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SYNTHESIS AND BIOLOGICAL ACTIVITY OF AMINOACYL PHENOTHIAZINE DERIVATIVES BASED ON THE ALKALOIDS CYTISINE, ANABASINE, AND *d*-PSEUDOEPHEDRINE

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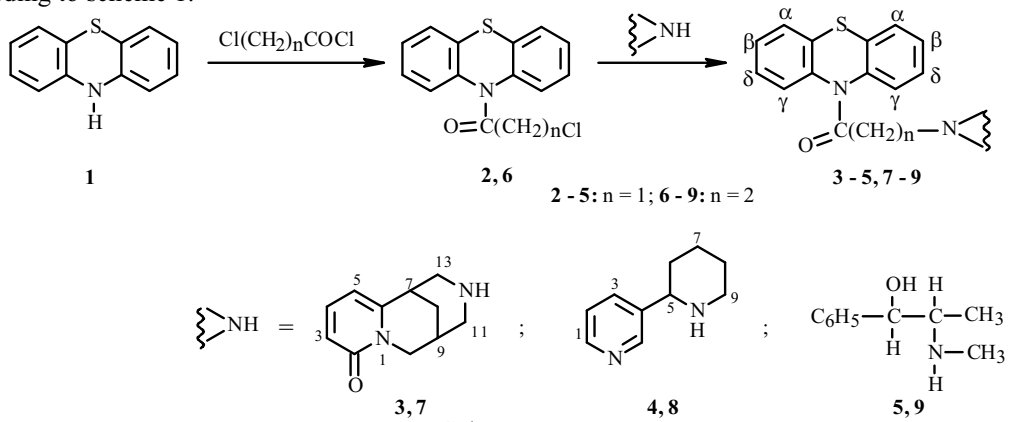
*10-(2-*N*-Cytisino-), (-anabasino-), and (-*d*-pseudoephedrino-)acetyl- and -propionylphenothiazines were synthesized by reaction of chloroacyl derivatives of phenothiazine with the alkaloids cytisine, anabasine, and* d*-pseudoephedrine. The compositions and structures of the products were confirmed by IR and PMR spectroscopy and mass spectrometry. One of several samples showed clearly pronounced antioxidant activity.*

Keywords: alkaloids, cytisine, anabasine, *d*-pseudoephedrine, phenothiazine, PMR spectroscopy, antioxidant activity.

The combination in a molecule of two and more pharmacophores is one of the principal approaches to the chemical design of new biologically active compounds, including natural alkaloids. Compounds with heterocyclic structural fragments are most numerous among all drugs [1]. Heterocyclic compounds containing S and N with a broad spectrum of biological activity occupy a special place among the many heterocycle derivatives. For example, phenothiazine (**1**) with a condensed tricyclic system is very valuable as an insecticide and antihelmintic drug [2]. Furthermore, phenothiazine, like many S-containing derivatives, has very low toxicity.

In the 1960s, compounds with high neuroleptic activity (aminazine, largactil) were found among 10-aminoalkylphenothiazine derivatives [3, 4]. This stimulated their synthesis and comprehensive study. Thus, 10-aminoacyl derivatives of phenothiazine were ineffective as neuroleptics, showed significant cholino- and adrenolytic activity, and exhibited pronounced anti-anginal and anti-arrhythmic activity [5]. Many phenothiazine derivatives are currently used widely in medical practice [6]. Definite trends in the relationship between the structure of substituents both on the N atom and in the cyclic system and the biological activity were found among over a thousand new phenothiazine compounds. 10-Aminopropionyl derivatives are known to be the most active whereas lengthening or shortening the acylalkyl chain decreases the activity [7]. However, despite the large number of synthesized phenothiazine derivatives, compounds combining in their structure the tricyclic phenothiazine nucleus and other physiologically active alkaloids as substituents have not been reported.

Therefore, a synthesis of previously unknown phenothiazine derivatives of certain alkaloids was developed and carried out according to scheme 1.



Scheme 1

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The starting phenothiazine (**1**) was synthesized as before [8]; chloroacetyl derivative **2**, by the literature method [9].

The alkaloids cytisine, anabasine, and *d*-pseudoephedrine were alkylated by 10-(2-chloroacetyl)phenothiazine in refluxing toluene in the presence of Et_3N. The products were purified by column chromatography (for **3**) and by conversion of the hydrochloride into the water-insoluble bases (for **4** and **5**).

The products **3**–**5** were white or gray crystalline compounds that were very soluble in most organic solvents except for saturated hydrocarbons.

10-(2-*N*-Cytisino-), (-anabasino-), and (-*d*-pseudoephedrino-)acetyl- and -propionylphenothiazines were also prepared for further studies of the structure–activity relationship because namely the 10-aminopropionyl phenothiazine derivatives exhibit high cholino- and adrenolytic activity in addition to anti-anginal and anti-arrythmic activity.

Products **7**–**9** were also white or gray crystalline compounds that melted lower than their acetyl analogs. They were soluble in most organic solvents except for saturated hydrocarbons.

IR spectra of all products showed a strong absorption band for the carbonyl (amide) at 1672–1650 cm^{-1} in addition to those for functional groups of the alkaloid fragments (hydroxyl at 3320–3280 for pseudoephedrine and carbonyl of the cytisine cyclic system at 1647).

Mass spectra of **3**, **4**, and **8** exhibited a molecular ion $[M]^+$ with intensity from 2 to 30%. Fragment ions with *m*/*z* and relative intensity I_{rel} (%), for example, for **4** at 175 (100), 199 (22), 132 (24), and 44 (58), and $>N–CH_2^+$ were assigned to the anabasine nucleus, phenothiazine, and other degradation products of the molecule.

PMR spectra of **3**–**5** and **7**–**9** showed phenothiazine protons as a doublet of triplets (multiplets) and doublets in the range 7.20–7.70 ppm and resonances for protons of the alkaloid fragments in their characteristic regions.

In order to determine the proposed biological activity of the synthesized phenothiazine derivatives, compounds **3** and **8** were selectively screened for antioxidant activity by the comprehensive study of an oxidant and the tested compound for total level of peroxide oxidation of lipids (POL) in an *in vitro* experiment using POL modeling by a yolk lipoproteide.

The tests found that **8** exhibited pronounced antioxidant activity (AOA = 8.10 ± 1.04%) whereas **3** had clearly pronounced antioxidant activity (AOA = 20.22 ± 2.3%) and was recommended for further *in vivo* tests.

EXPERIMENTAL

PMR spectra were recorded in DMSO-d_6 on a Bruker DRX500 spectrometer at operating frequency 500 MHz relative to TMS internal standard. IR spectra were taken in KBr disks on a Nicolet Avatar-320 Fourier-transform spectrometer. Mass spectra were obtained in a Finnigan Mat.Incos 50 instrument by direct sample introduction with ionization energy 70 eV. Melting points were determined on a Boetius stage. TLC analysis was performed on Sorbfil plates with development by iodine vapor. Elemental analyses of all compounds agreed with those calculated.

10-(2-*N*-Cytisinoacetyl)phenothiazine (3). A mixture of cytisine (0.95 g, 5 mmol) and 10-(2-chloroacetyl)phenothiazine (1.38 g, 5 mmol) in toluene (10 mL) was treated with Et_3N (1.01 g, 10 mmol) and refluxed for 3 h. The resulting solid triethylammonium chloride was filtered off. Solvent was distilled off. The oily residue was ground with petroleum ether. The product was purified by passage over a column of Al_2O_3:SiO_2 (1:1) with elution by benzene. Dilution of the resulting solution with a 3-fold excess of hexane precipitated a white crystalline solid that was filtered off to afford **3** (1.61 g, 75%), mp 99–100°C, $C_{25}H_{23}N_3O_2S$. Mass spectrum (EI, 70 eV, *m*/*z*, I_{rel}, %): 429 (29) $[M]^+$, 203 (100), 160 (27), 58 (98), 42 (44). PMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.68 (2H, dd, $J_{8,7}$ = 12.7, $J_{8,9}$ = 13.1, H-8), 2.32 (2H, m, H-11), 2.42 (1H, br.d, H-9), 2.73 (2H, m, H-13), 2.97 (1H, br.s, H-7), 3.17 (2H, dd, $J_{a,b}$ = 13.6, H-14), 3.66 (2H, m, H-10), 6.05 (1H, d, $J_{5,4}$ = 6.3, H-5), 6.23 (1H, d, $J_{3,4}$ = 9.5, H-3), 7.25 (4H, m, $2H_\beta$, $2H_\delta$), 7.35 (1H, dd, $J_{4,5}$ = 6.3, $J_{4,3}$ = 9.5, H-4), 7.47 (4H, m, $2H_\alpha$, $2H_\gamma$).

10-(2-*N*-Anabasinoacetyl)phenothiazine (4) was synthesized analogously to **3** from anabasine (0.81 g, 5 mmol), 10-(2-chloroacetyl)phenothiazine (1.38 g, 5 mmol), and Et_3N (1.01 g, 10 mmol). The toluene solution was washed with distilled water and extracted with HCl (10%). The acidic solution was diluted with water, clarified with activated charcoal, and neutralized with NH_4OH (25%) until neutral. The resulting precipitate was filtered off, washed with water, and dried in air to afford a light-beige powder (1.24 g, 62%), mp 75–76°C, $C_{24}H_{23}N_3OS$. Mass spectrum (EI, 70 eV, *m*/*z*, I_{rel}, %): 401 (2) $[M]^+$, 199 (25), 175 (100), 132 (22), 44 (57). PMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.20–1.70 (6H, m, H-6, H-7, H-8), 2.55 (2H, m, H-9), 2.94 (1H, t, $J_{5,6}$ = 9.5, H-5), 3.40 (2H, dd, $J_{a,b}$ = 11.5, H-14), 7.27 (1H, dd, $J_{2,1}$ = 4.5, $J_{2,3}$ = 3.5, H-2), 7.30 (4H, m, $2H_\beta$, $2H_\delta$), 7.47 (4H, m, $2H_\alpha$, $2H_\gamma$), 7.55 (1H, d, H-3), 8.33 (1H, s, H-4), 8.43 (1H, d, H-1).

10-(2-*N*-*d*-Pseudoephedrinoacetyl)phenothiazine (5) was synthesized analogously to **4** from *d*-pseudoephedrine (0.66 g, 4 mmol), 10-(2-chloroacetyl)phenothiazine (1.11 g, 4 mmol), and Et_3N (1.01 g, 10 mmol). The toluene solution was washed with distilled water and extracted with HCl (10%). The acidic solution was diluted with water, clarified with activated charcoal, and neutralized with NH_4OH (25%) until neutral. The resulting precipitate was filtered off, washed with water, and dried in air to afford a gray powder (1.13 g, 70.0%), mp 94–95°C, $C_{24}H_{24}N_2O_2S$. PMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.50 (3H, d, J = 7.0, C<u>H</u>$_3$–CH), 2.16 (3H, s, N–CH_3), 2.50 (1H, m, CHN), 3.53 (2H, dd, $J_{a,b}$ = 15.5, CH_2), 4.20 (1H, d, J = 8.5, C<u>H</u>(OH)), 4.88 (1H, s, OH), 7.24 (2H, t, J = 7.0, 2H$_\beta$), 7.30 (5H, m, H_{arom}), 7.41 (2H, t, J = 7.0, 2H$_\delta$), 7.57 (2H, d, J = 8.5, 2H$_\alpha$), 7.70 (2H, br.s, 2H$_\gamma$).

10-(3-*N*-Cytisinopropionyl)phenothiazine (7) was prepared analogously to **3** from cytisine (0.95 g, 5 mmol), 10-(3-chloropropionyl)phenothiazine (1.45 g, 5 mmol), and Et_3N (1.01 g, 10 mmol) to afford a white finely crystalline compound (1.59 g, 72.0%), mp 70–72°C, $C_{26}H_{25}N_3O_2S$. PMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.65 (2H, dd, $J_{8,7}$ = 11.8, $J_{8,9}$ = 12.7, H-8), 2.15 (2H, m, H-11), 2.30 (1H, br.d, H-9), 2.48 (4H, m, H-14, H-15), 2.66 (2H, m, H-13), 2.92 (1H, br.s, H-7), 3.65 (2H, m, H-10), 6.00 (1H, d, $J_{5,4}$ = 6.6, H-5), 6.13 (1H, d, $J_{3,4}$ = 9.5, H-3), 7.26 (1H, dd, $J_{4,3}$ = 9.5, H-4), 7.30, 7.36 (4H, dt, $J_{\beta\alpha} = J_{\delta\gamma}$ = 7.9, 2H$_\beta$, 2H$_\delta$), 7.48 (2H, br.d, 2H$_\alpha$), 7.54 (2H, d, J = 7.9, 2H$_\gamma$).

10-(3-*N*-Anabasinopropionyl)phenothiazine (8) was synthesized analogously to **4** from anabasine (0.65 g, 4 mmol), 10-(3-chloropropionyl)phenothiazine (1.16 g, 4 mmol), and Et_3N (1.01 g, 10 mmol) to afford a gray powder (1.11 g, 67%), mp 73–74°C, $C_{25}H_{25}N_3OS$. Mass spectrum (EI, 70 eV, *m*/*z*, I_{rel}, %): 415 (12) $[M]^+$, 199 (25), 175 (100), 161 (70), 132 (19), 44 (43). PMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.20–1.70 (6H, m, H-6, H-7, H-8), 1.92 (1H, t, $J_{14a,15}$ = 11.1, H-14a), 2.13 (1H, br.t, H-14b), 2.46 (2H, m, H-9), 2.56–2.75 (2H, m, H-15), 3.0 (1H, br.d, $J_{5,6}$ = 10.3, H-5), 7.25 (1H, m, H-2), 7.32, 7.40 (4H, dt, 2H$_\beta$, 2H$_\delta$), 7.55 (4H, m, 2H$_\alpha$, 2H$_\gamma$), 7.58 (1H, m, H-3), 8.36 (1H, s, H-4), 8.45 (1H, br.s, H-1).

10-(3-*N*-*d*-Pseudoephedrinopropionyl)phenothiazine (9) was synthesized analogously to **5** from *d*-pseudoephedrine (0.66 g, 4 mmol), 10-(3-chloropropionyl)phenothiazine (1.16 g, 4 mmol), and Et_3N (1.01 g, 10 mmol) to afford a powder (1.14 g, 68.0%), mp 69–70°C, $C_{25}H_{26}N_2O_2S$. PMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.54 (3H, d, J = 7.1, C<u>H</u>$_3$–CH), 2.15 (3H, s, N–CH_3), 2.55 (1H, m, CHN), 2.60 (4H, m, H-14, H-15), 4.35 (1H, d, J = 8.5, C<u>H</u>(OH)), 4.95 (1H, s, OH), 7.26 (2H, m, 2H$_\beta$), 7.28 (5H, m, H_{arom}), 7.42 (2H, t, J = 6.9, 2H$_\delta$), 7.55 (2H, d, J = 8.4, 2H$_\alpha$), 7.66 (2H, br.s, 2H$_\gamma$).

Antioxidant Activity of 3 for Level of POL *in vitro*. The *in vitro* experiment was conducted using a POL model with yolk lipoproteide. A solution of DMSO (0.4 mL), $FeCl_3$ (0.2 mL, $1\cdot10^{-4}$ M), ascorbic acid (0.2 mL, $5\cdot10^{-4}$ M), and **3** (0.1 mL, $1\cdot10^{-2}$ M) was treated with alcoholic phosphatidylcholine solution (0.01 mL, 10%), stirred vigorously for 2 min, and incubated at 37°C for 10, 15, 20, and 30 min. The reaction was stopped by cooling the solution and adding EDTA solution (0.2 mL, $1\cdot10^{-3}$ M). Phosphatidylcholine was removed from the reaction by adding $CHCl_3$:EtOH (1:1, 0.4 mL) and centrifuging for 1 min at 300 rpm. The upper layer (1 mL) was sampled, treated with thiobarbituric acid solution (1 mL, $5\cdot10^{-3}$ M) in acetic acid (10%), and heated for 15 min at 100°C. Optical density was measured at 530 nm. The control was **3** without the additives.

The study of the antioxidant activity of **3** on the level of POL gave AOA = 20.22 ± 2.3%.

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