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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 3,4-DIHYDROPYRIMIDIN-2-THIONE AMINOMETHYLENE DERIVATIVES BASED ON THE ALKALOID CYTISINE

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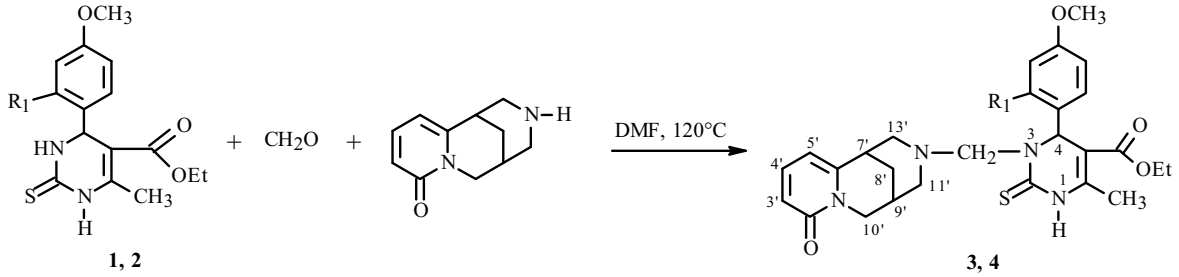
3,4-Dihydropyrimidin-2-thiones derivatized by the alkaloid cytisine were synthesized by Mannich aminomethylation. The compositions and structures of the products were confirmed by mass spectrometry and PMR and ^{1}H–^{1}H NOESY spectroscopy. One of the products showed pronounced antimicrobial activity.

Keywords: alkaloid cytisine, PMR and ^{1}H–^{1}H NOESY spectroscopy, antimicrobial activity.

Incorporation into the structure of plant alkaloids of other pharmacophores, including physiologically active heterocyclic compounds, many of which are analogs of natural nucleotides, is known to be a basic approach to the chemical design of new biologically active compounds. The alkaloid cytisine, which exhibits analeptic and antismoking activity, occupies a special place among a multitude of natural alkaloids [1]. Furthermore, the presence of a free secondary amine in cytisine facilitates the incorporation into its structure of various functional groups and heterocycles. The number of publications on the synthesis of heterocyclic *N*-derivatives of cytisine has increased in the last decade. Among these, compounds with types of biological activity that are uncharacteristic of cytisine itself are constantly being discovered. Until now many cytisine derivatives with various heterocylic groups such as coumarin [2, 3]; 1,2,3-triazole [4]; 1,2,4-thiadiazole [5]; 1,3-thiazoline [6]; 2,5-dimercapto-1,3,4-thiadiazole [7]; barbituric acid [8]; pyridine [9]; 1,4-dihydropyridine [10]; and phenothiazine [11] were prepared.

The Mannich reaction, which is widely used in organic chemistry to synthesize a variety of practically important compounds, is a convenient method for preparing new *N*-derivatives of cytisine in addition to the broadly used nucleophilic substitution and addition reactions of cytisine [2–7, 9–11].

The starting synthons for preparing new *N*-heterocyclic cytisine derivatives were 3,4-dihydropyrimidin-(1*H*)-2-thione derivatives, which were obtained via three-component condensation using a Biginelli reaction. The number of publications on the chemistry of 4-aryl-3,4-dihydropyrimidin-2-ones and 4-aryl-3,4-dihydropyrimidin-2-thiones has recently increased significantly in the scientific literature. This is due to not only their preparative availability but also the manifestation by them of a broad spectrum of pharmacological activity such as analgesic, antibacterial, antihypertensive, etc. [12–14].



1, 2 **3, 4**

R_1 = H (**1**, **3**), OCH_3 (**2**, **4**)

Because the starting 3,4-dihydropyrimidin-(1*H*)-2-thiones (**1** and **2**) had two reaction centers with nucleophilic N atoms (in the ring) and the S atom, which also exhibited a certain nucleophilicity and participated in possible thione–thiol tautomerism, it seemed interesting to us to study the possible involvement of these thiones in the Mannich synthesis as an

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N–H or S–H acidic component and the possibility of preparing from them Mannich mono- or *bis*-bases incorporating the pharmacologically important alkaloid. For this, the 3,4-dihydropyrimidin-(1*H*)-2-thiones (**1** and **2**) were derivatized by cytisine and aqueous formaldehyde solution (40%) using Mannich aminomethylation.

The reaction was carried out with heating of the starting materials in DMF solution at 120°C for 15–20 h with an excess of formalin and various thione:cytisine ratios (1:1, 1:1.5, 1:2). In every instance, TLC analysis showed the formation of a single product, acceptable yields of which after isolation from the reaction mixture were greatest for thione:cytisine ratios of 1:1.5 and 1:2.

It was supposed that both *N*- and *S*-aminomethylene derivatives of the Mannich bases formed. Furthermore, the possible formation of *bis*-bases with aminomethyl groups bonded to *N*(1)-, *N*(3)-, or an *N*-, *S*-dihydropyrimidine ring was also considered. It was also possible that the 3,4-dihydropyrimidin-(1*H*)-2-thione (**1** and **2**) did not undergo the aminomethylation reaction but a simple linking of two cytisine molecules to form dicytisinomethane, as reported previously [15].

Mass spectral analysis of **3** showed a weak molecular ion $[M]^+$ 508 (2%) that corresponded to the molecular weight of supposed structure **3** in addition to fragment ions at 306 (22), 277 (32), 233 (39), 203 (76), 58 (100), and 42 (59). Among these, the fragment ion with *m*/*z* 203 (76%) corresponded to the $>N–CH_2^+$ fragment of the cytisine framework.

PMR spectroscopy of the isolated products also showed the presence of protons from the starting 3,4-dihydropyrimidin-(1*H*)-2-thiones (**1** and **2**) and cytisine. Furthermore, an analysis of the PMR spectrum of **3** revealed a characteristic singlet for the C(4) –H proton of the dihydropyrimidine ring at 5.00 ppm, indicating a lack of coupling with the neighboring N(3)–H proton, in the presence of which, for example in the starting 3,4-dihydropyrimidin-(1*H*)-2-thiones (**1** and **2**), the C(4)–H resonance would be split into a doublet.

The free N(1) –H proton was observed as a singlet at 10.35 ppm. The aminomethylene protons of the $>NCH_2N<$ group were non-equivalent and appeared as two characteristic doublets in different spectral regions at 5.33 and 3.27 ppm with a difference of 2.06 ppm and the same SSCC $J_{a,b}$ = 11.7 Hz. This assignment of the aminomethylene protons was confirmed also by recording the 2D NOESY spectrum, which showed signals for coupled non-equivalent aminomethylene protons and a signal for coupling of the N(1)–H proton with the neighboring C(6)–CH_3 methyl.

Therefore, the PMR and $^1H–^1H$ NOESY spectra were consistent with N(3)-aminomethylation of the starting 3,4-dihydropyrimidin-(1*H*)-2-thiones (**1** and **2**).

Bioscreening of **3** for antimicrobial activity against strains of Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*, Gram-negative strains *Pseudomonas aeruginosa* and *Escherichia coli*, and yeast fungus *Candida albicans* was performed using the agar diffusion method (wells) in order to establish the biological activity of the synthesized derivatives (**3** and **4**). The reference drugs were gentamicin for bacteria and nystatin for the yeast fungus *C. albicans*. The bioscreening of **3** revealed its pronounced antibacterial activity only against the Gram-positive strains *S. aureus* and *B. subtilis* and weak activity against Gram-negative strains *P. aeruginosa* and *E. coli* in addition to the yeast fungus *C. albicans*.

EXPERIMENTAL

PMR spectra were recorded in DMSO-d_6 solution on a Bruker DRX500 spectrometer relative to TMS internal standard. Mass spectra were recorded on a Finnigan Mat.Incos 50 instrument at ionization energy 70 eV by direct sample introduction. Melting points were determined on a Boetius apparatus. TLC was performed on Sorbfil plates with detection by iodine vapor.

4-(4-Methoxyphenyl)-6-methyl-3-(*N*-cytisinomethyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic Acid Ethyl Ester (3). A solution of formalin (2.5 g, 40%) was treated with **1** (1.23 g, 4 mmol), diluted with DMF (10 mL), refluxed with stirring on a magnetic stirrer for 2 min, treated with cytisine (1.52 g, 8 mmol), heated for 25 h until **1** disappeared (TLC), cooled, and poured into a beaker with icewater (100 mL). The resulting gray precipitate was filtered off and washed several times with H_2O to afford a crude product (1.85 g, 90%). Two recrystallizations from benzene and benzene:hexane produced white needle-like crystals, mp 214–215°C. Elemental analysis of **3** agreed with the calculated formula $C_{27}H_{32}N_4O_4S$. Mass spectru (EI, 70 eV, *m*/*z*, I_{rel}, %): 508 (2) $[M]^+$, 306 (22), 277 (32), 233 (39), 203 (76), 58 (100), 42 (59).

PMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.02 (3H, t, J = 7.1, $CH_2C\underline{H}_3$), 1.73, 1.83 (2H, 2 br.d, J = 11.9, H-8′), 2.21 (3H, s, CH_3), 2.40 (1H, br.d, H-11′a), 2.46 (1H, br.s, H-9′), 2.54 (1H, br.d, H-13′a), 2.83 (2H, br.t, H-13′e, 11′e), 3.02 (1H, br.s, H-7′), 3.27 (1H, d, J = 11.7, N–CH_b), 3.71 (3H, s, OCH_3), 3.80 (1H, dd, J = 6.83, 14.91, H-10′a), 3.94 (1H, d, $J_{10'e,10'a}$ = 15.0, H-10′e), 3.97 (2H, q, $C\underline{H}_2CH_3$), 4.99 (1H, s, H-4), 5.33 (1H, d, J = 11.7, N–CH_a), 6.04 (1H, d, $J_{5',4'}$ = 6.90,

H-5′), 6.15 (1H, d, $J_{3',4'}$ = 8.90, H-3′), 6.86 (2H, d, J = 8.67, 2H–Ar), 7.00 (2H, d, J = 8.66, 2H–Ar), 7.23 (1H, dd, $J_{4',5'}$ = 6.90, $J_{4',3'}$ = 8.90, H-4′), 10.35 [1H, s, N(1) –H].

4-(2,4-Dimethoxyphenyl)-6-methyl-3-(*N*-cytisinomethyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl ester (4) was prepared by an analogous method in 70% yield, mp 227-228°C (benzene). Elemental analysis of **4** agreed with the calculated formula $C_{28}H_{34}N_4O_5S$.

PMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.96 (3H, t, J = 7.08, $CH_2C\underline{H}_3$), 1.73, 1.84 (2H, 2 br.d, J = 12.50, H-8′), 2.22 (3H, s, CH_3), 2.41 (1H, br.d, H-11′a), 2.47 (1H, br.s, H-9′), 2.57 (1H, br.d, H-13′a), 2.84 (2H, br.t, H-13′e, 11′e), 3.01 (1H, br.s, H-7′), 3.24 (1H, d, J = 11.48, N–CH_b), 3.72 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 3.82 (1H, m, H-10′a), 3.86 (2H, q, $C\underline{H}_2CH_3$), 3.96 (1H, d, $J_{10'e,10'a}$ = 15.59, H-10′e), 5.29 (1H, d, J = 11.44, N–CH_a), 5.44 (1H, s, H-4), 6.00 (1H, d, $J_{5',4'}$ = 6.88, H-5′), 6.09 (1H, d, $J_{3',4'}$ = 9.00, H-3′), 6.44 (1H, d, J = 8.40, 1H–Ar), 6.49 (1H, s, 1H–Ar), 6.92 (1H, d, J = 8.47, 1H–Ar), 7.19 (1H, dd, $J_{4',5'}$ = 6.88, $J_{4',3'}$ = 9.00, H-4′), 10.19 [1H, s, N(1) –H].

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