α-Adrenoreceptor Reagents. 3.[1,2] Synthesis of Some 2-Substituted 1,4-Benzodioxans as Selective Presynaptic α_2-Adrenoreceptor Antagonists

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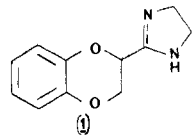
The synthesis and pharmacological activity of a series of 2-substituted derivatives of the selective α_2-adrenoreceptor antagonist idazoxan (RX 781094) is described. Substitution in this position by alkyl, alkenyl, cycloalkenyl, and alkoxy groups in many cases gives compounds whose potencies and selectivities are significantly greater than those of the parent compound.

We have recently reported[1] the synthesis and biological activity of the selective α_2-adrenoreceptor antagonist idazoxan (1, RX 781094). The effects of substitution in



(1)

the aromatic ring and modifications of the imidazoline ring have also been described, and it was shown that such manipulations resulted in a reduction or loss of both selectivity and potency compared to those of the parent compound. More recently, analogues in which the dioxan ring has been replaced by other ring systems have been examined[2] and the dihydrobenzofuran series proved to be of particular interest. We now report our investigations into the synthesis and pharmacological properties of compounds substituted in the dioxan ring, in particular those bearing a 2-substituent, many of which are more potent and selective than the parent compound idazoxan.

Chemistry. The chemistry can be conveniently divided into two main sections, the first dealing with those compounds having alkyl, alkenyl, cycloalkenyl, and aryl substituents and the second describing the synthesis of compounds substituted by alkoxy and aryloxy groups.

(i) Alkyl, Alkenyl, Cycloalkenyl, and Aryl Derivatives. The introduction of alkyl substituents at C-2 in 1 cannot be achieved by direct base-catalyzed alkylation. Reaction of the intermediate nitrile (Scheme I) with for example MeI/NaH results in abstraction of the proton at C-2 in **2** leading to immediate ring opening with the production of the unsaturated nitrile **3**.[3] The 2-phenyl **16** and 2-*n*-alkyl analogues **4–8** (Table I) can be prepared however from catechol and the appropriately substituted epichlorohydrin (Scheme II).

An alternative procedure is described in Scheme III. The product **18**, from the condensation of the chloro ketone and catechol, was chlorinated with HCl in dichloromethane. The resulting chloro compound **19** was treated with trimethylsilyl cyanide to give the key intermediate, nitrile **20**, which was converted to the imidazoline by using established procedures.

During the preparation of the unsubstituted nitrile **2** the carbinol byproduct **21** was isolated (Scheme IV), which arose from the base-catalyzed condensation of **2** with acetone, the solvent used in the reaction. It was later found that cycloalkanones could also be employed as the solvent to give the corresponding cycloalkylcarbinols, although surprisingly no reaction was observed when ethyl methyl ketone was used. The carbinols could then be converted to the corresponding cycloalkenyl and cycloalkyl imidazolines **12–15** and **9** and **10**, respectively, by conventional means. The initial base-catalyzed substitution reaction of nitrile **2** with acetone appears to contradict the statement made earlier, which suggests that treatment with base invariably leads to ring opening. In this case, however, it appears that the ring-opening reaction does occur but is reversible and this leads to a gradual buildup of the substituted product **21**. Attempts to introduce vinyl and alkenyl groups into this 2-position, by reaction of the nitrile **2** with potassium carbonate in the presence of aldehydes, were unsuccessful. The 2-vinyl compound **11** was finally obtained by using the route shown in Scheme V. Only one 3-substituted compound was prepared (Scheme VI) and this was achieved via a cyclization reaction between catechol and the appropriately substituted dibromo ester with subsequent elaboration to the imidazoline **17**.

(ii) Alkoxy and Aryloxy Derivatives. The intermediate in the synthesis of all of these compounds is the bromo nitrile **22** prepared via bromination of the nitrile **2** with a molar equivalent of *N*-bromosuccinimide (Scheme VII). Treatment of this with an excess of ethereal HCl containing 1 mol equiv of ethanol gave the bromo imidoate hydrochloride salt **23** (method A, Scheme VII), which, when dissolved in the alcohol of choice containing ethylenediamine, afforded the 2-alkoxy derivative in moderate yield. The possibility of using base-induced imidoate formation was also examined. Three products were identified from the reaction of the bromo nitrile **22** with 1 mol equiv of sodium methoxide in methanol (Scheme VII); these were the 2-bromo imidoate **24**, the benzodioxin imidoate **25**, and the 2-methoxy imidoate **26**. In contrast, reaction of **22** with a catalytic amount of sodium methoxide at room temperature in methanol resulted in a rapid and virtually quantitative conversion to the bromo imidoate **24**, which, on treatment with ethylenediamine followed by methanolic HCl, gave a good yield of the 2-methoxy imidazoline **31** (Table II). The reaction sequence (method B, Scheme VII) is now routinely carried out at 0 °C and the soluble nature of the imidoate free base makes it possible for solid alcohols and phenols to be used in the presence of a cosolvent (THF) although the yields in these reactions are considerably lower than those employing liquid alcohols.

The reaction of the bromo imidoate hydrochloride **23** with ethylenediamine in chloroethanol failed to give the desired product. Instead the spiro compound **28** was isolated, presumably arising from ring closure of the intermediate 2-chloroethoxy derivative **27** (Scheme VIII). The

(1) Part 1: Chapleo, C. B.; Myers, P. L.; Butler, R. C. M.; Doxey, J. C.; Roach, A. G.; Smith, C. F. C. *J. Med. Chem.* **1983**, *26*, 823.

(2) Part 2: Chapleo, C. B.; Myers, P. L.; Butler, R. C. M.; Davis, J. A.; Doxey, J. C.; Higgins, S. D.; Myers, M.; Roach, A. G.; Smith, C. F. C.; Stillings, M. R.; Welbourn, A. P. *J. Med. Chem.* **1984**, *27*, 570.

(3) Chapleo, C. B.; Davis, J. A.; Myers, P. L.; Readhead, M. J.; Stillings, M. R.; Welbourn, A. P.; Hampson, F. C.; Sugden, K. *J. Heterocycl. Chem.* **1984**, *21*, 77.

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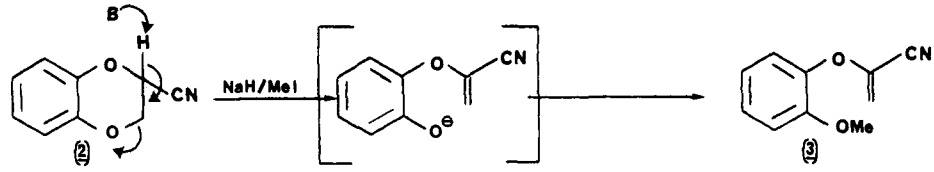
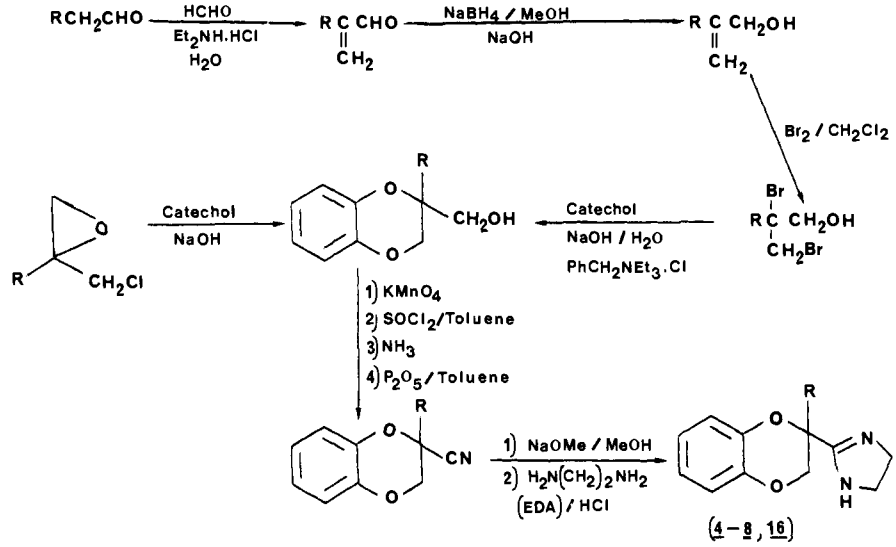
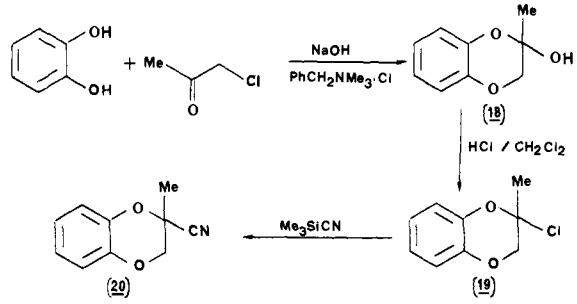
Scheme I



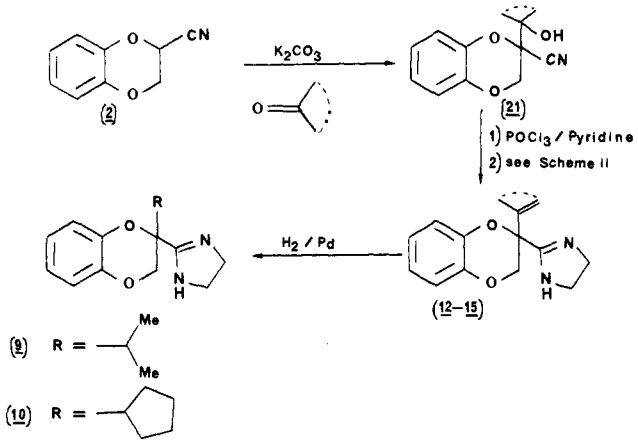
Scheme II



Scheme III



Scheme IV



compound was of no interest pharmacologically.

In contrast to the simple and high-yielding synthesis of the alkoxy compounds described above, the preparation of the corresponding thio compounds was much more difficult. Treatment of a solution of the bromo nitrile **22** in ethanethiol with a catalytic quantity of sodium methoxide resulted in a complex mixture of unidentified products. In addition, the bromo imidoate hydrochloride **23** was totally insoluble in the thiol. However, an ethereal solution of the bromo nitrile **22** and ethanethiol containing 1 mol equiv of triethylamine resulted in a mixture from which a basic product could be isolated after removal of the triethylamine. The hydrochloride salt was formed by treatment with ethereal HCl and although it was insoluble in ethanethiol, it could be dissolved in a small quantity of ethanol. Subsequent addition of ethylenediamine then afforded a mixture of the required 2-thioethyl imidazoline **55** contaminated with the 2-ethoxy derivative **32**. Further purification proved to be impossible. NMR analysis showed an approximate 3:1 mixture of the two compounds with the thio derivative predominating; this was later confirmed by HPLC though the separation between the two compounds was minimal.

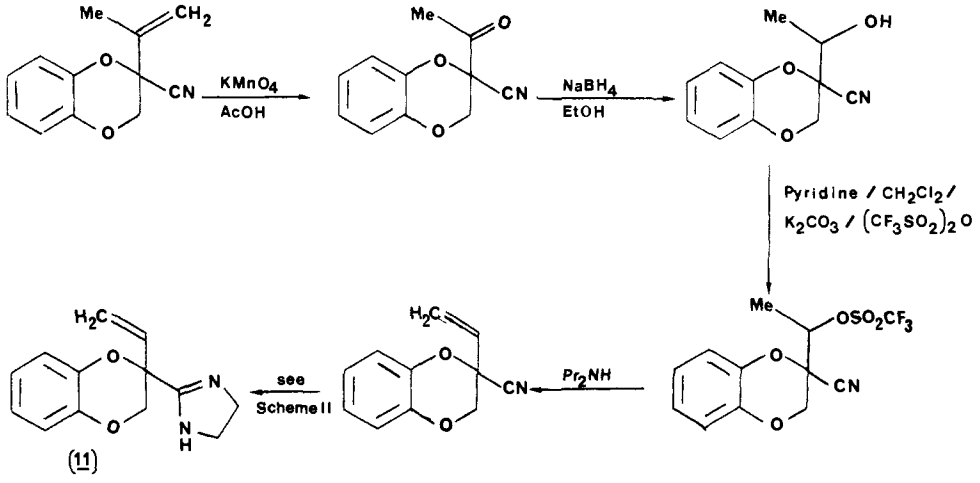
The hygroscopic nature of many of the compounds is shown by the varying amounts of water found during elemental analysis (see Tables I and II).

Results and Discussion

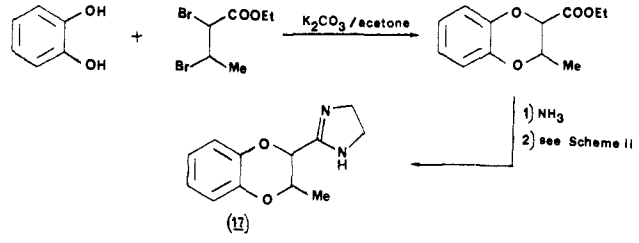
All compounds were examined for α_1- and α_2-adrenoreceptor agonist and antagonist properties by using standard testing procedures.[1,2] The results are summarized in Tables I and II with the antagonist values being quoted as potencies relative to idazoxan. Table III shows the pA_2 values and selectivities of the parent compound 1 and some of the more selective and potent antagonists identified from the primary screen. Calculation of the α_2-antagonist potencies of the derivatives relative to idazoxan using the pA_2 values shows a good correlation with the results presented in Tables I and II. In common with idazoxan, none of the compounds exhibited agonist properties at either α_2- or α_1-adrenoreceptors in the tests described in Tables I–III. In other test situations, however, idazoxan has been shown[4,5] to possess partial agonist activity at

(4) Paciorek, P. M.; Shepperson, N. B. *Br. J. Pharmacol.* **1983**, *79*, 12.
(5) Roach, A. G.; Doxey, J. C.; Berridge, T. L. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1983**, *42*, 636.
(6) Arunlakshana, O.; Schild, H. O. *Br. J. Pharmacol. Chemother.* **1959**, *14*, 48.

Scheme V



Scheme VI



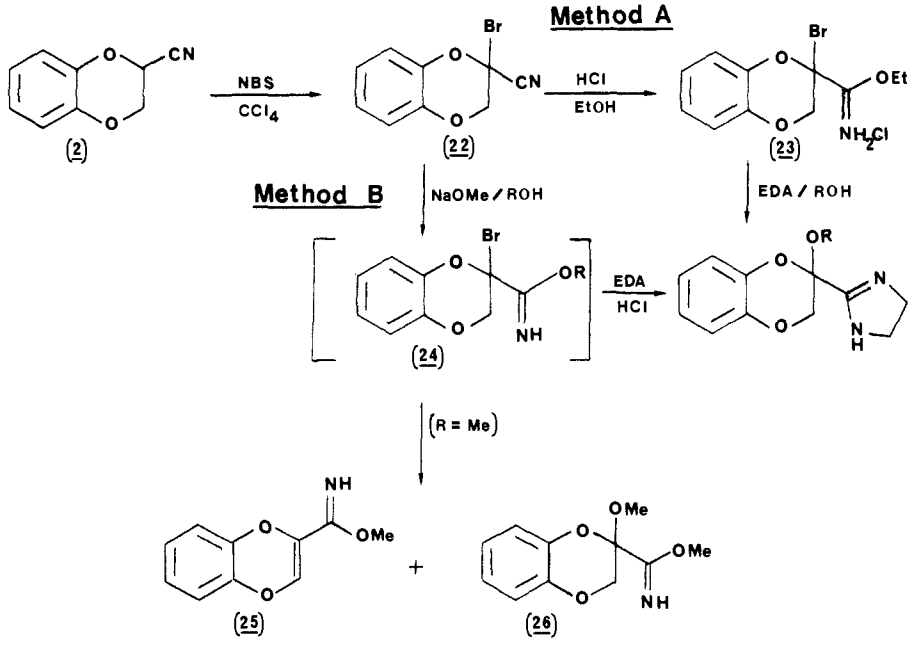
α_1-receptors. The compounds described in this paper demonstrate markedly reduced agonism in these tests.

For clarity of presentation the subsequent discussion is divided into two parts dealing with alkyl, alkenyl and phenyl derivatives followed by an examination of the results obtained for the alkoxy compounds. The implications of the results for both series are then discussed.

(i) 2-Alkyl, 2-Alkenyl, and 2-Aryl Derivatives. The very low level of α_2-antagonist activity of the 3-methyl derivative **17** compared with that of the 2-methyl isomer **4** demonstrates the uniquely important position of 2-substitution in the relationship of structure to activity in the idazoxan area. This is further emphasized when the low potencies of compounds containing methyl groups at other positions in the idazoxan molecule are considered.[1]

Within the 2-substituted area, low α_2-adrenoreceptor potency is associated with those compounds possessing phenyl **16** and branched-chain alkyl groups (**9** and **10**). The introduction of unsaturation into the branched com-

Scheme VII



Scheme VIII

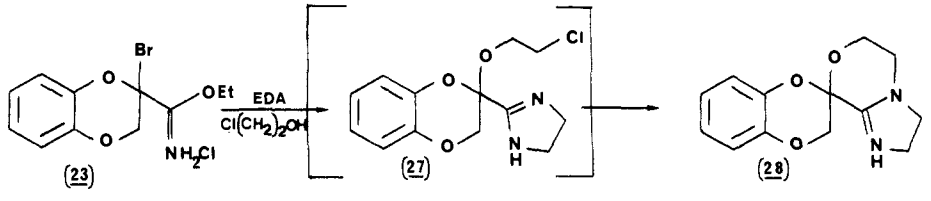
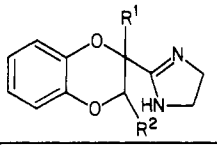


Table I



no.	R^1	R^2	mp, °C	formula	anal.	pharmacological testing results[a] presynaptic antagonism[b]	postsynaptic antagonism[c]	α_2/α_1[d]
4	CH_3	H	258–261	$C_{12}H_{14}N_2O_2 \cdot HCl \cdot {}^1/_4H_2O$	C, H, N	0.67	0.28	2.4
5	CH_2CH_3	H	98–100	$C_{13}H_{16}N_2O_2 \cdot {}^1/_8H_2O$	C, H, N	3.0	2.25	1.3
6	$(CH_2)_2CH_3$	H	113–115	$C_{14}H_{18}N_2O_2 \cdot {}^3/_4H_2O$	C, H, N[e]	4.8	3.2	1.5
7	$(CH_2)_3CH_3$	H	99–100	$C_{15}H_{20}N_2O_2$	C, H, N[f]	1.5	2.2	0.68
8	$(CH_2)_6CH_3$	H	52–57	$C_{18}H_{26}N_2O_2 \cdot {}^1/_4H_2O$	C, H, N	0.9	1.1	0.85
9	$CH(CH_3)_2$	H	124–125	$C_{14}H_{18}N_2O_2$	C, H, N	0.024	<0.006	
10		H	135–136	$C_{16}H_{20}N_2O_2$	C, H, N	0.015	0.4	0.04
11	$CH{=}CH_2$	H	180–182	$C_{13}H_{14}N_2O_2 \cdot HCl \cdot {}^1/_4H_2O$	C, H, N	1.0	0.3	3.3
12	$C(CH_3){=}CH_2$	H	285–287	$C_{14}H_{16}N_2O_2 \cdot HCl$	C, H, N	0.4	0.07	5.7
13		H	199–201	$C_{15}H_{16}N_2O_2 \cdot HCl$	C, H, N	0.3	0.13	2.3
14		H	83–85	$C_{16}H_{18}N_2O_2$	C, H, N	0.15	0.09	1.7
15		H	122–124	$C_{17}H_{20}N_2O_2$	C, H, N	0.026	0.75	0.035
16	Ph	H	115–116	$C_{17}H_{16}N_2O_2$	C, H, N	0.04	0.026	1.6
17	H	CH_3	191–193	$C_{12}H_{14}N_2O_2 \cdot HCl.H_2O$	C, H, N	0.002	0.023	0.087

[a] None of the compounds tested exhibited agonist activity at either the α_2- or α_1-adrenoreceptor. [b] Presynaptic antagonist potency (idazoxan = 1), dose–response curves of the standard were obtained before and after the dose response curve of the analogue. There was no significant difference between the two dose–response curves of the standard. [c] Postsynaptic antagonism concentration giving a dose response equal to 2 vs. phenylephrine (idazoxan = 1). A minimum of five dose–response curves was obtained for phenylephrine alone, followed by a minimum of four dose–response curves in the presence of the analogue. [d] Presynaptic selectivity ratio of antagonists (pre/post) (idazoxan = 1). [e] N: calcd, 10.78; found, 10.23. [f] H: calcd, 7.74; found, 8.15.

pounds results in a significant increase in potency (compare **9** with **12** and **10** with **14**), which decreases as the alkenyl group becomes larger (**13–15**). Selectivity also decreases in these compounds. The most potent of the unsaturated derivatives is the vinyl compound **11**; the most selective is the isopropenyl derivative **12**, which is significantly more selective than the parent compound **1** (Table I). In the *n*-alkyl subseries, α_2-antagonist potency increases on homologation from methyl (**4**) to *n*-propyl (**6**) but then decreases as the chain length is extended further (**7** and **8**). It is interesting, however, that even with a C-7 side chain (**8**) the α_2 and α_1 potencies are still equal to those of the parent compound **1**. The most selective compound in the *n*-alkyl series is the methyl derivative **4**; the other *n*-alkyl homologues all have selectivities close to that of **1**.

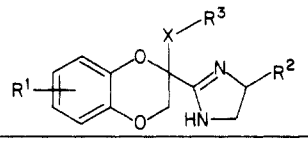
(ii) 2-Alkoxy, 2-Phenoxy, and 2-Benzyloxy Derivatives. The outstanding compound in this series both in terms of potency and selectivity is the methoxy derivative **31**. Homologation leads to a reduction in both of these parameters. Nevertheless, the majority of the compounds in the series do retain the α_2-antagonist potency of the parent compound **1**, the major exceptions being the phenoxy derivative **49** and the two compounds **53** and **54**, which possess substituents in the aromatic and imidazoline rings. In contrast to the alkyl series, the introduction of unsaturation into the side chain has no significant effect on either potency or selectivity (compare **33** with **37** and **38**) and also chain branching (**34** and **39**) does not seriously reduce potency. The spacing effect of the oxygen atom that removes the secondary carbon from the immediate vicinity of the 2-position may be responsible for these differences. In common with the alkyl series, the introduction of bulky groups in positions remote from the benzodioxan ring in general does not result in compounds (e.g., **40–45**) with α_2-antagonist potencies very different from that of the parent compound **1**, although a number of these derivatives do show enhanced α_1-antagonist activity (**42**, **45**). The decrease in selectivity shown by the *n*-butoxy compound (**35**, Table III) also reflects this trend.

Results from earlier work[1,2] suggest that three major binding sites exist on the α_2-adrenoreceptor. These are a planar hydrophobic area that interacts with the phenyl ring, a second site responsible for binding to one or both of the dioxanyl oxygen atoms, and an anionic site that binds to one of the amino functions of the imidazoline ring. The preceding papers in this series have shown that even minor modifications to the structure of the molecules interacting at these sites can cause a dramatic decrease in the potency of the compounds compared to that of the parent compound **1** and in many cases alter their agonist/antagonist and α_1/α_2 profiles. The results presented here indicate that the 2-position is unique in that (1) it can accommodate relatively large groups with little or no decrease in potency and (2) the introduction of smaller groups such as ethyl, *n*-propyl, methoxy, and ethoxy cause a significant increase in affinity.

It is possible that this increase could be due to the existence of a fourth binding site on the receptor which can interact with the 2-substituent. It is also possible, however, that the 2-substituent plays a major role in determining the overall conformation of the molecule and hence its antagonist activity. When the most active compound in the series, the 2-methoxy derivative **31**, was resolved, pharmacological investigation revealed that at least 98% of the α_2- and α_1-antagonist activity resided in the (+) enantiomer. This was subsequently shown by X-ray diffraction studies to possess the *S* absolute configuration.[7] An ORTEP representation is shown in Figure 1 and this

(7) Welbourn, A. P., unpublished results.

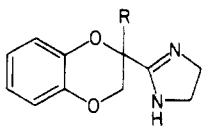
Table II



no.	X	R^1	R^2	R^3	mp, °C	formula	anal.	pharmacological testing results[a]: presynaptic antagonism[b]	pharmacological testing results[a]: postsynaptic antagonism[c]	α_2/α_1[d]
31	O	H	H	CH_3	90–91	$C_{12}H_{14}N_2O_3$	C, H, N	11.0	4.0	2.8
32	O	H	H	CH_2CH_3	222–224	$C_{13}H_{16}N_2O_3 \cdot HCl$	C, H, N	5.9	7.0	0.8
33	O	H	H	$(CH_2)_2CH_3$	95–97	$C_{14}H_{18}N_2O_3$	C, H, N	1.5	1.0	1.5
34	O	H	H	$CH(CH_3)_2$	100–102	$C_{14}H_{18}N_2O_3 \cdot {}^1/_4H_2O$	C, H, N	1.0	0.6	1.6
35	O	H	H	$(CH_2)_3CH_3$	92–93	$C_{15}H_{20}N_2O_3$	C, H, N	1.6	2.2	0.7
36	O	H	H	$(CH_2)_4CH_3$	188–190	$C_{16}H_{22}N_2O_3 \cdot HCl \cdot {}^1/_4H_2O$	C, H, N	0.72	0.6	1.2
37	O	H	H	$CH_2CH{=}CH_2$	73–76	$C_{14}H_{16}N_2O_3$	C, H, N	2.0	6.0	0.3
38	O	H	H	$CH_2C{\equiv}CH$	209–211	$C_{14}H_{14}N_2O_3 \cdot HCl$	C, H, N	1.2	2.1	0.57
39	O	H	H		107–109	$C_{16}H_{20}N_2O_3$	C, H, N	0.16	1.0	0.16
40	O	H	H	CH_2Ph	139–141	$C_{18}H_{18}N_2O_3 \cdot {}^1/_3H_2O$	C, H, N	2.0	3.0	0.7
41	O	H	H	$CH_2(2\text{-}MeOC_6H_4)$	109–111	$C_{19}H_{20}N_2O_4$	C, H, N[e]	1.7	2.3	0.75
42	O	H	H	$CH_2(4\text{-}MeOC_6H_4)$	257–258	$C_{19}H_{20}N_2O_4 \cdot HCl \cdot {}^1/_4H_2O$	C, H, N	0.6	4.8	0.13
43	O	H	H	$CH_2(2\text{-}ClC_6H_4)$	147–148	$C_{18}H_{17}ClN_2O_3 \cdot HCl \cdot {}^3/_4H_2O$	C, H, N	0.36	1.0	0.36
44	O	H	H	$CH_2(4\text{-}ClC_6H_4)$	255–260	$C_{18}H_{17}ClN_2O_3 \cdot HCl \cdot {}^3/_4H_2O$	C, H, N	1.0	2.46	0.4
45	O	H	H	$(CH_2)_2Ph$	165–166	$C_{19}H_{20}N_2O_3 \cdot HCl$	C, H, N	1.0	8.8	0.14
46	O	H	H	$(CH_2)_2OH$	142–145	$C_{13}H_{16}N_2O_4$	C, H, N	1.4	1.0	1.4
47	O	H	H	$(CH_2)_4OH$	oil	$C_{15}H_{20}N_2O_4$	NMR, MS	0.24	1.0	0.24
48	O	H	H	$(CH_2)_2OMe$	191–193	$C_{14}H_{18}N_2O_4$	C, H, N	0.2	1.0	0.2
49	O	H	H	Ph	124–125	$C_{17}H_{16}N_2O_3 \cdot {}^1/_4H_2O$	C, H, N	0.023	1.0	0.023
50	O	H	H	$3\text{-}MeC_6H_4$	223–225	$C_{18}H_{18}N_2O_3 \cdot HCl \cdot {}^1/_4H_2O$	C, H, N[f]	0.35	0.2	1.75
51	O	H	H	$4\text{-}MeOC_6H_4$	*g*	$C_{18}H_{18}N_2O_4 \cdot HCl \cdot {}^1/_2H_2O$	C, H, N	0.34	2.0	0.17
52	O	H	H	CH_2CCl_3	148–150	$C_{13}H_{13}Cl_3N_2O_3$	C, H, N	0.1	0.2	0.5
53	O	6,7-$(OMe)_2$	H	CH_3	121–123	$C_{14}H_{18}N_2O_5$	C, H, N	0.006	0.05	0.1
54	O	H	CH_3	CH_3	207–208	$C_{13}H_{16}N_2O_3$	C, H, N	0.09	1.0	0.09
55[h]	S	H	H	CH_2CH_3			NMR, HPLC	5.4	8.0	0.7

[a–d] See corresponding footnotes in Table I. [e] N: calcd, 8.23; found, 7.70. [f] N: calcd, 7.17; found, 7.81. [g] Indefinite, compound highly hygroscopic. [h] Contaminated with ca. 25–30% of compound **32**.

Table III. α_2- and α_1-Adrenoreceptor pA_2 Values[a] in in Vitro Experiments



no.	R	rat vas deferens: α_2 pA_2 values vs. UK 14,304	α_2-antag potency rel to idazoxan	rat anococcygeus: α_1 pA_2 values vs. noradrenaline	α_1-antag potency rel to idazoxan	α_2/α_1 selectivity[b] ratio
1	H	8.50 (8.38–8.64)	1.0	6.32 (6.14–6.57)	1.0	151
4	CH_3	7.96 (7.80–8.14)	0.3	5.30 (5.19–5.44)	0.1	457
5	CH_2CH_3	8.69 (8.54–8.87)	1.6	6.40 (6.35–6.44)	1.2	195
6	$(CH_2)_2CH_3$	8.92 (8.74–9.17)	2.6	6.56 (6.35–6.88)	1.7	229
12	$C(CH_3){=}CH_2$	8.21 (8.00–8.47)	0.5	5.42 (5.37–5.48)	0.1	617
13		7.96 (7.77–8.21)	0.3	5.84 (5.49–6.90)	0.3	132
31	OCH_3	9.41 (9.30–9.52)	8.1	6.91 (6.66–7.23)	4.0	316
32	OCH_2CH_3	8.92 (8.85–9.01)	2.6	6.74 (6.53–7.08)	2.6	151
33	$O(CH_2)_2CH_3$	8.64 (8.25–9.35)	1.4	6.26 (6.17–6.38)	0.9	239
34	$OCH(CH_3)_2$	8.67 (8.51–8.86)	1.5	6.01 (5.84–6.31)	0.5	457
35	$O(CH_2)_3CH_3$	8.37 (8.31–8.44)	0.7	6.70 (6.63–6.78)	2.4	47
37	$OCH_2CH{=}CH_2$	8.81 (8.42–9.51)	2.0	6.55 (6.50–6.62)	1.7	182
40	OCH_2Ph	8.68 (8.37–9.10)	1.5	6.39 (6.29–6.54)	1.2	195

[a] pA_2 values were calculated according to Arunlakshana and Schild[6] and are the means ± SEM of, in each case, a minimum of nine determinations. [b] Antilog of the difference between the pA_2 values at α_2- and α_1-adrenoreceptors.

reveals that the imidazoline ring is pseudoequatorial and is held in a skewed orientation with respect to the plane of the benzodioxan moiety. It is clear from a study of space filling models that in many of the compounds that are only weakly active as antagonists at the α_2-adrenoreceptor (e.g., **9**, **10**, **15**, and **16**) the imidazoline ring can be sterically constrained by the bulky 2-substituent in a coplanar orientation and one possible interpretation of their low activity is that in this conformation the imidazoline amino function cannot effectively interact with the anionic site on the receptor. Alternatively there is the possibility that the introduction of bulky groups at the 2-position may cause the imidazoline ring to adopt a pseudoaxial conformation, which again would lead to reduced activity.

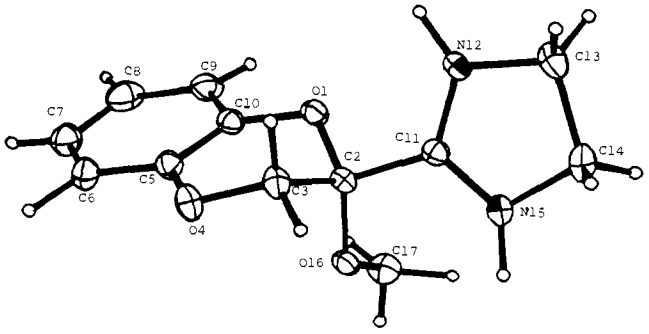


Figure 1. ORTEP diagram of the active (*S*) enantiomer of compound **31**.

Further work is being undertaken in an attempt to resolve this question.

Experimental Section

Chemistry. Melting points were determined in a Büchi apparatus in glass capillary tubes and are uncorrected. IR, NMR, and MS spectra were recorded for all compounds on Perkin-Elmer 700, Varian Associates T-60, and Varian Associates LKB-2091 instruments, respectively, and were consistent with the assigned structures. Where analyses are indicated only by symbols of the elements, results obtained were within ±0.4% of the theoretical values. Where purifications were carried out by column chromatography, silica refers to Kieselgel 60, 70–230-mesh ASTM.

(1) 2-Alkyl, 2-Cycloalkyl, 2-Alkenyl, 2-Cycloalkenyl, and 2-Phenyl Derivatives. 2-[2-(2-Methyl-1,4-benzodioxanyl)]-2-imidazoline Hydrochloride (4). A mixture of 2-(hydroxymethyl)-2-methyl-1,4-benzodioxan and 3-hydroxy-3-methyl-2*H*-1,5-benzodioxepin (23.7 g, 100 mmol of the benzodioxan; obtained as a 3:1 ratio according to the literature procedure[8]) was stirred with 1 N NaOH (135 mL) and cooled to 0–10 °C. A solution of potassium permanganate (42 g, 266 mmol) in water (165 mL) was added slowly with stirring so that the temperature was maintained below 10 °C. After 48 h at room temperature the mixture was filtered and the filtrate was acidified with 1 N H_2SO_4 and extracted with dichloromethane. The extracts were washed with aqueous $NaHCO_3$ solution, and the aqueous layer was acidified with 1 N H_2SO_4. Extraction with dichloromethane followed by washing, drying, and evaporation of the extracts gave 2-methyl-1,4-benzodioxan-2-carboxylic acid: yield 11.5 g (60%); mp 125–129 °C. A mixture of this acid (10.28 g, 53.0 mmol) and thionyl chloride (7.8 mL, 107 mmol) in anhydrous toluene (40 mL) was heated for 1 h at 90–100 °C. Removal of solvent and excess thionyl chloride in vacuo gave the crude acid chloride, which slowly solidified. A solution of this intermediate in anhydrous dioxan (25 mL) was added slowly to stirred aqueous ammonia (26 mL, 33% w/w) with cooling (0–10 °C). After 1 h water (300 mL) was added and the solid was collected by filtration, washed with water, and dried to yield the carboxamide: yield 8.3 g (81%); mp 127–129 °C. A stirred mixture of the amide (8.17 g, 42.3 mmol), phosphorus pentoxide (17 g, 120 mmol), and anhydrous toluene (175 mL) was heated under reflux for 4 h. After it had cooled, the supernatant was decanted from the residue, which was washed with more toluene. Filtration and evaporation of the solvent gave a solid residue (5.18 g), which was recrystallized from ethanol to give 2-cyano-2-methyl-1,4-benzodioxan (**20**): yield 4.4 g (59%); mp 88–89 °C. A mixture of the cyano compound (0.61 g, 3.49 mmol), sodium methoxide (0.016 g, 0.3 mmol), and methanol (2.3 mL) was stirred for 18 h to give an almost clear solution. On cooling to 0–10 °C, a solution of ethylenediamine (0.235 g, 3.91 mmol) in methanol (1 mL) was added dropwise with stirring. After a few minutes a solution of HCl in methanol (0.65 mL of 5.6 M solution, 3.64 mmol) was added dropwise and the mixture was allowed to warm to room temperature. After 16 h the mixture was made slightly acid with methanolic HCl and filtered. Addition of diethyl ether to the filtrate gave a solid, which was collected by filtration (0.84 g). Recrystallization from ethanol–diethyl ether containing HCl gave the imidazoline hydrochloride **4**: yield 0.59 g (67%); mp 258–261 °C. Anal. ($C_{12}H_{14}N_2O_2 \cdot HCl \cdot {}^1/_4H_2O$) C, H, N.

Other 2-substituted 2-hydroxymethyl-1,4-benzodioxans were obtained from the reaction of catechol with the corresponding substituted epichlorohydrin by using standard literature procedures.[9]

2-Cyano-2-methyl-1,4-benzodioxan (20) (via Scheme III). A mixture of the monosodium salt of catechol (9.3 g, 70 mmol), chloroacetone (7.3 g, 70 mmol), and benzyltrimethylammonium chloride (0.8 g, 35 mmol) in dry benzene (50 mL) was heated under reflux for 6 h. Powdered KBr (0.1 g, catalytic quantity) was added and reflux was continued for a further 4 h. The reaction mixture was cooled and partitioned between 2 N HCl solution and ether. The organic layer was separated, washed with brine, and dried ($MgSO_4$). Evaporation yielded a black viscous oil, which was chromatographed on silica eluting with petroleum ether (bp 40–60 °C) and dichloromethane to give 2-hydroxy-2-methyl-1,4-benzodioxan (**18**) as a white crystalline solid: yield 1.4 g (12.1%); NMR ($CDCl_3$) δ 6.9 (4 H, s, aryl H), 4.0 (2 H, AB, J = 11 Hz, CH_2), 3.5 (1 H, s, OH), 1.6 (3 H, s, CH_3). In the synthesis of the 2-ethyl and 2-*n*-propyl derivatives, the corresponding bromo ketones were used and yields were typically 75–85%.

The hydroxy compound (1.2 g, 7.2 mmol) was dissolved in dry dichloromethane (50 mL) and dry HCl gas was bubbled through the stirred solution for 0.5 h at 0 °C. The solution was evaporated to yield crude 2-chloro-2-methyl-1,4-benzodioxan (**19**) as a gray solid: yield 1.31 g (97.8%); IR ν_{max} ($CHBr_3$) 1500, 1270 cm^{-1}.

A mixture of the chloro compound (0.92 g, 5.0 mmol), stannic chloride (0.33 g, 1.25 mmol), and trimethylsilyl cyanide (0.7 g, 7 mmol) was stirred under an atmosphere of nitrogen at room temperature for 60 h. The mixture was then partitioned between water and dichloromethane and the organic layer was washed with $NaHCO_3$ solution, dried, and evaporated to yield a brown solid, which was purified via column chromatography on silica eluting with petroleum ether (bp 40–60 °C) and dichloromethane to give pure 2-cyano-2-methyl-1,4-benzodioxan, yield 0.65 g (74.3%), identical in all respects with that prepared previously.

2-(2-Isopropenyl-1,4-benzodioxan-2-yl)-2-imidazoline Hydrochloride (12). A suspension of 2-cyano-1,4-benzodioxan[10] (40 g, 248 mmol) and anhydrous potassium carbonate (176 g, 1274 mmol) in acetone (500 mL) was stirred and heated under reflux for 5 days. The mixture was cooled, and inorganic salts were removed by filtration. After evaporation of the acetone in vacuo, the residue was partitioned between dichloromethane and 2 N NaOH. The organic layer was washed with 2 N NaOH, 5% HCl, and water and then dried by passage through absorbent cotton wool. Evaporation in vacuo gave an orange oil (37 g), which was chromatographed on silica with dichloromethane as eluant to give 2-cyano-2-(1-hydroxy-1-methylethyl)-1,4-benzodioxan: yield 14.5 g (27%); mp 63–65 °C. A solution of the hydroxy compound (0.8 g, 3.65 mol) in anhydrous pyridine (8 mL) at room temperature was treated dropwise with phosphorus oxychloride (1 mL, 10.7 mmol) over 5 min. The solution was then heated at 60–70 °C for 18 h, cooled, and poured carefully onto ice–water. The mixture was extracted with dichloromethane, and the extracts were washed with saturated NaCl solution and dried. Evaporation in vacuo gave 2-cyano-2-isopropenyl-1,4-benzodioxan (0.62 g, 84%), which was converted by the procedures described above to the imidazoline hydrochloride **12**: mp 285–287 °C. Anal. ($C_{14}H_{16}N_2O_2 \cdot HCl$) C, H, N.

Compounds **13–15** were prepared by the methods described above for **12**, using the corresponding cycloalkanone in place of acetone.

Attempts to react 2-cyano-1,4-benzodioxan with acetaldehyde, propionaldehyde, formaldehyde in dioxane or methyl ethyl ketone using the above procedure all failed to give the required carbinol intermediate.

2-(2-Cyclopentyl-1,4-benzodioxan-2-yl)-2-imidazoline (10). A solution of 2-(2-cyclopent-1-enyl-1,4-benzodioxan-2-yl)-2-

(8) Salimberi, A.; Manghisi, E. *J. Heterocycl. Chem.* **1980**, *17*, 489.

(9) Johnson, F.; Panella, J. P.; Carlson, A. A. *J. Org. Chem.* **1962**, *27*, 2241. Stephenson, O. *J. Chem. Soc.* **1954**, 1571.

(10) Cook, M. J.; Katritzky, A. R.; Sewell, M. J. *J. Chem. Soc. B* **1970**, 1207. Martin, A. R.; Mallick, S. K.; Caputo, J. F. *J. Org. Chem.* **1974**, *39*, 1808.

imidazoline (14; 0.5 g, 1.85 mmol) in ethanol (10 mL) with 10% palladium on carbon (0.08 g) was hydrogenated at atmospheric pressure and room temperature for 3.5 h. The mixture was filtered and the filtrate evaporated to dryness to give a brown oil (0.5 g). The oil was partitioned between 2 N HCl and diethyl ether. The aqueous layer was basified with aqueous $NaHCO_3$ and extracted with diethyl ether. The dried extracts were evaporated in vacuo to leave a solid, which was recrystallized from hexane to give **10**: yield 0.1 g (20%); mp 135–136 °C. Anal. ($C_{16}H_{20}N_2O_2$) C, H, N.

2-(2-Vinyl-1,4-benzodioxan-2-yl)-2-imidazoline Hydrochloride (11). To a solution of 2-cyano-2-isopropenyl-1,4-benzodioxan (29.1 g, 150 mmol) in acetic acid (550 mL) and water (160 mL) was added potassium permanganate (53.2 g, 340 mmol) portionwise over a period of 1.5 h at room temperature. The mixture was stirred a further 3 h. Sodium metabisulfite solution was added and the aqueous mixture was extracted with dichloromethane. The organic layer was washed with 10% Na_2CO_3 solution, saturated brine, and dried. Evaporation of the solvent gave the 2-acetyl nitrile as a white solid: yield 19.6 g (67%); IR ($CHBr_3$) ν_{max} 1740 cm^{-1}. The acetyl nitrile (19.6 g, 96 mmol) was suspended in anhydrous ethanol (340 mL) and stirred during the portionwise addition of sodium borohydride (9.8 g, 260 mmol) over 10 min, the temperature being maintained at 20 °C. The mixture was stirred for a further 3 h at room temperature. Excess reducing agent was decomposed by the dropwise addition of 2 N HCl to the stirred solution at 0–5 °C. The mixture was poured into water and extracted with dichloromethane and the organic layer was washed with water, dried, and evaporated. The resulting green oil was purified by column chromatography using silica and eluting with chloroform/methanol (2%) to give the corresponding carbinol as a clear oil: yield 12.2 g (62%); IR ($CHBr_3$) ν_{max} 3600–3300 cm^{-1}. A solution of this alcohol (4.25 g, 21 mmol) and pyridine (1.7 mL, 21 mmol) in dichloromethane (90 mL) was added dropwise over 0.5 h to a stirred solution of trifluoromethanesulfonic anhydride (4.0 mL, 24 mmol) in dichloromethane (90 mL) maintained at 0 °C. The mixture was stirred at 0 °C for a further 1.5 h and anhydrous potassium carbonate (5.7 g, 41 mmol) was added and stirring at room temperature was continued for 24 h. Dipropylamine (2.8 mL, 20 mmol) was added and stirring was continued for a further 24 h. The mixture was poured into water and extracted with dichloromethane. The organic layer was washed with 2 N HCl, water, and saturated brine and dried. The crude product obtained by removal of the solvent was purified via column chromatography using silica and eluting with petroleum ether (bp 40–60 °C) containing successive 10% increments of diethyl ether. The 2-vinyl nitrile was obtained as a solid: yield 0.7 g (18%); IR ($CHBr_3$) ν_{max} 1640 cm^{-1}. The above vinyl compound was converted to the imidazoline hydrochloride 11 by using procedures already described: mp 180–182 °C. Anal. ($C_{13}H_{14}N_2O_2 \cdot HCl \cdot {}^1/_4H_2O$) C, H, N.

2-(3-Methyl-1,4-benzodioxan-2-yl)-2-imidazoline Hydrochloride (17). 3-Methyl-1,4-benzodioxan-2-carboxamide[11] was converted to the imidazoline hydrochloride 17 by using procedures already described (see 4 above). The NMR spectrum of 17 showed it to be a 1:4 mixture of the two diastereoisomers.

(2) 2-Alkoxy, 2-Cycloalkoxy, and 2-Phenoxy Derivatives. Method A (Scheme VII). 2-(2-Methoxy-1,4-benzodioxan-2-yl)-2-imidazoline (31). To a solution of benzodioxancarbonitrile (2;[10] 50.0 g, 310 mmol) in carbon tetrachloride (1250 mL) was added *N*-bromosuccinimide (57.0 g, 310 mmol) and a catalytic quantity of benzoyl peroxide (0.5 g). The resulting mixture was stirred and heated at reflux for 7 h. The mixture was allowed to cool and the resulting succinimide was filtered off and the filtrate was evaporated to yield an orange solid. The crude bromo nitrile was purified via column chromatography on silica eluting with petroleum ether (bp 40–60 °C) and ether to give 2-bromo-2-cyano-1,4-benzodioxan (22), yield 70.5 g (94%), as a white crystalline solid. This was subsequently found to contain a small quantity of 2,3-dibrominated material and an analytically pure sample of 22 was obtained by triturating the crystalline solid with cold petroleum ether (bp 40–60 °C). The pure material was filtered off and dried to give 22: mp 62–63 °C. Anal. ($C_9H_6BrNO_2$) C, H, N.

(11) Koo, J. *J. Org. Chem.* **1961**, *26*, 339.

A slow stream of HCl gas was passed through a solution of the above bromo nitrile (5.0 g, 21 mmol) and ethanol (1.16 mL, 21 mmol) in dry diethyl ether (150 mL) at 0–5 °C for 0.5 h. The reaction mixture was then kept at 0 °C for 14 h after which the crystalline product was filtered off, washed with diethyl ether, and dried to give ethyl 2-bromo-1,4-benzodioxan-2-carboximidoate hydrochloride (23): yield 5.3 g (80%); IR (Nujol) ν_{max} 2750, 2670 cm^{-1}.

A suspension of the imidoate hydrochloride salt (1.3 g, 4.0 mmol) in dry methanol (7.5 mL) was stirred and cooled at 0–5 °C during the dropwise addition of ethylenediamine (0.29 g, 4.8 mmol). The resultant solution was stirred at room temperature for 24 h before being poured into a saturated solution of $NaHCO_3$. The aqueous layer was extracted with dichloromethane, and the extract was dried and evaporated to yield a solid. Purification via column chromatography (on silica) eluting with methylene chloride gave 31: yield 0.25 g (26%); mp 90–91 °C. Anal. ($C_{12}H_{14}N_2O_7$) C, H, N.

All the compounds having alkoxy side chains originating from alcohols that are liquid at normal temperatures (31–42, 45–48, and 50–52) can also be obtained by using method B (Scheme VI). A representative example is given below.

Method B (Scheme VII). 2-(2-Ethoxy-1,4-benzodioxan-2-yl)-2-imidazoline Hydrochloride (32). A solution of 2-bromo-2-cyano-1,4-benzodioxan (22; 3.0 g 12.5 mmol) in dry ethanol (60 mL) was cooled to 0 °C and sodium methoxide (100 mg, catalytic quantity) was added. After stirring of the solution at 0–10 °C for 0.5 h, ethylenediamine (0.84 g, 14 mmol) was added followed by the dropwise addition of ethanolic HCl (5 M, 3 mL, 15 mmol). The solution was stirred for a further 0.5 h at 0–10 °C and then allowed to warm to room temperature and stirred overnight. The ethanol was evaporated at 40 °C and the resulting mixture was partitioned between dichloromethane and saturated $NaHCO_3$ solution. The organic layer was separated, dried by pouring it through absorbent cotton wool, and evaporated to give a light brown viscous oil. This was dissolved in diethyl ether (a small quantity of insoluble material was filtered off) and ethereal HCl was added. The precipitated hydrochloride salt was filtered off, washed with diethyl ether, and dried to yield a white solid, which was crystallized from ethanol/diethyl ether to give 32: yield 2.7 g (75%); mp 222–224 °C. Anal. ($C_{13}H_{16}N_2O_3 \cdot HCl$) C, H, N.

Compounds 43, 44, and 49 were prepared with use of a cosolvent during imidoate formation. The synthesis of 43 is a representative example.

2-[2-[(2-Chlorobenzyl)oxy]-1,4-benzodioxan-2-yl]-2-imidazoline (43). To a stirred solution of the bromo nitrile 22 (6.0 g, 25 mmol) and 2-chlorobenzyl alcohol (20.0 g, 141 mmol) in tetrahydrofuran (30 mL) was added a catalytic quantity of sodium methoxide at 0 °C. After 0.5 h, ethylenediamine (1.5 g, 25 mmol) was added followed by ethereal HCl (4 M, 7.0 mL, 28 mmol) at 0 °C. The reaction mixture was allowed to stir at 0 °C for 0.5 h and then at room temperature for 16 h. It was then poured into a large excess of aqueous $NaHCO_3$ solution and extracted with ethyl acetate. The organic layer was washed with 2 N HCl solution and the acid extract was basified with aqueous $NaHCO_3$ solution and extracted with ethyl acetate. The organic layer was washed with water, dried, and evaporated to give a brown oil, which was purified by column chromatography on silica eluting with chloroform/methanol to give pure material, which was converted to the hydrochloride salt 43: yield 0.03 g (0.3%); mp 147–148 °C. Anal. ($C_{18}H_{17}N_2O_3 \cdot HCl \cdot {}^3/_4H_2O$) C, H, N.

2′,3′,5′,6′-Tetrahydrospiro[1,4-benzodioxin-2(3*H*),8′-imidazo[2,1-*c*][1,4]oxazine] Hydrochloride (28). A solution of the bromo imidoate hydrochloride 23 (3.2 g, 9.9 mmol) in 2-chloroethanol (30 mL) was stirred at 0 °C during the addition of ethylenediamine (0.67 g, 11.1 mmol) over 1.5 min. The solution was stirred at this temperature for 1 h and then for 4.5 h at room temperature. The reaction mixture was partitioned between saturated brine and dichloromethane and the organic layer was washed with water, dried, and evaporated. The residue was purified via column chromatography on silica eluting with a mixture of dichloromethane/methanol. The major product was triturated with hexane/ether to give a white solid, which was purified further with an alumina column eluting with chloroform. This produced a colorless oil, which was converted to the hydrochloride salt with ethereal HCl, giving 28: yield 0.12 g (4.3%);

mp 226–232 °C; the IR spectrum of the free base showed no signal for NH; NMR (free base) ($CDCl_3$) δ 6.9 (4 H, s, aryl H), 4.4 (2 H, AB, J = 11 Hz, dioxan CH_2), 3.9–2.8 (8 H, m, oxazine CH_2, imidazo CH_2); MS, 246 (M^+) ($C_{13}H_{14}H_2O_3$ requires M^+ 246). Anal. ($C_{13}H_{14}N_2O_3 \cdot HCl \cdot {}^1/_4H_2O$) C, H, N.

2-[2-(Ethylthio)-1,4-benzodioxanyl]-2-imidazoline Hydrochloride (55). A solution of the bromo nitrile **22** (1.2 g, 5 mmol) in a mixture of ethanethiol (25 mL), dry ether (50 mL), and triethylamine (0.5 g, 5 mmol) was stirred at 0–5 °C for 16 h and then allowed to warm to room temperature and stirred for a further 8 h. The resulting precipitate was filtered off and the filtrate was evaporated to dryness at <40 °C. The residue was dissolved in dry ether and ethereal HCl was added. The resulting solid was filtered off and dried (1.0 g). This was dissolved in ethanol (5 mL) and ethylenediamine (0.22 g, 3.7 mmol) was added at 0–5 °C. The mixture was stirred at this temperature for 0.5 h and then overnight at room temperature. The reaction was worked up as described in method B above to give an oil, which was converted to the hydrochloride salt with ethereal HCl. The salt was crystallized from ethanol/ether to give a white crystalline solid **55**: yield 0.14 g. NMR suggested the presence of the 2-ethoxy derivative **32**; NMR (Me_2SO) δ 12.0 (2 H, br s, NH), 7.0 (4 H, s, aryl H), 4.8 (2 H, AB, J = 11 Hz, dioxanyl CH_2), 4.0 (4 H, s, imidazoline CH_2's), 3.7 (q, OCH_2), 2.8 (q, SCH_2), 1.2 (3 H, t, CH_3); the ratio of the SCH_2 signal to OCH_2 was approximately 3:1; MS, 264 (M^+) ($C_{13}H_{16}N_2O_2S$ requires M^+ 264). The mass spectrum showed very little evidence for the presence of the ethoxy derivative **32**. The impurity could be detected by HPLC and was estimated at 30%; the separation was minimal, however, and purification by this method was impractical. Successive crystallizations also failed to achieve any increase in the proportion of the ethylthio derivative.

Reaction of 2-Bromo-2-cyano-1,4-benzodioxan (22) with Sodium Methoxide. A solution of **22** (10.0 g, 47.1 mmol) and sodium methoxide (2.47 g, 45.7 mmol) in methanol (50 mL) was stirred at room temperature for 48 h. The resulting suspension was filtered and the solid washed with methanol. The combined methanol fractions were evaporated to give an oil, which was chromatographed on silica eluting with ether/petroleum ether (bp 40–60 °C). Three major fractions were recovered and were identified as follows. Methyl 2-bromo-1,4-benzodioxan-2-carboximidoate (**24**): yield 1.75 g (15.5%); NMR ($CDCl_3$) δ 8.4 (1 H, br s, NH), 7.1 (4 H, s, aryl H), 4.2 (2 H, AB, J = 11 Hz, CH_2), 3.9 (3 H, s, OCH_3); IR ($CHBr_3$) ν_{max} 3330, 1670 cm^{-1}; MS, 270 (M^+), 272, ($C_{10}N_{10}BrNO_3$ requires $M^+ - 1$, 270, 272); NMR, IR, and TLC indicated that this compound was contaminated with an impurity, probably the α-bromo or α-methoxy ester. Methyl 1,4-benzodioxin-2-carboximidoate (**25**): yield 1.05 g (13.2%); NMR ($CDCl_3$) δ 7.8 (1 H, br s, NH), 7.1–6.6 (5 H, m, aryl H, OH), 3.8 (3 H, s, OCH_3); IR ($CHBr_3$) ν_{max} 3350, 1640 cm^{-1}; MS 191 (M^+) ($C_{10}H_9NO_3$ requires M^+ 191). Methyl 2-methoxy-1,4-benzodioxan-2-carboximidoate (**26**): yield 1.05 g (11.3%); NMR ($CDCl_3$) δ 8.2 (1 H, br s, NH) 6.9 (4 H, s, aryl H), 4.1 (2 H, AB, J = 11 Hz, CH_2), 3.8 (3 H, s, imidoate OCH_3), 3.3 (3 H, s, benzodioxanyl OCH_3); IR ($CHBr_3$) ν_{max} 3340, 1660 cm^{-1}; MS 223 (M^+) ($C_{11}H_{13}NO_4$ requires M^+ 223).

Pharmacology. Preparations. Rat Vas Deferens. Vasa deferentia were removed from male Sprague–Dawley rats weighing 200–250 g. The prostatic half of the vas deferens was cleaned of connective tissue and suspended under an initial tension of 0.5 g in an organ bath of 8–10-mL capacity. The tissue was bathed in Krebs solution (NaCl, 118 mM; KCl, 4.7 mM; $CaCl_2$, 2.5 mM; KH_2PO_4, 1.2 mM; $MgSO_4$, 0.6 mM, $NaHCO_3$, 25 mM; dextrose, 11.1 mM), which was gassed with 95% O_2 and 5% CO_2 and maintained at a temperature of 30 °C. The intramural nerves of the vas deferens were stimulated by rectangular pulses of 3-ms duration, 40 V, at a frequency of 0.1 Hz, and the resultant contractions of the tissue were recorded isometrically.

Mouse Vas Deferens. Vasa deferentia from adult male mice (MFI > 30 g) were set up, under an initial tension of 0.5 g, in an organ bath of 50-mL capacity that contained magnesium-free Krebs solution. The physiological solution was maintained at 30 °C and gassed with 95% O_2 and 5% CO_2. The preparations were field stimulated between platinum electrodes at 0.1 Hz with rectilinear pulses of 3-ms duration. The voltage (100–140 V) was adjusted to give a twitch response of approximately 100-mg tension. Contractions of the tissue were recorded isometrically.

Rat Anococcygeus Muscle. The anococcygeus muscles of male Sprague–Dawley rats weighing 200–250 g were removed and suspended in a 50-mL organ bath under an initial tension of 0.5 g. The tissue was bathed in Krebs solution, which was gassed with 95% O_2 and 5% CO_2 and maintained at 30 °C.

In Vitro Screening. Presynaptic α_2-Adrenoreceptor Agonist Activity. Vas Deferens. Either the mouse or rat vas deferens was used in these studies. Repeated cumulative concentration–response curves were constructed to the presynaptic α_2-adrenoreceptor agonist clonidine until consistent ID_{50} values were obtained. The effect of the test compound was then examined, and if inhibition of the twitch was obtained, an ID_{50} value was determined; i.e., presynaptic potency of the new analogue was compared directly with that of clonidine in the same experiment. The compound was then removed from the bathing fluid and the responsiveness of the tissue to clonidine reassessed.

Presynaptic α_2-Adrenoreceptor Antagonist Properties. Vas Deferens. Tissues taken from either the rat or mouse were used to determine presynaptic α_2-adrenoreceptor antagonist potency. Contractions of the vas deferens were inhibited by including clonidine (110 nM) in the Krebs solution. The concentration of compound required to produce 50% reversal of the inhibitory effects of clonidine was determined and compared with the value determined for idazoxan in the same tissue. Presynaptic α_2-adrenoreceptor antagonist potency was therefore expressed with respect to idazoxan as the standard.

Postsynaptic α_1-Adrenoreceptor Agonist Activity. Rat Anococcygeus. Postsynaptic α_1-adrenoreceptor agonist activity was determined on the rat anococcygeus muscle. Cumulative concentration–response curves to the contractile effects of phenylephrine were constructed until the responses were reproducible. The effects of test compounds were then studied, and the potencies of compounds with agonist activity were compared directly with that of phenylephrine in the same tissue.

Postsynaptic α_1-Adrenoreceptor Antagonist Properties. Rat Anococcygeus. Cumulative concentration–response curves to phenylephrine were constructed in the absence and presence of a fixed concentration of idazoxan or one of the test compounds. From the dose ratios produced was calculated the concentration of agonist producing a dose ratio, and, thus, the α_1-antagonist potency relative to idazoxan was determined.

Determination of pA_2 Values for Competitive Antagonists. The pA_2 values of selected compounds were determined at presynaptic α_2-adrenoreceptors and postsynaptic α_1-adrenoreceptors. Antagonism of the inhibitory effect of UK 14,304 on the vas deferens and antagonism of noradrenaline contractions on the anococcygeus muscle were used to determine pA_2 values at presynaptic α_2-adrenoreceptors and postsynaptic α_1-adrenoreceptors, respectively. pA_2 is the negative log of the antagonist concentration required to maintain a constant response when the concentration of the agonist is doubled.

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Registry No. **2**, 1008-92-0; **4**, 96576-09-9; **4**·HCl, 84141-81-1; **5**, 84141-82-2; **6**, 84141-92-4; **7**, 96576-10-2; **8**, 96576-11-3; **9**, 84141-88-8; **10**, 84141-94-6; **11**, 96576-12-4; **11**·HCl, 96576-13-5; **12**, 84141-87-7; **12**·HCl, 96576-14-6; **13**, 96576-15-7; **13**·HCl, 84141-93-5; **14**, 84141-90-2; **15**, 84141-89-9; **16**, 84141-84-4; *cis*-**17**, 96576-16-8; *cis*-**17**·HCl, 96576-18-0; *trans*-**17**, 96576-17-9; *trans*-**17**·HCl, 96615-39-3; **18**, 5771-13-1; **18**(2-ethyl derivative), 96576-63-5; **18**(2-propyl derivative), 96576-64-6; **19**, 91933-95-8; **20**, 84141-80-0; **20**(2-ethyl derivative), 96576-67-9; **20**(2-propyl derivative), 96576-68-0; **21**, 84141-85-5; **22**, 96576-19-1; **23**, 96576-20-4; **24**(R = Me), 96576-21-5; **25**, 96576-22-6; **26**, 96576-23-7; **28**, 96615-40-6; **28**·HCl, 96615-41-7; (*S*)-(+)-**31**, 89195-34-6; (*R*)-(−)-**31**, 89195-31-3; **32**, 96576-24-8; **32**·HCl, 96576-25-9; **33**, 96576-26-0; **34**, 96576-27-1; **35**, 96576-28-2; **36**, 96576-29-3; **36**·HCl, 96576-30-6; **37**, 96576-31-7; **38**, 96576-32-8; **38**·HCl, 96576-33-9; **39**, 96576-34-0; **40**, 96615-42-8; **41**, 96576-35-1; **42**, 96576-36-2; **42**·HCl, 96576-37-3;

43, 96576-38-4; 43·HCl, 96576-39-5; 44, 96615-43-9; 44·HCl, 96576-40-8; 45, 96576-41-9; 45·HCl, 96576-42-0; 46, 96576-43-1; 47, 96576-44-2; 48, 96576-45-3; 49, 96576-46-4; 50, 96576-47-5; 50·HCl, 96576-48-6; 51, 96576-49-7; 51·HCl, 96576-50-0; 52, 96576-51-1; 53, 96576-52-2; 54, 96576-53-3; 55, 96576-54-4; 55·HCl, 96576-55-5; EDA, 107-15-3; $ClCH_2COCH_3$, 78-95-5; $BrCH_2COCH_2CH_3$, 816-40-0; $BrCH_2CO(CH_2)_2CH_3$, 817-71-0; $2\text{-}ClC_6H_4CH_2OH$, 17849-38-6; $Cl(CH_2)_2OH$, 107-07-3; EtSH, 75-08-1; MeCHBrCHBrCOOEt, 609-11-0; $CH_3(CH_2)_2OH$, 71-23-8; $(CH_3)_2CHOH$, 67-63-0; $CH_3(CH_2)_3OH$, 71-36-3; $CH_3(CH_2)_4OH$, 71-41-0; $CH_2{=}CHCH_2OH$, 107-18-6; $HC{\equiv}CCH_2OH$, 107-19-7; $PhCH_2OH$, 100-51-6; $2\text{-}MeOC_6H_4CH_2OH$, 612-16-8; $4\text{-}MeOC_6H_4CH_2OH$, 105-13-5; $4\text{-}ClC_6H_4CH_2OH$, 873-76-7; $Ph(CH_2)_2OH$, 60-12-8; $HO(CH_2)_2OH$, 107-21-1; $HO(CH_2)_4OH$, 110-63-4; $MeO(CH_2)_2OH$, 109-86-4; PhOH, 108-95-2; $3\text{-}MeC_6H_4OH$, 108-39-4; $4\text{-}MeOC_6H_4OH$, 150-76-5; Cl_3CCH_2OH, 115-20-8; $CH_3CH(NH_2)CH_2NH_2$, 78-90-0; 2-(hydroxymethyl)-2-methyl-1,4-benzodioxan, 16163-83-0; 3-hydroxy-3-methyl-2*H*-1,5-benzodioxepin, 68281-26-5; 2-methyl-1,4-benzodioxan-2-carboxylic acid, 68281-27-6; 2-methyl-1,4-benzodioxan-2-carbonyl chloride, 77156-57-1; 2-methyl-1,4-benzodioxan-2-carboxamide, 84141-79-7; catechol, 120-80-9; catechol monosodium salt, 34789-97-4; 2-cyano-2-isopropenyl-1,4-benzodioxan, 84141-86-6; 2-acetyl-2-cyano-1,4-benzodioxan, 96576-56-6; 2-cyano-2-(1-hydroxyethyl)-1,4-benzodioxan, 96576-57-7; 2-cyano-2-vinyl-1,4-benzodioxan, 96576-58-8; 3-methyl-1,4-benzodioxan-2-carboxamide, 96576-59-9; 2,3-dibromo-1,4-benzodioxan-2-carbonitrile, 96576-60-2; 2-(ethylthio)-1,4-benzodioxan-2-carbonitrile, 96576-61-3; 2-(chloromethyl)-2-methyloxirane, 598-09-4; 2-butyl-2-(chloromethyl)oxirane, 86488-91-7; 2-(chloromethyl)-2-heptyloxirane, 96576-62-4; 2-(chloromethyl)-2-phenyloxirane, 1005-91-0; 2-butyl-2-(hydroxymethyl)-1,4-benzodioxan, 96576-65-7; 2-heptyl-2-(hydroxymethyl)-1,4-benzodioxan, 96576-66-8; 2-(hydroxymethyl)-2-phenyl-1,4-benzodioxan, 84141-83-3; cyclobutanone, 1191-95-3; cyclopentanone, 120-92-3; cyclohexanone, 108-94-1; ethyl 3-methyl-1,4-benzodioxan-2-carboxylate, 67770-59-6; cyclopentanol, 96-41-3; 2-(chloromethyl)-2-vinyloxirane, 96576-69-1; 2-bromo-6,7-dimethoxy-1,4-benzodioxan-2-carbonitrile, 96576-70-4.

Synthesis and Biological Activity of Pentapeptide Analogues of the Potent Angiotensin Converting Enzyme Inhibitor 5(*S*)-Benzamido-4-oxo-6-phenylhexanoyl-L-proline

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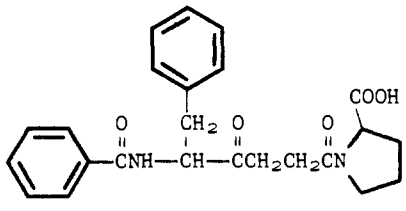
Two pentapeptide analogues (14 and 15) of the ketomethylene-containing angiotensin converting enzyme (ACE) inhibitor 5(*S*)-benzamido-4-oxo-6-phenylhexanoyl-L-proline (1) were synthesized and evaluated as ACE inhibitors and antihypertensive agents. Compounds 14 and 15 were very potent ACE inhibitors with I_{50} values of 7.0 and 3.0 nM, respectively, compared to an I_{50} value of 70 nM for 1. Neither 14 nor 15 showed significant blood pressure lowering activity in renal hypertensive rats. Investigations conducted on a tritiated analogue of 14 showed that 70% of an oral dose of this compound is absorbed but is rapidly excreted from the blood with a half life of 24 min. Thin-layer chromatography of bile and urine contents in rats given tritiated 14 orally showed that it is excreted in greater than 90% unchanged form. This implies that a ketomethylene linkage can stabilize peptide amide linkages adjacent to it to peptidase degradation.

The ketomethylene tripeptide analogue 5(*S*)-benzamido-4-oxo-6-phenylhexanoyl-L-proline (1) has been shown to be a potent in vitro angiotensin converting enzyme (ACE) inhibitor, I_{50} = 70 nM.[1] Unfortunately this compound has poor activity as a blood pressure lowering agent when given either orally or intravenously to renal hypertensive rats.[2]



1

Recent studies[3] of radiolabeled derivatives of 1 suggest two possible explanations for its poor in vivo activity. First, only 20% of an oral dose of 1 is absorbed into the blood stream over a 24-h period. Second, the half-life of 1 in the blood in rats was only 10 min because it is rapidly excreted into the bile. Compound 1, however, is only slowly metabolized by the rat in which 80% of the excreted radioactivity was in the form of unchanged 1.

One possible method to improve the antihypertensive activity of 1 in the rat would be to develop analogues of it that have greatly increased ACE inhibition activity. Such compounds would be expected to require lower blood levels than 1 to achieve total ACE inhibition in vivo. Therefore, even though these new compounds may also be rapidly excreted in the bile, the blood level required to achieve significant blood pressure reduction with them in the rat may be lower because of their increased potency.

An examination of the ACE inhibition data published by Cushman et al.[4] on various peptide analogues of the original snake venom peptide ACE inhibitors indicates that the pentapeptide pGlu-Lys-Phe-Ala-Pro is almost 30 times more potent than the tripeptide Phe-Ala-Pro. By analogy, extending 1 to a pentapeptide might greatly increase its

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(1) Almquist, R. G.; Chao, W.-R.; Ellis, M. W.; Johnson, H. L. *J. Med. Chem.* **1980**, *23*, 1392.
(2) Meyer, R. F.; Nicolaides, E. D.; Tinney, F. J.; Lunney, E. A.; Holmes, A.; Hoefle, M. L.; Smith, R. D.; Essenburg, A. D.; Kaplan, H. R.; Almquist, R. G. *J. Med. Chem.* **1981**, *24*, 964.
(3) Almquist, R. G.; Steeger, T.; Jackson, S.; Mitoma, C. *Life Sci.* **1985**, *37*, 299.
(4) Cushman, D. W.; Pluscec, J.; Williams, N. J.; Weaver, E. R.; Sabo, E. F.; Kocy, O.; Cheung, H. S.; Ondetti; M. A. *Experientia* **1973**, *29*, 1032.

0022-2623/85/1828-1062$01.50/0