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# INTERRELATIONSHIP OF CHEMICAL STRUCTURE AND ANTIACETYLCHOLINESTERASE ACTIVITY OF DISUBSTITUTED QUINAZOLONES

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**Abstract**—Antiacetylcholinesterase activity of quaternary ammonium compounds of 2-methyl-3(2′-)pyridyl-4-quinazolone [QZ-2′] and 2-methyl-3(4′-)pyridyl-4-quinazolone [QZ-4′] has been investigated. Acetylcholinesterase activity of rat brain homogenates was determined colorimetrically, with acetylthiocholine as substrate. Both series of these quaternary compounds inhibited acetylcholinesterase competitively. With QZ-4′ derivatives, it was found that an increased inhibition was dependent upon the number of carbon atoms of the alkyl chain at the pyridinium nitrogen, whereas no such effect was observed with QZ-2′ compounds. The possible mechanism of action for such dissimilarity in enzyme inhibition has been discussed.

It is well recognized that the negatively charged anionic site which binds and orients substituted ammonium ions, and the esteratic site binding the ester bond, are two subsites of the active center of acetylcholinesterase.[1–2] Many quaternary ammonium compounds have been shown to inhibit acetylcholinesterase[3] by competing with acetylcholine for the anionic site of the enzyme.[1] In the present study, rat brain homogenate was used to investigate the nature of the properties of some newer quaternary ammonium compounds of 2,3-disubstituted quinazolones that inhibit cholinesterase, where relationship between chemical structure and enzyme inhibition was evaluated.

## MATERIALS AND METHODS

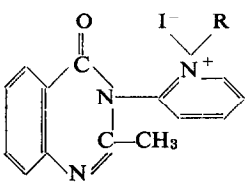
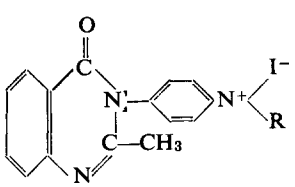
*Enzyme preparation.* Adult rats weighing approximately 150g were killed by decapitation. Brains were quickly removed and weighed in a balance (Roller-Smith type) and homogenized in ice-cold 0·25 M sucrose in a motor-driven Teflon Pyrex homogenizer. The final concentration of the homogenate (without further purification) used throughout these studies was 10% w/v.

*Determination of acetylcholinesterase activity.* Acetylcholinesterase activity was determined colorimetrically, with acetylthiocholine as substrate.[4] The reaction mixture, in final concentration, consisted of 43 mM Tris buffer, pH 7·4, 350 mM sodium chloride, and 0·3 ml of brain homogenate. Water and acetylthiocholine were added to adjust the final volume to a total of 2·0 ml. The reaction mixture, with or without inhibitor, was incubated at a constant temperature (37°) for varying lengths of time (10, 20, and 30 min respectively) prior to the addition of acetylthiocholine. The

simultaneous addition of substrate and quaternary compounds represents the zero-time experiment. At the end of 10-min incubation, subsequent to the addition of substrate, 0·5 ml of 25% (w/v) trichloroacetic acid was added and the resultant solution was centrifuged for 5 min at 500 *g*. An aliquot of the clear supernatant so obtained was withdrawn, and the enzymatically formed thiocholine content was determined colorimetrically. Each assay was done in triplicate where tissue and substrate blanks were subtracted to give the actual value for the hydrolysis of acetylthiocholine. Results are expressed as change in extinction per 100 mg fresh tissue.[5]

We have recently reported[6] the synthesis of 2-methyl-3(2′-)pyridyl-4-quinazolone (QZ-2′) and 2-methyl-3(4′)-pyridyl-4-quinazolone (QZ-4′). Quaternary ammonium derivatives of QZ-2′ and QZ-4′ were synthesized in our laboratory according to the method of Bogert and Geiger,[7] in which methyl, ethyl, n-propyl, and n-butyl iodides were used for quaternization.[8] Reagents were prepared in doubly glass-distilled water and were of the highest available purity. Acetylthiocholine was purchased from Mann Research Laboratories, New York, N.Y.

TABLE 1. INHIBITION OF RAT BRAIN ACETYLCHOLINESTERASE

| Compound No. | | R | Inhibition (%) |
|---|---|---|---|
|  (QZ-2′) | I | | None |
| | II | $CH_3$ | 22·2 ± 1·60* |
| | III | $C_2H_5$ | 22·7 ± 2·86 |
| | IV | n-$C_3H_7$ | 22·3 ± 1·13 |
| | V | n-$C_4H_9$ | 21·2 ± 1·53 |
|  (QZ-4′) | VI | | None |
| | VII | $CH_3$ | 27·1 ± 3·08 |
| | VIII | $C_2H_5$ | 33·7 ± 4·80 |
| | IX | n-$C_3H_7$ | 67·3 ± 2·36 |
| | X | n-$C_4H_9$ | 81·0 ± 1·60 |

* Mean ± S.D. Inhibition was calculated on the basis of the decrease in the enzyme activity estimated as ΔE/100 mg tissue/10 min. For assay procedure and contents of the reaction mixture see text. Compounds used at a final concentration of $1 \times 10^{-3}$ M were incubated with rat brain homogenates (0·3 ml of 10%, w/v) for 10 min prior to the addition of acetylthiocholine (1·5 mM final concentration).

## RESULTS

### *Inhibition of acetylcholinesterase*

Typical results obtained with QZ-2′ and QZ-4′ compounds at a final concentration of $1{\cdot}4 \times 10^{-3}$ M are summarized in Table 1. Disubstituted quinazolones (cf. compounds I and VI) were found to have no inhibitory effect. Generally, all the quaternary compounds produced inhibition. As is evident from Table 1, with QZ-4′ derivatives (cf. compounds VII-X) an increased inhibition was found to be dependent

upon the number of carbon atoms of the alkyl group attached to the pyridinium nitrogen, whereas with QZ-2′ derivatives (compounds II–V), the inhibition remained unchanged.

*Influence of the incubation period*

Rat brain homogenates were incubated with an appropriate representative of the quaternary ammonium compounds (e.g. V, IX, and X) for varying lengths of time before the addition of acetylthiocholine. In such preincubation studies the degree of acetylcholinesterase inhibition as shown in Table 2 remained constant, indicating presumably a reversible competitive nature of inactivation.

TABLE 2. PREINCUBATION STUDIES WITH QUATERNARY COMPOUNDS

| Compound No. | Final concentration (M) | Per cent inhibition Preincubation time (min) | | | |
|---|---|---|---|---|---|
| | | 0 | 10 | 20 | 30 |
| V | $3 \times 10^{-3}$ | 60·1 ± 1·2* | 61·1 ± 1·4 | 59·2 ± 0·0 | 60·1 ± 0·3 |
| IX | $1{\cdot}4 \times 10^{-3}$ | 59·2 ± 0·0 | 62·9 ± 0·0 | 62·0 ± 0·9 | 63·5 ± 1·5 |
| X | $7 \times 10^{-4}$ | 66·1 ± 0·51 | 66·0 ± 0·0 | 66·0 ± 0·5 | 66·6 ± 0·0 |

* Mean ± S.D. Assay procedure as in Table 1. Compound numbers refer to numbers in Table 1. "Zero-time experiments" indicate when these compounds and acetylthiocholine were added simultaneously.

*Nature of the enzyme inhibition*

The data obtained from inhibition studies were evaluated by conventional reciprocal plots.[9, 10] As is indicated in Fig. 1, truly competitive inhibition was found with

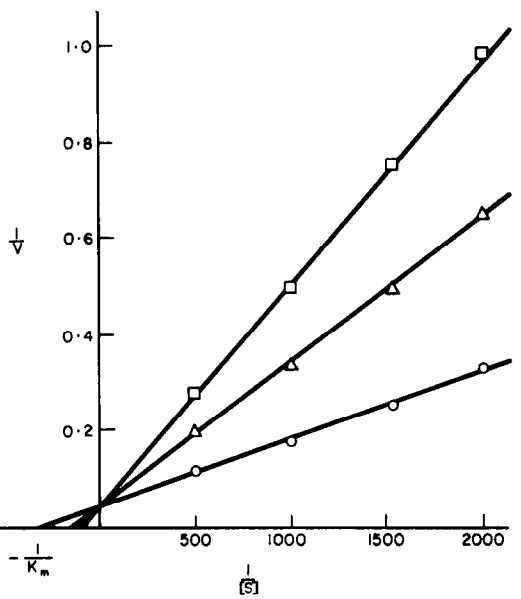



FIG. 1. Kinetic study showing competitive inhibition of rat brain acetylcholinesterase by compound IV (QZ-2′ derivative, Table 1). All reactions were carried out as described in the text. 1/V denotes reciprocal of ΔE/100 mg fresh tissue/10 min; [S] denotes molar concentration of acetylthiocholine; ○——○ = control; △——△ = $1 \times 10^{-3}$ M; and □——□ = $2 \times 10^{-3}$ M of the inhibitor (compound IV). $K_m = 3{\cdot}3 \times 10^{-3}$ M.

compound IV. From the intercept at the 1/[S] axis a $K_m$ (Michaelis constant) of $3{\cdot}3 \times 10^{-3}$ M was obtained for rat brain homogenate, with acetylthiocholine as substrate. These results confirmed previous studies under similar conditions.[11] The inhibition produced by compound X was of similar nature (Fig. 2). Other quaternary

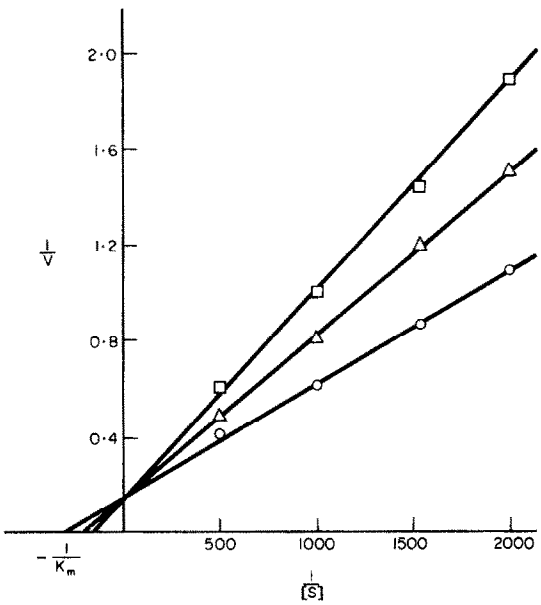



FIG. 2. Competitive inhibition of acetylcholinesterase by compound X (QZ-4′ derivative, Table 1). For other components and the assay procedure see the text. [S] denotes molar concentration of acetylthiocholine and 1/V, reciprocal of ΔE/100 mg fresh tissue/10 min. $K_m = 3{\cdot}3 \times 10^{-3}$ M; ○——○ = control; △——△ = $1{\cdot}5 \times 10^{-4}$ M, and □——□ = $3 \times 10^{-4}$ M concentrations, respectively, of compound X.

TABLE 3. INHIBITOR CONSTANTS OF QUATERNARY AMMONIUM COMPOUNDS

| | Compound no. | Inhibitor constant ($K_i$) $10^{-3}$ M |
|---|---|---|
| QZ-2′ | IV | 2·7 |
| | V | 2·65 |
| QZ-4′ | VII | 2·7 |
| | VIII | 2·5 |
| | IX | 0·52 |
| | X | 0·15 |

Assay procedure and the components of the reaction are described in the text. In all these experiments Michaelis constant was $3{\cdot}3 \times 10^{-3}$ M. Compound numbers as in Table 1.

derivatives of QZ-2′ and QZ-4′ were also found to inhibit acetylcholinesterase competitively. Data in Table 3 summarize the inhibitor constant ($K_i$) calculated by the graphic method of Dixon[10] for some of the compounds studied. Consistent with the per cent enzyme inhibition (cf. compounds VII–X, Table 1), a decrease in $K_i$

was also found to be dependent upon the number of carbon atoms in the alkyl chain of QZ-4′ derivatives.

## DISCUSSION

The effect of certain quaternary ammonium compounds, having an active quinazolone moiety,[12, 13] has been investigated for the interrelationship of their structural characteristics and their ability to inhibit acetylcholinesterase. The inhibition of acetylcholinesterase by 4-aminopyridine[14] led us to synthesize QZ-2′ and QZ-4′ and their quaternary derivatives. It was found that the quinazolone derivative completely lacks the inhibitory properties of 4-aminopyridine. Compounds obtained on quaternization of QZ-2′ and QZ-4′ were effective inhibitors. In the studies described, evidence is presented for an increased inhibition of acetylcholinesterase depending on the length of the alkyl chain attached to the pyridinium nitrogen atom. This effect was specific for only QZ-4′ derivatives. Thus the inhibitory properties of quaternary compounds of QZ-4′ (compounds VI–X, Table 1) differ from those of QZ-2′ derivatives (compounds II–V, Table 1) where such an effect was not dependent on the number of carbon atoms of the alkyl chain. The introduction of the methylene group *per se* in both QZ-2′ and QZ-4′ derivatives should have a similar effect on the van der Waals forces which have been shown to be responsible for the inhibition of acetylcholinesterase by quaternary ammonium compounds.[15]

It has also been demonstrated that the inhibitory effects of quaternary ammonium compounds are related to the total forces of adsorption composed of coulombic interaction of the positive charge on the nitrogen atom and the anionic site of the enzyme surface.[2, 16, 17] The observed changes, therefore, could conceivably be due to change in the total force of adsorption. A similar explanation was put forward by Thomas and Marlow[18] for differences in the properties of trimethylphenylalkylammonium compounds to inhibit acetylcholinesterase. These investigators have emphasized that the charge availability is an important factor in determining antiacetylcholinesterase activity.[19] On the basis of our results it seems that the presence of the quinazolone nucleus in the close vicinity of the positive nitrogen atom, such as in QZ-2′ derivatives, is more likely to be responsible for the interference with the charge availability. The quaternary nitrogen atom of QZ-4′ derivatives, being located at a greater distance, on the other hand, may not be equally influenced by the quinazolone nucleus.

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