Inhibition of Cyclic Adenosine-3′,5′-monophosphate Phosphodiesterase from Vascular Smooth Muscle by Rolipram Analogues

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Rolipram [(*R*,*S*)-4-[3-(cyclopentyloxy)-4-methoxyphenyl]-2-pyrrolidone] has been shown to inhibit selectively the cAMP phosphodiesterase (PDE) of vascular smooth muscle. In order to further explore the structural requirements for selective PDE inhibition, we synthesized a series of rolipram derivatives differently substituted either at the pyrrolidinone or at the aromatic ring. Among these compounds, rolipram was the most active compound. Semirigid analogues were prepared and used for an evaluation of the active conformation of rolipram. Structural comparison with two other potent and chemically different smooth muscle cAMP-PDE inhibitors, trequinsin and Ro 20-1724, allows us to propose a first topological model of the smooth muscle cAMP-PDE pharmacophore.

Interest in the field of phosphodiesterase (PDE) inhibitors has been renewed within the recent years.[1] Such agents may affect cardiac and smooth muscles and platelets, as well as the central nervous system and various secretory functions. Many PDE inhibitors are not selective and exert their inhibiting activity toward every or several subtypes of PDE. Rolipram [(*R*,*S*)-4-[3-(cyclopentyloxy)-4-methoxyphenyl]-2-pyrrolidone] is described as a neurotropic compound, which may exert its pharmacological actions through an increase of the cyclic AMP (cAMP) levels in noradrenergic nerve endings.[2] It has been proved to be a potent and selective cAMP phosphodiesterase inhibitor in the brain.[3,4] This rolipram-sensitive form has also been isolated from vascular smooth muscle,[5,6] but not from platelets. Recently another cAMP-specific phosphodiesterase, which is inhibited by cyclic GMP, has been characterized.[7] The two forms have been found in cardiac preparations, the latter being the target of cardiotonic drugs. The selectivity of rolipram toward a specific form of PDE present in different tissues led us to investigate structure–activity relationships in a series of analogues of rolipram. We examined successively the role played by the alkoxy groups in the 3- and 4-position of the phenyl ring and the effects of substitution at the pyrrolidone ring. The present work describes the synthesis and the inhibitory activity of these analogues on different forms of PDE separated from bovine arterial muscle preparations. A model of the pharmacophore for the rolipram-sensitive vascular smooth muscle cAMP-PDE inhibitors is proposed.

Chemistry

Most of the 4-aryl-2-pyrrolidones **1–24** were prepared following modified literature procedures.[8] Compounds **7**, **9–11**, and rolipram were generous gifts from Dr. R. Schmiechen from Schering A.G., Berlin, Germany. Ro 20-1724 and trequinsin were provided respectively by Hoffmann-La Roche (Nutley, NJ) and by Hoechst A.G. (Frankfurt, Germany).

The most convenient method (method A, Scheme I) involves 1,4-addition of nitromethane to the corresponding methyl cinnamate, followed by a reductive cyclization. Method B leads to the expected pyrrolidinones with high yields. It consists of a cyanide addition to a substituted benzylidenemalonate, followed by an hydrogenation step in acidic medium and thermal ring closure. Method C was used for the synthesis of the 3-substituted 4-arylpyrrolidones and involves the addition of diethyl malonate to 2-(3′,4′-dimethoxyphenyl)nitroethylene. Alkylation and reductive cyclization led to the 3-carbethoxypyrrolidones **19**. Compound **19b** was further saponified and decarboxylated, affording the α-methylpyrrolidone **17**. The phenolic derivative **5** was obtained by catalytic hydrogenation of the benzyloxy precursor **12**. Functionalized compounds **13–16** were prepared as described in Scheme II. Pyrrolidone **6** served as starting material for compounds **20–26**. By known precedures,[8] it was N-alkylated to compounds **20**, **22**, and **23** or N-acylated to yield compounds **21** and **24**. The conversion to thiolactam **25** was achieved by means of P_2S_5 and sodium bicarbonate in a DME–MeCN mixture. Pyrrolidine **26** was obtained by a LAH reduction of pyrrolidone **6**.

cis-3,3a,8,8a-Tetrahydro-5,6-dimethoxyindeno[2,1-*b*]-pyrrol-2(1*H*)-one (**27**) (Table II) was prepared according to Jung et al.,[9] and surprisingly, only the cis isomer was obtained. Thus ^{1}H NMR (200 Hz) shows the characteristic coupling constant of 6 Hz between the two methine protons. *cis*- and *trans*-1,2,3a,4,5,9b-hexahydro-7,8-dimethoxybenz[*e*]isoindol-3-ones (**28** and **29**) were obtained (Scheme III) by a regioselective Diels–Alder reaction between methyl acrylate and 1-cyano-4,5-dimethoxybenzocyclobutene.[10,11] The resulting two diastereomeric cyano esters were separated by chromatography. After catalytic hydrogenation in acidic medium, they led to the corresponding amino esters. The cis isomer (J = 4.5 Hz)[10] spontaneously cyclized to the lactam **28**, whereas the cyclization of the trans isomer (J = 9.5 Hz) needed a reflux in toluene.

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‡ Laboratoire de Pharmacologie Cellulaire et Moléculaire.

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(2) Wachtel, H. *Neuropharmacology* **1983**, *22*, 267.
(3) Schwabe, U.; Miyake, M.; Ohga, Y.; Daly, J. W. *Mol. Pharmacol.* **1976**, *12*, 900.
(4) Nemoz, G.; Prigent, A. F.; Moueqqit, M.; Fougier, S.; Macovschi, O.; Pacheco, H. *Biochem. Pharmacol.* **1985**, *34*, 2997.
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(6) Lugnier, C.; Schoeffter, P.; Lebec, A.; Strouthou, E.; Stoclet, J. C. *Biochem. Pharmacol.* **1986**, *35*, 1743.
(7) Harrison, S. A.; Chang, M. L.; Beavo, J. A. *Circulation* **1986**, *73*, Suppl. III, 109.
(8) Seidelman, D.; Schmiechen, R.; Paschelke, G.; Muller, B. German Patent 2 413 935/3 (Schering A.G., Berlin), March 20, 1974.
(9) Jung, E.; Vossius, V.; Schirdewahn, J. English Patent 32 120/70 (Allen and Handburrys Ltd., London), July 2, 1970.
(10) Kametani, T.; Ogasawara, K.; Takahashi, T. *Tetrahedron* **1972**, *29*, 73.
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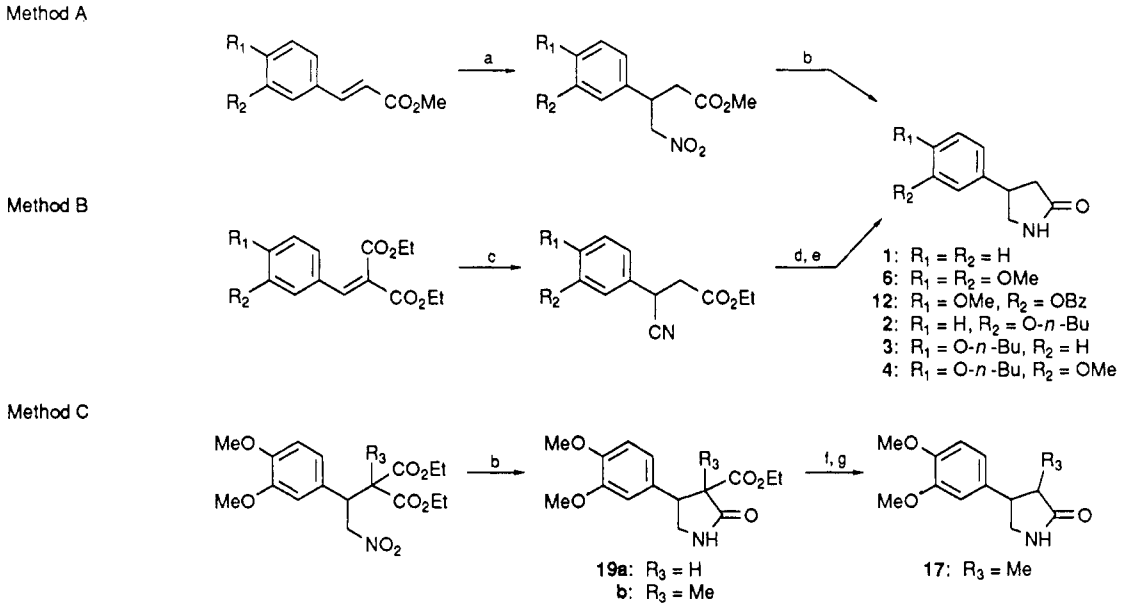
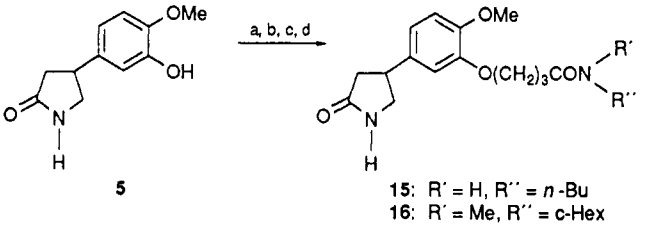
Scheme I[a]



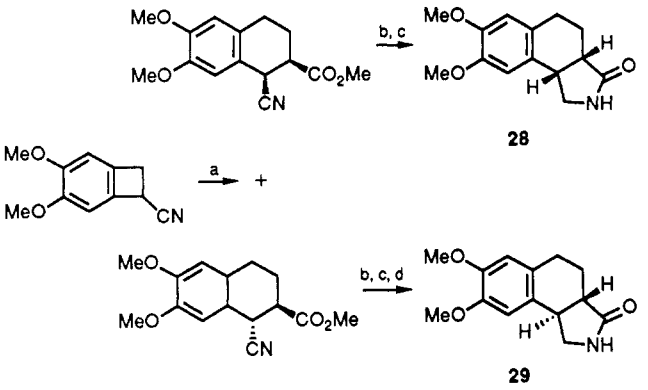
[a] (a) CH_3NO_2, TMG; (b) H_2–Raney Ni–MeOH, Δ; (c) KCN, piperidine; (d) H_2–Pd/C, H^+; (e) $(Et)_3N$, toluene, Δ; (f) KOH, EtOH–H_2O, Δ; (g) Δ, toluene.

Scheme II[a]



[a] (a) $Br(CH_2)_3CO_2Et$, DMF/K_2CO_3; (b) KOH, EtOH–H_2O, Δ; (c) $(Et)_3N$, isobutyl chloroformate; (d) R'R''NH.

Scheme III[a]



[a] (a) Methyl acrylate, Δ, pressure; (b) H_2–Pd/C, H^+; (c) $KHCO_3$; (d) Δ, toluene.

Phosphodiesterase Inhibition Studies

Compounds were tested on the three different forms of PDE isolated from bovine aorta.[6] The first form hydrolyzed preferentially cGMP and was activated 6–15-fold by calmodulin (CaM); it was named CaM-PDE. The second form hydrolyzed cGMP with a high degree of selectivity and was insensitive to CaM and cGMP; it was called cGMP-PDE. The third peak hydrolyzed preferentially cAMP and was not stimulated by CaM; it was named cAMP-PDE. The concentration of each drug that inhibited 50% of the enzymatic activity (IC_{50}) was determined at 1 μM cGMP in the presence of calcium (10 μM) and calmodulin (15 nM) for CaM-PDE, at 1 μM of cGMP and 1 μM cAMP respectively for cGMP-PDE and cAMP-PDE in the absence of calcium (1 mM EGTA). The curve obtained by plotting percent enzymatic activity versus the logarithmic concentration of the inhibitor included six concentrations in its linear portion. The IC_{50} was calculated by linear regression (correlation coefficient 0.95) and represents the mean of three determinations. The experimental error was about 15%.

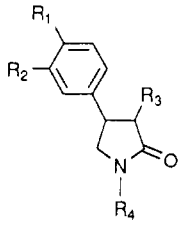
The physical and biochemical data for all the tested compounds are listed in Tables I and II.

Results and Discussion

Data given in Table I show that the unsubstituted (1) and the monoalkoxylated (2, 3) derivatives are inactive. Inactivity was also found for the free phenol 5. These results clearly indicate that alkoxy groups must be present in both the meta and para positions. However, the inactivity of the *p*-butoxy derivative 4 as well as that of other analogues with rather bulky substituents in the para position characterizes steric hindrance in the vicinity of this position. Various alkoxy aliphatic chains with three to five carbon atoms could be introduced in the meta position, leading to potent compounds (7–11), the highest potency being obtained with a cyclopentyloxy group (rolipram). A benzyloxy group (12) was less favorable. The dimethoxy analogue of rolipram, compound 6, still showed a significant activity (IC_{50} = 53 μM, compared to 5 μM for rolipram). Given its easy accessibility, the dimethoxy substitution was therefore kept during structural variations at the pyrrolidine ring. Introduction of a butyric ester or amide in the meta position (14–16) improved the activity, when compared with compound 6. In contrast polar groups or ionized chains attached to the meta position appear to be unfavorable, as illustrated by the inactivity of the meta phenolic derivative 5 and the free carboxylic acid 13. The good activity of amide 15 prompted us to use the carboxylic precursor 13 for affinity chromatography studies.[12] Improvement of activity with regard to the reference compound 6 was also observed for the 3-methyl

(12) Fougier, S.; Nemoz, G.; Prigent, A. F.; Marivet, M.; Bourguignon, J. J.; Wermuth, C. G.; Pacheco, H. *Biochem. Biophys. Res. Commun.* **1986**, *138*, 205.

Table I. Physical Data and PDE Inhibitory Capacities of Various Substituted Rolipram Analogues



no.	R_1	R_2	R_3	R_4	mp, °C (lit.)	recrystn[b] solvent	formula[c]	IC_{50}, μM CaM-PDE	IC_{50}, μM cGMP-PDE	IC_{50}, μM cAMP-PDE
1	H	H	H	H	78 (76–77)	A	$C_{10}H_{11}NO$	ns	ns	ns
2	H	O-*n*-Bu	H	H	134	C/D	$C_{14}H_{19}NO_2$	ns	ns	ns
3	O-*n*-Bu	H	H	H	132	C/D	$C_{14}H_{19}NO_2$	ns	ns	ns
4	O-*n*-Bu	OMe	H	H	118 (118)	C	$C_{15}H_{21}NO_3$	ns	ns	ns
5	OMe	OH	H	H	142 (144)	B	$C_{11}H_{13}NO_3$	ns	ns	ns
6	OMe	OMe	H	H	121 (120)	C	$C_{12}H_{15}NO_3$	ns	ns	53
7	OMe	$OCH_2C{=}CH$	H	H			$C_{14}H_{15}NO_3$	ns	ns	32
8	OMe	O-*n*-Bu	H	H	125 (125)	D	$C_{15}H_{21}NO_3$	ns	ns	13
9	OMe	O-*i*-Bu	H	H			$C_{15}H_{21}NO_3$	ns	ns	18
10	OMe	$OCH_2C(CH_3){=}CH_2$	H	H			$C_{15}H_{19}NO_3$	ns	ns	24
11	OMe	$OCH_2CH(CH_3)C_2H_5$	H	H			$C_{16}H_{23}NO_3$	ns	200	46
12	OMe	OBz	H	H	136 (132)	C	$C_{18}H_{19}NO_3$	ns	174	96
13	OMe	$O(CH_2)_3COOH$	H	H	106–110	C	$C_{15}H_{19}NO_5{\cdot}H_2O$	ns	ns	400
14	OMe	$O(CH_2)_3COOEt$	H	H	105	A	$C_{17}H_{23}NO_5$	ns	ns	30
15	OMe	$O(CH_2)_3CONH$-*n*-Bu	H	H	136	D	$C_{19}H_{28}N_2O_4$	ns	ns	16
16	OMe	$O(CH_2)_3CON(Me)$cHex	H	H	133	B	$C_{22}H_{32}N_2O_4$	ns	ns	34
17	OMe	OMe	Me	H	135	C/D	$C_{13}H_{17}NO_3$	ns	ns	22
18	OMe	OMe	COOH	H	110 dec	C	$C_{13}H_{15}NO_5$	ns	ns	ns
19	OMe	OMe	COOEt	H	108 (106)	A	$C_{15}H_{19}NO_5$	ns	ns	21
20	OMe	OMe	H	Me	69 (69)	D	$C_{13}H_{17}NO_3$	ns	ns	34
21	OMe	OMe	H	COMe	128 (135)	A	$C_{14}H_{17}NO_4$	ns	ns	25
22	OMe	OMe	H	Ph	100 (104)	D	$C_{18}H_{19}NO_3$	230	ns	19
23	OMe	OMe	H	Bz	67–68	D	$C_{19}H_{21}NO_3$	ns	ns	17
24	OMe	OMe	H	COPh	146	D	$C_{19}H_{19}NO_4$	ns	ns	58

[a] ns: $IC_{50} > 10^{-3}$ M. [b] A, EtOH; B, *i*-PrOH; C, AcOEt; D, *i*-Pr_2O. [c] Analytical data for new compounds were within ±0.4% of the theoretical values unless otherwise stated.

and the 3-carbethoxy derivatives **17** and **19**. The pyrrolidonecarboxylic acid **18** was totally devoided of activity. This inactivation may result from an intramolecular hydrogen bond between the carboxylic hydrogen and the carbonyl oxygen, rendering this latter unable to establish a hydrogen bond with the enzymatic site. The carbonyl group of the pyrrolidone in rolipram seems to be fundamental for the activity as illustrated by the total inactivity of compounds **25** and **26**, in which the carbonyl group was replaced by a thiocarbonyl or a methylene group, respectively, both being unable to act as hydrogen acceptors. Unexpectedly N-substitution at the pyrrolidone ring led to more potent and still specific derivatives. Thus the *N*-phenyl and the *N*-benzyl derivatives **22** and **23** were 3 times more active than the reference compound **6**. Among the three rigid rolipram derivatives **27–29**, the indanopyrrolidone **27** was by far the less active on the three phosphodiesterase forms, whereas the 2-bridged trans isomer **29** was only 2 times less active than the reference compound **6**. In addition to rolipram, two other reference PDE inhibitors, Ro 20-1724 [4-[3-(butyloxy)-4-methoxybenzyl]imidazolidin-2-one] and trequinsin, an isoquinoline derivative, were tested on the three PDE forms of vascular smooth muscle. Ro 20-1724 is described as a potent inhibitor of erythrocytes PDE with an IC_{50} value of 0.1 μM.[13] This compound presents also some activity on vascular smooth muscle PDE, with the same profile as that of rolipram,[2-4,6] and is 2 times less active than the corresponding *m*-butyloxy analogue of the rolipram series (compound **8**).

Trequinsin is a very potent inhibitor of the blood platelets PDE.[14] In our hands this compound was found highly active on the smooth muscle cAMP-PDE (IC_{50} = 0.4 μM), however, with a weak selectivity with regard to CaM-PDE and cGMP-PDE.

The data given in Table I illustrate substituent effects at the phenyl ring of rolipram: the replacement of the *p*-methoxy group by other alkoxy groups or its suppression lead to inactive compounds, whereas large lipophilic substituents are tolerated in the meta position. Similar structure–activity relationship (SAR) criteria are found in the Ro 20-1724 series, that is, the presence of a dialkoxyphenyl group with the same substituent effects as those found in the rolipram series and necessity of a carbonyl dipole that can be compared with the carbonyl of rolipram. These common requisites suggest that both series share a common mode of interaction with the enzymatic site. In addition the presence of a medium-sized substituent is allowed at the vicinity of the carbonyl group and rather large substituents at the lactam nitrogen were favorable as shown by the good activities of compounds **17**, **19**, and **20–24**.

In order to illustrate some conformational characteristics of the cAMP-PDE inhibitors presented here, a computer graphics study was undertaken. The structures represented in Figures 1 and 2 were built and compared by the SYBYL molecular modeling package[15] on a GOULD SEL.MPX 32/77 computer system. A geometry optimi-

(13) Sheppard, H.; Wiggan, G. *Mol. Pharmacol.* **1970**, *7*, 111.

(14) Lal, B.; Dohadwalla, A. N.; Dadkar, N. K.; D'Sa, A.; De Souza, N. J. *J. Med. Chem.* **1984**, *27*, 1470.

(15) Tripos Associates, Inc., St Louis, MO 63117.

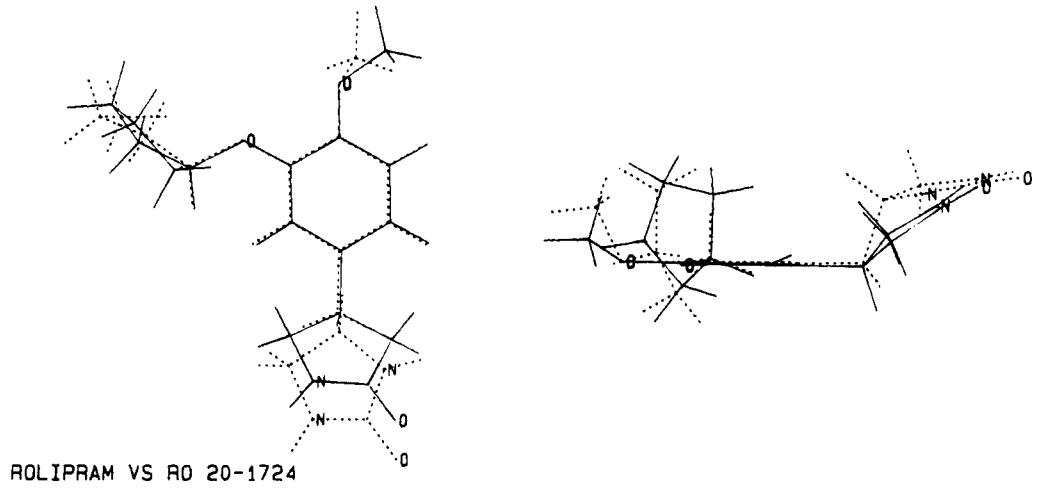


Figure 1. Computer-generated best fits of rolipram (torsion angle θ = 141°) and Ro 20-1724 (126° and 113°, dashed lines).

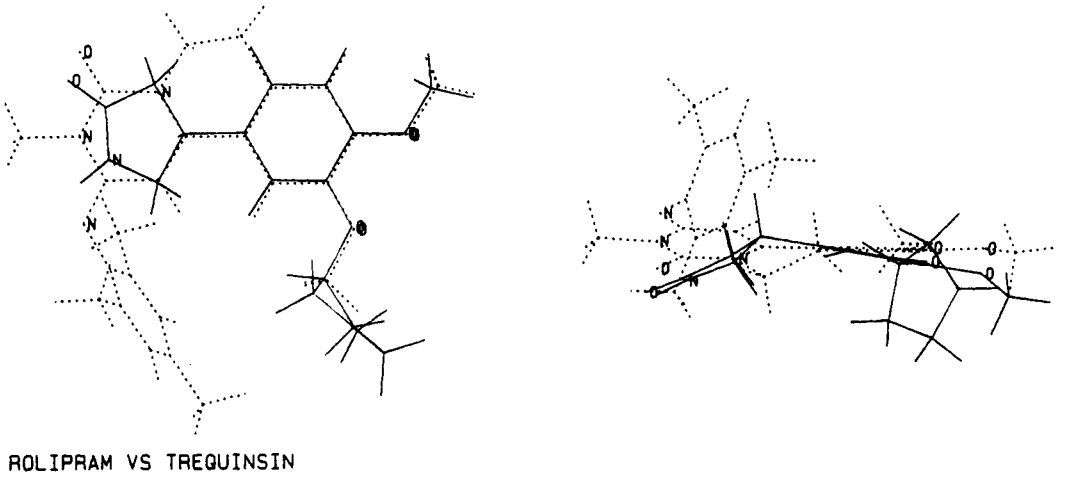


Figure 2. Superposition of rolipram (as compound 6) with trequinsin (dashed lines).

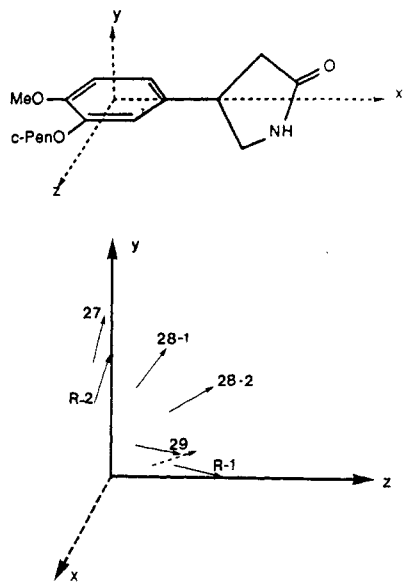


Figure 3. Graphic representation of carbonyl dipole traces of rolipram analogues in the *YOZ* plane: R-1 and R-2 are two singular conformations of rolipram (see text); dotted arrows refer to trequinsin.

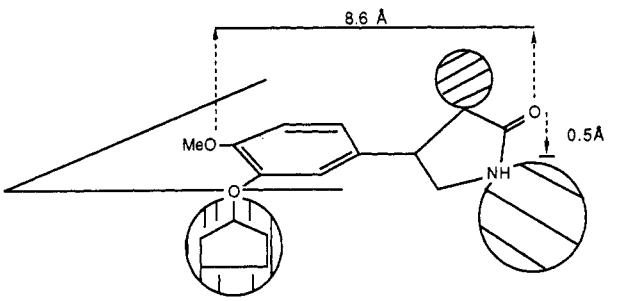


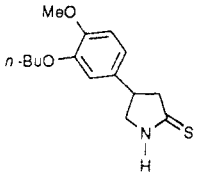
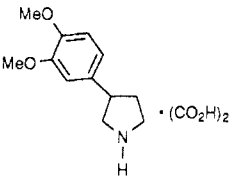
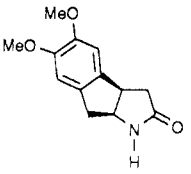
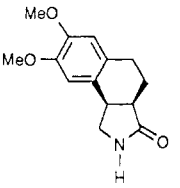
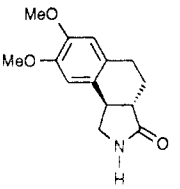
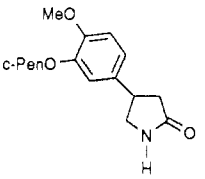
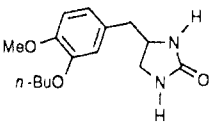
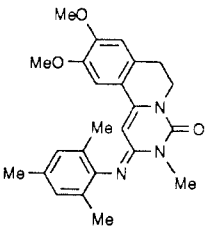
Figure 4. Structural and topological requirements associated with vascular smooth muscle cAMP-PDE inhibitors deriving from rolipram.

zation was carried out with MAXIMIN, a method available in SYBYL and which uses the Simplex algorithm. When necessary, a search of sterically allowed, low-energy conformations was performed, neglecting the charge distribution. This SEARCH option is a conformational analysis based on screening of van der Waals contact distances. For the optically active rolipram analogues, the same *R* configuration for the benzylic carbon was always selected.

As illustrated in Figure 1, the overlay of rolipram and Ro 20-1724 shows that the carbonyl dipoles of both structures in a given, energically favorable conformation occupy the same region of space and present a similar orientation. The distance measured between the carbonyl and the *p*-methoxy oxygen is 8.5 Å for rolipram, whereas the corresponding distance for Ro 20-1724 in the conformation represented in Figure 1 is 9.2 Å.

Trequinsin presents also a carbonyl function at the characteristic distance of 8.5 Å from the oxygen of the *p*-methoxy group and can be superposed on rolipram as illustrated in Figure 2. This comparison suggests for the free rotating rolipram an active conformation, in which the

Table II. Physical Data and PDE Intensity Capacities of Rolipram Derivatives and Reference Compounds

no.	structure	mp, °C	cryst solvent	formula	CaM-PDE	cGMP-PDE	cAMP-PDE
25		83	$EtOH/H_2O$	$C_{15}H_{21}NO_2S$	ns	195	500
26		134	THF	$C_{14}H_{19}NO_6$	ns	ns	ns
27		231 (231–232)	AcOEt	$C_{13}H_{15}NO_3$	ns	ns	800
28		180	AcOEt	$C_{14}H_{17}NO_3$	ns	ns	220
29		236	AcOEt	$C_{14}H_{17}NO_3$	ns	ns	138
rolipram					2700	246	5
Ro 20-1724					940	380	26
trequinsin					13	5	0.4

carbonyl group presents the antiorientation toward the *m*-alkoxy substituent. Additional information about the active conformation of rolipram and its analogues was gained by the comparison of rolipram with the three rigid structures **27**, **28**, and **29** and trequinsin.

For this purpose, we have chosen the coordinate system defined in Figure 3. For this representation, the phenyl ring of rolipram was placed in the plane defined by the *X* and *Z* axes, the C–C bond between the aromatic and the pyrrolidone ring corresponding to the *X* axis. The *Y* axis was chosen perpendicular to the phenyl ring in its center (origin of axes). Figure 3 represents projections of carbonyl dipole vectors of rolipram analogues in the YOZ plane. In this mode of representation, the carbonyl group can adopt a large number of orientations. Two singular conformations have been chosen as references. The first one called "R-1" places the carbonyl oxygen on the *X* axis and renders the carbonyl dipole nearly coplanar with the aromatic ring. The second one ("R-2") sets the carbonyl oxygen on the *Y* axis and represents rolipram in a twisted conformation.

With the same mode of representation, the inactive rigid indanopyrrolidone **27** presents a twisted conformation and shows an orientation of the carbonyl dipole similar to that observed for rolipram in the "R-2" conformation. For the

Table III. Conformational Data of Rolipram, Semirigid Analogues of Rolipram, and Trequinsin

compd	conformation	θ,[a] deg	d, Å	E,[b] kcal/mol
rolipram	R-1	26.5	8.5	6.4
	R-2	63	8.5	13.5
27		52.4	7.2	9
28	28-1	28.4	7.4	8
	28-2	9.1	7.9	4.7
29		31.2	8.8	8.3
trequinsin		15.3	8.6	3

[a] θ = dihedral angle between the aromatic and the mean plane of the pyrrolidine ring. [b] E is the global minimal energy as a sum of bond, bond angle, torsional angle, and van der Waals energy terms (SYBYL).

weakly active semirigid cis isomer **28**, two conformations called (28-1) and (28-2) were energetically accessible (Table III). As shown in Figure 3, these two conformations mimic two intermediate orientations of rolipram. The rigid trans isomer **29** observed in the same conditions can be compared with rolipram in its R-1 conformation. Moreover, the carbonyl dipole of the highly potent and rigid trequinsin coincides with those of rolipram (R-1) and **29**. These results suggest that in its active conformation, rolipram adopts an orientation of the carbonyl dipole as coplanar as possible with the phenyl ring. Such an orientation is present in the potent trequinsin and probably accounts for the relative activity of the trans isomer **29**. Thus the inactivity of the rigid analogues **27** and **28** may result from wrong orientations of the carbonyl vector. However interatomic distance criteria have to be considered. As discussed above, the measured distance between the carbonyl oxygen and the *p*-methoxy oxygen, which ranges between 8.5 and 8.8 Å for rolipram, trequinsin, and compound **29**, can be extended to 9.2 Å in the Ro 20-1724 series. Lesser distances are found for the inactive analogues **27** and **28** (7.2–7.9 Å). Thus the inactivity of these compounds may result from an unfavorable carbonyl orientation, as well from a too short interatomic distance d, or even from a combination of both factors. It is noteworthy that the particularly high activity of trequinsin can be attributed to the existence of additional binding sites due to the mesidine moiety.

Taken together, the present SAR studies emphasize some topological aspects of the enzymatic recognition site for the smooth muscle cAMP-PDE: (a) the pharmacophore is mainly characterized by the existence of a carbonyl dipole at a typical distance from a dialkoxyphenyl ring (d = 8.6 Å); (b) the dialkoxyphenyl ring is surrounded by a region sensitive to steric hindrance in the para position and a large hydrophobic pocket in the meta position; (c) a relatively large hydrophobic pocket close to the lactam nitrogen and a medium-sized space around the carbon atom adjacent to the carbonyl group characterize the environment of the lactam ring; (d) the active conformation of rolipram presents a relatively flat overall topography. Trequinsin and Ro 20-1724 fulfill the main criteria of this model.

The present model has to be compared with another model proposed for cardiac cAMP-PDE[16] and which was derived from 6-aryldihydropyridazinones. This model may also account for the PDE inhibitory activity of structurally related compounds, such as milrinone and piroximone. Both have in common the existence of an electron-rich aromatic group situated at approximately the same distance from an oxygen carbonyl group and a generally flat topography of the molecule. However, they present some differences, particularly the nature of substituents at the phenyl ring and the possibility of N-substitution of the pyrrolidone ring of rolipram, in opposition with the necessity in the cardiotonic pyridazinone series to maintain an NH acidic proton adjacent to the carbonyl group.[16] These differences could be related to the recent finding that cardiac cAMP-PDE can be dissociated in two enzymatic forms, one being the rolipram-sensitive[16] and the other being inhibited by cardiotonic drugs[7] such as the CI[17] or LY[18] series deriving from 6-aryldihydropyridazinones.

In conclusion, the effects of known inhibitors differ markedly on smooth muscle or on platelet PDE. Rolipram represents a good tool to differentiate both enzymatic systems. However, discrimination between cardiac and smooth muscle PDE seems more critical, as shown by the structural similarities found in both pharmacophore models.

Clinical use of PDE inhibitors requires the development of drugs with a high specificity.[5] Works are in continuation for a better knowledge of structural requirements for the design of potent and selective cAMP-PDE inhibitors in different tissues.

Experimental Section

Melting points were obtained on a calibrated Kofler hot-stage apparatus and are uncorrected. ^{1}H NMR spectra were recorded on Bruker WP80 (80 MHz) and WP 200 Sy (200 MHz) instruments, and chemical shifts are reported in parts per million (δ) relative to Me_4Si. All the compounds were analyzed for C, H, and N and gave results within ±0.4% of the theoretical values.

4-[3-(Benzyloxy)-4-methoxyphenyl]-2-pyrrolidinone (12) (Method A). To a solution of 9.0 g (31 mmol) of 3-benzylisoferulic acid methyl ester in 16 mL of nitromethane was added 0.8 mL of 1,1,3,3-tetramethylguanidine, and the mixture was heated with stirring at 70 °C for 27 h. After removal of the solvent, the residue was taken in AcOEt, washed with a 2 N HCl solution and then with water, and dried over Na_2SO_4. After evaporation of the solvent and chromatography (silica, AcOEt/hexane, 1:3), 9.6 g of methyl 3-[3-(benzyloxy)-4-methoxyphenyl]-4-nitrobutyrate was collected as a yellow oil (88%). The latter in 100 mL of MeOH was hydrogenated at 60 °C for 10 h in the presence of 1 g of Raney nickel catalyst. The crude pyrrolidone **12** was recovered and recrystallized from AcOEt (55%); mp 136 °C (lit.[8] mp 132 °C).

4-(3-Butoxy-4-methoxyphenyl)-2-pyrrolidinone (4) (Method B). To a solution of diethyl (4-butoxy-3-methoxybenzylidene)malonate (35.0 g, 0.1 mol) in 180 mL of MeOH was added dropwise 6.5 g (0.1 mol) of KCN dissolved in 25 mL of H_2O. The medium was heated at 60 °C for 7 h. The cold mixture was filtered, concentrated, and extracted with several portions of AcOEt. The combined organic layers were washed with brine, dried, and evaporated to dryness to give the crude ethyl 3-cyano-3-[4-*n*-(butyloxy)-3-methoxyphenyl]propionate. This latter was hydrogenated in the presence of 1.5 g of Pd/C (10%) in 120 mL of MeOH containing 5 mL of concentrated HCl over a 12-h period and under a 40-psi pressure. The crude recovered viscous oil was crystallized with a mixture of 2-propanol/diethyl ether (1:9); yield 75–85%. The hydrochloride of the corresponding 4-aminobutyrate (3.45 g, 10 mmol) was treated with $(Et)_3N$ (1.4 mL, 10 mmol) in 50 mL of toluene. The mixture was refluxed until the disappearance of the starting material. The cold suspension was evaporated under vacuum and the residue was taken up in CH_2Cl_2. The organic layer was washed with a 10% HCl solution and H_2O, dried, and removed in vacuo, affording crude pyrrolidone **4**, which was recrystallized from AcOEt (50–67%); mp 118 °C.

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4-(3,4-Dimethoxyphenyl)-3-methyl-2-pyrrolidinone (17) (Method C). The 4-(3,4-dimethoxyphenyl)-3-(ethoxycarbonyl)-3-methyl-2-pyrrolidinone was obtained from 2-(3,4-dimethoxyphenyl)nitroethylene and diethyl malonate[19] with an overall yield of 70%. The ester (15.4 g, 50 mmol) in 200 mL of EtOH and 60 mL of 1 N NaOH solution was refluxed for 30 min, cooled, and evaporated to dryness. The residue was triturated in AcOEt. After filtration the solid was taken in H_2O. The aqueous phase was saturated with NaCl and then carefully acidified. The precipitate was collected, washed with cold water, and dried, giving 11.9 g (90%) of an essentially pure acidic compound, which was decarboxylated by refluxing it in toluene under nitrogen for 6 h. After removal of the solvent, the crude in CH_2Cl_2 was washed with a 10% $KHCO_3$ solution and then with H_2O. After drying and evaporation of the solvent, the crude **17** was crystallized from a mixture of 2-propanol/diisopropyl ether (yield 70%); mp 135 °C.

4-[3-[[(Ethoxycarbonyl)propyl]oxy]-4-methoxyphenyl]-2-pyrrolidinone (14). One gram (3.36 mmol) of **12** in 150 mL of EtOH containing one drop of $HClO_4$ was hydrogenated in a Parr apparatus for 12 h in the presence of 200 mg of Pd as catalyst (Engelhard, 10% Pd/C). The crude pyrrolidone **5** was recrystallized from 2-propanol (75%); mp 142 °C (lit.[20] mp 145 °C). The phenolic derivative **5** (2.6 g, 12.5 mmol) in 25 mL of DMSO was reacted for 12 h at room temperature with 1.6 mL (12.5 mmol) of ethyl 4-bromobutyrate in the presence of 2.6 g (18.8 mmol) of powdered K_2CO_3 and a catalytic amount of KI. The mixture was poured into 150 mL of H_2O and extracted with AcOEt. The combined organic layers were washed with brine and dried. After removal of the solvent, the residue was crystallized from hexane, affording 3 g (75%) of **14**: mp 105 °C; NMR ($CDCl_3$) δ 1.30 (t, 3 H), 2.15–2.85 (m, 6 H), 3.2–4.2 (m, 7 H), 3.90 (s, 3 H), 6.85 (m, 3 H), 7.5 (s, 1 H).

4-[3-[[[(*N*-Cyclohexyl-*N*-methylamino)carbonyl]propyl]oxy]-4-methoxyphenyl]-2-pyrrolidinone (16). Ester **14** was first saponified as described in method C. The free acid corresponding to **14** was crystallized in EtOH (78%); mp 107–109 °C. The obtained compound (2.93 g, 10 mmol) in 25 mL of dry $CHCl_3$ was treated with 1.4 mL (10 mmol) of $(Et)_3N$. To the cold stirred solution was added dropwise 1.3 mL (10 mmol) of isobutyl chloroformate in 5 mL of $CHCl_3$. The mixture was maintained at 4 °C for 15 min. A solution of 1.6 mL (12 mmol) of *N*-methyl-*N*-cyclohexylamine in 5 mL of $CHCl_3$ was then added dropwise and the solution was kept at 4 °C for 15 min and then left at room temperature until consumption of the reagents. Another 70-mL $CHCl_3$ portion was added to the mixture, and the medium was extracted with a 2 N HCl solution and then with H_2O. After removal of the solvent, the crude product was crystallized in 2-propanol, affording 3.3 g (80%) of amide **16**: mp 133 °C; NMR (DMSO-d_6) δ 0.9–2.1 (m, 10 H), 1.90–2.35 (m, 2 H), 2.4–2.7 (m, 2 H), 2.70–3.05 (m, 2 H), 3.12 (s, 3 H), 3.2–4.0 (m, 4 H), 3.9 (s, 3 H), 4.1 (t, 2 H), 6.9 (s, 3 H), 7.15 (br s, 1 H).

1-Benzyl- and 1-Benzoyl-4-(3,4-dimethoxyphenyl)-2-pyrrolidinones (23 and 24). To 1.1 g (5 mmol) of **6** in 8 mL of DMF was added with cooling 135 mg (5.5 mmol) of NaH in one portion. The stirred mixture was carefully heated at 60 °C, until the gas evolution ceased. To the medium maintained at 4 °C was added 1.1 equiv of benzyl bromide (or benzoyl chloride) in 3 mL of DMF. The medium was heated at 50 °C for 15 min. The cold solution was poured into NaCl-saturated H_2O solution and extracted with AcOEt. After removal of the solvent, the product was crystallized in diisopropyl ether (55%): **23**, mp 68 °C; **24**, mp 146 °C.

3-(3,4-Dimethoxyphenyl)pyrrolidine (26). A solution of compound **6** (0.6 g, 2.71 mmol) in 10 mL of dry THF was carefully added dropwise to a suspension of 300 mg (7.91 mmol) of LAH in 10 mL of THF. The mixture was refluxed for 1 h. To the cold medium was gently added 0.6 mL of H_2O with vigorous stirring. The mixture was filtered and washed with benzene. After removal of the solvent, the residue was dissolved in a minimum of THF and added to a solution of 245 mg (2.71 mmol) of oxalic acid in a minimum of THF. The precipitated salt was filtered and recrystallized from THF, affording 0.46 g (50%) of pure **26**, mp 134 °C.

***cis*-3,3a,8,8a-Tetrahydro-5,6-dimethoxyindeno[2,1-*b*]pyrrol-2(1*H*)-one (27).** This compound was prepared according to a modified patent literature procedure[9] starting from 3-(3,4-dimethoxyphenyl)glutaric acid: mp 231 °C (AcOEt) (lit. mp 231–232 °C); NMR ($CDCl_3$) δ 2.49 (dd, 1 H), 2.77–2.92 (m, 2 H), 3.22 (m, 1 H), 3.86 (s, 6 H), 3.95 (m, 1 H), 4.5 (m, 1 H, J = 6 Hz), 6.73 (s, 2 H), 7.27 (s, 1 H).

***cis*- and *trans*-1,2,3a,4,5,9b-Hexahydro-7,8-dimethoxybenz[*e*]isoindol-3-ones (28 and 29).** The *cis*- and *trans*-methyl 1-cyano-6,7-dimethoxytetralin-2-carboxylates were obtained from 1,2 dihydro-4,5-dimethoxybenzocyclobutene-1-carbonitrile according to Kametani.[11] The two diastereoisomers were separated by chromatography on silica (AcOEt/hexane 1:4): trans isomer (minor), mp 111 °C; cis isomer, mp 124 °C. The *cis*-cyano ester (0.5 g, 1.6 mmol) in 80 mL of MeOH in the presence of 1 mL of concentrated HCl was hydrogenated over 100 mg of 10% Pd/C as catalyst in a Parr apparatus at 40 psi for 12 h. The catalyst was removed by filtration and the solvent was evaporated under reduced pressure. The crude *cis*-amino ester hydrochloride was dissolved in 10 mL of H_2O. After addition of 20 mL of saturated $KHCO_3$, the lactam **24** precipitated. It was extracted with $CHCl_3$, and the extracts were washed with H_2O and dried over Na_2SO_4. After evaporation of the solvent and recrystallization from AcOEt, the cis lactam **28** was obtained as white needles (300 mg, 64%): mp 180 °C; NMR ($CDCl_3$) δ 1.90 (m, 2 H), 2.80 (m, 3 H), 3.20 (m, 1 H), 3.70–3.90 (m, 2 H), 3.86 (s, 6 H), 5.90 (s, 1 H, NH), 6.60 (s, 2 H).

The trans tetralin isomer (0.5 g, 1.6 mmol) was hydrogenated as indicated above. The crude *trans*-amino ester hydrochloride was suspended in 50 mL of toluene and 180 mg (1.76 mmol) of $(Et)_3N$. The mixture was refluxed for 6 h. After cooling, the solvent was evaporated in vacuo. The residue was taken up in $CHCl_3$, and the organic layer was washed with dilute HCl and then with H_2O and dried over Na_2SO_4. After removal of the solvent, the resulting solid was recrystallized from AcOEt, yielding the trans lactam **29** (235 mg, 48%): mp 236 °C; NMR ($CDCl_3$) δ 1.60–1.84 (m, 2 H), 2.1–2.4 (m, 2 H), 2.9–3.0 (m, 2 H), 3.15–3.25 (m, 1 H), 3.30–3.40 (m, 1 H), 3.84 (s, 6 H), 5.95 (s, NH), 6.48 (s, 1 H), 6.68 (s, 1 H).

Phosphodiesterase Preparation. Bovine aorta media layers (10 g) were minced and homogenized at 4 °C with an Ultraturrax (6 × 15 s) and a glass pestle homogeneizer in 6 volumes (w/v) of buffer (20 mM Tris·Cl, 2 mM magnesium acetate, 1 mM dithiotreitol, 5 mM EDTA, 2000 units/mL aprotinin, pH 7.5). The homogenate was centrifuged at 105000*g* for 60 min, 4 °C. The supernatant was applied to a DEAE-trisacryl (IBF) column (15 cm × 2.4 cm) and eluted first with buffer A (20 mM Tris·HCl, 2 mM magnesium acetate, 1 mM dithiotreitol, pH 7.5) until no absorbance at 280 nm was detected in the column eluat (about 350 mL). Elution at 50 mL h^{-1} flow rate was then continued with a linear gradient (0–0.4 NaCl in buffer A, 600 mL). Seven-milliliter fractions were collected in test tubes containing 0.2 mL of 40 mg/mL BSA. The different peaks collected were dialyzed overnight with three changes of buffer B (20 mm Tris·HCl, 2 mM magnesium, pH 7.5) distributed in aliquots and stored at −80 °C. Three peaks of phosphodiesterase activities were eluted. The first preferentially hydrolyzes cGMP and was activable by Ca^{2+} calmodulin called CaM-PDE. The second hydrolyzes cGMP, was insensitive to Ca^{2+} calmodulin, and was named cGMP-PDE. The third preferentially hydrolyzes cAMP, was also insensitive to Ca^{2+} calmodulin, and was named cAMP-PDE.

Phosphodiesterase Assay. Phosphodiesterase activities were determined according to Keravis et al.[21] in the presence of calcium ($CaCl_2$, 10 mM) and an excess of calmodulin (15 mM) from bovine brain[22] or with 0.1 mM EGTA. Time and enzyme concentrations in the assay medium were adjusted so that no more than 15% of the substrate was hydrolyzed in the assay conditions. Drugs

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were tested on CaM-PDE at 1 μM cGMP in the presence of calmodulin, on cGMP-PDE at 1 μM cGMP in the presence of EGTA, and on cAMP-PDE at 1 μM cAMP in the presence of EGTA.

The concentration of the drug that inhibits 50% of the enzymatic activity (IC_{50}) was determined with a minimum of six concentrations of drugs in the linear part of the curve obtained by fitting percent enzymatic activity to the logarithmic concentration of the drug. IC_{50} was calculated by linear regression (correlation coefficient was >0.950). For the assay the drugs were dissolved in DMSO or DMF and diluted so that in the assay medium the final concentration of the organic solvent was not higher than 1%. Controls were run with the assay medium in the same conditions and did not modify enzymatic activity. Results were expressed as mean ± SEM derived from three determinations performed on different enzyme preparations.

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Synthesis and Antiviral Activity of the Nucleotide Analogue (*S*)-1-[3-Hydroxy-2-(phosphonylmethoxy)propyl]cystosine

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The acyclic nucleotide analogue (*S*)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine (**2**, HPMPC) was prepared on a multigram scale in 18% overall yield starting from (*R*)-2,3-*O*-isopropylideneglycerol. The key step in the nine-step synthetic route is coupling of cytosine with the side-chain derivative **8** which bears a protected phosphonylmethyl ether group. In vitro data showed that HPMPC has good activity against herpes simplex virus types 1 and 2, although it was 10-fold less potent than acyclovir [ACV, 9-[(2-hydroxyethoxy)methyl]guanine]. By comparison, HPMPC exhibited greater activity than ACV against a thymidine kinase deficient strain of HSV 1 and was more potent than ganciclovir [DHPG, 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine] against human cytomegalovirus. In vivo, HPMPC showed exceptional potency against HSV 1 systemic infection in mice, having an ED_{50} of 0.1 mg/kg per day (ip) compared with 50 mg/kg per day for ACV. HPMPC was also more efficacious than ACV in the topical treatment of HSV 1 induced cutaneous lesions in guinea pigs.

De Clercq and Holy recently described the novel acyclic nucleotide analogue (*S*)-9-[3-hydroxy-2-(phosphonylmethoxy)propyl]adenine (**1**, HPMPA) as a potent and selective antiviral agent with activity against a broad spectrum of DNA viruses.[1,2] Biochemical studies have shown that HPMPA inhibits HSV 1 DNA synthesis at a concentration much lower than required for inhibition of cellular DNA synthesis and that HPMPA is converted by cellular kinases to its mono- and diphosphate ester derivatives.[3] HPMPA thus appears to share a common mechanism of action with the nucleoside analogue acyclovir [ACV, 9-[(2-hydroxyethoxy)methyl]guanine] in that a triphosphate equivalent is formed which can act as an inhibitor of viral DNA polymerase and consequently viral replication. However, the biosynthesis of ACV-triphosphate requires initial conversion of ACV to its monophosphate by virus-encoded thymidine kinase (TK) prior to further phosphorylation by cellular kinases. Since HPMPA acts as a metabolically stable monophosphate equivalent, it does not rely on activation by the viral enzyme and, as a consequence, has activity against viruses that do not encode a thymidine kinase such as cytomegalovirus, as well as TK-deficient strains of herpes simplex virus. On the other hand, the selective action of acyclovir as an antiviral agent results in part from its preferential phosphorylation by virus-specified TK in infected cells; monophosphorylation by host enzymes does not occur to a significant extent in uninfected cells. Although HPMPA bypasses this level of selectivity, it does exhibit a significant preference for suppression of viral over cellular DNA synthesis and is a highly selective antiviral agent.[3]

The antiviral activity of a series of (phosphonylmethoxy)alkyl purine and pyrimidine derivatives related to HPMPA has been reported by De Clercq and co-workers.[4] Among the more promising of these acyclic nucleotide analogues is the cytosine derivative (*S*)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine (**2**, HPMPC), which has demonstrated in vitro activity against a wide range of DNA viruses and is particularly effective in the inhibition of cytomegalovirus.[5] Following our initial interest in HPMPA,[6] we have continued the evaluation of this class of nucleotide analogues and herein report our recent work on HPMPC. This paper provides details for a modified synthetic route[7] that allows the preparation of multigram quantities of HPMPC and describes our findings on the activity of HPMPC against DNA viruses in both in vitro and in vivo model systems. For comparison, the related acyclic cytosine derivative 1-[2-(phosphonylmethoxy)ethyl]cytosine (**3**, PMEC) was also synthesized.

Chemistry

Our strategy for the synthesis of the nucleotide analogues HPMPC and PMEC is based on alkylation of cytosine with an acyclic side-chain derivative already functionalized with a protected phosphonylmethyl ether group.

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