Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 17, pp. 1977-1982, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00336-3

Phosphodiesterase Type IV (PDE IV) Inhibition. Synthesis and Evaluation of a Series of 1, 3, 4-Trisubstituted Pyrrolidines

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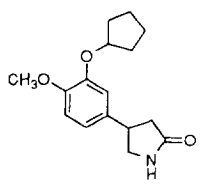
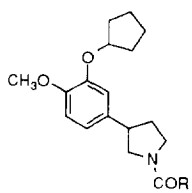
Abstract: Structure-activity relationships within a series of 1,3,4-trisubstituted pyrrolidines, novel and selective inhibitors of cAMP-specific phosphodiesterase (PDE IV), are discussed.

Phosphodiesterases comprise a family of enzymes whose role in mammalian cells is to regulate the levels of the ubiquitous second messenger cyclic nucleotides, cAMP and cGMP, by their degradation to inactive 5'-monophoshate metabolites.[1] Within inflammatory cells, such as monocytes and macrophages, the cAMP-specific phosphodiesterase type IV (PDE IV) has been shown to be the principal PDE isotype.[2] The observation that an elevation of cAMP in these proinflammatory cells can suppress their activation[3] has stimulated wide interest in developing therapeutic agents for chronic inflammatory diseases through selective inhibition of PDE IV.[4]

Rolipram [(*R, S*)-4-(3-cyclopentoxy-4-methoxyphenyl)-2-pyrrolidinone] (**1**) is a selective inhibitor of PDE IV[5] and has served as the starting point for a number of medicinal chemistry groups whose aim has been both to improve its potency against PDE IV and to moderate its dose-limiting side effects. A number of groups, including our own, have reported the synthesis of PDE IV inhibitors derived from rolipram, in which the modifications were made to the pyrrolidinone ring.[6,7] In particular, we have shown that selective PDE IV inhibition can be preserved by a simple modification to rolipram, namely transposing the rolipram lactam carbonyl to an exocyclic position, thereby generating a 1,3-substituted pyrrolidine as represented by the generic structure **2**.[6e] In this Letter we describe the synthesis and structure-activity relationships of a series of 1,3,4-substituted pyrrolidines, exemplified by the methyl ketone **3**. Lacking any structural data on our enzyme target, we reasoned that the added substitution at C3 of the pyrrolidine ring in **3** and related compounds might provide us with a better understanding of the steric and electronic requirements necessary for PDE IV inhibition.



Rolipram (1)



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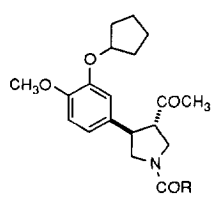
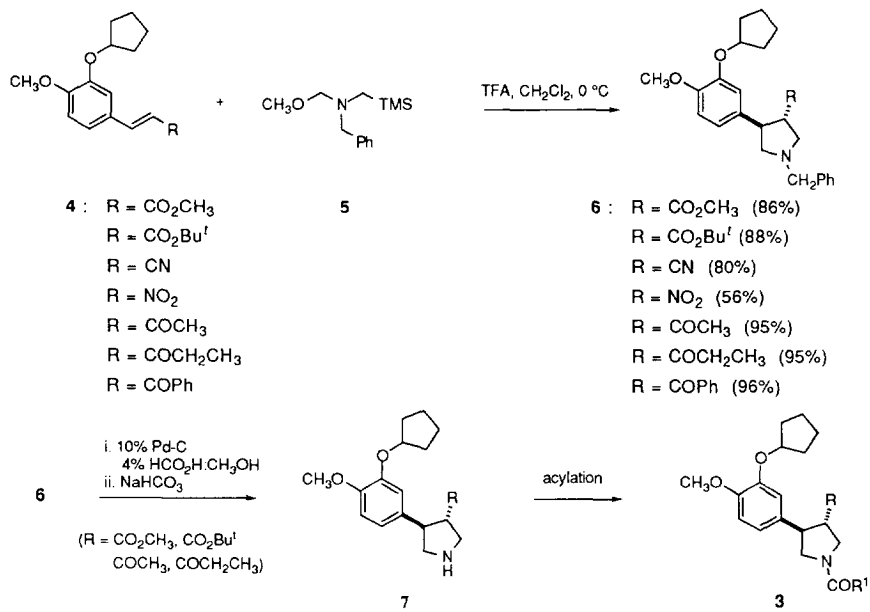


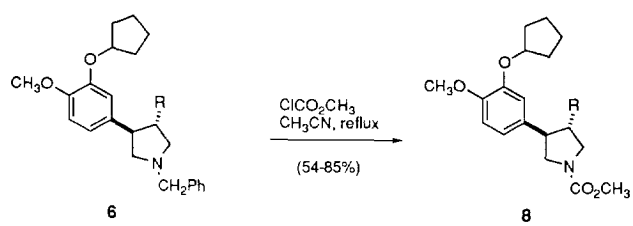
3

The 1,3,4-trisubstituted pyrrolidines described in this study were synthesized following the general route shown in Scheme 1. Dipolar cycloaddition between aryl-substituted olefins **4**[8] and the azomethine ylide that is generated by trifluoroacetic acid-mediated decomposition of *N*-benzyl-*N*-methoxymethyl-*N*-trimethylsilylmethylamine (**5**) afforded the *N*-benzylpyrrolidines **6** in good yields.[9] As anticipated, the olefin geometry in dipolarophile **4** is conserved in the cycloadduct. Unless the functional group at C3 was incompatible to reducing conditions, the benzyl group in **6** was removed by transfer hydrogenation. The derived free pyrrolidine **7** was then acylated under standard conditions to give the target compounds **3**.

Scheme 1



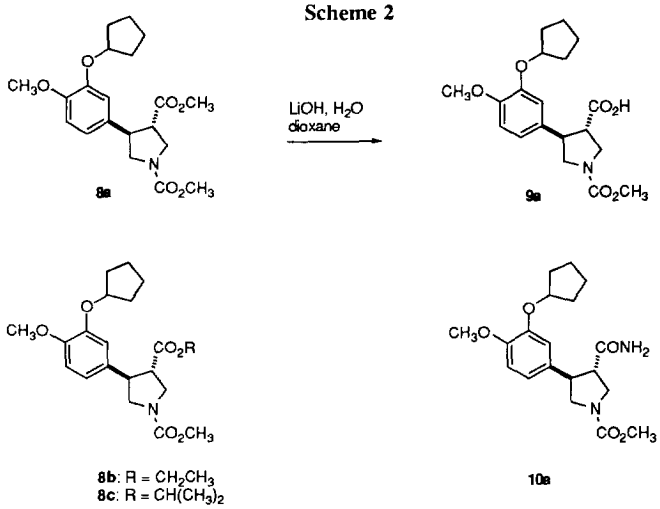
As an alternative to reductive debenzylation, direct acylation of the cycloadducts **6** could be accomplished by treatment with methyl chloroformate in refluxing acetonitrile, as shown below in equation 1. This method was particularly useful for compounds whose C3 functional group was competitively reduced under the conditions of transfer hydrogenation, (e. g., where R is cyano, nitro or phenylcarbonyl).



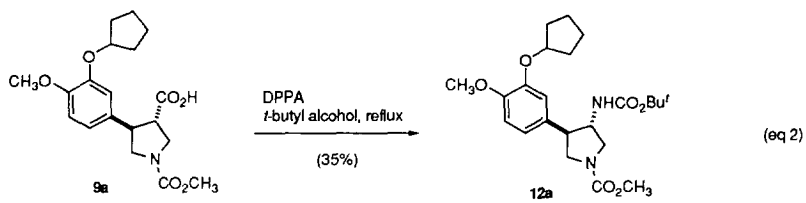
(eq. 1)

Carboxylic ester **8a** (R = CO_2CH_3) was identified early in our studies as a potent inhibitor of PDE IV (K_i = 0.16 μM). We therefore decided to pursue other carboxylic acid derivatives related to **8a**. Saponification of **8a** provided carboxylic acid **9a**, which then served to prepare either ester **8b** and **8c** via a Fischer esterification (alcohol, H_2SO_4) or amide **10** (i. 1,1'-carbonyldiimidazole (CDI) ii. NH_3) (Scheme 2).

Scheme 2



Treatment of **9a** with diphenylphosphoryl azide (DPPA)[10] in *t*-butyl alcohol affected a Curtius rearrangement to afford directly the *bis*-carbamate derivative **12a** (eq 2). It should be noted that **12a** possesses pseudo C_2-symmetry about the C4-C(aryl) bond. In the event that the enzyme is unable to discriminate between the two potential carbamate pharmacophores on binding, we reasoned that this compound symmetry might provide enhanced enzyme/inhibitor binding affinity (i. e., inhibition).



(eq 2)

PDE IV Inhibition and Structure-Activity Relationships

Test compounds were measured for their ability to inhibit the catalytic activity of the human PDE IV protein termed PDE IV_B (PDE IV_B is the human homolog of the rat PDE IV termed dpde4).[11] This protein was recently cloned from a human frontal cortex cDNA library, expressed in the yeast *S. cerevisiae*, and purified to functional purity.[12] The data are shown in the Table. Several conclusions can be made relating to the structure-activity of 1,3,4-trisubstituted pyrrolidines, the most important of which is that substitution at C3 is, in general, well tolerated. The diversity of functionality at C3 that preserves the PDE IV activity of the parent pyrrolidine[6c]

is exemplified by entries 1, 5, 6, 8, 10, 11, and 18. However, there are examples where the C3 substituent is clearly interfering with ability of the acyl pyrrolidine to inhibit the enzyme (entries 4, 13, 17, and 20). In each of these cases the C3 group is sterically demanding and/or aromatic. In addition, the C3 amino derivatives **12a-12e** (entries 13-17) were generally poor inhibitors.

Among the C3 carboxylic esters (entries 1-4), we observed greater than tenfold decreased potency with an increase in the size of the ester alkoxy group. In contrast, the exact opposite trend was observed at the N1 position of the pyrrolidine. Small and/or polar acyl groups are less tolerated than their larger, lipophilic counterparts. For example, comparing entries 21, 1, and 24-26, formamide **14a** is 0.81 μM, methyl carbamate **8a** is 0.16 μM, while the benzyl, isopropyl, and *t*-butyl carbamates are all less than 0.06 μM. Indeed, we observed that in all cases tested, *t*-butyl carbamates were approximately tenfold more potent inhibitors than the corresponding methyl carbamates (cf., entries 1 vs. 26, 7 vs. 8, 9 vs. 10, and 13 vs. 14). Additionally, the more lipophilic thiourea **14c** is about threefold more potent than the more polar urea **14b**. With respect to the alkoxy substitution on the aryl ring, we observe a significant loss in inhibitory activity when replacing the cyclopentoxy group (entries 26, 28, and 29). The importance of the N1 substituent is further highlighted by entries 1 and 27, in which a tenfold difference in inhibition potency is evidenced between the methyl carbamate and the isosteric propionamide.

Albeit not shown in the Table, we addressed the importance of the pyrrolidine C3-C4 relative stereochemistry. When R^2 is *cis* to the aromatic ring in both urea **14b** and carbamate **14f**, we measured K_i's of 9.75 μM and 4.70 μM, respectively.[13] We concluded from these two examples that C3-C4 relative stereochemistry is important and that *trans* C3-C4 stereochemistry is significantly preferred.

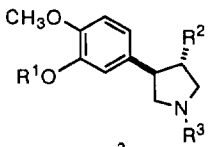
The most potent inhibitors from this series (K_i < 0.10 μM) were compounds **8e, 8f, 11a, 11b, 13a,** and **14d-f**. From this group, the methyl ketone **13a** was tested in our functional cellular assay, which measures the inhibition of TNF-α secretion from LPS-stimulated human monocytes, a biochemical effect that is sensitive to selective PDE IV inhibition.[15] In this assay **13a** had an IC_{50} = 457 nM (cf., rolipram IC_{50} = 320 nM). Additionally, its potency against PDE IV was at least 100-fold greater than other families (isotypes) of PDE.[16]

In summary we have described the synthesis and evaluation of a series of 1,3,4-trisubstituted pyrrolidines as novel inhibitors of human PDE IV. The synthesis of these inhibitors is efficient and relies on the use of an azomethine ylide cycloaddition reaction to rapidly assemble the pyrrolidine ring. The effect of C3 substitution on PDE IV inhibition is expressed by a number of structure-activity relationships, and this effect appears to be sensitive not only to the C3 substituent, *per se*, but also relative to the N1 substituent and the relative stereochemistry with C4. Methyl ketone **13a**, in particular, is a potent and selective PDE IV inhibitor (K_i = 0.03 μM) that demonstrated activity in a functional setting. Further studies within this general series of PDE IV inhibitors will be reported in due course.

Acknowledgement

The authors thank the ICOS Corporation (Bothell, WA) for supplying the PDE IV that was used in the enzyme experiments, and to Marcus F. Brackeen, Dr. Donald S. Karanewsky, and Dr. Frank J. Schoenen for helpful discussions throughout this study.

Table. PDE IV Inhibitory Activity of 1,3,4-Trisubstituted Pyrrolidines[a]



entry	cmpd	R^1	R^2	R^3	$K_i \pm s_m$ (μM)[b] (n)[c]
1	**8a**	cyclopentyl	CO_2CH_3	CO_2CH_3	0.16 ± 0.01
2	**8b**	cyclopentyl	$CO_2CH_2CH_3$	CO_2CH_3	0.55 ± 0.11
3	**8c**	cyclopentyl	$CO_2CH(CH_3)_2$	CO_2CH_3	2.29 ± 0.41
4	**8d**	cyclopentyl	$CO_2C(CH_3)_3$	CO_2CH_3	4.63 ± 1.76
5	**8e**	cyclopentyl	NO_2	CO_2CH_3	0.06 ± 0.002
6	**8f**	cyclopentyl	CN	CO_2CH_3	0.08 ± 0.01
7	**9a**	cyclopentyl	CO_2H	CO_2CH_3	1.88 ± 0.19
8	**9b**	cyclopentyl	CO_2H	$CO_2C(CH_3)_3$	0.15 ± 0.01
9	**10a**	cyclopentyl	$CONH_2$	CO_2CH_3	1.57 ± 0.26
10	**10b**	cyclopentyl	$CONH_2$	$CO_2C(CH_3)_3$	0.19 ± 0.04 (4)
11	**11a**	cyclopentyl	CH_2OH[d]	$CO_2C(CH_3)_3$	0.05 ± 0.01
12	**11b**	cyclopentyl	CH_2OCH_3[d]	$CO_2C(CH_3)_3$	0.08 ± 0.01
13	**12a**	cyclopentyl	$NHCO_2C(CH_3)_3$	CO_2CH_3	14.4 ± 2.67
14	**12b**	cyclopentyl	$NHCO_2C(CH_3)_3$	$CO_2C(CH_3)_3$	1.57 ± 0.18
15	**12c**	cyclopentyl	$NHCO_2C(CH_3)_3$	CO_2CH_2Ph	0.29 ± 0.06
16	**12d**	cyclopentyl	$NHSO_2CH_3$	CO_2CH_2Ph	1.47 ± 0.35 (4)
17	**12e**	cyclopentyl	$NHSO_2Ph$	CO_2CH_2Ph	4.42 ± 0.57
18	**13a**	cyclopentyl	$COCH_3$	CO_2CH_3	0.03 ± 0.002
19	**13b**	cyclopentyl	$COCH_2CH_3$	CO_2CH_3	0.35 ± 0.08
20	**13c**	cyclopentyl	COPh	CO_2CH_3	6.03 ± 1.58
21	**14a**	cyclopentyl	CO_2CH_3	CHO	0.81 ± 0.08
22	**14b**	cyclopentyl	CO_2CH_3	$CONH_2$	1.48 ± 0.19
23	**14c**	cyclopentyl	CO_2CH_3	$CSNH_2$	0.38 ± 0.03
24	**14d**	cyclopentyl	CO_2CH_3	CO_2CH_2Ph	0.05 ± 0.001
25	**14e**	cyclopentyl	CO_2CH_3	$CO_2CH(CH_3)_2$	0.04 ± 0.01 (4)
26	**14f**	cyclopentyl	CO_2CH_3	$CO_2C(CH_3)_3$	0.03 ± 0.01 (4)
27	**14g**	cyclopentyl	CO_2CH_3	$COCH_2CH_3$	1.13 ± 0.02 (4)
28	**14h**	methyl	CO_2CH_3	$CO_2C(CH_3)_3$	1.20 ± 0.13
29	**14i**	$PhOCH_2CH_2CH_2$	CO_2CH_3	$CO_2C(CH_3)_3$	0.24 ± 0.05
30	rolipram	—	—	—	0.22 ± 0.03 (9)

[a] All compounds are racemic. Satifactory spectral and analytical data (C, H, and N) were obtained on all compounds. [b] K_i values are expressed as the mean ± the standard deviation of the mean. [c] n is the number of experiments. In all cases, n = 3 unless noted otherwise. [d] See reference 14.

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8. Unsaturated esters used in the cycloaddition reaction were prepared by a Horner-Emmons condensation on the appropriate aryl aldehyde. The nitrostyrene derivative was prepared by a Henry condensation (0.1 eq $BnNH_2$, CH_3NO_2, 80 °C). Unsaturated ketones were prepared from cinnamate methyl ester by the following three-step procedure: 1. LiOH, dioxane/H_2O, 2. i. CDI, CH_2Cl_2 ii. *N, O*-dimethylhydroxylamine hydrochloride, 3. RLi or RMgBr, ether, 0 °C.

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13. The Still-Gennari modification of the Horner-Emmons condensation (*Tetrahedron Lett.* **1983**, *24*, 4405) was used on the appropriate isovanillin-derived aldehyde to prepare the (*Z*)-cinnamate ester (87% yield) for the azomethine ylide cycloaddition.

14. Alcohol **11a** was prepared by $LiAlH_4$ reduction of pyrrolidine **6** (R = CO_2CH_3) followed by the methods shown in Scheme 1. Ether **11b** was prepared from **11a** (NaH, CH_3I, DMF).

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16. Several compounds in this study were tested for PDE isotype selectivity, and all were found to be highly selective (≥ 10-fold) for PDE IV. The authors thank Dr. Timothy J. Martins and Carmen C. Hertel of ICOS, Co. for providing data from some of these experiments.

(*Received in USA* 3 *July* 1995; *accepted* 27 *July* 1995)