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THE SYNTHESIS OF NEW BIOLOGICALLY ACTIVE SUBSTANCES AMONG 4-AMINO-5-ALKYL-1,2,4-TRIAZOLE(4H)-3-YL-THIOACETANILIDES AND THEIR CHEMICAL MODIFICATION

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Key words: 3-mercapto-4-amino-5-alkyl-1,2,4-triazole; derivatives; synthesis; alkylation; Paal-Knorr reaction; antitumor activity

The synthesis of a series of new 4-amino-5-alkyl-1,2,4-triazole(4H)-3-yl thioacetanilides and 4-pyrrolyl-5-alkyl-1,2,4-triazole(4H)-3-yl thioacetanilides has been described in the article. The key intermediates – 4-amino-5-alkyl-3-mercapto-1,2,4-triazoles(4H) 4a-c were synthesized started from the corresponding carboxylic acids (acetic, propanoic and butanoic) after their esterification followed by hydrazinolysis, CS₂ reaction and cyclisation under hydrazine hydrate. The first group of substances 6a-p was obtained by alkylation of the key intermediate 4a-c with chloroacetic acid anilides in the presence of basic catalysts. The subsequent modification under conditions of Paal-Knorr reaction led to the corresponding pyrrolyl derivatives 7a-p. The structure of the compounds synthesized has been proven by the data of elemental analysis and NMR spectra. In NMR-spectra the result of alkylation has been confirmed by disappearance of the chemical shift of the mercapto group. All compounds both intermediate 4a-c and end products 6a-u and 7a-u contain signals of the alkyl protons of substituents in the triazole (methyl or methyl and methylene) ring; the 4-aminogroup protons are in the spectra of compounds 6 as singlet signals at 5.87-5.92 ppm. Modification of amino derivatives 6 into pyrrolyl substituted 7 is accompanied with the appearance of the characteristic signals of methyne protons of the pyrrole moiety instead of the signal of the amino group – triplet (positions 3,4) at 6.30-6.31 ppm and doublet (positions 2,5) at 7.21-7.24 ppm. Substances 7a, 7f, 7j and 7m were tested on the antitumour activity *in vitro*. As the result of this investigation it was noted that unfortunately all substances selected were not effective inhibitors of tumour cells in this dose.

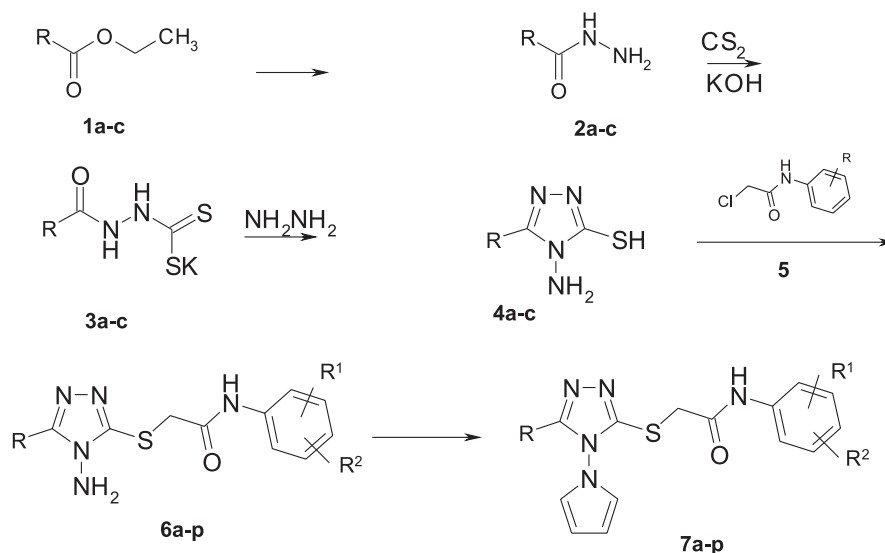
The synthesis of substituted derivatives of 1,2,4-triazole is well studied and described in many scientific articles. There are many convenient and simple methods for introduction of different substituent into this heterocycle. For several years we engaged in the synthesis of 3-mercapto-1,2,4-triazole derivatives. It has been found that some of these substances are very promising thanks to their pharmacological activity [12-14].

There is a lot of information about the synthesis of new promising medicines among 4-aminosubstituted derivatives of 1,2,4-triazole-3-thiol. Different methods for their synthesis are described. Thus, the following procedure is used as general: the ester of carboxylic acid (corresponding to the substituent in position 5) is transformed into hydrazide treated with carbon disulfide (for introduction of the mercapto group) and potassium hydroxide. Sodium dithiocarbazinate obtained is cyclized into the triazole ring with the excess of hydrazine hydrate (introduction of the amino group in position 4). Due to this methods aryl [5, 6], substituted benzyl [4], diphenylmethyl [3], pyridyl [8,9], phthalazine [7] moieties were introduced in position 5 of 3-mercapto-1,2,4-triazole. All compounds synthesized were tested on the biological activity after some chemical transformations

(usually the synthesis of Schiff bases or/and fused heterocycles). It has been shown that they can be used as antibacterial and antifungal agents.

We used this method before for the synthesis of similar cyclohexyl [11] and aryl [2] derivatives. The preliminary prognosis of the pharmacological activity using the PASS software [10] has shown that substances of such structure might have the anti-inflammatory, antiviral, membranoprotective action [1]. For alkyl (methyl, ethyl, and propyl) derivatives their activity as monoamine oxidase inhibitors is the most likely. Thus, the next synthesis was planned to be carried out in the group of 3-mercapto-4-amino-5-alkyl derivatives and their pyrrolyl products.

The key intermediate of 3-mercapto-4-amino-5-alkyl-1,2,4-triazole(4H) molecule was formed from initial carboxylic acid (acetic, propanoic and butanoic) ethyl esters **1a-c** by the procedure previously described (Scheme 1) [11]. The first step was hydrazinolysis leading to substances **2a-c**. Potassium dithiocarbazates **3a-c** formed after interaction of hydrazides with carbon disulfide in the alkaline medium were treated with an excess of hydrazine hydrate resulted in 3-mercapto-4-amino-5-alkyl-1,2,4-triazole(4H) **4a-c**. The key intermediates



Scheme

were alkylated with chloracetanilides **5** giving substances **6a-p**. The alkylation reaction was carried out under common alkylation conditions – in ethanol with addition of catalytic amounts of alkali (sodium hydroxide).

At next step Paal-Knorr condensation was used for modification of the chemical structure of the amino group into the pyrrolyl moiety. As a result of this reaction amides **7a-p** were obtained. Because of the absence of any activity predicted for amino compounds **6a-p** [1], we used them without additional purification for the next step in the synthesis. Their structure was proven by the NMR method. The absence of the initial triazole **4** impurity was assessed chromatographically. All compounds synthesized were obtained with good yields (Table 1).

The structure of the substances **7a-l** synthesized has been proven by the data of elemental analysis and NMR spectra (Table 2).

All compounds both intermediate **4** and end products **6a-u** and **7a-u** contain signals of the alkyl protons of substituents in the triazole ring (methyl or methyl and methylene); the 4-aminogroup protons are in the spectra of compounds **6** as singlet signals at 5.87-5.92 ppm. In NMR-spectra the results of alkylation were proven by disappearing the chemical shift of the mercapto group and signals of the acetamide moiety (CONH at about 10 ppm). Signals of aromatic protons of the phenyl ring can be found due to their intensity and multiplicity in accordance with the nature, positions and the number of substituents (Table 2).

Modification of amino derivatives **6** into pyrrolyl substituted **7** is accompanied by changes in the NMR spectra: instead of the amino group the triplet signals of methyne protons of the pyrrole ring (protons in positions 3,4) at 6.30-6.31 ppm appear. As to the second pair of the pyrrole ring protons (in positions 2, 5), they sometimes can not be distinguished among other signals of aromatic protons. In other cases they are doublet at 7.21-7.24 ppm.

Due to prognosis and logical analysis of the data the substances synthesized will be examined as possible antitumour agents and MAO inhibitors.

From the compounds synthesized such substances as **7a**, **7f**, **7j**, and **7m** (Table 1) were selected by the National Cancer Institute (NCI) within the Developmental Therapeutic Programme (www.dtp.nci.nih.gov) for *in vitro* cell line screening. Anticancer assays were performed according to the US NCI protocol [15]. Compounds were evaluated in one dose for the primary anticancer assay towards approximately 60 cell lines (with the concentration of 10⁻⁵ M). The human tumour cell lines represent all forms of cancer (such as non-small cell lung cancer, colon cancer, breast cancer, ovarian cancer, leukemia, renal cancer, melanoma, prostate cancer). In the screening protocol, each cell line was inoculated and pre-incubated for 24-48 h on a microtiter plate. Test agents were then added with the single concentration, and the culture was incubated for an additional 48 h. The end point determinations were made with a protein binding dye, sulforhodamine B (SRB). The results for each test agent were reported as the percent growth of the treated cells compared to the untreated control cells.

As the result of this investigation it was noted that unfortunately all substances selected were not effective inhibitors of tumour cells in this dose. Thus, the growth percent for more sensitive substances was: for cells of renal cancer UO-31 – 70.36 (**7a**), 76.31 (**7f**) and 81.50 (**7j**), 88.24 (**7m**); A498 – 82.12 (**7m**); melanoma UACC-62 – 86.17 (**7j**); leukemia SR – 80.92 (**7m**); leukemia RPMI-8226 – 81.31 (**7m**); prostate cancer PC-3 – 74.84 (**7m**) and 83.39 (**7a**); for non-small cell lung cancer HOP-92 – 74.36 (**7a**). Sensitivity of all other cancer cell lines and for colon, CNS, ovarian, and breast cancer were approximately at the control level.

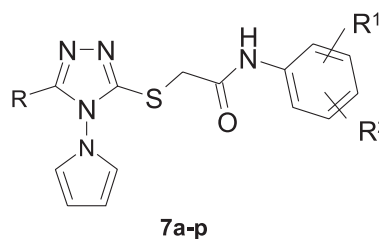
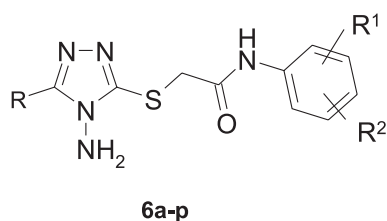
The next step of the study according to the PASS-prognosis for these compounds will be investigation of the CNS activity.

Experimental Part

Melting points were determined by an open capillary tube. NMR ¹H spectra were recorded on a Bruker WM spectrometer (300 MHz); solvents – CDCl₃ or DMSO-d₆; chemical shifts were in ppm, TMS was used as an internal standard. The purity of the compounds synthesized was monitored by TLC.

Table 1

Yields, melting points of the substances synthesized with general formulas:



Comp.	R	R ¹	R ²	Yield,%	M.p. °C
6a	CH ₃	4-OCH ₃	H	78.4	122-4
6b	CH ₃	3-CF ₃	H	81.2	184-6
6c	CH ₃	2-COOCH ₃	H	69.2	176-8
6d	CH ₃	2-CH ₃	3-CH ₃	77.4	166-8
6e	CH ₃	3-CH ₃	4-CH ₃	64.9	183-5
6f	CH ₃	2-CH ₃	4-Cl	78.1	173-5
6g	C ₂ H ₅	4-CH ₃	H	77.4	128-30
6h	C ₂ H ₅	3-OCH ₃	4-OCH ₃	76.6	164-6
6i	C ₃ H ₇	H	H	64.4	133-5
6j	C ₃ H ₇	3-OCH ₃	H	72.5	154-6
6k	C ₃ H ₇	4-OCH ₃	H	72.2	165-7
6l	C ₃ H ₇	2-OC ₂ H ₅	H	77.4	118-20
6m	C ₃ H ₇	3-CF ₃	H	76.5	155-7
6n	C ₃ H ₇	4-Br	H	74.9	170-2
6o	C ₃ H ₇	2-CH ₃	4-CH ₃	81.1	110-2
6p	C ₃ H ₇	2-OCH ₃	5-OCH ₃	77.5	116-8
7a	CH ₃	4-OCH ₃	H	77.1	127-9
7b	CH ₃	3-CF ₃	H	76.5	210-2
7c	CH ₃	2-COOCH ₃	H	74.4	151-3
7d	CH ₃	2-CH ₃	3-CH ₃	78.3	162-4
7e	CH ₃	3-CH ₃	4-CH ₃	72.1	205-7
7f	CH ₃	2-CH ₃	4-Cl	73.6	151-3
7g	C ₂ H ₅	4-CH ₃	H	75.2	192-4
7h	C ₂ H ₅	3-OCH ₃	4-OCH ₃	74.4	175-7
7i	C ₃ H ₇	H	H	78.5	132-4
7j	C ₃ H ₇	3-OCH ₃	H	81.1	102-4
7k	C ₃ H ₇	4-OCH ₃	H	83.8	149-51
7l	C ₃ H ₇	2-OC ₂ H ₅	H	79.3	127-9
7m	C ₃ H ₇	3-CF ₃	H	77.7	133-5
7n	C ₃ H ₇	4-Br	H	74.5	162-4
7o	C ₃ H ₇	2-CH ₃	4-CH ₃	76.3	161-3
7p	C ₃ H ₇	2-OCH ₃	5-OCH ₃	79.6	126-8

4-Amino-3-mercapto-5-alkyl-1,2,4-triazoles(4H) 4a-c. It was synthesized as previously described [12].

4-Amino-3-mercapto-5-methyl-1,2,4-triazole(4H) 4a. Yield – 65%. M.p. – 200-205°C. Spectrum NMR: 13.41, 1H, s, SH; 5.48, 2H, s, NH₂; 2.16, 3H, s, CH₃.

4-Amino-3-mercapto-5-ethyl-1,2,4-triazole(4H) 4b. Yield – 68%. M.p. – 149-150°C. The NMR spectrum: 13.40, 1H, s, SH; 5.46, 2H, s, NH₂; 2.50, 2H, q, CH₂, 1.12, 3H, s, CH₃.

4-Amino-3-mercapto-5-propyl-1,2,4-triazole(4H) 4c. Yield – 71%. M.p. – 108-110°C. The NMR spectrum:

13.40, 1H, s, SH; 5.45, 2H, s, NH₂; 2.61, 2H, t, CH₂, 1.52, 2H, m, CH₂, 0.86, 3H, t, CH₃.

4-Amino-5-alkyl-1,2,4-triazole(4H)-3-yl-thioacetanilides (6a-p, Table 1) (the general procedure). To the solution of 0.002 mol of 3-mercapto-4-amino-5-alkyl-1,2,4-triazole(4H) **5** in 20 ml ethanol 20 ml KOH add 0.002 M water solution. To the solution obtained add the solution of the corresponding chloroacetanilide (0.002 mol) while stirring. Reflux the reaction mixture for approximately 1 h, cool and place into 200 ml of water. Collect the precipitate and dry, recrystallize from ethanol.

Table 2

Chemical shifts (δ , ppm) at NMR ^1H spectra of substances 7a-p

Comp.	NH, c, 1H	Ar-H	Pyrrole 2.5, 2H, d	Pyrrole 3.4, 2H, t	S-CH ₂ , s, 2H	5-Alk	
7a	8.64, t	7.18, dd	7.12	6.28	4.21	2.15, 3H, s	3.73, 3H, s, OCH ₃
7b	10.69	8.01-7.56, 4H, m	7.17	6.32	4.12	2.16, 3H, s	-
7c	11.15	8.96-7.21, 6H, m		6.33	4.12	2.15. 3H. s	3.86, 3H, s, OCH ₃
7d	9.70	7.16, 5H, m		6.30	4.08	2.16. 3H. s	2.54, 3H, s, 2.49, 3H, s, 2x CH ₃
7e	10.13	7.17, 5H, m		6.30	4.05	2.18. 3H. s	2.54, 6H, s, 2x CH ₃
7f	9.73	7.61-7.24, 5H, m		6.31	4.10	2.15. 3H. s	2.51, 3H, s, CH ₃
7g	10.25	7.43, 7.10, 4H, dd	7.16	6.29	4.07	2.50, 2H, q, 1.12, 3H, t	2.25, 3H, s, CH ₃
7h	10.20	7.26, 1H, s, 7.06, 1H, d, 6.90, 1H, d	7.17	6.31	4.07	2.51, 2H, q, 1.11, 3H, t	3.73, 6H, s, 2xOCH ₃
7i	10.35	7.57-7.31. 7.08, 5H, m	7.17	6.30	4.10	2.49, 2H, t, 1.53, 2H, m, 0.86, 3H, t	-
7j	10.31	7.25-6.64, 6H, m		6.31	4.09	2.50. 2H, t, 1.52, 2H, m, 0.86. 3H, t	3.72, 3H, s, OCH ₃
7k	10.18	7.48. 6.90, 4H, dd	7.16	6.30	4.06	2.51, 2H, t, 1.54, 2H, m, 0.89, 3H, t	3.72, 3H, c, OCH ₃
7l	9.71	7.25-6.90, 6H, m		6.30	4.09	2.49, 2H, t, 1.56, 2H, m, 0.87, 3H, t	
7m	10.67	8.05-7.42, 4H, m	7.17	6.31	4.12	2.40, 2H, t, 1.54, 2H, m, 0.86, 3H, t	
7n	10.45	7.53, 4H, dd	7.16	6.30	4.09	2.51, 2H, t, 1.54, 2H, m, 0.86, 3H, t	-
7o	9.62	7.26-7.01, 5H, m		6.30	4.08	2.61, 2H, t, 1.52, 2H, m, 0.86, 3H, t	2.43, 6H, s, 2x CH ₃
7p	9.71	7.74. 6.94-6.61, 3H, m	7.17	6.30	4.12	2.50, 2H, t, 1.52, 2H, m, 0.88, 3H, t	3.79, 3H, s, 3.68, 3H, s, 2x OCH ₃

N-Phenyl-2-(5-alkyl-4-(1H-1-pyrrolyl)-4H-1,2,4-triazole-3-ylthio)acetanilides (7a-p, Table 1) (the general procedure). To the solution of 0.005 mol of N-phenyl-2-(4-amino-5-alkyl-4H-1,2,4-triazol-3-ylthio)acetanilide **2** [1] in 40 ml acetic acid add 0.005 mol of 2,5-dimethoxytetrahydrofuran. Reflux the mixture for approximately 1 h, cool and place into 200 ml of water. Collect the precipitate and dry, recrystallize from ethanol.

CONCLUSIONS

1. A series of new 4-phenyl-5-alkyl-3-mercapto-1,2,4-triazole(4H) derivatives has been synthesized started

from acetic, propanoic or butanoic acid, respectively. The compounds synthesized were transformed into 5-pyrrolyl-derivatives using Paal-Knorr condensation.

2. The structure of the compounds synthesized has been proven by the data of elemental analysis and NMR-spectra.

3. When studying *in vitro* four of the compounds synthesized have no high potential in cancer cell inhibition. Due to prognosis and logical analysis of the data the substances synthesized will be examined as possible MAO inhibitors.

REFERENCES

1. Саїдов Н.Б., Георгіяни В.А. // Фармація. – 2014. – №2. – С. 12-14.
2. Саїдов Н.Б., Георгіяни В.А., Ліпакова К.Ю. // Фармац. часопис. – 2014. – С. 15-18.
3. Akhter M.W., Hassan M.Z., Amir M. // Arab. J. Chem. – 2014. – Vol. 7, №9. – P. 955-963.
4. Anu Vipan K.K., Bhawana S., Prabhakar K.V. // Res. J. Pharm., Biol., Chem. Sci. – 2014. – Vol. 5, №6. – P. 866-872.
5. Ayah A.H., Firas H. // Int. J. Appl. Sci. Tech. – 2014. – Vol. 4, №2. – P. 202-211.
6. Chandrashekar A., Eswarappa B., Yadav D. et al. // Int. J. Pharm. Tech. Res. – 2014. – Vol. 6, №4. – P. 1245-1255.
7. Mahmoud M.R., Abou-Elmagd W.S.I., El-Shahawi M.M., Hekal M.H. // Eur. Chem. Bull. – 2014. – Vol. 3, №7. – P. 723-728.
8. Murti Y., Agnihotri R., Pathak D. // Amer. J. Chem. – 2011. – Vol. 1, №2. – P. 42-46.
9. Patel G.K., Patel H.S. // Int. J. Res. Pharm. Chem. – 2014. – Vol. 4, №4. – P. 928-935.

10. Poroikov V.V. // *Med. Chem. Res.* – 2010. – Vol. 19 (S1). – S. 30.
11. Saidov N.B., Georgiyants V.A., Garna N.V. // *ЖОФХ.* – 2013. – Т. 11, №4 (44). – С. 33-37.
12. Saidov N.B., Kadamov I.M., Georgiyants V.A. // *Вісник фармації.* – 2012. – №4 (72). – С. 22-26.
13. Saidov N.B., Kadamov I.M., Georgiyants V.A. // *ЖОФХ.* – 2012. – Т. 10, вип. 4 (40). – С. 25-28.
14. Saidov N.B., Kadamov I.M., Georgiyants V.A. // *ЖОФХ.* – 2013. – Т. 11, №1 (41). – С. 44-48.
15. Shoemaker R.H. // *Nat. Rev. Cancer.* – 2006. – Vol. 6, №8. – P. 813-823.

СИНТЕЗ НОВИХ БІОЛОГІЧНО АКТИВНИХ РЕЧОВИН У РЯДУ 4-АМІНО-5-АЛКІЛ-1,2,4-ТРИАЗОЛ(4Н)-3-ІЛТІОАЦЕТАНІЛІДІВ ТА ЇХ ХІМІЧНА МОДИФІКАЦІЯ

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Ключові слова: 3-меркапто-4-аміно-5-алкіл-1,2,4-триазол; похідні; синтез; алкілування; реакція Пааля-Кнорра; протипухлинна активність

Описано синтез серії нових 4-аміно-5-алкіл-1,2,4-триазол(4Н)-3-ілтїоацетанїлідів та 4-піроліл-5-алкіл-1,2,4-триазол(4Н)-3-ілтїоацетанїлідів. Ключові інтермедїати – 4-аміно-5-алкіл-3-меркапто-1,2,4-триазол(4Н) **4а-с** були синтезовані з вихідних карбонових кислот (оцтової, пропіонової та масляної) після їх етерифікації, наступного гїдразинолізу реакцією з сірководнем та циклізацією надлишком гїдрозин гїдрату. Перша група речовин **6а-р** була отримана алкілуванням проміжних речовин **4а-с** анілідами хлорацетатної кислоти в присутності основних каталїзаторів. Наступна модифікація в умовах реакції Пааля-Кнорра приводить до відповідних піролільних похідних **7а-р**. Структура синтезованих сполук доведена даними елементного аналізу та спектріє ЯМР¹Н. В ЯМР-спектрах результат алкілування було доведено зникненням хїмічного зсуву меркаптогрупи. Всі сполуки, як проміжні **4а-с**, так і цільові продукти **6а-р** і **7а-р** містять сигнали протонів алкільних замісників в триазольному кільці (метильних або метильних та метиленових); протони 4-аміногрупи в спектрах сполук **6** спостерігаються у вигляді синглетних сигналів при 5,87-5,92 м.ч. Модифікація амінопохідних **6** в піролілзаміщені **7** супроводжується появою характерних сигналів метинових протонів піролільного залишку замість сигналу аміногрупи – триплет (положення 3,4) при 6,30-6,31 м.ч. та дублет (положення 2,5) при 7,21-7,24 м.ч. Речовини **7а, 7f, 7j** і **7м** були протестовані на протипухлинну активність *in vitro*. В результаті цього дослідження було відзначено, що всі обрані речовини, на жаль, не були ефективними інгібіторами пухлинних клітин у цій дозі.

СИНТЕЗ НОВИХ БІОЛОГІЧЕСКИ АКТИВНЫХ ВЕЩЕСТВ В РЯДУ 4-АМИНО-5-АЛКИЛ-1,2,4-ТРИАЗОЛ(4Н)-3-ИЛТИОАЦЕТАНИЛИДОВ И ИХ ХИМИЧЕСКАЯ МОДИФИКАЦИЯ

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Ключевые слова: 3-меркапто-4-амино-5-алкіл-1,2,4-триазол; производные; синтез; алкилирование; реакция Пааля-Кнорра; противоопухолевая активность

Описан синтез серии 4-амино-5-алкіл-1,2,4-триазол(4Н)-3-илтїоацетанїлидов и 4-піроліл-5-алкіл-1,2,4-триазол(4Н)-3-илтїоацетанїлидов. Ключевые интермедїаты – 3-меркапто-4-амино-5-алкіл-1,2,4-триазол(4Н) **4а-с** синтезированы из исходных карбоновых кислот (уксусной, пропионовой и масляной) после их этерификации, последующего гїдразиноліза, реакции с сероводородом и циклізації избытком гїдрозин гїдрата. Первая группа веществ **6а-р** получена алкілированием промежуточных соединений **4а-с** анілідами хлоруксусной кислоты в присутствии основных каталїзаторов. Последующая модифікация в условиях реакции Пааля-Кнорра приводит к соответствующим піролільным производным **7а-р**. Структура синтезированных соединений доказана данными элементного анализа та спектров ЯМР¹Н. В ЯМР-спектрах результат алкілирования подтверждается исчезновением химического сдвига меркаптогруппы. Все вещества, как промежуточные **4а-с**, так и целевые продукты **6а-р** и **7а-р** содержат на спектрах сигналы протонов алкільных заместителей в триазольном кольце (метильных или метильных и метиленовых); протоны 4-аміногруппы в спектрах веществ **6** наблюдаются в виде синглетных сигналов при 5,87-5,92 м.д. Модифікация амінопроизводных **6** в піролілзамещенные **7** сопровождается появлением характерных сигналов метинових протонов піролільного остатка вместо сигнала аміногруппы – триплет (положения 3,4) при 6,30-6,31 м.д. и дублет (положения 2,5) при 7,21-7,24 м.д. Вещества **7а, 7f, 7j** и **7м** были протестированы на противоопухолевую активность *in vitro*. В результате этого исследования было отмечено, что все выбранные вещества, к сожалению, не были эффективными ингібиторами роста опухолевых клеток в этой дозе.