42-0; **45**·2HCl, 111028-24-1; **46**, 111027-43-1; **46**·2C$_4$H$_4$O$_4$, 111028-25-2; **47**, 111027-44-2; **48**, 111027-45-3; **49**, 111027-46-4; **49**·2C$_4$H$_4$O$_4$, 111028-26-3; **50**, 111027-47-5; **50**·2C$_4$H$_4$O$_4$, 111028-27-4; **51**, 111027-48-6; **51**·2HCl, 111028-28-5; **52**, 111059-97-3; **52**·2HCl, 111028-29-6; **53**, 111027-49-7; **53**·2HCl, 111028-30-9; **54**, 111027-50-0; **54**·2HCl, 111028-31-0; **55**, 111027-51-1; **55**·2HCl, 111028-32-1; **56**, 111027-52-2; **56**·2HCl, 111028-33-2; **57**, 111027-53-3; **57**·2HCl, 111028-34-3; **58**, 111027-54-4; **58**·2HCl, 111028-35-4; **59**, 111027-55-5; **59**·2HCl, 111028-36-5; **60**, 111027-56-6; **61**, 111027-57-7; **62**, 111027-58-8; **62**·C$_4$H$_4$O$_4$, 111028-37-6; **63**, 111027-59-9; **63**·2HCl, 111028-38-7; **64**, 111027-60-2; **64**·2HCl, 111060-01-6; **65**, 111027-61-3; **66**, 111027-62-4; **67**, 111027-63-5; **68**, 111059-98-4; **69**, 111027-64-6; **70**, 111027-65-7; **71**, 111027-66-8; **72**, 111027-67-9; **73**, 111027-68-0; **73**·2HCl, 111028-39-8; **74**, 111027-69-1; **74**·2HCl, 111028-40-1; **75**, 111027-70-4; **75**·2C$_4$H$_4$O$_4$, 111028-41-2; **76**, 111027-71-5; **76**·2HCl, 111028-42-3; **77**, 111027-72-6; **77**·2HCl, 111028-43-4; **78**, 111027-73-7; **78**·2C$_4$H$_4$O$_4$, 111028-44-5; **79**, 111027-74-8; **79** ($R_1$ = 7-C$_3$H$_5$, $R_2$ = $R_3$ = H), 111028-84-3; **79**·2C$_4$H$_4$O$_4$, 111028-45-6; **80**, 111027-75-9; **80**·2C$_4$H$_4$O$_4$, 111028-46-7; **81**, 111027-76-0; **81**·2HCl, 111028-47-8; **82**, 111027-77-1; **82**·2HCl, 111028-48-9; **83**, 111027-78-2; **83**·2C$_3$H$_4$O$_4$, 111060-02-7; **84**, 111027-79-3; **84**·2HCl, 111028-49-0; **85**, 111027-80-6; **85**·$^5/_4$C$_4$H$_4$O$_4$, 111028-50-3; **86**, 111027-81-7; **86**·2C$_4$H$_4$O$_4$, 111028-51-4; **87**, 111027-82-8; **87**·2HCl, 111028-52-5; **88**, 111027-83-9; **88**·2HCl, 111028-53-6; **89**, 111027-84-0; **89**·C$_4$H$_4$O$_4$, 111028-54-7; **90**, 111027-85-1; **90**·2HCl, 111028-55-8; **91**, 111027-86-2; **91**·2HCl, 111028-56-9; **92**, 111027-87-3; **92**·C$_4$H$_4$O$_4$, 111028-57-0; **93**, 111027-88-4; **93**·2HCl, 111028-58-1; **94**, 111027-89-5; **94**·2HCl, 111028-59-2; **95**, 111027-90-8; **95**·C$_4$H$_4$O$_4$, 111028-61-6; **96**, 111027-91-9; **96**·2HCl, 111028-62-7; **97**, 111027-92-0; **97**·C$_4$H$_4$O$_4$, 111028-63-8; **98**, 111027-93-1; **98**·2HCl, 111028-64-9; **99**, 111027-94-2; **100**, 111027-95-3; **100**·HCl, 111028-65-0; **101**, 111027-96-4; **101**·2HCl, 111028-66-1; **102**, 111059-99-5; **103**, 111027-97-5; **103**, 111028-67-2; **104**, 111027-98-6; **105**, 111027-99-7; **106**, 111028-00-3; **107**, 111028-01-4; **107**, 111060-03-8; $C_6H_5COCH_3$, 98-86-2; 4-$FC_6H_4CO(CH_2)_3Br$, 40132-01-2; 4-$CH_3OC_6H_4CH_2Br$, 2746-25-0; $C_6H_5COCH_2Br$, 70-11-1; 3-$F_3CC_6H_4CHO$, 454-89-7; 4-$F_3CC_6H_4CHO$, 455-19-6; piperazine, 110-85-0; *N*-methylpiperazine, 109-01-3; *N*-(2-hydroxyethyl)piperazine, 103-76-4; *N*-phenylpiperazine, 92-54-6; *N*-ethylpiperazine, 5308-25-8; *N*-benzylpiperazine, 2759-28-6; piperidine, 110-89-4; 4-hydroxy-4-(4-chlorophenyl)piperidine, 39512-49-7; 4-hydroxypiperidine, 110-89-4; 4-(4-fluorophenylcarbonyl)piperidine, 56346-57-7; morpholine, 110-91-8.

# Evaluation of the Side Arm of (Naphthylvinyl)pyridinium Inhibitors of Choline Acetyltransferase

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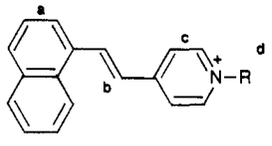
A number of quaternary salts of *trans*-4-(β-1-naphthylvinyl)pyridine (NVP) were synthesized and evaluated as inhibitors of the enzymes choline acetyltransferase (ChAT) and acetylcholinesterase (AChE). Structural variations in the side arm attached to the pyridine nitrogen atom demonstrate that an inductive effect is small but significant for activity. Inhibition of ChAT by alkylated derivatives decreases when electron-withdrawing groups are placed in the side chain. Substitution of a methyl group on the pyridine ring only slightly affects activities toward ChAT and AChE. When the pyridinium moiety is replaced by an imidazolium ring, no ChAT inhibition was observed. The imidazolium compound, however, was a weak inhibitor of AChE. For design of affinity columns for purification of ChAT, the data also supports the use of long chain alkylated amide derivatives of NVP.

Choline acetyltransferase (EC 2.3.1.6, ChAT) is an enzyme that catalyzes the formation of acetylcholine in neurons and other tissues. Interest in ChAT has intensified recently as the enzyme has been studied as a marker for cholinergic neurons especially in Alzheimer's disease and related symptoms of dementia.[1,2] Furthermore, methods of purifying ChAT are required for studying properties of the enzyme. Drugs that affect ChAT might also be useful in the investigations of several neurological disease states. There have been a number of successful attempts in developing in vitro inhibitors of this enzyme. However, the control of synthesis, tissue levels, and release of acetylcholine in vivo is a much more complex issue.[3] The more potent inhibitors of ChAT suffer from inability to cross the blood–brain barrier, poor absorption from the GI tract, or low selectivity between ChAT and its enzyme cousin acetylcholinesterase (EC 3.1.1.7, AChE).

Among the most potent synthetic inhibitors of ChAT are quaternary derivatives of *trans*-4-(β-1-naphthylvinyl)pyridine (NVP), I. Despite several drawbacks, the parent drug NVP is still utilized as a pharmacological tool for the inhibition of ChAT.[3,4] In 1967, Cavallito et al. reported the first in a series of papers on the NVP analogues.[5-8] Structure–activity studies on NVP derivatives indicated that naphthyl substitution at area a was superior to a phenyl group; a single bond at b resulted in loss of

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[§] Department of Pharmaceutical Sciences.

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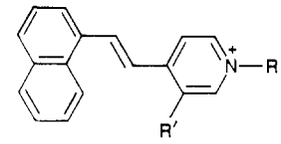
**Table I.** (Naphthylvinyl)pyridinium Derivatives




| compd[a] | R' | R | X | mp, °C | method[b] | yield,[c] % | recrystn solvent | $I_{50}$ ChAT × $10^7$ M | $I_{50}$ AChE × $10^7$ M | $I_{50}$ AChE/$I_{50}$ ChAT |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | H | H | Cl | ref 6, 14 | | | | 380 | NI[d] | >26 |
| 2 | $CH_3$ | H | Cl | ref 6, 14 | | | | 400 | NI[d] | >25 |
| 3 | H | $CH_3$ | I | ref 6 | | | | 3.9 | 300 | 77 |
| 4[e] | H | $CH_2CH_3$ | Br | 218–219 | A | 25 | MeOH–$Et_2O$ | 8.6 | 151 | 18 |
| 5 | H | $CH_2CH_2CH_3$ | Br | 206–207 | A | 27 | MeOH–$Et_2O$ | 1.9 | 175 | 92 |
| 6 | H | $CH_2CH_2CH_2CH_3$ | Br | 211–213 | A | 20 | MeOH–$Et_2O$ | 3.7 | 39 | 11 |
| 7 | H | $CH_2CH_2OH$ | Br | ref 7 | | | | 17 | 680 | 40 |
| 8 | H | $CH_2CH_2CH_2OH$ | Br | 245–246 | A | 22 | MeOH–$Et_2O$ | 13 | 360 | 28 |
| 9 | H | $CH_2CH_2NH_2$·HBr | Br | 307–308 | A | 12 | MeOH–$Et_2O$ | 39 | 640 | 16 |
| 10 | H | $CH_2CH_2CH_2NH_2$·HBr | Br | ref 14 | | | | 53 | 2200 | 42 |
| 11 | H | $CH_2COOH$ | Br | ref 10 | | | | 460 | NI[d] | >22 |
| 12 | H | $CH_2CH_2COOH$ | Br | 263–264 | A | 31 | EtOH | 400 | 10000 | 25 |
| 13[f] | H | $CH_2COCH_3$ | Br | 247–248 | B | 49 | MeOH–$Et_2O$ | 37 | 310 | 8 |
| 14 | H | $CH_2CONH_2$ | I | ref 7 | | | | 2.7 | 800 | 296 |
| 15 | H | $CH_2COOC_2H_5$ | Br | 193–194 | A | 40 | EtOH–$Et_2O$ | 10 | 320 | 32 |
| 16 | H | $CH(COOC_2H_5)_2$ | Br | 166–167 | B | 16 | EtOH–$Et_2O$ | 210 | 5000 | 24 |
| 17[g] | H | $CH_2COCH_2OH$ | Br | 280–281 | | 13 | MeOH–$H_2O$ | 30 | 6000 ($I_{10}$) | >200 |
| 18 | H | $CH_2COCH_2Br$ | Br | 219–220 | B | 23 | absolute EtOH | 100 | 400 | 4 |
| 19[h] | H | $CH_2CONHCONH_2$ | Br | 290–291 | B | 18 | MeOH–$Et_2O$ | 37 | 1400 | 38 |
| 20 | H | $CH_2CONHCONHC_2H_5$ | Br | 247–248 | B | 47 | MeOH | 39 | 1300 | 33 |
| 21[i] | H | $CH_2COCH_2COO$-2-Oct | Br | wax | B | 29 | MeOH–hexane | 500 | 3500 | 7 |
| 22 | $CH_3$ | $CH_2CH_2OH$ | Cl | 229–230 | A | 42 | EtOH–$Et_2O$ | 17 | 270 | 16 |
| 23 | $CH_3$ | $CH_2CH_2NH_2$·HBr | Br | 300–301 | A | 18 | EtOH–MeOH | 50 | 300 | 6 |
| 24 | $CH_3$ | $CH_2CH_2COOH$ | Br | 253–255 | B | 18 | MeOH | 450 | 7500 | 17 |
| 25 | $CH_3$ | $CH_2CONH_2$ | I | 227–228 | B | 11 | EtOH–$CHCl_3$ | 3.4 | 450 | 132 |
| 26 | $CH_3$ | $CH_2COOC_2H_5$ | Br | 155–156 | A | 25 | MeOH–$Et_2O$ | 12 | 100 | 8 |

[a] Elemental analyses obtained for C, H, and N were within 0.4% of theoretical except where noted. [b] Method A, reactants refluxed; method B, room temperature reaction. [c] Yields are of analytically pure material. Where no data appears, compounds have been reported previously. [d] No inhibition observed up to 0.001 M. [e] Monomethanolate; H: calcd 5.96; found 5.45. [f] H: calcd 4.93; found 4.39. [g] Formed by solvolysis of 18. [h] Monomethanolate. [i] Dimethanolate; H: calcd 7.19; found, 6.71.

potency.[5] A pyridine ring in position c was also shown to be superior to a phenyl nucleus, with the 4-substituted pyridine moiety most favorable. Baker and Gibson[9] later made an comprehensive study of the properties of a series of substituted styrylpyridines that contained no quaternary groups. More recently, we reported on the chemical activity at the vinyl group and the crystallographic structures of two NVP analogues.[10] Although rather extensive investigations on structure–activity relationships (SAR) were reported by Cavallito and Baker and their co-workers involving various moieties at regions a, b, c, only limited investigations were conducted involving quaternary substitutions on the pyridine nitrogen atom (site d). Cavallito et al.[7,8] suggested that variations in the substituents on the pyridine nitrogen atom were not as critical for ChAT inhibition relative to the inhibition of acetylcholinesterase. In view of the limited investigations on side arm substitution, the preparation and evaluation of a number of NVP derivatives were undertaken to examine in greater detail the structural parameters involving region d. With these compounds, similarities and differences between ChAT and AChE could also be explored. In addition, we sought to synthesize inhibitors having various sidearms with the eventual goal of preparing $NVP^+$ based affinity chromatography columns, suitable for purification of the ChAT enzyme. Several articles have appeared in recent years describing $NVP^+$-coupled Sepharose columns for purification.[11,12]

## Results and Discussion

As illustrated in Table I, most of the NVP analogues synthesized are potent ChAT inhibitors, with various degrees of specificity as measured by the $I_{50}$ AChE/$I_{50}$ ChAT ratio. Compounds **1–3**, **7**, **10**, **11**, and **14** have been reported previously; they are included here for comparative purposes. From a SAR viewpoint, the side arm on the pyridine nitrogen does play a small but significant role in affecting the inhibitory activity toward both enzymes. Certainly, the quaternary salts in almost all cases are more potent than the free bases. The parent hydrochlorides of **1** and **2** probably equilibrate with significant amounts of the free bases under the assay conditions employed (pH 7.7), which would account for their relatively lower activity when compared to the nonprotonated quaternary cationic species. The majority of compounds do not greatly affect the AChE/ChAT ratios. This is probably due to the quaternerized nitrogen, which is favorable for binding both enzymes. Among the most potent substances are the alkyl derivatives **3–6**, containing the lipophilic side arm. These activities are in line with that of **3** and with a bromohexane styryl analogue, both of which have been previously reported.[6] At the other extreme, the carboxylate analogues **11**, **12**, and **24** are among the least potent inhibitors of both enzymes. Since significant negative charge is associated with carboxy derivatives at the pH of the assay environment, the anionic portion of the molecule may be repelled

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by similar charges on the enzymes. Thus, attraction of a negative site on the enzymic surface for the cationic pyridinium species would be lessened by the zwitterion inhibitors. A similar decrease in activity was previously observed with a stilbene carboxylic acid and a stilbazole *N*-oxide.[6] Cozzari and Hartman[11] in preparing $NVP^+$ ligands also found the hexanoic acid derivatives to be about 60 times less active than the hydrocarbon analogue **3**. The much more lipophilic undecanoic acid analogue was only 4 times less potent. Interestingly, the propionic acid compounds **12** and **24** can be shown to undergo a reverse Michael reaction by stirring the compounds at room temperature in buffer pH 8. The isolated products were the parent free bases **1** (NVP) and **2** (NVL), respectively. Since the observed activities of the propionates and their dealkylated forms are quite similar, we questioned whether inhibition was primarily a result of this degradation. It is doubtful that this reverse Michael reaction was of significance during the assay since only a 30% conversion of **12** to NVP occurred after 5 days.[13] Furthermore, the *N*-acetate, **11**, which did not decompose, had an activity similar to that of the propionates, again suggesting that a negative charge in area d of the inhibitors is detrimental to the binding of inhibitor for both enzymes.

Various functional groups on the side arm of position d play a role in altering potencies. For example, comparisons of the $I_{50}$'s for the alkyl compounds and their biosteres, viz. **5**, **7**, and **9** (group I) and **6**, **8**, and **10** (group II), show that substitution of the hydrophilic moieties OH and $NH_2$ tends to decrease potency when compared to the more lipophilic side arm. The influence of these polar groups is felt by both ChAT and AChE. The data obtained for **7** and **10** is similar to that in previous reports,[8,14] whereby the alcohol and amine exhibited lower activity than the methyl analogue. In comparing alcohols **7**, **8**, and **22** with the corresponding biosteric amine analogues **9**, **10**, and **23**, respectively, the presence of a second cation, i.e., $NH_3^+$, diminishes potency about threefold for ChAT.

A carbonyl moiety, $\beta$ to the pyridine nitrogen, generally tends to decrease both AChE and ChAT inhibition. Evidence of this is found by comparison of the activities for the following pairs of compounds: **13** and **5**, **17** and **8**, **11** and **7**. In further support of this conclusion, compound **16**, which contains two carbonyl functions $\beta$ to the pyridine nitrogen, is much less active than the mono carbonyl containing ester **15**. The simple amides **14** and **25** on the other hand are as potent as the alkyl compounds, and their selectivities are the highest of all the inhibitors tested. Comparison of amides **14** and **25** with the corresponding esters **15** and **26**, as well as **14** vs its bioisostere **13**, demonstrate both the greater ChAT activities of the amides as well as their higher selectivities. Again, the data implicate the electronic effects near the charged pyridinium ion. The ketones and esters are more strongly electron withdrawing than the amides; therefore, the positive charge in the former would not be as intensified as in the amides. Thus, substituents that stabilize the positive charge are the most active.

Other types of compounds were prepared and tested in order to further elucidate the nature of the side arm. Previously, Persson et al.[15,16] had reported ChAT inhibition by choline acetyl halide derivatives with biological activity being attributed to alkylation by a thiol group at the active site on the enzyme. The thiol group seems to be an essential part of the ChAT enzyme, and nucleophilic attack on a haloacetyl group $COCH_2X$ would appear plausible. We thought it would be prudent to evaluate a NVP analogue having a reactive side chain. Compound **18**, containing a bromoacetyl function, was also a candidate for linkage to an affinity column. Surprisingly, **18** was one of the least effective inhibitors, and its specificity was the lowest of all the compounds tested. That **18** possessed a reactive side arm was demonstrated in our initial attempts to purify the compound. Quaternization of NVP with 1,3-dibromoacetone led to the expected "crude" **18**. Upon recrystallization from aqueous ethanol, the keto alcohol **17** was obtained. The displacement of the halide could be prevented by crystallization in absolute ethanol. These results on the chemically labile acetyl halide but the relatively low biochemical potency also corroborate our previous observation[10] that the NVP compounds are not irreversibly bound to a nucleophilic thiol function of ChAT. Biochemical results[14,17] have also shown that the $NVP^+$ inhibitors are not reacting at the active site. Overall, there has been no demonstrated proclivity of an active-site nucleophile for the double bond at region b, nor for alkylating arms at region d of the $NVP^+$ inhibitors.

For various reasons, we were intrigued by the naturally occurring halogen compound, 2-octyl-$\gamma$-bromoacetoacetate, which was reported[18,19] to be an inhibitor of AChE. The quaternary NVP derivative **21**, however, was only a moderately potent inhibitor with little specificity.

We further explored the effect of a methyl group in the area of the pyridinium nitrogen. It had been previously reported[7] that a C-3 methyl group on the heterocycic ring of (styrylvinyl)pyridinium inhibitors increased the potency toward both enzymes; only one compound was tested in the NVP series, however. As shown in Table I, substitution of a methyl group at C-3 of the pyridine ring to give the lutidine (NVL) analogues **2** and **22–26** resulted in increased inhibition of AChE. On the other hand, the lutidine series exhibited a very slight decrease in the inhibitory activity toward ChAT. In all instances however, the differentiation between the two enzymes (as described by the $I_{50}$ ratios) was less for the lutidine analogues than for the pyridine compounds. By inference from our X-ray analysis of the trans NVP analogue **11**, the methyl group at C-3 might be expected to slightly alter the conformation near the heterocycle–vinyl bond, resulting in decreasing coplanarity in the lutidine series. Since activities toward both enzymes are only slightly changed by the $NVL^+$ inhibitors, it appears that at least small spatial alterations in the area of the heterocycle–vinyl skeleton can be tolerated by ChAT and AChE, but the effects are felt more by AChE. Similar observations for ChAT were made by Cavallito et al.[7] when the pyridine nucleus was substituted by a bulkier quinoline ring.

Since previous studies[15,16] have shown that choline acetyl halides were inhibitory toward ChAT, the (bromoacetyl)ureas **27** and **28** were prepared.[20,21] A comparison

(13) Stirring a solution of **12** in phosphate buffer (pH 8) for 5 days and extraction with ether, followed by TLC analysis, resulted in a 30% conversion to **1**. DeBernardis, J. F.; Siuda, J. F., unpublished results.

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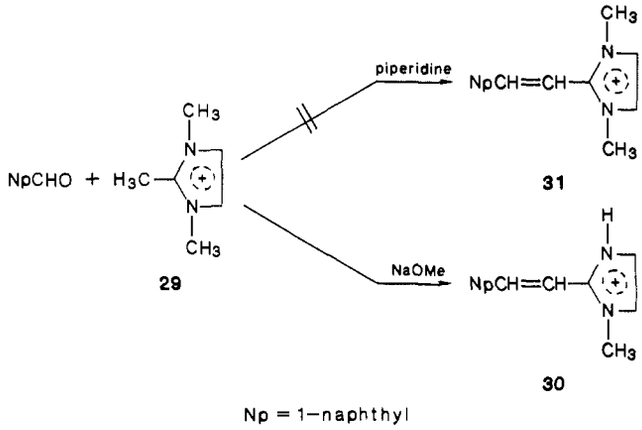
**Table II.** (Bromoacetyl)ureas

| compd | structure | $I_{50}$ ChAT × $10^7$ M | $I_{50}$ AChE × $10^7$ M |
|---|---|---|---|
| 27 | $NH_2CONHCOCH_2Br$ | 1300 | NI |
| 28 | $C_2H_5NHCONHCOCH_2Br$ | 2500 | NI |

of either end of the molecules in both **27** and **28** vs compounds **19**, **20**, and **18** demonstrates that the latter cationic species are better inhibitors of ChAT. As seen by the $I_{50}$'s, ChAT inhibition was enhanced approximately 50-fold by replacing the Br moiety with the (naphthylvinyl)pyridine group. The greater actvity of the pyridinium compounds does not negate the possibility that **27** and **28** are alkylating ChAT. However, if alkylation by **27** and **28** does occur, then this process does not appear to be as efficient as binding of the charged compounds **19** and **20**. On the other hand, the two types of inhibitors may be binding at different sites on the ChAT enzyme. These results provide further evidence that inhibition of ChAT by the bromoacetyl analogue **18** seems to be chiefly due to a noncovalent attraction by the a–b–c region of the inhibitor to the enzyme, rather than by alkylation. For AChE, alkylation by **27** and **28** is insignificant (Table II). However, a cation was essential for inhibition of AChE by the evaluated compounds.

Whereas previous studies[6,7] have shown that the more effective inhibitors of ChAT contained a nitrogen atom in a six-membered ring (pyridine, quinoline, etc.), there have been no reports on a five-membered nitrogen-containing ring in region c. An attempt to prepare **31** from 1-naphthaldehyde and 1,2,3-trimethylimidazolium iodide (**29**) in the presence of piperidine was not successful. However, condensation was achieved when NaOMe was used, but with an attending loss of a methyl group to give **30**. N-Dealkylations of 1,3-imidazoliums are not unusual in basic media or in heated solutions.[22] Inhibition assays are illustrated in Table III.




The imidazolium moiety in **30** may be considered a ring-equivalent isostere of the pyridinium group. In spite of the fact that **30** appears to have all the requirements in regions a–d for significant anti-ChAT activity, the presence of the imidazolium ring causes a drastic reduction of inhibitory action. This decrease in activity may be due to the much weaker basicity of **30** (relative to the pyridinium analogue), a difference in electron density, or a combination of these factors. Yet, the change in the heterocyclic moiety is not sufficient to alter the antiesterase properties. This observation further supports the significant differences between the two enzymes in the areas binding the cation.

In view of some of the conclusions of structure–activity, we used compounds **11**, **12**, and **24** in our preliminary investigation to purify ChAT by affinity chromatography. The most successful experiment occurred with a column made with **12**. With this support, 37% of the protein containing 67% of the total enzyme activity was recovered with a twofold increase in specific activity.[23] Our observations are similar to those of Husain and Mautner[12] who utilized a (styrylvinyl)pyridinium affinity column. To date, the most successful purification of ChAT by using an $NVP^+$-coupled affinity column appears to be an undecanoic acid–$NVP^+$ analogue bound to Sepharose via an amide linkage.[11,24] Our results demonstrate that unsubstituted amides are among the most selective of the $NVP^+$ types of ChAT inhibitors. Since extended hydrophobic functions in the d region do not drastically affect activity toward ChAT, the coupling of a fatty acid to an "inert" support via an amide bond would seem to offer the best choice for an affinity column of the NVP type.

## Summary

1. The most potent $NVP^+$ inhibitors of ChAT are alkyl and amide derivatives. Their effectiveness is directly related to the intensity of the positive charge in the d region. The significance of the inductive effect is further demonstrated by the fact that electron-withdrawing groups decrease activity.

2. Unsubstituted amide derivatives offer the greatest selectivity between ChAT and AChE.

3. Polar functions attached to the hydrocarbon terminus decrease potencies for both enzymes.

4. A thiol or other strong nucleophile at the active site of ChAT does not appear to bind alkylating groups at region d of the $NVP^+$ compounds. In conjunction with our previous paper, and by inference, the vinyl group also does not bind irreversibly to ChAT. A similar argument can be made for AChE.

5. The presence of a $\beta$-methyl group on the pyridine nucleus only slightly affects enzyme activities.

## Experimental Section

Preparative details are included only when specifically varied from the general procedure illustrated in Table I. No attempts were made to maximize yields. All synthesized compounds were subjected to IR, UV, NMR, and TLC procedures and satisfied criteria for structure and purity. IR data were obtained with a Perkin-Elmer Model 267 instrument with KBr pellets. NMR spectra were recorded on a Varian A-60 spectrometer or a Perkin-Elmer R-24 spectrometer with DMSO-$d_6$, $CD_3OD$, or $D_2O$ as solvents and TMS as a reference. UV spectra were obtained with a Beckman Model 25 spectrophotometer with 95% EtOH as the solvent. Melting points were determined with a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Compounds **27**–**29** have been described briefly elsewhere, but chemical analyses have not been reported. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN, and are within 0.4% of the

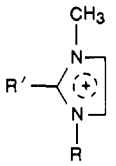
(21) Nuesslein, L.; Pieroh, E. A. S. African Patent 68 05,016, 1969; *Chem. Abstr.* **1969**, *71*, 123642*j*.

(22) *The Chemistry of Heterocyclic Compounds: Imidazole and Its Derivatives, Part I*; Hofmann, K., Ed.; Interscience: New York, 1953; pp 50–51.

(23) Habal, A.; Siuda, J. F., unpublished results. Condensation of the carboxylic acid side arm of **12** with the terminal amino group of AH-Sepharose 4B to form $NVP^+(CH_2)_2CONH(CH_2)_6NH$-Sepharose **4b** was achieved in greater than 85% yield.

(24) Other means of purification have become available recently. See: Peng, J. H.; McGeer, P. L.; McGeer, E. G. *Neurochem. Res.* 1986, *11*, 229; Gordon, B.; Wainer, B. H.; Hersh, L. B. *J. Neurochem.* **1985**, *45*, 611.

**Table III.** Imidazolium Analogues



| compd | R | R′ | X | mp, °C | yield, % | recrystn solvent | $I_{50}$ ChAT × $10^7$ M | $I_{50}$ AChE × $10^7$ M |
|---|---|---|---|---|---|---|---|---|
| **29** | $CH_3$ | $CH_3$ | I | 310–311 | 32 | MeOH–$Et_2O$ | NI[a] | 1200 |
| **30** | H | NV[b] | I | 257–258 | 11 | MeOH–$Et_2O$ | NI[a] | 510 |

[a] No inhibition observed up to 0.001 M. [b] NV = naphthylvinyl.

theoretical values except where noted.

Rat brain ChAT was partially purified by the method of Potter and Glover[25] carried through step 3, the batch elution from CM-Sephadex, and was stored in small aliquots at −20 °C. This preparation of unknown specific activity was diluted such that 2 μL contained sufficient enzyme activity to provide linear kinetics for the duration of the 3-min incubation at 37 °C when added to 20 μL of buffer/substrate having the following composition: [$^{14}$C]acetylcoenzyme A, 0.1 mM; choline chloride, 5 mM; $MgSO_4$, 5 mM; NaCl, 300 mM; physostigmine sulfate, 0.2 mM; phosphate buffer (pH 7), 15 mM; EDTA, 0.1 mM; and 5% albumin. The assay was completed according to the method of Schrier and Shuster.[26] For the acetylcholinesterase studies, the enzyme (Sigma Chemical Co.) was incubated (37 °C) for 10 min in phosphate buffer containing 1% Triton-X with [$^{14}$C]acetylcholine (New England Nuclear) as the substrate. The product ([$^{14}$C]-acetate) was separated by adsorption onto a Dowex 1-X8 chloride column (0.5 × 5 cm) followed by a wash and subsequent elution with dilute acetic acid. All radiolabeled samples were counted in a Packard Tri-Carb liquid scintillation counter. Each of the inhibitors was tested at a concentration range of $10^{-3}$–$10^{-8}$ M. Inhibition curves were obtained by plotting log concentration vs. percent inhibition. Biological results were averages of values obtained in triplicate.

**4-(β-1-Naphthylvinyl)pyridines.** A modified procedure of Cavallito's[6] was used to prepare the naphthylvinyl bases. 1-Naphthaldehyde was condensed by refluxing for 5 h a twofold excess of either 4-picoline or 3,4-lutidine in an equimolar of portion of $Ac_2O$. The reaction mixture was poured into an excess of 10% aqueous NaOH, the organic layer was separated, and a small volume of acetone was added. Concentrated HCl was added until the solution became acidic, at which time the reaction mixture was allowed to stand in an ice bath until precipitation occurred. Purification of the crude hydrochlorides was carried out by recrystallization from methanol. The hydrochlorides were then dissolved in water and converted back to the free bases by addition of NaOH. Extraction with ether followed by recrystallization from hexane–benzene afforded the free bases in good yield.

**Quaternized 4-(β-1-Naphthylvinyl)pyridines or Lutidines.** As a general procedure, NVP or NVL was dissolved in dioxane and quaternized with a half molar excess of the appropriate alkyl halide. The mixture was either refluxed (method A) or stirred at room temperature (method B), depending upon the reactivity of the alkyl halide. The methods employed for each compound are shown in Table I. The keto alcohol analogue **17** was obtained during the attempted crystallization of **18** with aqueous ethanol.

**1,2,3-Trimethylimidazolium Iodide (29).** The compound was prepared by a modified procedure of Radziszewski.[27] Five grams (0.061 mol) of 2-methylimidazole was dissolved in 30 mL of dioxane with 12.79 g (0.09 mol) of methyl iodide added slowly through the top of a condenser. Almost immediate precipitation occurred, and after approximately 10 min, the solid was filtered and recrystallized from methanol–ether, affording white needle crystals in 32% yield: mp 310–311 °C; NMR ($D_2O$) δ 7.25 (s, 2 H, Het *H*), 3.70 (s, 6 H, 2 $NCH_3$), 2.60 (s, 3 H, $CCH_3$).

***N*-Methyl-2-(β-1-naphthylvinyl)imidazolium Iodide (30).** To a solution containing 4.05 g (0.017 mol) of **29** in 30 mL of methanol was added 918 mg (0.017 mol) of NaOMe. 1-Naphthaldehyde (3.97 g, 0.026 mol) in 5 mL of methanol was added, and the reaction mixture was refluxed overnight. The solution was cooled in the refrigerator, resulting in precipitation of a yellow solid. Recrystallization from methanol–ether afforded **30** as yellow crystals in 11% yield: NMR (DMSO–$CD_3OD$), δ 8.38–6.77 (m, 11 H, Ar *H*, Het *H*, vinyl *H*), 3.08 (s, 3 H, $NCH_3$). Anal. ($C_{16}H_{15}N_2I$) C, H, N. The compound also gave a positive Hinsberg test, characteristic of secondary amines.

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**Registry No.** **1**, 16375-78-3; 1·HCl, 20111-29-9; **2**, 89711-14-8; **2**·HCl, 110852-52-3; **4**, 110852-53-4; **5**, 110852-54-5; **6**, 110852-55-6; **8**, 110852-56-7; **9**, 89711-17-1; 11, 89711-10-4; **12**, 110852-59-0; **13**, 110852-60-3; **15**, 110852-61-4; **16**, 110852-62-5; **17**, 110852-63-6; **18**, 110852-64-7; **19**, 110852-65-8; **20**, 110852-66-9; **21**, 110852-67-0; **22**, 110852-68-1; **23**, 110852-70-5; **24**, 110852-71-6; **25**, 110852-72-7; **26**, 110852-73-8; **27**, 6333-87-5; **28**, 25578-58-9; **29**, 36432-31-2; **30**, 110852-74-9; ChAT, 9012-78-6; AChE, 9000-81-1; $Br(CH_2)_3OH$, 627-18-9; $Br(CH_2)_2NH_2$·HBr, 2576-47-8; $Br(CH_2)_2CO_2H$, 590-92-1; $BrCH_2COCH_3$, 598-31-2; $BrCH_2C_2H_5$, 105-36-2; $BrCH(CO_2C_2H_5)_2$, 685-87-0; $BrCH_2COCH_2Br$, 816-39-7; $BrCH_2COCH_2CO_2$-2-Oct, 22509-90-6; $Br(CH_2)_2OH$, 540-51-2; $BrCH_2CONH_2$, 683-57-8; $BrCH_2CONHCONH_2$, 6333-87-5; $BrCH_2CONHCONHCH_2CH_3$, 25578-58-9; 1-naphthaldehyde, 66-77-3; 4-picoline, 108-89-4; 3,4-lutidine, 583-58-4; 2-methylimidazole, 693-98-1.

(25) Potter, L. T.; Glover, V. A. S. In *Methods in Enzymology*; Tabor, H., Tabor, C. W., Eds.; Academic: New York, 1970; Vol. 17B, pp 798–801.
(26) Schrier, B. K.; Shuster, L. *J. Neurochem.* **1967**, *14*, 977.
(27) Radziszewski, B. *Ber. Dtsch. Chem. Ges.* **1883**, *16*, 487.