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SAMARQAND DAVLAT UNIVERSITETI

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SYNTHESIS OF 2-METHYL-PYRAZOLO [3,4-d]PYRIMIDINONES

F.Ruzieva[1], D.Turgunov[1], Kh. Bozorov[1,2]
[1]Samarkand State University named after Sharof Rashidov, Samarkand, Uzbekistan.
[2]State Key Laboratory Basis of Xinjiang Indigenous Medicinal Plants Resource Utilization, Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, South Beijing Rd 40-1, Urumqi, 830011, P.R. China.

F.Ruzieva Samarkand State University named after Sharof Rashidov, Samarkand, Uzbekistan.

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Abstract. Current research in the field of medicinal and organic chemistry is focused on the study of heterocyclic synthons, which are the basis of complex organic molecules with biological activity. In this study, tri-, tetra-, and pentamethylenepyrazolo[3,4-*d*]pyrimidinones were synthesized from methyl 3-amino-1-methyl-1*H*-pyrazole-4-carboxylate in one-step cyclocondensation reactions, resulting in the synthesis of three new compounds. The results of molecular docking of the obtained compounds showed that they have a strong inhibitory potential against target proteins associated with neurodegenerative diseases. These results may serve as an important scientific basis for the development of new drug candidates for the treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's in the future.

Keywords: pyrazolo[3,4-*d*]pyrimidinone, $POCl_3$, cyclocondensation, lactams, 1,4-dioxane.

2-metil-pirazolo[3,4-*d*]pirimidinonlarning sintezi

Abstract. Tibbiy va organik kimyo sohasidagi dolzarb tadqiqotlar biologik faol bo‘lgan murakkab organik molekulalarning asosini tashkil etuvchi geterosiklik sintonlarni o‘rganishga qaratilgan. Mazkur tadqiqotda metil 3-amino-1-metil-1*H*-pirazol-4-karboksilatdan bir bosqichda siklokondensatsiya reaksiyalari asosida tri-, tetra- va pentametilenpirazolo[3,4-*d*]pirimidinonlar sintez qilinib, uchta yangi birikmalar olindi. Olingan birikmalarning molekulyar doking natijalari ularning neyrodegenerativ kasalliklar bilan bog‘liq nishon oqsillarga nisbatan kuchli ingibitorlik potensialiga ega ekanligini ko‘rsatdi. Ushbu natijalar kelajakda Alsgeymer va Parkinson kabi neyrodegenerativ kasalliklarini davolash uchun yangi dori nomzodlarini yaratishda muhim ilmiy asos bo‘lib xizmat qilishi mumkin.

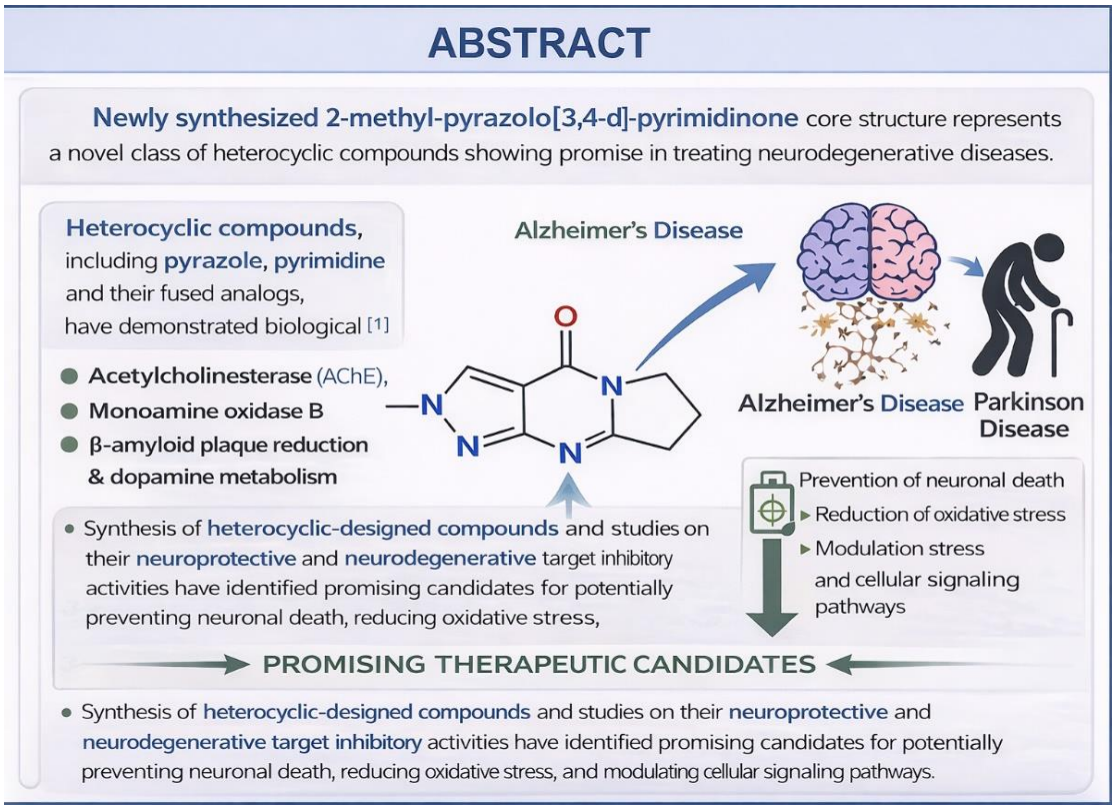
Kalit so‘zlar: pirazolo[3,4-*d*]pirimidinon, $POCl_3$, siklokondensatsiya, laktamlar, 1,4-dioksan.

Синтез 2-метил-пиразоло[3,4-*d*]пиримидинонов

Аннотация. Современные исследования в области медицинской и органической химии сосредоточены на изучении гетероциклических синтонов, которые лежат в основе сложных органических молекул с биологической активностью. В данном исследовании три-, тетра-, пентаметиленпиразоло[3,4-*d*]пиримидиноны были синтезированы из метил-3-амино-1-метил-1*H*-пиразол-4-карбоксилата в одностадийных реакциях циклоконденсации, в результате чего были получены три но-

вых соединения. Результаты молекулярного докинга полученных соединений показали, что они обладают сильным ингибирующим потенциалом в отношении целевых белков, связанных с нейродегенеративными заболеваниями. Эти результаты могут послужить важной научной основой для разработки новых лекарственных препаратов для лечения нейродегенеративных заболеваний, таких как болезнь Альцгеймера и болезнь Паркинсона, в будущем.

Ключевые слова: пиразоло[3,4-*d*]пиримидинон, $POCl_3$, циклоконденсация, лактамы, 1,4-диоксан.

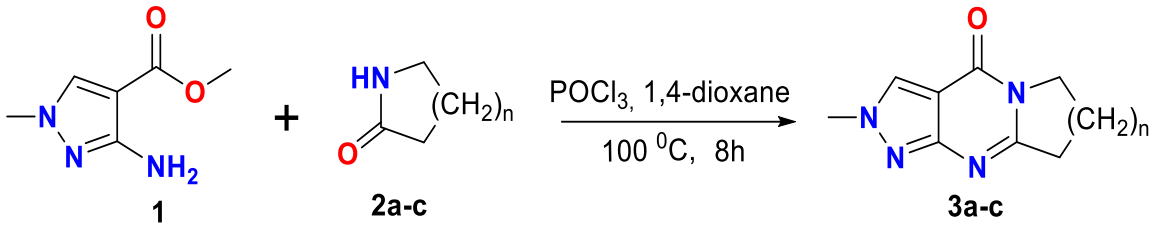


Introduction. Pyrazolo[3,4-*d*]pyrimidines are heterocyclic compounds that have attracted great interest in pharmaceutical chemistry and biological research [1]. This skeleton is the main source for various biological activities, including antitumor, antimicrobial, anti-inflammatory, kinase inhibitory, and other enzymatic activities [2]. Therefore, pyrazolo[3,4-*d*]pyrimidine compounds are widely used not only in the design of new drug molecules, but also in drug design and SAR analysis [3]. Many methods have been developed for the synthesis of heterocyclic rings. Synthesis using conventional Vilsmeier-Haack reagents yields 1-aryl pyrazolo[3,4-*d*]pyrimidinones in high yields [4]. However, several modern methods using $POCl_3$ allow for rapid, high-yield synthesis of the pyrazolo[3,4-*d*]pyrimidine nucleus [5]. This significantly facilitates rapid drug synthesis and preparation of new molecules in laboratories. Recent studies have also paid special attention to the biological evaluation of pyrazolo[3,4-*d*]pyrimidine derivatives. They have identified the antitumor activity of pyrazolo[3,4-*d*]pyrimidine derivatives as VEGFR-2 inhibitors and confirmed the efficacy of newly designed molecules through molecular dynamics analysis and biological validation [6]. However, subsequent studies have shown the antitumor and antimicrobial potential of new derivatives [7]. Pyrazolo[3,4-*d*]pyrimidines [8] have been extensively studied not only as kinase inhibitors, but also as inhibitors of EGFR-TK [9], PDE-5, CDK2, Bcr-Abl, and other kinases [10, 11]. SAR (structure-activity relationship) [12] analyses of the effects of structural modifications and substitutions on biological activity are an important tool in improving the efficacy of molecules [13]. These assays allow for the prediction and optimization of the activity profile of newly designed

derivatives. Pyrazolo[3,4-*d*]pyrimidine derivatives are also being investigated for their potential as dual kinase inhibitors and to overcome the problems of multidrug resistance (MDR) [3]. This further increases their importance in modern drug design, SAR analysis, and pharmacological research.

Thus, existing studies on the synthesis, biological activity, and structural analysis of pyrazolo[3,4-*d*]pyrimidines provide a solid scientific basis for new synthetic routes, tested derivatives, and modern methodologies [13].

Research methodology. New 2-methyl-pyrazolo[3,4-*d*]pyrimidinones were synthesized by the condensation reaction between 3-amino-1-methyl-1*H*-pyrazole-4-carboxylate (1) and the corresponding lactams (2a-c). This synthesis was carried out in the presence of phosphorus oxychloride and 1,4-dioxane at 100 °C for 8 h. The reaction resulted in the formation of compounds 3a-c. The resulting compounds were studied in the solvent system ethyl acetate/petroleum ether=3:1 and separated by column chromatography (Scheme 1) [14-16].



Scheme 1

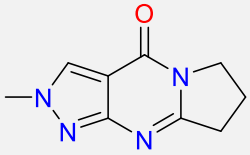
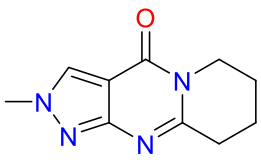
To optimize the cyclization process, the reaction was carried out in different solvents and time intervals. The results of the optimization of the reaction conditions are presented below (Table 1). The highest yield (62 %) of the product was obtained in the reaction carried out in 1,4-dioxane solvent at 100 °C for 8 h.

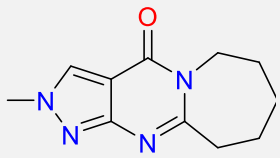
Table 1. Optimization of reaction conditions for compound 3a.

№	Cyclic reagent	Solvent	Temperature °C	Time	Yield %
1.	$POCl_3$	DCE	85 °C	8 h	22 %
2.	$POCl_3$	toluene	110 °C	8 h	13 %
4.	$POCl_3$	1,4-oxane	100 °C	8 h	62 %

The following table lists the main physicochemical parameters, efficiencies, thin-layer chromatography Rf values, and melting points (MP) of the synthesized compounds 3a–c (Table 2).

Table 2. Physical quantities of the synthesized compounds.

№		Structure	Yield %)	R_f	Melting point
1.	**3a**		55 %	EA/PE=3: 1, Rf=0.3	232-233 °C
2.	**3b**		61 %	EA/PE=3: 1, Rf=0.42	235-236 °C

3.	**3c**		62 %	EA/PE=3:1, Rf=0.48	194-195 C

Analysis results. *General procedure for the synthesis of compounds 3a.*

3-Amino-1-methyl-1*H*-pyrazole-4-carboxylate (1) (1 mmol) was dissolved in anhydrous 1,4-dioxane (5 mL). The corresponding lactams (2a-c) were added to the resulting solution. Then, the cyclizing reagent phosphorus oxychloride ($POCl_3$) (1.5 mmol) was added dropwise and heated at 80 °C for 8 h. TLC was completely monitored during the reaction. Then water was added to the mixture, and the mixture was worked up to pH=7. It was extracted with ethyl acetate (25 x 3 mL), dried over anhydrous sodium sulfate. The reaction gave product 3a. The resulting product was purified by column chromatography.

2-methyl-2,6,7,8-tetrahydro-4*H*-pyrazolo[3,4-*d*]pyrrolo[1,2-a]pyrimidin-4-one (3a). Yield 55%, white solid, m.p: 232-233 °C. Rf=0.3, EA/PE=3:1. ^{1}H YaMR (400 MHz, $CDCl_3$) δ 7.97 (s, 1H), 4.10 (t, 2H), 4.04 (s, 3H), 3.12 (t, *J* = 8.0 Hz, 2H), 2.30 – 2.18 (m, 2H).^{13}C YaMR (100 MHz, $CDCl_3$) δ 162.18, 160.64, 158.42, 128.31, 106.10, 46.01, 40.66, 32.48, 19.92. HRMS (ESI) calcd for $C_9H_{10}N_4O$ $[M+H]^+$ 191.0855, found 191.0922.

2-methyl-6,7,8,9-tetrahydropyrazolo[3,4-*d*]pyrido[1,2-a]pyrimidin-4(2*H*)-one (3b). Yield 61%, white solid, m.p: 235-236 °C. Rf=0.42, EA/PE=3:1. ^{1}H YaMR (400 MHz, $CDCl_3$) δ 7.91 (s, 1H), 3.98 (s, 3H), 3.95 (t, *J* = 6.0 Hz, 2H), 2.89 (t, *J* = 6.6 Hz, 2H), 1.93 – 1.81 (m, 4H). ^{13}C YaMR (100 MHz, $CDCl_3$) δ 159.48, 158.41, 157.56, 128.39, 105.94, 41.32, 40.54, 32.17, 22.28, 19.30. HRMS (ESI): calcd for $C_{10}H_{12}N_4O$ $[M + H]^+$: 205,1084, found 205.1076.

2-methyl-2,6,7,8,9,10-hexahydro-4*H*-pyrazolo[3',4':4,5]pyrimido[1,2-a]azepin-4-one (3c). Yield 62%, white solid, m.p: 194-195 °C. Rf=0.48, EA/PE=3:1. ^{1}H YaMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H), 4.27 – 4.20 (m, 2H), 3.98 (s, 3H), 3.02 – 2.95 (m, 2H), 1.88 – 1.60 (m, 6H). ^{13}C YaMR (100 MHz, $CDCl_3$) δ 162.15, 159.35, 158.46, 128.73, 105.62, 42.15, 40.53, 37.94, 29.57, 28.43, 25.54. HRMS (ESI) calcd for $C_{11}H_{14}N_4O$ $[M+H]^+$ 219.1168, found 219.1234.

Conclusion. In this study, the efficiency of the condensation-based cyclization method for the preparation of 2-methyl-pyrazolo[3,4-*d*]pyrimidinone derivatives was evaluated. During the optimization of the reaction conditions, it was found that the solvent environment, temperature, and reaction time significantly affect the product yield. Under the studied conditions, compound 3a was obtained with an efficiency of 62%. The results of thin-layer chromatography showed that the obtained compounds have individual properties. Considering the relevance of this class in medicinal chemistry, the obtained compounds may serve as promising candidates for enzyme inhibitors in the future, particularly against biological targets associated with neurodegenerative diseases.

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