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Molecular Modeling of (*E*)-1-Alkyl-4(3)-[2-(1*H*-azolyl)vinyl]-pyridinium Salts and Evaluation of their Behavior towards Choline Acetyltransferase

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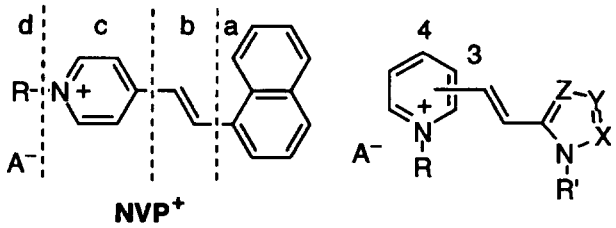
Abstract—A new type of extended π-system aza-analogue of (*E*)-4-[2-(1-naphthylvinyl)]-1-substituted pyridinium salts (**NVP**$^+$) has been designed and its inhibitory activity towards choline acetyltransferase (ChAT) has been evaluated in vitro. Among the several examples of the title quaternary salts synthesized **2** and **3**, the indolylvinylpyridinium salt **2e** is the only one to show a very low ChAT inhibition. The molecular modeling study is highly illustrative of their behavior towards ChAT and interaction with the recognition site. Thus, several selected cations together with the reference **NVP**$^+$ compound **1a** were studied at the PM3 and AM1 levels respectively. At the global minima, all the compounds are planar, which, from the electron charge distribution, shows a degree of polarization similar to the **NVP**$^+$ model compound **1a**. However, the fitting of all optimized structures indicated that only the indole derivative **2e** showed the same aromatic fragment orientation as **1a**, which allows us to define a volume that is not accessible to ligands in the enzyme and consequently to a refined model of the choline acetyltransferase recognition site. © 1997 Elsevier Science Ltd.

Introduction

Among the biological activities displayed by quaternary pyridinium compounds, several series have been shown to be specific enzyme inhibitors, for example, certain (*E*)-stilbazolium salts strongly inhibit choline acetyltransferase.[1] Synthetic inhibitors of this enzyme are of interest in the pharmacological study of aspects that are dependent on cholinergic systems (e.g., selectivity between choline acetyltransferase (ChAT) and acetylcholinesterase (AChE)),[2a,3] neuropathological states and specific cognitive functions.[2a] In 1988, de Bernardis et al.[3] summarized the investigations[4–6] on structure–activity relationships (SAR) on **NVP**$^+$ analogues, which indicate that there are four main structural moieties at regions *a*, *b*, *c* and *d*. This study[3] aimed to ascertain the influence of the substituents on the pyridine nitrogen atom (site *d*) on **NVP**$^+$ derivatives upon in vitro inhibition of ChAT, and some new **NVP**$^+$ analogues were found to be highly potent (e.g., compound **1d**).

Selected examples of the title extended π-systems **2** and **3** may also be considered as enzyme inhibitors,[7] for instance they might inhibit ChAT as they are aza analogues of **NVP**$^+$ derivatives (Chart 1). Thus, several examples of **2** and **3** have previously been designed and prepared by the authors, and a preliminary molecular modeling study at the PM3 level has been performed.[7,8] Some time later, Cavallito, Bowen and colleagues[9] also reported a molecular modeling study of some ChAT inhibitors related to **NVP**$^+$.



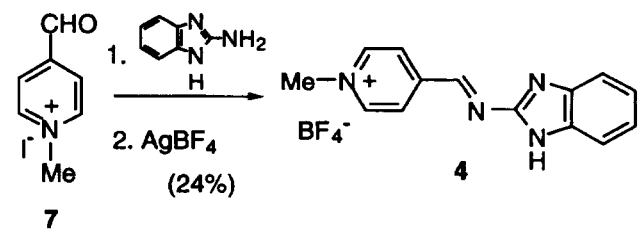
1a (R = Me; A^- = I^-)
1b (R = H; A^- = Cl^-)
1c (R = CH_2CONH_2; A^- = Cl^-)
1d (R = nPr; A^- = Br^-)

2 (4-pyridinio)
3 (3-pyridinio)
X, Y, Z: = CR–; = N–

Chart 1.

Chemistry and Biological Results

The selected azolylvinylpyridinium salts **2a–d** and **3a,b** together with the (*E*)-2-(2-pyridylvinyl)-1*H*-benzimidazoles **5** and **6** (Chart 2) and the model **NVP**$^+$ compounds **1a–d** were prepared.[3,10]



Scheme 1.

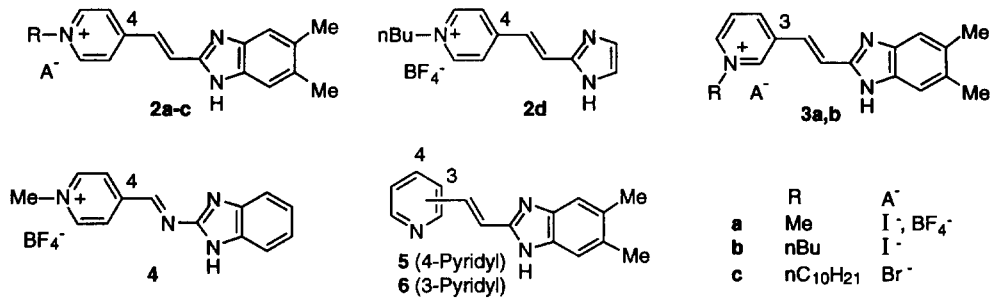
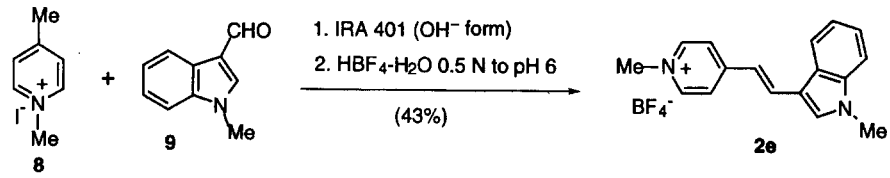


Chart 2.



Scheme 2.

The benzimidazolylpyridinium salt **4** with an azomethine interannular linkage was obtained by condensation of 2-aminobenzimidazole and 4-formyl-1-methylpyridinium iodide.

Compounds **1a–d**, **2a–d**, **3a,b**, **4–6** shown in Chart 2 were subsequently evaluated in vitro as inhibitors of ChAT. The results, the mean of two independent series of experiments, with triplicate chick optic tectum samples, using two preparations of each compound are gathered in Table 1 (see Experimental).[11] From the compounds tested, only **1a**, **1c**, and **1d** show significant inhibitory activity. These compounds were previously prepared and assayed for an inhibitory effect on ChAT by De Bernardis et al.,[3] and found to be among the most active in their series.[12,13] Moreover, none of the tested compounds **2a–d**, **3a**, **3b**, and **4–6** manifested inhibitory activity towards acetylcholinesterase (AChE).

In order to understand the lack of inhibitory activity of compounds **2a–d**, **3a**, **3b**, and **4–6**, a molecular modeling study was carried out (vide infra). On this basis, a new compound was designed—(*E*)-1-methyl-4-[2-(1-methylindol-3-yl)vinyl]pyridinium tetrafluoroborate **2e**. This was obtained following a modified protocol of a type of Knoevenagel condensation (Scheme 2), appropriately applied for the synthesis of several (*E*)-1-alkyl-[2-(1*H*-imidazol-2-yl)vinyl]pyridinium tetrafluoroborates (e.g., compound **2d**).[10c]

The ChAT inhibitory activity of compound **2e** was measured, and a value of 1% inhibition at 10^{-4} M was recorded. Considering that compound **2e** was the only azolylvinylpyridinium salt to be assayed, which showed slight activity, the very low inhibition of this product might be explained by its insolubility in solvents compatible with biological systems.

Table 1. Inhibition of chick optic lobe ChAT[11] by different compounds

Compd	% Inh. at 10^{-4} M	IC_{50} ($\times 10^{-6}$ M)
1a	73	40
1b	5	—
1c	78	37
1d	80	29
2a–d	<1	—
3a, 3b, 4–6	<1	—

The structures of the new quaternary salts **2e** and **4** were established on the basis of analytical and spectroscopic data. Their ^1H and ^{13}C NMR parameters were in agreement with data previously reported on related compounds.[10]

Molecular modeling study

Previous SAR studies on **NVP**$^+$ analogues have led to the proposal of two basic requirements for ChAT inhibition activity:[3,4] (1) coplanarity of the *a b c* system, and (2) polarization of the (*E*)-vinylic linkage *b* resulting in a partial positive charge on the carbon adjacent to the benzene ring and a partial negative charge on the carbon next to the pyridine ring (see Chart 1 and Fig. 1).

To confirm that the tested compounds fulfilled these requirements, molecular modeling of cations present in compounds **2a**, **2d**, **3a** and **4** together with **1a**, as **NVP**$^+$ reference compound were performed using the Chem-X molecular modeling program.[14] Semiempirical calculations were carried out in the preliminary study at the PM3[8,15] level as implemented in Stewart's computer program[16] and using the Chem QM interface.[17] The geometries of the five cations were fully optimized, and their structures were built using the standard bond lengths and angles within Chem-X.[14]

The results indicate[8] that the global minima of all the compounds are planar and, as can be seen from the electron charge distribution shown in Figure 1, the central double bond has a similar degree of polarization to that of the model **1a**.

However, if we consider the fitting of all the optimized structures of compounds **1a**, **2a**, **2d**, **3a** and **4**,[8] it has

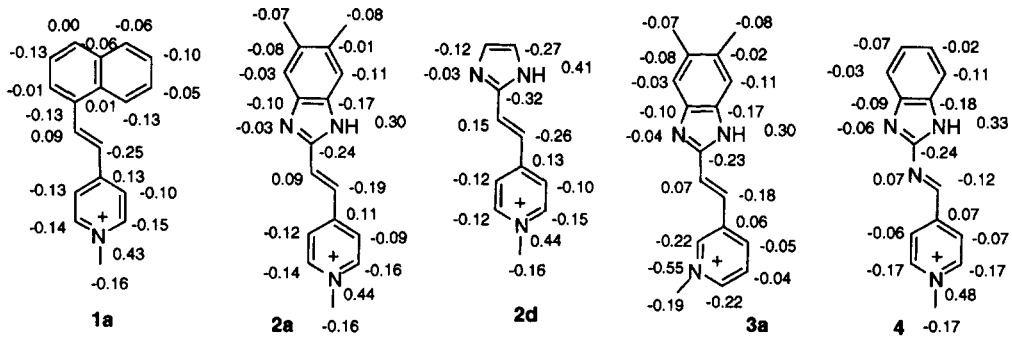


Figure 1. Electron charge distribution of **1a** and the cations **2a**, **2d**, **3a**, and **4** calculated by the PM3 method.[8]

been observed that the volume distribution of the aromatic portion *a* has a very different orientation in the azolylvinyl derivatives compared with that of the model **1a** (see later). Thus, we defined a volume that was not accessible to ligands in the enzyme.[8]

With these results in mind, a new compound was designed to fit the *steric* and *volume* prerequisites. This was the (*E*)-1-methyl-4-[2-(1-methylindol-3-yl)vinyl]-pyridinium tetrafluoroborate **2e** (see Scheme 2). The theoretical calculation of the cation showed a coplanarity of the systems *a b c* and a correct polarization of the (*E*)-vinylic bridge (Fig. 2) and the fitting of the optimized structure of **2e** with the model **1a** showed the same orientation of the aromatic(heteroaromatic) fragments *a*.[8]

AM1 SCF-MO Hamiltonians were then applied for a further semiempirical theoretical study.[18] Running the molecular orbital calculations in the same way as for related extended π-systems containing pyridinium and benzimidazolate fragments with a (*E*)-vinylene bridge,[10d] the geometries of the five cations **1a**, **2d**, **2e**, **3a** and **4** were fully optimized at the RHF, closed-shell, ground-state level using the AM1 SCF-MO[19] program as implemented in the MOPAC package (version 6.0).[20]

For compound **3a**, 16 conformations are possible (ϕ_1 = 180, 0, 90° and −90° and ϕ_2 = 180, 0, 90° and −90°) (Table 2). However, we considered only 10 conformations, since those with (ϕ_1, ϕ_2): (180, 90°), (0, 90°), and (90, 180:0:90:−90°) are chiral, and therefore, their enantiomers (180, −90°), (0, −90°), and (−90, 180:0:90:−90°) would have the same heats of formation. In the case of compounds **1a**, **2d**, **2e**, and **4** eight

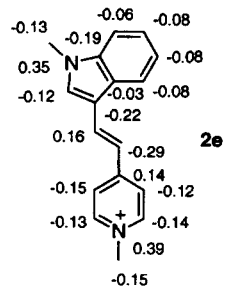


Figure 2. Electron charge distribution of the indolylvinylpyridinium **2e** calculated by the PM3 method.[8]

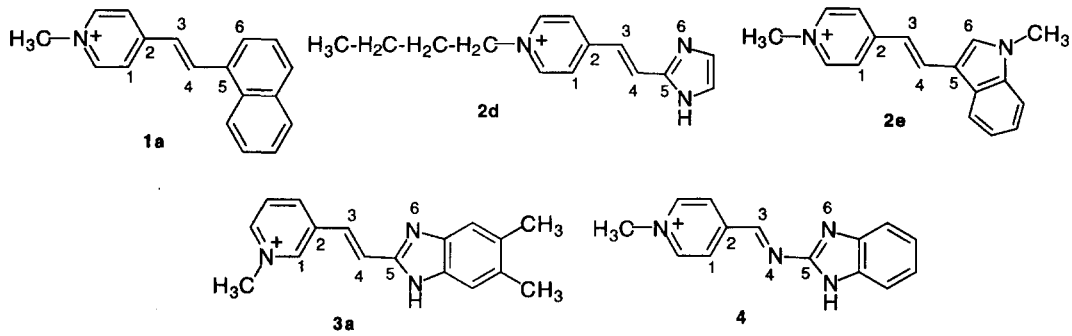
conformations are possible (ϕ_1 = 0 and 90° and ϕ_2 = 180, 0, 90, and −90°) (Table 2). However, those with (ϕ_1, ϕ_2): (0, 90°), and (90, 90°) are chiral, and therefore, their enantiomers (0, −90°), and (90, −90°) would give the same results. Thus, for these structures, we only considered six conformations. For the lateral *n*-butyl chain of compound **2d** the alternated conformation was taken in the starting geometry.

All the stationary states obtained for each compound were characterized by force calculations. The dihedral angles, heats of fomation, and nature of the stationary point obtained are gathered in Table 2 (Figs 3–5).

For all the compounds studied, coplanar structures (ϕ_1 = 0 or 180°, and ϕ_2 = 0 or 180°) were found to be global or local minima and, therefore, energetically more stable. The energy difference calculated by AM1 is 1.60–6.38 kcal mol^{-1} for compound **1a**, 0.82–8.14 kcal mol^{-1} for **2d**, 1.32–12.49 kcal mol^{-1} for **2e**, 0.79–6.32 kcal mol^{-1} for **3a**, and 2.07–8.14 kcal mol^{-1} for **4**. In all the coplanar conformations the (*E*)-vinylene spacer allowed a conjugated extended π-system that explains the stability of these structures in comparison with the nonplanar ones.

All those compounds in which one of the rings was twisted 90° with respect to the (*E*)-vinylene linkage (ϕ_1 = 90 or ϕ_2 = 90°) were characterized as saddle points since a negative frequency was found when the force calculation was carried out. In contrast, those compounds in which both rings were turned 90° with respect to the (*E*)-vinylene linkage exhibited two negative frequencies when the force calculation was performed, and they were characterized as second-order saddle points. These points would seem to be intermediate structures between two first-order saddle points in a rotational profile. For example, starting from a conformation ϕ_1 = 0, ϕ_2 = 0°, that is a minimum, a possible pathway could be ϕ_1 = 0, ϕ_2 = 90° (first-order saddle point), then, ϕ_1 = 90, ϕ_2 = 90° (second-order saddle point), then, ϕ_1 = 90, ϕ_2 = 180° (first-order saddle point), and then, ϕ_1 = 180, ϕ_2 = 180° (which, in all the compounds studied, is equivalent by symmetry to the minimum ϕ_1 = 0, ϕ_2 = 180°). In this way, all the (90, 90°) conformations correspond to the intermediate state between two first-order saddle points: (0, 90°) and (90, 0°).

Table 2. Results obtained from AM1 semiempirical calculations of the (*E*)-1-alkyl-4(3)-[2-(1*H*-azol-2-yl)vinyl]pyridinium cations **2d**, **2e**, **3a**, **4**, and the model compound **1a**



Compound	ϕ_1[a] (°)	ϕ_2[a] (°)	ΔH_f (k_{cal}/mol)	
1a-*a*	−10.25	137.00	239.74	minimum
b	−6.51	−27.78	238.14	minimum
c	−5.51	−95.16	241.19	saddle pt.
d	−89.86	−130.58	243.99	saddle pt.
e	−89.87	33.77	242.68	saddle pt.
f	−88.66	−104.23	244.52	s.p. 2nd o.
2d-*a*	0.54	−179.87	226.36	minimum
b	0.55	0.01	227.18	minimum
c	1.41	−88.02	232.16	saddle pt.
d	−89.84	−179.87	230.28	saddle pt.
e	−90.52	−0.13	231.66	saddle pt.
f	−92.38	−82.56	234.50	s.p. 2nd o.
2e-*a*	0.01	179.99	247.50	minimum
b	0.02	0.17	248.82	minimum
c	0.38	−90.23	256.32	saddle pt.
d	−89.54	179.90	255.02	saddle pt.
e	−89.59	−12.40	255.98	saddle pt.
f	−88.58	−91.63	259.99	s.p. 2nd o.
3a-*a*	179.97	179.99	253.67	minimum
b	177.85	−0.58	255.48	minimum
c	−157.87	−85.23	258.17	saddle pt.
d	−20.93	176.87	254.46	minimum
e	−19.28	−1.04	255.26	minimum
f	31.79	−87.13	258.19	saddle pt.
g	−90.34	175.63	255.95	saddle pt.
h	−90.89	5.83	257.47	saddle pt.
i	−92.58	−82.19	259.99	s.p. 2nd o.
j	−92.09	82.52	259.31	s.p. 2nd o.
4-*a*	−0.02	−180.00	284.59	minimum
b	−0.28	−0.40	289.64	minimum
c	−1.21	−74.82	291.37	saddle pt.
d	−92.56	179.72	286.66	saddle pt.
e	−92.57	20.44	292.43	saddle pt.
f	−93.84	−64.78	292.73	s.p. 2nd o.

[a] ϕ_1 = 1, 2, 3, 4 and ϕ_2 = 3, 4, 5, 6.

The molecular modeling results at the AM1 level are illustrated in Figures 4 and 5. They show a high degree of parallelism with the results obtained at the PM3 level and validate our first report. Figure 5 illustrates the fitting of the optimized structure of the design cation **2d** and the reference compound **1a**, showing the same orientation of the aromatic (heteroaromatic) moiety *a*.

In 1994, Cavallito and colleagues[9] indicated that the choice of the computational method AM1 for use on the **NVP**$^+$ type ChAT inhibitors (e.g., **1a**) was made on the basis of the results obtained with MNDO, AM1, and PM3 on biphenyls. However, we believe biphenyls are not comparable with the **NVP**$^+$ compounds. In the first place, the sterical hindrance that biphenyls present is not as pronounced within the **NVP**$^+$ series in which the aromatic rings are separated by an (*E*)-vinylene bridge which gives the planar structures greater stability. Second, the presence of a cationic charged nucleus in the **NVP**$^+$ molecules makes the comparison even less feasible.[7,8,10d]

In a previous study,[9] we pointed out[8] that by using the semiempirical method PM3, coplanar structures were

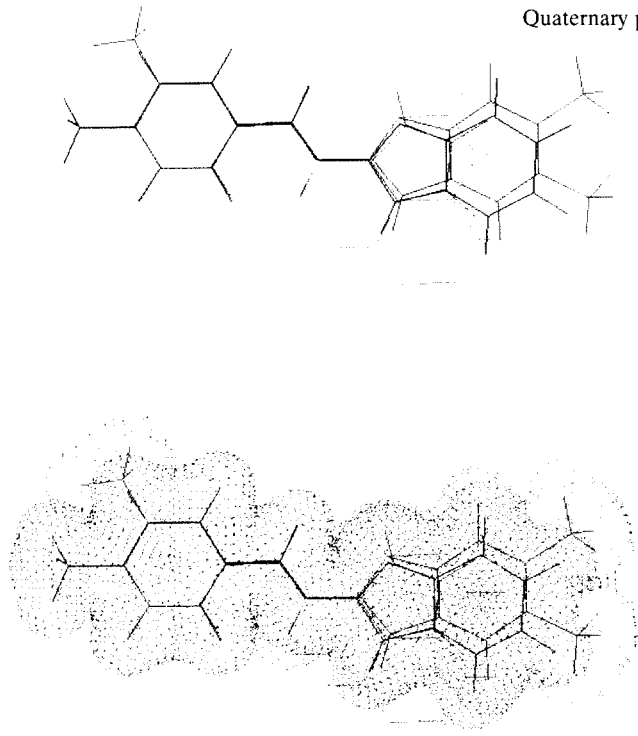


Figure 3. Superimposition and VDW surface of the NVP^+ compound **1a** (green) and the calculated cations **2d** (blue), **3a** (red), and **4** (navy blue) calculated by the AM1 method.

seen to be the most energetically more stable structures for the NVP^+ ChAT inhibitors (e.g., **1a**). Here, we confirm that the minima calculated by PM3 correspond exactly to the minima predicted at the AM1 level.

Conclusions

The present results suggest that previously established coplanarity and polarization criteria are not enough to account for the ChAT inhibitory activity of the ensemble constituted by (*E*)-aryl(heteroaryl)vinylpyridinium salts. The molecular modeling study sheds light on their structure and suggests that *steric* requirements may play a very important role in their enzyme interactions. Furthermore, it provides a definition of the *volume* that is inaccessible to ligands in the recognition site and, consequently to a reasonable refined binding mode of NVP^+ and their aza analogues to choline acetyltransferase.

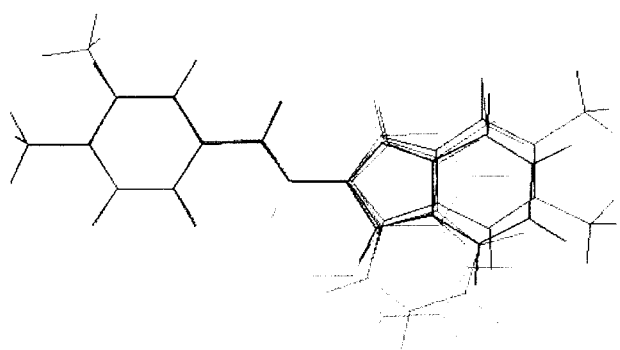


Figure 4. Rigid fitting of the AM1 optimized structures of **1a** (green) and the cations **2d** (blue), **2e** (pink), **3a** (red), and **4** (navy blue) calculated by the AM1 method.

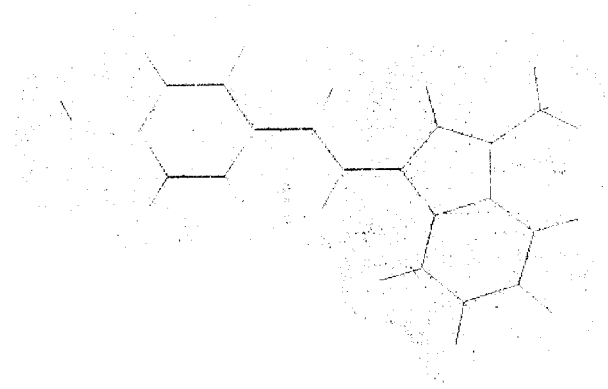


Figure 5. Superimposition and VDW surface of the NVP^+ compound **1a** (green) and the cation **2e** (red) calculated by the AM1 method.

Experimental

Melting point: CTPMP 300 hot-plate apparatus with ASTM 2C thermometer. IR (KBr disks): Perkin–Elmer 1430 spectrophotometer. ^1H NMR: Varian Gemini 200 (200 MHz). ^{13}C NMR: Varian Gemini 200 spectrometer (50.3 MHz). NMR spectra were determined in dimethyl-d_6 sulfoxide. TLC: Merck precoated silica gel 60 F_{254} plates, detection by UV light. Microanalyses were performed on a Carlo Erba 1106 analyzer.

Compounds **1a–d**,[3] **2a–c**,[10a,b] **2d**,[10c] **3a.b**,[10b] **5**,[10a] **6**,[10a] **7**,[21] **8**[22] and **9**[23] were prepared as in the literature.

(*E*)-1-Methyl-4-[2-(*N*-methylindol-3-yl)vinyl]pyridinium tetrafluoroborate 2e. A mixture of 1,4-dimethylpyridinium iodide **8** (1 g, 4.2 mmol) in anhydrous methanol (40 mL) and ion-exchange Amberlite resin IRA 401 (OH-form) (2.80 g, 10.7 mmol), previously filtered from its aqueous suspension[10c] and washed with anhydrous methanol, was energetically stirred at room temperature under an atmosphere of nitrogen for 5 min. The solution was transferred via a cannula to a solution of *N*-methyl-3-indolcarbaldehyde **9** (0.62 g, 4.25 mmol) in anhydrous methanol (40 mL) under an atmosphere of nitrogen. The reaction mixture was stirred at room temperature for 30 h. Then 0.5 N aqueous fluoroboric acid was added to pH $\approx$ 6, and on concentrating the solution a yellow solid precipitated. The crude product was filtered and then recrystallized from dichloromethane to afford 0.62 g (43%) of **2e**: mp 242–244 °C. Anal. ($C_{17}H_{17}N_2BF_4$) C, H, N.

4-[*N*-(1*H*-Benzimidazol-2-yl)iminomethyl]-1-methylpyridinium tetrafluoroborate 4. In a dry, N_2-filled three-necked flask fitted with a stirrer, 4-formyl-1-methylpyridinium iodide **7** (1 g, 4 mmol) and 2-aminobenzimidazole (0.53 g, 4 mmol) were dissolved in anhydrous ethanol and this solution was heated at 80 °C for 6 h. The mixture was evaporated and then

worked up. The crude product was dissolved in water (50 mL), silver tetrafluoroborate (4 mmol) was added and then the precipitate filtered. The aqueous solution was evaporated until dry and the residue triturated with dichloromethane to afford 0.31 g (24%) of **4**: mp 176–178 °C. Anal. ($C_{14}H_{13}N_4BF_4.3/2H_2O$) C, H, N.

Inhibition of choline acetyltransferase

Young (6–10 days old) chick tectal lobes were homogenized in 0.05 M potassium phosphate buffer, pH 6.8, containing 1 mM EDTA (K^+) and 0.05% Triton X-100, and the activity of choline acetyltransferase (ChAT) was measured in the homogenate, as previously described,[11] using [1-^{14}C]acetylcoenzyme A as acetate donor and choline iodide as substrate. The [1-^{14}C]acetylcholine generated during the incubation (10 min/37 °C) was separated from the nonreacted substrates by passing the reaction mixture through a Dowex 1X8 column. Blanks without choline were also assayed to measure acetylation of endogenous substrates, which amounted to less than 2% of the choline-dependent acetylation. When studying the possible inhibition of ChAT activity by different compounds, the homogenate samples were preincubated for 10 min, at 37 °C, in the presence of the potential inhibitor, before starting the reaction by the addition of [1-^{14}C]acetylcoenzyme A.

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12. Actually, the IC_{50} values for these compounds in De Bernardis et al. (ibid) are about two orders of magnitude lower than those recorded here. This discrepancy may reflect differences in the animal source of the enzyme, and also in the sample preparation and ChAT assay methods (see Experimental).[3,11,13a]

13. (a) Professor Cavallito has pointed out[13b] that during the course of his investigations on **NVP$^+$** derivatives,[5] the initial testing of these compounds showed weaker potency when the test was conducted under bright light—resulting in substantial isomerization of the (*E*)-isomer to the inactive (*Z*)-form. Subsequent tests were routinely performed in dim light or in the light of a red bulb. In the present work, while the reasons for the lower activity shown by the reference **NVP$^+$** derivatives **1a–d** are not clear, it is significant that the designed quaternary heteroaromatic salts **2** and **3** were highly stable in *daylight conditions* and no spontaneous (*E*)/(*Z*) isomerization was observed.[10] (b) Personal communication (November, 1994).

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18. The AM1 SCF-MO model is better suited to predicting experimentally observed trends of pyridinium azolate betaines with several interannular spacers[7] (e.g., (*E*)-vinylene).[10d] Moreover, in molecular mechanics, empirical force field calculations (MM2) with atomic point charges modeling AM1 surfaces can be used, as the MM2//AM1 approach has shown the feasibility of this method in several examples of pyridiniun azolate inner salts.[7,24]

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