A Novel Class of Conformationally Restricted Heterocyclic Muscarinic Agonists

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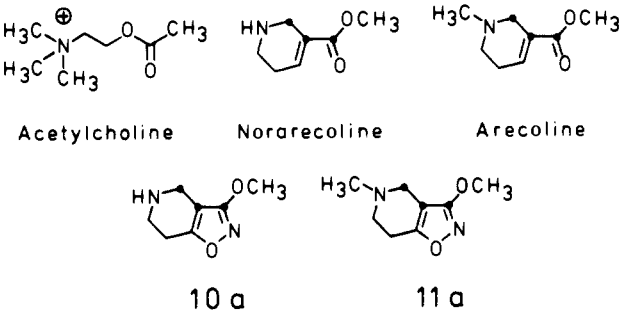
A series of conformationally restricted compounds containing the 4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol (THPO) skeleton, including *O*-methyl-THPO (**10a**) and *O*,5-dimethyl-THPO (**11a**), were synthesized. The compounds were designed by bioisosteric replacement of the methyl ester groups of the muscarinic cholinergic agonists norarecoline and arecoline by the 3-methoxyisoxazole group, and their interactions with central and peripheral muscarinic receptors were tested in vitro. The compounds **10a**, **11a**, *O*-ethyl-THPO (**10b**), *O*-propargyl-THPO (**10j**), and *O*-ethyl-5-methyl-THPO (**11b**) were inhibitors of the binding of the muscarinic mustard [^{3}H]PrBCM to rat brain membranes with an increasing order of potency. There was, however, a very low degree of correlation between these binding data and the effects of the compounds on peripheral (ileal) muscarinic receptors, where **11a**, **10j**, **11b**, and **10a** were agonists with a decreasing order of potency, whereas *O*-isopropyl-THPO (**10e**) showed antagonistic effects. The relatively low pK_a values of the compounds (7.5–7.7 for compounds with secondary and 6.1–7.0 for compounds with tertiary amino groups) are likely to allow the compounds to penetrate the blood–brain barrier.

A number of reports have consistently indicated major deficits in central cholinergic transmission in patients with the pathology characteristic of Alzheimer's disease (AD) and senile dementia of the Alzheimer's type (SDAT).[1-8] On the basis of clinical and animal behavioral studies, this cholinergic deficit may be of particular relevance to disturbances in learning and memory in the AD/SDAT patients.[6,9,10] This accumulating evidence is supported by neurochemical examination of autopsy and biopsy brain material from Alzheimer patients demonstrating loss of the presynaptic marker enzyme choline acetyltransferase (ChAT) correlating with dementia score and severity of neurohistopathology, whereas postsynaptic muscarinic receptors seem to survive the loss of cholinergic nerve endings.[11,12] Although the degree of functional integrity of these muscarinic receptor sites, detected by using receptor-binding techniques, is unknown, these observations have focused much interest on such receptors as therapeutic sites of attack in Alzheimer patients.[7,10,13]

These aspects have accelerated the pharmacological characterization of the M1 and M2 muscarinic receptors and, perhaps, further subtypes of such receptors, but this receptor "mapping" is still very incomplete, particularly in central nervous system tissues.[7] As part of our attempts to shed light on the muscarinic pharmacophore(s) relevant to AD/SDAT and to design therapeutically useful muscarinic agonists, we have synthesized and tested a series of bicyclic compounds, notably **10a** and **11a**, using the reverse ester acetylcholine isosteres norarecoline (guvacoline) and arecoline as leads.[14,15] The semirigid structures of these bicyclic compounds are particularly pertinent to the former objective of this project, whereas the resistance to hydrolysis of the 3-methoxyisoxazole unit[14-16] and the presence of secondary or tertiary amino groups with pK_a values close to physiological pH (Table IV) are of importance from a pharmacological and therapeutic point of view.

This paper describes the syntheses of **10a**, **11a**, and a number of related compounds and studies of their affinities for central muscarinic receptor sites and their effects on peripheral muscarinic receptors.

Chemistry. The compounds **10a–i** and **11a–d** were synthesized via alkylation of methyl 3-hydroxy-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridine-5-carboxylate (*N*-methoxycarbonyl-THPO, **1**).[17] The 3-alkoxyisoxazoles **3a–i** were separated from the concomitantly formed N-alkylated isomers **2a–i** and deprotected to give **10a–i**



(Scheme I). Analogously, **10j–l** containing more labile alkyl groups were synthesized with use of *N*-*tert*-butyloxycarbonyl-THPO (**5**) as starting material. The ratios between the O-alkylated (**3a–i** and **6j–l**) and the respective N-alkylated products (**2a–i** and **7j–l**) were dependent on the structures of the alkylating agents (Table I). An alternative synthetic route to the key compound **10a**, starting with **2a**, was developed by using a recently described reaction.[18] Thus, treatment of **2a** with neat

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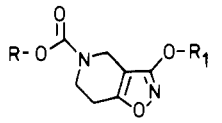
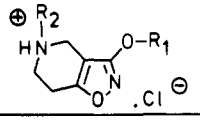
Table I. Alkyl 3-Alkoxy-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridine-5-carboxylates



compd	R	R_1	yield, %	mp, °C	recryst solvent	formula	method	ratio **3**/**2**[c]
3a	CH_3	CH_3	22	55–57[a]	Et_2O/light petr.		A	1:3
3b	CH_3	CH_2CH_3	62	96–98	C_6H_{12}	$C_{10}H_{14}N_2O_4$	B	3:2
3c	CH_3	$CH_2CH_2CH_3$	64	48–52	light petr.	$C_{11}H_{16}N_2O_4$	B	3:2
3d	CH_3	$CH_2CH_2CH_2CH_3$	62	41–42	light petr.	$C_{12}H_{18}N_2O_4$	C	2:1
3e	CH_3	$CH(CH_3)_2$	45	oil			A	3:2
3f	CH_3	$CH_2CH(CH_3)_2$	38	oil			A	1:3
3g	CH_3	$CH_2CH=CH_2$	40	39–41	light petr.	$C_{11}H_{14}N_2O_4$	A	2:3
3h	CH_3	*t*-$CH_2CH=CHCH_3$	41	67–69	light petr.	$C_{12}H_{16}N_2O_4$	C	2:3
3i	CH_3	$CH_2C_6H_5$	24	103–105	EtOAc/Et_2O	$C_{15}H_{16}N_2O_4$	A	1:3
6j	$C(CH_3)_3$	$CH_2C\equiv CH$	30	107–108	Et_2O	$C_{14}H_{16}N_2O_4$	F	2:3
6k	$C(CH_3)_3$	$CH_2CBr=CH_2$	18	62–66	Et_2O/light petr.	$C_{14}H_{19}BrN_2O_4$	G	1:1
6l	$C(CH_3)_3$	$CH_2C\equiv CCH_3$	8[b]	79–81	light petr.	$C_{15}H_{20}N_2O_4$	G	2:1

[a] See ref 14. [b] 1-Chloro-2-butyne was prepared from 2-butyn-1-ol by treatment with $SOCl_2$ and was used without isolation. [c] Ratios **3**/**2** are based on isolated compounds.

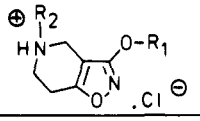
Table II. 3-Alkoxy-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridinium Chloride Derivatives



compd	R_1	R_2	yield, %	mp, °C	recryst solvent	formula	method
10a	CH_3	H	77	191 dec[a]	MeOH/EtOAc		J
10b	CH_2CH_3	H	62	190–193	CH_3CN	$C_8H_{13}ClN_2O_2$	J
10c	$CH_2CH_2CH_3$	H	59	185–186	CH_3CN	$C_9H_{15}ClN_2O_2$	J
10d	$CH_2CH_2CH_2CH_3$	H	80	178–180	CH_3CN/Et_2O	$C_{10}H_{17}ClN_2O_2$	J
10e	$CH(CH_3)_2$	H	57	185–188	CH_3CN/Et_2O	$C_9H_{15}ClN_2O_2$	J
10f	$CH_2CH(CH_3)_2$	H	63	195–196	CH_3CN/Et_2O	$C_{10}H_{17}ClN_2O_2$	J
10g	$CH_2CH=CH_2$	H	75	144–145	CH_3CN/Et_2O	$C_9H_{13}ClN_2O_2$	J
10h	*t*-$CH_2CH=CHCH_3$	H	35	156–157	MeOH/EtOAc/Et_2O	$C_{10}H_{15}ClN_2O_2$	J
10i	$CH_2C_6H_5$	H	49	196–197	CH_3CN/Et_2O	$C_{13}H_{15}ClN_2O_2$	J
10j	$CH_2C\equiv CH$	H	74	176–177	CH_3CN/Et_2O	$C_9H_{11}ClN_2O_2$	K
10k	$CH_2CBr=CH_2$	H	66	154–155	CH_3CN/MeOH/Et_2O	$C_9H_{12}BrClN_2O_2$	K
10l	$CH_2C\equiv CCH_3$	H	68	151–155	CH_3CN/Et_2O	$C_{10}H_{13}ClN_2O_2$	K
11a	CH_3	CH_3	73	210	MeOH/Et_2O	$C_8H_{13}ClN_2O_2$	M
11b	CH_2CH_3	CH_3	88	180–184	*i*-PrOH	$C_9H_{15}ClN_2O_2$	M
11c	CH_3	CH_2CH_3	24	180 dec	EtOH/EtOAc	$C_9H_{15}ClN_2O_2$	N
11d	CH_3	$CH_2C_6H_5$	65	202–204	MeOH/Et_2O	$C_{14}H_{17}ClN_2O_2$	O

[a] See ref 14.

Table III. ^1H NMR Spectral Data for 3-Alkoxy-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridinium Chloride Derivatives

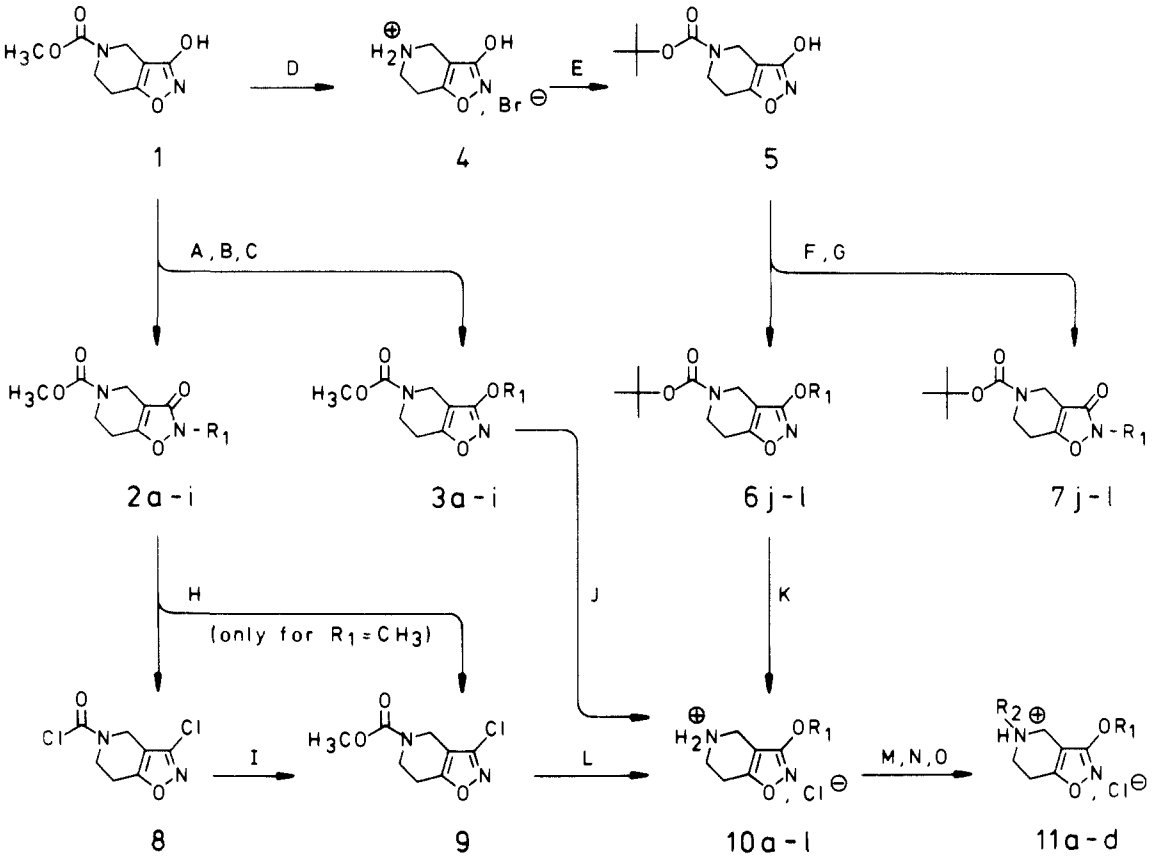


compd	R_1	R_2	chemical shifts,[a] δ
10a	CH_3	H	4.00 (s, OCH_3)
10b	CH_2CH_3	H	4.35 (q, J = 7 Hz, OCH_2), 1.40 (*t*, J = 7 Hz, CCH_3)
10c	$CH_2CH_2CH_3$	H	4.25 (t, J = 7 Hz, OCH_2), 2.0–1.5 (m, CCH_2C), 1.00 (t, J = 7 Hz, CCH_3)
10d	$CH_2CH_2CH_2CH_3$	H	4.30 (J = 6 Hz, OCH_2), 2.0–1.2 (m, CCH_2CH_2), 0.93 (t, J = 6 Hz, CCH_3)
10e	$CH(CH_3)_2$	H	5.00 (m, J = 7 Hz, OCH), 1.40 (d, J = 7 Hz, $C(CH_3)_2$)
10f	$CH_2CH(CH_3)_2$	H	4.10 (d, J = 7 Hz, OCH_2), 2.4–1.8 (m, OCCH), 1.00 (d, J = 7 Hz, $C(CH_3)_2$)
10g	$CH_2CH=CH_2$	H	4.85 (d, J = 6 Hz, OCH_2), 6.5–5.8 (m, CCH=), 5.7–5.2 (m, $C=CH_2$)
10h	*t*-$CH_2CH=CHCH_3$	H	4.55 (d, J = 6 Hz, OCH_2), 5.9–5.3 (m, CCH=CH), 1.60 (d, J = 6 Hz, CCH_3)
10i	$CH_2C_6H_5$	H	5.35 (s, OCH_2), 7.55 (s, C_6H_5)
10j	$CH_2C\equiv CH$	H	5.00 (d, J = 2 Hz, OCH_2), 3.10 (t, J = 2 Hz, C≡CH)
10k	$CH_2CBr=CH_2$	H	5.00 (s, OCH_2), 5.85 and 6.15 (d, J = 2 Hz, $C=CH_2$)
10l	$CH_2C\equiv CCH_3$	H	5.00 (q, J = 3 Hz, OCH_2), 1.93 (t, J = 3 Hz, CCH_3)
11a	CH_3	CH_3	4.02 (s, OCH_3), 3.10 (s, NCH_3)
11b	CH_2CH_3	CH_3	4.35 (q, J = 7 Hz, OCH_2), 1.40 (t, J = 7 Hz, CCH_3), 3.10 (s, NCH_3)
11c	CH_3	CH_2CH_3	4.10 (s, OCH_3), 3.50 (q, J = 7 Hz, NCH_2), 2.45 (t, J = 7 Hz, CCH_3)
11d	CH_3	$CH_2C_6H_5$	4.00 (s, OCH_3), 4.58 (s, NCH_2), 7.60 (s, C_6H_5)

[a] All spectra contained the following signals: δ 4.2 (s, 2 H, H-4), 3.7 (pert. t. J = 7 Hz, 2 H, H-6), 3.1 (pert. t, J = 7 Hz, 2 H, H-7).

phosphorus oxychloride gave the 3-chloroisoxazoles **8** and **9**. Quite surprisingly, displacement of the chloro atom of **9** by a methoxy group could only be accomplished with use of a solution of potassium hydroxide in aqueous methanol, whereas treatment with methanolic sodium methoxide gave very low yields of 3-methoxyisoxazole. The ^1H NMR

Scheme I[a]



[a] The letters A–O refer to the methods A–O, respectively, in the Experimental Section.

spectroscopic data for **2a–i**, **3a–i**, **6j–l**, and **7j–l** (supplementary material), of which only the 3-alkoxyisoxazoles (**3a–i**, **6j–l**) were purified to analytical purity (Table I), and for the final products **10a–l** and **11a–d** (Table III) were consistent with the structures indicated.

All of the 3-alkoxy-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]-pyridines (**10**) studied had very similar basic character (pK_a = 7.5–7.7). On the other hand, the pK_a values of N_5-methyl analogues **11a** and **11b** (pK_a = 6.6) of **10a** and **10b**, respectively, and the N_5-ethyl analogue **11c** (pK_a = 7.0) were significantly lower than those of the parent compounds containing secondary amino groups, probably reflecting that the steric hindrance to protonation caused by these groups more than offset the positive inductive effects of the alkyl groups.

Affinities for Central Muscarinic Receptor Sites. The ability of the compounds to inhibit the binding of radioactive *N*-propylbenziloylcholine mustard ([^{3}H]-PrBCM) to a crude preparation of rat brain membranes was used as an in vitro model for studies of the affinity of the compounds for central cholinergic muscarinic receptor sites.[19] While PrBCM is considered a selective ligand for muscarinic receptor sites, this compound does not seem to interact selectively with any particular subtype of such receptor sites.

In agreement with the relative potency of arecoline and norarecoline as inhibitors of [^{3}H]PrBCM binding, the *N*-methyl analogues **11a** and **11b** were significantly more potent than the respective secondary amines *O*-methyl-THPO (**10a**) and *O*-ethyl-THPO (**10b**). Replacement of the *N*-alkyl groups of **11a** or **11c** by the more bulky benzyl group to give *O*-methyl-5-benzyl-THPO (**11d**) did, however, result in complete loss of receptor affinity (Table IV). The relationship between muscarinic receptor affinity and structure of the *O*-alkyl groups of the compounds containing a secondary amino group was studied. While *O*-propyl-THPO (**10c**) and *O*-butyl-THPO (**10d**) were only slightly more potent than *O*-methyl-THPO (**10a**), replacement of the methyl group of **10a** by an ethyl (**10b**) or, in particular, by an isopropyl (**10e**) group gave substantially more effective inhibitors of [^{3}H]PrBCM binding (Table IV). Compound **10d** and its unsaturated analogue *O*-crotyl-THPO (**10h**) were virtually equipotent. Similarly, **10c** and its allyl analogue (**10g**) were equally effective, whereas *O*-propargyl-THPO (**10j**) was a more potent inhibitor (Table IV).

Effects of Peripheral Muscarinic Receptors. The guinea pig ileum was used as an in vitro model for studies of the efficacies of the compounds at peripheral muscarinic receptors.[19] There is some evidence that this in vitro biological system is particularly rich in cholinergic muscarinic receptors of the M2 type.[20,21]

In general, there is a low degree of correlation between the affinities of the compounds for central muscarinic receptor sites and their effects on muscarinic receptors in the guinea pig ileum (Table IV). *O*-Methyl-THPO (**10a**), *O*,5-dimethyl-THPO (**11a**), *O*-ethyl-THPO (**10b**), *O*-propargyl-THPO (**10j**), and *O*-ethyl-5-methyl-THPO (**11b**), which inhibited [^{3}H]PrBCM binding with an increasing order of potency, were agonists at peripheral muscarinic receptors, **11a** and **10j** being the most potent compounds. Similarly, the [^{3}H]PrBCM binding inhibitors *O*-allyl-THPO (**10g**) and *O*-methyl-5-ethyl-THPO (**11c**) were ca-

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Table IV. In Vitro Muscarinic Effects and pK_a Values

compound	[^{3}H]PrBCM binding to rat brain membranes in vitro: IC_{50}, μM	muscarinic effects[a] agonism: EC_{50}, μM	antagonism: IC_{50}, μM	pK_a
carbacholine	9.0	0.07		
norarecoline	30	0.3		8.7
arecoline	5.6	0.1		7.8
4	>50	>50	>50	4.3, 9.1[b]
10a	45	1.8		7.5
10b	6.0	3.4		7.6
10c	10	>50	35	7.6
10d	15	>50	>50	7.6
10e	3.5	>50	11	7.5
10f	>50	>50	49	7.7
10g	9.6	13		7.5
10h	14	>50	>50	7.7
10i	32	>50	48	7.5
10j	5.8	1.2		7.6
10k	13	>50	22	7.6
10l	5.2	4.5		7.6
11a	27	0.8		6.6
11b	2.4	1.6		6.6
11c	15	20		7.0
11d	>50	>50	>50	6.1

[a] On guinea pig ileum in vitro. [b] Referring to the hydroxy and the amino group, respectively.

pable of activating ileal muscarinic receptors, whereas *O*-isopropyl-THPO (**10e**), which binds quite effectively to central muscarinic receptor sites, blocked ileal muscarinic receptors without showing any sign of agonist activity (Table IV).

Discussion

The accumulating evidence for progressive impairments in central cholinergic neurotransmission in Alzheimer patients[1-8] has brought muscarinic cholinergic agonists into focus as potential therapeutic agents.[13] The alkaloid arecoline has been shown to produce significant cognitive improvements when given intravenously to Alzheimer patients,[22] and, furthermore, arecoline is capable of enhancing learning in normal young humans and aged nonhuman primates.[23,24] These effects of arecoline are, however, very short-lived, probably reflecting rapid hydrolysis of the ester group of arecoline in vivo.[25] These observations prompted us to design *O*,5-dimethyl-THPO (**11a**) and *O*-methyl-THPO (**10a**) by bioisosteric replacement of the methyl ester groups of arecoline and the somewhat weaker muscarinic agonist norarecoline[26,27] by the 3-methoxyisoxazole unit, which is resistant to hydrolytic cleavage in vitro.[14-16]

These bioisosteres and a number of structurally related compounds were synthesized and their affinities for central muscarinic receptor sites and effects on peripheral (ileal) muscarinic receptors tested (Table IV). In spite of lack of correlation between the effects of the compounds on central and peripheral cholinergic receptors, the structure–activity relationship illustrated in Table IV does not necessarily reflect different pharmacological characteristics of central and peripheral muscarinic receptors with respect to the present class of compounds. The effects observed on the ileum preparation probably are primarily mediated by M2 muscarinic receptors,[20,21] whereas the binding data have been obtained with use of [^{3}H]PrBCM, a ligand assumed to interact nondiscriminately with all types of muscarinic receptor sites.[19]

A structure–activity analysis of the compounds having *O*-alkyl groups containing three carbon atoms is interesting. *O*-Propargyl-THPO (**10j**) as well as *O*-allyl-THPO (**10g**) were muscarinic agonists, **10j** being 1 order of magnitude more potent than **10g**. On the other hand, the corresponding saturated analogue *O*-propyl-THPO (**10c**) as well as *O*-(2-bromoallyl)-THPO (**10k**) showed weak and the isopropyl analogue (**10e**) relatively potent antagonistic properties at ileal muscarinic receptors (Table IV). Interestingly, **10j** and **10l**, which have *O*-alkyl groups containing triple bonds, were potent muscarinic agonists (Table IV) in agreement with earlier findings for arecoline analogues containing the same *O*-alkyl groups.[28] In light of this structure–activity relationship the possibility that some of the compounds under study are partial agonists/antagonists at muscarinic receptors cannot be excluded.

A more precise description of the pharmacological profiles and selectivities with respect to muscarinic receptor subtypes of the compounds must await further pharmacological and receptor binding studies. Some of the compounds listed in Table IV, notably **10a** and **11a**, show potent and relatively long-lasting central effects, including analgesic effects, as well as peripheral actions after systemic administration to animals.[29] Further studies in a variety of animal models and, ultimately, in humans are, however, necessary before the therapeutic prospects of the compounds can be estimated. In any case the present compounds are likely to be useful leads for the development of new types of muscarinic cholinergic agonists of therapeutic interest.

Experimental Section

Chemistry. Melting points were determined in capillary tubes and are uncorrected. Column chromatography (CC) was performed on silica gel 60 (70–230 mesh, ASTM, Merck). Elemental analyses were performed by Mr. Cornali, Microanalytical Laboratory, Leo Pharmaceutical Products, Denmark, and were within

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±0.4% of the calculated values. ^{1}H NMR spectra were recorded on a Varian (60 MHz) EM-360L NMR spectrometer in either $CDCl_3$ or D_2O with Me_4Si or sodium 3-(trimethylsilyl)propanesulfonate, respectively, as internal standards. The pK_a values (H_2O, 25 °C) were determined following a published procedure.[30]

General Procedures for the Syntheses of Methyl 3-Alkoxy-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridine-5-carboxylates (3). (Table I). Method A (3a, 3e, 3f, 3g, 3i). To a solution of 1[17] (1.98 g, 10 mmol) in acetone (180 mL) was added K_2CO_3 (3.5 g, 25 mmol), and the mixture was stirred at 50 °C for 1 h. The alkyl halide (30 mmol) was added dropwise, and the mixture was stirred for 20 h at 50 °C. The reaction mixture was filtered and the filtrate evaporated in vacuo. Water (6 mL) was added to the residue, and the mixture was extracted with $CHCl_3$ (3 × 10 mL). The combined extracts were dried and evaporated in vacuo. The resulting oil contained both **2** and **3**. The two compounds were separated by CC using toluene–ethyl acetate mixtures as eluents. The first fractions contained **3** and the latter fractions contained **2**.

Method B (3b, 3c). The alkylation was performed as described in method A, but instead of separation by CC, water (10 mL) was added to the resulting oil containing both **2** and **3**. After stirring of the mixture for 1 h at room temperature, **3** crystallized.

Method C (3d, 3h). The alkylation was performed as described in method A. Instead of separation by CC, **3** was extracted with light petroleum (2 × 60 mL) from a mixture of water (60 mL) and **2** and **3**.

3-Hydroxy-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridinium Bromide (4). Method D. A solution of **1** (10.0 g, 50.5 mmol) in AcOH containing 33% HBr (75 mL) was left in the dark at room temperature for 7 days. The mixture was evaporated in vacuo and the residue recrystallized (MeOH–Et_2O), yielding 9.15 g of **4** (82%). The procedure for the synthesis of **4** has been described earlier.[17]

***tert*-Butyl 3-Hydroxy-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]-pyridine-5-carboxylate (5). Method E.** To a solution of **4** (3.0 g, 13.5 mmol) and K_2CO_3 (1.86 g, 13.5 mmol) in water (25 mL) was added a solution of pyrocarbonic acid di-*tert*-butyl ester (5.2 mL, 22.5 mmol) in THF (25 mL), and the mixture was stirred at room temperature for 20 h. The reaction mixture was evaporated in vacuo and the residue dissolved in water (25 mL). The aqueous solution was washed with EtOAc (2 × 25 mL), cooled in an ice bath, and covered with EtOAc (150 mL). The mixture was carefully acidified with 2 N HCl to pH 3, and the phases were separated. The aqueous phase was further extracted with EtOAc (50 mL). The combined extracts were dried and evaporated in vacuo. Recrystallization of the residue (toluene–light petroleum) yielded 1.85 g (57%), mp 151–152 °C. Anal. ($C_{11}H_{16}N_2O_4$) C, H, N.

General Procedures for the Syntheses of *tert*-Butyl 3-Alkoxy-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridine-5-carboxylates (6) (Table I). Method F (6j). The alkylation was performed as described in method A, using **5** (2.40 g, 10 mmol) as starting material. Instead of separation by CC, **6j** was extracted from the resulting oil containing **6j** and **7j** with light petroleum containing 5% $CHCl_3$ (3 × 80 mL).

Method G (6k, 6l). The alkylation of **5** and the separation of the two products was performed as described in method A.

Methyl 3-Chloro-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]-pyridine-5-carboxylate (9). Method H. A solution of **2a** (1.0 g, 4.7 mmol) in $POCl_3$ (5 mL) was refluxed for 20 h and evaporated in vacuo. The residue was dissolved in $CHCl_3$ (10 mL) and washed with ice-cooled water (10 mL). The aqueous phase was extracted with $CHCl_3$ (2 × 10 mL), and the combined and dried extracts were evaporated in vacuo. The residue contained both **8** and **9**. The two compounds were separated by CC using toluene–ethyl acetate (4:1) as eluent. Compound **8** was isolated as an oil: 73 mg (7%). IR (film): 1740 (s), 1645 (m), 1460 (m) cm^{-1}. ^{1}H NMR (CCl_4): δ 4.55 (s, 2 H), 4.05 (pert. s, 2 H), 2.95 (t, 2 H, J = 7 Hz). Compound **9** was recrystallized (EtOAc–light petroleum), yielding 28 mg (3%), mp 41–43 °C. ^{1}H NMR ($CDCl_3$): δ 4.40 (t, 2 H, J = 2 Hz), 3.85 (t, 2 H, J = 7 Hz), 3.80 (s, 3 H), 2.85 (t, 2 H, J = 7 Hz). Anal. ($C_8H_9ClN_2O_3$) C, H, N, Cl.

Method I. A solution of **8** (60 mg, 0.3 mmol) in MeOH (4 mL) was refluxed for 3 h and evaporated in vacuo. The residue contained **9** and a small amount of **8**. The compounds were separated by CC using toluene–ethyl acetate (4:1) as eluent. Yield of **9**: 24 mg (40%).

General Procedures for the Syntheses of 3-Alkoxy-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridinium Chlorides (10) (Tables II and III). Method J (10a–i). A solution of **3** (10 mmol) and KOH (5.3 g, 100 mmol) in MeOH (25 mL) and water (5 mL) was refluxed for 15 h. The mixture was evaporated in vacuo. Upon addition of water (15 mL), the residue was extracted with $CHCl_3$ (3 × 30 mL). After drying, the extracts were evaporated in vacuo, and the residue was dissolved in EtOAc (50 mL). An excess of 2 N HCl in EtOAc was added and the precipitate collected.

Method K (10j–l). To a solution of **6** (10 mmol) in EtOAc (125 mL) was added an excess of 2 N HCl in EtOAc. After the mixture was stirred for 24 h at room temperature the precipitate was collected.

Method L (10a). A solution of **9** (640 mg, 3.0 mmol) in MeOH (7.6 mL) was refluxed with a solution of KOH (3.0 g, 53 mmol) in water (1.4 mL) for 3 h. After evaporation in vacuo, the residue was dissolved in water (10 mL) and extracted with $CHCl_3$ (3 × 15 mL). The combined and dried extracts were evaporated in vacuo and dissolved in EtOAc. An excess of 2 N HCl in EtOAc was added to the solution. The precipitate was collected and recrystallized (MeOH–EtOAc) to give **10a** (262 mg, 46%).

General Procedures for the Syntheses of 3-Alkoxy-5-alkyl-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridinium Chlorides (11) (Tables II and III). Method M (11a,b). To a solution of K_2CO_3 (1.38 g, 10 mmol) in water (15 mL) was added **10a** or **10b** (7 mmol), and the mixture was extracted with $CHCl_3$ (3 × 15 mL). The extracts were dried and evaporated in vacuo. Formic acid (7 mL) and a 35% aqueous solution of formaldehyde (7 mL) were added to the residue, and the mixture was stirred at 100 °C for 1 h and evaporated in vacuo. A solution of K_2CO_3 (1.38 g, 10 mmol) in water (15 mL) was added to the residue and the mixture was extracted with $CHCl_3$ (3 × 15 mL). The combined and dried extracts were evaporated in vacuo. A solution of 2 N HCl in EtOAc (10 mL) was added to the residue and the product was collected.

Method N (11c). Compound **10a** (381 mg, 2 mmol) was heated with a solution of K_2CO_3 (553 mg, 4 mmol) in water (5 mL), and the mixture was extracted with $CHCl_3$ (3 × 15 mL). The combined and dried extracts were evaporated in vacuo, and the residue was dissolved in dry pyridine (1.5 mL). Acetic anhydride (0.75 mL) was added and the solution was heated at 100 °C for 15 min. After evaporation in vacuo water (15 mL) was added to the oily residue, and the mixture was extracted with $CHCl_3$ (3 × 15 mL). The combined and dried extracts were evaporated to give 3-methoxy-5-acetyl-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridine (433 mg). An analytical sample was recrystallized (light petroleum), mp 40–42 °C. Anal. ($C_9H_{12}N_2O_3$) C, H, N. To a solution of the crude acetyl compound (392 mg, 2 mmol) in dry ether (6 mL) was added $LiAlH_4$ (304 mg, 3 mmol). The mixture was refluxed for 1 h, after which water (2 mL) and then NaOH (1 mL, 33%) were added. The ether phase was isolated and dried, and upon addition of a solution of 2 N HCl in EtOAc (5 mL), **11c** (105 mg, 24%, based on **10a**) was collected.

Method O (11d). A solution of **10a** (1.5 g, 7.9 mmol) and K_2CO_3 (2.8 g, 20 mmol) in acetone (70 mL) was stirred at 50 °C for 1 h. Benzyl chloride (2.05 mL, 17.8 mmol) was added dropwise, and the mixture was stirred for 20 h at 50 °C. The mixture was filtered and the filtrate was evaporated in vacuo. Water (15 mL) was added to the residue and the mixture was extracted with EtOAc (3 × 70 mL). The combined and dried extracts were concentrated to 20 mL, and an excess of 2 N HCl in EtOAc was added, precipitating **11d**.

Muscarinic Cholinergic Agonism and Antagonism in Guinea Pig Ileum. Guinea pig ileum pieces were prepared as described earlier in detail.[19] Guinea pigs were killed by a blow on the head and exsanguinated. A segment (30 mm long) of ileum was removed and placed isotonically in Tyrode solution at 37 °C in a 10-mL organ bath. In studies of agonistic activity, the effect on muscle contraction was examined by adding the test drug to

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the bath in three to five different concentrations. The drug-induced contraction was checked for atropine antagonism. In antagonistic studies muscle contractions were induced by acetylcholine (0.22 μM). Comparisons were made between the acetylcholine-induced contractions before and 3 min after addition of the test drug in three to five different concentrations. EC_{50} values and IC_{50} values were calculated by log probit analysis.

Inhibition of [^{3}H]PrBCM Binding to Rat Brain Homogenates. Rat brain homogenates were prepared as described earlier in detail.[19] Whole brain minus cerebellum of a rat was homogenized in 10 vol ice-cold 0.32 M sucrose, pH 7.4. The homogenate was centrifuged at 600*g* for 10 min and the supernatant at 25000*g* for 55 min at 4 °C with rehomogenization of the pellet in 0.32 M sucrose. Incubation tubes in triplicate received at 30 °C test substance and tissue suspension. After 10 min of incubation, ligand (New England Nuclear, Boston, MA; 28–44 Ci/mmol) was added (final concentration of [^{3}H]PrBCM: 1.5 nM). After 15 min of incubation, the reaction was stopped by addition of sodium thiosulfate. The samples were filtered through Whatman GF/B filters (25 mm). The tubes and filters were rinsed twice with buffer. Nonspecific binding was determined in the presence of 20 μM atropine. IC_{50} values were calculated by log probit analysis.

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Registry No. 1, 53601-98-2; **2a**, 95628-20-9; **2b**, 101697-33-0; **2c**, 101697-34-1; **2d**, 101697-35-2; **2e**, 101697-36-3; **2f**, 101697-37-4; **2g**, 101697-38-5; **2h**, 101697-39-6; **2i**, 101697-40-9; **3a**, 82988-64-5; **3b**, 95597-29-8; **3c**, 95597-30-1; **3d**, 95597-40-3; **3e**, 95597-36-7; **3f**, 95597-42-5; **3g**, 95597-38-9; **3h**, 95597-44-7; **3i**, 95597-31-2; **4**, 53601-99-3; **5**, 95579-09-2; **6j**, 95579-10-5; **6k**, 101697-41-0; **6l**, 101697-42-1; **7j**, 101697-43-2; **7k**, 101697-44-3; **7l**, 101697-45-4; **8**, 101697-46-5; **9**, 101697-47-6; **10a**, 82988-65-6; **10a** (free base), 95579-17-2; **10b**, 95597-32-3; **10b** (free base), 95579-18-3; **10c**, 95597-33-4; **10c** (free base), 95579-19-4; **10d**, 95597-41-4; **10d** (free base), 95597-21-0; **10e**, 95597-37-8; **10e** (free base), 95579-20-7; **10f**, 95597-43-6; **10f** (free base), 95597-22-1; **10g**, 95597-39-0; **10g** (free base), 95597-23-2; **10h**, 95597-45-8; **10h** (free base), 95628-19-6; **10i**, 95597-34-5; **10i** (free base), 95579-21-8; **10j**, 95579-11-6; **10j** (free base), 95578-97-5; **10k**, 101697-48-7; **10k** (free base), 101697-50-1; **10l**, 101697-49-8; **10l** (free base), 101697-51-2; **11** (free base) (R^1 = Me, R^2 = Ac), 95597-27-6; **11a**, 95597-35-6; **11b**, 95578-99-7; **11c**, 95597-28-7; **11d**, 95579-08-1.

Supplementary Material Available: ^{1}H NMR spectral data for the 3-alkoxyisoxazoles **3a–i** and **6j-l** (Table Is) and for the isomeric 2-alkyl-4-isoxazolin-3-ones **2a–i** and **7i–l** (Table I's) (2 pages). Ordering information is given on any current masthead page.

Indole–Phenol Bioisosterism. Synthesis and Antihypertensive Activity of a Pyrrolo Analogue of Labetalol

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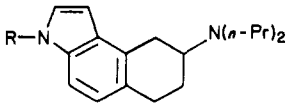
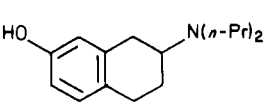
The synthesis of 5-[hydroxy-2-[(1-methyl-3-phenylpropyl)amino]ethyl]-1*H*-indole-7-carboxamide, **5**, a pyrrolo analogue of labetalol, is described. Compound **5** was found to reduce blood pressure in spontaneously hypertensive rats with an ED_{50} of 5 mg/kg po, without causing any decrease in heart rate. Isolated tissue studies with **5** shows that it is a nonselective β-adrenoceptor antagonist and that it is a weaker α-adrenoceptor antagonist with a relative selectivity for α_1-receptors. Additionally, the compound displayed significant β-adrenoceptor intrinsic sympathomimetic activity. Evidence is presented that the β-adrenoceptor antagonist and agonist properties of **5** are mediated via hydrogen-bond formation with the receptor.

We have recently demonstrated[1] the bioisosterism of the pyrrolo ring and the phenolic hydroxyl group at the dopamine receptor. Thus, the benz[*e*]indole **1**, a pyrrolo analogue of the dopaminergic agonist **3**,[2-4] was found to display potent dopaminergic properties, to be orally active, and to have a longer duration of action than the phenol **3**. The bioisosterism of the pyrrolo ring and the phenolic hydroxyl group was ascribed to the ability of both groups to function as hydrogen-bond donors to a common acceptor nucleus on the dopamine receptor macromolecule, and evidence supporting this contention derived from the inactivity of the *N*-methylpyrrolo analogue **2**.[1]



1, R = H
2, R = CH_3



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To examine the generality of the "bioisofunctionality" of the pyrrolo ring and the phenolic hydroxyl group, we were attracted to the structure of labetalol (**4**),[5] an agent that is an antagonist at both α- and β-adrenergic receptors[6,7] and thus has a mechanism of action that is unique among clinically used antihypertensive agents. This paper describes the synthesis and pharmacological evaluation of 5-[hydroxy-2-[(1-methyl-3-phenylpropyl)amino]ethyl]-1*H*-indole-7-carboxamide, **5** (AY-28,925), the pyrrolo analogue of labetalol, and **6**, the *N*-methylpyrrolo analogue.

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