described.[9] Briefly, reaction mixtures containing supercoiled DNA, topoisomerase I, and drug were incubated for 30 min at 37 °C followed by treatment with proteinase K to remove covalently bound enzyme. The resultant nicked DNA plasmids were separated from supercoiled and covalently closed circular relaxed DNA by ethidium bromide/agarose gel electrophoresis. Negatives of the gel photographs were scanned with a densitometer to quantitate DNA cleavage. The pH profile for topoisomerase I catalytic activity was determined by the relaxation of supercoiled pDPT2789 DNA as described,[20] substituting 50 mM HEPES buffer of the appropriate pH for Tris·HCl.

Growth-Inhibition Assay. L1210 murine leukemia cells were incubated at 37 °C for 60 min in the presence of various concentrations of drug. Treated cells were diluted into soft agar (0.6% noble agar, 20% fetal calf serum in Fischer's medium) and incubated for 7 days to permit development of macroscopic colonies. Colonies were stained with tetrazolium salts (0.1% for 1–2 days and enumerated with a Biotran III automatic totalizer (New Brunswick Scientific). Cloning efficiency of L1210 cells in this assay is 8–10%. The IC_{50} is the concentration of drug (in the soft agar during the 7-day incubation) that resulted in a 50% reduction in the number of cells that survive to form colonies.

(20) Liu, L. F. *Methods Enzymol.* **1983**, *100*, 133.

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Registry No. 1, 7689-03-4; **2a**, 69203-72-1; **2b**, 69181-16-4; **3**, 118514-58-2; **4**, 118514-59-3; **5**, 118514-60-6; **6**, 118514-61-7; **7**, 118514-62-8; **8**, 118514-63-9; **9**, 118514-64-0; **10**, 118514-65-1; (±)-**11**, 118514-66-2; (±)-**12**, 118514-67-3; **13**, 55854-89-2; (±)-**14**, 118514-68-4; **15**, 25387-67-1; **16**, 7688-64-4; (±)-**17**, 34141-35-0; **18**, 26862-74-8; **19**, 118514-69-5; **20**, 35903-44-7; CH_2=C(CH_2Br)C-$O_2C_2H_5$, 17435-72-2; topoisomerase I, 80449-01-0.

2*H*-[1]Benzopyrano[3,4-*b*]pyridines: Synthesis and Activity at Central Monoamine Receptors

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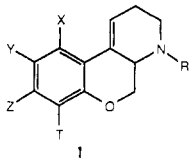
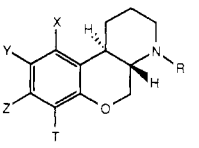
Two general synthetic approaches to a novel series of 2*H*-[1]benzopyrano[3,4-*b*]pyridines are described together with their receptor binding profile at a variety of monoamine receptors in mammalian brain tissue. The biologically active members of this series fall into into one of two broad classes: 3,4,4a,5-tetrahydro-2*H*-[1]benzopyrano[3,4-*b*]pyridines or *trans*-1,3,4,4a,5,10b-hexahydro-2*H*-[1]benzopyrano[3,4-*b*]pyridines. By appropriate pharmacophoric modification potent selective ligands for D_2, α-2, $5HT_{1A}$, and $5HT_2$ receptors may be obtained. The previously published in vivo data on certain key representatives of these series are also summarized.

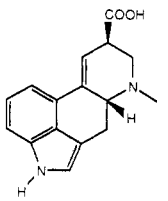
During the course of investigations directed toward the discovery of novel, direct-acting modulators of central monoamine receptor function, we have synthesized a series of 2*H*-[1]benzopyrano[3,4-*b*]pyridines and studied their binding to a variety of monoamine receptors in mammalian brain tissue. Most of the biologically active members of this series fall into one of two broad classes: 3,4,4a,5-tetrahydro-2*H*-[1]benzopyrano[3,4-*b*]pyridines such as **1** or *trans*-1,3,4,4a,5,10b-hexahydro-2*H*-[1]benzopyrano-[3,4-*b*]pyridines such as **2**. The former class of compounds may be viewed as tricyclic analogues of lysergic acid (**3**) in which the pyrrole ring has been eliminated and an oxygen has been introduced at the former 3-position of the indole moiety. Lysergic acid derivatives have a long history of central nervous system (CNS) activity. They are also known to have affinity for central monoamine receptors.[1] The hexahydro analogues **2** are structurally related to **4** (PHNO, MK 458)[2] and to the 1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinolines, which have been previously described by Cannon[3] and Carlsson.[4] All of these derivatives are rigid structures that lock the phenethylamine pharmacophore in the extended presumed bioactive conformation.[5] It was envisioned that such templates could be



1



2



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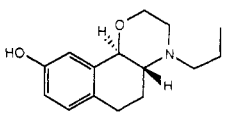

4

modified to give selective binders to a variety of monoamine receptors as well as being effective probes to explore the relative similarities and differences in the topology of a number of central monoamine receptors. In practice, the modification of the benzopyranopyridine template **2** has led to the discovery of four compounds that have been selected for extensive biological evaluation and toxicological assessment, namely, **2k** (CGS 15855A),[6] **2m** (CGS 15873A),[6,7] and **2u** (CGS 19845)[8] as selective presynaptic

(1) A. Hoffman In *Die Mutterkornalkaloide*; Ferdinand Enke Verlag: Stuttgart, 1964.
(2) Martin, G. E.; Williams, M.; Pettibone, D. J.; Yarbrough, G. G.; Clineschmidt, B. V.; Jones, J. H. *J. Pharmcol. Exp. Ther.* **1984**, *230*, 569.
(3) Cannon, J. G. *Annu. Rev. Pharmacol. Toxicol.* **1983**, *23*, 103.
(4) Carlsson, A.; Wikstrom, H.; Sanchez, D.; Lindberg, P.; Arvidsson, L.; Hacksell, U.; Johansson, A.; Nilsson, J.; Hjorth, S. *J. Med. Chem.* **1982**, *25*, 925.
(5) Kaiser, C.; Jain, T. *Med. Res. Rev.* **1985**, *5*, 145.
(6) Glaeser, B. S.; Berry, J. C.; Boyar, W. C.; Lovell, R. A.; Braunwalder, A.; Loo, P.; Stone, G.; Kalinsky, H.; Hutchison, A. J. *Soc. Neurosci. Abstr.* **1985**, *11*, 500. Glaeser, B. S.; Liebman, J. M.; Sills, M. A.; Hutchison, A. L.; Lovell, R. A.; Bennett, D. A.; Jarvis, M. F.; Berry, J. C.; Boyar, W. C.; Wood, P. L.; Welch, J. M.; Barbaz, B. J.; Williams, M. *Drug Dev. Res.* Submitted.

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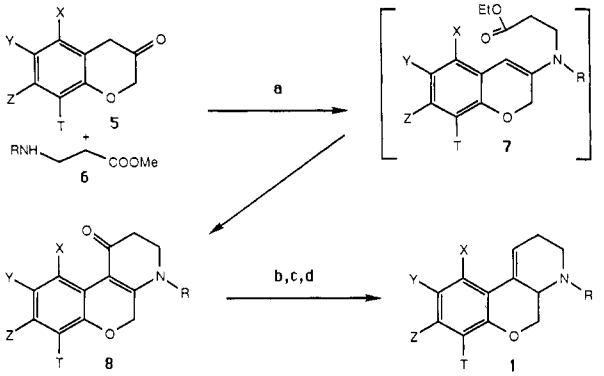
Scheme I[a]



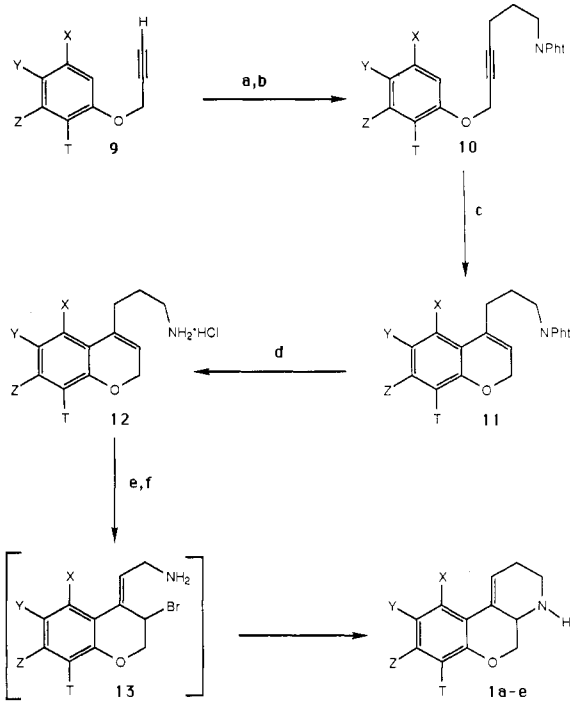
[a] Method A: (a) TFA, toluene; (b) LAH, pyridine; (c) LAH, THF; (d) $POCl_3$, pyridine.

dopamine agonists and **2g** (CGS 18102)[9] as a combined $5HT_2$ antagonist/$5HT_{1A}$ receptor agonist. The present manuscript describes the synthesis and biological activity of a number of analogues from these series in six central monoamine receptor binding assays.

Chemistry

Two approaches to the tetrahydrobenzopyranopyridine system **1** were developed. The first is outlined in Scheme I. The key step involves the condensation of a 3-chromanone **5** with an *N*-alkyl-β-alanine methyl ester **6**[10] to afford the vinylogous amide **8**. It was found that trifluoroacetic acid was the best catalyst for this transformation. Yields ranged from 40 to 80%. The vinylogous amides **8** can be directly reduced with LAH in ether[10] to the desired tetrahydrobenzopyranopyridines **1a–c,f**; however, yields were low, and the desired product was difficult to isolate. This transformation apparently results from an initial 1,2 addition of hydride to the carbonyl of the vinylogous amide followed by elimination and a subsequent 1,2 reduction of the resulting immonium species to give the allylic amines **1a–c,f**. An improved procedure was developed that employs a two-step reduction (LAH in pyridine, LAH in THF) followed by elimination of the resulting alcohol with phosphorus oxychloride in pyridine. This procedure can be carried out without isolation of intermediates and affords the desired tetrahydro derivatives **1a–c,f** in yields of 27–60%. The use of pyridine as solvent[11] assures an initial 1,4 addition to the vinylogous amide system. The resulting mixture of β-amino ketone and alcohol is quantitatively converted to the β-amino alcohol with LAH in THF. Whereas this approach has been successfully applied to prepare many of the tetrahydro derivatives described in this paper, it suffers from two major drawbacks. First the required 3-chromanones are often difficult to prepare and relatively unstable, possessing a marked tendency to dimerize.[12] Second the formation of the vinylogous amide **8** fails when X is methoxy, presumably because of steric compression in the transition state necessary for the cyclization of the enamine **7**.

Scheme II[a]



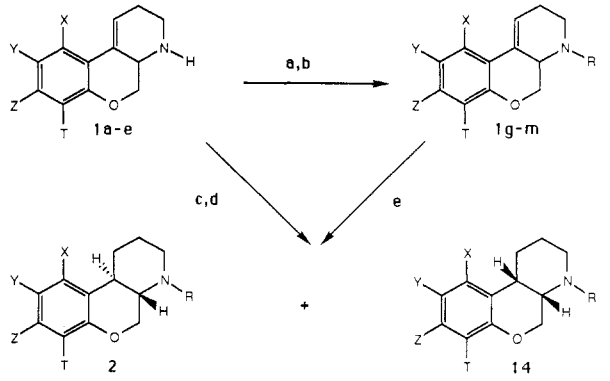
[a] Method B: (a) *n*-butyllithium, THF; DMSO, 1-bromo-3-chloropropane; (b) potassium phthalimide, DMSO; (c) heat, *N*-methylpyrrolidinone; (d) hydrazine, EtOH; (e) bromine, ethyl acetate; (f) triethylamine.

The need to prepare large quantities of **2k** as well as a desire to synthesize analogues in which X is not hydrogen stimulated the development of an alternate approach to the synthesis of the benzopyranopyridine systems **1** and **2**. This approach is outlined in Scheme II. The propargyl ethers **9**, which were prepared by alkylation of the corresponding phenols with propargyl bromide,[13] are monoalkylated with 1-bromo-3-chloropropane in DMSO after metalation with *n*-butyllithium in THF. The resulting chloride is not purified but directly reacted with potassium phthalimide in DMSO to afford the propargyl ethers **10** in overall yields of 50–75%. These compounds are cyclized to the chromenes **11** in high yield by thermolysis in diethylaniline or 1-methyl-2-pyrrolidinone in the presence of 1 equiv of diethylaniline. In the cases where two modes of cyclization are possible both regioisomers are obtained in about a 1:1 ratio. The mixtures of regioisomers were separated at the tricyclic amine stage to prepare the analogues **1b**, **1d**, and **1e**. The phthalimido protecting group is removed with hydrazine and the hydrochloride salt of the resulting primary amine is prepared. The amine **12** is treated with 1 equiv of bromine in ethyl acetate followed by 3 equiv of triethylamine to afford the tricyclic amines **1a–e** in overall yields of 66–90%. This useful transformation presumably occurs by addition of bromine to the chromene double bond followed by base-catalyzed elimination of hydrogen bromide to give the allylic bromide **13**, which spontaneously cyclizes to **1a–e**. In fact when the phthalimido-protected amine **11c** is subjected to the above conditions, an intermediate corresponding to **13** can be isolated, although it has only limited stability.

The hexahydro derivatives **2** are prepared either by hydrogenation of the tricyclic amines **1a–e** followed by in situ reductive amination with the appropriate aldehyde or by hydrogenation of the tertiary tricyclic amines **1f–j**.

(7) Boyar, W. C.; Wood, P. L.; Hutchison, A.; Altar, C. A. *Soc. Neurosci. Abstr.* **1985**, *11*, 501.
(8) Berry, J. C.; Boyar, W. C.; Hutchison, A. J.; Altar, C. A.; Wood, P. L.; Lovell, R. A. *Soc. Neurosci. Abstr.* **1986**, *12*, 140. Vinick, F. J.; Heyn, J. H. *Annu. Rep. Med. Chem.* **1987**, *22*, 4.
(9) Hutchison, A. J.; et al. Manuscript in preparation.
(10) Hirano, H.; Kurihara, T.; Nakamura, K. *Chem. Pharm. Bull.* **1974**, *22*, 1839.
(11) Lansbury, P. T. *J. Am. Chem. Soc.* **1961**, *83*, 429.
(12) See for example: *The Chemistry of Heterocyclic Compounds*; Ellis, G. P., Ed.; Wiley-Interscience: New York, Vol. 31, p 200.
(13) Anderson, W. K.; LaVoie, E. J. *J. Org. Chem.* **1973**, *38*, 3832.

Scheme III[a]



[a] Method C: (a) R'COCl, TEA, dichloromethane; (b) LAH, THF. Method D: (c) H_2, Pd/C, aq EtOH, K_2CO_3; (d) R'CHO, H_2, Pd/C. Method E: (e) H_2, Pd/C, EtOH.

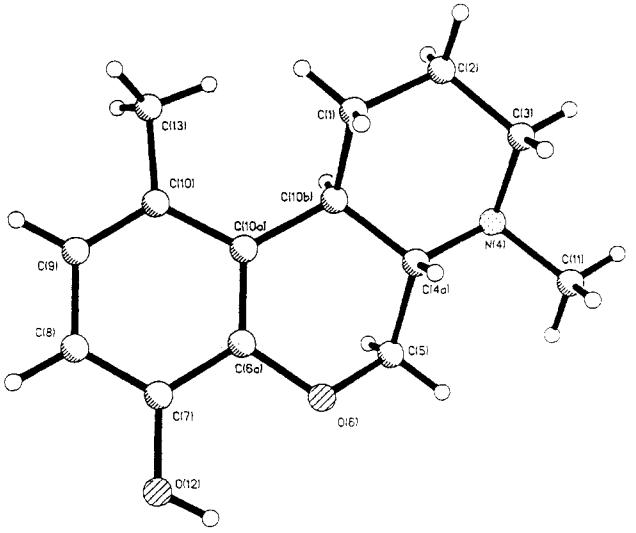


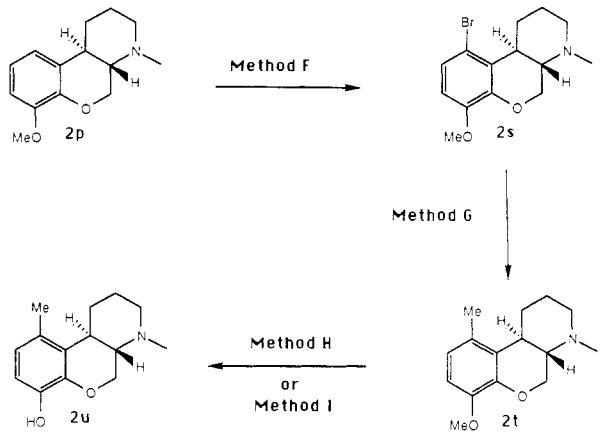
Figure 1.

These in turn were prepared by acylation of **1a–e** with propionyl chloride or ethyl chloroformate followed by reduction with LAH. These reactions are outlined in Scheme III. The hydrogenations give a mixture of cis and trans derivatives **14** and **2**, respectively, in all cases studied. The best yield of trans isomers **2k–p** was obtained by reduction of the secondary amines **1a–c** with 10% palladium on carbon catalyst in aqueous ethanol at 50 psi, which gave a 3:1 trans/cis ratio. However for the preparation of **2g–j** it was better to hydrogenate the tertiary amines, and in these cases the isomer ratio was approximately 1:1. In most cases the trans isomers were isolated by fractional crystallization of the corresponding hydrochloride salts. However in some cases flash chromatography was necessary. The relative stereochemistry was assigned on the basis of high-field NMR. This assignment has been confirmed in one case, **2u**, by X-ray crystallography (see Figure 1). The phenolic analogues of **1** and **2** were prepared by demethylation of the appropriate methyl ethers with either aqueous HBr or lithium diphenylphosphide[14] in THF as shown in Scheme IV.

Finally the analogues **2s–u** were prepared as outlined in Scheme IV. The amine **2p** was reacted with bromine in acetic acid to afford a mixture of bromides from which **2s** could be separated by fractional crystallization. Metal halogen exchange with *tert*-butyllithium followed by methylation of the resulting aryllithium species gave the

(14) Mann, F. G.; Pragnell, M. J. *J. Chem. Soc.* **1965**, 4120.

Scheme IV[a]



[a] Method F: bromine, acetic acid. Method G: *tert*-butyllithium, THF, iodomethane. Method H: lithium diphenylphosphide, THF. Method I: Concentrated HBr, heat.

corresponding methyl analogue **2t**, which was demethylated to afford **2u**. The structure of **2u** has been confirmed by X-ray crystallography to be as shown in Figure 1. The original assignment of the structure previously reported[8] in which the aromatic methyl group was assigned to position 8 (Z = Me) was based on NMR studies and is incorrect. A compilation of all of the characterized derivatives **1** and **2** along with melting points and method of preparation is found in Table I.

Methodology

The in vitro biological activity of the various benzopyranopyridines was measured by using previously published radioligand binding methods, which are documented below. All assays were validated and appropriate reference standards run. Compounds were run at 5–10 concentrations in triplicate in each experiment and IC_{50} values determined with a log–logit transformation of specific binding data using the program RS/1.[15] Each compound was evaluated in three separate assays to determine activity. Binding at dopamine recognition sites was measured with [^{3}H]ADTN[16] and [^{3}H]spiperone[17] as radioligands. For binding to serotonin sites, [^{3}H]-8-hydroxy(dipropylamino)tetralin (8-OH-DPAT) and [^{125}I]iodocyanopindolol (ICYP) were used to define $5HT_{1A}$ and $5HT_{1B}$ receptor sites, respectively.[18,19] Binding to $5HT_2$ receptors was measured with [3H]ketanserin[20] while α_2-adrenoceptor binding was measured with [^{3}H]clonidine.[21]

Results and Discussion

The in vitro binding data for selected tetrahydro analogues **1** and trans hexahydro analogues **2** are shown in Table II along with appropriate reference standards.

At the dopamine binding site defined by ADTN, several of the analogues tested showed biphasic inhibition curves. In all cases the IC_{50}'s reported are either for the high-affinity component of binding or for inhibition at a single site.[6]

(15) Bolt, Beranek and Newman Software, Cambridge, MA, 1985.
(16) Seeman, P.; Woodruff, G. N.; Poat, J. A. *Eur. J. Pharmacol.* **1979**, *55*, 137.
(17) Creese, I.; Snyder, S. H. *Eur. J. Pharmacol.* **1978**, *49*, 201.
(18) Gozlan, H.; El Mestikawy, S.; Pichat, L.; Glowinski, J.; Hamon, M. *Nature (London)* **1983**, *305*, 140.
(19) Hoyer, D.; Engel, G.; Kalkman, H. O. *Eur. J. Pharmacol.* **1985**, *118*, 1.
(20) Battaglia, G.; Shannon, M.; Borgundvaag, B.; Titler, M. *Life Sci.* **1983**, *33*, 2011.
(21) Braunwalder, A.; Stone, G.; Lovell, R. A. *J. Neurochem.* **1981**, *37*, 70.

Table I. Physical and Analytical Data for the Analogues 1 and 2

no.	X	Y	Z	T	R	method[a]	% yield[b]	mp,[c] °C	formula	anal.[d]
1a	H	H	H	OMe	H	B	31%	193–195	$C_{13}H_{16}ClNO_2$	C,H,N
1b	H	H	OMe	H	H	B	15%	224–228	$C_{13}H_{16}ClNO_2$	C,H,N
1c	H	OMe	H	H	H	B	26%	285–287	$C_{13}H_{16}ClNO_2$	C,H,N
1d	OMe	H	H	H	H	B	17%	262–264	$C_{13}H_{16}ClNO_2$	C,H,N
1e	OMe	H	Me	H	H	B	15%	*e*	$C_{14}H_{17}NO_2$	N.D.
1f	H	H	H	H	*n*-Pr	A	11%	257–259	$C_{15}H_{20}ClNO$	C,H,N
1g	OMe	H	H	H	*n*-Pr	C	47%	252–254	$C_{16}H_{22}ClNO_2$	C,H,N
1h	OMe	H	H	H	Me	C	51%	247–250	$C_{14}H_{18}ClNO_2$	C,H,N
1i	OMe	H	Me	H	*n*-Pr	C	85%	*e*	$C_{17}H_{23}NO_2$	C,H,N
1j	OMe	H	Me	H	Me	C	45%	244–245	$C_{15}H_{20}ClNO_2$	C,H,N
1k	H	OH	H	H	*n*-Pr	C, I	51%	265–266	$C_{15}H_{20}ClNO_2$	C,H,N
1l	H	OH	H	H	Me	C, I	47%	268–271	$C_{13}H_{16}ClNO_2$	C,H,N
1m	H	H	H	OH	*n*-Pr	C, I	40%	195–196	$C_{15}H_{20}ClNO_2$	C,H,N
2f	H	H	H	H	*n*-Pr	E	41%	257–262	$C_{15}H_{22}ClNO$	C,H,N
2g	OMe	H	H	H	*n*-Pr	E	57%	262–264	$C_{16}H_{24}ClNO_2$	C,H,N
2h	OMe	H	H	H	Me	E	42%	231–234	$C_{14}H_{20}ClNO_2$	C,H,N
2i	OMe	H	Me	H	*n*-Pr	E	57%	251–253	$C_{17}H_{26}ClNO_2$	C,H,N
2j	OMe	H	Me	H	Me	E	41%	228–232	$C_{15}H_{22}ClNO_2$	C,H,N
2k	H	OH	H	H	*n*-Pr	D, I	51%	292–294	$C_{15}H_{22}ClNO_2$	C,H,N
2l	H	OH	H	H	Me	E, H	32%	>260	$C_{13}H_{18}ClNO_2$	C,H,N
2m	H	H	H	OH	*n*-Pr	D, I	31%	262–265	$C_{15}H_{22}ClNO_2$	C,H,N
2n	OH	H	H	H	*n*-Pr	H	96%	309–310	$C_{15}H_{22}ClNO_2$	C,H,N
2o	H	H	OH	H	*n*-Pr	E, I	30%	275–278	$C_{15}H_{22}ClNO_2$	C,H,N
2p	H	H	H	OMe	Me	D	50%	236–238	$C_{14}H_{20}ClNO_2$	C,H,N
2q	H	H	H	OH	Me	H	81%	275–277	$C_{13}H_{18}ClNO_2$	C,H,N
2r	H	H	H	OH	*n*-Bu	C, E, H	23%	252–253	$C_{16}H_{24}ClNO_2$	C,H,N
2s	Br	H	H	OMe	Me	F	44%	265–267	$C_{14}H_{19}BrClNO_2$	C,H,N
2t	Me	H	H	OMe	Me	G	52%	277–278	$C_{15}H_{22}ClNO_2$	C,H,N
2u	Me	H	H	OH	Me	H	86%	252–254	$C_{14}H_{20}ClNO_2$	C,H,N

[a] Refers to the general method used to synthesize this compound as described in the Experimental Section. [b] Overall yield for the method(s) utilized. [c] As the HCl salt. [d] Combustion analyses were within ± 0.4% of the theoretical value. [e] Isolated by chromatography of the free base.

The basic *n*-propyl tetrahydro template **1f** shows virtually no affinity for either central dopamine receptors or the $5HT_2$ receptor, having only moderate (IC_{50} = 74 nM) activity for the $5HT_{1A}$ receptor and weak affinity for the α-2 adrenoreceptor. Addition of methoxy at position 10 (X = OMe: **1g**) results in a potent selective ligand for the $5HT_{1A}$ receptor (IC_{50} = 1.8 nM) with only a slight increase in α-2 activity as compared to that of **1f**. The methoxy substitution also increases $5HT_2$ activity with some evidence of activity at the $5HT_{1B}$ receptor. Addition of a hydroxyl to position 9 (Y = OH: **1k, 1l**) produced compounds with high affinity (IC_{50} = 15–20 nM) at dopaminergic binding sites labeled by [^{3}H]ADTN. It is interesting to note that unlike the octahydrobenzo[*f*]quinoline series[4] and the hexahydrobenzopyranopyridine analogues **2**, the *N*-propyl substituent produces no enhancement of dopamine receptor affinity over methyl. Apparently the presence of the additional double bond in the tetrahydro analogues **1** alters the topology of the tricyclic ring system so that optimal binding to the now famous *N*-propyl auxiliary binding site[5] on the dopamine D-2 receptor does not occur. This alteration in topology also prevents binding to dopamine receptors as well as to the other five monoamine receptors examined when hydroxyl is added to position 7 (T = OH: **1m**), whereas the corresponding analogue in the hexahydro series **2** is quite active (**2m**: IC_{50} = 3 nM) at [^{3}H]ADTN sites as well as expressing moderate activity (IC_{50} = 77 nM) at the α-2 adrenoreceptor.

The SAR of the hexahydro series was more extensively studied because of its greater chemical stability and the fact that analogues in this series showed good in vivo efficacy. The basic N-propylated template **2f** had significant activity at α-2 (IC_{50} = 88 nM), $5HT_2$ (IC_{50} = 178 nM), and $5HT_{1A}$ (IC_{50} = 219 nM) receptor sites, but was devoid of activity at dopamine recognition sites. However addition of a hydroxyl to positions 7, 8, or 9 (T, Z, or Y = OH) resulted in potent selective activity at dopamine receptors whereas the addition of hydroxyl to position 10 (X = OH) resulted in selective affinity for the $5HT_{1A}$ receptor. These observations are consistent with the findings of Carlson[4] in the octahydrobenzo[*f*]quinoline series.

In the 7-hydroxy series (T = OH: **2m, 2q, 2r, 2u**) the identity of the N substituent does not appear to be important for potent dopamine receptor affinity although larger N substituents appear to enhance affinity for α-2 adrenoreceptors. Alternately in the 9-hydroxy series the *N*-propyl substituent was essential for high-affinity binding to central monoamine receptors (**2k**: ADTN IC_{50} = 9 nM) while the corresponding *N*-methyl analogue (**2i**: ADTN IC_{50} = 114 nM) was only weakly active.

It is interesting to note that a similar SAR picture with regards to N substitution is seen for the 10-hydroxy (X = OH) analogue **2n** where the *N*-propyl substituent is essential for potent binding to the $5HT_{1A}$ receptor and the corresponding *N*-methyl derivative **2v** is about 50-fold less active. However this preference for the *N*-propyl substituent is not exhibited for the $5HT_2$ receptor since in all cases studied the corresponding *N*-methyl analogues show similar affinities to their *N*-propyl counterparts. Finally, addition of a 10-methyl (X = Me) substituent to **2q** results in retention of affinity for dopamine receptors.

As previously mentioned, addition of a 10-hydroxy or -methoxy substituent (X = OH or OMe) to the hexahydro template **2** results in potent and selective ligands for serotonin receptors, particularly $5HT_{1A}$ but also $5HT_2$. A similar SAR pattern exists for 7-substituted compounds (T = OH or OMe). Addition of a 7-methoxy or -hydroxyl substituent particularly in combination with a 10-methyl substituent can result in potent affinity for $5HT_{1A}$ and/or $5HT_2$ receptors. For example **2t** (X = Me, T = OMe) shows high affinity for $5HT_{1A}$ sites (IC_{50} = 19 nM). In the 10-methoxy series addition of a 8-methyl substituent (**2i, 2j**: X = OMe, Z = Me) has the effect of enhancing $5HT_2$ affinity (IC_{50} = 42–86 nM) while reducing $5HT_{1A}$ activity.

Table II. Affinity of the Analogues 1 and 2 at Selected Monoamine Receptors

compound	IC_{50} (μM) or % change at 1 μM (SD)[a]					
	ADTN[b,c]	SPIP[d]	CLON[e]	$5HT_{1A}$[f]	$5HT_{1B}$[g]	$5HT_2$[h]
apomorphine	0.004 (0.0003)	0.182 (0.066)	-	-	-	-
clonidine	-	-	0.002 (0.0001)	-	-	-
8-OH-DPAT	-	-	-	0.003 (0.0005)	-	-
TFMPP	-	-	-	-	0.051 (0.004)	
cinanserin	-	-	-	-	-	0.002 (0.0003)
1f	−15% (15%)	−13% (4%)	0.208 (0.078)	0.074 (0.003)	−11% (4%)	−22% (9%)
1g	−13% (4%)	−36% (22%)	0.151 (0.023)	0.0018 (0.0005)	1.32 (0.06)	0.839 (0.28)
1k	0.015[i] (0.0025)	4.19 (2.4)	0.634 (0.072)	0.244 (0.085)	0% (6%)	−14% (4%)
1l	0.020[i] (0.004)	−3% (0.6%)	0.169 (0.009)	−42% (1%)	−6% (2%)	−32% (12%)
1m	−10% (2%)	−17% (7%)	−34% (6%)	−36% (5%)	−1% (3%)	−14% (4%)
2f	−24% (7%)	−19% (17%)	0.088 (0.016)	0.219 (0.06)	−10% (4%)	0.178 (0.252)
2g	−9% (11%)	−53% (13%)	0.071 (0.018)	0.009 (0.003)	−19% (5%)	0.114 (0.032)
2i	−15% (3%)	−36% (15%)	0.504 (0.145)	0.186 (0.050)	−31% (5%)	0.086 (0.010)
2j	−11% (9%)	−39% (8%)	0.946 (0.069)	0.075 (0.017)	0.825 (0.055)	0.042 (0.004)
2k	0.009[i] (0.002)	1.26 (0.71)	0.559 (0.039)	1.21 (0.283)	−1% (3%)	−30% (4%)
2l	0.114[i] (0.029)	4.08 (2.29)	−64% (5%)	−13% (7%)	6% (4%)	−27% (7%)
2m	0.003[i] (0.001)	0.26 (0.11)	0.077 (0.020)	1.00 (0.190)	−2% (3%)	1.10 (0.17)
2n	−12% (4%)	−23% (13%)	0.362 (0.052)	0.004 (0.001)	−4% (4%)	0.384 (0.115)
2o	0.016 (0.003)	4.83 (2.85)	0.249 (0.019)	−17% (7%)	−1% (2%)	−14% (6%)
2p	−23% (14%)	−22% (6%)	−24% (4%)	0.253 (0.100)	−7% (2%)	0.920 (0.292)
2q	0.011 (0.003)	−46% (1%)	−41% (2%)	−45% (6%)	−2% (1%)	1.26 (0.253)
2r	0.008 (0.003)	0.2600 (0.062)	0.039 (0.006)	1.20 (0.450)	0% (2%)	−40% (2%)
2t	−6% (3%)	−15% (1%)	−12% (6%)	0.019 (0.002)	−35% (2%)	−44% (6%)
2u	0.026 (0.003)	0.170 (0.003)	1.02 (0.17)	0.076 (0.006)	−10% (1%)	0.097 (0.025)

[a] Numbers refer to the concentration necessary to achieve half-maximal inhibition of specific binding (IC_{50}'s); percents refer to the percent inhibition of specific binding at 1 μM. Standard deviations are in parentheses. [b] Compounds were run at 5–10 concentrations in triplicate. [c] [^{3}H]-(+)-23-Amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene binding.[16] [d] [^{3}H]Spiperone binding.[17] [e] [^{3}H]Clonidine binding.[21] [f] [^{3}H]-8-Hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) binding.[18] [g] [^{125}I]ICYP (iodocyanopindolol) binding.[19] [h] [^{3}H]Ketanserin binding.[20] [i] IC_{50} reported is for the high-affinity site.

As alluded to in the introduction, several of the analogues **2** have been extensively evaluated in in vivo functional assays. Compound **2k** has the profile of a presynaptic dopamine autoreceptor agonist being active in the γ-butyrolactone (GBL) model of autoreceptor function by both the oral and ip routes.[6] Compound **2m** has a similar in vivo profile[6,7] with an α-2 adrenoreceptor component similar to that seen for many putative autoreceptor agonists.[6] Compound **2u** also has a dopamine autoreceptor agonist profile in vivo with a relatively long duration of action. Compound **2g** is a long-acting $5HT_{1A}$ agonist/$5HT_2$ antagonist active in both the 5-HTP accumulation and 5-HTP-induced head twitch model at similar doses.[4,9] The compound is under evaluation as a novel serotonergic anxiolytic.[9]

In conclusion, it is clear that the basic benzopyranopyridine templates **1** and **2** with the appropriate substitution can possess potent and selective affinities for dopamine, $5HT_{1A}$, and $5HT_2$ receptors. This series demonstrates clear topological similarities between the six monoamine receptors studied as well as illustrating the principle of modifying a relatively nonselective pharmacophore to obtain selective binders to a variety of central monoamine receptors. With use of modified azaspirodecanedione derivatives, similar conclusions have been derived in regard to the inherent spectrum of monoaminergic activity in a single pharmacophore which can be selectively enhanced (or deemphasized) by appropriate modification.[22]

Experimental Section

Binding Studies: Dopamine Receptor Binding. The binding of [^{3}H]ADTN ((+)-2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene) to dopamine receptors was measured as de-

(22) Hibert, M. F.; Girros, M. W.; Middlemiss, D. N.; Mir, A. K.; Fozard, J. R. *J. Med. Chem.* **1988**, *31*, 1087.

scribed by Seeman et al.[16] Washed calf caudate membranes were incubated in a 50 mM Tris·HCl buffer, pH 7.7, of the following composition: 0.1% ascorbate, 10 μM pargyline, 120 mM NaCl; 5 mM KCl; 2 mM $CaCl_2$; 1 mM $MgCl_2$ with 0.2 nM [^{3}H]ADTN (sp act. 15–40 Ci/mmol) at 25 °C for 1 h. Bound radioactivity was isolated by vacuum filtration onto glass fibre filters. Nonspecific binding was determined in the presence of 1 μM dopamine. The binding of [^{3}H]spiperone was measured as described by Creese and Snyder[17] with the same tissue as that used for measuring the binding of [^{3}H]ADTN. The buffer used in the latter was supplemented with 10 mM dithiothreitiol. Tissue was incubated with 0.2 nM spiperone (sp act. 20–40 Ci/mmol) at 37 °C for 10 min and bound radioactivity isolated by vacuum filtration. Nonspecific binding was measured in the presence of 1 μM (+)-butaclamol. Radioactivity was determined as described above.

Serotonin Receptor Binding. Binding to $5HT_{1A}$ receptors was measured with [^{3}H]-8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin) as described by Gozlan et al.[18] Rat cortical membranes were freshly prepared in 50 mM Tris·HCl buffer, pH 7.4, containing 2.5 mM $MgCl_2$ and after incubation for 20 min at 37 °C to remove endogenous 5HT, centrifuged at 48000*g* and resuspended in Tris–ascorbate buffer, pH 7.4, of the following composition: 50 mM Tris·HCl, 10 μM pargyline, 0.1% ascorbate. Tissue was incubated with 1 nM [^{3}H]-8-OH-DPAT (sp act. 160 Ci/mmol) for 15 min at 37 °C and bound radioactivity isolated on glass fibre filters by vacuum filtration. Nonspecific binding was measured in the presence of 10 μM 5HT. Binding to the $5HT_{1B}$ receptor was measured with [^{125}I]ICYP (iodocyanopindolol) by the method of Hoyer et al.[19] Tissue was prepared as described for $5HT_{1A}$ binding except that it was 10-fold more dilute than that used for [3H]-8-OH-DPAT binding.[18] Tissue was incubated with 50 pM [^{125}I]ICYP (sp act. 2200 Ci/mmol) at 37 °C for 90 min and bound radioactivity isolated by vacuum filtration. Binding of ICYP to β-adrenergic receptors was blocked by the inclusion of 600 μM (±)-isoproterenol.[19] Nonspecific binding was measured in the presence of 10 μM 5HT. Binding to $5HT_2$ receptors was measured by the method of Battaglia et al.[20] using [^{3}H]ketanserin. Washed rat cortex membranes were suspended in 50 mM Tris·HCl buffer, pH 8.2, containing 10 mM $MgCl_2$ and incubated with 0.4 nM [^{3}H]ketanserin (sp act. 60–70 Ci/mmol) for 15 min at 37 °C. Bound radioactivity was isolated on glass fibre filters by vacuum filtration. Nonspecific binding was determined in the presence of 1 μM cinanserin.

α_2-Adrenergic Receptor Binding. Binding to α_2-adrenergic receptors was measured by the method of Braunwalder et al.[21] Washed calf cerebral cortex membranes were resuspended in 50 mM Tris-HCl buffer, pH 7.7, of the following composition: 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and incubated with 0.4 nM [^{3}H]clonidine (sp act. 40–60 Ci/mmol) for 30 min at 23 °C. Bound radioactivity was isolated on glass fibre filters by vacuum filtration. Nonspecific binding was determined in the presence of 1 μM unlabeled clonidine.

Data Calculations. Bound radioactivity was determined for tritium by conventional scintillation spectrometry at an efficiency of 45–55% and for iodine by gamma spectrometry. IC_{50} values (concentration of compound required to displace total specific binding by 50%) derived from specific binding data were determined by using an iterative, nonlinear regression analysis program, RS/1.[15] All binding assays were validated by determination of binding characteristics and the inclusion of appropriate reference standards.

Compound Sources. All radioligands were obtained from DuPont-NEN, Boston, MA. Reference compounds were obtained as generous gifts from their respective manufacturers or from Research Biochemicals, Natick, MA.

Chemistry. All melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. All 60-MHz NMR spectra were recorded on a Perkin-Elmer R-12 spectrometer and all 300-MHz NMR spectra were recorded on a Varian XL 300 spectrometer. All chemical shifts are expressed in ppm relative to a TMS internal standard. All reactions were carried out under a nitrogen atmosphere.

Method A. 4-Propyl-1,3,4,5-tetrahydro-2*H*-[1]benzopyrano[3,4-*b*]pyridin-1-one (8f). A mixture of 3-chromanone (8.6 g, 58 mmol) and *N*-propyl-β-alanine methyl ester (9.3 g, 64 mmol) in toluene (80 mL) was treated with trifluoroacetic acid (0.9 mL, 11.6 mmol) and refluxed for 16 h in a Dean–Stark apparatus. The solvent was removed in vacuo and the residue dissolved in ether, and the desired product was extracted with 6 N hydrochloric acid. The aqueous layer was neutralized with 3 N sodium hydroxide and the product was extracted with dichloromethane. After drying over magnesium sulfate, the solvent was removed in vacuo and the residue was triturated with ether to afford 6.0 g (42%) of the title compound **8f** as an amorphous solid: NMR ($CDCl_3$, 60 MHz) 1.0 ppm (3 H, t, J = 6 Hz), 1.55 (2 H, m), 2.60 (2 H, t, J = 8 Hz), 3.30 (2 H, t, J = 7 Hz), 3.60 (2 H, t, J = 8 Hz), 4.75 (2 H, s), 6.95 (3 H, m), 8.42 (1 H, m).

4-Propyl-3,4,4a,5-tetrahydro-2*H*-[1]benzopyrano[3,4-*b*]pyridine Hydrochloride (1f). A mixture of **8f** (6.0 g, 24.7 mmol) and pyridine (30 mL) was treated with lithium aluminum hydride (700 mg, 18.5 mmol) added in small portions at room temperature. After 0.5 h the reaction mixture was quenched wtih 10% sodium hydroxide, magnesium sulfate was added and the reaction mixture was filtered. The filter cake was washed with ethyl acetate and the solvents were removed in vacuo from the combined filtrates. This residue was dissolved in THF (30 mL) and treated again with lithium aluminum hydride (700 mg, 18.5 mmol). After 0.5 h the reaction was worked up as above and the residue was dissolved in pyridine (20 mL) and treated with phosphorus oxychloride (6.65 g, 43.5 mmol). After 0.5 h at 65 °C the reaction mixture was poured onto an ice-cold sodium carbonate solution and the product was extracted with ethyl acetate. After drying over magnesium sulfate, the solvent was removed in vacuo. The resulting oil was treated with ethanolic hydrochloric acid to afford 1.8 g (27%) of the title compound **1f** melting at 257–259 °C: NMR (free base) (60 MHz, $CDCl_3$) 0.90 ppm (3 H, t, J = 7 Hz), 1.5 (2 H, m), 1.90–2.35 (7 H, m), 3.68 (1 H, dd, J = 10 Hz), 4.50 (1 H, dd, J = 3, 10 Hz), 6.15 (1 H, m), 7.05 (4 H, m). Anal. (CHNOCl) C, H, N.

Method B. 4-Methoxyphenyl 6-Phthalimido-2-hexynyl Ether (10c). To a solution of 4-methoxyphenyl 2-propynyl ether (**9c**) (10 g, 61.5 mmol) in THF (100 mL) cooled to −40 °C was added a 10.2 M solution of *n*-butyllithium in hexane (6.12 mL, 61.5 mmol) in such a manner so as to keep the temperature below −25 °C. After the addition was complete, dry DMSO (26 mL) was added followed by 1-bromo-3-chloropropane (11.5 mL, 92 mmol) while the temperature was kept below −15 °C. The reaction mixture was kept at room temperature overnight and concentrated in vacuo. Water was added and the product extracted with dichloromethane. After drying over magnesium sulfate, the solvent was removed in vacuo to afford an oil. This material was treated with potassium phthalimide (17.5 g, 90 mmol) in DMF (225 mL) and stirred for 20 h at 60 °C. The reaction mixture was concentrated in vacuo, water was added, and the product was extracted with ether. After drying over magnesium sulfate, the solvent was removed in vacuo and the residue was triturated with ether to afford 14.5 g (67%) of the title compound **10c** melting at 50–52 °C: NMR ($CDCl_3$, 60 MHz) 2.15 ppm (4 H, m), 3.78 (3 H, s), 3.79 (2 H, t, J = 7 Hz), 4.50 (2 H, t, J = 2 Hz), 6.87 (4 H, s), 7.70 (4 H, m). Anal. (CHNO) C, H, N.

4-(3-Phthalimidopropyl)-6-methoxy-2*H*-[1]benzopyran (11c). A mixture of 4-methoxyphenyl 6-phthalimido-2-hexynyl ether (**10c**) (14 g, 40 mmol) and diethylaniline (6.0 g, 40 mmol) in 1-methyl-2-pyrrolidinone (50 mL) was heated at 195–200 °C under argon for 30 h. After cooling, the reaction mixture was diluted with water and the product extracted with toluene. After drying over sodium sulfate, the solvent was removed in vacuo and the residue was triturated with 2-propanol to afford 11.9 g (85%) of the title compound **11c** melting at 72–76 °C: NMR ($CDCl_3$, 60 MHz) 2.30 ppm (4 H, m), 3.78 (3 H, s), 3.78 (2 H, t, J = 7 Hz), 4.70 (2 H, d, J = 3 Hz), 5.71 (1 H, t, J = 3 Hz), 6.70 (3 H, m), 7.80 (4 H, m). Anal. (CHNO) C, H, N.

4-(3-Aminopropyl)-6-methoxy-2*H*-[1]benzopyran Hydrochloride (12c). A mixture of 4-(3-phthalimidopropyl)-6-methoxy-2*H*-[1]benzopyran (**11c**) (11.5 g, 33 mmol), hydrazine hydrate (3.34 g, 57.5 mmol), and ethanol (84 mL) was refluxed for 2 h. The reaction mixture was diluted with 10% potassium hydroxide solution (50 mL) and most of the ethanol was distilled in vacuo. The product was extracted with toluene, the organic layer was dried over sodium sulfate, and the solvent was removed in vacuo. The residue was dissolved in toluene (50 mL) and treated with 10 N ethanolic hydrochloric acid (3.3 mL, 33 mmol) to afford 5.8

g (70%) of the title compound **12c** melting at 141–142 °C: NMR ($CDCl_3$, 60 MHz) 2.30 ppm (4 H, m), 3.10 (2 H, t, J = 7 Hz), 3.75 (3 H, s), 4.62 (2 H, d, J = 3 Hz), 5.71 (1 H, t, J = 3 Hz), 6.70 (3 H, m), 8.32 (3 H, bs). Anal. (CHNOCl) C, H, N.

9-Methoxy-2,3,4a,5-tetrahydro-2*H*-[1]benzopyrano[3,4-*b*]pyridine Hydrochloride (1c). To a stirred suspension of 4-(3-aminopropyl)-6-methoxy-2*H*-[1]benzopyran hydrochloride (**12c**) (5.8 g, 22.6 mmol) in ethyl acetate (50 mL) was added bromine (3.61 g, 22.6 mmol) in a dropwise fashion at room temperature. After 1 h at room temperature, triethylamine (6.85 g, 67.8 mmol) was added and the reaction mixture heated at 76 °C for 4 h. The reaction mixture was washed with 10% potassium hydroxide solution and dried over magnesium sulfate and the solvent removed in vacuo. The resultant residue was dissolved in ethanol and treated with 10 N ethanolic hydrogen chloride to afford 3.81 g (66%) of the title compound **1c** melting at 285–287 °C: NMR (free base) ($CDCl_3$, 300 MHz) 1.60 ppm (1 H, bs), 2.18 (1 H, m), 2.48 (1 H, m), 2.97 (1 H, m), 3.21 (1 H, m), 3.68 (1 H, dd, J = 10 Hz), 3.77 (3 H, s), 3.78 (1 H, m), 4.24 (1 H, dd, J = 4, 10 Hz), 6.22 (1 H, m), 6.74 (2 H, m), 6.95 (1 H, d, J = 2.5 Hz). Anal. (CHNOCl) C, H, N.

Method C. 10-Methoxy-4-propyl-2,3,4a,5-tetrahydro-2*H*-[1]benzopyrano[3,4-*b*]pyridine Hydrochloride (1g). To a stirred mixture of 10-methoxy-2,3,4a,5-tetrahydro-2*H*-[1]-benzopyrano[3,4-*b*]pyridine hydrochloride (**1d**) (850 mg, 3.3 mmol) and triethylamine (1.1 mL, 8 mmol) in dichloromethane (10 mL) was added propionyl chloride (340 mg, 3.66 mmol). After 0.5 h the reaction mixture was washed with 1 N sodium hydroxide and dried over magnesium sulfate and the solvent removed in vacuo. The residue was dissolved in THF (10 mL) and treated with lithium aluminum hydride (250 mg, 6.6 mmol). After refluxing for 1 h the reaction mixture was quenched with 10% sodium hydroxide and filtered and the solvent removed in vacuo. The residue was dissolved in ethanol and treated with 10 N ethanolic hydrogen chloride to afford 467 mg (47%) of the title compound **1g** melting at 252–254 °C: NMR (free base) ($CDCl_3$, 90 MHz) 0.90 ppm (3 H, t, J = 6 Hz), 1.54 (2 H, m), 2.18–3.32 (7 H, m), 3.74 (1 H, dd, J = 10 Hz), 4.47 (1 H, dd, J = 3.10 Hz), 6.48 (2 H, dd, J = 2, 8 Hz), 6.82 (1 H, m), 7.08 (1 H, dd, J = 8 Hz). Anal. (CHNOCl) C, H, N.

Method D. *trans*-7-Methoxy-4-methyl-1,2,3,4a,5,10b-hexahydro-2*H*-[1]benzopyrano[3,4-*b*]pyridine Hydrochloride (2p). A mixture of 7-methoxy-2,3,4a,5-tetrahydro-2*H*-[1]benzopyrano[3,4-*b*]pyridine hydrochloride (**1a**) (5.9 g, 23.4 mmol), anhydrous sodium carbonate (1.24 g, 11.7 mmol), ethanol (70 mL), water (5 mL), and 10% palladium on carbon catalyst (590 mg) was shaken under a hydrogen atmosphere (50 psi) at room temperature for 16 h. Aqueous formaldehyde (37%, 17.4 mL, 232 mmol) was added and the hydrogenation continued for 24 h. After filtration the solvent was removed in vacuo and the residue dissolved in ethanol. Acidification with ethanolic hydrogen chloride afforded 3.1 g (50%) of the title compound **2p** melting at 277–278 °C: NMR (free base) ($CDCl_3$, 300 MHz) 1.20 ppm (1 H, m), 1.80 (2 H, m), 2.13 (2 H, m), 2.30 (3 H, s), 2.37 (1 H, m), 2.68 (1 H, m), 2.92 (1 H, m), 3.82 (3 H, s), 3.89 (1 H, dd, J = 10 Hz), 4.68 (1 H, dd, J = 3, 10 Hz), 6.80 (3 H, m). Anal. (CHNOCl) C, H, N.

Method E. *trans*-10-Methoxy-4-propyl-1,3,4,4a,5,10b-hexahydro-2*H*-[1]benzopyrano[3,4-*b*]pyridine Hydrochloride (2g). A mixture of the free base derived from 10-methoxy-3,4,4a,5-tetrahydro-2*H*-[1]benzopyrano[3,4-*b*]pyridine hydrochloride (**1g**) (8.0 g, 30.9 mmol), 10% palladium on carbon catalyst (2.0 g), and ethanol (200 mL) was shaken under a hydrogen atmosphere (50 psi) at room temperature for 8 h. After filtration the solvent was removed in vacuo to give an oil, which was subjected to flash chromatography on silica gel with ether/hexane (1:4) as the eluent to afford 4.6 g (57%) of the pure trans isomer. The hydrochloride **2g** was prepared by treating this material with ethanolic hydrochloric acid and melted at 262–264 °C: NMR (free base) (90 MHz, $CDCl_3$) 0.89 ppm (3 H, t, J = 8 Hz), 1.0–3.3 (12 H, m), 3.70 (1 H, dd, J = 10 Hz), 3.85 (3 H, s), 4.40 (1 H, dd, J = 3, 10 Hz), 6.50 (2 H, dd, J = 4, 7 Hz), 7.10 (1 H, t, J = 7 Hz). Anal. (CHNOCl) C, H, N.

Method F. *trans*-10-Bromo-7-methoxy-4-methyl-1,3,4,4a,5,10b-hexahydro-2*H*-[1]benzopyrano[3,4-*b*]pyridine Hydrochloride (2s). A mixture of the free base derived from *trans*-7-methoxy-4-methyl-1,3,4,4a,5,10b-hexahydro-2*H*-[1]-benzopyrano[3,4-*b*]pyridine hydrochloride (**2p**) (5.72 g, 24.4 mmol) and acetic acid (60 mL) was treated with bromine (3.91 g, 24.4 mmol) in a dropwise manner at room temperature. After 16 h the reaction mixture was diluted with sodium bicarbonate solution and the product extracted with ether. After drying over magnesium sulfate, the solvent was removed in vacuo. The residue was treated with ethanolic hydrochloric acid to afford 3.72 g (44%) of the title compound **2s**, which melted at 265–267 °C after one recrystallization from ethanol: NMR (free base) ($CDCl_3$, 300 MHz) 1.03 ppm (1 H, m), 1.85 (2 H, m), 2.24 (2 H, m), 2.35 (3 H, s), 2.78 (1 H, m), 3.03 (1 H, m), 3.28 (1 H, m), 3.64 (1 H, dd, J = 10 Hz), 3.84 (3 H, s), 4.64 (1 H, dd, J = 3, 10 Hz), 6.59 (1 H, d, J = 8 Hz), 7.09 (1 H, d, J = 8 Hz). Anal. (CHNOBrCl) C, H, N.

Method G. *trans*-4,10-Dimethyl-7-methoxy-1,3,4,4a,5,10b-hexahydro-2*H*-[1]benzopyrano[3,4-*b*]pyridine Hydrochloride (2t). To a solution of the free base of *trans*-10-bromo-7-methoxy-4-methyl-1,3,4,4a,5,10b-hexahydro-2*H*-[1]-benzopyrano[3,4-*b*]pyridine hydrochloride (**2s**) (5.79 g, 18.7 mmol) in THF (60 mL) cooled to −78 °C was added 1.7 M *tert*-butyllithium in hexane (22 mL, 37.4 mmol) in a dropwise manner. After 5 min at −78 °C, methyl iodide (2.7 g, 19 mmol) was added and the resulting mixture was stirred for 30 min at −78 °C and then warmed to 0 °C. The reaction mixture was diluted with water, and the products were extracted with ether. After drying over magnesium sulfate, the solvent was removed in vacuo. The resulting oil was chromatographed on a Waters Prep 500 with isooctane/ethyl acetate/methanol 65:35:1 as the eluent to afford 2.1 g (52%) of the free base of **2t**, which was quantitatively converted to its hydrochloride salt **2t** melting at 277–278 °C with ethanolic hydrochloric cid: NMR (free base) ($CDCl_3$, 300 MHz) 1.12 ppm (1 H, m), 1.82 (2 H, m), 2.24 (2 H, m), 2.28 (3 H, s), 2.35 (3 H, s), 2.60 (1 H, m), 2.82 (1 H, m), 3.02 (1 H, m), 3.65 (1 H, dd, J = 10 Hz), 3.84 (3 H, s), 4.59 (1 H, dd, J = 3, 10 Hz), 6.63 (1 H, d, J = 8 Hz), 6.67 (1 H, d, J = 8 Hz). Anal. (CHNOCl) C, H, N.

Method H. *trans*-4,10-Dimethyl-1,3,4,4a,5,10b-hexahydro-2*H*-[1]benzopyrano[3,4-*b*]pyridin-7-ol Hydrochloride (2u). To a solution of diphenylphosphine (1.92 g, 10.4 mmol) in THF (20 mL) was added 2.4 M *n*-butyllithium in hexane (3.6 mL, 8.8 mmol) at 0 °C. To the resulting mixture was added the free base of *trans*-4,10-dimethyl-7-methoxy-1,3,4,4a,5,10b-hexahydro-2*H*-[1]benzopyrano[3,4-*b*]pyridine hydrochloride (**2t**) (1.28 g, 5.2 mmol) in 10 mL of THF and the reaction was refluxed for 30 min. The resulting mixture was diluted with ether and the product was extracted with chloroform. After drying over magnesium sulfate, the solvent was removed in vacuo. The residue was treated with ethanolic HCl to afford 1.2 g (86%) of the title compound **2u** melting at 252–254 °C: NMR (free base) ($CDCl_3$, 300 MHz) 1.14 ppm (1 H, m), 1.85 (2 H, m), 2.26 (3 H, s), 2.26 (2 H, m), 2.35 (3 H, s), 2.65 (1 H, m), 2.83 (1 H, m), 3.03 (1 H, m), 3.69 (1 H, dd, J = 10 Hz), 4.55 (1 H, dd, J = 3, 10 Hz), 6.03 (1 H, bs), 6.62 (1 H, d, J = 8 Hz), 6.68 (1 H, d, J = 8 Hz). Anal. (CHNOCl) C, H, N.

X-ray Structure Determination of 2u. The X-ray structure determination was performed with a Nicolet R3m/V diffractometer with the SHELXTL PLUS software on a Microvax II computer. The crystal was monoclinic, space group $P21/n$ with two identical molecules in each asymmetric unit. The cell constants were a = 6.579 (4) Å, b = 14.518 (8) Å, c = 26.052 (11) Å, and β = 90.41 (4)°.

Method I. *trans*-4-Propyl-1,3,4,4a,5,10b-hexahydro-2*H*-[1]benzopyrano[3,4-*b*]pyridin-7-ol Hydrochloride (2m). A mixture of 45% hydrobromic acid (160 mL) and *trans*-7-methoxy-4-propyl-1,3,4,4a,5,10b-hexahydro-2*H*-[1]benzopyrano[3,4-*b*]pyridine hydrochloride (**2v**) (16.3 g, 55.5 mmol) was heated at 120 °C for 5 h. The reaction mixture was poured onto ice and neutralized with 50% NaOH. The product was extracted with ethyl acetate, and after drying over magnesium sulfate, the solvent was removed in vacuo. The residue was treated with ethanolic HCl to afford 13.9 g (88%) of the title compound **2m** melting at 262–264 °C: NMR (free base) (CD_3OD, 300 MHz) 0.92 ppm (3 H, t, J = 7 Hz), 1.1–1.90 (6 H, m), 2.38 (3 H, m), 2.68 (2 H, m), 3.06 (1 H, m), 3.72 (1 H, dd, J = 10 Hz), 4.50 (1 H, dd, J = 4, 10 Hz), 6.57 (2 H, m), 6.64 (1 H, d, J = 2.4 Hz). Anal. (CHNOCl) C, H, N.

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Registry No. **1a**, 100746-45-0; **1a**·HCl, 100746-46-1; **1b**, 118715-84-7; **1b**·HCl, 118715-94-9; **1c**, 100745-90-2; **1c**·HCl, 100776-81-6; **1d**, 118715-85-8; **1d**·HCl, 118715-95-0; **1e**, 118715-86-9; **1f**, 100746-09-6; **1f**·HCl, 100746-10-9; **1g**, 100746-47-2; **1g**·HCl, 100746-48-3; **1h**, 100746-29-0; **1h**·HCl, 100746-30-3; **1i**, 118715-87-0; **1j**, 100746-23-4; **1j**·HCl, 100746-24-5; **1k**, 100745-19-5; **1k**·HCl, 100745-18-4; **1l**, 100745-52-6; **1l**·HCl, 100745-51-5; **1m**, 100745-34-4; **1m**·HCl, 100745-33-3; **2f**, 100746-17-6; **2f**·HCl, 100746-18-7; **2g**, 100746-35-8; **2g**·HCl, 100746-36-9; **2h**, 100746-37-0; **2h**·HCl, 100746-38-1; **2i**, 100746-53-0; **2i**·HCl, 100746-54-1; **2j**, 100746-27-8; **2j**·HCl, 118715-96-1; **2k**, 100745-13-9; **2k**·HCl, 100745-12-8; **2l**, 118715-88-1; **2l**·HCl, 118715-97-2; **2m**, 100745-36-6; **2m**·HCl, 100745-35-5; **2n**, 100746-39-2; **2n**·HCl, 100746-40-5; **2o**, 101392-20-5; **2o**·HCl, 101392-21-6; **2p**, 100745-46-8; **2p**·HCl, 100745-45-7; **2q**, 100745-59-3; **2q**·HCl, 100745-58-2; **2r**, 100745-48-0; **2r**·HCl, 100745-47-9; **2s**, 118715-89-2; **2s**·HCl, 118715-98-3; **2t**, 118715-90-5; **2t**·HCl, 118715-99-4; **2u**, 118715-91-6; **2u**·HCl, 118716-00-0; **2v**, 100746-42-7; **8f**, 118715-92-7; **9c**, 17061-86-8; **10c**, 100746-90-5; **11c**, 100746-91-6; **12c**, 100746-87-0; **14** (X = Y = Z = T = H; R = *n*-Pr), 118716-01-1; **14** (X = OMe; Y = Z = T = H; R = *n*-Pr), 118716-02-2; **14** (X = OMe; Y = Z = T = H; R = Me), 118716-03-3; **14** (X = OMe; Y = T = H; Z = Me; R = *n*-Pr), 118716-04-4; **14** (X = OMe; Y = T = H; Z = R = Me), 118716-05-5; **14** (X = Z = T = H; Y = OH; R = *n*-Pr), 118716-06-6; **14** (X = Z = T = H; Y = OH; R = Me), 118716-07-7; **14** (X = Y = Z = H; T = OH; R = *n*-Pr), 100745-38-8; **14** (X = Y = T = H; Z = OH; R = *n*-Pr), 118716-08-8; **14** (X = Y = Z = H; T = OMe; R = Me), 118716-09-9; chromanone, 19090-04-1; *N*-propyl-β-alanine methyl ester, 5036-62-4; 2-methoxyphenyl 2-propynyl ether, 41580-71-6; 3-methoxyphenyl 2-propynyl ether, 41580-72-7; 3-methoxy-5-methylphenyl 2-propynyl ether, 118715-93-8; 1-bromo-3-chloropropane, 109-70-6; propionyl chloride, 79-03-8.

Supplementary Material Available: Details of the structure determination of **2u**, the numbering system of the two molecules, and tables of the atomic coordinates, bond distances and angles, anisotropic displacement coefficients (10 pages); tables of observed and calculated structure factors (12 pages). Ordering information is given on any current masthead page.

Evaluation of Glycolamide Esters and Various Other Esters of Aspirin as True Aspirin Prodrugs

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A series of glycolamide, glycolate, (acyloxy)methyl, alkyl, and aryl esters of acetylsalicylic acid (aspirin) were synthesized and evaluated as potential prodrug forms of aspirin. N,N-Disubstituted glycolamide esters were found to be rapidly hydrolyzed in human plasma, resulting in the formation of aspirin as well as the corresponding salicylate esters. These in turn hydrolyzed rapidly to salicylic acid. The largest amount of aspirin formed from the esters were 50 and 55% in case of the *N,N*-dimethyl- and *N,N*-diethylglycolamide esters, respectively. Similar results were obtained in blood with the *N,N*-dimethyl- and *N,N*-diethylglycolamide esters. Unsubstituted and monosubstituted glycolamide esters as well as most other esters previously suggested to be aspirin prodrugs were shown to hydrolyze exclusively to the corresponding salicylic acid esters. Lipophilicity parameters and water solubilities of the esters were determined, and structural factors favoring ester prodrug hydrolysis at the expense of deacetylation to yield salicylate ester are discussed. The properties of some N,N-disubstituted glycolamide esters of aspirin are highlighted with respect to their use as potential aspirin prodrugs.

For several years many attempts have been made to develop bioreversible derivatives or prodrugs of aspirin (acetylsalicylic acid) in order to depress its side effects in the form of gastric irritation and bleedings.[1] Since the gastric irritation and ulcerogenicity associated with oral dosing of aspirin is largely a local phenomenon possibly involving accumulation of the acid within gastric mucosal cells,[2-4] a promising approach to minimize these side effects may be masking the acidic carboxyl group of aspirin via prodrug formation. Thus, esterification of aspirin as well as various other nonsteroidal antiinflammatory carboxylic acids to produce methyl esters has been shown to greatly suppress the gastric ulcerogenic activity.[5,6]

The aspirin prodrug derivatives developed so far can be classified in two groups according to their mechanism of conversion: derivatives that undergo enzymatic cleavage to regenerate the parent drug and derivatives being hydrolyzed nonenzymatically. The former group consists of several ester derivatives including simple alkyl or aryl esters,[5-14] triglycerides,[15-17] (acyloxy)alkyl esters,[18,19] certain

(1) Jones, G. In *Design of Prodrugs*; Bundgaard, H., Ed.; Elsevier: Amsterdam, 1985; pp 199–241.
(2) Ivey, K. J.; Paone, D. D.; Krause, W. J. *Dig. Dis. Sci.* **1980**, *25*, 97–99.
(3) Rainsford, K. D. *Agents Actions* **1975**, *5*, 326–344.
(4) McCormack, K.; Brune, K. *Arch. Toxicol.* **1987**, *60*, 261–269.
(5) Rainsford, K. D.; Whitehouse, M. W. *J. Pharm. Pharmacol.* **1976**, *28*, 599–600.
(6) Whitehouse, M. W.; Rainsford, K. D. *J. Pharm. Pharmacol.* **1980**, *32*, 795–796.
(7) Rainsford, K. D.; Schweitzer, A.; Green, P.; Whitehouse, M. W.; Brune, K. *Agents Actions* **1980**, *10*, 457–464.
(8) Rainsford, K. D.; Whitehouse, M. W. *Agents Actions* **1980**, *10*, 451–456.
(9) Cousse, H.; Casadio, S.; Mouzin, G. *Trav. Soc. Pharm. Montpellier* **1978**, *38*, 71–76.
(10) Davis, A. F.; Dixon, G. J. A. Brit. Patent 1,518,622, 1978.
(11) Croft, D. N.; Cuddigan, J. H. P.; Sweetland, C. *Br. Med. J.* **1972**, *3*, 545–547.
(12) Sunkel, C.; Cillero, F.; Armijo, M.; Pina, M.; Alonso, S. *Arzneim.-Forsch.* **1978**, *28*, 1692–1694.
(13) Warolin, C.; Foussard-Blampin, O. *Thérapie* **1966**, *21*, 245–259.
(14) Tedeschi, S.; Spano, R. Eur. Patent 70,049, 1982.
(15) Kumar, R.; Billimoria, J. D. *J. Pharm. Pharmacol.* **1978**, *30*, 754–758.
(16) Paris, G. Y.; Garmaise, D. L.; Cimon, D. G. *J. Med. Chem.* **1979**, *22*, 683–687.

0022-2623/89/1832-0727$01.50/0