Current Trends in Pharmacology and Clinical Trials
ISSN: 2642-0848

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*Research Article* *Volume 5 Issue 1*

# Modeling of the Relationship of the Anticholinesterase Activity of Aminostigmin Derivatives Series with their Molecular Structure

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**Received Date:** February 15, 2022; **Published Date:** March 28, 2022

## Abstract

Statistically significant molecular features have been identified that determine the anticholinesterase activity of a number of analogs of aminostigmine. It is shown that the affinity constant is closely related to the pseudopotential of the molecule. Regression equations were obtained that determine changes in the bimolecular inhibition constant and carbamylation constant. The conditions for the possibility of creating effective drugs with low toxicity have been established.

**Keywords:** Aminostigmine; Regression; Inhibitor; Cholinesterase; Enzyme; Affinity Constant; Statistical Criteria; Carbamylation Constant; Hydrophobicity; Information

**Abbreviations:** ChE: Cholinesterase; AChE: Anticholinesterase.

## Introduction

Pyridylcarbamates are known [1-6] to be used for the treatment of glaucoma, cuts, paralysis as anti-curative and anti-atropic agents, among which are hydrostigmine (calamine), physostigmine, proserine, demicarius bromide. The reversible cholinesterase inhibitor aminostigmine (N,N-dimethyl - (2 - N',N' - dimethylamino-methyl-pyridyl - 3) carbamate hydrochloride) is widely used in medical practice (Figure 1) [2,7-10]. The biological effect of aminostigmine is due to its ability to inhibit the activity of cholinesterase (ChE), an enzyme that controls the hydrolysis of acetylcholine. To date, it has been established that ChE is an oligomeric protein complex that includes two active centers carrying an anionic charge and an esteric charge. The ester charge, due to the presence of a hydroxoserine fragment in it, cleaves the esters of acetic (in the case of acetylcholine) and carbamic (in the case of carbamate inhibitors) acids.

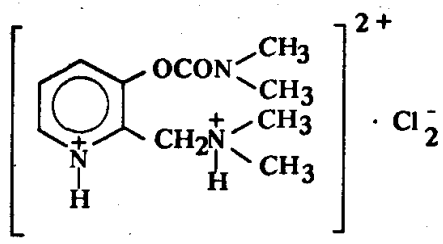

**Figure 1:** Structural formula (Hill's gross formula $C_{11}H_{19}Cl_2N_3O_2$) of the aminostigmine molecule.

Unfortunately, aminostigmine (number I in Table 1) has an insufficient breadth of therapeutic action. Statistical modeling is presented here in order to identify the relationship between anticholinesterase activity and toxicity in a series of new derivatives of aminostigmine under the condition of varying the structure of the molecule [7]. In addition, molecular features of a number of pyridylcarbamates, which are responsible for the biological activity of chemical compounds, are revealed.

Mukhomorov VK. Modeling of the Relationship of the Anticholinesterase Activity of Aminostigmin Derivatives Series with their Molecular Structure. Curr Trends Pharma Clinical Trials 2022, 5(1): 180045.

## Results and Discussion

It is known [8] that the interaction of an inhibitor with an enzyme (ChE) proceeds according to the following scheme:

$$E + I \underset{K_1}{\overset{K_{-1}}{\rightleftarrows}} E \xrightarrow{K_c} E' \xrightarrow{K_{2c}} E + \text{inhibitor conversion products.} \quad (1)$$

Here $E$ is an enzyme (ChE); $I$ is an inhibitor (pyridyl carbamate); $EI$ is an intermediate i.e. intermediate reversible Michaelis complex, the formation of which is characterized by an affinity constant $K_a = K_1 / K_{-1}$. $EI'$ is a carbamylated enzyme. Its formation is characterized by the carbamylation constant $K_c$. The overall rate of complex formation $EI'$ is measured by the bimolecular rate constant $K_2^{'} = K_c.K_{2c}$. $K_{2c}$ is the decarbamylation constant, which characterizes the ability of the formed carbamylated enzyme $EI'$ to be hydrolyzed during washing, dilution or dialysis.

Aminostigmin and its analogues have two centres capable of ionisation, namely the nitrogen atom of the pyridine ring and the amino group at the second position of this heterocycle. According to earlier data [9,10], in aminostigmine the ring constant is $pK_a^{(1)} = 2.4$ and in the side radical is $pK_a^{(2)} = 7.8$, respectively. Consequently, the nitrogen of the aminomethyl group at pH = 7.4 (the pH value at which the inhibitor reacts with ChE) acquires a larger positive charge and is more important for the electrostatic interaction. Therefore, it can be assumed that the substituent at this nitrogen should have a significant effect on the degree of protonation and the implementation of the first stage of the reaction. In addition, it is possible that hydrophobic radicals at the nitrogen of the aminomethyl group can also contribute to the formation of the Michaelis complex.

| *N* | $R^1$ | $R^2$ | $T_m$, C | Gross-formula | Ionization constant | | *Z*, arb. units | *H*, *bits* | $Z_m$, arb. units |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | $pK_a^{(1)}$ | $pK_a^{(2)}$ | | | |
| I | $CH_3$ | $CH_3$ | - | $C_{11}H_{19}Cl_2N_3O_2$ | 2.4 | 7.8 | 2.811 | 1.763 | 2.667 |
| II | $C_4H_9$ | $CH_3$ | 93-95 | $C_{16}H_{25}N_3O_6$ | 4.37 | 8.54 | 2.8 | 1.637 | 2.524 |
| III | $C_5H_{11}$ | $CH_3$ | 79-81 | $C_{17}H_{27}N_3O_6$ | 4.29 | 8.36 | 2.755 | 1.612 | 2.489 |
| IV | *i*-$C_5H_{11}$ | $CH_3$ | 91-92 | $C_{17}H_{27}N_3O_6$ | - | - | 2.755 | 1.612 | 2.489 |
| V | $C_6H_{13}$ | $CH_3$ | 94-97 | $C_{18}H_{29}N_3O_6$ | 4.41 | 8.83 | 2.714 | 1.59 | 2.458 |
| VI | $C_7H_{15}$ | $CH_3$ | 94-98 | $C_{19}H_{31}N_3O_6$ | - | - | 2.678 | 1.568 | 2.431 |
| VII | $C_8H_{17}$ | $CH_3$ | 93-96 | $C_{20}H_{33}N_3O_6$ | 4.79 | 9.43 | 2.645 | 1.548 | 2.407 |
| VIII | $C_6H_{11}$ | $CH_3$ | 51-54 | $C_{18}H_{27}N_3O_6$ | - | - | 2.778 | 1.612 | 2.522 |
| IX | $C_6H_5$ | $CH_3$ | 68-72 | $C_{18}H_{21}N_3O_6$ | - | - | 3 | 1.673 | 2.759 |
| X | $C_2H_5$ | $C_2H_5$ | 81-83 | $C_{15}H_{23}N_3O_6$ | 4.4 | 8.44 | 2.851 | 1.663 | 2.564 |
| XI | $-(CH_2)_5-$ | | 168-171 | $C_{16}H_{25}N_3O_6$ | 4.39 | 8.65 | 2.8 | 1.637 | 2.524 |

**Table 1:** Physico-chemical properties of a number of pyridylcarbamates. (Figure 2)

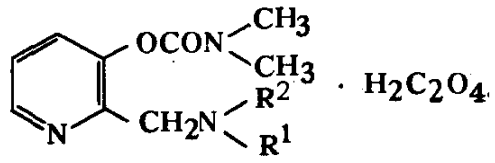


**Figure 2:** Aminostigmine analogues. $R^1$ and $R^2$ are substituents.

The ionization constants of aminostigmine derivatives were determined in Albert A, et al. [11] by potentiometric titration with a glass electrode [7]. For aminostigmine, the ratio $pK_a^{(2)} / pK_a^{(1)} = 3.25$, which is more than one and a half times higher than for other chemical compounds of the analyzed series of chemical compounds (Table 1). That is, for aminostigmine the highest asymmetry of the positive charge is observed, whereas for the other compounds this ratio lies in the range 1.95 - 2.00.

As shown by the statistical analysis of the studied series of analogues of aminostigmine, the ionization constants $pK_a^{(1)}$ and $pK_a^{(2)}$ are very closely interconnected:

$$pK_a^{(2)} = a_0^{(1)} + a_1^{(1)} \cdot pK_a^{(1)}, \qquad N_1 = 6, \qquad R_1 = 0.95 \pm 0.15,$$

$R_1^* = 0.97 > R_{0.05^{cr}}(N_1 - 2) = 0.811$; the minimum sample size sufficient for the reliability of the correlation coefficient: $N_{0.05}^{min} < 5$ [12]; criterion of significance of the correlation coefficient based on the normalizing Fisher *z*-transform (Hotelling corrections is taken into account [13]): $u_H = 1.56 > u_{0.05}(N_1) = z_{0.975^*}(N_1 - 1)^{-0.05} = 0.88$;

$a_0^{(1)} = -0.66 \pm 1.51$; $a_1^{(1)} = 2.11 \pm 0.34$,

$t(a_1^{(1)}) = 6.19 > t_{0.05^{cr}}(f = N_1 - 2) = 2.77$; standard error (*RMSE*) of the regression estimate: $S_1 = 0.134$;

$F = 38.31 > F = 114.5 > F_{0.05^{cr}}(f_1 = 1; f_2 = 4) = 7.71$,

straightness feature:

$K = \left(N\left(1 - R_{1^2}\right)\right)^{0.5} = 0.77 < K^{thr} = 3.00$ [12]; $\sum = 0.0719$, AIC = -4.091, SC = -3.827, SS = 0.0536 (2).

Here we present information tests of the quality of linear regression: AIC [14], SC [15], as well as the relation $SS = \sum^{0.05} / (N - m)$. For small samples, it is recommended [13] to use the corrected correlation coefficient: $R^* = R \cdot \left[1 + 0.5 \cdot \left(1 - R^2\right) / (N - 4)\right]$. According to the Chaddock scale [16,17], the correlation coefficient characterizes the relationship between the features as "very close". The regression (2) indicates that, within the statistical method, an increase in the ionisation constant $pK_a^{(1)}$ is accompanied by an increase in the other ionisation constant $pK_a^{(2)}$, this increase having a constant value determined by the slope of the regression line $a_1^{(1)} = 2.21 \pm 0.34$. For aminostigmine the ratio $\Upsilon_{max} = pK_a^{(2)} / pK_a^{(1)} = 3.25$ has a maximum value, which is markedly higher than the regression coefficient $a_1^{(1)}$. The statistical significance of regression (2) indicates that, apparently, for the series of pyridylcarbamates presented in (Table 2), we cannot expect values of γ greater than $\Upsilon_{max}$.

The following statistics for sets $pK_a^{(1)}$ and $pK_a^{(2)}$ are obtained: $pK_a^{(1)}: N_1 = 6$, $pK_a^{(1)av} = 4.44 \pm 0.07$; the 95% confidence interval is 4.26 - 4.63; $pK_a^{(1)min} = 4.29$, $pK_a^{(1)max} = 4.79$, $S_{11} = 0.176$, $\tau^{min} = 0.86 < \tau^{max} = 1.98 < \tau_{0.05^{cr,2}}(N_1) = 1.996 < \tau_{0.05^{cr,1}}(N_1) = 2.184$ [18-20]; Wilk-Shapiro normality test: $W = 0.719 < W_{0.05^{cr}}(N_1) = 0.788$, David-Hartley-Pearson normality test: $U1 = {}_{0.05^{cr}}(N_1) = 2.200 < U = \left[\left(pK_a^{(1)max} - pK_a^{(1)min}\right) / S_{11}\right] = 2.84 < U2_{0.05^{cr}}(N_1) = 3.012$;

$pK_a^{(2)}: N_1 = 6$; $pK_a^{(2)av} = 8.71 \pm 0.16$; the 95% confidence interval is 8.30-9.12; $pK_a^{(2)min} = 8.36$, $pK_a^{(2)max} = 9.43$, $S_{12} = 0.390$, $\tau^{min} = 0.89 < \tau^{max} = 1.85 < \tau_{0.05}^{cr,2}(N_1) = 1.996 < \tau_{0.05}^{cr,1}(N_1) = 2.184$, Wilk-Shapiro normality test: $W = 0.856 > W_{0.05}^{cr}(N_1) = 0.788$, David-Hartley-Pearson normality test: $U1_{0.05}^{cr}(N_1) = 2.200 < U = [(pK_a^{(2)max} - pK_a^{(2)min})/S_{12}] = 2.74 < U2_{0.05}^{cr}(N_1) = 3.012$ (3).

It follows from inequalities (3) that the sets $pK_a^{(1)}$ and $pK_a^{(2)}$ are homogeneous and close to a normal distribution. The statistical validity of the linear correlation coefficient for a small sample can also be checked using the following inequality [12]: $t = 0.5 \cdot \ln[(1 + |R_1|)/(1 - |R_1|)] \cdot (N_1 - 3)^{0.5} = 3.17 > t_{0.05}^{cr}(f = N_1 - 2) = 2.78$. It follows from this inequality that the correlation coefficient is statistically significant at the 95% confidence level. The aminostigmine molecule has a $pK_a^{(1)}$ value iqual to 2.4, which falls out of the set of elements (Table 1) because the homogeneity of the population is broken (Grubbs-Romanovsky test [18-20]: $\tau_{0.05}^{cr,1}(N_2 = 7) = 2.267 > \tau^{min} = 2.22 > \tau_{0.05}^{cr,2}(N_2 = 7) = 2.003 > \tau^{max} = 0.81$). Indeed, if aminostigmine is added to sample (2) (sample volume $N = 7$), the linear correlation coefficient for the relationship between $pK_a^{(1)}$ and $pK_a^{(2)}$ drops to $R = 0.82 \pm 0.26$. Linear regression linking features $pK_a^{(1)}$ and $pK_a^{(2)}$ in this case will be as follows:

$pK_a^{(2)} = a_0^{(2)} + a_1^{(2)} \cdot pK_a^{(1)}$, $N_2 = 7$, $R_2 = 0.82 \pm 0.26$, $R_2^* = 0.85 > R_{0.05}^{cr}(N_2 - 2) = 0.754$; the minimum sample size sufficient for the reliability of the correlation coefficient: $N_{0.05}^{min} = 6$; criterion of significance of the correlation coefficient based on the normalizing Fisher *z*-transform (Hotelling corrections is taken into account): $u_H = 1.16 > u_{0.05}(N_2) = z_{0.975} \cdot (N_2 - 1)^{-0.5} = 0.80$; $a_0^{(2)} = 6.45 \pm 0.68$, $a_1^{(2)} = 0.51 \pm 0.16$, $t(a_1^{(2)}) = 3.19 > t_{0.05}^{cr}(f = N_2 - 2) = 2.571$; standard error (*RMSE*) of the regression estimate: $S_2 = 0.311$; $F = 10.20 > F = 114.5 > F_{0.05}^{cr}(f_1 = 1; f_2 = 5) = 6.61$; straightness feature: $K = 1.5 < K^{thr} = 3.00$; Σ = 0.4829, AIC = -2.388, SC = -2.188, SS = 0.1182 (4).

The statistics of sets $pK_a^{(1)}$ and $pK_a^{(2)}$:

$pK_a^{(1)}$: $N_2 = 7$, $pK_a^{(1)av} = 4.15 \pm 0.30$; the 95% confidence interval is 3.42 - 4.88; $pK_a^{(1)min} = 2.40$, $pK_a^{(1)max} = 4.79$, $S_{21} = 0.788$, $\tau^{max} = 0.842 < \tau_{0.05}^{cr,2}(N_2) = 2.093 < \tau^{min} = 2.22 < \tau_{0.05}^{cr,1}(N_2) = 2.267$; Wilk-Shapiro normality test: $W = 0.628 < W_{0.05}^{cr}(N_2) = 0.803$, David-Hartley-Pearson normality test: $U1_{0.05}^{cr}(N_2) = 2.400 < U = [(pK_a^{(1)max} - pK_a^{(1)min})/S_{21}] = 3.03 < U2_{0.05}^{cr}(N_2) = 3.222$;

$pK_a^{(2)}$: $N_2 = 7$, $pK_a^{(2)av} = 8.58 \pm 0.19$; the 95% confidence interval is 8.12 - 9.04; $pK_a^{(2)min} = 7.80$, $pK_a^{(2)max} = 9.43$, $S_{22} = 0.495$, $\tau^{min} = 1.57 < \tau^{max} = 1.72 < \tau_{0.05}^{cr,2}(N_2) = 2.093 < \tau_{0.05}^{cr,1}(N_1) = 2.267$; Wilk-Shapiro normality test: $W = 0.961 > W_{0.05}^{cr}(N_2) = 0.803$, David-Hartley-Pearson normality test: $U1_{0.05}^{cr}(N_2) =$

$2.400 < U = [(pK_a^{(2)max} - pK_a^{(2)min})/S_{12}] = 3.29 < U2_{0.05}^{cr}(N_2) = 3.222$.

The AIC, SC and SS information tests of regression quality (4) are significantly reduced, indicating higher quality than regression (2). An additional statistical test can also be performed to determine the significance of the difference between the aminostigmine molecule and the set of other compounds in (Table 1). Let us compare whether the regressions (2) and (4) differ significantly. Let us first check the significance of the difference in the variances of the residuals for these regressions:

$$F = \left(S_2 / S_1\right)^2 = 5.39 < F_{0.05}^{cr}\left(f_1 = 5; f_2 = 4\right) = 6.26 \qquad (5)$$

Since the variances of the residuals for the regressions do not differ significantly, it is possible to test the null hypothesis about the statistical identity of the regression coefficients $a_1^{(1)}$ and $a_1^{(2)}$, which determine the slope of the regression lines. A *t*-statistics is used to test the null hypothesis. Let's use the following inequality [21]:

$$t = \frac{|a_1^{(2)} - a_1^{(1)}|}{\left[\frac{(N_1-2)S_1^2 + (N_2-2)S_2^2}{N_1+N_2-4}\left(\frac{1}{(N_1-1)S_1^2} + \frac{1}{(N_2-1)S_2^2}\right)\right]^{0.5}}$$

$$= 4.55 > t_{0.05}^{cr}(f = N_1 + N_2 - 4) = 2.262 \qquad (6)$$

Since $t > t^{cr}$, therefore, the slope of the regression lines differs significantly at a significance level of $\alpha = 0.05$. Thus, the addition of aminostigmine to the sample leads to a significant change in the relationship equation (2).

There are data Mikhelson MYa, et al. and Hobbiger F [22,23] according to which, at the initial stage of the interaction of the enzyme with aminostigmine derivatives, the ionized part of the pyridylcarbamate molecule is sorbed on the anionic site of the enzyme due to electrostatic forces of attraction. Next, the orientation of the inhibitor molecule on the ChE surface is assumed. As shown in van der Waals interactions ($\sim R^{-6}$) and dipole interactions ($\sim R^{-3}$) between carbon radicals in the ammonium part of the molecule and, probably, hydrophobic regions of the enzyme surface also play an important role in this process [24]. Here R is the effective distance between the molecules. (Table 1) also lists the molecular factors *Z* and *H*. It was also shown Mukhomorov VK et al. [25] that the greater the value of the *Z* factor of a molecule, the stronger the energy of the pair dispersion interaction. Here *Z* is the average number of electrons in the outer shell of atoms in a molecule: $Z = \Sigma_i n_i Z_i / N$ [26,27]. Here $n_i$ is the number of atoms of the *i*-th sort with the number of electrons $Z_i$ on the outer electron shell. The summation is performed on all atoms in the molecule; $\Sigma_i n_i = N$ is the total number of atoms. The electronic factor *Z* is related to the pseudopotential of the molecule [28]. The information function *H* [29], for a discrete data set, is quantified as follows: $H = -\Sigma_j p_j \log_2 p_j$. The ratio $p_i = n_i/N$ satisfies the following conditions: $0 \le p_i \le 1$, $\Sigma_i p_i = 1$. In which connection, $p_i = 0$ means the impossibility of the occurrence of the *i*-th event; $\Sigma_i n_i = N$; *N* is the number of atoms in the molecule. The ratio $n_k/N$ determines the shareholding of the *k*th kind of atom in the molecule. As a result, mutual "conformational adaptation" (according to Wills' terminology) occurs, which leads to the fixation of the inhibitor with the subsequent formation of a reversible Michaelis complex *EI*.

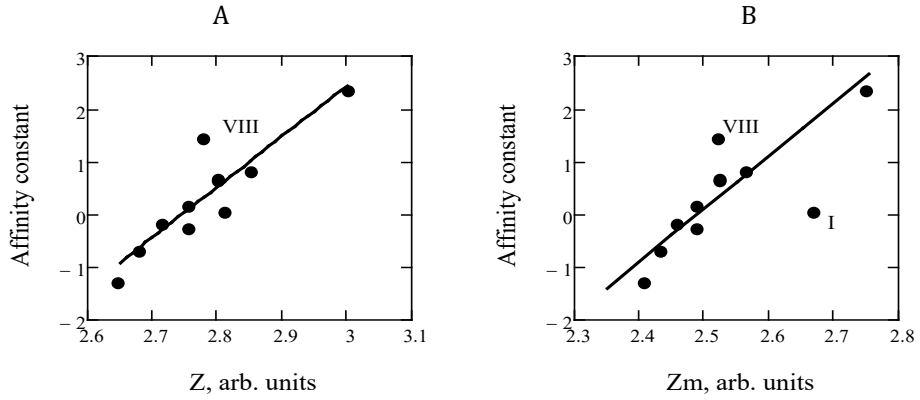


**Figure 3:** Scatter diagrams. Relationship between the affinity constant and the value of molecular factors *Z* and $Z_m$.

The indices I and VIII indicate the position relative to the regression line of aminostigmine and the aminostigmine analogue VIII. A. Equation of the regression line: $\lg(K_a \cdot 10^{-7}) = a_0 + a_1 \cdot Z$, $N = 11$, $R_1 = 0.90 \pm 0.15$, $RMSE\ (S_1) = 0.370$; $a_0 = -26.30 \pm 4.35$, $a_1 = 9.58 \pm 1.57$; $F = 37.38 > F_{0.05}^{cr}(1;9) = 5.12$; $m_1 = 1$; $\Sigma = 1.973$, AIC = -1.5365, SC = -1.2824, SS = 0.1405. B. Equation of the regression line (excluding I and VIII): $\lg(K_a \cdot 10^{-7}) = a_0 + a_1 \cdot Z_m$, $N = 9$, $R_1 = 0.96 \pm 0.10$; $RMSE = 0.296$;

$a_0$ = -25.00 ± 2.62, $a_1$ = 10.04 ± 1.04, $F$ = 92.92 > $F_{0.05}^{cr}(1;7)$ = 5.59; Σ = 0.6629, AIC = -2.3861, SC = -2.1201, SS = 0.1018.

Orientation dipole-dipole interactions contribute to the conformational adaptation of the inhibitor molecule. As a result of the subsequent hydrolysis of *EI'* (decarbamylation), free enzyme *E* and carbamic acid are released.

It was suggested in Prozorovsky VB et al. [7] that molecules of a number of pyridylcarbamates (general molecular formula $C_{n\text{-}2}H_{m\text{-}2}N_3O_2$, $Z_m$ is a molecular feature factor (without $H_2C_2O_4$)) can contribute to the formation of the Michaelis complex. (Figure 3) shows the relationship between the affinity constant lg($K_a$·$10^{-7}$) and the molecular factors $Z_m$ and $Z$. The AIC, SC and SS regression quality tests are also given here. It can be noted that compound VIII appears to be an outlier (Figure 3A). To quantitatively find out, let's estimate the confidence interval of the forecast for an individual value $Z$ = 2.778 arb. units corresponding to preparation VIII. To do this, we use the following relation for the confidence interval of the predicted value lg($K_a$·$10^{-7}$) (for brevity, the dependent variable will be denoted by $Y$) [13]:

$$Y_{regr} \pm t_{0.05}^{cr}(N-2)S_1[1+\frac{1}{N}+(Z-Z^{av})/S_Z^2(N-1))]^{0.5} = 0.313 \pm 0.707 \quad (7)$$

The following values have been used here: $Z^{av}$ = 2.781, $Z$ = 2.778, $S_Z$ = 0.095, $S_1$ = 0.370, $N$ = 11. Thus, the observed value of lg($K_a$·$10^{-7}$) = 1.4472 is noticeably outside the 95% confidence range. A similar test can be performed for (Figure 3B), which shows the relationship between the affinity constant and the value of the substituent factor $Z_m$.

Figure 3B shows that molecules (I) and (VIII) clearly deviate from the linear relationship, and in opposite directions from the regression line. Apparently, such a deviation is associated with the spatial dimensions of the substituents. It can also be noted that the linear relationship persists even if the $Z_m$ factor is used as an explanatory variable (Figure 3B). The regression lines presented in (Figures 3A and 3B) have almost the same slope. Statistical insignificance of the difference in regression coefficients a1 is obtained if we use relation (7): $t$ = 0.315 < $t_{0.05}^{cr}$ ($f$ = 16) = 2.12.

The interaction of the inhibitor with the enzyme was studied [7] at pH = 7.4 (the pH value at which the inhibitor reacts with cholinesterase). The UV spectrum of aminostigmine derivatives has a clear maximum at 265 ± 2 nm and a minimum at 240 ± 2 nm. The IR spectrum has an absorption band in the range of 1750-1730 $cm^{-1}$, which indicates the presence of a carbomoyl group (–OCON<) [7].

Comparison of the structure of the synthesized aminostigmine derivatives with their anticholinesterase activity is consistent with known literature data [24]. These data indicate that the van der Waals interaction between the enzyme surface and the pyridylcarbamate molecule plays a significant role at the stage of formation of the Michaelis complex.

It was shown in Mukhomorov VK et al. [25] that the energy of intermolecular dispersion interaction correlates with the value of the molecular feature *Z*. For compounds of a number of aminostigmine derivatives (compounds I – XI from Table 2), a close linear relationship was found (Figure 3A) between the lg($K_a$·$10^{-7}$) value and the value of the molecular factor *Z*.

| Compound | Anticholinesterase activity | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $K_a$•$10^{-7}$, $mol^{-1}$ | $K_c$, $min^{-1}$ | $K_2'$·$10^{-5}$, $mol^{-1}$·$min^{-1}$ | $K_{2c}$•$10^2$, $min^{-1}$ | $LD_{50}$ mg/kg, mice | $\pi$ ($R^1$,$R^2$), arb. units | $B_4$, arb. units | $L$, arb.units |
| I | 1.11 | 0.34 | 31 | 3.35 | 0.23 | 1.404 | 6.40*) | 3.86*) |
| II | 4.6 | 0.64 | 14.3 | 3.6 | 0.82 | 2.985 | 6.46 | 9.17 |
| III | 1.4 | 0.45 | 33.3 | 4.17 | 1.8 | 3.512 | 6.5 | 10.11 |
| IV | 0.55 | 0.23 | 43.5 | - | 2.58 | 3.512 | 6.5 | 10.11 |
| V | 0.67 | 0.52 | 76.9 | 1.8 | 2.6 | 4.039 | 7.93 | 11.22 |
| VI | 0.2 | 0.3 | 154 | 3.09 | 1.03 | 4.566 | 8.43 | 12.16 |
| VII | 0.05 | 0.3 | 667 | - | 2.05 | 5.093 | 8.50*) | 12.92*) |
| VIII | 28 | 0.11 | 0.4 | - | 137 | 3.871 | 4.53 | 9.17 |
| IX | 220 | 0.5 | 0.2 | - | 200 | 2.615 | 5.6 | 9.28 |
| X | 6.5 | 0.77 | 11.6 | 3.95 | 0.48 | 2.464 | 5.94 | 8.22 |
| XI | 4.5 | 0.41 | 8.7 | 5.23 | 1.63 | 2.65 | 6.33 | 9.07 |

**Table 2:** Properties of aminostigmine and its derivatives [7]. Anticholinesterase activity was determined in experiments.
*Approximate estimates obtained using the regression equation. For an aliphatic chain, the information function *H* can be used as an explanatory variable in the regression equation.

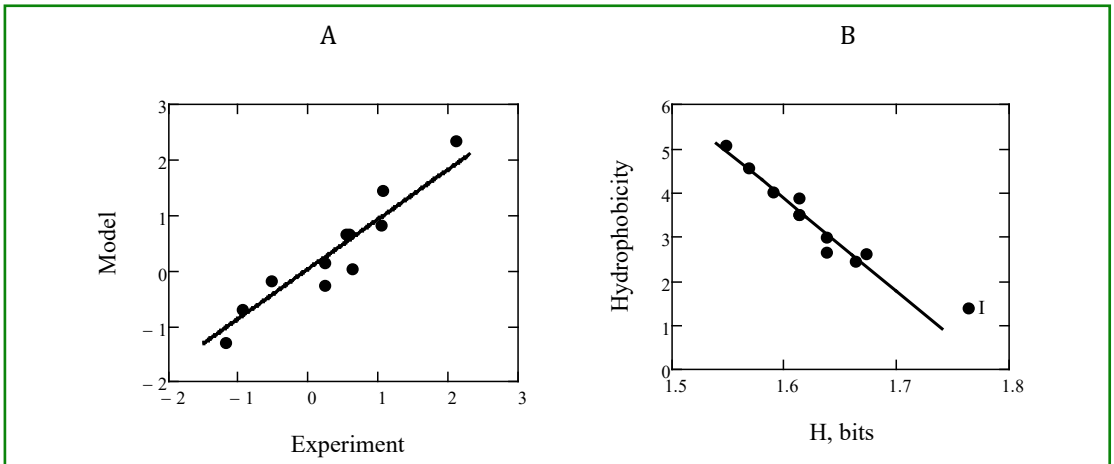


**Figure 4:** Scatter diagrams. A. Interrelation of affinity constants for model (8) and experiment.

Regression line: $\lg(K_a \cdot 10^{-7})_{model} = a_0 + a_1 \cdot \lg(K_a \cdot 10^{-7})_{exp}$, $N = 11$, $R = 0.95 \pm 0.11$, $RMSE = 0.324$; $a_0 = 0.04 \pm 0.10$, $a_1 = 0.90 \pm 0.10$, $t(a_1) = 8.82 > t_{0.05}^{cr}(f = 9) = 2.262$; $F = 77.87 > F_{0.05}^{cr}(1;9) = 5.12$. B. Relationship between hydrophobicity $\pi(R^1,R^2)$ and information function $H$. Index I corresponds to the aminestigmine molecule.

For aminostigmine and cyclohexyl radical (compound VIII), a noticeable deviation of the initial data from the regression line can be noted. This deviation may be due to the geometric dimensions of the substituent. In order to take into account in the statistical analysis the geometric dimensions of the substituent and their influence on the variability of the kinetic constant $K_a$, the five-dimensional steric parameters of the substituents were analyzed: $L$, $B_1$, $B_2$, $B_3$ и $B_4$ [30,31]. The following two-factor regression was obtained, taking into account the combined effect of two molecular parameters $B_4$ and $Z$ on the change in the resulting trait $\lg(K_a \cdot 10^{-7})$:
$\lg(K_a \cdot 10^{-7}) = a_0 + a_1 \cdot B_4 + a_2 \cdot Z$, $N = 11$, $m_2 = 2$, $R_2 = 0.95 > R_{0.05}^{cr}(\nu = 8;\ m_2 = 2) = 0.726$, $R_2^2 = 0.90$, $R_2^{*2} = 0.89$; standard error (*RMSE*) of the regression estimate: $S_{lg} = 0.364$: $a_0 = -15.12 \pm 5.43$, $a_1 = -0.35 \pm 0.13$, $a_2 = 6.40 \pm 1.71$, $t(a_2) = 3.74 > t(a_0) = 2.79 > |t(a_1)| = 2.64 > t_{0.05}^{cr}(f = 8) = 2.306$; $F = 34.63 > F_{0.05}^{cr}(f_1 = 2; f_2 = 8) = 4.46$; standardized regression coefficients [12]: $a_1^* = -0.42$, $a_2^* = 0.60$; $\Sigma = 1.0542$, AIC = -1.981, SC =-1.691, SS = 0.1141 (8).

Here $R_{0.05}^{cr}(\nu = N - m_2 - 1;\ m_2)$ is the critical value of the sample multiple correlation coefficient [32].

Thus, it follows from inequalities (8) that the effect of explanatory variables $B_4$ and $Z$ on the resultant variable is statistically significant. In accordance with the information tests AIC, SC and SS, the joint consideration of the variables $Z$ and $B_4$ in the regression equation improves the quality of the regression (8) compared to the regression presented in (Figure 3A).

Explanatory variables $B_4$ and $Z$ are interrelated. The correlation coefficient is equal to $|r_{12}| = 0.70$. The experience of using statistical methods shows [21] that if the correlation coefficient between explanatory variables is less than the boundary value of 0.8, then the collinearity of explanatory variables, which in their meaning characterize different (do not duplicate each other) properties of molecules, is neglected. In this case, it is acceptable to keep both explanatory variables in the regression equation. The factor $B_4$ determines one dimension of the geometric size of the substituent $R^1$, whereas the attribute $Z$ is related to the electronic properties of the molecule (pseudopotential of the molecule). This result does not contradict the assumption of mutual "conformational adaptation" (according to Wills terminology), which can fix the inhibitor on the enzyme surface with subsequent formation of a reversible Michaelis complex (*EI*). Both characteristics $B_4$ and $Z$ are important in this process. The first determines the complementarity of the substituent, and the second characterizes the intensity of intermolecular interaction.

Now we can check whether the variables $B_4$ and $Z$ together make a significant contribution to explaining the variation of the resulting feature $\lg(K_a 10^{-7})$. Let's compare the regression (8) (Figure 4A) with the regression (Figure 3A), which takes into account only the variability of the resulting trait depending on the change in the molecular factor $Z$. To compare regressions, we use the following statistics, which has an $F$-distribution with $f_1 = m_2 - m_1$ and $f_2 = N - m_2 - 1$ degrees of freedom [21]:

$$F = (R_{2^2} - R_{1^2})(N - m_2 - 1) / (m_2 - m_1) / (1 - R_{2^2}) = 7.59 > F_{0.05^{cr}}(f_1; f_2) = 5.32 \quad (9)$$

Since $F > F_{\alpha}^{cr}$, then at the significance level $\alpha = 0.05$, the joint

action of the explanatory variables $B_4$ and $Z$ has a significant impact on the resulting sign $\lg(K_a \cdot 10^{-7})$. The fact that there is a "very close relationship" (Cheddock scale) between the $K_a$ constant and the $Z$ value confirms the importance of intermolecular interactions in the fixation of the molecule on the ChE surface. The relationship between the molecular factor $Z$ and the value of the pairwise dispersion interaction energy has been demonstrated in [25]. All the aminostigmine derivatives analysed have close values of ionisation constants and are therefore ionised approximately equally, but to a greater extent than aminostigmine. Therefore, the difference between the compounds due to a change in factor $Z$ seems to be due to their ability to participate in intermolecular interactions.

The statistics of the sets $\lg(K_a \cdot 10^{-7})$, $B_4$ and $Z$ are as follows: $\lg(K_a \cdot 10^{-7})$: $N = 11$, $\lg(K_a \cdot 10^{-7})^{av} = 0.334 \pm 0.304$, 95% confidence interval: -0.344, 1.012; $\lg(K_a \cdot 10^{-7})^{min} = -1.30$, $\lg(K_a \cdot 10^{-7})^{max} = 2.34$, $S_{lg} = 1.018$, $\tau^{min} = 1.61 < \tau^{max} = 1.97 < \tau_{0.05}^{cr,2}(N)$ 2.343 $< \tau_{0.05}^{cr,1}(N) = 2.484$; Wilk-Shapiro normality test: $W = 0.979 > W_{0.05}^{cr}(N) = 0.850$, David-Hartley-Pearson normality test: $U1_{0.05}^{cr}(N) = 2.740 < U = [(\lg(K_a \cdot 10^{-7})^{max} - \lg(K_a \cdot 10^{-7})^{min})/S_{lg}] = 3.57 < U2_{0.05}^{cr}(N) = 3.800$;

$B_4$: $N = 11$, $B_4^{av} = 6.65 \pm 0.36$; 95% confidence interval: 5.830 - 7.458; $B_4^{min} = 4.53$, $B_4^{max} = 8.50$, $S_{B4} = 1.207$, $\tau^{max} = 1.54 < \tau^{min} = 1.75 < \tau_{0.05}^{cr,2}(N) = 2.343 < \tau_{0.05}^{cr,1}(N) = 2.484$; Wilk-Shapiro normality test: $W = 0.910 > W_{0.05}^{cr}(N) = 0.850$, David-Hartley-Pearson normality test: $U1_{0.05}^{cr}(N) = 2.740 < U = [(B_4^{max} - B_4^{min})/S_{B4}] = 3.29 < U2_{0.05}^{cr}(N) = 3.800$;

$Z$: $N = 11$; $Z^{av} = 2.78 \pm 0.03$; 95% confidence interval 2.717 - 2.844; $Z^{min} = 2.65$, $Z^{max} = 3.00$, $S_Z = 0.095$, $\tau^{min} = 1.43 < \tau^{max} = 2.31 < \tau_{0.05}^{cr,2}(N) = 2.343 < \tau_{0.05}^{cr,1}(N) = 2.484$; Wilk-Shapiro normality test: $W = 0.925 > W_{0.05}^{cr}(N) = 0.850$, David-Hartley-Pearson normality test: $U1_{0.05}^{cr}(N) = 2.740 < U = [(Z^{max} - Z^{min})/S_Z] = 3.68 < U2_{0.05}^{cr}(N) = 3.800$ (10).

From the inequalities (10) it follows that the resulting attribute and explanatory variables are homogeneous and normally distributed. The regression coefficients $a_1$ and $a_2$ (8) differ significantly in absolute value from each other. Therefore, in order to compare the proportionate effect of factor $Z$ and geometric size $B_4$ on the variability of the resultant trait, it is necessary to switch to standardized (normalized) [18] regression coefficients $a_1^*$ and $a_2^*$:

$$a_1^* = -0.42, a_2^* = 0.60 \quad (11)$$

The standardised regression coefficients are dimensionless, so it becomes possible to compare them. It follows from relations (11) that the intensities of influence of factor $B_4$ and factor $Z$ on the variability of the resultant trait are comparable (in absolute value). The considered together of these two explanatory variables makes it possible to explain 90.3% of the variability of the resulting trait. Only 9.7% of the unexplained variation can be attributed either to some unaccounted for factors or random variation in the original data. Let us determine the share of factors $B_4$ and $Z$ in the change in the response function. The approximate coefficient of determination [18] is equal to:

$$R_{appr^2} = a_1^* \cdot r_{1g-B4} + a_2^* \cdot r_{1g-Z} = 0.357 + 0.539 = 0.896 \quad (12)$$

Here $r_{B4,lgK} = -0.845$ and $r_{Z,lgK} = 0.898$ are the pair correlation coefficients of the resultant feature $\lg(K_a \cdot 10^{-7})$ with explanatory variables $B_4$ and $Z$, respectively. The approximate multiple coefficient of determination is close to the coefficient of determination $R_2^2 = 0.903$ (8). From (12) it follows that the share contribution of the explanatory variables $B_4$ and $Z$ in explaining the variability of the resulting attribute is 35.7% and 53.9%, respectively.

Since parameter $B_4$ was estimated approximately for chemical compounds I and VII, we will check the significance of the regression equation for a sample that does not contain these drugs. The following regression was obtained: $\lg(K_a \cdot 10^{-7}) = a_0 + a_1 \cdot B_4 + a_2 \cdot Z$, $N = 9$, $R_3 = 0.96 > R_{0.05}^{cr}(\nu = N - m - 1; m = 2) = 0.795$ [32], $R_3^2 = 0.91$, $R_3^{*2} = 0.90$; standard error of the regression estimate: $S_{lg} = 0.332$: $a_0 = -16.39 \pm 5.00$, $a_1 = -0.33 \pm 0.13$, $a_2 = 6.48 \pm 1.60$, $t(a_2) = 4.06 > |t(a_0)| = 3.08 > |t(a_1)| = 2.61 > t_{0.05}^{cr}(f = 6) = 2.447$, $F = 28.81 > F_{0.05}^{cr}(f_1 = 2; f_2 = 6) = 5.14$ (13)

It follows from inequalities (13) that at the 95% confidence level, the significance of the regression and explanatory variables has not practically changed compared to (8). The standardized regression coefficients were as follows: $a_1^* = -0.41$, $a_2^* = 0.64$, which does not contradict the results of (11). Statistical criteria (3), (8) and (13) indicate that the relationship between the resultant factor $\lg(K_a \cdot 10^{-7})$ and the explanatory variables $B_4$ and $Z$ is not random.

The data in Table 2 show that the affinity increases by a factor of 22 when the aliphatic chain length of the radical is increased to $C_8$ (chemical compound VII). At the same time, the molecular feature $Z$ for the analyzed series reaches its minimum value of 2.645 arb. units. Such an increase in the length of the aliphatic chain is accompanied by a monotonic change in the hydrophobic properties of the molecules. (Figure 3A) demonstrates that an increase in the affinity constant of molecules for AChE correlates with the value of the factor feature $Z$.

The hydrophobicity value $\pi(R^1,R^2)$ for substituents (Table 2) was determined by the additive method [33,34]. There is a close linear relationship between the hydrophobicity $\pi(R^1,R^2)$ and the information function of the $H$ molecules (excluding aminostigmine) (Figure 4B).

$\pi(H) = a_0 + a_1 \cdot H$, $N = 10$, $R = -0.97 \pm 0.08$, $|R^*| = 0.974 > R_{0.05}^{cr}(N - 2) = 0.632$ [13]; standard error (*RMSE*) of the regression estimate: $S_{l\pi} = 0.2203$; the minimum sample size sufficient for the reliability of the correlation coefficient: $N_{0.05}^{min} < 5$ [12]; correlation coefficient significance test based on the normalizing Fisher *z*-transform (with Hotelling corrections taken into account): $u_H = 2.02 > u_{0.05}(N) = z_{0.975} \cdot (N - 1)^{-0.5} = 0.65$; $a_0 = 37.56 \pm 3.23$, $a_1 = -21.06 \pm 2.00$, $t(a_0) = 12.79 > |t(a_1)| = 11.6 > t_{0.05}^{cr}(f = N - m - 1) = 2.306$, $F = 134.95 > F_{0.05}^{cr}(f_1 = 1; f_2 = 8) = 5.32$; $\Sigma = 0.3882$; straightness index: $K = 0.82 < K^{thr} = 3.0$ [12] (14)

Applying the Student's *t*-test, you can additionally check the reliability of the correlation coefficient: $t = 0.5 \cdot \ln[(1 + R)/(1 - R)] \cdot (N - 3)^{0.5} = 5.54 > t_{0.05}^{cr}(f = N - 2) = 2.306$.

The statistics of π and *H* sets (excluding aminostigmine):

π: $N = 10$, $\pi^{av} = 3.53 \pm 0.28$; 95% confidence interval: 2.902 - 4.159; $\pi^{min} = 2.46$, $\pi^{max} = 5.09$, $S_\pi = 0.878$, $\tau^{min} = 1.22 < \tau^{max} = 1.78 < \tau_{0.05}^{cr,2}(N) = 2.294 < \tau_{0.05}^{cr,1}(N) = 2.441$; Wilk-Shapiro normality test: $W = 0.942 > W_{0.05}^{cr}(N) = 0.842$, David-Hartley-Pearson normality test: $U1_{0.05}^{cr}(N) = 2.670 < U = [(\pi^{max} - \pi^{min})/S_\pi] = 3.00 < U2_{0.05}^{cr}(N) = 3.685$.

*H*: $N = 10$, $H^{av} = 1.617 \pm 0.013$; 95% confidence interval: 1.587 - 1.644; $H^{min} = 1.548$, $H^{max} = 1.678$, $S_H = 0.040$, $\tau^{max} = 1.525 < \tau^{min} = 1.725 < \tau_{0.05}^{cr,2}(N) = 2.294 < \tau_{0.05}^{cr,1}(N) = 2.441$; Wilk-Shapiro normality test: $W = 0.971 > W_{0.05}^{cr}(N) = 0.842$, David-Hartley-Pearson normality test: $U1_{0.05}^{cr}(N) = 2.670 < U = [(H^{max} - H^{min})/S_H] = 3.25 < U2_{0.05}^{cr}(N) = 3.685$ (15)

Therefore, the samples π and *H* are homogeneous and normally distributed. Figure 4B shows that the aminostigmine (I) molecule deviates markedly from the linear relationship. This can be checked quantitatively [13] by determining the confidence interval for the response function π(*H*). We will use the following relation (7) to determine the predictive value:

$$\pi_{pred} = \pi_{regr} \pm S_\pi t_{0.05}^{\hat{a}\hat{e}\hat{o}} \left(N - 2\right)[1 + 1/N + (H - H)^2 / \left(S_H \left(N - 1\right)\right)]^{0.5}$$

(16)

Here the variance of the residuals is $S_\pi = [\Sigma/(N - 2)]^{0.5} = 0.2202$; $t_{0.05}^{cr}(f = N - 2) = 2.306$, $H^{av} = 1.617$, $S_H = 0.04$, $N = 10$; for aminostigmine the tabulated value is $H = 1.763$ *bits* and accordingly the predicted value obtained from the regression equation (14) taking into account (16) will be as follows: $\pi_{pred} = (0.33 \pm 0.55)$ arb. units. Value π = 1.454 arb. units from (Table 1) is outside the 95% confidence interval, that is, the aminostigmine molecule falls out of the linear relationship.

The ongoing changes in AChE activity can be attributed to the fact that a hydrophobic zone of indeterminate length is located on ChE at a close distance from the "anion cup" [35]. Then, it can be assumed that the initial decrease in the activity of the chemical compound (II), noted in [7], is associated not only with electrostatic interactions, but also with a violation of the complementarity of the drug. The subsequent increase in activity is due to the additional influence of the hydrophobic effects of the substituents.

The five-dimensional steric parameters of the substituents were again used to characterise the geometric dimensions of the substituents [31,36]. The linear dimensions (along the bond axis) of the substituents of a number of aminostigmine derivatives (Table 2) are at least twice as large as the linear dimensions of the methyl group in aminostigmine ($L = 4.92$ arb. units). This can significantly affect the complementarity of molecules to the region of interaction with the local area of the biophase. That is, the effectiveness of the interaction is related to the "conformational adaptation" of the molecules.

At the next stage, the covalent binding of pyridylcarbamate to the esterase site of the enzyme occurs and the formation of the carbamylated enzyme *EI'*. The process of formation of the carbamylated enzyme *EI'* is characterized by the carbamylation constant $K_c$. Statistical analysis showed that the $K_c$ constant is related to the linear dimension *L* characterising the length of substituent along the chemical bond axis and the hydrophobic properties $\pi(R^1,R^2)$. The following two-factor regression was obtained:

$\lg(1/K_c) = a_0 + a_1 \cdot L + a_2 \cdot \pi$, $N = 11$, $m = 2$; $R = 0.732 > R_{0.05}^{cr}(\nu = N - m - 1; m = 2) = 0.725$ [32], $R^2 = 0.535$; standard error (*RMSE*) of the regression estimate: $S_{lg} = 0.178$; $a_0 = 0.59 \pm 0.25$, $a_1 = -0.17 \pm 0.06$, $a_2 = 0.43 \pm 0.14$, $|t(a_2)| = 3.01 > t(a_1) = 2.64 > |t(a_0)| = 2.39 > t_{0.05}^{cr}(f = N - m - 1) = 2.306$; $F = 4.61 > F_{0.05}^{cr}(f_1 = m; f_2 = N - m - 1) = 4.46$; $a_1^* = -1.69$, $a_2^* = 1.927$ (17)

From inequalities (17) for *t* values of the regression coefficients it follows that the explanatory variables *L* and π are significant at the 95% confidence level. The smaller the value of the resulting variable $\lg(1/K_c)$, the greater the carbamylation constant. It also follows from regression (17) that the carbamylation constant decreases with increasing hydrophobicity $\pi(R^1,R^2)$ and increases with increasing linear size *L*. The statistics of the sets π, *L* and $\lg(1/K_c)$ will be as follows:

π: $N = 11$, $\pi^{av} = 3.34 \pm 0.32$; 95% confidence interval: 2.631-4.043; $\pi^{min} = 1.40$, $\pi^{max} = 5.09$, $S_\pi = 1.051$, $\tau^{max} = 1.671 < \tau^{min} = 2.261 < \tau_{0.05}^{cr,2}(N) = 2.343 < \tau_{0.05}^{cr,1}(N) = 2.484$; Wilk-Shapiro normality test: $W = 0.983 > W_{0.05}^{cr}(N) = 0.850$, David-Hartley-Pearson normality test: $U1_{0.05}^{cr}(N) = 2.740 < U = [(\pi^{max} - \pi^{min})/S_\pi] = 3.51 < U2_{0.05}^{cr}(N) = 3.800$ (18)

*L*: $N = 11$, $L^{av} = 9.57 \pm 0.72$; 95% confidence interval: 7.98 - 11.2; $L^{min} = 3.86$, $L^{max} = 12.92$, $S_L = 2.373$, $\tau^{max} = 1.411 < \tau_{0.05}^{cr,2}(N)$ 2.343 $< \tau^{min} = 2.407 < \tau_{0.05}^{cr,1}(N) = 2.484$; Wilk-Shapiro normality test: $W = 0.887 > W_{0.05}^{cr}(N) = 0.850$, David-Hartley-Pearson normality test: $U1_{0.05}^{cr}(N) = 2.740 < U = [(L^{max} - L^{min})/S_L] = 3.81 \approx U2_{0.05}^{cr}(N) = 3.800$; (19)

$\lg(1/K_c)$: $N$ = 11, $\lg(1/K_c)^{av}$ = 0.43 ± 0.07; 95% confidence interval: (0.27-0.59); $\lg(1/K_c)^{min}$ = 0.1135, $\lg(1/K_c)^{max}$ = 0.9586, $S_{lg}$ = 0.233, $\tau^{min} = 1.36 < \tau^{max} = 2.27 < \tau_{0.05}^{cr,2}(N) < 2.343 < \tau_{0.05}^{cr,1}(N) = 2.484$; Wilk-Shapiro normality test: $W = 0.939 > W_{0.05}^{cr}(N) = 0.850$, David-Hartley-Pearson normality test: $U1_{0.05}^{cr}(N) = 2.740 < U = [(\lg(1/K_c)^{max} - \lg(1/K_c)^{min})/S_{lg}] = 3.62 < U2_{0.05}^{cr}(N) = 3.800$ (20)

In accordance with inequalities (18) - (20), the samples π, $L$ and $\lg(K_c)$ are homogeneous and normally distributed. For comparative quantification of the effects of explanatory variables, it is necessary to move to standardized dimensionless regression coefficients:

$$a_1^* = -1.69, a_2^* = 1.93 \quad (21)$$

Therefore, the explanatory variables have a comparable effect (in absolute value) on the resulting feature, with some predominant influence of the hydrophobic properties of the substituents. The analysis of the regression equation (18) also showed that only the joint accounting of explanatory variables in the regression equation has a significant impact on the variability of the resulting attribute. Checking for collinearity of the explanatory variables π and $L$ showed that they are closely related: $r_{12} = 0.93 > 0.8$. For normally distributed residuals, collinearity follows from the test proposed by Farrar and Glauber [37]:

$$\chi^2 = [N-1-(2m+5)/6].\ln(1-r_{12}^2) = 22.4 >> x_{0.05}^{2,cr}(f=1) = 3.841 \quad (22)$$

Inequality (22) implies a strong relationship of explanatory variables. It is known that the presence of collinearity "causes difficulties associated with a decrease in the accuracy of estimation or even with the impossibility of assessing the influence of variables" [21]. One possible way to eliminate collinearity is to linearly transform the explanatory variables. For example, instead of the variable $L$, you can use the new variable $\Delta_{L\pi} = L - \pi$. The following regression was obtained:

$\lg(1/K_c) = a_0 + a_1 \cdot \Delta_{L\pi} + a_2 \cdot \pi$, $N$ = 11, $R$ = 0.732 > $R_{0.05}^{cr}(\nu = N-m-1; m=2)$ = 0.726; $R^2$ = 0.53; standard error ($RMSE$) of the regression estimate: $S_{lg}$=0.178; $a_0$=0.59±0.25, $a_1$=-0.17±0.06, $a_2$=-0.26±0.09, $t(a_2)$=3.01>$|t(a_1)|$=2.64>$t(a_0)$=2.40>$t_{0.05}^{cr}(f=N-m-1)$=2.306, $F$=4.61>$F_{0.05}^{cr}(f_1=2; f_2=8)$=4.46 (23)

The statistics of $\Delta_{L\pi}$ set:

$N$ = 11, $\Delta_{L\pi}^{av}$ = 6.28 ± 0.45, 95% confidence interval: 5.277-7.288; $\Delta_{L\pi}^{min}$ = 2.456, $\Delta_{L\pi}^{max}$ = 7.827, $S_\Delta$=1.497, $\tau^{max}$= 1.03<$\tau_{0.05}^{cr,2}(N)$<2.343<$\tau_{0.05}^{cr,1}(N)$= 2.484< $\tau^{min}$= 2.555; Wilk-Shapiro normality test: $W$= 0.826 ≈ $W_{0.05}^{cr}(N)$ = 0.850, David-Hartley-Pearson normality test: $U1_{0.05}^{cr}(N)$ = 2.740<$U$ = [($\Delta_{L\pi}^{max}$ – $\Delta_{L\pi}^{min}$)/$S_\Delta$] = 3.58 < $U2_{0.05}^{cr}(N)$ = 3.800 (24)

It follows that the minimum value of the variable $\Delta_{L\pi}^{min}$, which corresponds to aminostigmine, violates the homogeneity ($\tau^{min} > \tau^{cr}$) of the sample. After excluding this element from the population, the following significant regression was obtained:

$\lg(1/K_c) = a_0 + a_1 \cdot \Delta_{L\pi} + a_2 \cdot \pi$, $N$= 10, $R$ = 0.77>$R_{0.05}^{cr}(\nu = N-m-1; m=2)$= 0.758, $R^2$= 0.59, $R^{*2}$= 0.54; $u_H$ = 0.924 > $u_{0.05}(N) = z_{0.975} \cdot (N-1)^{-0.5}$ = 0.693; standard error ($RMSE$) of the regression estimate: $S_{lg}$ = 0.178: $a_0$= 1.05±0.52, $a_1$= -0.25 ± 0.10, $a_2$= 0.28 ± 0.09, $t(a_2)$= 3.16 > $|t(a_1)|$= 2.44 > $t_{0.05}^{cr}(f = N_2 - m - 1) = 2.365$, $F$ = 5.10 > $F_{0.05}^{cr}(f_1 = m; f_2 = N-m-1) = 4.74$ (25)

The statistics of $\Delta_{L\pi}$ and $\lg(1/K_c)$ sets:

$\Delta_{L\pi}$: $N$ = 10, $\Delta_{L\pi}^{av}$ = 6.67 ± 0.26; 95% confidence interval: 6.067 - 7.263; $\Delta_{L\pi}^{min}$ = 5.299, $\Delta_{L\pi}^{max}$ = 7.827, $S_\Delta$ = 0.836, $\tau^{max} = 1.39 < \tau^{min} = 1.633 < \tau_{0.05}^{cr,2}(N) < 2.294 < \tau_{0.05}^{cr,1}(N) = 2.441$; Wilk-Shapiro normality test:$W$ = 0.971 > $W_{0.05}^{cr}(N)$ = 0.842, David-Hartley-Pearson normality test: $U1_{0.05}^{cr}(N) = 2.670 < U = [(\Delta_{L\pi}^{max} - \Delta_{L\pi}^{min})/S_\Delta] = 3.02 < U2_{0.05}^{cr}(N) = 3.685$; (26)

$\lg(1/K_c)$: $N$ = 10, $\lg(1/K_c)^{av}$ = 0.43 ± 0.08; 95% confidence interval: 0.251- 0.603; $\lg(1/K_c)^{min}$ = 0.114, $\lg(1/K_c)^{max}$ = 0.959, $S_{lg}$ = 0.246, $\tau^{min} = 1.29 < \tau^{max} = 2.15 < \tau_{0.05}^{cr,2}(N) < 2.294 < \tau_{0.05}^{cr,1}(N) = 2.441$; Wilk-Shapiro normality test:$W$ = 0.932 > $W_{0.05}^{cr}(N)$ = 0.842, David-Hartley-Pearson normality test: $U1_{0.05}^{cr}(N) = 2.670 < U = [(\lg(1/K_c)^{max} - \lg(1/K_c)^{min})/S_{lg}] = 3.44 < U2_{0.05}^{cr}(N) = 3.685$ (27)

The statistics of the explanatory variable π for $N$ = 10 are available in (15). Thus, the sets are homogeneous and normally distributed. A collinearity check of the explanatory variables shows that the correlation between them drops significantly (compared to (17)) to a value of $r$ = 0.64 < 0.8. Since the regression residuals (25) are normally distributed ($W$= 0.931 > $W_{0.05}^{cr}(N = 10)$ = 0.842), the Farrar-Glauber test can be used. The result is an inequality which also indicates that there is no significant collinearity between the linearly transformed explanatory variables: $\chi^2$ = 3.95 ≈ $\chi_{0.05}^{2,cr}(f = 1)$ = 3.841.

For a comparative quantitative assessment of the influence of explanatory variables, let's move on to standardized dimensionless regression coefficients:

$$a_1^* = -0.85, a_2^* = 0.999 \quad (28)$$

The contribution of the variables in explaining the variability of the resulting variable can be determined using equation (21):

$$R_{appr}^2 = a_1^* \cdot r_{\Delta,lgK} + a_2^* \cdot r_{\pi,lgK} = 0.10 + 0.50 = 0.60 \quad (29)$$

The pair correlation coefficients are equal to $r_{\Delta,logK}$= -0.117 and $r_{\pi,logK}$= 0.498, respectively. The approximate coefficient of determination (29) is very close to the value $R^2$ = 0.59 (25). The analysis of the regression equation also showed that only the combined consideration of explanatory variables in the regression equation has a significant effect on the

variability of the resultant variable. The carbamylation constant decreases with an increase in the factor π of substituents and increases with an increase in the geometric size of substituents *L*.

The bimolecular inhibition constant $K_2'$ has a very close linear relationship with the value of the affinity constant $K_a$:
$\lg(K_2'\cdot 10^{-5}) = a_0 + a_1\cdot\lg(K_a\cdot 10^{-7})$, $N = 11$, $R = -0.98 \pm 0.07$, $|R| > R_{0.05}^{cr}(N-2) = 0.602$; $a_0 = 1.57 \pm 0.08$, $a_1 = -1.00 \pm 0.08$; $t(a_1) = 12.5 > t_{0.05}^{cr}(f = N - m - 1) = 2.62$; the minimum sample size sufficient for the reliability of the correlation coefficient: $N_{0.05}^{min} < 5$; standard error (*RMSE*) of the regression estimate: $S_{lg} = 0.24$; $F = 179.0 > F_{0.05}^{cr}(f_1 = 1; f_2 = 9) = 5.12$ (30)

According to the Chaddock scale, this relationship is characterized as "very close".
The bimolecular inhibition constant $\lg(K_2')$ turned out to be significantly related to the geometric parameter $B_4$ of the substituent $R^1$:
$\lg(K_2'\cdot 10^{-5}) = a_0 + a_1\cdot B_4$, $N = 11$, $R = 0.88$, $R^* = 0.89 > R_{0.05}^{cr}(f = N - 2) = 0.602$; standard error (*RMSE*) of the regression estimate: $S_{lg} = 0.511$ the minimum sample size sufficient for the reliability of the correlation coefficient: $N_{0.05}^{min} = 5$; criterion of significance of the correlation coefficient based on the normalizing Fisher *z*-transform (taking into account the Hotelling corrections): $u_H = 1.26 > u_{0.05}(N) = z_{0.975}\cdot(N-1)^{-0.5} = 0.62$; $a_0 = -3.79 \pm 0.90$, $a_1 = 0.76 \pm 0.13$, $t(a_1) = 5.65 > |t(a_0)| = 4.19 > t_{0.05}^{cr}(f = N - 2) = 2.262$, $F = 31.86 > F_{0.05}^{cr}(f_1 = 1; f_2 = 9) = 5.12$ (31)

The statistics of $\lg(K_2'\cdot 10^{-5})$ set:
$N = 11$, $\lg(K_2'\cdot 10^{-5})^{av} = 1.24 \pm 0.31$, 95% confidence interval: 0.54 - 1.93; $\lg(K_2')^{min} = -0.70$, $\lg(K_2'\cdot 10^{-5})^{max} = 2.82$, $S_{lg} = 1.035$, $\tau^{max} = 1.53 < \tau^{min} = 1.87 < \tau_{0.05}^{cr,2}(N) < 2.343 < \tau_{0.05}^{cr,1}(N) = 2.484$; Wilk-Shapiro normality test: $W = 0.929 > W_{0.05}^{cr}(N) = 0.850$, David-Hartley-Pearson normality test: $U1_{0.05}^{cr}(N) = 2.740 < U = [(\lg(K_2'\cdot 10^{-5})^{max} - \lg(K_2'\cdot 10^{-5})^{min})/S_{lg}] = 3.40 < U2_{0.05}^{cr}(N) = 3.800$ (32)

The statistics of the explanatory variables $B_4$ is given in (10). The relationship of the residuals $\delta\lg(K_2'\cdot 10^{-5})$ of the regression (31) with the explanatory variables π, *L*, *Z*, *H*, $Z_m$ and $Z_{sub}$ was tested. As shown by statistical analysis, the set of molecular parameters $Z_{sub}$ for substituents in positions $R^1$ and $R^2$ significantly correlates with the set of regression residues (31). The pair correlation coefficient is equal to $|R| = 0.85 > R_{0.05}^{cr}(f = N - 2) = 0.602$. The pair correlation coefficient between $Z_{sub}$ and $B_4$ is equal to 0.29. Farrar-Glauber test (22) is equal to $\chi^2 = 0.48 < \chi_{0.05}^{2,cr}(f = 1) = 3.841$, i.e. there is no collinearity. Therefore, in regression (31), you can use an additional explanatory variable $Z_{sub}$. The following statistics were obtained for multiple regression:
$\lg(K_2'\cdot 10^{-5}) = a_0 + a_1\cdot B_4 + a_2\cdot Z_{sub}$, $N = 11$, $R = 0.98 > R_{0.05}^{cr}(\nu = N - m - 1; m = 2) = 0.697$ [32], $R^2 = 0.96$; the standard error (*RMSE*) of the regression estimate is equal to $S_{lg} = 0.254$; $a_0 = 2.28 \pm 1.22$, $a_1 = 0.65 \pm 0.07$, $a_2 = -2.74 \pm 0.51$, $t(a_1) = 9.28 > |t(a_2)| = 5.33 > t_{0.05}^{cr}(N-2) = 2.262$; $F = 76.62 > F_{0.05}^{cr}(f_1 = 2; f_2 = 8) = 4.46$ (33)
The standardized regression coefficients (33) are respectively:

$$a_1^* = 0.71, a_2^* = -0.47 \quad (34)$$

Regression (33) can explain 96% of the variability of the resultant variable. Figure 5 shows a scatter diagram of the experimental values of the bimolecular inhibition constant with respect to the values obtained from the regression (33).

Comparison of the structure of the synthesized aminostigmine derivatives with their anticholinesterase activity is consistent with known literature data [38]. At the stage of formation of the Michaelis complex, the electrostatic interaction between the anionic site of the enzyme and the inhibitor plays a certain role, but less significant than previously thought [39]. All the chemical compounds obtained have close values of ionisation constants, therefore ionized to approximately the same extent, but to a greater extent than aminostigmine. However, for compounds II and VIII, a decrease in affinity for ChE is observed. This decrease seems to be due to the presence of relatively short alkyl and, especially, cyclic substituents on nitrogen in the second position of the pyridine ring (preparations X and XI). The steric complementarity of the $N^+H(CH_3)_2$ cationic group to the hypothetical "cup" of the anionic site of the enzyme is thereby broken.

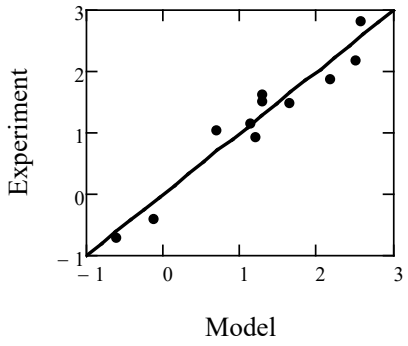


**Figure 5:** Scatter diagram. Bimolecular inhibition constant $\lg(K_2'\cdot 10^{-5})$. The model is determined by regression (33).

A different situation is observed when alkyl radicals with more than four carbon atoms are introduced. Chemical compounds III–VII are of the greatest interest among the synthesized derivatives of aminostigmine. Since the $K_a$ values of these preparations are close to each other, they also differ little in the degree of ionization at pH = 7.4. However, the values of affinity constants fluctuate in a wide range from

$4.6 \cdot 10^7$ to $0.05 \cdot 10^7$. It should be noted that their affinity for AChE increases with an increase in the number of $CH_2$ atomic groups. It is compounds III–VII that are characterized by low values of the molecular features *Z* and *H* and an increase in the value of $\pi(R^1,R^2)$ for substituents. There is some closeness of changes in anticholinesterase activity with dependence on the molecular factor features *Z* and *H* of the carcinogenic properties of substances [40] and the activity of sulfur-containing radioprotectors [41].

It can be assumed that close to the "anion cup" there is a conformationally flexible hydrophobic zone of some length on the ChE [35]. The initial decrease in activity for chemical compound II is probably due to a disturbance in the complementarity of the molecule to the size of the "anion cup". The subsequent increase in activity in a number of compounds (up to the chemical compound VII) is due to an increase in the hydrophobicity of substituents, with a simultaneous monotonous decrease in the value of the feature *Z*. As the chain length increases, the hydrophobic interaction becomes more and more important, and already compound VII is 20 times more active than aminostigmine.

The final stage of the studied reaction of the interaction of pyridylcarbamates with AChE is the breaking of the putative covalent bond of the carbamic acid residue with the enzyme, characterized by the decarbamylation constant $K_{2c}$. According to Table 2, the rates of this process for all chemical compounds are close and therefore depend little on the structure of the "leaving" part of the molecule (the 2-substituted pyridine ring), despite the fact that some of them interact hydrophobically with AChE. All drugs can therefore be characterized as reversible inhibitors.

The toxicity of the synthesized aminostigmine derivatives was estimated in Prozorovsky VB et al. [7] from the average lethal doses ($LD_{50}$) determined by the Prozorovsky tabular method [42,43]. The method is based on the introduction of a series of standard doses to groups of animals of the same size, followed by finding the dose and its error.

A statistical analysis showed that the toxicity of aminostigmine and the synthesised pyridylcarbamate derivatives was closely related to the value of the bimolecular inhibition constant $K_2'$, which characterises the total anticholinesterase activity, and the hydrophobicity value $\pi(R^1,R^2)$:

$\lg(1/LD_{50}) = a_0 + a_1 \cdot \lg(K_2' \cdot 10^{-5}) + a_2 \cdot \pi(R^1,R^2)$, $N = 11$, $R = 0.95 > R_{0.05}^{cr}(\nu = N - m - 1; m = 2) = 0.726$, $R^2 = 0.90$, $R^{*2} = 0.89$; $a_0 = 0.34 \pm 0.36$, $a_1 = 0.94 \pm 0.12$, $a_2 = -0.59 \pm 0.12$, $t(a_1) = 8.00 > |t(a_2)| = 5.10 > |t_{0.05}^{cr}(f = N - m - 1)| = 2.37$; the standard error (*RMSE*) of the regression estimate: $S_{lg} = 0.34$; $F = 33.34 > F_{0.05}^{cr}(f_1 = m; f_2 = N - m - 1) = 4.5$; standardized regression coefficients: $a_1^* = 1.04, a_2^* = -0.66$ (35)

The statistics of $\lg(1/LD_{50})$ set:

$N = 11$, $\lg(1/LD_{50})^{av} = -0.46 \pm 0.28$, 95% confidence interval: -1.08, 0.17; $\lg(1/LD_{50})^{min} = -2.30$, $\lg(1/LD_{50})^{max} = 0.638$, $S_{lg} = 0.93$, $\tau^{max} = 1.18 < \tau^{min} = 1.98 < \tau_{0.05}^{cr,2}(N) < 2.343 < \tau_{0.05}^{cr,1}(N) = 2.484$; Wilk-Shapiro normality test: $W = 0.805 < W_{0.05}^{cr}(N) = 0.850$, David-Hartley-Pearson normality test: $U1_{0.05}^{cr}(N) = 2.740 < U = [\lg(1/LD_{50})^{max} - \lg(1/LD_{50})^{min})/S_{lg}] = 3.16 < U2_{0.05}^{cr}(N) = 3.800$ (36)

The regression equation (35) shows that an increase ($a_1 > 0$) in the bimolecular inhibition constant leads to a significant increase in drug toxicity. At the same time, an increase in the hydrophobicity of $R^1$ and $R^2$ substituents affects the toxicity of pyridylcarbamate derivatives in the opposite direction ($a_2 < 0$). Thus, in order to obtain less toxic compounds, it is necessary to synthesize compounds with a higher distribution coefficient or a lower value of the bimolecular inhibition constant $K_2'$. Apparently, the distribution coefficient creates the prerequisites for the manifestation of toxicity, and the anticholinesterase action activates the toxicity of chemical compounds of the series.

The approximate coefficient of determination allows us to indicate the influence of the explanatory variables on the variability of the resultant variable:

$$R_{\text{ППП}}^2 = a_1^* r_{\pi,\lg K_2'} + a_2^* r_{\lg K_2',\text{ЛД}}, \quad R_{\text{ППП}}^2 = 0.126 + 0.770 = 0.896 \quad (37)$$

Here, the pair correlation coefficients are, respectively, $r_{\pi,\lg\text{ЛД}} = -0.19$, $r_{\lg K,\lg\text{ЛД}} = 0.7$. It follows from relation (37) that the explanatory variable $\lg(K_2' \cdot 10^{-5})$ makes the dominant contribution to the explanation of the variability of the resulting attribute. The correlation coefficient between the explanatory variables is equal to

$$r_{1,2} = 0.46 < 0.8 \text{ [21]} \quad (38)$$

Therefore, it can be assumed that there is no significant collinearity between the variables. Given that the residuals satisfy a normal distribution ($W = 0.941 > W_{0.05}^{cr}(N) = 0.850$), we can also use the Farrar-Glauber relation (22) to quantify collinearity: $\chi^2 = 2.10 < \chi_{0.05}^{2,cr}(f = 1) = 3.841$. This inequality does not contradict relation (38).

Since the value of $K_2'$ is related to the geometric size of the substituent $B_4$ (33), let us check the relationship of drug toxicity with the explanatory variable $B_4$. The following significant regression was obtained, which additionally takes into account also the explanatory variable π:

$\lg(1/LD_{50}) = a_0 + a_1 \cdot B_4 + a_2 \cdot \pi$, $N = 11$, $m_1 = 2$, $R_1 = 0.76 > R_{0.05}^{cr}(\nu = N - m_1 - 1; m_1 = 2) = 0.726$; the standard error (*RMSE*) of the regression estimate: $S_{lg} = 0.672$: $a_0 = -2.99 \pm 1.19$, $a_1 = 0.70 \pm 0.22$, $a_2 = -0.63 \pm 0.25$, $t(a_1) = 3.24 > |t(a_2)| = 2.54 > |t(a_0)| = 2.52 > t_{0.05}^{cr}(N - m_1 - 1) = 2.306$, $F = 5.59 > F_{0.05}^{cr}(f_1 = m_1; f_2 = N - m_1 - 1) = 4.46$; standardized regression coefficients: $a_1^*$

= 1.26, $a_2^*$ = -0.99; Σ = 3.6128, AIC = -0.7498, SC = -0.4594, SS = 0.2112 (39)

It follows that the regression coefficients on the explanatory variables $B_4$ and π are statistically significant, and that these variables significantly affect the variability of toxicity of chemical compounds. The pair correlation coefficient between the explanatory variables is $r_{1,2}$ = 0.57 < 0.8 [21]. This inequality assumes that the factors are not significantly related and therefore collinearity can be neglected. The following inequality can be used as a test for lack of collinearity:

$$t = r_{1,2} \cdot \left(N - m_1\right)^{0.5} / \left(1 - r_{1,2}^{\ 2}\right)^{0.5} = 2.08 < t_{0.05}^{\ cr}\left(f = 9\right) = 2.262 \quad (40)$$

Since the inequality $t < t^{cr}(f)$ (40) holds, it is assumed that the explanatory variables are not significantly collinear. In addition, there is the following inequality: $\chi^2 = 3.34 < \chi_{0.05}^{\ 2,cr}(f = 1) = 3.841$ (22), which does not contradict (40).

Experimental determination of the distribution value for a number of aminostigmine derivatives is difficult [7]. This is due to the fact that for some drugs the distribution coefficient turned out to be higher than the upper limit of instrumental resolution. Therefore, it is of interest to use calculated molecular descriptors in the regression equation. Since the bimolecular inhibition constant is closely related to the affinity constant (4), the toxicity of chemical compounds should also be related to the affinity constant. At the same time, the affinity constant is associated with the molecular features *Z* and *H*. The following multiple regression can be written relating the toxicity properties of aminostigmine derivatives to their molecular parameters:

$\lg(1/LD_{50}) = a_0 + a_1 \cdot B_4 + a_2 \cdot \pi + a_3 \cdot Z$, $N = 11$, $m_2 = 3$, $R_2 = 0.92 > R_{0.05}^{\ cr}(\nu = 7; m_2 = 5) = 0.807$, $R_2^{\ 2} = 0.846$, $R^{*2} = 0.81$; the standard error (*RMSE*) of the regression estimate is equal to $S_{lg} = 0.448$: $a_0 = 23.27 \pm 7.98$, $a_1 = 0.41 \pm 0.17$, $a_2 = -0.99 \pm 0.20$, $a_3 = -8.37 \pm 2.52$, $|t(a_2)| = 5.00 > |t(a_3)| = 3.32 > t(a_0) = 2.93 > t(a_1) = 2.49 > t_{0.05}^{\ cr}(f = N - m_2 - 1) = 2.365$; $F = 12.02 > F_{0.05}^{\ cr}(f_1 = 3; f_2 = 7) = 4.35$; standardized regression coefficients are equal to: $a_1^* = 1.12$, $a_2^* = -2.33$, $a_3^* = -1.77$; Σ = 1.406, AIC = -1.5127, SC = -1.1863, SS = 0.1481; $R_{appr}^{\ 2} = a_1^* \cdot r_{B4,lgЛД} + a_2^* \cdot r_{\pi,lgЛД} + a_3^* \cdot r_{Z,lgЛД} = 0.266 + 0.212 + 0.366 = 0.843$ (41)

For all three informational tests AIC, SC and SS the quality of the regression (41) is higher than the quality of the regression (39). The statistical significance of the multiple correlation coefficient is determined by the inequality [21]: $t = R \cdot (N - m - 1)^{0.5} / (1 - R^2)^{0.5} = 6.21 > t_{0.05}^{\ cr}(f = N - m - 1) = 2.365$. In accordance with the values of the standardized coefficients (41), the greatest contribution to the variability of the toxicity of aminostigmine derivatives comes from the *Z* factor and, to the least extent, there is a dependence on the hydrophobic properties (π) of the substituents.

Check whether the addition of the explanatory variable *Z* to the regression equation (41) is statistically significant. To test the hypothesis, we use a statistic (9) that has an *F*-distribution:

$$F = \left(R_2^2 - R_1^2\right)\left(N - m_2 - 1\right) / \left(m_2 - m_1\right) / \left(1 - R_2^2\right) = 12.25 > F_{0.05}^{\ cr}\left(f_1 = m_2 - m_1; f_2 = N - m_2 - 1\right) = 5.59 \quad (42)$$

It follows from inequality (42) that the combined consideration of the explanatory variables $B_4$, π and *Z* in the regression equation has a significant effect on the variation in the toxicity of pyridylcarbamate derivatives.

To assess multicollinearity between explanatory variables, pairwise correlation coefficients between explanatory variables were calculated: $r_{1,2}$ = 0.57 < $|r_{1,3}|$ = 0.70 < $|r_{2,3}|$ = 0.72. Let us find out which explanatory variable leads to the highest values (in absolute value) of the pair correlation coefficient. For this, the relation [21] is used, the values of which have a t-distribution with $f = N - m$ degrees of freedom:

$$t_{ij} = r_{ij} \bullet \left(N - m\right)^{0.5} / \left(1 - r_{ij}^{\ 2}\right)^{0.5} \quad (43)$$

Here *i* and *j* are the numbers of explanatory variables in equation (41); *m* is the number of explanatory variables. From relation (43) we obtain the following inequalities: $t_{1,2} = 1.99 < t_{0.05}^{\ cr}(N - m) = 2.306 < |t_{1,3}| = 2.77 < |t_{2,3}| = 2.96$. Since the values $|t_{1,3}|$ and $|t_{2,3}|$ more than the tabular $t^{cr}(f)$, then collinearity arises mainly due to the third explanatory variable, namely *Z*. However, this variable should not be excluded from the regression equation. The fact is that the variables $B_4$, π and *Z* are not duplicative in their content. Coefficient $B_4$ is one of the geometric dimensions of substituents, and feature π determines the total hydrophobicity of substituents $R^1$ and $R^2$. Both of these signs characterize the local region of the molecule (the region of substituents), and the feature *Z* characterizes the electronic properties of the molecule as a whole.

Regression residuals (41) are normally distributed: $W = 0.945 > W_{0.05}^{\ cr}(N = 11) = 0.850$. Therefore, multicollinearity is quantified using the Farrar-Glauber relation:

$$\chi^2 = -(N - 1 - (2m + 5)/6) \cdot \ln \begin{vmatrix} 1 & r_{12} & r_{13} \\ r_{21} & 1 & r_{23} \\ r_{31} & r_{32} & 1 \end{vmatrix} = 11.49 > \chi_{0.01}^{2,cr}(f = 3) = 11.34 \quad (44)$$

Inequality (44) indicates that there is weak multicollinearity between the explanatory variables.

To lower the relationship of the explanatory variables, we perform a linear transformation by introducing the modified variables π - *Z* and $B_4$ - *Z* into the equation. As a result, the pair correlation coefficients noticeably decrease in absolute

value: $r_{1,2}$ = 0.17, $r_{1,3}$ = -0.80 and $r_{2,3}$ = 0.082. Farrar-Glauber test would be next: $\chi^2 = 9.75 < \chi_{0.05}^{2,cr}(f = 3) = 11.34$. The following regression was obtained:

$\lg(1/LD_{50}) = a_0 + a_1 \cdot (\pi - Z) + a_2 \cdot (B_4 - Z) + a_3 \cdot Z$, $N = 11$, $m_2 = 3$, $R = 0.92$, $R^2 = 0.84$; standard error of the regression estimate: $S_{lg} = 0.448$: $a_0 = 23.33 \pm 7.99$, $a_1 = 0.42 \pm 0.17$, $a_2 = -0.99 \pm 0.20$, $a_3 = -8.37 \pm 2.53$, $t(a_2) = 5.00 > |t(a_3)| = 3.31 > t(a_1) = 2.49 > t_{0.05}^{cr}(f = 7) = 2.365$, $F = 12.00 > F_{0.05}^{cr}(f_1 = m_2; f_2 = N - m_2 - 1) = 4.35$; standardized regression coefficients: $a_1^* = -0.62$, $a_2^* = 0.79$, $a_3^* = -0.42$; Σ = 1.407, AIC = -1.511, SC = -1.186, SS = 0.148 (45)

## Conclusion

The values of the determination coefficients (41) and (45) and the information quality of the regressions remained virtually unchanged. Thus, a decrease in the toxicity of drugs can be achieved by reducing the molecular feature *Z*. At the same time, a decrease in the feature *Z* leads to an increase in the bimolecular inhibition constant in accordance with equations (3) and (4). The noted feature of the studied series of ChE inhibitors allows us to hope for obtaining chemical compounds with a wider therapeutic effect in the series of analogs of aminostigmine, compared with aminostigmine, that is, with high anticholinesterase activity, but less toxic.

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