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(*E*)-1-ALKYL-[2-(1*H*-AZOL-2-YL)VINYL]PYRIDINIUM SALTS: THEORETICAL ANALYSIS, SYNTHESIS AND EVALUATION OF THEIR INTERACTION WITH CHOLINE ACETYLTRANSFERASE.

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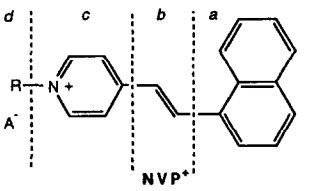
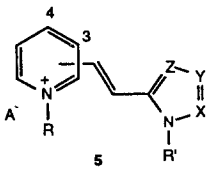
Abstract: A new type of aza-analogues of (*E*)-4-[2-(1-naphthylvinyl)]-1-substituted pyridinium salts (**NVP$^+$**) has been designed in order to study their *in vitro* inhibitory activity towards ChAT. A theoretical analysis has been performed and several examples of (*E*)-1-alkylazolylvinylpyridinium salts have been synthesized. Among them, the indolylvinylpyridinium salt is the only one showing some ChAT inhibition.

Among the variety of biological activities found for quaternary pyridinium compounds, several series have been shown to be specific enzyme inhibitors as is the case of certain (*E*)-stilbazolium salts with a high inhibitory potency for choline acetyltransferase.[1] Synthetic inhibitors of this enzyme are of interest to study pharmacological aspects that are dependent on cholinergic systems, e.g. selectivity between ChAT and AChE[2,3], neuropathological states and specific cognitive functions.[2a] In 1988, de Bernardis *et al.*[3] summarized 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] was mainly focused on the possible influence of the substituents at the pyridine nitrogen atom (site *d*) of **NVP$^+$** derivatives on the *in vitro* inhibition of ChAT, and some new **NVP$^+$** analogues turned out to be very potent (e.g. compound **4**).

In the present work, several examples of (*E*)-1-alkylazolylvinylpyridinium salts **5** have been designed in order to investigate their *in vitro* behaviour towards ChAT.



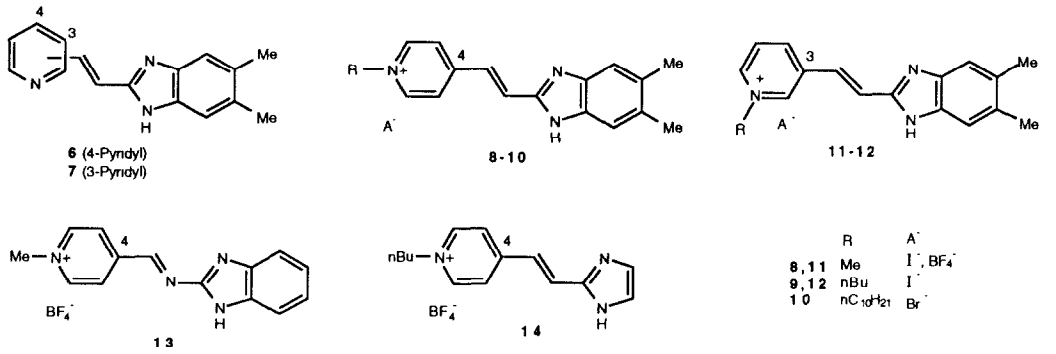
1 (R= Me; A$^-$= I$^-$) 2 (R= H; A$^-$= Cl$^-$)
3 (R= CH_2CONH_2; A$^-$= Cl$^-$) 4 (R= nPr; A$^-$= Br$^-$)



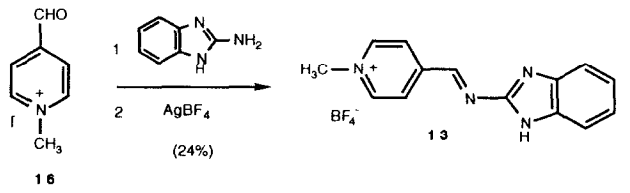
5

X, Y, Z: =CR-, =N-

The selected azolylvinylpyridinium salts **8-14** together with the (*E*)-2-(2-pyridylvinyl)-1*H*-benzimidazoles **6, 7** and the model NVP$^+$ compounds **1-4** have been prepared[7] and then evaluated as inhibitors of ChAT.



The benzimidazolylvinylpyridinium salts **9**, **10** and **12** were obtained by *N*-alkylation under neutral conditions of the 2-(2-pyridylvinyl)-1*H*-benzimidazoles **6** and **7** (*ca* 40% yield).[8a] The benzimidazolylpyridinium salt **13**, with an azomethine interannular linkage, was synthesized by condensation of 2-aminobenzimidazole and 4-formyl-1-methylpyridinium iodide **16** (Scheme 1). The structures of the new quaternary salts **9**, **10**, **12**, **13** and **15** were established according to their analytical and ^{1}H NMR and ^{13}C NMR spectroscopic data.[10] Their ^{1}H and ^{13}C NMR parameters were in good agreement with data previously reported in related compounds.[8,9]



Scheme 1

All the prepared compounds **1-4** and **6-14** were evaluated as inhibitors of ChAT. The results are summarized in Table 1, with only compounds **1**,**3** and **4** showing some significant inhibitory activity. These compounds were also synthesized and assayed for inhibitory effect on ChAT by De Bernardis *et al.*[3], and found to be among the most active in their series. Actually, IC_{50}s for these compounds in the paper by De Bernardis *et al.* are about two orders of magnitude lower than in our hands. This discrepancy prompted us to check the effect of our most active compound, *i.e.* **4**, on samples of chick and rat ChAT partially purified by the method of Potter and Glover, as described by De Bernardis *et al.*[3]. IC_{50}s for compound **4** were then estimated as 58±7 x 10^{-6}M for the chick enzyme and 30±4 x 10^{-6}M for the rat enzyme (mean±S.D. for three assays[11]), in close agreement with our results using a crude chick optic lobe preparation. On the other hand, it is noteworthy that none of the synthesized compounds **6-14** have shown inhibitory activity towards AChE.

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

Compounds	% Inhibition at 10^{-4}M	IC_{50} (x 10^{-6}M)
1	73	40
2	5	---
3	78	37
4	80	29
6-14	<1	---

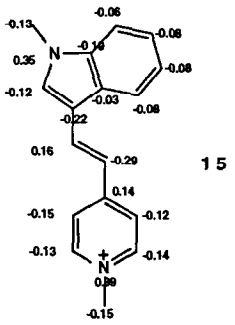
In order to understand the lack of inhibitory activity of the prepared compounds a theoretical study was carried out. Previous SAR studies had lead to the proposal of two basic requirements for ChAT inhibition

activity:[3,4] i-coplanarity of the *a b c* system; ii-polarization of the 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.

To confirm that the synthesized compounds fulfilled these requirements PM3 calculations of cations present in compounds **8**, **11**, **13** and **14** together with **1**, as **NVP**$^+$ model compound, were performed. The theoretical studies were carried out using the Chem-X molecular modeling program.[13] Semiempirical calculations were carried out at the PM3[14,15] level and using the Chem QM interface.[16] The geometries of all cations studied were fully optimized using the Fletcher-Powell algorithm.[17]

The results obtained with the PM3 method indicate that the final minima of all the compounds are planar and that, from the electron charge distribution the central double bond shows a similar degree of polarization to that of the model compound **1**. However, the fitting of all the optimized structures of compounds **1**, **8**, **11**, **13** and **14**, clearly indicates the volume distribution of the aromatic portion *a* has a very different orientation in the azolylvinyl compounds compared to **NVP**$^+$. This might lead us to define a volume not accesible to ligands in the enzyme and consequently to a refined model of the ChAT recognition site.

With all these results in mind, a new compound was designed. This was the (*E*)-1-methyl-4-[2-(1-methylindol-3-yl)vinyl]pyridinium tetrafluoroborate (**15**). The theoretical calculation of the cation showed a coplanarity of the systems *a b c* and a correct polarization of the vinylic bridge (Figure 1). Moreover, the fitting of the optimized structure **15** with the model compound **NVP**$^+$ shows the same orientation of the aromatic fragments *a* (Figure 2).



15

Figure 1. Electron charge distribution of the indolylvinylpyridinium **15** calculated by the PM3 method.

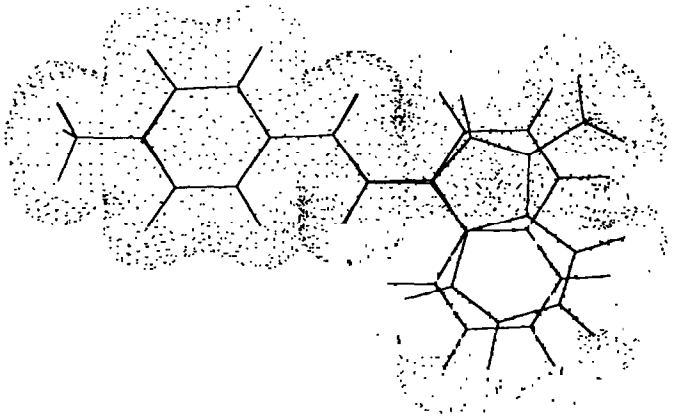
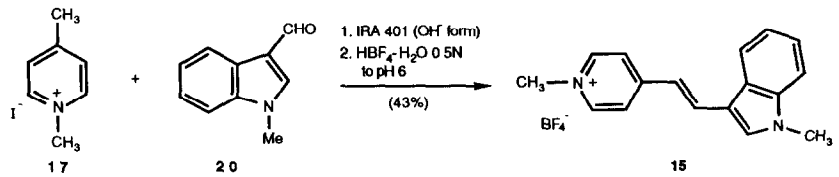


Figure 2. Superimposition and VDW surface of the NVP$^+$ compound **1** and the designed cation **15**.

The synthesis of **15** was achieved using a modified protocol of the classical Knoevenagel condensation (Scheme 2), recently applied[9] for the synthesis of several (*E*)-1-alkyl-[2-(1*H*-imidazol-2-yl)vinyl]pyridinium tetrafluoroborates, i.g. compound **14**.



Scheme 2

The inhibition of ChAT by compound **15** was measured, and a value of 1% inhibition at 10^{-4} M was found. Compound **15** is the only one showing some activity of all the azolylvinylpyridinium salts assayed; however, the insolubility in solvents compatible with biological systems should also be taken into account to explain the very low inhibitory activity of this product.

In conclusion, the results described in this note suggest that the previously established coplanarity and polarization criteria may not be enough to account for the ChAT inhibitory activity of aryl(heteroaryl)vinylpyridinium salts and that steric requirements might play a very important role in their interaction with the enzyme.

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