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A comparative study of the effect of phenothiazine derivatives and their S-oxides on cholinesterase investigated by a new kinetic spectrophotometric method

Abstract

Aim. To develop a new kinetic spectrophotometric method for determining acetylcholinesterase (AChE) inhibitors – phenothiazine antipsychotic drugs (PhT) and their sulfoxide metabolites (S-oxides) without adding an exogenous catalyst to obtain a chromogenic agent.

Materials and methods. The bases of S-oxides of promethazine (PMZ), chlorpromazine (CPM) and thioridazine (THZ) were obtained by oxidizing the corresponding PhT hydrochlorides with diperoxyadipic acid. The structure of the S-oxides of the corresponding PhT synthesized was proven by melting points, spectral characteristics (^{1}H NMR and IR methods) and oscillopolarography results. ^{1}H NMR spectra were recorded on a Varian XL-200 spectrometer. IR spectra were recorded within the range of 4000-400 cm^{-1} on a SPECORD M-80 spectrometer (Zeiss, Jena, Germany). To register polarograms, a "PO 03 CLA" oscillopolarograph with a three-electrode cell was used. The purity of S-oxides was determined by the high-performance liquid chromatography method on a Zorbax SB, C-18 (250×4.6) mm column. Measurements of absorbance of solutions were performed in a 1 cm cuvette on an Evolution 60S UV-Visible Thermo-Scientific Spectrophotometer (USA) (λ = 358 nm).

Results and discussion. Acetylcholine (ACh) was found to mimic the activity of peroxidase; based on it, a spectrophotometric system containing ACh–H_2O_2–p-phenetidine for a sensitive and selective assessment of the AChE activity and determination of its inhibitors was developed. According to the plots of inhibition efficiency *vs* inhibitors concentration, the inhibiting ability of chlorpromazine, promethazine and thioridazine and their S-oxides was determined. The IC_{50} values of CPM, PMZ and THZ and their metabolites in relation to the AChE activity were estimated as 11 ng mL^{-1} (CPM) and 1.8 ng mL^{-1} (CPM S-oxide), 17 ng mL^{-1} (PMZ) and 2.5 ng mL^{-1} (PMZ S-oxide) and 27 ng mL^{-1} (THZ 2S,5S-dioxide). The results obtained indicate that S-oxides of the corresponding PhT are selective and potent inhibitors of AChE. The values of the inhibition efficiency obtained for S-oxides of PhT derivatives were an order of magnitude lower than those of the corresponding PhT derivatives.

Conclusions. The spectrophotometric method proposed without the addition of other exogenous catalysts holds promise for the on-site determination of PhT antipsychotics and can be additionally used for sensory applications in areas related to environmental protection and food safety, as well as in the chemical-toxicological analysis.

Keywords: acetylcholine; acetylcholinesterase; phenothiazine derivatives; S-oxides; photometric methods of analysis

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Порівняльне вивчення впливу похідних фенотіазину та їх S-оксидів на холінестеразу за допомогою нового кінетичного спектрофотометричного методу

Анотація

Мета. Розробити новий кінетико-спектрофотометричний метод визначення інгібіторів ацетилхолінестерази (AChE), фенотіазинових антипсихотичних засобів (PhT) та їх сульфоксидних метаболітів (S-оксидів) без додавання екзогенного каталізатора для отримання хромогенного агента.

Матеріали та методи. Основи S-оксидів прометазину, хлорпромазину та тіоридазину синтезували шляхом окиснення відповідних гідрохлоридів PhT дипероксиадипіновою кислотою. Будову синтезованих S-оксидів відповідних PhT доводили за температурами плавлення, спектральними характеристиками (^{1}Н ЯМР- та ІЧ-методи) та результатами осцилополярографії. Спектри ^{1}Н ЯМР записували на спектрометрі Varian XL-200. ІЧ-спектри реєстрували в діапазоні

4000-400 см$^{-1}$ на спектрометрі SPECORD M-80 (Zeiss, Jena, Німеччина). Для реєстрації полярограм використовували осцилополярограф ПО, модель 03 ЦЛА з триелектродним елементом. Чистоту S-оксидів визначали методом ВЕРХ на колонці Zorbax SB, C-18 (250 × 4,6) мм. Вимірювання поглинання випробуваних розчинів S-оксидів відповідних PhT проводили за довжини хвилі λ = 358 нм у кюветі завтовшки 1 см на спектрофотометрі Evolution 60S UV-Visible Thermo-Scienticfic (США).

Результати та їх обговорення. З'ясовано, що ацетилхолін (ACh) імітує активність пероксидази, на основі чого було розроблено спектрофотометричну систему, що містить ACh-H$_2$O$_2$-*п*-фенетидин для чутливого селективного оцінювання активності AChE та визначення інгібіторів ферменту. Відповідно до графіків залежності інгібувальної здатності від концентрації інгібіторів визначено інгібувальну здатність хлорпромазину, прометазину, тіоридазину та їх S-оксидів. *IC*$_{50}$ хлорпромазину, прометазину, тіоридазину та їх метаболітів щодо активності AChE оцінено так: 11 нг мл$^{-1}$ – хлорпромазин, 1,8 нг мл$^{-1}$ – хлорпромазин S-оксид, 17 нг мл$^{-1}$ – прометазин, 2,5 нг мл$^{-1}$ – прометазин S-оксид та 27 нг мл$^{-1}$ – тіоридазин 2S,5S-діоксиду. Одержані результати свідчать про те, що S-оксиди відповідних PhT є селективними та потужними інгібіторами AChE. Отримані значення ефективності інгібування для S-оксидів похідних PhT були на порядок нижчими, ніж у відповідних похідних PhT.

Висновки. Розроблена спектрофотометрична методика без додавання інших екзогенних каталізаторів має перспективи для визначення PhT нейролептиків на місці і може бути додатково використана для сенсорних застосувань у сферах, пов'язаних із захистом навколишнього середовища та безпекою харчових продуктів, а також у хіміко-токсикологічному аналізі.

Ключові слова: ацетилхолін; ацетилхолінестераза; похідні фенотіазину; S-оксиди; фотометричні методи аналізу

■ Introduction

Acetylcholinesterase (AChE) is an important enzyme in the central and peripheral nervous system [1]. Its primary biological function is to catalyze the breakdown of acetylcholine (ACh) and some other choline esters that function as neurotransmitters [2]. Nowadays, it is accepted that Alzheimer's disease (AD), common dementia for older people worldwide, is related to a low level of ACh in the hippocampus and cortex [3]. AChE inhibitors can penetrate the blood-brain barrier and have been suggested to increase the level of ACh to treat AD [4]. However, ACh excess results in neuromuscular paralysis or fatal consequences [5].

Various analytical techniques, such as Ellman's method [6], electrochemical methods [7–10], the liquid crystals-based method [11] and chemiluminescent and fluorescent methods [12–19], have been developed for the AChE activity and screening of its inhibitors.

However, some of these methods suffer from drawbacks of the time-consuming sample pretreatment, sophisticated instrument manipulation or high costs. Moreover, it is reported that Ellman's method may lead to a false-positive effect [20].

Hence, developing a simple, highly sensitive and selective method for probing the AChE activity and screening for its potential inhibitors is highly needed.

Photometric methods have attracted significant attention because of their simplicity and low-cost advantages. Moreover, they can circumvent the relative complexity inherent in other detection methods by relying on unaided visual readouts instead of complicated instruments, which is especially useful for on-site detection in real-time [21, 22].

Earlier, we proposed a new kinetic spectrophotometric method for determining the AChE activity. It consists of spectrophotometric measurement of the rate of the ACh enzymatic hydrolysis through two conjugated reactions – perhydrolysis of the non-hydrolyzed ACh residue with a hydrogen peroxide excess and oxidation of the indicator substance *p*-phenetidine (*p*-Ph) by peroxyacetic acid formed to 4,4'-azoxyphenetol (λ$_{max}$ = 358 nm, lgε 4.2) [23]. The rate of the enzymatic hydrolysis of ACh was measured by the tangent method using the linear part of the

kinetic curve in the light absorbance (*A*, 358 nm) *vs* time (*t*, min) coordinates. The linear inversely proportional dependence of the conditional reaction rate on the enzyme concentration was observed. The same principle has been used to quantify compounds capable of inhibiting AChE enzyme as a change in the activity of AChE causes a change in the amount of unreacted ACh in the enzymatic hydrolysis reaction. The latter is quantified by the kinetic-spectrophotometric method as mentioned above (Scheme) [24].

■ Materials and methods

Acetylcholine chloride (pharmaceutical grade), 0.02 g per amp/5 mL, produced by "VECTOR", State Science Centre of Virology and Biotechnology, Russia.

Disodium hydrogen phosphate dodecahydrate, $Na_2HPO_4 \cdot 12H_2O$ (puriss. p.a.), produced by "ReaChem", Kharkiv, Ukraine.

A dry acetylcholinesterase enzyme from horse serum – 80 mg per vial (VI class) with the known specific activity of 27 AU mg^{-1} (according to the certificate) produced by SMU "Biomed" (Russia). The catalytic activity of 1 activity unit (AU) is manifested by such an amount of this enzyme preparation that converts 1 µmole of this substrate in 1 min under given reaction conditions.

A stabilized hydrogen peroxide 30–40% solution, puriss. p.a. (LLC Inter-Synthes, Boryslav, Ukraine). The precise content of hydrogen peroxide was determined according to the State Pharmacopoeia of Ukraine [25].

Chlorpromazine hydrochloride ≥98% (TLC); CAS 69-09-0, Sigma-Aldrich. $C_{17}H_{19}ClN_2S \cdot HCl$ (CPZ), 2-chloro-10-(3-dimethylaminopropyl)phenothiazine hydrochloride.

Promethazine hydrochloride 98%; CAS 58-33-3, Sigma-Aldrich. $C_{17}H_{20}N_2S \cdot HCl$ (PMZ), 10-[2-(dimethylamino)propyl]phenothiazine hydrochloride.

Thioridazine hydrochloride ≥99%; CAS 130-61-0, Sigma-Aldrich. $C_{21}H_{26}N_2S_2 \cdot HCl$ (THZ), 10-[2-(1-methyl-2-piperidyl)ethyl]-2-(methylthio)-10*H*-phenothiazine hydrochloride.
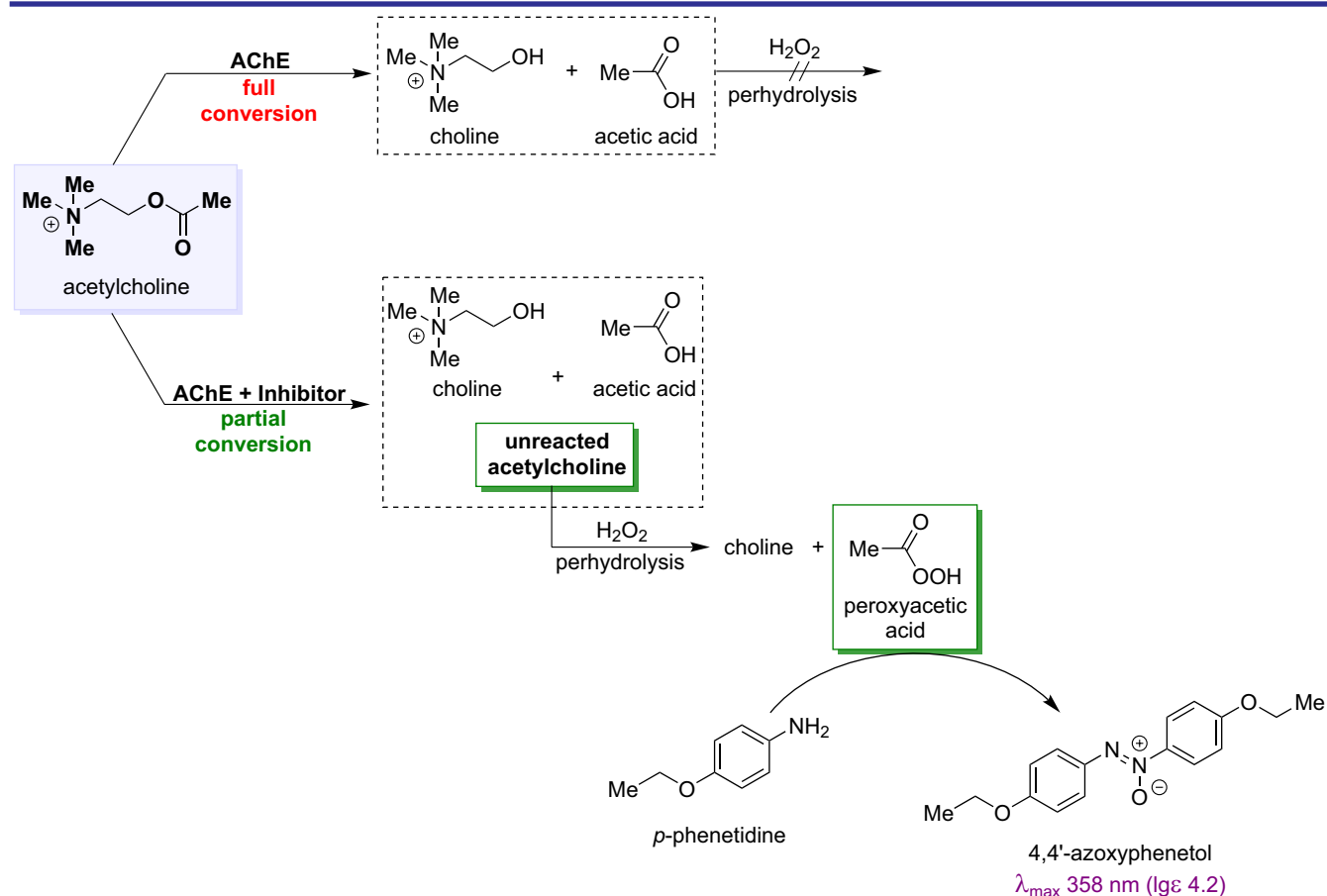
Scheme. An analytical system for the determination of AChE inhibitors using coupled reactions of ACh perhydrolysis and peroxyacid oxidation of *p*-Ph as an indicator substance
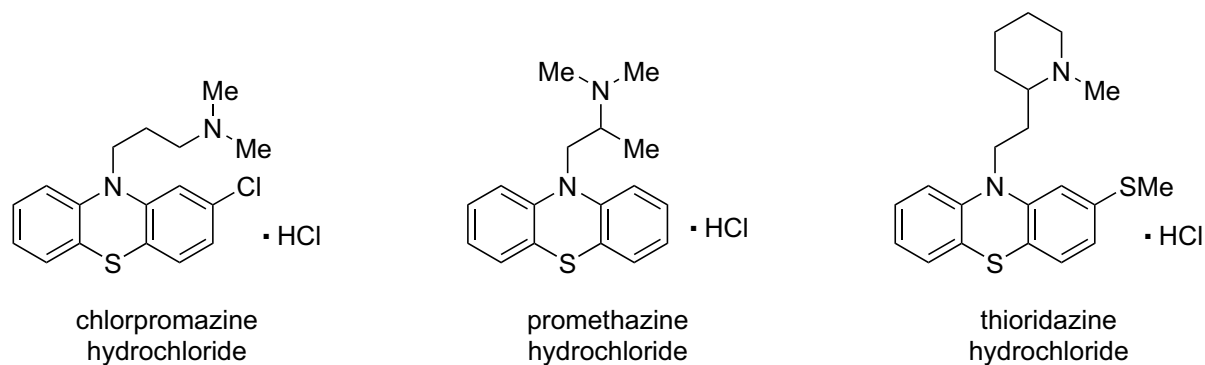
Figure 1. The structures of the phenothiazines tested

High purity double distilled water was used in all experiments.

Chlorpromazine hydrochloride, promethazine hydrochloride, thioridazine hydrochloride and thioridazine 2S,5S-disulfoxide were obtained from commercial sources and used without further purification.

The structures of the phenothiazine antipsychotic drugs and the corresponding S-oxides are given in Figure 1.

^{1}H NMR spectra were recorded on a Varian XL-200 Spectrometer (200 MHz) using DMSO-d_6 as a solvent and TMS as an internal standard.

IR spectra were recorded within the range of 4000–400 cm^{-1} on a SPECORD M-80 spectrometer (Zeiss, Jena, Germany) in KBr pellets (200 mg of KBr and 2 mg of the test compounds).

Voltammograms were recorded on a PO 03 CLA oscilllographic polarograph (Rostov-on-Don Research Institute, Russia) in a three electrode thermostated cell at 20°C; the indicator microelectrode was a dropping mercury one, the reference electrode was a saturated calomel electrode, and the auxiliary electrode was a platinum electrode. The potentials of peak maxima were measured with a V7-21 digital voltmeter with the precision of ±1 mV. Triangular-shaped polarizing voltage was applied to the cell electrodes at a scanning rate of = 0.5 V s^{-1}. The potential was varied in the range from –0.2 to –1.4 V. Dissolved oxygen was removed from solutions by blowing purified argon over 20 min. The polarography conditions were selected in each case.

The pH measurements were performed with a combined glass electrode (SP20B) together with an EAL-1M3.1 reference standard silver chloride electrode.

The measurements of absorbance (*A*) of solutions were performed in a 1.0 cm cuvette on an Evolution 60S UV-Visible Thermo-Scientific Spectrophotometer (USA) against the buffer solution with double distilled water (compensation solution).

The preparation procedure for the promethazine S-oxide base

0.64 g (0.002 mol) of PMZ was dissolved in 15 mL of distilled water; 0.44 g (0.0025 mol) of diperoxyadipic acid was added with stirring and left at room temperature for 15 min. The mixture was poured with 2 mL of 50% sodium hydroxide solution, and the resulting precipitate was extracted with diethyl ether (3×10 mL). The combined organic phases were washed with water cooled to 10°C (3×20 mL), dried over anhydrous sodium sulfate, and the solvent was evaporated at room temperature. Acetone was added to the residue, and the mixture was cooled. In 2 days, white crystals precipitated; then they were filtered and dried at room temperature. The yield of PMZ S-oxide was close to the quantitative one.

Diperoxyadipic acid was obtained by the interaction between hydrogen peroxide and adipic acid in the presence of sulfuric acid according to the known method [24]. Diperoxyadipic acid, HO$_3$C(CH$_2$)$_4$CO$_3$H, 98%, m. p. 114.5°C (dec.), active oxygen content, % (theor.), 17.5 (17.9).

Promethazine S-oxide base. M. p. 118-119°C. ^{1}H NMR (200 MHz, DMSO-d_6), δ, ppm: 0.75 (3H, d, CH$_3$); 2.42 (6H, s, 2NCH$_3$); 2.81–2.95 (1H, m, CH); 4.18–4.60 (2H, t, CH$_2$); 7.38–7.92 (8H, m; ArH). IR (KBr), ν, cm^{-1}: 1028 (S=O).

CPZ S-oxide base was synthesized by the similar procedure as for PMZ S-oxide base.

Chlorpromazine S-oxide base. M. p. 111-112°C. ^{1}H NMR (200 MHz, DMSO-d_6), δ, ppm: 1.82 (2H, q, CH$_2$); 2.15 (6H, s, 2×NCH$_3$); 2.35 (2H, t, CH$_3$NC$\underline{H}_2$); 4.38 (2H, t, NCH$_2$); 7.22–8.11 (7H, m, ArH).

Analysis of oxidation products of phenothiazine derivatives by HPLC

In addition, the purity of S-oxides of CPZ and PMZ was determined by the high-performance liquid chromatography method on a Zorbax SB, C-18 (250×4.6) mm column. The mobile phase was the solution of camphorsulfonic acid (2.9 g L^{-1}), which pH was adjusted to 5.4 with 5 mol L^{-1}

sodium hydroxide solution. The flow rate was 1 mL min⁻¹. The volume of the injected sample was 20 µL. Detection was performed by UV-spectrophotometry at 262 nm. The sulfoxides of PhT derivatives studied were dissolved in the mobile phase. The concentration of S-oxides, mg mL⁻¹, was: CPZ – 0.402, PMZ – 0.406. The percentage of the active substance calculated by the normalization method was: for CPZ S-oxide – 94.66%, S-oxide PMZ – 97.87% with the purity of the prominent peaks of 99.85%, 99.84%, respectively.

Oscillopolarograms (0.1 mol L⁻¹ KCl, pH 5.5), E_p^k (V): CPM S-oxide (1.8×10^{-5} mol L⁻¹) –0.785, –1.058 (E_p^A –0.935); PMZ S-oxide (1.8×10^{-5} mol L⁻¹) –1.140, –1.385 (E_p^A –1,13); THZ 2S,5S-disulfoxide (1.8×10^{-5} mol L⁻¹) –1.10, –1.295; (0.1 mol L⁻¹ KCl, 0.01 M HCl), E_p^k (V): CPM S-oxide (1.8×10^{-5} mol L⁻¹) –0.865, –1.084 (E_p^A –0.938); PMZ S-oxide (1.8×10^{-5} mol L⁻¹) –0.937; THZ 2S,5S-disulfoxide (1.8×10^{-5} mol L⁻¹) –0.841, –0.975.

Preparation of the solutions

Stock solutions of PhT derivatives

Daily 5.0×10^{-3} mol L⁻¹ of PhT derivatives stock solutions were prepared by dissolving accurately weighed samples of PhT derivatives in 0.01 mol L⁻¹ HCl solution (THZ in 96 % ethanol) and diluting the solution to 100 mL with double distilled water in volumetric flasks. The corresponding aliquots of the stock solutions of PhT derivatives were transferred into separate glass-stopper tubes.

Working standard solutions of PhT derivatives

Working standard solutions (WSS) of PhT derivatives were prepared from the stock solutions by the corresponding dilution with double distillate water or 0.01 M hydrochloric acid. All solutions were stored at room temperature in a cool dark place.

Preparation of 0.2 mol L⁻¹ phosphate buffer solution (pH 8.35)

Disodium hydrogen phosphate dodecahydrate (35.75 g) was dissolved in a 500 mL flask using double-distilled water. Then 19 mL of 0.1 mol L⁻¹ solution of the hydrochloric acid solution was added. The pH of the final solution was controlled by potentiometry.

Preparation of 10% hydrogen peroxide solution

The solution was prepared by the corresponding high-test hydrogen peroxide dilution with double-distilled water. The hydrogen peroxide content in 10% working solution was determined by the permanganatometric method.

Preparation of 1% p-phenetidine hydrochloride solution

p-Phenetidine hydrochloride (*p*-Ph) was prepared by dissolution of *p*-phenetidine in chloroform followed by precipitation of the salt by gaseous HCl. 1.00 g of *p*-phenetidine hydrochloride was dissolved in 80 mL of double-distilled water in a 100 mL volumetric flask and diluted to the volume with the same solvent.

Preparation of acetylcholine chloride solution

The ampoule content (0.02 g of ACh) was dissolved in 20 mL of double-distilled water. The ampoule was opened, 4.0 mL of water was pipetted and added to the ampoule, then shaken until acetylcholine was completely dissolved. Then the ACh solution was transferred into a 20 mL volumetric flask and diluted to the volume with double-distilled water.

Preparation of acetylcholinesterase solution

The ampoule content of 80 mg of AChE was dissolved in 20 mL of double-distilled water. The ampoule was opened, 4.0 mL of water was pipetted, and shaken until AChE was completely dissolved. Then the AChE solution was transferred into a 20 mL volumetric flask, and diluted to the volume with double-distilled water.

The procedure generally recommended

The first part: 10.0 mL of 0.2 M phosphate buffer solution (pH = 8.35) was transferred into a 20 mL graduated test tube with a ground stopper, and 1.0 mL of 1 mg mL⁻¹ ACh solution was added. Then 1.6 mL of 10% hydrogen peroxide solution was added, and the stopwatch was started. After that the solution was shaken thoroughly and thermostated for 10 min. Then 0.5 mL of 1% *p*-Ph solution was added, and the solution was diluted to the volume with double distilled water. The stopwatch was switched on again, and the solution was scanned photometrically every minute over the period of 15 min (λ = 358 nm). The solution containing only 0.2 M phosphate buffer (10.0 mL, pH = 8.3) was used as a reference solution. The rate of the reaction [(ACh + H₂O₂) + *p*-Ph] was determined as a slope of the kinetic curve *A vs t*, (tgα₀, min⁻¹).

The second part: 10.0 mL of 0.2 M phosphate buffer solution (pH = 8.35) was transferred into a 20 mL graduated test tube with a ground stopper, and 1.0 mL of 1 mg mL⁻¹ ACh solution was added. After that a 0.5 mL accurate portion of ChE was added, then 1.6 mL of 10% hydrogen peroxide solution was added while stirring, shaken up thoroughly and kept for 10 min in a thermostat. Then 0.5 mL of 1% *p*-Ph solution was added, and the mixture was diluted to the volume with double distilled water. The stopwatch was switched on, and the solution was scanned photometrically every minute over the period of 15 min (λ = 358 nm). The solution containing only 0.2 M phosphate buffer (10.0 mL, pH = 8.3) was used as a reference solution. The rate of the reaction

[(ChE + ACh) + H_2O_2 + *p*-Ph] was determined as a slope of the kinetic curve *A vs t*, (tgα, min⁻¹).

The third part: 10.0 mL of 0.2 M of phosphate buffer solution (pH = 8.35) was transferred into a 20 mL graduated test tube with a ground stopper. The accurate volumes (from 0.40 to 3.20 mL) of WSS of PhT derivatives (Inh) were added to the tube. Then 0.5 mL of ChE was added while stirring, the stopwatch was switched on, every solution was shaken thoroughly and thermostated for 10 min. After that 1.0 mL of 1 mg mL⁻¹ ACh solution was quickly added, and the stopwatch was switched on, the content was shaken thoroughly and thermostated for 10 min again. Then 1.6 mL of 10% hydrogen peroxide solution was added, the tube was kept for 10 min in a thermostat, 0.5 mL of 1% *p*-Ph solution was added and diluted to the volume with double distilled water. The stopwatch was switched on, and the solution was scanned photometrically every minute over the period of 15 min (λ = 358 nm). The buffer solution with double distilled water was used as a reference solution. The rate of the reaction [(ChE + Inh) + ACh] +H_2O_2 + *p*-Ph] was determined as a slope of the kinetic curve *A vs t* (tgα$_i$, min⁻¹).

Screening of AChE inhibitors

The inhibiting efficiency (*IE*, %) of the enzymatic hydrolysis of acetylcholine in the presence of PhT derivatives was determined by the following equation:

$$IE\ (\%) = \frac{tg\alpha_i - tg\alpha}{tg\alpha_0 - tg\alpha} \times 100\ \%$$

where tgα$_i$ (min⁻¹) is the slope tangent of the linear part of the kinetic curve in the *A vs t* coordinates (a slope of the kinetic curve) for the reaction [(ChE + Inh) + ACh] +H_2O_2 + *p*-Ph] in the presence of AChE and an inhibitor;

tgα$_0$ is the slope tangent of the linear part of the kinetic curve in the *A vs t* coordinates (a slope of the kinetic curve) for the reaction [(ACh + H_2O_2) + *p*-Ph] in the absence of AChE and an inhibitor;

tgα is the slope tangent of the linear part of the kinetic curve in the *A vs t* coordinates (a slope of the kinetic curve) for the reaction [(ChE + ACh) + H_2O_2 + *p*-Ph] in the presence of AChE and the absence of an inhibitor.

■ Results and discussion

Figures 2–4 show the dependence of *IE* on the concentration of PhT derivatives and their S-oxides for the reaction [(ChE + Inh) + ACh] + H_2O_2 + *p*-Ph]. The inhibition ability of CPZ, PMZ and THZ and their metabolites is evaluated by the IC_{50} value, which is the concentration of the inhibitor needed for 50% inhibition of the AChE activity. The IC_{50} value of CPZ, PMZ and THZ and their metabolites in relation to the AChE activity was estimated as 11 ng mL⁻¹ (CPM) and 1.8 ng mL⁻¹ (CPM S-oxide), 17 ng mL⁻¹ (PMZ) and 2.5 ng mL⁻¹ (PMZ S-oxide) and 27 ng mL⁻¹ (THZ 2S,5S-dioxide) from the plots of *IE* versus the concentration of inhibitors (Figures 2–4), which were similar to the previous reports [27, 28].

Thus, to summarize, a new kinetic spectrophotometric method for determining the activity of AChE and its inhibitors of PhT antipsychotic drugs and their sulfoxides metabolites is attractive because of its convenience, without adding an exogenous catalyst to obtain a chromogenic
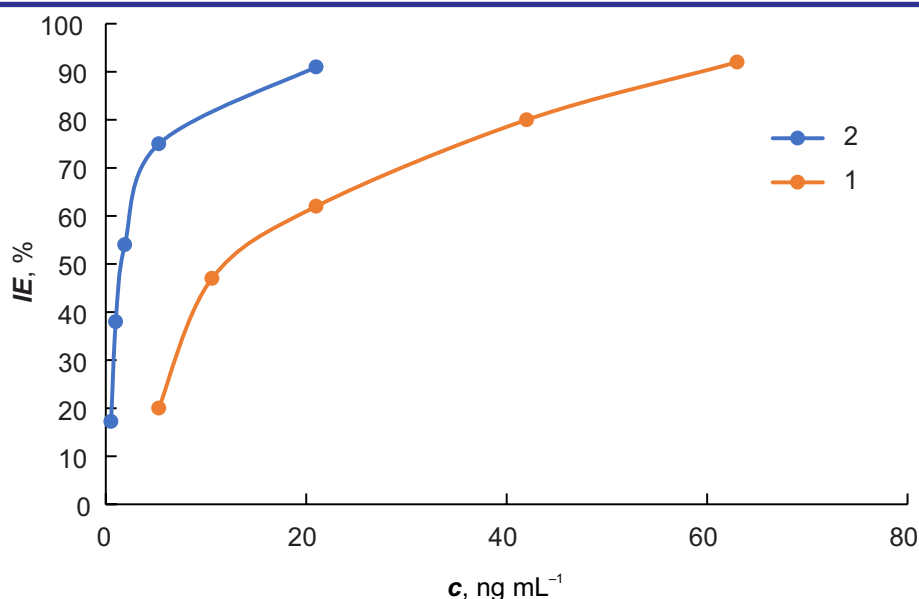

Figure 2. The effect of the CPZ (1) and its S-oxide (2) concentration on the AChE inhibition efficiency
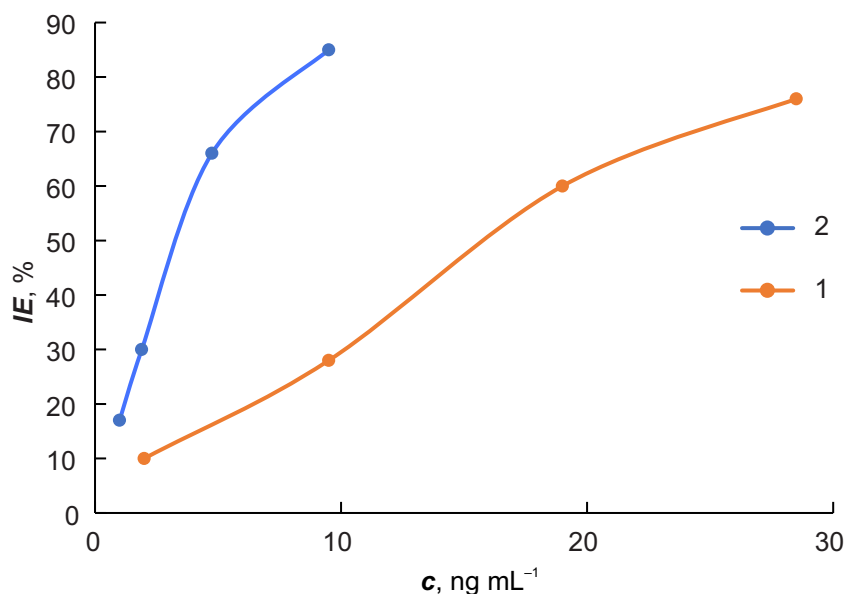
Figure 3. The effect of the PMZ (1) and its S-oxide (2) concentration on the AChE inhibition efficiency
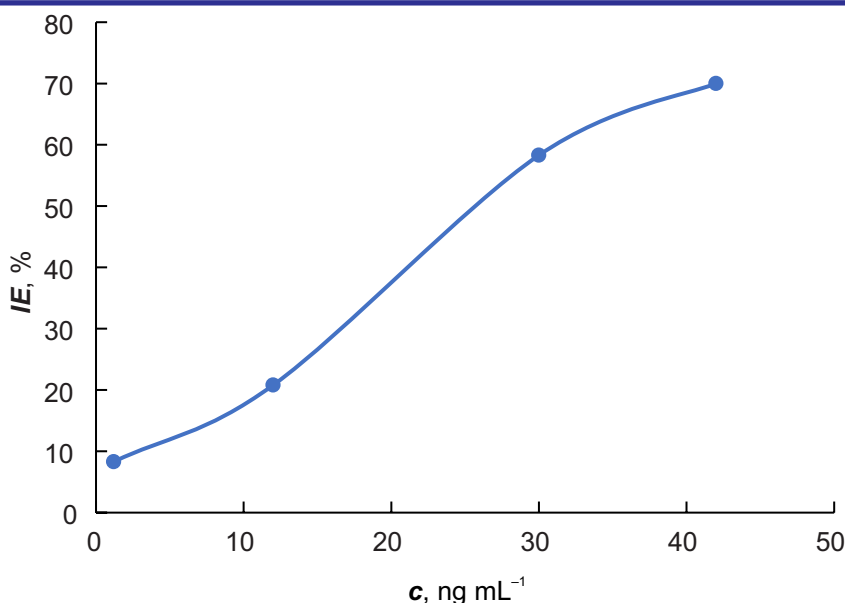

Figure 4. The effect of the THZ 2S,5S-dioxide concentration on the AChE inhibition efficiency

agent, which can lead to complexity and interferences. In this study, for the first time we discovered that ACh itself mimicked the activity of peroxidase, and based on it, a simple and reliable spectrophotometric system containing ACh–H_2O_2–p-Ph for a sensitive and selective assessment of the AChE activity and determination of its inhibitors was developed.

PhT derivatives and their S-oxides inhibit the activating action of AChE in the hydrolysis reaction of acetylcholine. As seen, the values of the inhibition efficiency IC_{50} obtained for sulfoxides of the corresponding PhT derivatives were an order of magnitude lower than those of the corresponding PhT derivatives.

This result displays that the detection method proposed provides a sensitive and rapid strategy for screening AChE inhibitors.

It has also been demonstrated that this strategy can be applied to the determination of PhT antipsychotic drugs in real samples. Meanwhile, the sensor platform can also be implemented on test sensors for fast PhT monitoring. Thus, this extremely simple spectrophotometric strategy without the addition of other exogenous catalysts holds promise for the on-site determination of PhT antipsychotics and can be additionally used for sensory applications in areas related to environmental protection and food safety, as well as in the chemical-toxicological analysis.

■ Conclusions

A new kinetic spectrophotometric method for determining AChE inhibitors – phenothiazine antipsychotic drugs and their sulfoxide metabolites without adding an exogenous catalyst to obtain a chromogenic agent has been developed. The ACh itself mimics the activity of peroxidase, and based on it, a simple and reliable spectrophotometric system containing ACh–H_2O_2–*p*-Ph for a sensitive and selective assessment of the AChE activity and determination of its inhibitors has been developed. The values of the inhibition efficiency IC_{50} obtained for sulfoxides of the corresponding PhT derivatives are an order of magnitude lower than those of the corresponding PhT derivatives. It has also been demonstrated that this strategy can be applied to the determination of PhT antipsychotic drugs in real samples.

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