Choline Acetyltransferase Inhibitors. Dimensional and Substituent Effects among Styrylpyridine Analogs

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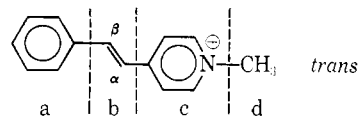
Among styrylpyridine analogs, choline acetylase (ChA) inhibitory potency is diminished by highly electronegative substituents (CN, NO_2) on the 3- or 4-position of the phenyl ring but is enhanced by halogens (Cl, Br) less electronegative than F. Substituents inducing deviation from coplanarity of the two ring systems are unfavorable for inhibitory activity. 3-Methyl substitution on the pyridine ring enhances potency. The nature of the pyrido-*N*-attached quaternizing group is noncritical and a hydrophilic substituent can provide potent, more water-soluble, derivatives. A naphthylvinylquinoline system provides a high order of potency, but the same mass distributed as in phenanthrylvinylpyridine is unfavorable. ChA inhibitory activity among these compounds seems favored by thin flat molecules, one end of which tends to have π-electron-excessive, the other end π-electron-deficient, characteristics separated by a conjugating exocyclic bond. The photolability of some of these compounds in solution requires appropriate precautionary measures in their evaluation.

In an earlier report,[1] derivatives and analogs of styrylpyridine were described that included some unusually potent inhibitors of choline acetylase (ChA) (choline acetyltransferase). The favorable influences of structural coplanarity, presence of a cationic charge, as well as some other features were related. The present report describes additional molecular modifications that provide the basis for more refined correlations of structural features with activities. These permit some speculations on the nature of possible interactions of these inhibitors with the enzyme at the molecular level. A further aim was to prepare highly potent, selective, water-soluble inhibitors of ChA. For the most part, these compounds are relatively poor inhibitors of acetylcholinesterase (AChE).

The target compounds in this report are quaternary ammonium salts which may be considered as variations of prototype I.



I

It was reported earlier[1] that the nonquaternary amine analogs were influenced in the same directions as the quaternaries by structural variations, but were less potent and of limited solubility under the test conditions.

Electronegativity of Substituents on a.—This influence is particularly evident from compounds in Table I. The distinctly unfavorable influences of NO_2 (VII, XI) and CN (VI) substituents, and progressive increases in potency in the order of F < Cl < Br, implicate unfavorable effects of highly electronegative substituents which can withdraw electron density from the aromatic system, and potency enhancing characteristics of less electronegative atoms that can supply π-electron density to this system. The fluoro derivative (III) is comparable to the prototype in potency. The intermediate electronegativity of F in this series may be such as to produce minimal resonance change in the aromatic system. In the halogen series, 3-substitution is slightly better than 4-; there is no advantage in disubstitution. Substitution in the 2-position is least desirable; however, here a steric influence may cause some deviation from coplanarity and reduction in potency. Third dimensional bulk (thickness) contributed by these substituents probably is insufficient to be a negative factor (note II *vs.* I). The NO_2 and CN groups, of course, are coplanar.

Heteroaromatic Systems at a.—In the earlier report[1] it was noted that analogs in which either a or c was fused bicyclic and the other monocyclic yielded compounds of greater potency than that of bismonocyclic I. Compounds are now included in which a is an indolyl and c a pyridinium group (compare XXIV with XVIII) and where a is 3-pyridyl (XXX) or 2-thienyl (XXIX) and c is quinolinium (compare with XXVI). In each of these the heteroaromatic substituent at a is unfavorable compared with the carbocyclic prototype in influencing potency. The pyrido component of XXX has a pK_a of the order of 3.8[2] and that moiety is essentially unionized under the test conditions. The nonbonding pair of n electrons on the hetero atoms may be an unfavorable influence in a.

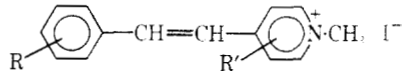
Substitution on b.—It was shown previously[1] that an interannular double or triple bond at b was a critical feature in providing active inhibitors. It now is reported that replacement of H by CH_3 on the carbon of b linked to the pyridine ring yields a completely inactive compound (XXXI). This is further support for a requirement of coplanarity of the a–b–c system. Methyl substitution in this position sterically prevents coplanarity.

Substitution at c.—Most of the compounds in this and in the previous reports are 4-substituted pyrido systems. A few examples of 2-isomers were compared and found to be active inhibitors but less potent than the 4-derivatives.[1] The 3-stilbazole methiodide (XXXII) was prepared for comparison (with I) and found to have an I_{50} of 4×10^{-5} against ChA. Although somewhat less potent, the activity of the 3-isomer makes it

(1) C. J. Cavallito, H. S. Yun, J. C. Smith, and F. F. Foldes, *J. Med. Chem.*, **12**, 134 (1969).

(2) Estimated by titration with HCl.

TABLE I
CHOLINE ACETYLASE AND CHOLINESTERASE INHIBITORY ACTIVITIES



No.	R	R′	Cor mp, °C	% yield[a]	Formula[b]	ChA I_{50}, M	AChE[c] I_{50}, M
I[d]					$C_{14}H_{14}IN$	1.5×10^{-5}	1.8×10^{-3}
II	4-Me		230–234	38	$C_{15}H_{16}IN$	2×10^{-5}	9.5×10^{-4}
III	4-F		268–270	34	$C_{14}H_{13}FIN$	2.1×10^{-5}	22% 6×10^{-3}
IV	4-Cl		245–248	70	$C_{14}H_{13}ClIN$	7×10^{-6}	9×10^{-4}
V	4-Br		260–263	47	$C_{14}H_{13}BrIN$	2.6×10^{-6}	6.2×10^{-4}
VI	4-CN		245–249 dec	17	$C_{15}H_{13}IN_2$	22% 10^{-4}	5.4×10^{-4}
VII	4-NO_2		222–224	10	$C_{14}H_{13}IN_2O_2$	2×10^{-4}	2.8×10^{-3}
VIII	3-Cl		239–244	49	$C_{14}H_{13}ClIN$	2.3×10^{-6}	4.5×10^{-4}
IX	2-Cl		214–217	36	$C_{14}H_{13}ClIN$[e]	2.8×10^{-5}	4.2×10^{-3}
X	3-Br		247–249	50	$C_{14}H_{13}BrIN$	1.5×10^{-6}	3.3×10^{-4}
XI	3-NO_2		309–311 dec	65	$C_{14}H_{13}IN_2O_2$	1.5×10^{-4}	3×10^{-5}
XII	3,4-diCl		276–278	27	$C_{14}H_{12}Cl_2IN$[f]	3.7×10^{-6}	1×10^{-4}
XIII[d]		3-Me			$C_{15}H_{16}IN$	5×10^{-6}	1×10^{-3}
XIV	3-Cl	3-Me	267–270	48	$C_{15}H_{15}ClIN$	1.6×10^{-6}	2.9×10^{-4}
XV	2-Cl	3-Me	239–241	30	$C_{15}H_{15}ClIN$	1.7×10^{-5}	3.5×10^{-3}
XVI	3,4-diCl	3-Me	238–240	11	$C_{15}H_{14}Cl_2IN$	1.7×10^{-6}	8.4×10^{-5}
XVII		3-Et	224–226	50	$C_{16}H_{18}IN$	1.9×10^{-5}	22% 6×10^{-4}

[a] Of analytically satisfactory material. [b] Where figures are not given, found analyses for C, H conform to within 0.3% of the calculated values. [c] Crude rat brain enzyme preparation. [d] Compound reported earlier,[3] included to facilitate comparisons. [e] H found = 0.40% above calcd. [f] C found = 0.41% below calcd.

unlikely that resonance species possible for the 2- or 4-isomers, but not for the 3-derivative, would be participating in the enzyme inhibition reaction.

Only a few variations in substituents on ring c were evaluated at this time. Replacement of H by CH_3 on the 3-position of the pyridine ring led to variable but consistent increases in inhibitory potency (XIII *vs.* I, XIV *vs.* VIII, XV *vs.* IX, XVI *vs.* XII, XIX *vs.* XVIII). Ethyl substitution was less desirable than methyl (XVII *vs.* XIII). Although electronic influences might be comparable for methyl and ethyl, the latter may contribute somewhat unfavorably to third dimensional bulk. Methyl substitution increases basicity of the parent pyridine,[3] and in these pyridinium derivatives, may have a favorable influence on electron distribution.

Variations in d.—The observation[1] that derivatives in which d was benzyl or *n*-hexyl were essentially equipotent inhibitors of ChA, but the latter was a much weaker inhibitor of AChE, suggested that this substituent was relatively nonspecific for ChA inhibition and might be varied to create greater specificity of enzyme inhibition. If the nature of substituent d were sufficiently optional, it also might be possible to prepare more water-soluble quaternary salts (some of the larger a–b–c systems provide methohalides of limited solubility). Use of more polar, hydrophilic groups at d such as hydroxyethyl and acetamido yielded more water-soluble derivatives (particularly hydroxyethyl), but had no deleterious effects on ChA inhibitory activity.

Mass and Configuration of a + c.—Earlier examples[1] had shown that greater ChA inhibitory potency was associated with molecules in which a or c was bicyclic, and that where a was tricyclic, potency was apparently lower and comparable to the bis-monocyclic prototype I. This conclusion was tentative because of the limited aqueous solubility of the methohalide in which a is tricyclic. Utilization of the water-solubilizing hydroxyethyl group at d permitted the clearer demonstration that indeed a tricyclic 9-phenanthryl moiety as a (XXV) was unfavorable for ChA inhibition. Interestingly, XXVIII, in which a and c both are bicyclic, and which has the same total mass as XXV, is a very potent inhibitor. Thus, a bicyclic fused system at a or c, or at both, favors ChA inhibition, but a tricyclic mass at a is unfavorable. Although appreciable latitude appears permissible in the shape of the a–b–c coplanar mass relative to the heterocyclic nitrogen atom, there appears to be a distinct upper limit in the area of a conducive to ChA inhibition.

The requirement of coplanarity and minimal "thickness" of the system a–b–c was inferred from earlier work.[1] The compounds herein reported have spectral characteristics of the expected *trans* isomers, excepting that XXXI cannot be coplanar (and is inert). Most of these compounds are very light sensitive, and uv spectral evidence is compatible with a conversion into *cis* isomers.[4] Such conversion is accompanied by a marked loss in ChA inhibitory activity. The *cis* isomers cannot be coplanar, and this observation further confirms the need for coplanarity. More intense light exposure (direct sunlight) of an aqueous solution of XVIII yields a derivative (XXXIII) which appears to have formed by addition of water to the interannular double bond. This compound is not a ChA inhibitor. Its spectrum is consistent with that expected of such an adduct and differs from that of the *cis* isomer. Under the illumination conditions yielding *cis* isomers, it is unlikely that a significant amount of water adduct would form.

Discussion of Bonding Interactions.—From the structure–activity relations evident at this time, what might be the nature of likely interactions of these inhibitors with ChA? A working hypothesis which is further being tested proposes that ChA inhibitory activity among these compounds is associated with a structure comprising a π-electron-excessive (a) and a

(3) L. Jaris and P. von R. Schleyer, *Tetrahedron*, **24**, 5991 (1968).

(4) H. L. White and C. J. Cavallito, *Biochim. Biophys. Acta*, in press.

TABLE II: CHOLINE ACETYLASE AND CHOLINESTERASE INHIBITORY ACTIVITIES

No.	R[a]	R′	R″	X⁻	Cor mp, °C	% Yield[b]	Formula[c]	ChA I_{50}, M	AChE[d] I_{50}, M
				A. Pyridyl Derivatives					
XVIII[e]	1-Np		Me	I			$C_{18}H_{16}IN$	4.7×10^{-7} (3.3×10^{-6})[f]	2×10^{-4}
XIX	1-Np	3-Me	Me	I	263–266	77	$C_{19}H_{18}IN$	4×10^{-7}	13%/4.5×10^{-6}
XX	1-Np		CH_2CH_2OH	Br	252–255	14	$C_{19}H_{18}BrNO$	6×10^{-7} (1×10^{-6})[f]	1.4×10^{-3}
XXI	1-Np		CH_2CONH_2	I	260–263	62	$C_{19}H_{17}IN_2O$	3.8×10^{-7}	5%/4.5×10^{-6}
XXII	3-ClPh	3-Me	CH_2CH_2OH	Br	222–225	34	$C_{16}H_{17}BrClNO$	2.4×10^{-6}	5.3×10^{-4}
XXIII	3-BrPh	3-Me	CH_2CH_2OH	Br	228–230	50	$C_{16}H_{17}Br_2NO$	4.2×10^{-6}	3.5×10^{-4}
XXIV	3-Ind		Me	I	268–271	35	$C_{16}H_{15}IN_2$	4.3×10^{-5}	13%/1.4×10^{-4}
XXV	9-Phthr		CH_2CH_2OH	Br	284–286	15	$C_{23}H_{20}BrNO$	6%/2×10^{-4}	
				B. Quinolinyl Derivatives					
XXVI[e]	Ph		Me	I			$C_{18}H_{16}IN$	3.5×10^{-6}	2×10^{-4}
XXVII	Ph		CH_2CH_2OH	Br	229–232 dec	13	$C_{19}H_{18}BrNO$	2.9×10^{-6}	4×10^{-4}
XXVIII	1-Np		CH_2CH_2OH	Br	260–263	20	$C_{23}H_{20}BrNO$	5.2×10^{-7}	38%/5.5×10^{-5}
XXIX	2-Thp		Me	I	248–251	36	$C_{16}H_{14}INS$	2.5×10^{-4}	2.8×10^{-4}
XXX	3-Py		Me	I	223–226	19	$C_{17}H_{15}IN_2$[g]	6×10^{-4}	3×10^{-5}

[a] Section A refers to pyridyl derivatives; for structure see compound A. Section B refers to quinolinyl derivatives; for structure see compound B. Abbreviations: Np = naphthyl, Ph = phenyl, Ind = indolyl, Phthr = phenanthryl, Thp = thienyl, Py = pyridyl. [b] Of analytically satisfactory material. [c] Where figures are not given, found analyses for C, H conform to within 0.3% of the calculated values. [d] Crude rat brain enzyme preparation. [e] Compound reported earlier,[1] included to facilitate comparisons. [f] Value in parentheses obtained by conducting tests in ordinary fluorescent light illuminated area in which compound is photosensitive. All other values are derived with appropriate light protection. [g] C found = 0.45% below calcd.

A

B

π-electron-deficient (c) moiety linked by an exocyclic bond (b) which can maintain coplanarity. The *trans* ethylenic and the acetylenic analogs could be represented schematically as XXXIV and XXXV in which

XXXIV

XXXV

π-donor (D) and π-acceptor (A) moieties are present in the same molecule. The favorable influence on activity of the less electronegative halogens and unfavorable effects of very strong electron-withdrawing groups is compatible with, although no proof of, the need for a D_π structure at a. Although ring atoms (N, S) with nonbonding n electrons may enrich the π-electron atmosphere, they may also maintain a more localized n-donor feature. In any event, these compounds are poorer ChA inhibitors (XXX, XXIX). For inhibitory activity an indole group as a (XXIV) is less favorable than an aromatic ring system (XVIII) but not as deleterious as thiophene (XXIX *vs.* XXVI). In addition to the unshared electrons on N, and a possible H-bonding NH feature, indole has been attributed to serve as a donor in charge transfer complexes in which the C-3 carbon has been suggested[5,6] as a region of localization of charge. In XXIV, the conjugated linkage through the C-3 of the indole group may not make the latter suggestion pertinent to this compound.

The greater potencies of the pyridinium derivatives than of the less ionized base forms is compatible with A_π characteristics of c associated with the quaternary or the protonated onium moiety. The favorable influence of cationic character of the pyridinium group may not be related to a need for bonding interaction at an enzyme anionic site, but the pyridinium form can provide the π-electron-acceptor characteristics. This still would be compatible with the relative inactivities reported[1] for the N-oxide form and of stilbenecarboxylic acid. The apparent noncompetitive character of the inhibition does not require involvement of cation bonding competition with choline. The wide latitude in dimensional and hydrophilic or hydrophobic characteristics permissible for d further suggests that the pyridinium nitrogen locus would not be called upon to react with a structurally discriminating site of the enzyme. The somewhat lower ChA inhibitory potency of 2- relative to 4-stilbazole isomers[1] need not be the result of increased steric hindrance to pyridinium bonding to a receptor anionic group but could derive from a

(5) A. Szent-Györgi, I. Isenberg and J. McLaughlin, *Proc. Natl. Acad. Sci. U. S.*, **47**, 1089 (1961).

(6) Several review chapters on charge transfer complexes are provided in "Molecular Associations in Biology," B. Pullman, Ed., Academic Press, New York, N. Y., 1968.

less favorable steric disposition of nonparticipating group d relative to interacting structure a–b–c.

The distinct upper limit in optimum area of a suggests the possibility that the inhibitors insert the a–b–c coplanar mass into a trough or trench-like structure of limited dimensions with the d group projecting away from the enzyme–inhibitor complex. The inactivity of phenanthridinium or benzoquinolinium methohalides[1] as ChA inhibitors might be related both to unfavorable size of the fused tricyclic systems and to the absence of separate donor–acceptor components in the molecule.

The functional participation of the interannular conjugated bond is uncertain. Sterically, this bond maintains coplanarity of the a and c components; electronically, the bond provides a π-electron bridge between the two ring systems. Coplanarity of the a–b–c system should facilitate transmission of electronic delocalization influences from one ring to the other. Structures such as XXXIV and XXXV might be viewed as thin, flat molecular π-electron dipoles subject to shifts in charge distribution with substitution. If the ChA inhibitory properties are related to electronic features represented schematically by XXXIV and XXXV, it becomes tempting to seek components in the enzyme system potentially capable of a charge-transfer type of interaction with such structures. Hydrophobic and van der Waals' bonding could be reinforcing participating factors. These features also may favor some degree of bimolecular association in solution. These and other matters are subjects of further investigations.

Experimental Section

Preparation, Characterization, and Testing of Compounds.—Most of the tabulated compounds were prepared conventionally[1] by condensation of an appropriate arylaldehyde with quaternized 4-methyl-substituted pyridinium or quinolinium salt. Only previously unreported intermediates are described here and these are not assigned numbers. The final compounds usually were dried *in vacuo* at 80°. Melting points were taken with a Fisher–Johns apparatus for temperatures up to 280° and with a Mel-Temp apparatus at higher temperatures; reported values are corrected. Analyses were performed by M–H–W Laboratories, Garden City, Mich.

Inhibition measurements against rat brain ChA and AChE enzyme preparations were conducted as described previously.[1] Although ionic strength can influence I_{50} values with some enzyme preparations, there was little difference in I_{50} values with this AChE preparation and these compounds in 0.1 *M* NaCl or in 0.5 *M* NaCl (the latter used generally here). With a purified eel AChE (Worthington, 1000 units/mg) compounds (XVIII, XX) showed greater inhibitory activities with I_{50} of about 10^{-8} *M*. Impurities in crude enzyme preparations presumably can compete in binding inhibitor. The observation that some of these quaternary stilbazole derivatives were very light sensitive in solution[4] led to use of subdued or pink light illumination during experimental exposures. Some of the earlier reported values,[1] particularly for bicyclic derivatives such as XVIII, will be conservative figures (see Table II). The acetylenic analogs,[1] of course, are not photolabile.

***N*-(2-Hydroxyethyl)-4-methylpyridinium Bromide.**—A solution of 9.3 g (0.1 mol) of 4-methylpyridine and 12.4 g (0.1 mol) of 2-bromoethanol in 20 ml of MeCN was refluxed overnight. Et_2O was added to the cooled reaction mixture, and the hygroscopic precipitate was washed with dry Et_2O, dried *in vacuo*, and used directly in further reactions.

***N*-(2-Hydroxyethyl)-3,4-dimethylpyridinium Bromide.**—A mixture of 10.7 g (0.1 mol) of 3,4-dimethylpyridine and 12.4 g (0.1 mol) of 2-bromoethanol in 20 ml of MeCN was refluxed overnight. After cooling, the material which separated was collected and recrystallized from *i*-PrOH to yield 19.8 g (85%) of product, mp 125–128°. *Anal.* ($C_9H_{14}BrNO$) C, H.

***N*-(2-Hydroxyethyl)-4-methylquinolinium Bromide.**—A mixture of 7.2 g (0.05 mol) of lepidine and 6.2 g (0.05 mol) of 2-bromoethanol in 15 ml of CH_3CN was refluxed overnight. The crude material which separated on cooling was filtered off and recrystallized from EtOH to yield 11.2 g (84%) of product, mp 196–197°. *Anal.* ($C_{12}H_{14}BrNO$) C, H.

***N*-(Carboxamidomethyl)-4-methylpyridinium Iodide.**—A mixture of 9.25 g (0.05 mol) of iodoacetamide and 6.9 g (0.075 mol) of 4-methylpyridine in 15 ml of PhH was heated to reflux for 3 hr. The crude material which precipitated on cooling was collected by filtration and recrystallized from EtOH to yield 10.1 g (73%) of product, mp 197–198°. *Anal.* ($C_9H_{11}IN_2O$) C, H.

3-Stilbazole Methiodide (*N*-Methyl-3-styrylpyridinium Iodide) (XXXII).—3-Stilbazole was prepared by reported procedures[7] and treated with MeI to yield the product which was recrystallized from EtOH, mp 210–211°. *Anal.* ($C_{14}H_{14}IN$) C, H.

***N*-Methyl-4-(α-methyl-β-phenylethenyl)pyridinium Iodide (XXXI).**—The condensation of PhCHO with 4-ethylpyridine yielded the α-methyl-4-stilbazole,[8] mp 70°. *Anal.* ($C_{14}H_{13}N$) C, H. Treatment with excess MeI by refluxing for 2 hr provided a 66% yield of methiodide, recrystallized from *i*-PrOH, mp 169–171°. *Anal.* ($C_{15}H_{16}IN$) C, H.

Light-Induced Reaction of XVIII with H_2O–XXXIII.—A solution of 0.5 g of *N*-methyl-4-(1-naphthylvinyl)pyridinium iodide (XVIII) in 100 ml of warm H_2O (50°, to facilitate solution) in a Pyrex flask was placed in direct, spring afternoon sunlight for 2 hr. The solution became lighter in color. The more soluble reaction product was recovered by evaporation of the H_2O under reduced pressure. The residue was dissolved in hot EtOH and filtered clear, and dry Et_2O added slowly to precipitate a cream-colored product, dried at 95°, mp 170° dec (XVIII, mp 275° dec). *Anal.* Calcd for a H_2O adduct, $C_{15}H_{15}NIO$: C, 55.54; H, 4.14. Found: C, 55.37; H, 4.34. The uv and ir spectra were consistent with a structure formed by addition of H–OH across the interannular bond.

A similar solution in the dark did not show this reaction.

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(7) J. A. T. Beard and A. R. Katritzky, *Rec. Trav. Chim.*, **78**, 592 (1959).
(8) A. P. Phillips, *J. Amer. Chem. Soc.*, **76**, 3986 (1954).