Conformational Effects on the Activity of Drugs. 7.[1] Synthesis and Pharmacological Properties of 2-(*p*-Nitrophenyl)-Substituted Morpholines[2]

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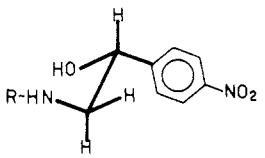
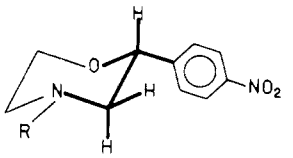
A series of 1-(*p*-nitrophenyl)-2-aminoethanol derivatives and their morpholine analogues have been synthesized and pharmacologically investigated in order to confirm some pharmacological observations made with the *N*-isopropyl-substituted compounds. In agreement with the previously obtained results, the weak α-adrenergic-stimulating activity and the potentiating effect on the responses to norepinephrine found in the open-chain compounds persist in their corresponding semirigid cyclic analogues. The results are discussed in the light of common knowledge of the structure–activity relationships of α-adrenergic drugs.

In preceding papers in this series[3,4] we described the synthesis and biological activity of 2-(*p*-nitrophenyl)-4-isopropylmorpholine (**7**), a conformationally restrained



1, R = H
2, R = CH_3
3, R = $CH(CH_3)_2$
4, R = $C(CH_3)_3$



5, R = H
6, R = CH_3
7, R = $CH(CH_3)_2$
8, R = $C(CH_3)_3$

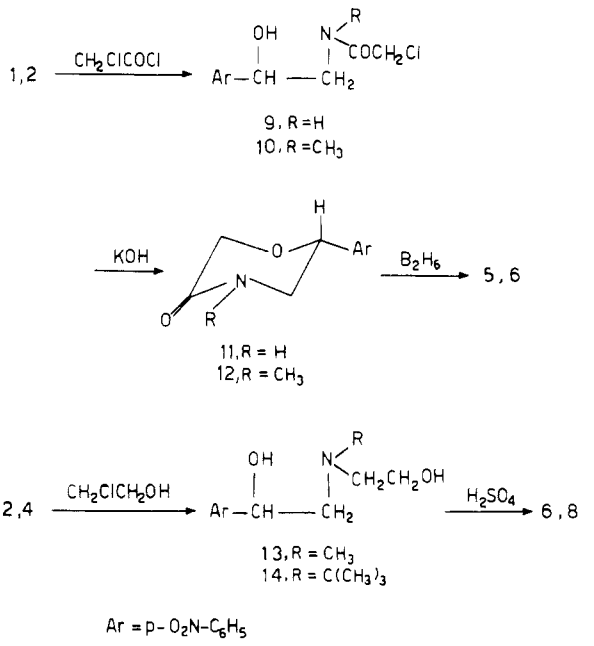
analogue of 1-(*p*-nitrophenyl)-2-aminoethanol (INPEA, **3**), in which the OCCN chain of **3** is locked in the morpholine ring, in the same preferred conformation[5] as in the open-chain compound. These studies showed that **7** does not possess the principal pharmacological property of **3**, i.e., β-receptor blocking activity,[6,7] but maintains some of the secondary effects on the adrenergic system; the potentation of catecholamines[7-9] and the weak intrinsic α-sympathomimetic activity[7] of **3** are still present in **7**. The structure of the basic group of the adrenergic agents is known[10] to determine the direction of their biological response: increasing the size of the alkyl substitution at the nitrogen increases affinity for the β-receptor and correspondingly decreases affinity for the α-adrenergic receptor.

Our aim was to confirm that the weak α-adrenergic-stimulating activity of a 1-aryl-2-aminoethanol derivative, **3**, persisted in its semirigid morpholine analogue **7**.

On the basis of the above data, we synthesized amino alcohols **1**, **2**, and **4** and their corresponding[11] morpholine analogues **5**, **6**, and **8** and studied their pharmacological properties to evaluate the α-adrenergic activity in comparison to that of **3** and **7**, which appears to be conditioned by the presence of the *N*-isopropyl group.

Chemistry. Amino alcohols **1**, **2**, and **4** were prepared[12] by reacting *p*-nitrostyrene oxide with the corresponding amines. Treatment of **1** and **2** with $CH_2ClCOCl$ and NaOH in CH_2Cl_2–H_2O (Scheme I) gave the corresponding *N*-chloroacetyl derivatives **9** and **10**, which were converted into morpholinones **11** and **12**, respectively, by base-catalyzed (KOH) cyclization. Reduction of the latter compounds with B_2H_6 yielded morpholine derivatives **5** and **6**. Reaction of amino alcohols **2** and **4** with CH_2ClCH_2OH yielded the corresponding *N*-(2-hydroxyethyl) derivatives

Scheme I



13 and **14**, which were cyclized to morpholines **6** and **8** by treatment with H_2SO_4.

Amino alcohols **1**–**4** preferentially exist in solution in the conformation shown, as indicated[13] by the values of the vicinal coupling constant (4.0 and 7.4 Hz for **1**, 3.6 and 9.6 Hz for **2**, 3.7 and 9.0 Hz for **3**,[5] and 3.8 and 8.7 Hz for **4**) of the signal of the benzylic proton in their ^1H NMR spectra and by the strong absorption (at 3460, 3459, 3447,[5] and 3441 cm^{-1}, respectively) in their IR spectra in dilute solution, attributable[14] to an intramolecular OH--N hydrogen bond.

The values of the vicinal coupling constants of the proton α to the aryl group in the ^1H NMR spectra of **5**–**8** (2.5 and 10.4 Hz for **5**, 2.7 and 10.4 Hz for **6**, 2.5 and 10.1 Hz for **7**,[5] and 2.3 and 10.1 Hz for **8**) are consistent[13] with a preferred axial position of this proton and, therefore, with the existence of these compounds in solution in the expected[3,4] conformation with the aryl group in an equatorial position.

Pharmacology. Methods. Vas deferens from male (ICEM: CER SPF Caw) rats (200–250 g body weight) were suspended in a 10-mL organ bath containing Krebs so-

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Table I. Isolated Rat Vas Deferens Tests Results

no.	A[a]	B[b]	C[c]
1	7.5×10^{-3} (4.2×10^{-3}-1.3×10^{-2})	1.66	179 ± 10.2
2	4.7×10^{-3} (2.9×10^{-3}-7.7×10^{-3})	1.04	253 ± 16.8
3	4.5×10^{-3} (1.9×10^{-3}-1.04×10^{-2})	1.00	209 ± 21.3
4	1.2×10^{-3} (0.7×10^{-3}-1.9×10^{-3})	0.27	127 ± 14.2
5	6.3×10^{-3} (4.3×10^{-3}-9.05×10^{-3})	1.40	203 ± 13.4
6	1.4×10^{-3} (1.1×10^{-3}-1.8×10^{-3})	0.31	192 ± 16.5
7	4.5×10^{-3} (2.7×10^{-3}-7.6×10^{-3})	1.00	223 ± 15.6
8	2.9×10^{-3} (1.9×10^{-3}-4.5×10^{-3})	0.64	169 ± 11.3

[a] α-Adrenergic-stimulating activity ratio of the compounds to NE. Each value represents the mean of eight experiments. In parentheses are the 95% confidence limits. [b] α-Adrenergic-stimulating activity of the compounds as indicated in A taking the activity of INPEA as 1. [c] Potentiating effect on the responses to NE (8×10^{-6} M): percent increase ± SE of control responses in the presence of the compounds (5×10^{-5} M) (six experiments).

lution aerated with an O_2/CO_2 mixture (95:5%) and thermostatically controlled at 36 °C. A 0.5-g tension was applied to the suspended tissue, which was allowed to stabilize for 30 min. Changes in muscle tone, expressed as millimeters of contraction, were recorded by means of a strain-gauge-equipped semiisometric lever connected to a high-gain amplifier and a galvanometer (Microdynamometer Basile).

The test compounds remained in contact with the tissue long enough to obtain the maximum effect (2–3 min).

The dose–effect curve for norepinephrine (NE) and the test compound was plotted for each rat vas deferens, by using the single-dose technique. The dose scale was adjusted each time, according to the sensitivity of the preparation. Various doses (at least four) were tested for each preparation, and the responses to those doses which gave maximum regression were used for statistical analysis.

Data were analyzed to evaluate the potency of each compound, compared to NE as standard. The value of M (log potency ratio) was calculated for each organ tested, as an expression of the potency of the compounds. The M values for each compound were then used to calculate the mean and fiducial limits ($p = 0.95$); finally, the antilogarithm of these values was calculated to obtain the potency ratio (R) and its fiducial limits ($p = 0.95$).

Parallelism of the dose–effect curves for NE and the test compounds was also tested, by comparing the b values (angle of the log dose–effect curve) for the standard and for the test compounds by means of Student's t test for paired data. Compounds 1–8 were used as hydrochlorides and NE was used as the bitartrate.

Results

Table I shows the results obtained on isolated rat vas deferens with both the open-chain compounds 1–4 and their cyclic derivatives 5–8 (A).

All the compounds showed a moderate degree of α-mimetic activity in comparison with NE. It was not possible to obtain the dose–response curves due to the fact that, when employed at a high concentration, the compounds showed α-blocking properties. As previously pointed out,[4] the same effect has also been demonstrated in the case of INPEA (3) itself.[15] No evidence against parallelism was found for any of the compounds tested. As the parallelism was not significant, there was no evidence to affirm that the intrinsic activity of the tested compounds was significantly different among them. The potency of the tested compounds relative to INPEA (3) is also shown in Table I (B).

The contractions induced by all the compounds were inhibited by an α-blocking drug, such as dihydroergotamine (DHE), at doses ranging from 1.10^{-5} to 4.10^{-5} M and maintaining a contact time of 20 min ($ID_{50} = 1.5 \times 10^{-5}$ M, six experiments); the range of the doses of DHE corresponded to that previously used as an α-adrenergic receptor antagonist.[4]

These effects were unchanged when vas deferens from reserpine-pretreated rats (1 mg/kg, 24 h before the experiment) was employed. Similar results were obtained[4,8] for INPEA (3) and its morpholine derivative 7.

In agreement with previous results,[4] compounds 1–8 significantly increased the responses of norepinephrine, mainly with submaximal doses of catecholamine, when the uptake processes were not saturated (Table I). Dose–response curves to NE were significantly shifted to the left in the presence of INPEA analogues, $1–5 \times 10^{-5}$ M (six experiments).

The potentiating effect of the drugs on the responses to norepinephrine is shown in Table I (C); this effect had previously been observed for 3 and 7.

Discussion

Within the range of doses tested, all the compounds gave a dose–effect curve on rat vas deferens contraction showing significant regression, parallel to that for NE; this indicates that they have a similar activity, although the potency is considerably lower than that of NE.

The compounds still showed activity on vas deferens from animals pretreated with reserpine; the activity seems, therefore, to be direct and not mediated by NE release.

That the drugs act directly on α receptors is confirmed by the fact that the activity is inhibited by an α blocker, such as dihydroergotamine.

On the basis of previous observations,[4] the high potentiation of response to NE in the presence of INPEA derivatives appears to be due to a block on the neuronal uptake of amines.

Comparing the α-adrenergic stimulating activity (see Table I, A and B) of the amino alcohols 1–4 and their cyclic analogues 5–8, it can be observed that the activity of the open-chain compounds 1–4 do not differ too much from that of the corresponding cyclic derivatives 5–8, except for the N-methyl-substituted compounds 2 and 6. In both series there is a decrease of the effect by moving from the N-unsubstituted derivative (1 and 5, respectively) to the N-*tert*-butyl-substituted one (4 and 8, respectively). The trend of the activity is not, however, strictly in accordance with the variation expected on the basis of the directing effect of the substituent on the amine nitrogen on α activity,[10] i.e., a regular decrease of activity moving from the N-unsubstituted compounds to those N substituted with alkyl groups of increasing steric hindrance: the N-methyl-substituted amino alcohol 2 is almost exactly equipotent to its N-isopropyl homologue 3; the N-methyl-substituted morpholine 6 is less active of not only its N-isopropyl homologue 7 but also of the N-*tert*-butyl one (8). The causes of these apparent disagreements[16] are to be sought in the complex balance of all the chemical and physical factors, unfortunately not yet completely clarified,[17,19] that directly or indirectly influence the fundamental processes in α activation. For example, proton transfer and formation of an ionic couple in the interaction of the cationic head of the drug molecule with α receptors

may be involved.[10a,20,21] The subtle effects on the mechanism of proton dissociation[19] and the importance of the spatial distribution of the masses around the basic nitrogen of adrenergic drugs in the drug–receptor interaction[22] have been recently pointed out.

In conclusion this study seems to confirm the results obtained[4] with **3** and **7** and the stereoelectron hypothesis previously advanced[4] as regards the structure–activity relationship. In particular, the conformational freedom of the amino alcohols when they are constrained in the framework of the morpholine cyclic system seems to be sufficient for them to show intrinsic α-adrenergic activity.

Experimental Section

All compounds were routinely checked for their structure by IR and ^{1}H NMR spectroscopy. Melting points were determined on a Kofler apparatus and are uncorrected. IR spectra for comparison between compounds were taken with a Perkin-Elmer Infracord Model 137 as Nujol mulls in the case of solid substances or as liquid film in the case of liquids, and IR spectra for the determination of OH--N stretching bands were taken with a Perkin-Elmer Model 257 double-beam grating spectrophotometer in dried (P_2O_5) CCl_4, using the indene band at 3110 cm^{-1} as a calibration standard; a quartz cell of 1-cm optical length was employed, and the concentration of the solutions was 5×10^{-3} M or lower to prevent intermolecular association. ^{1}H NMR spectra were obtained on ~10% $CDCl_3$ [for the free bases (Me_4Si)] and D_2O [for the HCl salts ($Me_3SiCD_2CD_2COONa$)] solutions with a JEOL C-60 HL spectrometer. ^{1}H NMR spectra for the determination of the coupling constants of the benzylic protons have been also measured on a JEOL PS-100 spectrometer. Evaporations were made in vacuo (rotating evaporator). Magnesium sulfate was always used as the drying agent. Elemental analyses were performed by our analytical laboratory and agreed with theoretical values to within ±0.4%.

Amino alcohols 1, 2, and 4,[12] 2-(*p*-nitrophenyl)-4-isopropylmorpholine (7),[3] and their HCl salts were prepared as previously described.

2-(*p*-Nitrophenyl)morpholin-5-one (11). A solution of NaOH (0.80 g, 20.0 mmol) in H_2O (60 mL) was added to a solution of **1** [^{1}H NMR δ 4.78 (m, 1, CHO); 3.0 g, 16.5 mmol] in CH_2Cl_2 (80 mL). The mixture was stirred, cooled to 0 °C, and treated dropwise with $CH_2ClCOCl$ (2.62 g, 23.2 mmol). After completion of the addition, the mixture was stirred at room temperature for 4 h. The layers were separated, and the CH_2Cl_2 solution was washed with dilute HCl and H_2O, filtered, and evaporated to give a semisolid residue (3.3 g) consisting essentially of **1-(*p*-nitrophenyl)-2-[*N*-(chloroacetyl)amino]ethanol (9)** [IR 1650 cm^{-1} (C=O)], which was directly used in the following transformation.

To a solution of **9** (3.3 g, 12.8 mmol) in EtOH (80 mL) was added in portions a solution of KOH (0.87 g, 15.5 mmol) in EtOH (30 mL). The resulting mixture was stirred at room temperature for 24 h and then diluted with H_2O and extracted with CH_2Cl_2. Evaporation of the washed (H_2O) and filtered CH_2Cl_2 extracts yielded a solid residue (2.23 g) which was crystallized from MeOH to give **11** (1.43 g, 39% calculated on **1**): mp 237–238 °C; IR 1665 cm^{-1} (C=O). Anal. ($C_{10}H_{10}N_2O_4$) C, H, N.

2-(*p*-Nitrophenyl)-4-methylmorpholin-5-one (12). A solution of NaOH (0.85 g, 21.3 mmol) in H_2O (50 mL) was added to a solution of **2** [^{1}H NMR δ 4.86 (m, 1, CHO); 3.5 g, 17.8 mmol] in CH_2Cl_2 (80 mL). The resulting mixture was treated, as described above, with $CH_2ClCOCl$ (2.8 g, 24.8 mmol), yielding a crude residue (4.25 g) which was crystallized from C_6H_6 to afford **1-(*p*-nitrophenyl)-2-[*N*-(chloroacetyl)-*N*-methylamino]ethanol (10)** (3.7 g, 76%): mp 122–123 °C; IR 1630 cm^{-1} (C=O). Anal. ($C_{11}H_{13}ClN_2O_4$) C, H, Cl, N.

A solution of **10** (3.5 g, 12.8 mmol) in EtOH (50 mL) was treated, as described above for the preparation of **11**, with a solution of KOH (0.87 g, 15.5 mmol) in EtOH (12 mL). After workup a solid residue (2.9 g) was obtained. Recrystallization from MeOH at −20 °C yielded **12** (2.6 g, 86%): mp 114–115 °C; IR 1650 cm^{-1} (C=O). Anal. ($C_{11}H_{12}N_2O_4$) C, H, N.

2-(*p*-Nitrophenyl)morpholine (5). A stirred solution of $NaBH_4$ (0.72 g, 19 mmol) in anhydrous THF (20 mL) was cooled at 0 °C and treated, under external cooling, dropwise with a solution of BF_3·Et_2O (3.2 mL, 25.3 mmol) in anhydrous THF (20 mL) and then with a solution of **11** (0.7 g, 3.2 mmol) in anhydrous THF (40 mL). After completion of the addition, the reaction mixture was stirred at room temperature for 10 min, refluxed for 1.5 h, cooled, treated with H_2O (10 mL) and 10% aqueous HCl (30 mL), and stirred for 20 min. After evaporation of THF, the aqueous solution was washed with CH_2Cl_2, basified with solid KOH, and extracted with CH_2Cl_2. Evaporation of the washed (H_2O) and dried CH_2Cl_2 extracts gave a residue which was dissolved in anhydrous Et_2O. Treatment of filtered Et_2O solution with an excess of Et_2O solution of HCl gave a solid (0.22 g), which was collected by suction filtration and crystallized from Me_2CO to yield **5**·HCl (0.14 g, 18%): mp 205–206 °C. Anal. ($C_{10}H_{13}ClN_2O_3$) C, H, Cl, N.

The HCl salt of **5** was converted to the free base by treating an aqueous solution of the salt with 50% aqueous KOH and extracting the free base into CH_2Cl_2. The CH_2Cl_2 layer was filtered and evaporated to give **5** as an oily residue. Anal. ($C_{10}H_{12}N_2O_3$) C, H, N.

2-(*p*-Nitrophenyl)-4-methylmorpholine (6). Method A. Following the procedure that was used for the preparation of **5**, a solution of $NaBH_4$ (0.97 g, 25.6 mmol) in anhydrous THF (30 mL) was allowed to react with a solution of BF_3·Et_2O (4.3 mL, 34.0 mmol) in anhydrous THF (20 mL) and then with a solution of **12** (1.0 g, 4.2 mmol) in anhydrous THF (50 mL). Crystallization of the product (0.50 g) precipitated by treatment with Et_2O–HCl afforded **6**·HCl (0.40 g, 37%): mp 226–227 °C (EtOH). Anal. ($C_{11}H_{15}ClN_2O_3$) C, H, Cl, N. **6** free base is an oil. Anal. ($C_{11}H_{14}N_2O_3$) C, H, N.

Method B. A mixture of **2** (0.33 g, 1.7 mmol) and 2-chloroethanol (1.4 g, 17.4 mmol) was heated at 90 °C for 7 days and then diluted with Et_2O to precipitate an hygroscopic solid, which was filtered and rapidly crystallized from EtOH–Et_2O to give the HCl salt of **1-(*p*-nitrophenyl)-2-[*N*-(2-hydroxyethyl)-*N*-methylamino]ethanol (13·HCl)** (0.20 g, 42%) as an hygroscopic solid, which was directly used in the next step.

Concentrated H_2SO_4 (2.0 mL) was added to **13**·HCl (0.20 g, 0.7 mmol) and the solution was left at room temperature for 24 h, poured into ice, made alkaline with 50% aqueous KOH, and extracted with $CHCl_3$. Evaporation of the dried $CHCl_3$ extracts yielded practically pure **6** (0.060 g, 38%).

2-(*p*-Nitrophenyl)-4-*tert*-butylmorpholine (8). A mixture of **4** [^{1}H NMR δ 4.67 (m, 1, CHO); 1.0 g, 4.2 mmol) and 2-chloroethanol (6.0 g, 75 mmol) was heated at 90 °C for 5 days and then diluted with Et_2O to precipitate a solid, which was crystallized from EtOH–Et_2O to yield the HCl salt of **1-(*p*-nitrophenyl)-2-[*N*-(2-hydroxyethyl)-*N*-*tert*-butylamino]ethanol (14·HCl)** (0.95 g, 71%). To **14**·HCl (0.60 g, 1.9 mmol) was added concentrated H_2SO_4 (6.0 mL). The resulting mixture was let stand at room temperature for 19 h and then treated as described above for the preparation of **6** (method B) to give **8** (0.25 g, 50%), which was crystallized from MeOH: mp 117–119 °C. Anal. ($C_{14}H_{20}N_2O_3$) C, H, N. The HCl salt of **8** had mp 237–239 °C (EtOH–Et_2O). Anal. ($C_{14}H_{21}ClN_2O_3$) C, H, Cl, N.

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Synthesis and Biological Activity of 8-Oxadihydropteridines

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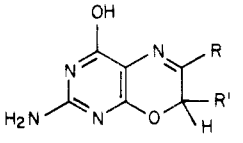
A series of 6-substituted and 6,7-disubstituted pyrimido[4,5-*b*][1,4]oxazines (8-oxadihydropteridines) was synthesized through the condensation of an α-halo ketone and 2,5-diamino-4,6-pyrimidinediol. The resulting 8-oxadihydropteridines were assayed as potential antifolates in a dihydrofolate reductase enzyme system. The 2-amino-4-hydroxyoxadihydropteridines were found to possess greater biological activity than the corresponding 2,4-diamino compounds. The pteroic acid homeostere 2-amino-4-hydroxy-6-phenethyl-8-oxadihydropteridine was the most potent of the compounds tested.

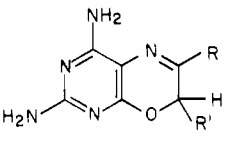
The use of folic acid analogues in the chemotherapy of cancer is well established,[1,2] and alterations in the vitamin structure have produced both classical and nonclassical antimetabolites.[3,4]

Homeosteric[5] substitutions in the pteridine ring system have been primarily limited to carbon–nitrogen interchanges,[6] and their activities have been studied as inhibitors of dihydrofolate reductase,[7] thymidylate synthetase,[8] or growth of microbial systems.[9] A recent report has described the preparation of some pyrimidothiazines which strongly inhibit dihydrofolate reductase.[10] The replacement of nitrogen by oxygen, however, has received little study in this system.[11]

The reactions of 2,5-diamino-4,6-pyrimidinediol hydrochloride with the appropriately substituted α-chloro ketones were effected in refluxing aqueous ethanol with sodium bicarbonate added to maintain a basic medium. The reaction products were the corresponding 6-substituted or 6,7-disubstituted 8-oxadihydropteridines **1** (Table I). Several previously unreported derivatives were synthesized and characterized by elemental analysis and by UV and NMR spectra (Table II). Since the pyrimidine used has a plane of symmetry through the 2 and 5 positions, the structures of the products (**1**) were unequivocal. The analogous 4-amino derivatives were prepared by the method described by Mirza et al. using 2,5,6-triamino-6-pyrimidol.[12]



1, R = alkyl, aryl, or hydrogen
R' = hydrogen, alkyl, or aryl



2, R = CH_3 or C_6H_5
R' = H, CH_3, or C_6H_5

None of the compounds prepared in this study were toxic to the growth of *Streptococcus faecalis* (ATCC 8043) at the limit of their solubilities (ca. 10 μg/mL), whereas amethopterin completely inhibited bacterial growth at a concentration of 1 μg/mL. It is possible that the bicyclic

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