terminated by the addition of 4 mL of ice-cold saline and filtration through Whatman GF/B filters which were presoaked in 0.05% polyethylenimine. Filters were washed once with 4 mL of ice-cold saline and the radioactivity remaining on the filters was counted.

[^{3}H]Dopamine Uptake. Uptake of [^{3}H]dopmine [(dihydroxyphenyl)ethylamine-3,4-t_2, 30 Ci/mmol; New England Nuclear, Boston MA] into rat striatal synaptosomes was measured at 37 °C by using a previously described method.[13] In brief, 50 μL of striatal synaptosomes (100 μg of protein) were preincubated with 5 μL of cocaine analogue in DMSO or solvent alone for 10 min in a final volume of 500 μL; uptake was initiated by the addition of 4 nM [^{3}H]dopamine and terminated after 5 min by the addition of 4 mL of ice-cold saline and filtration through Whatman GF/B filters. Nonspecific uptake was measured at 37 °C using 100 μM cocaine in buffer in which choline was substituted equimolar for sodium, which defined sodium-dependent, cocaine-sensitive [^{3}H]dopamine uptake. Nonspecific uptake was typically 3-5% of the total (total and nonspecific dpm were 38600 and 1360, respectively).

Data Analysis. All assays were performed in triplicate and the mean values of at least three separate experiments were used. The IC_{50} values for inhibition of [^{3}H]cocaine binding were determined with ligand EBDA, an iterative nonlinear curve-fitting routine for an IBM-PC.[26] The IC_{50} values for inhibition of [^{3}H]dopamine uptake were determined from the inhibition curves.

Acknowledgment. We thank Patrick McNeilly for assisting in obtaining the infrared spectra and Dr. Donald H. Chace for providing the thermospray mass spectra. This investigation was supported in part by Grant DA03680 from the National Institute on Drug Abuse.

Registry No. 1, 7143-09-1; 2, 29364-08-7; 3, 131013-13-3; 4, 131013-14-4; 4·2HCl, 131100-30-6; 5, 131013-15-5; 6, 131013-16-6; 4-nitrophenyl isocyanate, 100-28-7; 3-nitrophenyl isocyanate, 3320-87-4.

(26) Munson, P. J.; Rodbard, D. *Anal. Biochem.* **1980**, *107*, 220.

Structure-Affinity Relationships of 12-Sulfonyl Derivatives of 5,8,8a,9,10,11,12,12a,13,13a-Decahydro-6*H*-isoquino[2,1-*g*][1,6]naphthyridines at α-Adrenoceptors[1]

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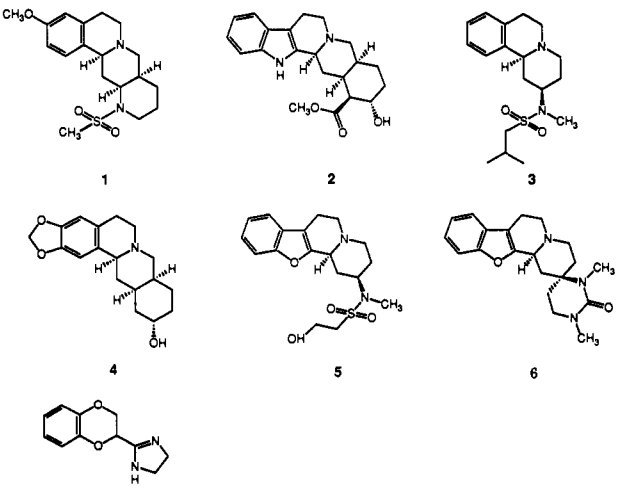
Analogues of the potent α_2-adrenoceptor antagonist (8a*R*,12a*S*,13a*S*)-5,8,8a,9,10,11,12,12a,13,13a-decahydro-3-methoxy-12-(methylsulfonyl)-6*H*-isoquino[2,1-*g*][1,6]naphthyridine (**1b**) were prepared and evaluated for α_1- and α_2-adrenoceptor affinity. Affinity for α_2-adrenoceptors was assessed by displacement of [^{3}H]yohimbine from rat cerebral cortical membranes and although **1b** and close structural analogues demonstrated high affinity, none were selective for the α_{2A} or α_{2B} subtypes reputedly present in this tissue. All of the high affinity α_2-adrenoceptor ligands were, however, selective with respect to [^{3}H]prazosin (α_1) binding. Affinity for [^{3}H]yohimbine-labeled α_2-adrenoceptors was found to be highly dependent on the stereochemistry of the tetracyclic system. The 8aβ,12aα,13aα diastereomer of **1** (**56**) had moderate affinity for α_2-adrenoceptors while the 8aβ,12aβ,13aα diastereomer (**55**) had very low affinity. The affinity and selectivity of these agents for α_2-adrenoceptors was found to correspond to that observed for several isomeric yohimbine analogues which have similar relative and absolute stereochemistries. Deviation from the structure of **1** by opening the B ring, changing the position of the sulfonamide nitrogen, or changing the attachment of the D ring led to a dramatic decrease in α_2-adrenoceptor affinity. High binding affinity was found to correlate with functional antagonism in the guinea pig ileum. The reversal of clonidine-induced mydriasis in the rat was used to assess bioavailability and indicated that **1b** was a potent α_2-adrenoceptor antagonist in vivo.

We have previously described the synthesis and preliminary pharmacological profile of the potent and selective α_2-adrenoceptor antagonist **1**.[2] This compound is a representative of a class of α_2-adrenoceptor antagonists related to the early prototype rauwolscine (**2**).[3] Other members of this class include the hexahydrobenzo[*a*]quinolizine WY-26703 (**3**),[4] the berbane derivative CH-38083 (**4**),[5] and the hexahydro-2*H*-benzo[*b*]furo[2,3-*a*]quinolizines L-654,284 (**5**)[6] and L-657,743 (**6**).[7] These agents are structurally dissimilar to the imidazoline α_2-adrenoceptor antagonists typified by idazoxan (**7**).[8] The potential therapeutic utility of selective α_2-adrenoceptor antagonists, which may be principally in the treatment of depression, has been reviewed.[9]

We based the design of compound **1** on the structures of rauwolscine (**2**) and **3**. Of primary interest were the conformational constraints imposed by the D ring in **1** which would determine the orientation of the sulfonamido group. The sulfonamido group would therefore occupy the same region of space as the carbomethoxy group of rauwolscine. The critical importance of the spatial orientation of the carbomethoxy group in determining α_2-adrenoceptor



† Syntex Research, Palo Alto.
‡ Syntex Research, Edinburgh.

(1) Contribution no. 801 from the Institute of Organic Chemistry.

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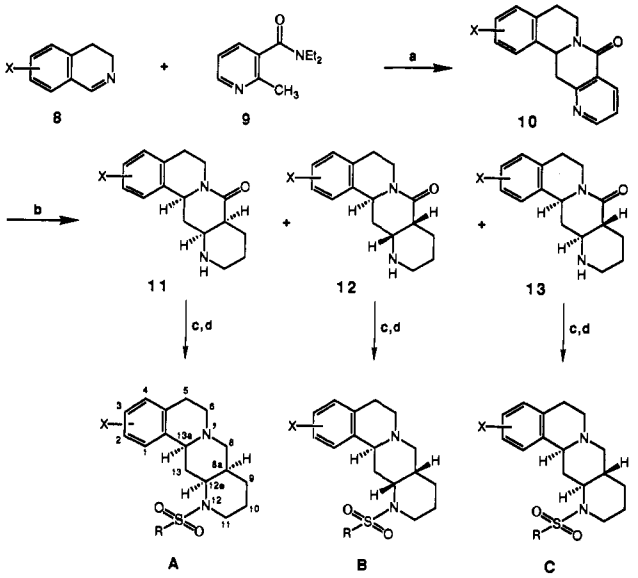
Table I. Physical Properties of Intermediates

compd	scheme	yield, %	mp, °C	recryst solvent	formula[a]	anal.
10 X = H	I	58	72–73	Et_2O	$C_{16}H_{14}N_2O$	C,H,N
10 X = 3-CH_3O	I	84	115–116	Et_2O	$C_{17}H_{16}N_2O_2$	C,H,N
10 X = 1,4-$(CH_3O)_2$	I	41	161–162	Et_2O–heptane	$C_{18}H_{18}N_2O_3$	C,H,N
10 X = 2,3-$(CH_3O)_2$	I	65	238–240	MeOH	$C_{18}H_{18}N_2O_3$	C,N,H[b]
10 X = 3,4-$(CH_3O)_2$	I	39	131–132	EtOAc–Et_2O	$C_{18}H_{18}N_2O_3$	C,H,N
10 X = 2,3-(OCH_2O)	I	57	177–179	Et_2O	$C_{17}H_{14}N_2O_3$	C,H,N
10 X = 2,3-(OCH_2CH_2O)	I	35	172–173	EtOAc	$C_{18}H_{16}N_2O_3$	C,H,N
10 X = 3-CH_3	I	57	108–109	Et_2O	$C_{17}H_{16}N_2O$	C,H,N
10 X = 2-F	I	40	121–122	Et_2O	$C_{16}H_{13}FN_2O$	C,H,N
19	IV	58	150–152	EtOAc	$C_{16}H_{14}N_2O$	C,H,N
22a	IV	96	189–191	EtOH	$C_{16}H_{15}N_3 \cdot 2HCl \cdot 0.25H_2O$	C,H,N
22b	IV	41	168–169	EtOAc	$C_{16}H_{14}N_2O$	C,H,N
25	V	30	87–88	EtOAc–hexane	$C_{16}H_{16}N_2O$	C,H,N
27[c]	V	65	147–148	EtOAc	$C_{16}H_{14}N_2O_3$	C,H,N
36	VII	36	249–250	EtOAc	$C_{18}H_{15}N_3O$	C,H,N

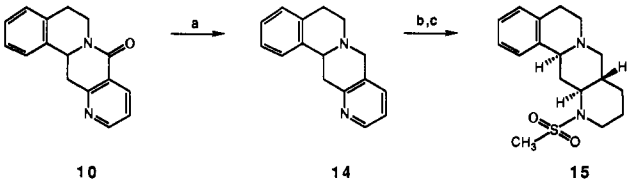
[a] All compounds were analyzed for C, H, and N, and analytical values were within ±0.4% of calculated values unless otherwise indicated. [b] H: calcd, 5.85; found 6.44. [c] Reference 12.

Scheme I[a]



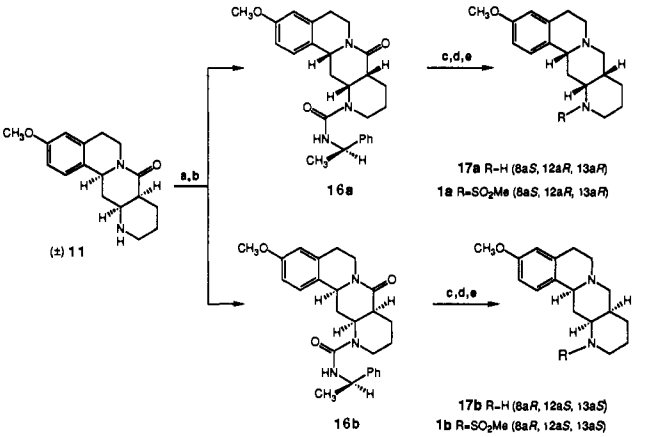
[a] (a) LDA, THF, −40 °C; (b) H_2, Rh–Al_2O_3; (c) LAH, THF; (d) RSO_2Cl, TEA, CH_2Cl_2.

Scheme II[a]



[a] (a) BH_3·DMS, THF; (b) Na, EtOH; (c) MsCl, TEA, CH_2Cl_2.

Scheme III[a]



[a] (a) (*R*)-α-Methylbenzyl isocyanate; (b) separation by chromatography; (c) LAH, THF; (d) NaOBu, *n*-BuOH; (e) MsCl, TEA, CH_2Cl_2.

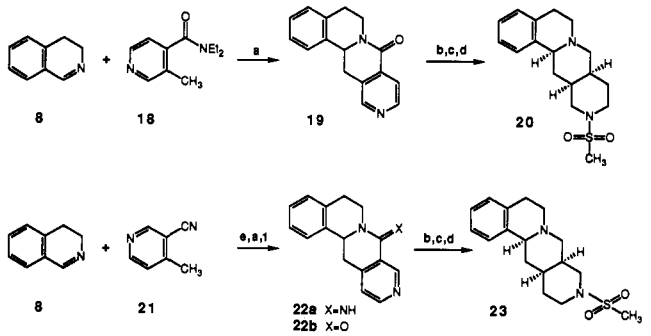
binding of the yohimbine alkaloids, including rauwolscine, has been noted.[10] We were also interested in evaluating the indole analogue of 1 (**37**) which would give information on the contribution of the indole moiety to the α_2-adrenoceptor binding in this structural class. Reported herein are the syntheses and α-adrenoceptor binding affinities of analogues of 1, including diastereomers and seco derivatives, and the corresponding indole congeners. The functional and in vivo α_2-adrenoceptor antagonist activity of a number of these agents is also reported.

Chemistry

Derivatives in the racemic 8aα,12aα,13aα series which includes 1 (series A), as well as of the two related diastereomers (series B and C), were prepared by the four-step

(2) Clark, R. D.; Repke, D. B.; Kilpatrick, A. T.; Brown, C. M.; MacKinnon, A. C.; Clague, R. U.; Spedding, M. *J. Med. Chem.* **1989**, *32*, 2034.
(3) Weitzell, R.; Tanaka, T.; Starke, K. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1979**, *308*, 127.
(4) (a) Lattimer, N.; McAdams, R. P.; Rhodes, K. F.; Sharma, S.; Turner, S. J.; Waterfall, J. F. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1984**, *327*, 312. (b) Ward, T. J.; White, J. F.; Lattimer, N.; Rhodes, K. F.; Sharma, S.; Waterfall, J. F. *J. Med. Chem.* **1988**, *31*, 1421.
(5) (a) Vizi, E. S.; Harsing, L. G.; Gaal, J.; Kapocsi, J.; Bernath, S.; Somogyi, G. T. *J. Pharmacol. Exp. Ther.* **1986**, *238*, 701. (b) Vizi, E. S.; Toth, I.; Somogyi, G. T.; Szabo, L.; Harsing, L. G.; Szantay, C. *J. Med. Chem.* **1987**, *30*, 1355.
(6) Huff, J. R.; Anderson, P. S.; Baldwin, J. J.; Clineschmidt, B. V.; Guare, J. P.; Lotti, V. J.; Pettibone, D. J.; Randall, W. C.; Vacca, J. P. *J. Med. Chem.* **1985**, *28*, 1756. (b) Pettibone, D. J.; Clineschmidt, B. V.; Lotti, V. J.; Martin, G. E.; Huff, J. R.; Randall, W. C.; Vacca, J.; Baldwin, J. J. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1986**, *333*, 110.
(7) Pettibone, D. J.; Clineschmidt, B. V.; Lotti, V. J.; Baldwin, J. J.; Huff, J. R.; Randall, W. C.; Vacca, J.; Young, S. D. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1987**, *336*, 169.
(8) 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.
(9) Clark, R. D.; Michel, A. D.; Whiting, R. L. In *Progress in Medicinal Chemistry*; Ellis, G. P., West, G. B., Eds.; Elsevier: Amsterdam, 1986; Vol. 23, p 1.
(10) Baldwin, J. J.; Huff, J. R.; Randall, W. C.; Vacca, J. P.; Zrada, M. M. *Eur. J. Med. Chem.* **1985**, *20*, 67.

Scheme IV[a]



[a] (a) LDA, THF, −40 °C; (b) H_2, Rh–Al_2O_3; (c) LAH, THF; (d) MsCl, TEA, CH_2Cl_2; (e) $CF_3SO_3SiMe_3$; (f) KOH, dioxane/H_2O.

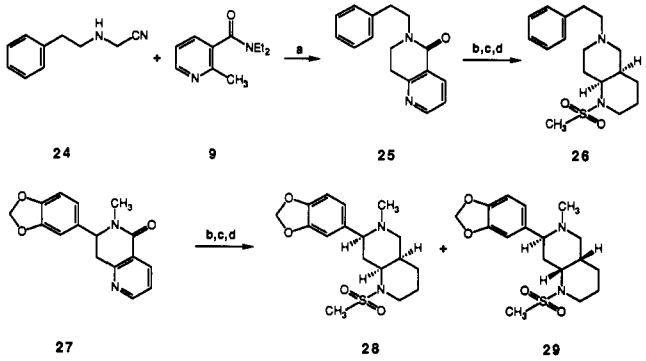
sequence depicted in Scheme I.[11] Tetracyclic pyridine amides **10** (Table I) were readily available by condensation of 3,4-dihydroisoquinolines **8** and nicotinamide **9** in the presence of lithium diisopropylamide (LDA).[12] Catalytic hydrogenation of **4** afforded the cis-syn (**11**), cis-anti (**12**), and trans (**13**) diastereomers in a ratio of ca. 65:35:5, respectively. The three isomers could be separated by chromatography and subsequently reduced with lithium aluminum hydride followed by treatment with the requisite sulfonyl halide to afford final products A, B, and C. Alternatively, the mixture **11–13** could be carried through the two-step sequence followed by chromatographic separation of A, B, and C. It was noted that all compounds of series A were dramatically less polar than those of series B and C which greatly facilitated isolation by chromatography of these more active diastereomers (Table II).

The trans compound **15** was also prepared by an alternate route involving sodium–ethanol reduction of pyridine **14**, albeit in rather low yield, followed by mesylation (Scheme II). Sodium–ethanol reductions of 5,6,7,8-tetrahydroquinolines are known to give the more stable (trans) isomer[13] and hence this sequence was used to confirm the stereochemistry of series C.[14]

The stereochemistries of **11** and **12** were initially based on ^1H NMR spectral data. Particularly diagnostic was a trans diaxial coupling between H-12a and H-13 (J = 9.5 Hz) in **11** which was not observed for **12** (J = 2.9 Hz). These structural assignments were subsequently confirmed by single-crystal X-ray analyses of derivative **16b** (Scheme III) and the derived final product **1b** (vide infra). It was also found that compounds of series B could be epimerized at H-13a[15] to afford series A by conversion to the imminium ion (trifluoroacetic anhydride treatment of the N-oxide), followed by reduction from the less hindered face with sodium borohydride.[16]

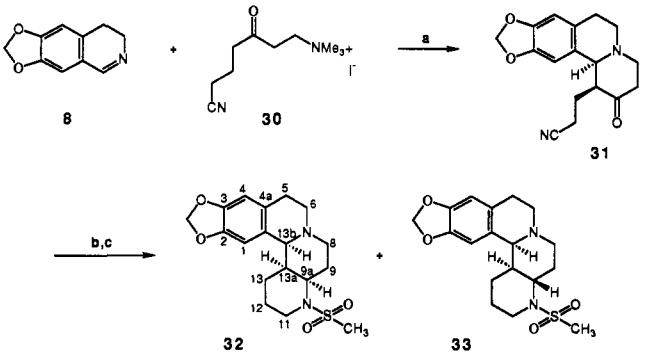
The enantiomers **1a** and **1b** were initially prepared as previously described[2] by chromatographic separation of the (*R*)-1-phenylethylureas **16a** and **16b** (Scheme III).[17] The absolute stereochemistry of **16b** was determined by

Scheme V[a]



[a] (a) **24** and *n*-BuLi; LDA, THF; (b) H_2, Rh–Al_2O_3; (c) LAH, THF; (d) MsCl, TEA, CH_2Cl_2.

Scheme VI[a]



[a] (a) CH_3OH, reflux; (b) H_2, Raney nickel; (c) MsCl, TEA, CH_2Cl_2.

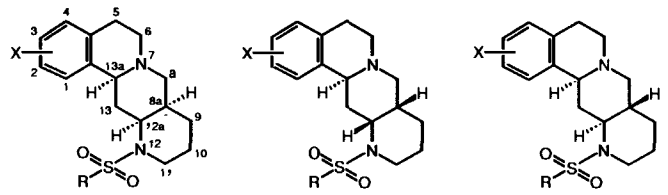
single-crystal X-ray analysis (supplementary material). It was found that the urea group of **16a** and **16b** was stable to conditions for lactam reduction (LAH, THF, reflux). Thus, removal of the chiral auxiliary could be accomplished subsequent to reduction of the lactam thereby avoiding the possibility of epimerization under the strongly basic conditions required for the urea cleavage. Enantiomers **1a** and **1b** derived from this sequence were found to be >99% pure by chiral HPLC analysis (Experimental Section). An asymmetric synthesis of **1b** was subsequently developed.[16]

Regioisomeric tetracyclic sulfonamides **20** and **23** (Scheme IV) were prepared by a sequence of reactions analogous to that described in Scheme I except that nitrile **21** was used instead of the amide. Catalytic hydrogenation of **19** and **22b** afforded product mixtures similar to those obtained by reduction of **10**. The chemical shifts and multiplicities of H-13a in **20** and **23** (Experimental Section) were characteristic for the cis-syn stereochemical series (A in Scheme I). The nonpolar nature of these products was further evidence that they belonged to the same stereochemical series.

B-ring seco derivatives **26**, **28**, and **29** were prepared as shown in Scheme V. The cyanomethylamine **24** was treated with *n*-BuLi to generate the corresponding formaldimine to which a solution of the lithio derivative of **9** was then added.[18] This afforded the condensation product **25** in 30% yield. Attempts to improve the yield, e.g. by

(11) All structural formulae represent racemates unless otherwise designated.
(12) Clark, R. D.; Jahangir. *J. Org. Chem.* **1987**, *52*, 5378.
(13) Vierhapper, F. W.; Eliel, E. L. *J. Org. Chem.* **1975**, *40*, 2734.
(14) The possibility of the alternative trans isomer (8aα,12aβ,13aα) was ruled out since a C ring boat conformation would be required.
(15) This is more easily seen if B is depicted as its enantiomer (8aα,12aα,13aβ) so that the epimerization of H-13a gives the 8aα,12aα,13aα isomer A.
(16) Clark, R. D.; Kern, J. R.; Kurz, L. J.; Nelson, J. T. *Heterocycles* **1990**, *31*, 353.
(17) Schonenberger, B.; Brossi, A. *Helv. Chim. Acta* **1986**, *69*, 1486.
(18) For generation of formaldehyde imines by treatment of *N*-(cyanomethyl)amines with strong bases and subsequent trapping, see: Overman, L. E.; Burk, R. M. *Tetrahedron Lett.* **1984**, *25*, 1635. Overman, L. E.; Osawa, T. *J. Am. Chem. Soc.* **1985**, *107*, 1698.

Table II. Physical Properties and Radioligand Binding Data



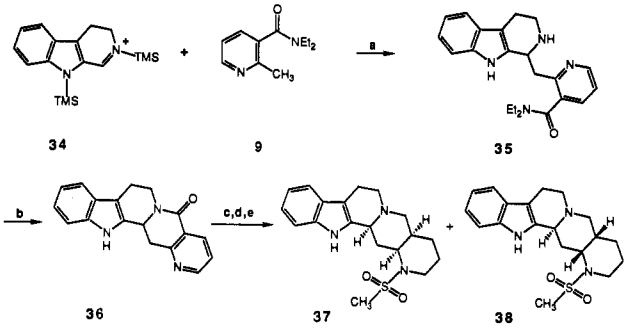
A B C

compd	series	X	R	recryst solvent	mp, °C	formula[a]	radioligand binding: pK_i[b] [^{3}H]prazosin (α_1)	[^{3}H]yohimbine (α_2)	selectivity ratio[c] (α_2/α_1)
39	A	H	CH_3	EtOH-Et_2O	234–235	$C_{17}H_{24}N_2O_2S\cdot HCl\cdot 0.5H_2O$	5.26 ± 0.23	8.77 ± 0.12	3200
40	B	H	CH_3	EtOH-Et_2O	230–235	$C_{17}H_{24}N_2O_2S\cdot HCl\cdot 0.5H_2O$	5.58 ± 0.14	5.96 ± 0.10	2
15	C	H	CH_3	EtOH	238–240	$C_{17}H_{24}N_2O_2S\cdot HCl$	5.65 ± 0.10	7.25 ± 0.25	40
41	A	H	CH_2CH_3	EtOH-Et_2O	203–204	$C_{18}H_{26}N_2O_2S\cdot HCl\cdot 0.25H_2O$	4.97	8.82 ± 0.03	7100
42	A	H	$CH_2CH_2CH_3$	EtOH-Et_2O	235–236	$C_{19}H_{28}N_2O_2S\cdot HCl\cdot 0.25H_2O$	5.29 ± 0.03	8.73 ± 0.22	2800
43	A	H	$CH_2CH_2CH_2CH_3$	EtOH-Et_2O	220–222	$C_{20}H_{30}N_2O_2S\cdot HCl\cdot 0.5H_2O$	5.64	8.90 ± 0.08	1800
44	B	H	$CH_2CH_2CH_2CH_3$	EtOH-Et_2O	215–216	$C_{20}H_{30}N_2O_2S\cdot HCl\cdot 0.5H_2O$	5.76	5.82 ± 0.32	1
45	C	H	$CH_2CH_2CH_2CH_3$	EtOH-Et_2O	205–206	$C_{20}H_{30}N_2O_2S\cdot HCl\cdot 0.5H_2O$	5.30	7.42 ± 0.04	130
46	A	H	$CH_2CH(CH_3)_2$	EtOH-Et_2O	220–221	$C_{20}H_{30}N_2O_2S\cdot HCl$	5.37	8.77 ± 0.16	2500
47	A	H	CH_2CH_2OH	EtOH-Et_2O	215–216	$C_{18}H_{26}N_2O_3S\cdot HCl$	4.89	8.16	1900
48	A	H	$CH_2CH_2OCH_3$	EtOH-Et_2O	173–174	$C_{19}H_{28}N_2O_3S\cdot HCl$	5.13	8.63 ± 0.34	3200
49	A	H	C_6H_5	EtOH-Et_2O	247–248	$C_{22}H_{26}N_2O_2S\cdot HCl\cdot 0.25H_2O$	5.31 ± 0.11	8.81 ± 0.09	3200
50	A	H	4-$OCH_3C_6H_4$	EtOH-MeOH	256–257	$C_{23}H_{28}N_2O_3S\cdot HCl$	5.31	9.34	11000
51	A	H	4-ClC_6H_4	EtOH-Et_2O	261–263	$C_{22}H_{25}ClN_2O_2S\cdot HCl$	5.19	8.30	1300
52	A	H	4-$NH_2C_6H_4$	EtOH-Et_2O	245–247	$C_{22}H_{27}N_2O_2S\cdot HCl\cdot H_2O$[d]	5.33	8.66	2100
53	A	H	4-FC_6H_4	EtOH-Et_2O	258–259	$C_{22}H_{25}FN_2O_2S\cdot HCl$	5.38	8.69 ± 0.35	2000
54	A	1-CH_3O	CH_3	EtOH-Et_2O	268–269	$C_{18}H_{26}N_2O_3S\cdot HCl\cdot 0.25H_2O$	5.48	8.65 ± 0.26	1500
1	A	3-CH_3O	CH_3	EtOH-Et_2O	265–266	$C_{18}H_{26}N_2O_2S\cdot HCl$	4.99 ± 0.10	9.18 ± 0.12	15000
1b	(8a*R*,12a*S*,13a*S*)	3-CH_3O	CH_3	EtOH-Et_2O	256–258	$C_{18}H_{26}N_2O_3S\cdot HCl$	5.29 ± 0.10	9.45 ± 0.16	14000
1a	(8a*S*,12a*R*,13a*R*)	3-CH_3O	CH_3	EtOH-Et_2O	256–258	$C_{18}H_{26}N_2O_3S\cdot HCl$	<5	6.32 ± 0.08	>21
55	B	3-CH_3O	CH_3	EtOH-Et_2O	238–239	$C_{18}H_{26}N_2O_3S\cdot HCl$	4.85	6.88 ± 0.06	110
56	C	3-CH_3O	CH_3	EtOH-Et_2O	260–261	$C_{18}H_{26}N_2O_3S\cdot HCl$	5.20	7.84 ± 0.04	440
57	(8a*R*,12a*S*,13a*S*)	3-CH_3O	CH_2Cl	EtOH	265–267	$C_{18}H_{25}ClN_2O_3S\cdot HCl$	5.13	8.95 ± 0.13	6600
58	A	3-CH_3O	$CH_2CH(CH_3)_2$	EtOH-Et_2O	127–130	$C_{21}H_{32}N_2O_3S\cdot HCl\cdot H_2O$	5.41	8.77 ± 0.20	2300
59	A	3-CH_3O	$CH_2CH_2OCH_3$	EtOH-Et_2O	218–220	$C_{20}H_{30}N_2O_4S\cdot HCl$	5.03	9.22 ± 0.24	15000
60	A	3-CH_3O	$CH{=}CH_2$	EtOH-Et_2O	185–187	$C_{19}H_{26}N_2O_3S\cdot HCl\cdot 0.25H_2O$	5.33	8.82 ± 0.11	3100
61	(8a*R*,12a*S*,13a*S*)	3-CH_3O	$CH_2C_6H_5$	EtOH-Et_2O	186–187	$C_{24}H_{30}N_2O_3S\cdot HCl\cdot 0.25H_2O$	5.13	8.82 ± 0.15	4900
62	(8a*R*,12a*S*,13a*S*)	3-CH_3O	2-thienyl	EtOH	302–304	$C_{21}H_{26}N_2O_3S_2\cdot HCl\cdot H_2O$	5.01	8.83 ± 0.18	6600
63	A	1,4-$(CH_3O)_2$	CH_3	EtOH-Et_2O	194–195	$C_{19}H_{28}N_2O_4S\cdot HCl\cdot H_2O$	5.63	7.37 ± 0.31	55
64	A	2,3-$(CH_3O)_2$	CH_3	EtOH-Et_2O	175–177	$C_{19}H_{28}N_2O_4S\cdot HCl\cdot 0.5H_2O$	4.46	8.01 ± 0.03	3500
65	A	2,3-$(CH_3O)_2$	$CH_2CH(CH_3)_2$	EtOH-Et_2O	155–156	$C_{22}H_{34}N_2O_4S\cdot HCl\cdot H_2O$	4.46	8.10 ± 0.08	4400
66	A	2,3-$(CH_3O)_2$, 6-CH_3	CH_3	EtOH-Et_2O	162–163	$C_{20}H_{30}N_2O_4S\cdot HCl\cdot 0.25H_2O$	4.93	7.08 ± 0.17	140
67	A	3,4-$(CH_3O)_2$	CH_3	EtOH-Et_2O	222–224	$C_{19}H_{28}N_2O_4S\cdot HCl$[e]	4.42	7.94 ± 0.09	3300
68	A	2,3-(OCH_2O)	CH_3	EtOH	279–280	$C_{18}H_{24}N_2O_4S\cdot HCl$	5.14	9.18 ± 0.22	11000
68a	(8a*R*,12a*S*,13a*S*)	2,3-(OCH_2O)	CH_3	EtOH-Et_2O	263–265	$C_{18}H_{24}N_2O_4S\cdot HCl$	5.32	9.19 ± 0.22	7400
69	B	2,3-(OCH_2O)	CH_3	EtOH-Et_2O	270–271	$C_{18}H_{24}N_2O_4S\cdot HCl$	5.95	7.24 ± 0.25	19
70	C	2,3-(OCH_2O)	CH_3	EtOH-Et_2O	262–263	$C_{18}H_{24}N_2O_4S\cdot HCl\cdot 0.25H_2O$	5.65 ± 0.10	7.56 ± 0.19	81
71	A	2,3-(OCH_2O)	$CH_2CH(CH_3)_2$	EtOH-Et_2O	172–174	$C_{21}H_{30}N_2O_4S\cdot HCl\cdot H_2O$[f]	5.04	8.17 ± 0.08	1300
72	A	2,3-(OCH_2CH_2O)	CH_3	EtOH	279–280	$C_{19}H_{26}N_2O_4S\cdot HCl$	5.02	8.80 ± 0.13	6000
73	A	3-OH	CH_3	EtOH-Et_2O	251–253	$C_{17}H_{24}N_2O_3S\cdot HCl\cdot 0.5H_2O$	5.27	8.20 ± 0.16	850
74	A	3-CH_3	CH_3	EtOH	269–271	$C_{18}H_{26}N_2O_2S\cdot HCl$	4.89	9.15 ± 0.14	18000

75	A	2-F	CH_3	EtOH	288–290	$C_{17}H_{23}FN_2O_2S$·HCl	5.21	8.04 ± 0.13	680
76	(8aR,12aS,13aS)	2-Br, 3-CH_3O	CH_3	EtOH–Et_2O	228–230	$C_{18}H_{25}BrN_2O_3S$·HCl	5.25	8.30 ± 0.28	1 100
77	(8aR,12aS,13aS)	4-Br, 3-CH_3O	CH_3	EtOH–Et_2O	250–252	$C_{18}H_{25}BrN_2O_3S$·HCl	5.35	8.30 ± 0.31	890
78	A	3-CH_3O	NH_2	EtOH	199–200	$C_{17}H_{25}N_3O_3S$·HCl·0.5H_2O	4.92	8.43 ± 0.11	3 200
79	A	3-CH_3O	$NHC(CH_3)_3$	EtOH–Et_2O	180–182	$C_{21}H_{33}N_3O_3S$·HCl·0.5H_2O	5.26	7.78 ± 0.23	330
80	A	H	$N(CH_3)_2$	EtOH–Et_2O	237–238	$C_{18}H_{27}N_3O_2S$·HCl	6.03	8.74 ± 0.24	510
81	A	3-CH_3O	$N(CH_3)_2$	EtOH–Et_2O	228–230	$C_{19}H_{29}N_3O_3S$·HCl	5.25	8.66 ± 0.27	2600
81a	(8aR,12aS,13aS)	3-CH_3O	$N(CH_3)_2$	EtOH–Et_2O	242–243	$C_{19}H_{29}N_3O_3S$·HCl[g]	5.57	8.97 ± 0.04	2500
20				EtOH	274–275	$C_{17}H_{24}N_2O_2S$·HCl	5.80	7.54 ± 0.47	55
23				EtOH	278–279	$C_{17}H_{24}N_2O_2S$·HCl	5.20	6.40 ± 0.05	16
26				EtOH–Et_2O	244–245	$C_{17}H_{26}N_2O_2S$·HCl[h]	5.51	6.12 ± 0.18	4
28				EtOH	272–273	$C_{17}H_{24}N_2O_4S$·HCl	4.82	5.38 ± 0.14	4
29				EtOH	284–185	$C_{17}H_{24}N_2O_4S$·HCl	5.55	6.13 ± 0.12	4
32				EtOH	280–281	$C_{18}H_{24}N_2O_4S$·HCl[e]	5.82	6.13 ± 0.12	2
33				EtOH	208–209	$C_{18}H_{24}N_2O_4S$·HCl[e]	5.06	6.40 ± 0.39	22
37				EtOH	273–274	$C_{19}H_{25}N_3O_2S$·HCl	4.80 ± 0.01	7.78 ± 0.07	950
38				EtOH	288–289	$C_{19}H_{25}N_3O_2S$·HCl·0.5H_2O	5.15 ± 0.15	6.26 ± 0.05	13
7 (idazoxan)							6.10 ± 0.08	7.96 ± 0.04	72
82 (yohimbine)							6.40 ± 0.03	7.90 ± 0.03	32
2 (rauwolscine)							6.50 ± 0.10	8.15 ± 0.10	45
3 (WY-26703)							5.70 ± 0.10	8.04 ± 0.10	220

[a] Elemental analyses for C, H, and N were within 0.4% of the theoretical values unless otherwise noted. [b] Determined in rat cerebral cortical membranes with [^{3}H]prazosin and [^{3}H]yohimbine to label α_1- and α_2-adrenoceptors, respectively. Values are means of three separate determinations ± SEM. Values without SEM are means of two determinations. [c] Antilog of the [^{3}H]yohimbine pK_i minus the [^{3}H]prazosin pK_i. [d] C: calcd, 58.45; found, 58.88. [e] Analysis was obtained on the free base. [f] C: calcd, 54.71; found, 55.36. [g] C: calcd, 54.86; found, 53.94. [h] N: calcd, 7.81; found, 7.05.

Scheme VII[a]



[a] (a) LDA, THF; (b) NaOBu, *n*-BuOH; (c) H_2, Rh–Al_2O_3; (d) LAH, THF; (e) MsCl, TEA, CH_2Cl_2.

attempted in situ generation of the formaldimine in the presence of the lithiated nicotinamide, were unsuccessful. Sequential reduction of **25** followed by mesylation afforded **26**.

The previously described naphthyridine **27**[12] was converted to diastereomers **28** and **29** which were separated by chromatography. The stereochemistry of major product **28** was assigned on the basis of the previously observed preference for hydrogenation to form cis-syn isomers and on the similarity of the ^{1}H NMR spectrum with compounds of type A (Scheme I). Consistent with previous findings, isomer **28** was less polar than isomer **29**.

The decahydro-6*H*-isoquino[1,2-*g*][1,6]naphthyridine sulfonamides **32** and **33** were obtained by hydrogenation (Raney nickel) of the 1-(2-cyanoethyl)hexahydrobenzo[*a*]quinolizinone **31**[19] followed by mesylation (Scheme VI). ^{13}C NMR spectral data was especially useful for the assignment of stereochemistry of **32** and **33**. Upfield shifts of 3–11 ppm were observed for carbons 5, 8, 9a, 13a, and 13b in minor isomer **33** relative to major isomer **32**. Analysis of molecular models indicated that ring D of **33** may adopt a pseudo-boat conformation to avoid peri interaction of H-13 and H-1. The upfield shifts observed for **33** would therefore be consistent with increased steric compression through γ-gauche interactions[20] particularly since ring C also must be a pseudo-boat. Further evidence for this conformation of **33** is an unusually low field chemical shift for one H-13 (δ 2.28) in **33** relative to isomer **32** (δ 1.18 and 1.65). Molecular models indicated that this hydrogen would be deshielded by the anisotropic effect of the benzene ring in **33** but not in **32**.

Pentacyclic indole sulfonamides **37** and **38** were similarly prepared from **36** (Scheme VII). This intermediate was obtained by addition of the lithio derivative of **9** to the 2,9-bis(trimethylsilyl)-3,4-dihydropyrido[3,4-*b*]indole triflate **34** to afford adduct **35** followed by cyclization.[21] The ^{1}H NMR spectra of **37** and **38** were nearly identical in relevant detail with those of **1** and **55**, respectively.

(19) This material was obtained as a minor product in the synthesis of the corresponding 3-cyanoethyl derivative.[16] It derives from the presence of ca. 15% of **24** in the trimethyl(2-acetyl-4-cyanobutyl)ammonium iodide used in that synthesis. The β-orientation of the cyanoethyl group was assigned on the basis of the 0.5-Hz coupling constant for H-1 and H-11b. The side chain presumably adopts a pseudoaxial orientation to avoid peri interactions with H-11.

(20) Tourwe, D.; Van Binst, G. *Heterocycles* **1978**, *9*, 507, and references cited therein.

(21) Similar methodology, in which the TMS group is used both to protect and to activate 3,4-dihydro-β-carboline, has been used to synthesize the alkaloid nauclefine which is regioisomeric with **36**: Repke, D. B.; Jahangir; Clark, R. D.; Nelson, J. T.; Maclean, D. B. *Tetrahedron* **1989**, *45*, 2541.

Hydroxyethanesulfonamide **47** was prepared by boron tribromide demethylation of **48**. The *p*-aminobenzenesulfonamide **52** was prepared by catalytic hydrogenation of the nitro derivative. Sulfonylation with 2-chloroethanesulfonyl chloride furnished the ethenesulfonamide **60**. Treatment of **1** with HBr afforded the phenol **73**. Bromination of **1b** (Br_2, HOAc) afforded regioisomers **76** and **77** which were separated by chromatography.

Results and Discussion

Structure-Affinity Relationships Based on α-Adrenoceptor Binding. Relative affinities of the sulfonyl derivatives for α-adrenoceptors were determined by measurement of radioligand displacement from rat cerebral cortical membrane binding sites (Table II). [^{3}H]Prazosin was used to label α_1-adrenoceptors and it can be noted that the test compounds displayed uniformly low affinity (pK_i < 6) for this site. [^{3}H]Yohimbine was used to label α_2-adrenoceptors and it is clear from the data that many compounds displayed high activity in displacement of this ligand. On this basis, these agents can be regarded as selective α_2-adrenoceptor ligands in the classical sense.

However, recent evidence indicates that heterogeneity exists within the α_2-adrenoceptor.[22] Two subtypes have been shown in the rat cerebral cortex: the α_{2A} subtype which has low affinity for prazosin and high affinity for oxymetazoline and the α_{2B} subtype for which the selectivities are reversed. The subtypes appear to be present in equal proportions in this tissue.[22a] [^{3}H]Yohimbine labels both subtypes[22b] whereas we have demonstrated that [^{3}H]idazoxan labels an α_2-adrenoceptor consistent with the α_{2A} subtype.[23] In addition, [^{3}H]idazoxan labels an imidazoline binding site in the rat cortex that is distinct from α_2-adrenoceptors.[23] Therefore, in the present study α_2-adrenoceptor affinity was assessed by using [^{3}H]yohimbine binding to rat cerebral cortex. As previously reported by Bylund,[22a] we found that prazosin and oxymetazoline show biphasic inhibition curves which can be resolved into two components, the α_{2A} subtype (prazosin $pK_i = 6.00 \pm 0.12$; oxymetazoline $pK_i = 8.25 \pm 0.20$) and the α_{2B} subtype (prazosin $pK_i = 7.30 \pm 0.11$; oxymetazoline $pK_i = 6.82 \pm 0.13$).[23] However, all of the high affinity sulfonamide derivatives in the present study (Table II) fully displaced the specific binding and showed Hill coefficients close to unity which suggested that they have similar affinity for both the α_{2A} and α_{2B} subtypes in the rat cortex.[24] The structure–affinity relationships which follow must therefore be viewed with this α_2-adrenoceptor subtype nonselectivity in mind and must be regarded in a general or qualitative sense.

The affinity of stereoisomers of **1** for [^{3}H]yohimbine labeled α_2-adrenoceptors was influenced dramatically by the relative stereochemistry of the tetracyclic system. For the racemic diastereomers, affinity was dependent on the relative stereochemistry of the C–D ring juncture. For the four sets of diastereomers examined (Table II), the cis-syn derivatives (series A) were the highest affinity α_2-adrenoceptor ligands. The corresponding trans analogues (series C) had intermediate affinity while the cis-anti analogues (series B) had the lowest affinity. The same trend was noted in selectivity for the α_2-adrenoceptor over the α_1-adrenoceptor and compounds in series A were remarkably selective for α_2-adrenoceptors with selectivity ratios greater than 1000. Although not as selective, the trans analogues still maintained a reasonable degree of selectivity compared to the standards idazoxan (**7**) and rauwolscine (**2**) (Table II). Affinity for α_2-adrenoceptors was also dependent on absolute stereochemistry. For the one enantiomer pair evaluated, the 8a*R*,12a*S*,13a*S* enantiomer **1b** had over 1000× the affinity of the 8a*S*,12a*R*,13a*R* enantiomer **1a**. Deviation from the structure of tetracycle A (Scheme I) in a regioisomeric sense (**20**, **23**) or by ring opening to form seco derivatives (**26**, **28**) led to significant reductions in affinity.

The α_2-adrenoceptor affinity of derivatives in series A appeared to be relatively independent of the nature of the sulfonamide moiety.[25] Lower alkyl, phenyl, and alkylamino sulfonamides all had affinity constants > 8. Substitution with alkoxy groups in the aromatic (A) ring was also well tolerated, although there was a trend toward reduced affinity among the dimethoxy derivatives, particularly those substituted in position **4** (**63**, **67**). Substitution of methyl at the 6-position also led to reduced affinity (**66** vs **64**).

The structure–affinity relationships within these tetracyclic sulfonamides can be compared with those of the isomeric yohimbine alkaloids. The structures of these agents are represented in Figure 1 and affinity data is given in Table III. The stereostructure of **1b** was determined by single-crystal X-ray analysis (Figure 2), and the absolute stereochemistry, determined earlier from X-ray analysis of **16b**, corresponds to that of rauwolscine. The representations for **37**, **55**, and **56** were generated with MODEL (version 2.94, Serena Software) while those of the yohimbine alkaloids are from the literature.[10,26]

The same trend in α_2-adrenoceptor affinity and selectivity is present in the isomers **1b**, **56**, and **55** as was found for rauwolscine (α-yohimbine), yohimbine, and 3-*epi*-α-yohimbine, respectively. However, the sulfonamides are significantly more selective for α_2-adrenoceptors than the

Table III. α-Adrenoceptor Affinity of Tetracyclic Sulfonamides and Yohimbine Stereoisomers

compd[a]	radioligand binding, pK_i[b] [^{3}H]prazosin (α_1)	[^{3}H]yohimbine (α_2)	selectivity ratio α_2/α_1
1b	5.29 ± 0.10	9.45 ± 0.16	14000
rauwolscine (**2**)	6.50 ± 0.10	8.15 ± 0.10	45
37	4.80 ± 0.01	7.78 ± 0.07	950
56	5.20	7.84 ± 0.04	440
yohimbine (**82**)	6.40 ± 0.03	7.90 ± 0.03	32
55	4.85	6.88 ± 0.06	110
3-*epi*-α-yohimbine (**83**)	5.80[c]	5.86[d]	1

[a] See Figure 1. Compounds **37**, **55**, and **56** are racemic. [b] From Table II unless otherwise noted. [c] Determined in rat liver membranes.[27] [d] Determined in human platelet membranes.[27]

(22) (a) Bylund, D. B. *Pharmacol. Biochem. Behav.* **1985**, *22*, 835. (b) Bylund, D. B.; Ray-Prenger, C.; Murphy, T. J. *J. Pharmacol. Exp. Ther.* **1988**, *245*, 600. (c) Bylund, D. B. *Trends Pharm. Sci.* **1988**, *9*, 356. (d) Boyajian, C. L.; Loughlin, S. E.; Leslie, F. M. *J. Pharmacol. Exp. Ther.* **1987**, *241*, 1079. (e) Boyajian, C. L.; Leslie, F. M. *J. Pharmacol. Exp. Ther.* **1987**, *241*, 1092. (f) Daly, R. N.; Sulpizio, A. C.; Levitt, B.; DeMarinis, R. M.; Regan, J. W.; Ruffolo, R. R., Jr.; Hieble, J. P. *J. Pharmacol. Exp. Ther.* **1988**, *241*, 122. (g) Young, P.; Berge, J.; Chapman, H.; Cawthorne, M. A. *Eur. J. Pharmacol.* **1989**, *168*, 381.

(23) Brown, C. M.; MacKinnon, A. C.; McGrath, J. C.; Spedding, M.; Kilpatrick, A. T. *Br. J. Pharmacol.* **1990**, *99*, 803.

(24) This was confirmed for **1b** in subtype selective assays:[22a] human platelet (α_{2A}) pK_i 9.53 ± 0.14 (nH 1.01) and rat neonate lung (α_{2B}) pK_i 9.77 ± 0.25 (nH 0.98). Brown, C. M.; Spedding, M. Manuscript in preparation.

(25) The sulfonamide group itself may be replaced with other moieties, e.g. amide, urea, thiourea, and carbamate, with little effect on affinity or selectivity. Clark, R. D.; Spedding, M. Unpublished results.

(26) Morrison, G. A. *Fortsch. Chem. Org. Naturst.* **1967**, *25*, 269.

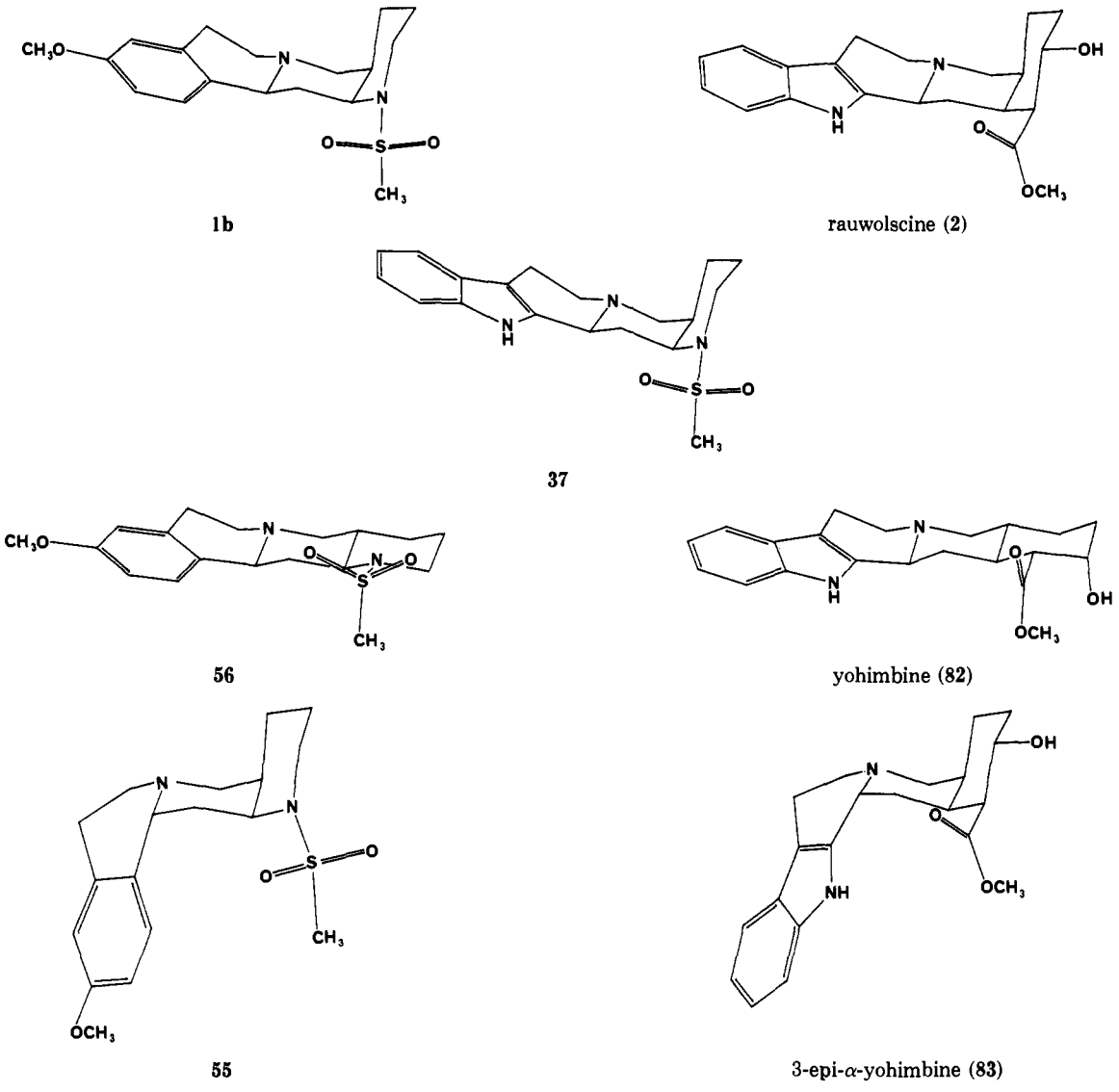


Figure 1. Structures of sulfonamides and yohimbine stereoisomers.

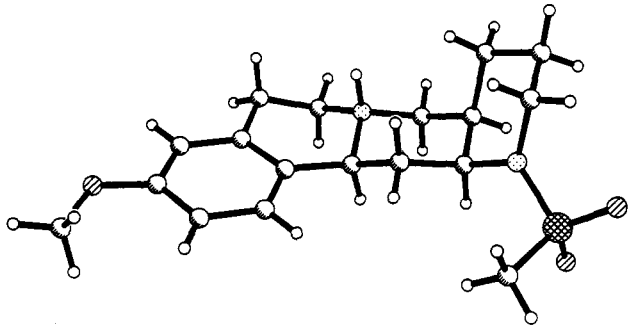


Figure 2. X-ray structure of **1b**·HCl (8a*R*,12a*S*,13a*S*-1).

corresponding alkaloids. The α_2-adrenoceptor selectivity of rauwolscine and yohimbine has been ascribed to the coplanarity of the carbomethoxy group and the indole nucleus.[27] The carbomethoxy group appears to provide an important dipole–dipole interaction at the α_2-adrenoceptor which is less important for binding to the α_1-adrenoceptor.[10] The substantial decrease in α_1-adrenoceptor affinity of the sulfonamide analogue (**37**) of rauwolscine (**2**) indicates that the sulfonamide moiety contributes even less to α_1-adrenoceptor affinity than the carbomethoxy group.

The enhanced α_2-adrenoceptor binding affinity of these tetracyclic sulfonamides, as typified by **1b** may be rationalized by considering the binding profile of indole analogue **37** (Figure 1). Consistent with the other sulfonamides, **37** is more α_2-adrenoceptor selective than its congener rauwolscine. However, the binding affinity of **37** is approximately the same as that of rauwolscine, taking into account that **37** is racemic, but is $^1/_{25}$th that of **1**. A reasonable explanation for these results is a negative influence of the polar indole nitrogen of **37** on α_2-adrenoceptor binding. In a structure–activity relationship study on binding of arylquinolizines to α_2-adrenoceptors, it was noted that incorporation of a basic nitrogen atom in the 1-position of the aryl system resulted in decreased affinity.[28] On the other hand, replacement of indole with benzofuran or benzothiophene led to an increase in α_2-adrenoceptor affinity. The high α_2-adrenoceptor affinity and selectivity (α_2 vs α_1) of **1b** relative to rauwolscine (**2**) would therefore appear to be a result of at least two major factors: (1) reduced binding to α_1-adrenoceptors of the

(27) Ferry, N.; Goodhart, M.; Hanoune, J.; Sevenet, T. *Br. J. Pharmacol.* **1983**, *78*, 359.

(28) Huff, J. R.; Baldwin, J. J.; de Solms, S. J.; Guare, J. P.; Hunt, C. A.; Randall, W. C.; Sanders, W. S.; Smith, S. J.; Vacca, J. P.; Zrada, M. M. *J. Med. Chem.* **1988**, *31*, 641.

Table IV. Functional in Vitro and in Vivo α_2-Adrenoceptor Antagonism

compd	X[a]	R[a]	pA_2, guinea pig ileum[b]	rat mydriasis assay IC_{50},[c] μg/kg iv	rat mydriasis assay IC_{50},[c] μg/kg oral	ratio oral/iv
39	H	CH_3	–	12.5	1200	96
41	H	CH_2CH_3	8.3 ± 0.06	19	1100	58
42	H	$CH_2CH_2CH_3$	9.4 ± 0.10	19	1400	74
43	H	$(CH_2)_3CH_3$	8.9 ± 0.10	36	900	25
46	H	$CH_2CH(CH_3)_2$	9.5 ± 0.10	34	850	25
47	H	CH_2CH_2OH	–	46	1340	29
48	H	$CH_2CH_2OCH_3$	–	62	240	4
49	H	C_6H_5	–	53	>10000	>190
50	H	4-$OCH_3C_6H_5$	8.8 ± 0.10	180	>3000	>16
1	3-CH_3O	CH_3	9.7 ± 0.10	13	210	16
1b	3-CH_3O	CH_3	9.7 ± 0.04	6.5	95	15
1a	3-CH_3O	CH_3	6.5 ± 0.10	>10000	>10000	–
58	3-CH_3O	$CH_2CH(CH_3)_2$	–	22	410	19
81a	3-CH_3O	$N(CH_3)_2$	9.3 ± 0.10	27	170	6
59	3-CH_3O	$CH_2CH_2OCH_3$	–	90	300	3
68	2,3-OCH_2O	CH_3	9.0 ± 0.10	60	760	13
71	2,3-OCH_2O	$CH_2CH(CH_3)_2$	–	62	440	7
72	2,3-OCH_2CH_2O	CH_3	–	130	1400	11
64	2,3-$(CH_3O)_2$	CH_3	–	380	1600	4
7 (idazoxan)			8.4 ± 0.03	17	1200	71
82 (yohimbine)			7.9 ± 0.06	185	2800	15

[a] Structure A, Table II. [b] Reversal of the inhibitory effect of UK-14304 on the contractile response to field stimulation. [c] Dose required to reduce pupillary diameter to 50% of the maximum induced by administration of clonidine. Values are means of five determinations and standard deviations were generally less than 20%.

sulfonamide group relative to the carbomethoxy group and (2) increased binding to α_2-adrenoceptors of the A-ring phenyl group relative to the indole moiety.[29]

Functional and in Vivo α_2-Adrenoceptor Antagonist Activity. As previously reported,[2] 1 is an extremely potent functional α_2-adrenoceptor antagonist in vitro in reversing the inhibitory effect of UK-14304 on the contractile response of the guinea pig ileum to field stimulation.[30] Results obtained in this assay with a number of analogues of 1 are presented in Table IV. The pA_2 values from this assay were in general agreement with the pK_i values obtained in the rat cerebral cortex (Table II).

To determine central α_2-adrenoceptor antagonism in vivo, selected compounds were tested for reversal of clonidine-induced mydriasis in the rat[31] (Table IV). This assay served to differentiate compounds on the basis of oral bioavailability. For derivatives of the A-ring unsubstituted parent **39**, there was a trend toward decreased potency upon iv administration as the size of the sulfonamide group increased. Potency upon oral administration reached a maximum with the butyl (**43**), isobutyl (**46**), and methoxyethyl (**48**) derivatives while the benzenesulfonamides (**49**, **50**) were virtually inactive. Among the A-ring-substituted analogues, the 3-methoxy analogue 1 was equipotent to the unsubstituted parent (**39**) upon iv administration. However, 1 was significantly more potent upon oral administration. With the exception of **48** and **59**, there was a general trend toward increased oral bioavailability, as manifested by lower oral/iv ratios, among A-ring-substituted analogues. This may indicate a lower rate of metabolism in these derivatives relative to the A-ring-unsubstituted congeners.[32] Thus, on the basis of the results in Table IV, **1b** was identified as the most potent orally active α_2-adrenoceptor antagonist in vivo in the series.

Conclusion

Tetracyclic sulfonamides as exemplified by 1 were found to be selective, high affinity α_2-adrenoceptor ligands as defined by displacement of [^{3}H]prazosin and [^{3}H]yohimbine from rat cerebral cortex. These compounds were not selective for the α_{2A} and α_{2B} subtypes in this tissue. Binding to α_2-adrenoceptors was shown to be critically dependent on the relative and absolute stereochemistry of these molecules. This series of compounds provides information on the structural requirements for α_2-adrenoceptor binding that is complementary to that previously reported for the yohimbine alkaloids.[10]

Functional α_2-adrenoceptor antagonism for these high affinity compounds was confirmed in vitro in the guinea pig ileum. Of the analogues reported in this paper, **1b** was found to be the most potent central α_2-adrenoceptor antagonist in vivo in the rat upon oral administration.[33] In addition to its low α_1-adrenoceptor affinity, **1b** has low affinity ($pK_i \leq 6.5$) at β_1- and β_2-adrenergic, D_1- and D_2-dopaminergic, and 5-HT_{1A}, and 5-HT_2 receptors.[34] This agent should be a useful tool for defining the pharmacological roles of α_2-adrenoceptors and the clinical potential of this class of α_2-adrenoceptor antagonist.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Silica gel chromatography was

(29) Pertinent to this point is the high affinity of 4 (pK_i 8.7[5]), a despyrollo analogue of rauwolscine which lacks the carbomethoxy group. Enhanced binding of the (methylenedioxy)-phenyl moiety of 4 relative to the indole of rauwolscine may compensate for the absence of the carbomethoxy dipole–dipole interaction.

(30) Drew, G. M. *Br. J. Pharmacol.* **1978**, *64*, 293.

(31) Berridge, T. L.; Gadie, B.; Roach, A. G.; Tulloch, I. F. *Br. J. Pharmacol.* **1983**, *78*, 507.

(32) Evidence for this was obtained from an in vitro rat liver microsomal preparation in which the rate of degradation of test compounds was measured. Rate constants of 4.2, 0.56, 0.21, and 0.0 h^{-1} were determined for **39**, **1**, **68**, and **64**, respectively. These rate constants are in the same rank order as the corresponding oral/iv ratios. Rush, R. Unpublished results.

(33) Additional in vivo activity of **1b** has been reported: Brown, C. M.; Clague, R. U.; Kilpatrick, A. T.; MacKinnon, A.; Martin, A. B.; Spedding, M. *Br. J. Pharmacol.* **1990**, *98*, 272P.

(34) Clark, R.; Spedding, M.; MacFarlane, C. B. *Br. J. Pharmacol.* **1990**, *99*, 123P.

performed with 70–230 mesh (Merck) silica gel. Medium-pressure chromatography was performed with 230–400 mesh Merck Kieselgel. Microanalyses were performed by the Syntex Analytical Department and where analyses are indicated only by symbols of the elements, results obtained were within ±0.4% (for C, H, N) of the theoretical values unless otherwise noted.

^1H and ^{13}C NMR spectra were measured on a Bruker WM 300 and AM 500 spectrometer in $CDCl_3$ or Me_2SO-d_6 solution referenced to internal tetramethylsilane. Proton assignments were confirmed by ^1H–^1H homonuclear shift correlation experiments and ^1H–^{13}C heteronuclear shift correlation was used to assign the protonated carbons in the ^{13}C NMR spectra.

5,8,13,13a-Tetrahydro-8-oxo-6*H*-isoquino[2,1-*g*][1,6]naphthyridine (10, X = H). A solution of LDA was prepared at −70 °C by addition of 62.5 mL (100 mmol) of 1.6 M *n*-butyllithium in hexane to 14 mL (100 mmol) of diisopropylamine in 300 mL of THF. A solution of *N,N*-diethyl-2-methylnicotinamide (**9**)[35] (19.2 g, 100 mmol) in 50 mL of THF was added at such a rate as to maintain the internal temperature below −60 °C. A solution of 3,4-dihydroisoquinoline[36] (**8**, X = H) (13.1 g, 100 mmol) in 50 mL of THF was added and the dark mixture was allowed to warm to −20 °C. The resulting semisolid mixture was treated with 5% aqueous HCl (250 mL), and the layers were separated. The aqueous layer was basified with NH_4OH and extracted 2× with ether. The ether extracts were combined, dried (Na_2SO_4), and evaporated to a residue which was purified by medium-pressure chromatography (EtOAc) to afford 14.5 g (58%) of **10** (X = H): mp 72–73 °C (ether); IR (KBr) 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.84–3.10 (m, 3 H), 3.16 (dd, 1 H, J = 13.6, 16.1 Hz, H-13_{ax}), 3.56 (dd, 1 H, J = 3.9, 16.1 Hz, H-13_{eq}), 4.90 (m, 2 H), 5.06 (dd, 1 H, J = 3.9, 13.6 Hz, H-13a), 7.20–7.40 (m, 5 H), 8.40 (dd, 1 H, J = 1.7, 7.8 Hz, H-9), 8.64 (dd, 1 H, J = 1.7, 4.8 Hz, H-11).

5,8,13,13a-Tetrahydro-3-methoxy-8-oxo-6*H*-isoquino[2,1-*g*][1,6]naphthyridine (10, X = 3-CH_3O). A solution of LDA (500 mmol) was prepared as above and cooled to −70 °C. A solution of **9** (96 g, 500 mmol) and 6-methoxy-3,4-dihydroisoquinoline[36] (**8**, X = 6-CH_3O) (80.5 g, 500 mmol) in 200 mL of THF was added over 15 min. The resulting dark solution was allowed to warm to −25 °C over 30 min at which point a semisolid suspension had formed. A solution of 200 mL of concentrated HCl in 500 mL of water was slowly added, and the layers were separated. The aqueous layer was basified with NH_4OH and extracted 2× EtOAc. The dried (Na_2SO_4) EtOAc extract was concentrated in vacuo and the resulting semisolid was treated with ether. Filtration afforded 93 g (66%) of **10** (X = 3-CH_3O). An additional 25 g (84% total yield) of product was obtained by medium-pressure chromatography (EtOAc) of the mother liquor: mp 115–116 °C; IR (KBr) 1640 cm^{-1}; ^1H NMR ($CDCl_3$) 2.85 (m, 1 H), 3.00 (m, 2 H), 3.16 (dd, 1 H, J = 13.8, 16.4 Hz, H-13_{ax}), 3.52 (dd, 1 H, J = 3.8, 16.4 Hz, H-13_{eq}), 3.82 (s, 3 H), 4.90 (m, 2 H), 5.00 (dd, 1 H, J = 3.8, 13.8 Hz, H-13a), 6.75 (d, 1 H, J = 2.6 Hz, H-4), 6.86 (dd, 1 H, J = 2.6, 8.6 Hz, H-2), 7.20 (d, 1 H, J = 8.6 Hz, H-1), 7.36 (dd, 1 H, J = 4.9, 7.8 Hz, H-10), 8.38 (dd, 1 H, J = 1.6, 7.8 Hz, H-9), 8.64 (dd, 1 H, J = 1.6, 4.9 Hz, H-11).

(8aα,12aα,13aα)-, (8aβ,12aβ,13aα)-, (8aβ,12aα,13aα)-5,8,8a,9,10,11,12,12a,13,13a-Decahydro-8-oxo-6*H*-isoquino[2,1-*g*][1,6]naphthyridine (11,12,13, X = H). A mixture of **10** (X = H) (37 g, 148 mmol) and 9.25 g of 5% rhodium on alumina in 350 mL of acetic acid was shaken under 50 psi of hydrogen for 48 h. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. The residue was partitioned between 200 mL of 2 N Na_2CO_3 and dichloromethane, and the organic layer was dried ($MgSO_4$) and evaporated to 38.8 g of crude product. TLC analysis (5% MeOH–CH_2Cl_2, 0.1% NH_4OH) showed two major products (R_f 0.5 and 0.3) and a minor product (R_f 0.4). The three components were separated by medium-pressure chromatography (5–10% MeOH–CH_2Cl_2, 0.1% NH_4OH).

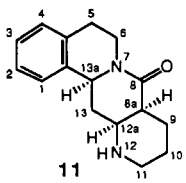
The first component eluted was the 8aβ,12aβ,13aα isomer **12** (X = H): 10 g (26%), mp 105–106 °C; IR (KBr) 3400, 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40–1.66 (m, 3 H, H-9_{ax},10,10), 1.90 (ddd, 1 H, J = 2.4, 12, 12 Hz, H-13_{ax}), 2.42 (ddd, 1 H, J = ca. 2, 2, 2 Hz, H-8a), 2.54–2.80 (m, 5 H, H-13_{eq},9_{eq},11_{ax},5_{ax}), 3.92 (m, 2 H, H-5_{eq},6_{ax}), 3.14 (m, 1 H, H-11_{eq}), 3.26 (ddd, 1 H, J = 2.9, 2, 2 Hz, H-12a), 4.85 (m, 1 H, H-6_{eq}), 5.02 (dd, 1 H, J = 5.1, 12 Hz, H-13a), 7.10–7.25 (m, 4 H); MS m/e 256 (M^+), 239, 211, 198, 146. Anal. ($C_{16}H_{20}N_2O$) C, H, N.

The second component eluted was the 8aβ,12aα,13aα isomer **13** (X = H): 1.5 g (4%); oil; ^1H NMR ($CDCl_3$) δ 1.30 (m, 2 H), 1.60 (m, 2 H), 1.82 (m, 2 H), 2.06 (ddd, 1 H), 2.48 (m, 1 H), 2.60–3.00 (m, 4 H), 3.16 (m, 1 H), 3.50 (br s, NH), 4.75 (m, 2 H), 7.10–7.25 (m, 4 H); MS m/e 256 (M^+), 228, 198, 146, 132.

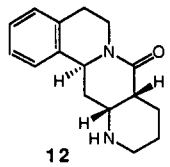
The third component eluted was the 8aα,12aα,13aα isomer **11** (X = H): 20 g (53%); mp 91–92 °C; ^1H NMR ($CDCl_3$) δ 1.58 (m, 1 H), 1.72 (m, 1 H), 1.90 (m, 2 H), 2.00 (m, 1 H, H-13_{ax}), 2.60 (ddd, 1 H, J = 5, 5, 13.4 Hz, H-13_{eq}), 2.68 (ddd, 1 H, J = 5, 5, 5 Hz, H-8a), 2.70–3.00 (m, 5 H), 3.56 (ddd, 1 H, J = 5, 5, 9.5 Hz, H-12a), 3.75 (s, NH), 4.70 (m, 2 H, H-6_{eq},13a), 7.12–7.26 (m, 4 H); MS m/e 256 (M^+), 228, 200, 146, 132. Anal. ($C_{16}H_{20}N_2O$) C, H, N.

^{13}C NMR spectral data for **11**, **12**, and **13** are presented in Table V.

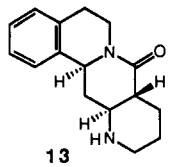
Table V. ^{13}C NMR Data for 11–13[a]



11



12



13

carbon	^{13}C chemical shift (multiplicity), δ		
	11	12	13
1	125.36* (d)	124.43* (d)	124.60* (d)
2	126.67* (d)	126.41* (d)	126.68* (d)
3	126.87* (d)	126.47* (d)	126.95* (d)
4	129.00* (d)	128.97* (d)	129.06* (d)
4a	134.97 (s)	135.31 (s)	134.95 (s)
5	29.03 (t)	29.16 (t)	28.86 (t)
6	39.33 (t)	36.61 (t)	36.78 (t)
8	171.33 (s)	170.06 (s)	169.31 (s)
8a	41.27 (d)	41.15 (d)	46.29 (d)
9	24.48 (t)	25.52 (t)	25.98 (t)
10	24.22 (t)	22.89 (t)	25.87 (t)
11	42.45 (t)	46.76 (t)	46.18 (t)
12a	50.21 (d)	51.66 (d)	54.96 (d)
13	34.67 (t)	39.62 (t)	39.92 (t)
13a	54.38 (d)	52.69 (d)	55.28 (d)
13b	136.45 (s)	138.14 (s)	137.00 (s)

*May be interchanged. [a]Spectra were run in $CDCl_3$.

(8aα,12aα,13aα)-5,8,8a,9,10,11,12,12a,13,13a-Decahydro-3-methoxy-8-oxo-6*H*-isoquino[2,1-*g*][1,6]naphthyridine (11, X = 3-CH_3O). Catalytic hydrogenation of **10** (X = 3-CH_3O) (96 g, 343 mmol) with 20 g of 5% rhodium on alumina in 750 mL of acetic acid as described above afforded 46 g (47%) of **11** (X = 3-CH_3O) after chromatographic separation from the other diastereomers, mp 118–119 °C. Anal. ($C_{17}H_{22}N_3O_2$) C, H, N.

(8aα,12aα,13aα)-, (8aβ,12aβ,13aα)-, and (8aβ,12aα,13aα)-5,8,8a,9,10,11,12,12a,13,13a-Decahydro-3-methoxy-12-(methylsulfonyl)-6*H*-isoquino[2,1-*g*][1,6]naphthyridine (1, 55, and 56). Hydrogenation of 50 g (178 mmol) of **10** (X = 3-CH_3O) and 12.5 g of 5% rhodium on alumina in 300 mL of acetic acid as described above afforded 51 g of crude product. A solution of this material in 250 mL of THF was added dropwise to a stirred suspension of lithium aluminum hydride (6 g, 158 mmol) in 300 mL of THF. After the addition was complete the resulting solution was heated under reflux for 30 min. The mixture was cooled to room temperature and carefully treated with 6 mL of water, 6 mL of 15% NaOH, and 18 mL of water. Filtration and evaporation afforded ca. 50 g of a thick oil. This material was dissolved in 800 mL of CH_2Cl_2 and 35 mL of triethylamine and the resulting solution was cooled in an ice bath. Methanesulfonyl chloride (17 mL, 220 mmol) was added over 5 min, and the mixture was stirred at room temperature for 30 min. The mixture was added to water and the CH_2Cl_2 was separated and concentrated in vacuo. The

(35) Baumgarten, P.; Dornow, A. *Ber. Dtsch. Chem. Ges. B.* **1939**, *72*, 563.

(36) Prepared by Bischler–Napieralski cyclization of the formamide of the requisite phenethylamine: Whaley, W. M.; Grovindachari, T. R. *Org. React.* **1951**, *6*, 74.

residue was partitioned between dilute aqueous HCl and EtOAc. The aqueous layer was basified with NH_4OH and extracted 2× with CH_2Cl_2. The CH_2Cl_2 was concentrated in vacuo and TLC analysis (EtOAc) indicated two major products at R_f 0.8 and 0.1 and a minor product at R_f 0.2.

Purification by medium-pressure chromatography (50% EtOAc–hexane) afforded 20 g (32% overall) of 1[37] which was converted to the HCl salt in EtOH–ether: ^{1}H NMR (Me_2SO-d_6) 1.60 (m, 2 H), 1.70 (m, 1 H), 2.10–2.40 (m, 3 H), 2.90 (m, 3 H), 3.08 (s, 3 H), 3.36 (s, exchanges with D_2O), 3.30–3.70 (m, 6 H), 3.76 (s, 3 H), 4.34 (m, 1 H), 4.52 (m, 1 H), 6.80 (d, 1 H, J = 2.5 Hz), 6.84 (dd, 1 H, J = 2.5, 8.7 Hz), 7.34 (d, 1 H, J = 8.7 Hz).

Further elution with EtOAc afforded 1.5 g (2.4%) of the trans isomer **56** and 9.0 g (15%) of isomer **55**.[37]

(8aβ,12aα,13aα)-5,8,8a,9,10,11,12,12a,13,13a-Decahydro-12-(methylsulfonyl)-6*H*-isoquino[2,1-*g*][1,6]naphthyridine (15). To a solution of **10** (X = H) (2.5 g, 10 mmol) in 100 mL of THF was added 15 mL of 1 M borane–THF and the resulting solution was heated under reflux for 4 h. Methanol (5 mL) was cautiously added to the cooled mixture and solvents were evaporated in vacuo. The residue was suspended in 50 mL of 5% HCl and stirred at 80 °C for 1 h to give a clear solution. This solution was cooled to room temperature, washed with EtOAc, and basified with NH_4OH. The resulting suspension was extracted 2× with EtOAc (insoluble residue was filtered off after initial addition of EtOAc) and the combined EtOAc was dried (Na_2SO_4) and evaporated to afford 1.2 g (51%) of pyridine **14** as an oil. A dihydrochloride salt was prepared from CH_3OH–ether: mp 240–242 °C. Anal. ($C_{16}H_{16}N_2$·2HCl·H_2O) C, H, N.

A solution of the free base of **14** (1.1 g, 4.7 mmol) in 50 mL of EtOH was heated under reflux and 5 g of sodium was gradually added along with 50 mL of additional EtOH. After all the sodium had reacted the mixture was partitioned between water and CH_2Cl_2. The CH_2Cl_2 layer was washed with water and dried (Na_2SO_4). Triethylamine (1 mL) was added followed by 1 mL (13 mmol) of methanesulfonyl chloride. After 30 min the mixture was concentrated in vacuo, and the residue was taken up in dilute HCl and washed with EtOAc. The HCl layer was basified with NH_4OH and extracted with EtOAc. After drying (Na_2SO_4) the EtOAc was evaporated and purification of the residue by chromatography (5% MeOH–CH_2Cl_2) afforded 0.1 g (7%) of **15**. This was identical (TLC, NMR) with material prepared from intermediate **13** (X = H) in Scheme I. Neither of the other diastereomers were present (TLC) in the crude product.

(8a*R*,12a*R*,13a*R*)- and (8a*S*,12a*S*,13a*S*)-5,8,8a,9,10,11,12,12a,13,13a-Decahydro-3-methoxy-12-[(*R*)-(1-phenylethyl)carbamoyl]-8-oxo-6*H*-isoquino[2,1-*g*][1,6]naphthyridine (16a and 16b). To a solution of racemic amine **11** (X = 3-CH_3O) (30 g, 105 mmol) in 250 mL of CH_2Cl_2 was added (+)-(*R*)-(1-phenylethyl) isocyanate (15.4 g, 105 mmol). After 30 min at room temperature the solution was concentrated in vacuo. TLC analysis showed the diastereomers at R_f 0.5 and 0.36 (10% CH_3OH–EtOAc). Medium-pressure chromatography afforded ca. 12 g each of the two products along with mixed fractions.

The less polar isomer was **16b**: mp 198–199 °C; ^{1}H NMR ($CDCl_3$) δ 1.50 (m, 2 H), 1.52 (d, 3 H, J = 7 Hz), 1.74 (m, 1 H), 2.02 (m, 1 H), 2.02 (m, 1 H), 2.16 (m, 1 H), 2.36 (m, 1 H), 2.62 (m, 1 H), 2.70 (m, 1 H), 2.80–2.95 (m, 3 H), 3.56 (m, 1 H), 3.78 (s, 3 H), 4.65–4.80 (m, 3 H), 4.88 (d, 1 H, J = 7 Hz), 5.05 (dq, 1 H, J = 7, 7 Hz), 6.64 (d, 1 H, J = 2.6 Hz), 6.78 (dd, 1 H, J = 2.6, 8.7 Hz), 7.10 (d, 1 H, J = 8.7 Hz), 7.25 (m, 1 H), 7.35 (m, 4 H); $[\alpha]^{25}_D$ +36.6° (*c* 0.3, $CHCl_3$). Anal. ($C_{26}H_{31}N_3O_3$) C, H, N.

The more polar isomer was **16a**: mp 220–221 °C; ^{1}H NMR ($CDCl_3$) 1.50 (m, 2 H), 1.52 (d, 3 H, J = 7 Hz), 1.74 (m, 1 H), 2.02 (m, 1 H), 2.16 (m, 1 H), 2.34 (m, 1 H), 2.60–2.74 (m, 2 H), 2.80–2.95 (m, 3 H), 3.54 (m, 1 H), 4.64–4.84 (m, 4 H), 5.04 (dq, 1 H, J = 7, 7 Hz), 6.64 (d, 1 H, J = 2.7 Hz), 6.78 (dd, 1 H, J = 2.7, 8.7 Hz), 7.08 (d, 1 H, J = 8.7 Hz), 7.25 (m, 1 H), 7.35 (m, 4 H); $[\alpha]^{25}_D$ −11.4° (*c* 0.3, $CHCl_3$). Anal. ($C_{26}H_{31}N_3O_3$) C, H, N.

(8a*R*,12a*S*,13a*S*)-5,8,8a,9,10,11,12,12a,13,13a-Decahydro-3-methoxy-6*H*-isoquino[2,1-*g*][1,6]naphthyridine (17b). A solution of **16b** (11.5 g, 26.5 mmol) in 50 mL of THF was slowly added to a stirred suspension of LAH (2 g, 53 mmol) in 150 mL of THF. The mixture was heated under reflux for 2 h and cooled, and 2 mL of water, 2 mL of 15% NaOH, and 6 mL of water were added. After filtration the solvents were removed in vacuo to afford the 1-(phenylethyl)urea derivative of **17b** as a foam. This material was dissolved in 125 mL of 2 N NaOBu in *n*-BuOH and heated under reflux for 4 h. The cooled mixture was diluted with water, acidified with HCl, and washed 2× with EtOAc. The aqueous layer was basified with NH_4OH and extracted 5× with CH_2Cl_2. The combined CH_2Cl_2 extract was evaporated, and the residue was purified by silica gel chromatography (15% MeOH–CH_2Cl_2, 0.1% NH_4OH) to afford 5.3 g (74%) of **17b** as a thick oil: $[\alpha]^{25}_D$ −151° (*c* 0.7, MeOH). Anal. ($C_{17}H_{24}N_2O$) C, H, N.

Compound **17a** was similarly prepared (77%): mp 125–127 °C; $[\alpha]^{25}_D$ +155° (*c* 0.5, MeOH). Anal. ($C_{17}H_{24}N_2O$) C, H, N.

(8a*S*,12a*R*,13a*R*)- and (8a*R*,12a*S*,13a*S*)-5,8,8a,9,10,11,12,12a,13,13a-Decahydro-3-methoxy-12-(methylsulfonyl)-6*H*-isoquino[2,1-*g*][1,6]naphthyridine (1a and 1b). Mesylation of **17a** as described for the racemate afforded **1a**. Free base: mp 165–166 °C; $[\alpha]^{25}_D$ +56° (*c* 0.3, $CHCl_3$). Anal. ($C_{18}H_{26}N_2O_3S$) C, H, N. HCl salt: $[\alpha]^{25}_D$ −13° (*c* 0.4, MeOH).

Compound **1b** was similarly obtained. Free base: mp 165–166 °C; $[\alpha]^{25}_D$ −56° (*c*, 0.3, $CHCl_3$). Anal. ($C_{18}H_{26}N_2O_3S$) C, H, N. HCl salt: $[\alpha]^{25}_D$ +13° (*c* 0.3, MeOH).

Determination of the Enantiomeric Purities of 1a and 1b. HPLC analysis of the enantiomers was done with a 100× 4-mm Chiral AGP, α_1-acid glycoprotein 5 μM column (LKB). The mobile phase was 2-propanol–phosphate buffer (pH 7) 14:86 v/v and the flow rate was 0.5 mL/min. The retention times for **1a** and **1b** were 4.7 and 7.5 min, respectively. The detection limit was ca. 1%.

Both compounds were found to be enantiomerically pure.

(8aα,12aα,13aα)-5,8,8a,9,10,11,12,12a,13,13a-Decahydro-12-[(2-hydroxyethyl)sulfonyl]-6*H*-isoquino[2,1-*g*][1,6]-naphthyridine (47). Reduction of **11** (X = H) with LAH followed by treatment with (2-methoxyethyl)sulfonyl bromide afforded **48**. To a −70 °C solution of **48** (0.8 g, 2.2 mmol) in 25 mL of CH_2Cl_2 was added 2 mL of 1 M BBr_3 in CH_2Cl_2. The mixture was allowed to warm to room temperature over 2 h. Water was added and the mixture was basified with NH_4OH and extracted with CH_2Cl_2. The CH_2Cl_2 was evaporated, and the residue was purified by silica gel chromatography (2% MeOH–CH_2Cl_2, 0.1% NH_4OH) to afford 0.7 g (90%) of the free base of **47** as an oil. The HCl salt was prepared from EtOH–ether: ^{1}H NMR (Me_2SO-d_6) δ 1.40–1.60 (m, 2 H), 1.70 (m, 1 H), 2.14–2.40 (m, 3 H), 2.56 (m, 1 H), 2.84–3.12 (m, 2 H), 3.34 (dt, 2 H, CH_2OH), 3.30–3.70 (m, 7 H, includes OH), 3.80 (t, 2 H, J = 7 Hz), 4.32 (m, 1 H), 4.62 (br dd, 1 H, collapses with D_2O to br d, J = 9 Hz), 7.20–7.40 (m, 4 H), 9.90 (br s, exchanges with D_2O).

(8aα,12aα,13aα)-5,8,8a,9,10,11,12,12a,13,13a-Decahydro-3-hydroxy-12-(methylsulfonyl)-6*H*-isoquino[2,1-*g*][1,6]-naphthyridine (73). A solution of 1·HCl (1.0 g, 2.6 mmol) in 25 mL of 48% HBr was stirred at 80 °C for 6 h. The mixture was concentrated in vacuo, diluted with water, and neutralized with NH_4OH. Extraction 3× with CH_2Cl_2 and evaporation of the extracts afforded a residue that was purified by silica gel chromatography (7% MeOH–CH_2Cl_2, 0.1% NH_4OH) to afford the free base of **73**. The HCl salt was prepared from EtOH–HCl, 0.4 g (42%): ^{1}H NMR (Me_2SO-d_6) δ 1.40–1.60 (m, 2 H), 1.72 (m, 1 H), 2.14–2.34 (m, 3 H), 2.48 (m, 1 H), 3.16 (s, 3 H), 3.30–3.70 (m, 6 H), 4.35 (m, 1 H), 4.48 (br dd, 1 H, J = ca. 9, 9 Hz, collapses to br d with D_2O, H-13a), 6.62 (d, 1 H, J = 2.6 Hz), 6.74 (dd, 1 H, J = 2.6, 8.7 Hz), 7.18 (d, 1 H, J = 8.7 Hz), 9.60 and 9.85 (br s, 1 H, exchanges with D_2O).

(8aα,12aα,13aα)-12-(Aminosulfonyl)-5,8,8a,9,10,11,12,-12a,13,13a-decahydro-3-methoxy-6*H*-isoquino[2,1-*g*][1,6]-naphthyridine (78). A solution of **11** (X = 3-CH_3O) (1.1 g, 4 mmol) in 20 mL of CH_2Cl_2 and 2 mL of triethylamine was cooled in an ice bath and (*tert*-butylamino)sulfonyl chloride (0.8 g, 5 mmol) was added. After stirring for 3 h at room temperature the mixture was diluted with additional CH_2Cl_2 (50 mL) and washed with dilute NH_4OH. The CH_2Cl_2 was concentrated in vacuo, and the residue was purified by medium-pressure chromatography (60% EtOAc–hexane) to afford 1.0 g (63%) of **79** as a foam. A solution of **79** (0.8 g, 2 mmol) in 5 mL of trifluoroacetic acid was stirred at room temperature for 12 h. The mixture was concen-

(37) Detailed ^{1}H and ^{13}C NMR data for this compound have been reported.[16]

trated in vacuo, and the residue was dissolved in water. The resulting solution was neutralized with NH_4OH and extracted with CH_2Cl_2. The CH_2Cl_2 was dried (Na_2SO_4) and evaporated to a crystalline residue which was redissolved in CH_2Cl_2 and treated with EtOH–HCl. The resulting solid was filtered and washed with ether to afford 0.6 g (78%) of the HCl salt of **78**: ^1H NMR (Me_2SO-d_6) δ 1.52 (m, 2 H), 1.70 (m, 1 H), 2.10–2.30 (m, 3 H), 2.76 (m, 1 H), 2.95 (m, 1 H), 3.30–3.70 (m, 7 H), 3.77 (s, 3 H), 4.26 (m, 1 H), 4.50 (br dd, 1 H, J = ca. 9, 9 Hz, collapses to br d with D_2O, H-13a), 6.78 (d, 1 H, J = 2.6 Hz), 6.84 (dd, 1 H, J = 2.6, 8.7 Hz), 6.92 (br s, 2 H, exchanges with D_2O, NH_2), 7.37 (d, 1 H, J = 8.7 Hz), 9.90 (br s, 1 H, exchanges with D_2O).

(8aα,12aα,13aα)-5,8,8a,9,10,11,12,12a,13,13a-Decahydro-11-(methylsulfonyl)-6*H*-isoquino[2,1-*b*][2,6]naphthyridine (20). Hydrogenation of **19** followed by LAH reduction and mesylation of the crude product as described above afforded a mixture which by TLC analysis contained a major product with several more polar minor products. Silica gel chromatography (2% MeOH–CH_2Cl_2) gave the free base of **20** (20% overall yield): ^1H NMR ($CDCl_3$) δ 1.50 (m, 1 H), 1.75 (m, 1 H), 1.94–2.14 (m, 3 H), 2.30–2.70 (m, 5 H), 2.74 (s, 3 H), 2.75–2.90 (m, 3 H), 3.14 (m, 2 H), 3.68 (dd, 1 H, J = 1, 12 Hz, H-13a), 3.80 (m, 1 H), 7.05–7.20 (m, 4 H). HCl salt: δ 4.52 (br t, 1 H, collapses to br d, J = 10.5 Hz, with D_2O, H-13a).

(8aα,12aα,13aα)-5,8,8a,9,10,11,12,12a,13,13a-Decahydro-10-(methylsulfonyl)-6*H*-isoquino[2,1-*b*][2,7]naphthyridine (23). Addition of lithiated 3-cyano-4-methylpyridine (**21**) to the trimethylsilyl trifluoromethanesulfonate complex of imine **8** according to the reported general procedure[21] afforded amidine **22a** which was subsequently hydrolyzed to amide **22b**. Hydrogenation, LAH reduction, and mesylation afforded the free base of **23** (31% overall yield): mp 108–110 °C: ^1H NMR ($CDCl_3$) δ 1.78 (m, 1 H), 1.90 (m, 1 H), 2.10 (m, 1 H), 2.30 (m, 2 H), 2.46 (m, 1 H), 2.72 (m, 1 H), 2.86 (s, 3 H), 2.96 (m, 1 H), 3.30–3.75 (m, 7 H), 4.52 (br dd, 1 H, collapses to d with D_2O, J = 9, 9 Hz, H-13a), 7.20–7.44 (m, 4 H), 10.0 (br s, 1 H, exchanges with D_2O).

5,6,7,8-Tetrahydro-5-oxo-6-(2-phenylethyl)-1,6-naphthyridine (25). To a −70 °C solution of (cyanomethyl)amine **24** (2.0 g, 12.7 mmol) in 100 mL of THF was added 7.9 mL (12.7 mmol) of 1.6 M *n*-BuLi in hexane, and the resulting solution was stirred for 1 h at −70 °C. In a separate flask, a −70 °C solution of LDA (22 mmol) was prepared and treated dropwise with a solution of amide **9** (3.9 g, 20 mmol) in 30 mL of THF. The dark purple solution of the lithiated nicotinamide was then transferred via cannula into the first solution. The mixture was stirred at −70 °C for 1 h and allowed to warm to room temperature and stirred an additional 12 h. Saturated NH_4Cl was added and the mixture was extracted with EtOAc. The EtOAc was washed with water, dried ($MgSO_4$), and evaporated. Silica gel chromatography (3% MeOH–CH_2Cl_2) of the residue afforded 0.9 g (30% based on **24**) of **25**: ^1H NMR ($CDCl_3$) δ 2.98 (t, 2 H, J = 7 Hz), 3.05 (t, 2 H, J = 7 Hz), 3.47 (t, 2 H, J = 7 Hz), 3.80 (t, 2 H, J = 7 Hz), 7.20–7.35 (m, 6 H), 8.34 (dd, 1 H, J = 1.8, 7.8 Hz), 8.58 (dd, 1 H, J = 1.8, 5 Hz).

(4aα,8aα)-1,2,3,4,4a,5,6,7,8,8a-Decahydro-1-(methylsulfonyl)-6-(2-phenylethyl)-1,6-naphthyridine (26). Hydrogenation of **25** followed by LAH reduction and mesylation afforded **26** (36% overall yield): ^1H NMR ($CDCl_3$) δ 1.55 (m, 3 H), 1.75 (m, 1 H), 1.85 (m, 1 H), 1.95–2.25 (m, 4 H), 2.45–2.60 (m, 2 H), 2.70–2.85 (m, 2 H), 2.85 (s, 3 H), 2.95 (m, 3 H), 3.64 (m, 1 H), 3.95 (m, 1 H), 7.16–7.32 (m, 5 H).

(4aα,7α,8aα)- and (4aβ,7α,8aβ)-1,2,3,4,4a,5,6,7,8,8a-Decahydro-6-methyl-7-[3,4-(methylenedioxy)phenyl]-1-(methylsulfonyl)-1,6-naphthyridine (28 and 29). Hydrogenation of **27**[12] followed by LAH reduction and mesylation afforded a mixture which was purified by silica gel chromatography (1% MeOH–CH_2Cl_2) to afford **28** as the major (less polar) product in 42% yield after conversion to the HCl salt from EtOH–HCl: ^1H NMR (Me_2SO-d_6) δ 1.40–1.60 (m, 2 H), 1.75 (m, 2 H), 2.15 (m, 1 H), 2.40 (d, 3 H, NCH_3), 2.64 (m, 1 H), 2.95 (s, 3 H), 3.02 (m, 1 H), 3.30–3.56 (m, 4 H), 4.18 (m, 1 H), 4.28 (m, 1 H), 6.08 (s, 2 H), 6.92 (s, 1 H), 6.96 (s, 2 H), 10.5 (br s, 1 H, exchanges with D_2O).

The minor (more polar) isomer **29** was also obtained (6% after conversion to the HCl salt).

3-[1,3,4,6,7,11bα-Hexahydro-9,10-(methylenedioxy)-2-oxo-2*H*-benzo[*a*]quinolizinyl]-(1β)-propionitrile (31). This was obtained as a minor product from the synthesis of the (racemic) corresponding 3-substituted isomer.[16] The methanol mother liquor from filtration of the major product was concentrated in vacuo and the residue was partitioned between CH_2Cl_2 and water. The CH_2Cl_2 was evaporated and the residue was crystallized from EtOAc to afford **31** in 5% yield: mp 186–187 °C; ^1H NMR ($CDCl_3$) δ 1.70 (m, 1 H, H-3′), 2.04 (m, 1 H, H-3′), 2.15 (m, 2 H, H-2′), 2.40 (m, 1 H, H-3_{ax}), 2.46 (m, 1 H, H-6_{ax}), 2.56 (m, 1 H, H-4_{ax}), 2.60 (m, 1 H, H-7_{ax}), 2.74 (m, 1 H, H-1), 2.80 (m, 1 H, H-3_{eq}), 3.0 (m, 2 H, H-6_{eq},7_{eq}), 3.20 (m, 1 H, H-4_{eq}), 3.60 (d, 1 H, J = 0.5 Hz, H-11b), 5.92 (AB, 2 H), 6.52 (s, 1 H), 6.62 (s, 1 H). Anal. ($C_{17}H_{18}N_2O_3$) C, H, N.

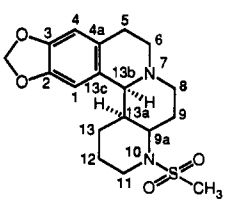
(9aα,13aα,13bα)- and (9aβ,13aα,13bα)-5,8,9,9a,10,11,12,13,13a,13b-Decahydro-2,3-(methylenedioxy)-10-(methylsulfonyl)-6*H*-isoquino[1,2-*g*][1,6]naphthyridine (32 and 33). A mixture of **31** (1.0 g, 3.4 mmol) and 5 g of Raney nickel in 150 mL of EtOH and 25 mL of THF was stirred under 1 atm of H_2 for 16 h. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel chromatography (5% MeOH–CH_2Cl_2, 1% NH_4OH) to afford 0.6 g (62%) of a foam. This material was homogeneous by TLC analysis but ^1H NMR indicated a diastereomeric mixture. This material (ca. 2.1 mmol) was converted to the mesylates as described above. Silica gel chromatography afforded 0.27 g (35%) of **32** as the less polar component: mp 191–193 °C (EtOH); ^1H NMR ($CDCl_3$) δ 1.18 (m, 1 H, H-13), 1.48 (m, 1 H, H-12), 1.65 (m, 3 H, H-9, 12, 13), 2.30 (m, 3 H, H-6, 8, 9), 2.40 (m, 1 H, H-13a), 2.52 (m, 1 H, H-5), 2.86 (m, 2 H, H-5, 6), 2.90 (s, 3 H), 2.98 (m, 1 H, H-8), 3.04 (m, 1 H, H-11), 3.28 (br s, 1 H, H-13b), 3.65 (m, 1 H, H-11), 4.12 (m, 1 H, H-9a), 5.89 (s, 1 H, J = 1.4 Hz), 6.53 (s, 1 H), 6.58 (s, 1 H). Anal. ($C_{18}H_{24}N_2O_4S$) C, H, N.

The second component eluted was **33** (0.19 g, 25%): mp 193–194 °C (EtOH); ^1H NMR ($CDCl_3$) δ 1.48 (m, 1 H, H-9), 1.66 (m, 1 H, H-13), 1.72 (m, 1 H, H-12), 1.84 (m, 1 H, H-12), 2.22 (m, 1 H, H-9), 2.28 (m, 1 H, H-13), 2.40 (m, 1 H, H-5), 2.45 (m, 1 H, H-13a), 2.62 (m, 1 H, H-8), 2.80 (m, 1 H, H-8), 2.83 (s, 3 H), 3.00 (m, 1 H, H-5), 3.04 (m, 1 H, H-11), 3.14 (m, 2 H, H-6, 6), 3.70 (m, 1 H, H-11), 3.86 (d, 1 H, J = 0.5 Hz, H-13b), 3.88 (m, 1 H, H-9a), 5.89 (d, 1 H, J = 1.5 Hz), 5.90 (d, 1 H, J = 1.5 Hz), 6.55 (s, 1 H), 6.81 (s, 1 H). Anal. ($C_{18}H_{24}N_2O_4S$) C, H, N.

^{13}C NMR spectral data for **32** and **33** are given in Table VI.

5,7,8,13,13b,14-Hexahydro-5-oxoindolo[2′,3′:3,4]pyrido[1,2-

Table VI. ^{13}C NMR Data for **32** and **33**[a]



32: 9aα-H
33: 9aβ-H

carbon	^{13}C chemical shift (multiplicity), δ	
	32	**33**
1	108.83 (d)	109.72 (d)
2	146.50* (s)	146.70* (s)
3	147.16* (s)	147.31* (s)
4	105.43 (d)	105.80 (d)
4a	129.71** (s)	129.11** (s)
5	30.54 (t)	23.83 (t)
6	52.48 (t)	51.48 (t)
8	55.81 (t)	44.97 (t)
9	24.71 (t)	23.79 (t)
9a	55.23 (d)	50.40 (d)
11	40.86 (t)	40.55 (t)
12	25.68 (t)	26.34 (t)
13	19.50 (t)	25.38 (t)
13a	40.68 (d)	37.49 (d)
13b	66.70 (d)	63.61 (d)
13c	129.84** (s)	128.95** (s)
SO_2Me	40.33 (q)	40.26 (q)
OCH_2O	101.44 (d)	101.40 (d)

*,** May be interchanged. [a] Spectra were run in $CDCl_3$.

g]-1,6-naphthyridine (36). To a stirred solution of 3,4-dihydro-β-carboline (0.63 g, 3.7 mmol) in 10 mL of THF at −65 °C was added 2.3 mL (3.7 mmol) of 1.6 M *n*-BuLi in hexane. Trimethylsilyl trifluoromethanesulfonate (1.43 mL, 7.4 mmol) was added, and the resulting mixture was stirred 15 min at −65 °C, 15 min at −20 °C, and 60 min at 0 °C. The solution was recooled to −65 °C, and a solution of amide **9** (0.88 g, 4.4 mmol) in 15 mL of THF was added. A previously prepared solution of LDA (from 0.62 mL of diisopropylamine and 2.78 mL of 1.6 M *n*-BuLi, 4.4 mmol) in 10 mL of THF was added via cannula. After 5 min, water was added and the mixture was warmed to room temperature and extracted with EtOAc. The EtOAc was dried ($MgSO_4$) and evaporated to 1.3 g (97%) of crude adduct **35**: ^{1}H NMR ($CDCl_3$) δ 1.05 and 1.25 (t, 3 H), 2.5–3.4 (m, 6 H), 3.10 and 3.50 (q, 2 H), 4.50 (br t, 1 H), 7.00–7.50 (m, 6 H), 8.60 (dd, 1 H), 9.80 (s, 1 H, NH).

A solution of adduct **35** (3.8 g, 10.5 mmol) in 40 mL of 1 M NaOBu in *n*-BuOH was heated under reflux for 3 h. The mixture was concentrated in vacuo, and the residue was partitioned between water and CH_2Cl_2. The CH_2Cl_2 was dried (Na_2SO_4) and evaporated. Crystallization of the residue from EtOAc afforded 1.1 g (36%) of **36**: mp 249–250 °C; ^{1}H NMR ($CDCl_3$) 3.00 (m, 3 H), 3.20 (dd, 1 H, J = 13.5, 15 Hz), 3.70 (dd, 1 H, J = 3, 15 Hz), 5.00–5.30 (m, 2 H), 7.00–7.60 (m, 5 H), 8.45 (dd, 1 H, J = 1.5, 7.5 Hz), 8.60 (dd, 1 H, J = 1.5, 6 Hz), 8.90 (br s, 1 H, NH): MS m/e 289 (M^+). Anal. ($C_{18}H_{15}N_3O$) C, H, N.

(4aα,13bα,14aα)- and (4aβ,13bα,14aα)-1,2,3,4,4a,5,7,8,13,13b,14,14a-Dodecahydro-1-(methylsulfonyl)indolo[2′,3′:3,4]pyrido[1,2-*g*]-1,6-naphthyridine (37 and 38). Catalytic hydrogenation, LAH reduction, and mesylation of **36** as described above furnished **37** (31% overall yield) and **38** (16%) after chromatographic separation (silica gel, 2% MeOH–CH_2Cl_2). The less polar isomer was **37**: foam; ^{1}H NMR ($CDCl_3$) δ 1.45–1.60 (m, 2 H, H-3, 4), 1.70 (m, 1 H, H-3), 1.92 (m, 1 H, H-4a), 2.05 (m, 2 H, H-4, 14), 2.14 (ddd, 1 H, J = 12, 12, 12 Hz, H-14$_{ax}$), 2.45 (m, 1 H, H-7), 2.56 (dd, 1 H, J = 3.3, 11.8 Hz, H-5), 2.68 (dd, 1 H, J = 4, 14 Hz, H-8), 2.80 (dd, 1 H, J = 1.7, 11.8 Hz, H-5), 2.80–3.00 (m, 3 H, H-2, 7, 8), 2.96 (s, 3 H), 3.14 (dd, 1 H, J = 1.8, 12 Hz, H-13b), 3.60 (m, 1 H, H-2), 4.14 (ddd, 1 H, J = 4, 4, 12 Hz, H-14a), 7.00–7.16 (m, 2 H), 7.32 (dd, 1 H, J = 1.3, 7.6 Hz), 7.42 (d, 1 H, J = 7.3 Hz), 8.30 (broad s, 1 H, NH).

The more polar isomer was **38**: foam; ^{1}H NMR ($CDCl_3$) δ 1.40–1.65 (m, 2 H), 1.70–1.90 (m, 2 H), 2.00–2.20 (m, 2 H), 2.55–2.80 (m, 3 H), 2.82 (s, 3 H), 3.00 (m, 2 H), 3.12 (m, 3 H), 3.50 (m, 1 H, H-2), 3.72 (m, 1 H, H-14a), 4.46 (br s, 1 H, H-13b), 7.06–7.20 (m, 2 H), 7.38 (dd, 1 H, J = 1.2, 7.6 Hz), 7.48 (d, 1 H, J = 7.3 Hz), 8.46 (br s, 1 H, NH).

X-ray Crystallography. X-ray crystallographic data were obtained on **1b**·HCl, $C_{18}H_{27}ClN_2O_3S$, FW = 386.93. Crystals were obtained from EtOH–ether. A clear, colorless prism, 0.4 × 0.4 × 0.4 mm was used for the structural determination. Preliminary diffraction photographs showed only triclinic symmetry, and accurate lattice constants of a = 6.0593 (11) Å, b = 7.9695 (14) Å, c = 11.134 (2) Å, α = 109.313 (14)°, β = 93.901 (15)°, and γ = 107.886 (13)° were determined from a least-squares fit of 15 diffractometer measured 2θ values. The space group was $P1$, Z = 1, V = 474.18 (15) Å^3, and D_{calcd} = 1.355 g/cm^{-3}. All unique diffraction maxima with 2θ ≤ 115° were collected on a computer controlled four-circle diffractometer (Nicolet, SHELXTL PLUS, MicroVax II) with graphite monochromated Cu Kα radiation (λ = 1.54184 A) and variable speed 2θ − θ scans. After correction for Lorentz, polarization, and background effects, 1455 of the 1550 independent reflections were judged observed. A phasing model was found by using direct methods, and full-matrix least-squares refinements with anisotropic heavy atoms and fixed isotropic riding hydrogens converged to give final R factors of R = 0.0373 and R_w = 0.055.

Adrenoceptor Binding Assays. Membrane Preparation. Male Sprague-Dawley rats (150–200 g) were killed by cervical dislocation: the brains were rapidly removed and dissected on ice. Cerebral cortices were homogenized in 25 volumes of Tris buffer (50 mM Tris-HCl, 5 mM EDTA; pH 7.4 at 4 °C) by using a polytron PT 10 tissue disrupter. The homogenate was then centrifuged at 38000*g* for 15 min. The pellet obtained was washed 3× by resuspension and centrifugation in Tris assay buffer (50 mM Tris-HCl, 0.5 mM EDTA; pH 7.4 at 4 °C). The final pellet was resuspended in assay buffer to a protein concentration of 1.0 mg/mL and stored under liquid nitrogen until required.

[^{3}H]Yohimbine Binding. Competition α_2-adrenoceptor binding assays were performed by incubating washed rat cerebral membranes (500 μg of protein) with 1.5 nM [^{3}H]yohimbine (New England Nuclear, 80.9 Ci/mmol) in the presence or absence of a range of 12 concentrations of the competing ligand in a total volume of 500 μL of Tris assay buffer (50 mM Tris HCl, 0.5 mM EDTA; pH 7.4 at 25 °C). Nonspecific binding was defined as the concentration of bound ligand in the presence of 10 μM phentolamine. Specific binding represented ~70% of total binding at 1.5 nM [^{3}H]yohimbine. Following equilibrium (30 min at 25 °C), bound radioactivity was separated from free by filtration through a Brandel cell harvester. Bound radioactivity on the glass fiber filters was determined by liquid-scintillation counting.

[^{3}H]Prazosin Binding. EDTA washed membranes (250 μg) were incubated with 0.5 nM [^{3}H]prazosin (New England Nuclear 85 Ci/mmol) for 30 min at 25 °C in Tris buffer (50 mM Tris-HCl, 0.5 mM EDTA; pH 7.4) in a volume of 0.5 mL. Nonspecific binding was defined by 10 μM phentolamine and represented ~15% of total binding.

Inhibition of specific ligand binding by the test compound was analyzed graphically to estimate the IC_{50} (concentration of test compound that displaced 50% specific binding). The inhibitory constant (K_i) was calculated from the IC_{50} by using the equation of Cheng and Prusoff.[38,39] Hill coefficients were essentially equal to unity for all compounds tested.

Functional α_2-Adrenoceptor Antagonism in the Guinea Pig Ileum. Ileum preparations were taken from female Dunkin-Hartley guinea pigs in the weight range of 250–300 g and set up in 30 mL isolated organ baths containing physiological Tyrode solution of the following composition, (mM): NaCl 136.89; KCl 2.68; $MgCl_2·6H_2O$ 1.05; $NaH_2PO_4·2H_2O$ 0.42; glucose 5.55; $NaHCO_3$ 11.9; $CaCl_2·6H_2O$ 1.8; gassed with 100% O_2 and maintained at 37 °C. An initial tension of 1 g was applied. The preparations were field stimulated at 0.1 Hz (1-ms pulse durations, supramaximal voltage) via a stainless steel electrode passing through the lumen. The resulting contractions were recorded isometrically on a LectroMed oscillograph. After an equilibrium period of 1 h an initial cumulative response curve to the agonist UK-14304 was obtained. The preparations were then washed thoroughly to remove all of the agonist and left to equilibrate for a further 40 min, after which, a second cumulative-response curve to the agonist was obtained and measured as the control. Preparations were treated with antagonists for 40 min prior to subsequent concentration–response curves to the agonist.

Reversal of Clonidine-Induced Mydriasis in the Rat. Male Sprague-Dawley rats were anesthetized with sodium pentobarbitol (60 mg/kg ip) and a catheter was inserted into the jugular vein for drug administration. A subcutaneous dose of clonidine (0.3 mg/kg) was administered and after 20 min the pupil diameter was measured. Increasing doses of test compound were administered at 10-min intervals and pupil diameter was measured every 5 min with an illuminated inspection glass with 7× magnification held close to the corneal surface. The dose required to reduce pupillary diameter to 50% of maximum induced by clonidine (IC_{50}) was determined as the mean of five determinations. For oral administration, compounds were administered by oral intubation. Increasing doses of test compound were given every 25 min and pupil diameter was measured 20 min post dose.

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(38) Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.

(39) The Cheng–Prusoff equation[38] is dependent on the K_D for the radioligand used and the accurate determination of the affinity of the test compound is limited to ca. 10-fold better than the K_D of the radioligand. The K_D for [^{3}H]yohimbine in our studies was 5.2 ± 0.9 nM. Therefore, for some of the compounds, e.g. **1b**, which are at this 10-fold limit the reported affinity may be an underestimation of the true affinity. This emphasizes the need for better α_2-adrenoceptor radioligands, a need that may be filled by compounds such as [^{3}H]**1b** Brown, C. M.; Spedding, M. Unpublished results.

Duyne, Cornell University, for the X-ray crystallographic data on compounds **16b** and **1b**, respectively. HPLC determinations by John Kern, Syntex Analytical Research, Palo Alto, and Isabelle Jullien, Syntex Research, France, are gratefully acknowledged. The manuscript was expertly prepared by Lani Russell and Jordan Singer.

Supplementary Material Available: X-ray structure of compound **16b** and tables of atomic coordinates, bond lengths, and bond angles for **16b** and **1b** (10 pages). Ordering information is given on any current masthead page.

Conformationally Defined Neurotransmitter Analogues. Selective Inhibition of Glutamate Uptake by One Pyrrolidine-2,4-dicarboxylate Diastereomer

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In order to determine the conformational requirements for binding of L-glutamate to the proteins involved in the process of neurotransmission, rigid analogues containing an embedded glutamate moiety have been prepared. These "conformer mimics", the pyrrolidine-2,4-dicarboxylates **4**, **7**, **11**, and **14**, were synthesized from commercially available *trans*-4-hydroxy-L-proline and *cis*-4-hydroxy-D-proline, and then were tested for their ability to inhibit the high-affinity transport of [^{3}H]-L-glutamate into synaptosomes and to block the binding of radioligands to the NMDA (*N*-methyl-D-aspartate), KA (kainate), and QA (quisqualate) glutamate neurotransmitter receptor sites. While none of the four analogues binds effectively to the excitatory receptors, the L-*trans*-isomer **7** is a potent and selective competitive inhibitor of L-glutamate transport. These results delineate a specific structural/conformational preference for binding to the uptake system that is distinct from that required for binding to the NMDA, KA, and QA receptors.

L-Glutamate is one of the major excitatory neurotransmitters in the mammalian brain, mediating much of the synaptic transmission in the central nervous system.[1] The process of synaptic transmission requires the interaction of glutamate with a variety of proteins, including receptors, transport systems, and enzymes. Although the pharmacological specificity of these sites has been characterized to some extent, a detailed understanding of the structural requirements of glutamate binding is lacking.[2] We have therefore attempted to assess preferred binding geometries by preparing a series of conformationally well-defined acidic amino acids that are designed to mimic different glutamate conformers. In this report we describe the enantioselective synthesis of the four diastereomeric pyrrolidine-2,4-dicarboxylates (2,4-PDCs), each of which contains an embedded glutamate moiety, and demonstrate that one is a potent and selective inhibitor of a transport system that acts to remove L-glutamate from the synaptic cleft.

Considerable evidence has firmly established the existence of several classes of postsynaptic receptors[1] through which the excitatory action of L-glutamate is mediated, as well as the presence of a high-affinity transport system[3] that is thought to terminate the resultant excitatory signal. In vivo, endogenous L-glutamate binds to each of these sites, while in vitro, various glutamate analogues exhibit selective affinities and thus can pharmacologically differentiate among individual receptor classes. Specifically, three major types of excitatory amino acid transmitter receptors have been distinguished by their selective interaction with various agonists (Figure 1): *N*-methyl-D-aspartate (NMDA), kainate (KA), and quisqualate (QA).[2] Despite the fact that these analogues have been invaluable in identifying and characterizing the various classes of transmitter receptors empirically, a unified model to explain the observed binding specificities in terms of specific molecular conformations has been elusive.

Unlike systems that depend upon rapid chemical degradation for transmitter signal termination, L-glutamate appears to be removed from the synaptic cleft by high-affinity transport. Again, little is known about the conformational requirements of substrate binding to this transport system, although several competitive inhibitors have been identified.[3] Interest in the functional characteristics of this uptake mechanism has dramatically increased with the recent finding that excessive levels of glutamate (as well as other excitatory agonists) are neurotoxic and may play a significant role in neurological disorders such as ischemia, hypoglycemia, epilepsy, Huntington's disease, and Alzheimer's disease.[4]

The fact that the receptors and the uptake system exhibit selective binding of various glutamate analogues implies that glutamate itself may bind to each in a characteristic conformation that is mimicked by the appropriate analogue. For glutamate in solution there are nine conformers within 2.5 kcal/mol of the ground state,[5] any of which might correspond to the bound form at a specific site.[6] In attempting to relate binding preferences to

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(1) For a review, see: Monaghan, D. T.; Bridges, R. J.; Cotman, C. W. *Annu. Rev. Pharmacol. Toxicol.* **1989**, *29*, 365.
(2) (a) Watkins, J. C.; Olverman, H. J. *Trends Neurosci.* **1987**, *10*, 265. (b) Watkins, J. C.; Krogsgaard-Larsen, P.; Honore, T. *Trends Pharmacol. Sci.* **1990**, *11*, 25.
(3) (a) Balcar, V. J.; Johnston, G. A. R. *J. Neurobiol.* **1972**, *3*, 295. (b) Balcar, V. J.; Johnston, G. A. R. *J. Neurochem.* **1972**, *19*, 2657. (c) Debler, E. A.; Lajtha, A. *Ibid.* **1987**, *48*, 1851.
(4) (a) Meldrum, B. *Clin. Sci.* **1985**, *68*, 113. (b) Wieloch, T. *Science* **1985**, *230*, 681. (c) Rothman, S. M.; Olney, J. W. *Ann. Neurol.* **1986**, *19*, 105. (d) Bridges, R. J.; Geddes, J.; Monaghan, D. T.; Cotman, C. W. In *Excitatory Amino Acids in Health and Disease*, Lodge, D., Ed.; Wiley & Sons, Limited: England, 1987; pp 321-335.
(5) Ham, N. S. *Molecular and Quantum Pharmacology*, Bergman, E., Pullman, B., Eds., D. Reidel Publishing Co.: Dordrecht-Holland, 1974; pp 261-268.
(6) It is of course possible that a substrate would bind in a conformation that does not correspond to a solution energy minimum; however, it is unfavorable to do so because relatively small deviations away from energy minima are energetically costly and would result in low binding energies.

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