M Tris-HCl, pH 7.5, and the thymidine kinase isozymes were separated essentially as described by Lee and Cheng,[17] except that the cytoplasmic thymidine kinase (which was used in this study) was eluted in a buffer containing 10% glycerol, 2 mM dithiothreitol, 0.3 M Tris-HCl, pH 7.5, and 200 μM thymidine. The deoxycytidine kinase and cytoplasmic thymidine kinase were separately passed through a G-25 Sephadex column equilibrated with buffer (25 mM Hepes, pH 7.5, 10% glycerol, and 2 mM dithiothreitol) at 4 °C. These enzyme preparations were stored at −70 °C.

The assay procedures used were similar to those described for thymidine kinase[17] and deoxycytidine kinase.[18] Both assay mixtures contained 40 mM Hepes, pH 7.5 (37 °C), 5.6 mM phosphocreatine, 0.5 unit of phosphocreatine kinase, 75 μg of bovine serum albumin, 2 mM dithiothreitol, 2 mM ATP (all of which were obtained from Sigma Chemical Co.), and 2 mM $MgCl_2$. In addition, the deoxycytidine kinase and thymidine kinase mixtures respectively contained 0.1 mM [2-^{14}C]CdR (9.4 mCi/mmol) and 0.1 mM [2-^{14}C]TdR (14 mCi/mmol) with the appropriate enzyme preparation. The labeled nucleosides were obtained from Moravek Biochemicals, Inc.

(17) Lee, L.-S.; Cheng, Y.-C. *J. Biol. Chem.* **1976**, *251*, 2600.
(18) Cheng, Y.-C.; Domin, B.; Lee, L.-S. *Biochim. Biophys. Acta* **1977**, *481*, 481.

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Registry No. 1, 84472-85-5; **2**, 87190-74-7; **3**, 85236-95-9; **4a**, 84472-87-7; **4b**, 66323-42-0; 4c, 87190-75-8; **5a**, 87190-76-9; **5b**, 87190-77-0; **5c**, 87190-78-1; **6a**, 84472-89-9; **6b**, 87190-79-2; **6c**, 87190-80-5; **7a**, 84472-90-2; **7b**, 87190-81-6; **7c**, 87190-82-7; **8**, 85236-92-6; **9**, 87190-83-8; **10**, 87190-89-4; 11, 87190-84-9; 12, 87190-85-0; **13**, 84472-86-6; **14**, 85236-89-1; **15**, 30516-87-1; **16**, 87190-86-1; **17**, 87190-87-2; **18**, 87190-88-3; thymidine kinase, 9002-06-6; deoxycytidine kinase, 9039-45-6; 1,2,4-triazole, 288-88-0.

Pyridinylpiperazines, a New Class of Selective α_2-Adrenoceptor Antagonists

Walfred S. Saari,*[†] Wasyl Halczenko,[†] Stella W. King,[†] Joel R. Huff,[†] James P. Guare, Jr.,[†] Cecilia A. Hunt,[†] William C. Randall,*[†] Paul S. Anderson,[†] Victor J. Lotti,*[‡] David A. Taylor,*[‡] and Bradley V. Clineschmidt[‡]

Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486, and Merck Institute for Therapeutic Research, West Point, Pennsylvania 19486. *Received April 11, 1983*

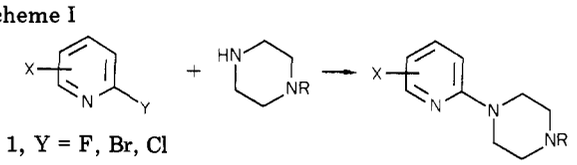
A series of 1-(2-pyridinyl)piperazine derivatives was synthesized and evaluated for adrenergic activity. In vitro activity was assessed through the antagonism of clonidine's effect in the rat, isolated, field-stimulated vas deferens and by the displacement of [^{3}H]clonidine from membrane binding sites of calf cerebral cortex. Antagonism of clonidine-induced mydriasis in the rat was used as an in vivo assay. Several members of the series proved to be potent, selective α_2-adrenoceptor antagonists. 1-(3-Fluoro-2-pyridinyl)piperazine was more potent than either yohimbine or rauwolscine in displacement of [^{3}H]clonidine and had a higher affinity for this binding site (α_2) than for the [^{3}H]prazosin site (α_1). In vivo, the 3-F derivative was more potent than the reference standards in reversing clonidine-induced mydriasis. None of the members of this series was more selective or potent than rauwolscine in antagonizing clonidine in the rat vas deferens.

In addition to the α_1-adrenoceptors of effector cells that mediate postjunctional responses to the neurotransmitter norepinephrine, other adrenoceptors are now known to be present at both pre- and postjunctional sites. These latter receptors, α_2-adrenoceptors, can be characterized and distinguished from α_1-adrenoceptors by their relative activities toward agonists and antagonists. The recently recognized potential of selective agonists and antagonists of α_2-adrenoceptors as therapeutic agents has stimulated the search for novel agents that will interact with these receptors.

Previous work[1] from these laboratories reported on the affinities of some piperazinylimidazo[1,2-*a*]pyrazines for α_1- and α_2-adrenoceptors. In this paper we describe the syntheses of some pyridinylpiperazine derivatives and their evaluation as selective α_2-adrenoceptor antagonists.

Chemistry. Most of the pyridinylpiperazines of Table I were synthesized by reaction of the appropriate 2-halopyridine **1** with either *N*-methylpiperazine or piperazine (Scheme I). In the latter case, the use of excess piperazine was preferred in order to circumvent formation of bis(pyridinyl)piperazines. The *N*-benzyl analogue **2h** was prepared by alkylation of **2c** with benzyl bromide, and the 3-amino derivative **2m** was obtained through catalytic reduction of the corresponding nitro compound **21**. All of the intermediate 2-halopyridines have either been reported previously in the literature or were prepared by established procedures.

Scheme I



1, Y = F, Br, Cl

2

Results and Discussion

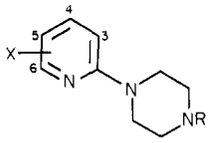
Relative affinities of the pyridinylpiperazine derivatives of Table I for central α-adrenergic binding sites were determined by measurement of radioligand displacement from membrane binding sites of calf cerebral cortex. Displacement of [^{3}H]clonidine was used as a measure of interaction with α_2-adrenoceptor binding sites, while [^{3}H]prazosin displacement served as an assay for α_1-adrenoceptor affinity.

[†] Merck Sharp & Dohme Research Laboratories.
[‡] Merck Institute for Therapeutic Research.

(1) Lumma, W. C.; Randall, W. C.; Cresson, E. L.; Huff, J. R.; Hartman, R. D.; Lyon, T. F. *J. Med. Chem.* **1983**, *26*, 357.

0022-2623/83/1826-1696$01.50/0 © 1983 American Chemical Society

Table I. Physical Properties of 1-(2-Pyridinyl)piperazines



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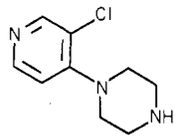


3

compd	X	R	formula	mp, °C	method[a]	yield, %	recrystn solvent
2b	3-F	H	$C_9H_{12}FN_3 \cdot 2HCl$	210-211	A	50	MeOH-Et_2O
			$C_9H_{12}FN_3 \cdot C_4H_4O_4$ [b]	165-166	A	58	MeOH-Et_2O
2c	3-Cl	H	$C_9H_{12}ClN_3 \cdot HCl$	142-144	A	71	MeOH-Et_2O
2d	3-Br	H	$C_9H_{12}BrN_3 \cdot HCl \cdot {}^1/_2H_2O$	180 dec	A[c]	79	MeOH-Et_2O
2e	3-I	H	$C_9H_{12}IN_3 \cdot 2HCl$	185-189 dec	B	69	MeOH-EtOH-EtOAc
2f	3-F	CH_3	$C_{10}H_{14}FN_3 \cdot C_4H_4O_4$ [d]	148-149	A[e]	27	MeOH-Et_2O
2g	3-Cl	CH_3	$C_{10}H_{14}ClN_3 \cdot HCl$	203-205	A[e,f]	22	EtOH-Et_2O
2h	3-Cl	$CH_2C_6H_5$	$C_{16}H_{18}ClN_3 \cdot HCl$	191-193	C	34	*i*-PrOH-Et_2O
2i	3-Br	CH_3	$C_{10}H_{14}BrN_3 \cdot HCl$	210-212 dec[g]	A[c,h]	49	MeOH-Et_2O
2j	3,5-Cl_2	H	$C_9H_{11}Cl_2N_3 \cdot HCl$	225-227	B[i]	63	EtOH
2k	3-Cl, 4-Me	H	$C_{10}H_{14}ClN_3 \cdot 2HCl$	205 dec	B[j]	83	EtOH-Et_2O
2l	3-NO_2	H	$C_9H_{12}N_4O_2$	83.5-86.5	D	38	$CHCl_3$-hexane
2m	3-NH_2	H	$C_9H_{14}N_4 \cdot 2HCl$	220-235 dec	E	56	EtOH-EtOAc
2n	3-CF_3	H	$C_{10}H_{12}F_3N_3 \cdot HCl$	174-178	B[k]	50	EtOH-Et_2O
2o	3-CN	H	$C_{10}H_{12}N_4 \cdot 2HCl$	210-218 dec	B[l]	52	MeOH-EtOH-EtOAc
2p	3-$CONH_2$	H	$C_{10}H_{14}N_4O \cdot 2HCl$	245-248 dec	B[m]	25	EtOH-Et_2O
2q	5-Cl	H	$C_9H_{12}ClN_3 \cdot HCl$	237-247 dec	F	17	EtOH-Et_2O
3			$C_9H_{12}ClN_3 \cdot 2HCl$	268-272 dec	B[n]	74	MeOH-EtOH-EtOAc

[a] The intermediate halopyridines were obtained commercially unless otherwise noted. [b] Hydrogen maleate. [c] The 3-bromo-2-chloropyridine intermediate was prepared by diazotization of 3-amino-2-chloropyridine.[16] [d] Hydrogen fumarate. [e] Five equivalents of *N*-methylpiperazine was used in place of piperazine. [f] Free base was purified by flash chromatography over silica gel and elution with 10% MeOH-90% $CHCl_3$. [g] Literature[16] mp 212-213 °C for 2HCl salt. [h] One equivalent of *N*-methylpiperazine was used in place of piperazine. [i] Five equivalents of piperazine at reflux for 48 h. The intermediate 2-bromo-3,5-dichloropyridine was prepared by diazotization of 2-amino-3,5-dichloropyridine.[18] [j] Heated at reflux for 4 days. The intermediate 2,3-dichloro-4-methylpyridine was prepared by methylation[17] of 2,3-dichloropyridine. [k] The intermediate 2-fluoro-3-(trifluoromethyl)pyridine was prepared by reaction of 2-chloronicotinic acid with SF_4.[19] [l] The intermediate 2-chloronicotinonitrile was obtained from reaction of nicotinamide *N*-oxide with PCl_5 and $POCl_3$.[20] [m] Heated at reflux for 48 h. [n] From reaction of piperazine with 3,4-dichloropyridine.[21]

The unsubstituted parent **2a** was found to be slightly more potent than the reference α_2-antagonist yohimbine in displacing [^{3}H]clonidine and approximately 14 times more selective than yohimbine for this α_2-binding site compared to the α_1 [^{3}H]prazosin site (Table II). Introduction of halogen, **2b–d**, into the 3-pyridinyl position of **2a** led to a pronounced enhancement of α_2 binding. However, since α_1 binding was not affected porportionately, α_2/α_1 selectivity also improved significantly for these compounds. Table II shows that these halogen derivatives, **2b–d**, have a greater affinity and selectivity for [^{3}H]clonidine binding sites than either yohimbine or rauwolscine. The corresponding 3-NO_2 (**21**) and 3-CN (**20**) congeners also exhibited improved α_2 binding and selectivity compared to **2a**, while substitution of NH_2 (**2m**), CF_3 (**2n**), and $CONH_2$ (**2p**) functions into the 3-position produced an opposite effect.

The 6-Cl derivative **2r** was found to have lower affinity and lower selectivity for the α_2-binding site than the 3-Cl analogue **2c**, while the 5-Cl compound **2q** was nonselective. This 5-Cl effect is also evident in the 3,5-dichloro derivative **2j**, which also showed little preference for either of the α-adrenergic binding sites. Introduction of a 4-Me group (**2k**) into **2c** significantly reduced α_2 affinity and selectivity.

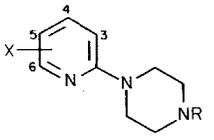
Although methylation of the piperazine nitrogen led to increased affinity for the α_2-binding site (compare **2f,g,i** with **2b–d**), α_1 binding also improved appreciably. The corresponding *N*-benzyl homologue **2h** was found to be even less selective for the α_2 site. Therefore although the 3-Cl, *N*-Me analogue **2g** shows greater affinity for α_2-adrenoceptors, the 3-F, NH derivative **2b** is the most selective.

Affinity for α_2-binding sites was reduced with the 4-pyridinyl isomer **3**. While the corresponding phenyl analogue of **2c** (**4**) retained some α_2 affinity, α_2 selectivity was much reduced.

Antagonistic activities of these compounds upon pre-(α_2) and postsynaptic (α_1) adrenoceptors were determined in the rat, isolated, field-stimulated vas deferens. In this tissue, presynaptic adrenergic agonists, such as clonidine, characteristically inhibit stimulation-induced contractions, whereas postsynaptic agonists, such as methoxamine, enhance contractions. These pre- and postsynaptic adrenergic agonist effects are preferentially blocked by known selective inhibitors of α_2- and α_1-receptors.[2-4] Of the reference agents reported in Table II, rauwolscine proved to be the most potent and selective α_2-antagonist in the rat vas deferens, while mianserin was found to be less potent and nonselective.

(2) Drew, G. M. *Eur. J. Pharmacol.* **1977**, *42*, 123.
(3) Doxey, J. C.; Smith, C. F. C.; Walker, J. M. *Br. J. Pharmacol. Chemother.* **1977**, *60*, 91.
(4) Eltze, M. *Eur. J. Pharmacol.* **1979** *59*, 1.

Table II. Radioligand Binding, Rat Vas Deferens and Rat Mydriasis Results



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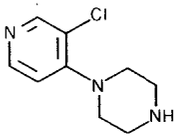

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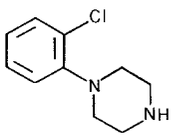

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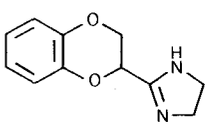

RX781094

			calf cerebral cortex radioligand binding, K_I, nM[a]			rat vas deferens pA_2[c] for antagonism of			
compd	X	R	[^{3}H]clonidine	[^{3}H]prazosin	selectivity ratio,[b] α_2/α_1	clonidine	methoxamine	selectivity ratio,[d] α_2/α_1	reversal of clonidine-induced mydriasis, rat AD_{50},[e] mg/kg, iv
2a[f]	H	H	37 ± 3	2 400 ± 600	65	6.4 ± 0.1	5.7 ± 0.1	5	1.28 (0.96-1.71)
2b	3-F	H	8.2 ± 0.7	2 500 ± 140	305	6.90 ± 0.12[g]	5.18 ± 0.12[g]	53	0.23 (0.22-0.25)
2c	3-Cl	H	7.9 ± 0.5	1 800 ± 90	228	6.90 ± 0.04	5.7 ± 0.1	16	0.44 (0.42-0.46)
2d	3-Br	H	11 ± 1	1 480 ± 90	135	6.6 ± 0.1	5.8 ± 0.1	6	0.68 (0.63-0.74)
2e	3-I	H	42 ± 2	1 600 ± 200	38	6.2 ± 0.1	6.0 ± 0.1	2	1.95 (1.59-2.37)
2f	3-F	CH_3	5 ± 0.3	490 ± 50	98	7.4 ± 0.1	6.20 ± 0.04	16[h]	0.67 (0.64-0.71)
2g	3-Cl	CH_3	2.7 ± 0.1	215 ± 21	80	7.4 ± 0.1	6.50 ± 0.03	8	0.56 (0.53-0.60)
2h	3-Cl	$CH_2C_6H_5$	120 ± 10	700 ± 80	5.8	6.5 ± 0.1	7.0 ± 0.1	0.3	>3.0
2i	3-Br	CH_3	2.9 ± 0.2	160 ± 10	55	7.6 ± 0.1	6.50 ± 0.03	13	0.74 (0.59-0.92)
2j	3,5-Cl_2	H	500 ± 40	800 ± 70	1.6	<5.8			>3.0
2k	3-Cl, 4-Me	H	74 ± 7	4 300 ± 300	58	5.90 ± 0.01			13.96 (10.03-19.44)
2l	3-NO_2	H	26 ± 3	3 800 ± 200	146	6.3 ± 0.1			1.53 (1.34-1.74)
2m	3-NH_2	H	440 ± 40	31 000 ± 2500	70	<5.8			>3.0
2n	3-CF_3	H	97 ± 12	2 850 ± 190	29	<5.8			3.63 (3.1-4.3)
2o	3-CN	H	14 ± 3	2 000 ± 180	143	6.1 ± 0.1			1.07 (0.99-1.17)
2p	3-$CONH_2$	H	5200 ± 600	62 000 ± 19 000	12	<5.8			>3.0
2q	5-Cl	H	1340 ± 70	900 ± 70	0.67	<5.8			>3.0
2r[i]	6-Cl	H	18 ± 1	375 ± 20	21	6.80 ± 0.03	6.00 ± 0.07	6	0.3-1.0[j]
3			390 ± 40	6 800 ± 450	17	<5.8			>3.0
4[f]			25 ± 7	160 ± 35	6.4	6.0 ± 0.3	6.5 ± 0.2	0.3[k]	1.03 (0.97-1.10)
yohimbine			49 ± 1	220 ± 10	4.5	7.65 ± 0.13[g]	6.52 ± 0.29[g]	14	1.04 (0.94-1.15)
rauwolscine			18 ± 0.7	940 ± 40	52	7.90 ± 0.21[g]	6.00 ± 0.17[g]	79	1.1 (0.97-1.24)
mianserin			17 ± 3.0	43 ± 7	2.5	7.27 ± 0.31[g]	7.24 ± 0.31[g]	1.1	>10.0[l]
RX 781094[m]			1.5 ± 0.2	500 ± 100	333	7.73 ± 0.09[g]	6.10 ± 0.14[g]	43	0.05 (0.043-0.054)

[a] Reported values are the mean of at least two independent determinations plus or minus the range. [b] Ratio of K_I(prazosin)/K_I(clonidine). [c] Reported values are the mean of at least three tissues per determination plus or minus the standard deviation. [d] Ratio of −log methoxamine pA_2/−log clonidine pA_2. [e] AD_{50} values are derived from at least three determinations. Numbers in parentheses are 95% confidence limits. [f] Purified commercial sample. [g] From Schild plot evaluation. [h] This compound also produced a 29% reduction of contractions at 1.5×10^{-6} M. [i] Prepared by the procedure of W. Lumma and W. Saari, U.S. Patent 4 078 063 (1978). [j] This compound reversed clonidine-induced mydriasis with an AD_{50} value of between 0.3 and 1.0 mg/kg iv. An accurate value could not be determined because of toxicity at these doses. [k] This compound also produced a 50% reduction of contractions at 7.5×10^{-7} M. [l] At higher doses, mianserin produces pupillary dilatation by itself. [m] Reference 8.

Table III. Correlations of Receptor Binding, Vas Deferens and Mydriasis Results of Table II

	pK_I, [^{3}H]prazosin binding	vas deferens pA_2		mydriasis pAD_{50} [c]
		clonidine	methoxamine	
pK_I, [^{3}H]clonidine binding	0.593,[a] $n = 24$	0.583,[b] $n = 18$	−0.208, $n = 15$	0.795,[a] $n = 16$
pK_I, [^{3}H]prazosin binding		0.519,[b] $n = 18$	0.827,[a] $n = 15$	0.435, $n = 16$
vas deferens pA_2, clonidine			0.147, $n = 15$	0.646,[b] $n = 15$
vas deferens pA_2, methoxamine				−0.189, $n = 12$

[a] $P < 0.01$. [b] $P < 0.05$. [c] Moles per kilogram.

As was observed in the radioligand binding procedure, N-methylation increased potency of the pyridinylpiperazines as α_2-adrenoceptor antagonists. The most potent member of this series in the vas deferens proved to be the 3-Br, *N*-Me derivative **2i**, which was comparable to yohimbine in terms of activity and selectivity. In accord with the binding data, the 3-F, NH compound **2b** was found to be the most selective α_2-adrenoceptor antagonist of this series. Although **2b** was a less potent α_2-adrenoceptor antagonist than the reference agents in the vas deferens, only rauwolscine was more selective.

Clonidine produces mydriasis in anesthetized cats and rats by activation of postsynaptic α_2-adrenoceptors located in the central nervous system.[5-7] Therefore, reversal of an established clonidine-induced mydriasis reflects the ability of a compound to penetrate the blood–brain barrier and act as a central α_2-adrenoceptor antagonist.

The mydriasis results reported in Table II show clearly that pyridinylpiperazines can function as centrally active α_2-adrenoceptor antagonists. The more potent members of this series are again those containing halogen in the 3-position. Most of the 3-halo derivatives, with the exception of the 3-I derivative (**2e**), the *N*-benzyl derivative (**2h**), and the disubstituted compounds **2j,k**, are more effective than rauwolscine in reversing clonidine-induced mydriasis in the rat. However, none of the compounds in this series were more potent than RX 781094.[8] The relatively poor activity of the adrenergic antagonist mianserin in the mydriasis assay compared to its good activity in the vas deferens and binding protocols is surprising, since mianserin has been shown to readily penetrate the central nervous system blood–brain barrier.[9]

The receptor-binding inhibition constants, rat vas deferens pA_2 responses, and mydriasis results of Table II were examined further for possible relevant relationships. Values designated as greater or less than were not included in the calculations. Inspection of the derived correlations for the entire set of compounds used, Table III, reveals that the in vivo [^{3}H]clonidine radioligand inhibition data correlate best with the in vivo mydriasis results ($r = 0.795$, $p < 0.01$), while inhibition of [^{3}H]prazosin binding correlates best with the vas deferens methoxamine pA_2 values ($r = 0.827$, $p < 0.01$). Displacement of [^{3}H]clonidine from membrane-binding sites of calf cerebral cortex therefore appears to be a useful predictor of in vivo antagonism of clonidine-induced mydriasis. A relatively poor correlation ($r = 0.583$) was obtained for displacement of [^{3}H]clonidine from calf cerebral cortex membrane binding sites and the rat vas deferens pA_2 values, both in vitro procedures. However, it should be noted that a significant correlation ($r = 0.99$, $p < 0.05$) has been reported[10] for activity in the isolated field-stimulated guinea pig ileum and displacement of [^{3}H]clonidine in homogenates of calf frontal cortex in the 2-aminotetralin and benzo[*f*]quinoline series of α_2-adrenoceptor agonists.

In summary, several members of this pyridinylpiperazine series have been found to be more potent and more selective α_2-adrenoceptor antagonists than either yohimbine or rauwolscine using in vitro and in vivo measures of α_2-adrenoceptor activity. The 3-fluoro derivative, **2b**, has been selected for in-depth pharmacological studies as a selective α_2-adrenoceptor antagonist.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus using open capillaries and are uncorrected. ^{1}H NMR spectra were recorded for all intermediate and final products and are consistent with the assigned structures. Microanalytical results on new compounds are indicated by atomic symbols and are within ±0.4% of theoretical values unless otherwise noted.

Pyridinylpiperazines. Method A. 1-(3-Fluoro-2-pyridinyl)piperazine Dihydrochloride (2b). A solution of 2-chloro-3-fluoropyridine[11] (18 g, 153 mmol) and piperazine (138 g, 1.6 mol) in *n*-BuOH (1 L) was stirred at reflux under N_2 for 3 days. After the solution was concentrated under reduced pressure, the residue was slurried with EtOAc (200 mL) and washed with H_2O. The EtOAc extract was dried (Na_2SO_4) and filtered, and the filtrate was concentrated under reduced pressure. The residual oil was dissolved in $CHCl_3$ (200 mL), saturated with NH_3, and passed through a pad of silica gel, which was then washed with 5% MeOH–95% $CHCl_3$ saturated with NH_3 (2 L). Removal of solvent under reduced pressure gave a light yellow oil, which was further purified by conversion to the salts listed in Table I.

Method B. 1-(3-Iodo-2-pyridinyl)piperazine Dihydrochloride (2e). A solution of 2-chloro-3-iodopyridine[12] (2.39, 10 mmol) and piperazine (8.61 g, 100 mmol) in *n*-BuOH (100 mL) was stirred at reflux under N_2 for 18 h. After most of the *n*-BuOH was removed under reduced pressure, the residue was partitioned between toluene and 10% NaOH. The toluene layer was washed further with H_2O, dried (Na_2SO_4), and filtered, and the filtrate was concentrated to an oil. The pyridinylpiperazine was purified by recrystallization of the 2HCl salt.

Method C. 4-Benzyl-1-(3-chloro-2-pyridinyl)piperazine Hydrochloride (2h). A solution of 1-(3-chloro-2-pyridinyl)-piperazine (600 mg, 2.4 mmol), benzyl bromide (408 mg, 2.4 mmol), and Et_3N (486 mg, 4.8 mmol) in MeCN (30 mL) was heated at reflux for 6 h. After the solution was concentrated under reduced pressure, the residue was partitioned between EtOAc and saturated Na_2CO_3 solution. The organic phase was separated, dried (Na_2SO_4), and filtered, and the filtrate was concentrated under reduced pressure. The residual oil was dissolved in *i*-PrOH and acidified with anhydrous EtOH–HCl, and the HCl salt was precipitated by the addition of Et_2O.

Method D. 1-(3-Nitro-2-pyridinyl)piperazine (2l). A solution of 2-chloro-3-nitropyridine (4.76 g, 30 mmol) and piperazine (5.9 g, 69 mmol) in MeCN (75 mL) was stirred at reflux for 5 h.

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After the solution was concentrated under reduced pressure, the residue was partitioned between EtOAc and 10% NaOH. The EtOAc extract was washed with H_2O, dried (Na_2SO_4), and filtered, and the filtrate was concentrated. Flash chromatography over Al_2O_3 and elution with 2% MeOH–98% $CHCl_3$ afforded **2k**, mp 82–87 °C. An analytical sample, mp 83.5–86.5 °C, was obtained upon recrystallization from $CHCl_3$–hexane.

Method E. 1-(3-Amino-2-pyridinyl)piperazine Dihydrochloride (2m). Catalytic hydrogenation of 1-(3-nitro-2-pyridinyl)piperazine (1.1 g, 5.3 mmol) in EtOH (50 mL) over a 5% Pt/C catalyst (0.5 g) at atmospheric pressure and room temperature resulted in the theoretical uptake of H_2 in 6 h. After the solution was filtered through a pad of diatomaceous earth, the filtrate was treated with excess anhydrous EtOH–HCl, and the dihydrochloride salt was precipitated with EtOAc. Anal. ($C_9H_{14}N_4 \cdot 2HCl$) H, N; C: calcd, 43.04; found, 43.54.

Method F. 1-(5-Chloro-2-pyridinyl)piperazine Hydrochloride (2q). 5-Chloro-2-pyridinol (11.6 g, 90 mmol) was added in portions to a well-stirred suspension of 50% NaH–mineral oil (4.32 g, 90 mmol) in dry dioxane (75 mL). After formation of the sodium salt was complete, the mixture was cooled in an ice bath while a solution of trifluoromethanesulfonyl chloride (15.2 g, 90 mmol) in dry THF (25 mL) was added over 10 min. The reaction mixture was stirred at room temperature overnight and then filtered. After the filtrate was concentrated under reduced pressure, the residue was distilled to give 5-chloro-2-[[(trifluoromethyl)sulfonyl]oxy]pyridine (16.2 g, 69%), bp 66–67 °C (1.5 mm).

A solution of 5-chloro-2-[[(trifluoromethyl)sulfonyl]oxy]pyridine (5.23 g, 20 mmol), piperazine (1.72 g, 20 mmol), and Et_3N (2.0 g, 20 mmol) in MeCN (50 mL) was stirred at reflux for 4 days. After the solution was concentrated under reduced pressure, the residue was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 extract was washed with brine, dried (Na_2SO_4), and filtered, and the filtrate was concentrated. Flash chromatography of the residue over silica gel and elution with $CHCl_3$ saturated with NH_3 afforded the product base as a light yellow oil. The base was converted to the HCl salt with anhydrous EtOH–HCl in EtOH–Et_2O for further purification.

Radioligand Binding. Assays for the competitive binding of selected compounds to central α-adrenergic binding sites employed radiolabelled clonidine or radiolabeled prazosin, which were obtained from New England Nuclear and Amersham, respectively. [^{3}H]Clonidine (specific activity 22.2–23.8 Ci/mmol) was stored in EtOH–H_2O (7:2) at 0 °C, and [^{3}H]prazosin (specific activity 33 Ci/mmol) was stored in a solution of 1% Et_2NH in EtOH at 0 °C. The radiochemical purity of these ligands was periodically checked by TLC.

Binding assays were conducted with frozen sections of calf cerebral cortex (−70 °C). A Brinkmann Polytron PT-10, at setting 6 for 10 s, was used to homogenize the frozen tissue in 20 vol (w/v) of ice-cold 50 nM pH 7.7 Tris-HCl buffer. The resultant homogenate was centrifuged twice at 48000*g* (Sorvall SS-34 rotor, 2000 rpm, RC-5 centrifuge) for 10 min at 40 °C, with rehomogenization of the intermediate pellet in 20 vol of fresh buffer. This final pellet was resuspended in 50 vol of ice-cold buffer.

Standard displacement assays were run with either 0.20 nM [^{3}H]clonidine or 0.14 nM [^{3}H]prazosin. Triplicate assay tubes contained ^{3}H-labeled ligand, 100 μL of various concentrations of the compound being investigated, 1 mL of tissue homogenate, and 50 mM pH 7.7 Tris-HCl buffer to a final volume of 2 mL. The reaction was initiated by the addition of tissue, and incubation continued for 30 min at 25 °C, at which time it was terminated by rapid filtration through Whatman GF/B glass-fiber filters under vacuum. Each filter was immediately rinsed with 3 × 5 mL aliquots of ice-cold buffer. The filters were removed into 10 mL of PCS (Amersham) and counted on either a Packard Model 2425 or Packard Model 460C scintillation spectrophotometer at approximately 35% efficiency.

Specific binding was defined as the difference between samples with and without 1 μM clonidine or 1 μM prazosin for [^{3}H]clonidine and [^{3}H]prazosin assays, respectively.

Data from binding assays were plotted as log concentration vs. percent inhibition and analyzed by nonlinear least-squares techniques in which 100% maximal inhibition was assumed at high test compound concentrations. The IC_{50} values obtained from such data treatment were used to calculate apparent inhibition constants from eq 1, where [C] is the concentration of

$$K_i = \frac{IC_{50}}{1 + ([C]/K_D)} \quad (1)$$

radioligand employed in the binding assay and K_D is its receptor dissociation constant (K_D = 0.48 nM for [^{3}H]clonidine and 0.14 nM for [^{3}H]prazosin).

Rat Vas Deferens. Rat vas deferens were extirpated from Sprague–Dawley rats (250–350 g) and prepared for field stimulation as described elsewhere.[13] pA_2 values were estimated on the basis of one or two concentrations of antagonists and a minimum of three tissues for each concentration[14] or by Schild plot analysis[15] using a minimum of three concentrations of antagonists and at least three tissues for each concentration. Clonidine and methoxamine were used as α_2- and α_1-adrenergic agonists, respectively, according to the protocols described elsewhere.[13]

Rat Mydriasis. Adult male Sprague–Dawley rats (250–350 g) were anesthetized with chloral hydrate (250 mg/kg, ip), and a femoral vein was cannulated for drug administration. Body temperature was monitored and maintained at 37 °C via a heating pad. Pupil diameter was measured under conditions of constant illumination by using a dissecting microscope fitted with an ocular micrometer having a resolution of 0.1 mm. Pupillary dilation was produced by a single iv injection of clonidine (100 μg/kg). This dose of clonidine produces a maximal mydriatic response that persists for at least 1 h. After a 5-min period of response stabilization, test compounds were administered in cumulatively increasing concentrations (0.1–3.0 mg/kg, iv), and pupil diameter was measured. The intradose time interval was 5 min with pupil diameter being recorded immediately prior to administration of the next higher concentration.

The dose of test compound required to reduce the pupil diameter to one-half that achieved in the presence of clonidine was calculated by linear regression analysis. Geometric mean AD_{50} values were then determined for each compound for purposes of comparison. In all cases, geometric mean AD_{50} values are derived from no less than three animals per compound. In those instances where a test compound failed to alter the mydriatic response to clonidine, an AD_{50} value of >3.0 mg/kg was assumed.

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Registry No. **2a**, 34803-66-2; **2b**, 85386-83-0; **2b** (free base), 85386-84-1; **2b** hydrogen maleate, 85386-89-6; **2c**, 85386-86-3; **2c** (free base), 87394-55-6; **2d**, 85386-85-2; **2d** (free base), 87394-56-7; **2e**, 87394-42-1; **2e** (free base), 85386-98-7; **2f**, 85386-91-0; **2g**, 87394-43-2; **2g** (free base), 87394-57-8; **2h**, 87394-44-3; **2h** (free base), 87394-58-9; **2i**, 87394-45-4; **2i** (free base), 87394-59-0; **2j**, 87394-46-5; **2j** (free base), 87394-60-3; **2k**, 87394-47-6; **2k** (free base), 87394-61-4; **2l**, 87394-48-7; **2m**, 87394-49-8; **2m** (free base), 87394-62-5; **2n**, 87394-50-1; **2n** (free base), 87394-63-6; **2o**,

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87394-51-2; **2o** (free base), 84951-44-0; **2p**, 87394-52-3; **2p** (free base), 87394-64-7; **2q**, 87394-53-4; **2q** (free base), 87394-65-8; **2r**, 87394-54-5; **3**, 87394-41-0; **3** (free base), 87394-66-9; **4**, 39512-50-0; 2-chloro-3-fluoropyridine, 17282-04-1; piperazine, 110-85-0; 2-chloro-3-iodopyridine, 78607-36-0; benzyl bromide, 100-39-0; 2-chloro-3-nitropyridine, 5470-18-8; 5-chloro-2-pyridinol, 4214-79-3; trifluoromethanesulfonyl chloride, 421-83-0; 5-chloro-2-[[(trifluoromethyl)sulfonyl]oxy]pyridine, 87412-10-0.

Estrogen Receptor Binding and Estrogenic/Antiestrogenic Effects of Two New Metabolites of Nitromiphene, 2-[*p*-[2-Nitro-1-(4-methoxyphenyl)-2-phenylvinyl]phenoxy]-*N*-ethylpyrrolidine

Peter C. Ruenitz,* Jerome R. Bagley, and Corwin M. Mokler

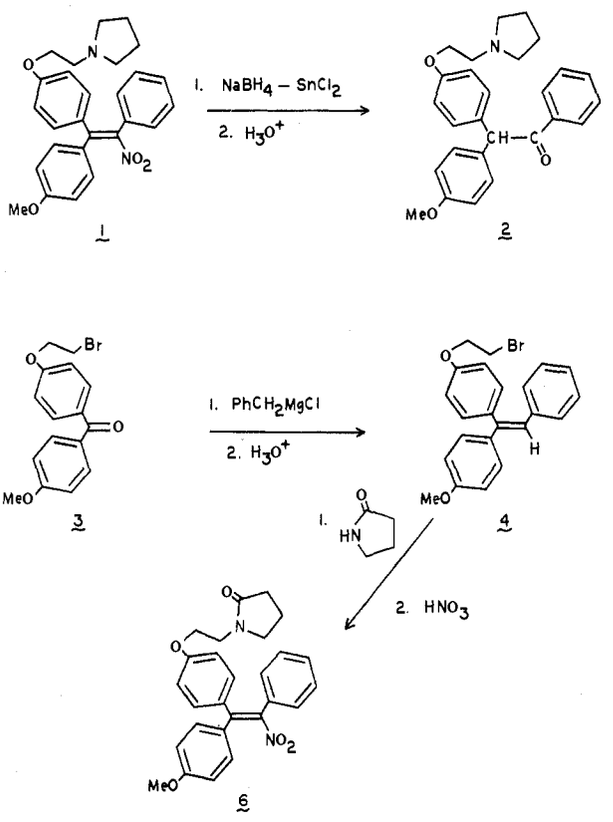
College of Pharmacy, University of Georgia, Athens, Georgia 30602. *Received May 2, 1983*

Reduction of the triarylethylene antiestrogen 2-[*p*-[2-nitro-1-(4-methoxphenyl)-2-phenylvinyl]phenoxy]-*N*-ethylpyrrolidine (1) with sodium borohydride–stannous chloride afforded 2-(*p*-methoxyphenyl)-*p'*-(2-pyrrolidin-1-ylethoxy)deoxybenzoin (2). Incubation of 1 with rat cecal content suspension under aerobic or anaerobic conditions also resulted in the generation of 2. The lactam analogue of 1 (6) was prepared by condensation of 4-(2-bromoethoxy)-4'-methoxybenzophenone (3) with benzylmagnesium chloride, followed by dehydration, amidation with 2-pyrrolidinone, and nitration. A metabolite with chromatographic and spectral properties identical with those of 6 was found in extracts from incubation mixtures of 1 with phenobarbital-induced rat liver 9000*g* supernatant. Compound 2 did not exhibit appreciable binding to the rat uterine cytosol estrogen receptor at concentrations of up to 1×10^{-6} M and had no estrogenic or antiestrogenic activity in the 3-day rat uterotropic assay. By contrast, 6 had estrogen receptor affinity somewhat greater than that of 1 and slightly greater estrogenic activity accompanied by reduced antiestrogenic activity in comparison with those of 1.

Triarylethylene antiestrogens, including 1[1] (CI 628, nitromiphene), appear to exert their effects through binding to cytosol estrogen receptors. The resulting antiestrogen–estrogen receptor complexes are much less effective in promoting estrogenic responses than is that involving estradiol.[2] The ability of triarylethylene antiestrogens to antagonize the growth-promoting effect of estradiol in target tissue has focused attention on the potential application of these compounds as therapeutic alternatives to surgery in estrogen-dependent cancers.[3] Also, compounds such as 1 have been of value in studies of the molecular mechanism of action of estrogens in target tissues.[4,5]

As is the case with other triarylethylenes, 1 undergoes biotransformation to a phenolic metabolite, *O*-demethyl-1. This metabolite binds more strongly to estrogen receptors than does 1[6,7] and appears to contribute substantially to the biological effects seen on administration of 1.[7] In addition to the *O*-methyl group, the structure of 1 contains several other moieties, in particular the pyrrolidine ring and the nitro group, that are major sites of biotransformation in other drugs. Thus, the pyrrolidine rings in nicotine and tremorine undergo hepatic *N*-methylene oxidation to afford the lactam metabolites cotinine and oxotremorine, respectively.[8-10] And numerous aromatic and heteroaromatic nitro compounds have been demonstrated to undergo reduction to the corresponding amino compounds in the presence of gastrointestinal microflora.[11-13]

Scheme I



(1) For convenience, the "trans" configurations of 1 and other triarylethylenes are shown in Scheme I. Actually, each of these compounds is an approximately equal mixture of both geometric isomers.

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