ether (**10**) (900 mg, 1.97 mmol) in MeOH (60 ml) was hydrogenolysed for 18 h under 1 atm at room temperature in the presence of 10% Pd–C (90 mg). Filtration through hyflo and concentration afforded a light-gray solid (730 mg, 100%). A portion of this product was recrystallized from *i*-PrOH/*i*-Pr_2O to give the pure phenol (**7**) as white needles: mp 158—160 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.96 (1H, dd, J=1, 1 Hz), 7.78 (1H, s), 7.22 (1H, dd, J=7, 7 Hz), 6.86—6.66 (5H, m), 4.76 (1H, m), 4.20 (1H, dd, J=9, 7 Hz), 3.87 (1H, dd, J=9, 7 Hz), 3.84 (3H, s), 3.63 (1H, dddd, J=9, 9, 9, 9 Hz), 3.05 (1H, dd, J=16, 9 Hz), 2.83 (1H, dd, J=16, 9 Hz), 1.98—1.51 (8H, m). IR (KBr) cm^{-1}: 3650, 1669. MS m/z: 367 (M^+). *Anal.* Calcd for $C_{22}H_{25}NO_4$: C, 71.91; H, 6.86; N, 3.81. Found: C, 71.82; H, 6.79; N, 4.10.

4-(3-Cyclopentyloxy-4-methoxyphenyl)-1-(3-benzoyloxyphenyl)-pyrrolidin-2-one (14) To the phenol (**7**) (160 mg, 0.44 mmol) in dry pyridine (2 ml) was added benzoyl chloride (74 mg, 0.52 mmol, 1.2 eq). After stirring at room temperature for 2 h, the reaction mixture was poured into 3 N HCl (15 ml) and extracted with EtOAc (3×). The organic phases were washed with water, sat. $NaHCO_3$ and brine. Drying (Na_2SO_4), filtration and concentration provided a yellow solid which was recrystallized from diisopropyl ether to yield a white crystalline solid (148 mg, 72%): mp 144—146 °C: ^{1}H-NMR ($CDCl_3$) δ: 8.20 (2H, m), 7.63 (2H, m), 7.50 (3H, m), 7.43 (1H, m), 7.03 (1H, m), 6.83—6.77 (3H, m), 4.76 (1H, m), 4.19 (1H, dd, J=9, 7 Hz), 3.85 (1H, dd, J=9, 7 Hz), 3.82 (3H, s), 3.61 (1H, m), 3.00 (1H, dd, J=16, 9 Hz), 2.78 (1H, dd, J=16, 9 Hz), 1.95—1.51 (8H, m). IR (KBr) cm^{-1}: 1726, 1699. MS m/z: 471 (M^+). *Anal.* Calcd for $C_{29}H_{29}NO_5$: C, 73.87; H, 6.20; N, 2.97. Found: C, 73.60; H, 6.30; N, 3.00.

4-(3-Cyclopentyloxy-4-methoxyphenyl)-1-[3-(*p*-methoxybenzoyloxyphenyl)]-pyrrolidin-2-one (15) To a mixture of NaH (18 mg, 0.45 mmol, 60% dispersion in oil) in dry DMF (2 ml) under argon was added the phenol (**7**) (150 mg, 0.41 mmol) dissolved in dry DMF (2 ml). The resulting gray-brown suspension was stirred for 1 h at room temperature, during which time the solid material slowly went into solution, and then *p*-methoxybenzoyl chloride (91 mg, 0.53 mmol, 1.3 eq) was added. After stirring at room temperature for 1 h, more NaH (5 mg, 0.12 mmol) and *p*-methoxybenzoyl chloride (20 mg, 0.12 mmol, 0.3 eq) were added and stirring continued for 30 min. The solvent was removed *in vacuo* and the oily residue dissolved in EtOAc, washed with water, 2 N aq. NaOH and brine. Drying (Na_2SO_4), filtration and concentration provided an opaque yellow oil (240 mg) which was chromatographed on silica (eluent: hexane/EtOAc, 2/1) to give a colorless oil that crystallized slowly. Recrystallization from 2-propanol afforded white needles in two crops (150 mg, 73 %): mp 90—92 °C: ^{1}H-NMR ($CDCl_3$) δ: 8.15 (2H, d, J=7 Hz), 7.60 (1H, dd, J=1, 1 Hz), 7.52 (1H, ddd, J=7, 1, 1 Hz), 7.41 (1H, dd, J=7, 7 Hz), 7.01 (1H, ddd, J=7, 1, 1 Hz), 6.99 (2H, d, J=7 Hz), 6.87—6.78 (3H, m), 4.77 (1H, m), 4.19 (1H, dd, J=9, 7 Hz), 3.90 (3H, s), 3.86 (1H, dd, J=9, 7 Hz), 3.83 (3H, s), 3.62 (1H, dddd, J=9, 9, 9, 9 Hz), 3.00 (1H, dd, J=16, 9 Hz), 2.78 (1H, dd, J=16, 9 Hz), 1.98—1.51 (8H, m). IR (KBr) cm^{-1}: 1731, 1701. MS m/z: 501 (M^+). *Anal.* Calcd for $C_{30}H_{31}NO_6$: C, 71.84; H, 6.23; N, 2.79. Found: C, 71.60; H, 6.04; N, 2.76.

4-(3-Cyclopentyloxy-4-methoxyphenyl)-1-[3-(*p*-methoxybenzamidophenyl)]-pyrrolidin-2-one (16) A solution of the nitro compound (**9**) (2 g, 5.05 mmol) in MeOH (100 ml) containing three drops of NEt_3 was hydrogenated for 2 h under 1 atm at room temperature in the presence of 10% Pd–C (200 mg). Filtration through hyflo and concentration afforded a light-gray foam (1.85 g, 100%). To a portion of this crude aniline (200 mg, 0.546 mmol) in dry pyridine (2 ml) was added, all at once, *p*-methoxybenzoyl chloride (121 mg, 0.71 mmol, 1.3 eq). After stirring at room temperature for 2 h, the reaction mixture was poured into 3 N HCl (15 ml) and extracted with EtOAc (3×). The organic phases were washed with water, sat. $NaHCO_3$ and brine. Drying (Na_2SO_4), filtration and concentration provided an opaque, light-gray semi-solid residue (320 mg) which was chromatographed twice on silica (eluent: hexane/EtOAc, 1/1) to give a colorless foam (210 mg, 99%): ^{1}H-NMR ($CDCl_3$) δ: 8.04 (1H, br s), 7.87 (1H, br s), 7.85 (2H, d, J=9 Hz), 7.55 (1H, m), 7.37 (2H, m), 6.98 (2H, d, J=9 Hz), 6.88—6.79 (3H, m), 4.78 (1H, m), 4.21 (1H, dd, J=9, 7 Hz), 3.88 (1H, dd, J=9, 7 Hz), 3.88 (3H, s), 3.84 (3H, s), 3.62 (1H, dddd, J=9, 9, 9, 9 Hz), 3.00 (1H, dd, J=16, 9 Hz), 2.78 (1H, dd, J=16, 9 Hz), 1.98—1.51 (8H, m). IR (KBr) cm^{-1}: 3300, 1700, 1674. MS m/z: 501 (MH^+). *Anal.* Calcd for $C_{30}H_{32}N_2O_5$: C, 71.98; H, 6.44; N, 5.60. Found: C, 71.74; H, 6.32; N, 5.77.

4-(3-Cyclopentyloxy-4-methoxyphenyl)-1-[3-(*p*-methoxybenzyloxy)-phenyl]-pyrrolidin-2-one (17) To a 60% dispersion of NaH (18 mg, 0.45 mmol, 1.1 eq) in dry DMF (2 ml) under argon was added during 5 min a solution of the phenol (**7**) (150 mg, 0.41 mmol) in dry DMF (2 ml). The resulting gray-brown suspension was stirred for 1 h at room temperature during which time the solid material slowly went into solution. This was now treated all at once with *p*-methoxybenzyl chloride (91 mg, 0.53 mmol, 1.3 eq) and stirred for 1 h. More NaH (5 mg, 0.12 mmol, 0.3 eq) and *p*-methoxybenzyl chloride (20 mg, 0.12 mmol, 0.3 eq) were added sequentially before stirring for another 30 min. The reaction mixture was then concentrated *in vacuo*, taken up in EtOAc and washed with water, 2 N NaOH (2×) and brine. Drying (Na_2SO_4), filtration and concentration provided an opaque, yellow oil (240 mg). Chromatography on silica (eluent: hexane/EtOAc, 2/1) gave a colorless oil which was crystallized from *i*-PrOH in two crops affording the ether **17** (150 mg, 78%) as white needles: mp 130—133 °C; ^{1}H-NMR ($CDCl_3$) δ: 7.52 (2H, d, J=7 Hz), 7.41 (1H, dd, J=7, 7 Hz), 7.01 (1H, ddd, J=7, 1, 1 Hz), 6.99 (2H, d, J=7 Hz), 6.88—6.78 (5H, m), 5.04 (2H, s), 4.77 (1H, m), 4.19 (1H, dd, J=9, 7 Hz), 3.90 (3H, s), 3.86 (1H, dd, J=9, 7 Hz), 3.83 (3H, s), 3.62 (1H, dddd, J=9, 9, 9, 9 Hz), 3.00 (1H, dd, J=16, 9 Hz), 2.78 (1H, dd, J=16, 9 Hz), 1.98—1.51 (8H, m). IR (KBr) cm^{-1}: 1685. MS m/z: 487 (M^+). *Anal.* Calcd for $C_{30}H_{33}NO_5$: C,

73.89; H, 6.77; N, 2.87. Found: C, 73.60; H, 6.61; N, 2.83.

3-Benzyloxy-5-[4-(3-cyclopentyloxy-4-methoxyphenyl)-2-oxo-pyrrolidin-1-yl]-benzoic acid (18) The methyl ester (**12**) (110 mg, 0.2 mmol) was dissolved in methanol (5 ml) and excess 2 N NaOH (0.5 ml) was added. The resulting solution was stirred for 48 h at room temperature and the reaction mixture was then evaporated to dryness. The residue was dissolved in CH_2Cl_2 and washed with 2 N HCl and brine. Drying (Na_2SO_4), filtration and concentration gave a white solid (75 mg, 75%), mp 200—202 °C: ^{1}H-NMR ($CDCl_3$) δ: 7.92 (1H, s), 7.60—7.30 (7H, m), 6.98 (1H, s), 6.87 (2H, m), 5.18 (2H, s), 4.80 (1H, m), 4.10 (1H, m), 3.83 (1H, m), 3.70 (3H, s), 3.64 (1H, m), 2.86 (1H, dd, J=16, 9 Hz), 2.72 (1H, dd, J=16, 9 Hz), 1.90—1.50 (8H, m). IR (KBr) cm^{-1}: 1735. MS m/z: 502 (MH^+).

Methyl 5-[4-(3-Cyclopentyloxy-4-methoxyphenyl)-2-oxo-pyrrolidin-1-yl]-3-(3-methoxybenzyloxy)-benzoate (20) This compound was prepared according to the abovementioned alternative procedure described for **11** (from **10**), starting in this case from methyl 3-benzyloxy-5-[4-(3-cyclopentyloxy-4-methoxyphenyl)-2-oxo-pyrrolidin-1-yl]-benzoate (**12**), *via* hydrogenolysis to **19** (cf. **10**→**7**) followed by 3-methoxybenzylation to **20** (cf. **7**→**11**). Amorphous solid: ^{1}H-NMR (DMSO-d_6) δ: 7.98 (1H, m), 7.56 (1H, m), 7.50—7.20 (2H, m), 7.10—6.80 (6H, m), 5.15 (2H, s), 4.71 (1H, m), 4.18 (1H, t, J=8 Hz), 3.83 (3H, s), 3.83 (1H, t, J=8 Hz), 3.75 (3H, s), 3.71 (3H, s), 3.64 (1H, m), 2.85 (1H, dd, J=16, 8 Hz), 2.74 (1H, dd, J=16, 8 Hz), 1.90—1.50 (8H, m). IR (KBr) cm^{-1}: 1705. MS m/z 546 (MH^+). *Anal.* Calcd for $C_{32}H_{35}NO_7$: C, 70.44; H, 6.47; N, 2.57. Found: C, 70.22; H, 6.45; N, 2.40.

5-[4-(3-Cyclopentyloxy-4-methoxyphenyl)-2-oxo-pyrrolidin-1-yl]-3-(3-methoxy-benzyloxy)benzoic Acid (21) Compound (**20**) (390 mg, 0.71 mmol) was dissolved in ethanol (15 ml) and treated with 2 N NaOH (1.25 ml, 2.5 mmol). After 5 h of stirring at room temperature the volume of the reaction mixture was reduced to *ca.* 1 ml and 2 N HCl was added (50 ml). The mixture was extracted with CH_2Cl_2 (2×) and the combined organic extracts dried (Na_2SO_4), filtered and concentrated to afford a yellow solid which was recrystallized from 2-propanol/diisopropylether (260 mg, 68%): ^{1}H-NMR (DMSO-d_6) δ: 13.0 (1H, s), 7.93 (1H, s), 7.56 (1H, s), 7.30 (2H, m), 7.1—6.8 (6H, m), 5.12 (2H, s), 4.80 (1H, m), 4.18 (1H, m), 3.83 (1H, m), 3.74 (3H, s), 3.71 (3H, s), 3.63 (1H, m), 2.85 (1H, dd, J=16, 9 Hz), 2.71 (1H, dd, J=16, 9 Hz), 1.90—1.50 (8H, m). IR (KBr) cm^{-1}: 3300—2700, 1712, 1660. MS m/z: 532 (MH^+). *Anal.* Calcd for $C_{31}H_{33}NO_7$: C, 70.04; H, 6.26; N, 2.63. Found: C, 69.76; H, 6.33; N, 2.52.

5-[4-(3-Cyclopentyloxy-4-methoxyphenyl)-2-oxo-pyrrolidin-1-yl]-3-(3-methoxybenzyloxy)benzoic Acid *N′*,*N′*-Dimethylhydrazide (22) The acid (**21**) (200 mg, 0.38 mmol) was dissolved in DMF (20 ml) and 1,1-dimethylhydrazine (0.11 ml, 1.5 mmol), 4-hydroxybenzotriazol (62 mg, 0.46 mmol), *N*-methyl morpholine (0.05 ml, 0.46 mmol) and EDC (180 mg, 0.93 mmol) were added. The yellow solution was stirred overnight and the solvent evaporated to dryness. The residue was dissolved in EtOAc, washed with 2 N HCl and brine, dried (Na_2SO_4), filtered and concentrated to afford a brown solid which was purified by column chromatography (silica gel; EtOAc/MeOH 9 : 1) to give **22** as an amorphous solid (156 mg, 72%): ^{1}H-NMR (DMSO-d_6) δ: 9.39 (1H, s), 7.66 (1H, s), 7.55 (1H, s), 7.33—6.80 (8H, m), 5.13 (2H, s), 4.82 (1H, m), 4.16 (1H, m), 3.82 (1H, m), 3.73 (3H, s), 3.71 (3H, s), 3.62 (1H, m), 2.85 (1H, dd, J=16, 9 Hz), 2.73 (1H, dd, J=16, 9 Hz), 2.58 (6H, s), 1.90—1.50 (8H, m). IR (KBr) cm^{-1}: 1701, 1646. MS m/z: 596 ($[M+Na]^+$), 574 (MH^+). *Anal.* Calcd for $C_{33}H_{39}N_3O_6$: C, 69.09; H, 6.85; N, 7.32. Found: C, 69.41; H, 6.97; N, 7.54.

References and Notes

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