The study of the neurotropic activity of the pyrrolopyrimidin-4-ones rearrangement products under the action of phosphorus oxychloride

Aim. To synthesize the annelated 4-aminopyridines and study the biological activity of one of products.

Results and discussion. In the laboratory of the Research Institute of Biomedical Problems of the Dnipropetrovsk Medical Academy the studies of the effect of 2,3,3-trimethyl-2,3,5,6,7,8-hexahydro-1H-pyrrolo[3,4-b]quinolin-9-amine on the neuroactivity in the "open field" model have been conducted. According to the results of the experiment it has been found that in two hours after the administration of the oil solution of the compound the indices of the motor activity of mice are significantly reduced.

Experimental part. 2,3,3-Trimethyl-2,3,5,6,7,8-hexahydro-1H-pyrrolo[3,4-b]quinolin-9-amine and 2,3,6,7,7-pentamethyl-6,7-dihydro-5H-pyrrolo[3,4-b]pyridin-4-amine were obtained by the rearrangement of the corresponding pyrrolopyrimidin-4-ones under the action of the excess of phosphorus oxychloride in toluene. The initial pyrrolopyrimidin-4-ones were synthesized by the condensation of 4-amino-1,2,2-trimethyl-2,5-dihydro-1H-pyrrole-3-carbonitrile with ketones. The structure of all compounds obtained was confirmed by 1H NMR-spectroscopy, mass spectrometry and elemental analysis.

Conclusions. The neurotropic activity has been detected for the oil solution of 2,3,3-trimethyl-2,3,5,6,7,8-hexahydro-1H-pyrrolo[3,4-b]quinolin-9-amine on the "open field" model. It has been found that the aqueous solution of this compound does not exhibit the neurotropic activity regardless of the administered dose. Taking into account the presence of the neurotropic activity further research in this field is a promising way to search novel bioactive molecules among 4-aminopyridine derivatives, which are structural analogs of the drug Tacrine.

Key words: rearrangement; pyrrolopyrimidin-4-ones; aminohydroacridines; neurotropic activity; Tacrine
One of the most promising approaches to the creation of new drugs is the synthesis of molecules close in structure to those natural compounds that play a key role in certain biochemical processes. To a large extent, this applies to various condensed systems with a pyridine nucleus, for example, such as derivatives of acridine and quinoline. Research in this area is carried out mainly in two directions: modification of the pyridine skeleton with pharmacophore fragments or annulation of new heteronuclei to the pyridine ring.

In medical practice, for the treatment of Alzheimer’s disease, 1,2,3,4-tetrahydroacridine-9-amine is used, a vivid representative of the condensed system with the pyridine core, it is better known as the drug Tacrine [1]. However, along with the effectiveness of the drug, a strongly pronounced hepatotoxic effect is observed, which forces us to conduct new studies in this area. In neurology, more attention is paid to the concept of mixed dementia, according to which both the neurodegenerative and ischemic components are present in the development of vascular and neurodegenerative cognitive impairment. In Alzheimer’s disease, concomitant vascular pathology leads to the formation of a more pronounced cognitive deficiency and intensification of the degenerative process. One of the directions of the treatment may be a decrease in the presence of these metabolic cerebral disorders [2]. In this regard, the efforts of many researchers are aimed at finding analogs of Tacrine among derivatives of acridines, which would have a higher pharmacological activity with low toxicity [3–5].

Earlier, we proposed the one-stage method for the synthesis of hard-to-reach derivatives of hydroacridines [6] – structural analogs of Tacrine, and also studied their reactivity [7–10]. Taking into account the prospects of this area, the aim of our work is the synthesis of compounds that are structural analogs of Tacrine and the study of the biological activity of one of them.

2,2-Disubstituted pyrimidin-4-ones 3a–d were selected as model compounds. Compounds 3a–d were synthesized by condensation of 4-amino-1,2,2-trimethyl-2,5-dihydro-1H-pyrrole-3-carbonitrile 2 with ketones. Enaminonitrile 2 was obtained by Torp–Ziegler cyclization of dinitrile 1 [11] with a good yield when using sodium isopropylate as a catalyst, in contrast to the published data where stronger bases were used for such cyclizations (Scheme 1) [12–15].

The structure of the compounds synthesized was confirmed by 1H NMR-spectroscopy and mass spectrometry, as well as by elemental analysis. The signals of amide NH (6.68 ppm) and amine NH (6.53 ppm) protons in 1H NMR-spectra of compounds 3a–d were characteristic for the derivatives of 2,2-disubstituted pyrimidin-4-ones [16].

The rearrangement of substituted pyrimidin-4-ones under the action of acid agents leading to the formation of bisannelated ones was previously described in literature [17–21]. The compounds containing the 4-aminopyridine cycle, like 4-aminopyridine itself, are modulators of ion channels. These compounds stimulate the formation of acetylcholine, its synthesis in the body is necessary for the treatment of Alzheimer’s disease [22].

From the analysis of the published data, it follows that overcoming the energy barrier to the opening of
the pyrimidine cycle occurs at high temperatures in the strong acid medium [21]. Similar reaction conditions became the basis of our synthetic procedure. To expand the range of bisannelated 4-aminopyridines and study their biological activity pyrrolopyrimidin-4-ones 3a,c were rearranged with an excess of POCl₃ by boiling in toluene. As a result of the reaction, the expected aneled 4-aminopyridines 4a,c were isolated (Scheme 2).

In the case of compound 4a we proposed the following scheme for the mechanism of this reaction (Scheme 3).

The prospect of substituted 4-aminopyridines as biologically active substances prompted us to test compounds 4a for the presence of the neuroactivity. In the laboratory of the Research Institute of Biomedical Problems of the Dnipropetrovsk Medical Academy under the supervision of prof. Drozdov O.L. the studies of the effect of compound 4a on the neuroactivity in the “open field” model were conducted. The experiment was carried out on 20 white adult mice weighing 150.0–200.0 g. The substance was administered in the form of the oil solution in the doses of 10, 20, 100 mg/kg. Compound 4a was administered to mice intraperitonea]lly on an empty stomach. Before the introduction of the compound solution the “open field” test was performed for each group of mice to determine the baseline. After that mice were injected with the oil solution of compound 4a in an equivalent dose, and the “open field” test was performed in 2 hours after the administration. A day later, the group of mice was re-tested to record the dynamics of the indicators. The behavior indicators of mice were changed on a setup of 100×100 cm in size with a distance between false burrows of 10 cm. The data obtained were mathematically processed using Student’s t-test.

The number of crossed squares for 2 minutes was indicative of the horizontal motor activity (HMA), the number of lifts on the hind legs was indicative of the vertical motor activity (VMA), the number of acts of defecation boluses (ADB) was indicative of the emotional reactivity, the number of burrows examined (NBE) was indicative of the unconditioned reflex activity. Along with these indicators, the continuation of grooming (Gr.) in seconds was recorded during testing. The test results are presented in Table and Fig.

According to the results of the experiment it was found that in two hours after the administration of the oil solution of compound 4a the indices of the motor activity of mice, namely horizontal and vertical, were significantly reduced. The percent changes in the parameters were the same, regardless of the dose used. It was found that an hour after the administration of the aqueous solution of compound 4a the indices of the motor activity did not change.

**Experimental part**

^1^H NMR-spectra were obtained on a Bruker Avance II 400 spectrometer in DMSO-d₆ with TMS as an internal standard. Mass spectra (FAB ionization) were registered on a VG-7070 spectrometer. Ion desorption from m-nitrobenzyl alcohol was done by a beam of argon atoms with an energy of 8 keV. Mass spectra (EI ionization, 70 eV) for compounds 3c and 4a were recorded on a MX1321 apparatus with direct sample injection at 200°C of ionization chamber temperature. Elemental analysis was performed on a LECO CHN-900 elemental analyzer. Melting points were determined on an Electrothermal 9100 digital apparatus. Monitoring of the reaction progress and assessment of
the purity of the compounds synthesized was done by TLC on Silica gel 60 F254 plates (Merck), the eluent – CHCl₃–iPrOH (10:1), visualization in the iodine chamber. Compounds 1 and 2 were obtained according to the literature methods [11].

6',7',7'-Trimethyl-1',5',6',7'-tetrahydrospiro-
[cyclohexane-1,2'-pyrrolo[3,4-d]pyrimidin]-4'-(3'H)-one 3a. Dissolve enamionitrile 2 (0.01 mol) in cyclohexanone (0.015 mol), and add 5 mL of 2 M solution of NaOH/MeOH. Heat the reaction mixture

| Series of observations | Static indicators | HMA | VMA | Gr. | NBE | ADB |
|------------------------|-------------------|-----|-----|-----|-----|-----|
| 1 The study background | M                  | 40.40 | 7.40 | 0.20 | 4.33 | 1.20 |
|                        | ±m                 | 7.65 | 0.51 | 0.20 | 0.61 | 0.80 |
| 2 Two hours after injection; the dose of 10 mg/kg | M                  | 14.40 | 2.60 | 6.60 | 1.00 | 0.40 |
|                        | ±m                 | 4.85 | 1.03 | 3.74 | 0.32 | 0.40 |
|                        | % measurement     | -64.36% | -64.87% | 32.00% | -76.91% | -66.67% |
| 3 The study background | M                  | 39.40 | 8.00 | 2.80 | 3.20 | 1.60 |
|                        | ±m                 | 4.87 | 1.14 | 0.86 | 0.80 | 0.51 |
| 4 Two hours after injection; the dose of 20 mg/kg | M                  | 21.20 | 4.40 | 0.40 | 1.60 | 0.80 |
|                        | ±m                 | 5.88 | 1.21 | 0.40 | 0.51 | 0.00 |
|                        | % measurement     | -46.19% | -45.00% | -85.71% | -50.00% | -50.00% |
| 5 The study background | M                  | 29.60 | 6.50 | 6.20 | 2.80 | 0.90 |
|                        | ±m                 | 4.17 | 1.15 | 2.38 | 1.06 | 0.28 |
| 6 Two hours after injection; the dose of 100 mg/kg | M                  | 12.90 | 2.10 | 4.30 | 1.80 | 0.30 |
|                        | ±m                 | 2.23 | 0.57 | 2.59 | 0.61 | 0.15 |
|                        | % measurement     | -56.42% | -67.69% | -30.65% | -35.71% | -66.67% |

Fig. The plot of the effect of compound 4a in the form of the oil solution on mice

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under reflux for 20 min. Then pour the reaction mixture onto ice, filter the precipitate and recrystallize from acetonitrile. A colorless powder. Yield – 78%. M.p. 185–190°C. Anal. Calcd. for C₇H₆N₂O₆: C 67.43, H 9.30, N 16.85. Found, %: C 67.61, H 9.19, N 16.98. ¹H NMR (400 MHz, DMSO-d₆), δ, ppm: 6.68 (1H, s, CONH), 6.53 (1H, s, NH), 3.26 (2H, s, CH₂), 2.18 (3H, s, N–CH₃). 1.25–1.72 (9H, m, 4CH₂OCH₃), 1.06 (6H, s, 2CH₂). 0.97–1.04 (1H, m, CH₃). Mass spectrum (FAB), m/z (I, %): 252 [M+H]⁺ (100).

2,2,6,7,7-Pentamethyl-1,2,3,5,6,7-hexahydro-4H-pyrrolo[3,4-d]pyrimidin-4-one 3b. Dissolve enaminonitrile 2 (0.01 mol) in a excess of acetonitrile (15 mL), then add a catalyst – 5 mL of 2 M NaOH aqueous solution. Allow the mixture to stand at room temperature for 3–4 days, filter the crystals formed and recrystallize from methanol or acetonitrile. A white powder. Yield – 74%. M. p. 265–267°C. Anal. Calcd. for C₂₈H₂₅N₃O: C 72.69, H 9.15, N 18.16. Found, %: C 72.83, H 9.28, N 18.05. ¹H NMR (400 MHz, DMSO-d₆), δ, ppm: 6.81 (1H, s, CONH), 6.69 (1H, s, NH), 3.27 (2H, s, CH₂), 2.19 (3H, s, N–CH₃). 1.31 (6H, s, 2CH₂). 1.03 (6H, s, 2CH₂). Mass spectrum (FAB), m/z (I, %): 210 [M+H]⁺ (100).

The synthesis of compounds 3c,d. Dissolve enaminonitrile 2 (0.01 mol) in an excess of 15 mL of the appropriate ketone, then add a catalyst – 5 mL of 2 M KOH solution in methyl alcohol. Boil the mixture for 3 h and pour onto ice, filter the crystals formed and recrystallize from methanol.

2-Ethyl-2,6,7,7-tetramethyl-1,2,3,5,6,7-hexahydro-4H-pyrrolo[3,4-d]pyrimidin-4-one 3c. A white powder. Yield – 89%. M. p. 200–202°C. Anal. Calcd. for C₂₄H₂₃N₃O: C 64.54, H 9.48, N 18.82. Found, %: C 64.67, H 9.60, N 18.65. ¹H NMR (400 MHz, DMSO-d₆), δ, ppm: 6.70 (1H, s, CONH), 6.59 (1H, s, NH), 3.26 (2H, s, CH₂), 2.19 (3H, s, N–CH₃). 1.59 (2H, q, J = 7.0 Hz, CH₂CH₂), 1.26 (3H, s, CH₃). 1.04 (6H, s, 2CH₂). 0.81 (3H, t, J = 7.0 Hz, CH₃CH₂). Mass spectrum (EI), m/z (I, %): 223 [M]+ (20).

2-Butyl-2,6,7,7-tetramethyl-1,2,3,5,6,7-hexahydro-4H-pyrrolo[3,4-d]pyrimidin-4-one 3d. A white powder. Yield – 20%. M. p. 200–202°C. Anal. Calcd. for C₂₅H₂₅N₃O: C 66.89, H 10.02, N 16.72. Found, %: C 66.93, H 10.22, N 16.53. ¹H NMR (400 MHz, DMSO-d₆), δ, ppm: 6.73 (1H, s, CONH), 6.58 (1H, s, NH), 3.27 (2H, s, N–CH₃). 2.21 (3H, s, N–CH₃). 1.22–1.64 (6H, m, 3CH₂). 1.25 (3H, s, CH₃). 1.04 (6H, s, 2CH₂). 0.82 (3H, t, J = 7.0 Hz, CH₃). Mass spectrum (FAB), m/z (I, %): 252 [M+H]⁺ (100).

The general procedure for preparation of compounds 4a-c. In a round bottom flask mix 0.01 mol of the corresponding pyrrolypyrimidine–4-one 3 and 0.04 mol of POCl₃ in 50 mL of toluene. Heat the reaction mixture for 3 h. After cooling decant the toluene layer and add aqueous methanol to dissolve the precipitate. After neutralizing the aqueous 15% NaOH solution to pH = 9–10, a white precipitate is formed, which is further recrystallized from methanol.

2,3,3-Trimethyl-2,3,5,6,7,8-hexahydro-1H-pyrrolo[3,4-b]quinolin-9-amine 4a. A white powder. Yield – 67%. M. p. 155–157°C. Anal. Calcd. for C₂₁H₁₉N₃O: C 72.69, H 9.15, N 18.16. Found, %: C 72.83, H 9.28, N 18.05. ¹H NMR (400 MHz, DMSO-d₆), δ, ppm: 5.43 (2H, br s, NH₂), 3.57 (2H, s, N–CH₂), 2.61 (3H, s, N–CH₃). 2.22–2.42 (4H, m, 2CH₂). 1.55–1.83 (4H, m, 2CH₂). 1.04 (6H, s, 2CH₂). Mass spectrum (EI), m/z (I, %): 231 [M]+ (2), 216 [M–CH₃]+ (100).

2,3,6,7,7-Pentamethyl-6,7-dihydro-5H-pyrrolo[3,4-b]pyridin-4-amine 4c. A white powder. Yield – 45%. M. p. 170–172°C. Anal. Calcd. for C₂₃H₂₅N₃O: C 70.20, H 9.33, N 20.47. Found, %: C 70.32, H 9.19, N 20.38. ¹H NMR (400 MHz, DMSO-d₆), δ, ppm: 5.52 (2H, br s, NH₂), 3.64 (2H, s, CH₂), 2.37 (3H, s, N–CH₃). 2.34 (3H, s, CH₃), 1.97 (3H, s, CH₃), 1.15 (6H, s, 2CH₂). Mass spectrum (FAB), m/z (I, %): 206 [M+H]⁺ (100).

Conclusions

As a result of the interaction of 4-amino-1,2,2-trimethyl-2,5-dihydro-1H-pyrrole-3-carbonitrile and ketones, 2,2-disubstituted 6,7,7-trimethyl-1,2-pyrolo[3,4-d]pyrimidine–4(3H)-ones have been synthesized. It has been found that 6,7,7-trimethylpyrrolo[3,4-d]-pyrimidine–4(3H)-ones undergo the rearrangement under the action of POCl₃ to form pyrrolo[3,4-b]quinolin (pyridine) derivatives. The results of the “open field” test have shown that the oil solution of acridine 4a exhibits the neurotropic activity, which is independent of the volume of the administered dose.

Conflict of interests: authors have no conflict of interests to declare.

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