required to inhibit acetycholinesterase activity in vitro by 50% (IC_{50}) were compared to that of (-)-Phy (9). A preincubation of 30 min was used for each assay prior to initiation of the reaction (addition of the substrate). The production of a yellow 5-mercapto-2-nitrobenzoate anion was followed spectrophotometrically at a wavelength of 412 nm.

Registry No. 1, 104069-11-6; 1·fumarate, 116181-31-8; 2, 116103-15-2; 3, 116103-16-3; 4, 116103-17-4; 5, 116103-18-5; 5·fumarate, 116181-32-9; 6, 6091-57-2; 7, 116103-19-6; 7·salicylate, 116181-33-0; 8, 114546-08-6; 8·salicylate, 116181-34-1; 9, 57-47-6; 9·salicylate, 57-64-7; 10, 107485-89-2; $Br(CH_2)_2Ph$, 103-63-9; $BrCH_2CH{=}CH_2$, 106-95-6.

Structure-Activity Relationships in 1,4-Benzodioxan-Related Compounds. Investigation on the Role of the Dehydrodioxane Ring on α_1-Adrenoreceptor Blocking Activity

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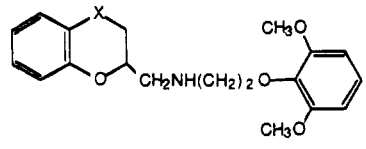
Several analogues of 2-[[[2-(2,6-dimethoxyphenoxy)ethyl]amino]methyl]-1,4-benzodioxan (WB 4101, 1) were prepared and evaluated for their blocking activity on α_1- and α_2-adrenoreceptors in the isolated rat vas deferens. The results were compared with those obtained for 1 and benoxathian (2). It was shown that the two oxygens at positions 1 and 4 may have a different role in receptor binding. It seems that the oxygen at position 4 as such does not contribute to the binding while it is important in stabilizing an optimal conformation for drug-receptor interaction mechanism. On the other hand, the oxygen at position 1 might interact with a receptor polar pocket of reduced size by way of a donor-acceptor dipolar interaction. Furthermore, it was shown that replacement of the dehydrodioxane ring of 1 by a phenyl or a pyrrole nucleus causes a significant decrease in activity.

Benzodioxans represent one of the oldest and best known classes of α-adrenoreceptor antagonists whose chemical structure incorporates a 1,4-benzodioxan-2-yl moiety as a basic feature.[1] Structure-activity relationship studies have revealed that the preferential selectivity toward α_1- or α_2-adrenoreceptors depends on the 2-substituent of the benzodioxan moiety. In fact, 2-[[[2-(2,6-dimethoxyphenoxy)ethyl]amino]methyl]-1,4-benzodioxan (WB 4101, 1)[2] and 2-(1,4-benzodioxan-2-yl)-2-imidazoline (idazoxan, RX 781094)[3] both carrying a benzodioxan nucleus but having a different 2-substituent proved to be potent and highly selective α_1- and α_2-adrenoreceptor antagonists, respectively. Several investigations were devoted to improving both activity and selectivity of 1.[1] Both benzodioxan and (2,6-dimethoxyphenoxy)ethyl moieties were reported to be essential for activity.[4] In contrast with this view, however, we showed that opening of the dehydrodioxane ring of 1 did not result in a loss of α_1-adrenoreceptor blocking activity.[5,6] Furthermore, the replacement of the ring oxygen at position 4 of the dehydrodioxane ring of 1 with a sulfur atom, giving benoxathian (2), did not modify the biological profile but rather gave a potent and highly selective α_1-adrenoreceptor antagonist.[7,8]



1 (WB4101): X=O
2 (benoxathian): X=S

The objectives of this study were to clarify the role of the dehydrodioxane ring and in particular of the oxygens at positions 1 and 4 of 1 in drug-receptor interaction. To this end, we describe here the synthesis and the pharmacological profile in the isolated rat vas deferens of several compounds (3-12) related to 1 and 2. Furthermore, to evaluate a possible role of π electrons, the dehydrodioxane moiety of 1 was replaced by a phenyl ring as in 13 and 14. Since the indole system is present in the structure of some α_1-adrenoreceptor antagonists such as corynanthine[9] and indoramin,[10] we included in this study compound 15, which bears that moiety instead of a benzodioxan nucleus.

Chemistry. All the compounds were synthesized by standard procedures and were characterized by IR, ^1H NMR, and elemental analysis. Their structure is shown in Scheme I and Table I.

Oxidation of 2[7] with hydrogen peroxide, in different conditions, gave sulfone 5 or a mixture of the corresponding sulfoxides 3 and 4, which were separated by column chromatography. The structure of the isomeric sulfoxides was attributed on the basis of their ^1H NMR spectra with measurement of the deshielding effect caused by the heteronuclear substituent on the proton at position 2 of the dehydrodioxane ring. Thus, a trans relation between the sulfoxide function and the side chain was as-

(1) For example, see: Melchiorre, C.; Belleau, B. *Adrenoceptors and Catecholamine Action*, Part A; Kunos, G., Ed.; Wiley: New York, 1981; pp 131-179.
(2) Mottram, D. R.; Kapur, H. *J. Pharm. Pharmacol.* 1975, *27*, 295.
(3) Chapleo, C. B.; Doxey, J. C.; Myers, P. L.; Roach, A. G. *Br. J. Pharmacol.* 1981, *74*, 842P.
(4) Kapur, H.; Mottram, D. R.; Green, P. N. *J. Pharm. Pharmacol.* 1978, *30*, 259.
(5) Melchiorre, C.; Giardinà, D.; Gallucci, P.; Brasili, L. *J. Pharm. Pharmacol.* 1982, *34*, 683.
(6) Giardinà, D.; Angeli, P.; Brasili, L.; Gulini, U.; Melchiorre, C.; Strappaghetti, G. *Eur. J. Med. Chem.* 1984, *19*, 411.
(7) Melchiorre, C.; Brasili, L.; Giardinà, D.; Pigini, M.; Strappaghetti, G. *J. Med. Chem.* 1984, *27*, 1535.
(8) Cassinelli, A.; Quaglia, W.; Brasili, L.; Giardinà, D.; Gulini, U.; Melchiorre, C. *Eur. J. Med. Chem.* 1987, *22*, 23.
(9) McGrath, J. C. *Biochem. Pharmacol.* 1982, *31*, 467.
(10) Archibald, J. L.; Alps, B. J.; Cavalla, J. F.; Jackson, J. L. *J. Med. Chem.* 1971, *14*, 1054.

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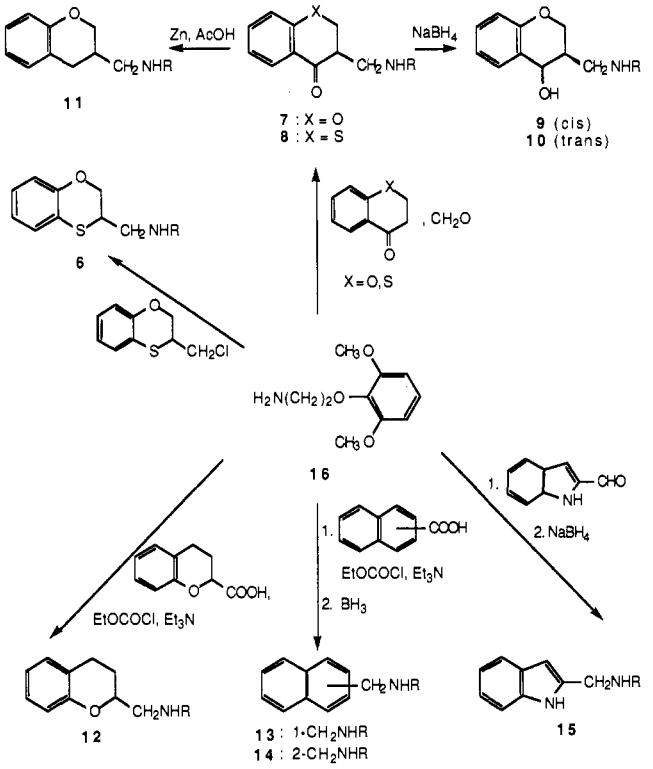
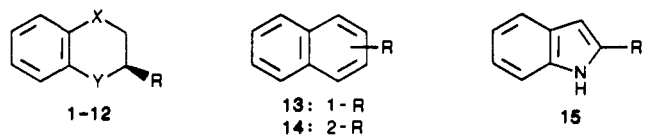
Scheme I[a]



[a] R = 2,6-$(CH_3O)_2C_6H_3O(CH_2)_2$.

Table I. α_1- and α_2-Adrenoreceptor pA_2 Values in the Isolated Rat Vas Deferens[a]



R = $CH_2NH(CH_2)_2O$-2,6-$(CH_3O)_2C_6H_3$

no.	X	Y	α_1 pA_2 against norepinephrine	α_2 pA_2 against clonidine	α_1/α_2[b] selectivity ratio
1	O	O	9.24 ± 0.05	6.63 ± 0.07	407
2	S	O	9.05 ± 0.09	6.15 ± 0.06	469
3	S◀O	O	7.54 ± 0.05	6.24 ± 0.10	20
4	S···O	O	7.71 ± 0.06	6.24 ± 0.09	30
5	SO_2	O	6.60 ± 0.10	5.80 ± 0.08	6
6	O	S	7.94 ± 0.03	6.09 ± 0.10	71
7	O	C=O	8.93 ± 0.06	6.52 ± 0.06	257
8	S	C=O	8.89 ± 0.03	6.53 ± 0.06	229
9	O	CH◀OH	7.22 ± 0.03	6.10 ± 0.06	13
10	O	CH···OH	7.33 ± 0.03	6.09 ± 0.12	17
11	O	CH_2	7.97 ± 0.03	6.28 ± 0.09	49
12	CH_2	O	9.20 ± 0.04	7.05 ± 0.06	141
13			7.33 ± 0.04	6.41 ± 0.08	8
14			7.75 ± 0.04	6.40 ± 0.08	22
15			6.60 ± 0.08	6.04 ± 0.11	4

[a] pA_2 values plus or minus standard error of estimate were calculated according to Arunlakshana and Schild[19] constraining the slope to −1.[25] pA_2 is defined as the negative logarithm to the base 10 of that dose of antagonist that requires a doubling of the agonist dose to compensate for the action of the antagonist. [b] The α_1/α_2 selectivity ratio is the antilog of the difference between pA_2 values at α_1- and α_2-adrenoceptors.

signed to compound 4 because the 2-H signal was found at lower field (δ 4.88) than the corresponding one in the cis isomer **3** (δ 4.34). This stereochemical attribution was confirmed by the spectra in C_6H_6. In fact, that solvent, owing to its association with the positive end of the sulfoxide function, causes a greater shielding effect on the 2-H which is trans to this function.[11] The difference between the signal of the hydrogen at position 2 in $CDCl_3$ and C_6D_6, respectively, was greater for **3** (0.24 Hz) than for **4** (0.0 Hz), in agreement with a stereochemical relation between the side chain and the sulfoxide oxygen which is cis for **3** and trans for **4**.

Compound **6** was prepared by alkylation of 2-(2,6-dimethoxyphenoxy)ethylamine (**16**)[12] with 3-(chloromethyl)-1,4-benzoxathian.[13]

Analogues **7** and **8** were synthesized by a Mannich reaction starting from chromanone or thiochromanone, respectively, **16**,[12] and formaldehyde. Clemmensen reduction of **7** gave the deoxa compound **11** whereas reduction of **7** yielded a mixture of the two isomeric alcohols **9** and **10**, which were separated by column chromatography. The stereochemical relation between the side chain and hydroxyl group in **9** and **10** was attributed on the basis of the coupling constant for the hydrogens at positions 3 and 4, respectively. Thus, a trans relation was assigned to **10** since the coupling constant (J = 8.2 Hz) was greater than that found for **9** (J = 4.4 Hz) for which a cis relation should be attributed.

Chroman-2-carboxylic acid[14] and 1- and 2-naphthoic acids were amidated with **16** followed by reduction of the intermediate amides to give **12**,[15] **13**, and **14**, respectively. Indole analogue **15** was synthesized by condensation of **16** with indole-2-carboxaldehyde[16] and subsequent reduction of the intermediate Schiff base.

Pharmacology. The biological profile of the compounds listed in Table I at α_1- and α_2-adrenoreceptors was assessed on isolated rat vas deferens with the prostatic and epididymal portions of this tissue because of their different properties.[17,18]

α_1-Adrenoreceptor blocking activity was assessed by antagonism of (−)-norepinephrine-induced contractions of the epididymal portion of the vas deferens. α_2-Adrenoreceptor blocking activity was determined by antagonism of the clonidine-induced depression of the twitch responses of the field-stimulated prostatic portion of the vas deferens. The potency of the drugs was expressed as dissociation constants (pA_2 values) calculated according to Arunlakshana and Schild.[19]

Results and Discussion

In the present study, the α_1- and α_2-adrenoreceptor blocking properties of **3**–**15** were studied in comparison with those of **1** (WB 4101) and **2** (benoxathian). The results obtained are shown in Table I. It can be seen that all the compounds proved to be effective α-adrenoreceptor antagonists with a slight to marked α_1-selectivity. It is also evident that potency at α_1-adrenoreceptors ranged within at least 3 orders of magnitude whereas activity at α_2-adrenoreceptors varied within 1 order of magnitude. This

(11) For example, see: Angeli, P.; Bacchiocchi, D.; Giannella M.; Pigini, M.; Romanelli, M. N. *Drug Des. Delivery* **1988**, *2*, 263.
(12) Woolley, D. W. *Nature (London)* **1957**, *180*, 630.
(13) Muhlstadt, M.; Stransky, N.; Seifert, A.; Kleinpeter, E.; Meinhold, H. *J. Prakt. Chem.* **1978**, *320*, 113.
(14) Augstein, J.; Monro, A. M.; Potter, G. W. H.; Scholfield, P. *J. Med. Chem.* **1968**, *11*, 844.
(15) Compound **12** was included in a general formula reported by: Green, P. N.; Shapero, M. Brit. 1296431; *Chem. Abstr.* **1973**, *78*, P111124y.
(16) Taylor, W. I. *Helv. Chim. Acta* **1950**, *33*, 164.
(17) McGrath, J. C. *J. Physiol.* **1978**, *23*, 283.
(18) Brown, C. M.; McGrath, J. C.; Summers, R. J. *Br. J. Pharmacol.* **1979**, *66*, 553.
(19) Arunlakshana, O.; Schild, H. O. *Br. J. Pharmacol.* **1959**, *14*, 48.

is in agreement with the view[6,20] that structural changes that markedly affect the binding of benzodioxan-bearing compounds at α_1 sites do not alter the affinity for α_2 sites.

An inspection of the results reveals that the two oxygens at positions 1 and 4 of the dehydrodioxane moiety of **1** may have different roles in drug–receptor interaction. In fact, the two isomers **11** and **12** in which the oxygens at positions 1 and 4, respectively, of **1** were replaced by a methylene displayed a different biological profile toward α_1- and α_2-adrenoreceptors as **12**, in contrast with previous findings,[21] was as active as **1** whereas **11** was 17-fold less active. This may allow the conclusion that the oxygen at position 4 of **1** does not contribute to receptor binding by way of hydrogen-bond formation or electronic effects. Rather it would seem that it may be important in stabilizing an optimal conformation for drug–receptor interaction, and to this end, a sulfur atom or a methylene group are effective as well. Furthermore, replacement of the sulfur atom of **2** with a cis- or trans-oriented function, as in **3** and **4**, or with a bulkier group, as in **5**, resulted in a significant decrease in activity, which suggests that these moieties may interfere sterically in receptor binding. It is worth noting that the two sulfoxide isomers **3** and **4** did not show any stereodiscrimination toward the α-adrenoreceptor.

On the contrary, the oxygen at position 1 of **1** seems to play a more definite role in receptor binding. In fact, its replacement with a sulfur or a methylene, as in **6** and **11**, respectively, resulted in a significant decrease in activity whereas its replacement by a carbonyl group did not modify the biological profile since **7** and **8** were as active as the parent compound. The significant decrease in activity observed following reduction of **7**, giving the two isomeric alcohols **9** and **10**, might indicate that the oxygen at position 1 of **1** interacts with a receptor polar pocket which is coplanar to the site for the benzodioxan nucleus.

Replacement of the dehydrodioxane ring by a phenyl one gave a significant decrease in activity. In fact, **13** and **14** displayed an α_1-blocking activity comparable to that of **11** and that was almost 2 orders of magnitude lower than that of **1**. Although this may suggest that π electrons can have hardly a role in drug–receptor interaction, the possibility that the low activity of **13** and **14** might be due to the planarity of the naphthalene and indole systems, which would cause a reorientation of the side chain such that its interaction with the receptor is unfavorable, cannot be excluded. The peculiar role of the dehydrodioxane ring is indeed confirmed by the low activity displayed by **15**, in agreement with a previous observation[21] that the furan analogue of **1** proved to be a very weak α_1-adrenoreceptor antagonist.

Experimental Section

Chemistry. Melting points were taken in glass capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. IR and NMR spectra were recorded on Perkin-Elmer 297 and Varian VXR-300 instruments, respectively. Although the IR and NMR spectra data are not uniformly included (because of the lack of unusual features), they were obtained for all compounds reported and were consistent with the assigned structures. The microanalyses were performed by the Microanalytical Laboratory of our department, and the elemental compositions of the compounds agreed to within ±0.4% of the calculated value. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040–0.063 mm, Merck) by flash chromatography. Petroleum ether refers to the fraction with a boiling point of bp 40–60 °C. The term "dried" refers to the use of anhydrous sodium sulfate.

Table II. Physical Characteristics of 1,4-Benzodioxan-Related Compounds

compd	mp,[a] °C	recrystn solvent	formula[b]
3	184–185	EtOH–Et_2O	$C_{19}H_{23}NO_5S\cdot HCl\cdot 0.5H_2O$
4	197–198	EtOH–Et_2O	$C_{19}H_{23}NO_5S\cdot HCl\cdot 0.5H_2O$
5	184–185	EtOH–Et_2O	$C_{19}H_{23}NO_6S\cdot HCl\cdot 0.5H_2O$
6	188–190	MeOH–Et_2O	$C_{19}H_{23}NO_4S\cdot H_2C_2O_4\cdot H_2O$
7	110–112	*i*-PrOH–Et_2O	$C_{20}H_{23}NO_5\cdot H_2C_2O_4$
8	90–92	*i*-PrOH–Et_2O	$C_{20}H_{23}NO_4S\cdot H_2C_2O_4\cdot H_2O$
9	160–161	EtOH–Et_2O	$C_{20}H_{25}NO_5\cdot H_2C_2O_4$
10	174–175	EtOH–Et_2O	$C_{20}H_{25}NO_5\cdot H_2C_2O_4$
11	190–191	*i*-PrOH	$C_{20}H_{25}NO_4\cdot H_2C_2O_4$
12	129–132	EtOH–Et_2O	$C_{20}H_{25}NO_4\cdot HCl\cdot H_2O$
13	143–145	*i*-PrOH	$C_{21}H_{23}NO_3\cdot HCl\cdot H_2O$
14	138–139	*i*-PrOH	$C_{21}H_{23}NO_3\cdot HCl$
15	187–188	MeOH	$C_{19}H_{22}N_2O_3\cdot H_2C_2O_4$

[a] The heating rate was 1 °C/min. [b] Analyses for C, H, N were within ±0.4% of the theoretical value required.

***cis*- and *trans*-2-[[[2-(2,6-Dimethoxyphenoxy)ethyl]amino]methyl]-1,4-benzoxathian *S*-Oxide Hydrochlorides (3 and 4) and 2-[[[2-(2,6-Dimethoxyphenoxy)ethyl]amino]methyl]-1,4-benzoxathian *S,S*-Dioxide Hydrochloride (5).** A solution of **2**[7] (free base) (0.3 g, 0.89 mmol) and 36% hydrogen peroxide (0.4 mL) in AcOH (2.5 mL) was left at room temperature for 30 min, and then it was made basic with NaOH pellets and extracted with chloroform (3 × 40 mL). The extracts were dried and then evaporated to give a residue, which was purified by column chromatography with ethyl acetate–methanol (1:3) as eluent. The first fraction was the cis isomer **3** as the free base: 9% yield; NMR ($CDCl_3$) δ 6.58–7.05 (m, 7 H, aromatics), 4.34 (m, 1 H, 2-*H*), 4.14 (m, 2 H, $NHCH_2CH_2O$), 3.83 (s, 6 H, OCH_3), 3.03 (m, 4 H, 3-CH_2 and CH_2NH), 2.92 (m, 2 H, $NHCH_2CH_2O$), and 2.01 (br s, 1 H, N*H*); NMR (C_6D_6) δ 6.32–7.17 (m, 7 H, aromatics), 4.10 (m, 1 H, 2-*H*), 4.18 (m, 2 H, $NHCH_2CH_2O$), 3.37 (s, 6 H, OCH_3), 2.64 (m, 2 H, CH_2NH), 2.70–2.86 (m, 4 H, 3-CH_2 and $NHCH_2CH_2O$), and 2.32 (br s, 1 H, N*H*).

The second fraction was the trans isomer **4** as the free base: 30% yield; NMR ($CDCl_3$) δ 6.55–7.55 (m, 7 H, aromatics), 4.88 (m, 1 H, 2-*H*), 4.13 (m, 2 H, $NHCH_2CH_2O$), 3.80 (s, 6 H, OCH_3), 3.22 (m, 2 H, CH_2NH), 3.11 (m, 2 H, 3-CH_2), 2.96 (m, 2 H, $NHCH_2CH_2O$), and 2.06 (br s, 1 H, N*H*); NMR (C_6D_6) δ 6.32–7.43 (m, 7 H, aromatics), 4.88 (m, 1 H, 2-*H*), 4.18 (m, 2 H, $NHCH_2CH_2O$), 3.40 (s, 6 H, OCH_3), 2.59–2.86 (m, 6 H, 3-CH_2 and $CH_2NHCH_2CH_2O$), and 1.80 (br s, 1 H, N*H*).

Compounds **3** and **4** were characterized as the hydrochloride salts (Table II).

Sulfone **5** was obtained by oxidation of **2** with hydrogen peroxide in AcOH for 20 h. The reaction mixture was worked up as described for **3** and **4** and the residue was purified by column chromatography, with ethyl ether–methylene chloride–petroleum ether–ethanol–28% ammonia (2:4:12:2:0.1) as eluent, to give **5** as the free base in 40% yield, which was characterized as the hydrochloride salt (Table II).

3-[[[2-(2,6-Dimethoxyphenoxy)ethyl]amino]methyl]-1,4-benzoxathian Oxalate (6). A mixture of 3-(chloromethyl)-1,4-benzoxathian[13] (0.44 g, 2.19 mmol), **16**[12] (2.0 g, 10.1 mmol), and a catalytic amount of potassium iodide in 2-methoxyethanol (25 mL) was stirred for 7 h at 140 °C. The solvent was evaporated to give a residue, which was taken up in 2 N HCl (10 mL). The solution was washed with ether and then was made basic with NaOH pellets and extracted with chloroform (3 × 50 mL). The extracts were dried and then evaporated to give an oil, which was purified by column chromatography with petroleum ether–chloroform–methanol (10:4:2) as eluent, yielding **6** as the free base in 23% yield, which was transformed into the oxalate salt and crystallized (Table II).

3-[[[2-(2,6-Dimethoxyphenoxy)ethyl]amino]methyl]-4-chromanone Oxalate (7) and 3-[[[2-(2,6-Dimethoxyphenoxy)ethyl]amino]methyl]thiochroman-4-one Oxalate (8). A solution of 4-chromanone (0.96 g, 6.4 mmol), **16**·HCl[12] (1.28 g, 5.6 mmol), paraformaldehyde (0.6 g, 19.9 mmol), and a few drops of ethanol saturated with HCl gas in absolute ethanol (5 mL) was heated at reflux for 6 h. Removal of the solvent gave an oil that was washed several times with anhydrous ether and then was

(20) Kapur, H.; Mottram, D. R. *Biochem. Pharmacol.* **1978**, *27*, 1879.
(21) Kapur, H.; Green, P. N.; Mottram, D. R. *J. Pharm. Pharmacol.* **1979**, *31*, 188.

taken up in water, made basic with NaOH pellets, and finally extracted with ether. The extracts were dried and then evaporated to give 7 as the free base in 75% yield, which was transformed into the oxalate salt and recrystallized (Table II).

The thio analogue 8 was similarly obtained from thiochroman-4-one in 68% yield (Table II).

***cis*- and *trans*-3-[[[2-(2,6-Dimethoxyphenoxy)ethyl]amino]methyl]-4-chromanol Oxalate (9 and 10).** Sodium borohydride (0.75 g, 19.8 mmol) was added portionwise to a stirred and cooled (0 °C) solution of 7 (free base) (1.0 g, 2.8 mmol) in methanol (13 mL). The resulting mixture was stirred at room temperature for 6 h, made acidic with 6 N HCl, and then extracted with chloroform. The extracts were dried and then evaporated to give a solid that was dissolved in water. The solution was made basic with NaOH pellets and then extracted with chloroform. The extracts were dried and then evaporated to give a mixture of the two alcohols, which were separated by column chromatography with ethyl acetate–methanol (1:3) as eluent. The first fraction was the trans isomer 10 as the free base: 40% yield; NMR ($CDCl_3$) δ 6.55–7.50 (m, 7 H, aromatics), 4.83 (d, J = 8.2 Hz, 1 H, 4-*H*), 4.12 (m, 4 H, 2-H_2 and $NHCH_2CH_2O$), 3.85 (s, 6 H, OCH_3), 3.02 (q, 2 H, $CH_2NHCH_2CH_2O$), 2.93 (m, 2 H, $NHCH_2CH_2O$), 2.19 (m, 1 H, 3-*H*), and 3.10 (br s, 1 H, N*H*).

The second fraction was the cis isomer 9 as the free base: 20% yield; NMR ($CDCl_3$) δ 6.55–7.49 (m, 7 H, aromatics), 5.02 (d, J = 4.4 Hz, 1 H, 4-*H*), 4.18 (d, 2 H, 2-H_2), 4.12 (t, 2 H, $NHCH_2CH_2O$), 3.84 (s, 6 H, OCH_3), 3.06 (d, 2 H, CH_2NH), 2.89 (m, 2 H, $NHCH_2CH_2O$), 2.36 (m, 1 H, 3-*H*), and 2.89 (m, 1 H, N*H*).

Compounds 9 and 10 were characterized as the oxalate salts (Table II).

3-[[[2-(2,6-Dimethoxyphenoxy)ethyl]amino]methyl]chromane Oxalate (11). A solution of 7 (free base) (0.5 g, 1.4 mmol) in AcOH (1.5 mL) was added to a suspension of Zn dust (2.0 g) in AcOH (5 mL). The mixture was heated at 100 °C for 6 h and then was filtered, and the solvent was evaporated to give a residue, which was taken up in water. The solution was made basic with NaOH pellets and then extracted with ethyl acetate. The extracts were dried and then evaporated to give an oil, which was purified by chromatography with ethyl acetate as eluent, yielding 11 as the free base in 12% yield, which was transformed into the oxalate salt and recrystallized (Table II).

2-[[[2-(2,6-Dimethoxyphenoxy)ethyl]amino]methyl]chromane Hydrochloride (12). Ethyl chloroformate (0.26 g, 2.36 mmol) was added to a stirred and cooled (0 °C) solution of chroman-2-carboxylic acid[14] (0.4 g, 2.25 mmol) and triethylamine (0.24 g, 2.36 mmol) in chloroform (30 mL). Stirring and cooling were continued for an additional 20 min, and then 16[12] (0.44 g, 2.25 mmol) in chloroform (10 mL) was added. The resulting reaction mixture was stirred at room temperature for 14 h and then was washed with 2 N NaOH, 2 N HCl, and finally water. Removal of the dried solvent gave a residue, which was purified by column chromatography, with petroleum ether–chloroform–methanol (5:2:1) as eluent, to afford 0.65 g of the intermediate amide as an oil. A solution of 10 M borane–methyl sulfide complex (1.09 mL, 10.9 mmol) in dry diglyme (2.5 mL) was added dropwise at room temperature to a solution of the above amide (0.65 g, 1.8 mmol) in dry diglyme (30 mL) with stirring under a stream of dry nitrogen. When the addition was completed, the reaction mixture was heated at 80 °C for 12 h. After the mixture was cooled to 0 °C, excess borane was destroyed by cautious addition of methanol (5 mL). The resulting mixture was left to stand for 1 h at room temperature, treated with HCl gas for 10 min, and then heated at 80 °C for 3 h. Removal of the solvent under reduced pressure gave a white residue that was recrystallized to give 12 in 48% yield (Table II).

Compounds 13 and 14 were similarly obtained in 25–30% yields from 1- and 2-naphthoic acids, respectively, and 16[12] (Table II).

2-[[[2-(2,6-Dimethoxyphenoxy)ethyl]amino]methyl]indole Oxalate (15). A solution of indole-2-carboxaldehyde[16] (0.5 g, 3.44 mmol) and 16[12] (0.57 g, 2.87 mmol) in benzene (20 mL) was heated at reflux and the formed water was continuously removed for 6 h. The cooled mixture was filtered and the filtrate evaporated to dryness, yielding the corresponding Schiff base, which was dissolved in absolute ethanol (50 mL) and treated with sodium borohydride (0.15 g, 3.96 mmol). The mixture was stirred at room temperature for 3 h and then acidified with concentrated HCl (ice added). The solvent was evaporated and the residue was dissolved in the minimum amount of water. The solution was washed with chloroform, made basic with 2 N NaOH (20 mL), and finally extracted with chloroform (4 × 30 mL). Extracts were dried and then evaporated to give a residue, which was purified by column chromatography with 28% ammonia–methanol–chloroform–petroleum ether (0.1:1.5:8:9.9) as eluent, yielding 15 as the free base in 49% yield, which was transformed into the oxalate salt and recrystallized (Table II).

Pharmacology. Male albino rats (175–200 g) were killed by a sharp blow on the head and both vasa deferentia were isolated, freed from adhering connective tissue, and transversely bisected. Prostatic, 12 mm in length, and epididymal portions, 14 mm in length, were prepared and mounted individually in baths of 20 mL working volume containing Krebs solution of the following composition (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.52; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; glucose, 11.1 $MgSO_4$ concentration was reduced to 0.6 mM when twitch response to field stimulation was studied. The medium was maintained at 37 °C and gassed with 95% O_2–5% CO_2. The loading tension used to assess α_1- or α_2-blocking activities was 0.4 or 0.5–0.8 g, respectively, and the contractions were recorded by means of force transducers connected to a two-channel Gemini 7070 polygraph.

Field stimulation of the tissue was carried out by means of two platinum electrodes, placed near the top and bottom of the vas deferens, at 0.1 Hz with square pulses of 3-ms duration at a voltage of 10–35 V. The stimulation voltage was fixed throughout the experiments.

Propranolol hydrochloride (1 μM) and cocaine hydrochloride (10 μM) were present in the Krebs solution throughout the experiments outlined below to block β-adrenoreceptors and neuronal uptake mechanisms, respectively.

α_1-Adrenoreceptor Blocking Activity. Postsynaptic α_1-adrenoreceptor blocking activity was determined on the epididymal portion of the vas deferens. The tissues were allowed to equilibrate for at least 1 h before the addition of any drug. Norepinephrine dose–response curves were obtained cumulatively, the first one being discarded and the second one taken as a control. After incubation with the antagonist for 30 min, a third dose–response curve was obtained. Responses were expressed as a percentage of the maximal response obtained in the control curve. Parallel experiments in which tissues did not receive any antagonist were run in order to correct for time-dependent changes in agonist sensitivity.[22] It was generally verified that the third dose–response curve was identical with the second because the change in dose–ratio is less than 2, which usually represents a minimal correction.

The antagonist potency of compounds at α_1-adrenoreceptors was expressed in terms of their dissociation constants.

α_2-Adrenoreceptor Blocking Activity. This was assessed on the prostatic portion of the vas deferens by antagonism to clonidine, which inhibits twitch responses of the field-stimulated vas deferens by acting on the α_2-adrenoreceptor.[23,24]

The tissues were allowed to equilibrate for at least 1 h before the addition of any drug. A first clonidine dose–response curve taken as a control was obtained cumulatively avoiding the inhibition of more than 90% of twitch responses. Under these conditions it was possible to obtain a second dose–response curve which is not significantly different from the first one. Thus, after incubation with the antagonist for 30 min, a second dose–response curve was obtained and dose–ratio (DR) values were determined from the concentration causing 50% inhibition of the twitch response in the absence and presence of antagonist. Parallel experiments, in which tissues did not receive any antagonist, were run in order to correct for time-dependent changes in agonist sensitivity and to determine the concentration of agonist causing 100% inhibition of twitch responses.

The results are expressed as dissociation constants.

Determination of Dissociation Constants. Dose ratios at the EC_{50} values of the agonists were calculated at three antagonist

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concentrations, and each concentration was tested five times. Dissociation constants (pA_2 values, Table I) were estimated by Schild plots[19] constrained to slope -1.0, as required by the theory.[25] When applying this method, it was always verified that the experimental data generated a line whose derived slope not significantly different from unity ($p > 0.05$).

Data are presented as means ± SE of n experiments. Differences between mean values were tested for significance by the Student's t test.

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Synthesis and Antiherpetic Activity of (±)-9-[[(*Z*)-2-(Hydroxymethyl)cyclopropyl]methyl]guanine and Related Compounds[1]

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A series of analogues of acyclovir and ganciclovir were prepared in which conformational constraints were imposed by incorporation of a cyclopropane ring or unsaturation into the side chain. In addition, several related base-modified compounds were synthesized. These acyclonucleosides were evaluated for enzymatic phosphorylation and DNA polymerase inhibition in a staggered assay and for inhibitory activity against herpes simplex virus types 1 and 2 in vitro. Certain of the guanine or 8-azaguanine derivatives were good substrates for the viral thymidine kinase and were further converted to triphosphate, but none was a potent inhibitor of the viral DNA polymerase. Nevertheless, one member of this group, (±)-9-[[(*Z*)-2-(hydroxymethyl)cyclopropyl]methyl]guanine (**3a**), displayed significant antiherpetic activity in vitro, superior to that of the corresponding cis olefin **4a**. Another group, typified by (±)-9-[[(*E*)-2-(hydroxymethyl)cyclopropyl]methyl]adenine (**17b**), possessed modest antiviral activity despite an apparent inability to be enzymatically phosphorylated. The relationship of side-chain conformation and flexibility to biological activity in this series is discussed.

The potent antiherpetic activity of acyclovir (ACV, **1a**)[2] has stimulated great interest in the area of acyclic nucleoside analogues. These efforts have intensified with the identification of the closely related ganciclovir (GCV, **2a**; also known variously as DHPG, 2′NDG, BW B759U, and BIOLF-62) as an agent with superior in vivo potency and somewhat broader spectrum of antiviral utility.[3-5] The corresponding carba analogues HBG (**1b**)[6] and carba-GCV (c-GCV, **2b**; also known as carba-DHPG, carba-2′NDG, 3HM-HBG, and BRL 39123)[7-10] have also demonstrated good inhibition of herpes simplex virus (HSV) in cell culture. All of the above compounds are believed to act by the same general mechanism.[3,5,6,11-14] The broadened substrate specificity of the HSV thymidine kinase enables these guanine acyclonucleosides to be selectively converted to monophosphate derivatives in infected cells. Further phosphorylation by host cell enzymes generates the acyclonucleoside triphosphate, which inhibits HSV DNA polymerase, thus blocking viral replication.

The effectiveness of an acyclic nucleoside analogue as a substrate (for phosphorylating enzymes) or inhibitor (for DNA polymerase) is likely to be dependent on the ability of the acyclic side chain to mimic the interaction of the

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