PRODUCTS OF INTERACTION OF SUBSTITUTED 5-AMINOPYRAZOLES WITH α-HALOKETONES AS POTENTIAL PHARMACEUTICAL AGENTS

© P. Tkachenko, O. Tkachenko, K. Netosova, O. Borisov, I. Zhuravel

1. Introduction

Substituted pyrazoles are one of the most important heterocyclic compounds, which are mentioned and emphasized in the biological, medical and technical fields. The structural fragment pyrazole as a monomer, and as a segment of polynuclear systems is part of many essential compounds of natural or synthetic origin. For example, L-a-amino-b-pyrazolyl-N)-propanolic acid, isolated from Citrullus vulgaris, demonstrates antiadipogenic effects; withasomnine from Withania somnifera demonstrates analgesic, anti-inflammatory effects, appears depressant to CNS, circulatory system; pyrazofurin functions as an antimutagen and antiviral agent [1]. Pyrazoles showed potent antibacterial and antifungal activity against different strains including gram-positive strains (Staphylococcus aureus [2], Streptococcus mutans [2], Bacillus subtilis [3]), gram-negative strains (Salmonella typhimurium [2] and Pseudomonas aeruginosa [4]) and fungus (Candida albicans [5], Gaeumannomyces graminis var. graminis [6], Aspergillus niger[3]). Pyrazoles are known to possess numerous chemical and biological activities such as anti-inflammatory [7], antiproliferative [8], antiepileptic [9].

2. Formulation of the problem in a general way, the relevance of the theme and its connection with important scientific and practical issues

The search for new methods of synthesis of biologically active substances [10].

3. Analysis of recent studies and publications in which a solution of the problem are described and to which the author refers

The important ability of functionally substituted pyrazoles to enter into a variety of heterocyclic reactions, as it includes the formation of binuclear compounds, which represent the most trending class of substances for biological screening [11]. For example, synthesized N2-substituted 5-amino-4-arylsulfonyl-3-phenylamino-pyrazoles are of certain interest as potential pharmaceutical agents and can be used to develop new antifungal agents against Candida albicans [12]. 5-(N,N-Diacylamino)-derivatives of N2-substituted 4-alkyl/arylsulfonyl-5-amino-3-alkylthiopyrazoles are of great interest for further biological screening in order to find substances with the properties associated with the action on fungal cells among them [13].

4. The field of research considering the general problem, which is described in the article

Taking into account the considerable pharmacological potential of substituted aminoazoles, we drew attention to the development of approaches to the synthesis of poly substituted imidazo[1,2-b]pyrazoles, in particular the interaction of 5-aminopyrazoles with α-halogenocetones.

5. Formulation of tasks of article

Optimization of reaction of substituted 5-aminopyrazoles with α-haloketones to form annealed 2,6,7-trisubstituted 1H-imidazo[1,2-b]pyrazoles.

6. Presentation of the main research material (methods and objects) with the justification of the results

6.1. Methods and objects

All reagents and solvents were obtained from the commercial sources. Melting points were obtained by a Buchi B-520 device. The NMR-spectra were recorded with a Bruker 170 Avance spectrometer at 200 MHz, 500 MHz (DMSO-d6); TMS was used as an internal standard; chemical shifts were reported in ppm. The TLC
was performed on the aluminum plates covered with a silica gel (Merck, Kiesegel 60 F-254).

5-Amino-4-phenylsulfonyl-3-methylthiopyrazole 1a and 5-Amino-4-(4'-methoxy phenyl)sulfonyl-3-methylthiopyrazole 1b were obtained according to the methods previously reported [11, 13].

The general methods for preparation of imidazo[1,2-b]pyrazoles 4 a-c. Method A. To a flask with 4 ml DMF add 5-amino-4-phenylsulfonyl-3-methylthiopyrazole (0.001 mmol, 0.27 g), chloroacetone (0.0011 mmol, 0.1 g) and potassium carbonate (0.004 mmol, 0.55 g). The mixture is stirred at 50 °C for 3 hours and diluted with water (10 ml). The oily precipitate, which was formed, was filtered, washed with ethanol, and 0.5 ml of concentrated hydrochloric acid. Then to the crude butter precipitate add 10 ml of ethanol and 0.5 ml of concentrated hydrochloric acid. The mixture is boiled for 2–4 hours and cooled. The formed precipitate was filtered, washed with ethanol, transferred into an aqueous solution of sodium bicarbonate, mix thoroughly. The precipitate is filtered, dried.

Method B. To a flask with 15 ml propanol-1 add 5-amino-4-phenylsulfonyl-3-methylthiopyrazole (0.001 mmol, 0.27 g), chloroacetone (0.00011 mmol, 0.12 g). The mixture is boiled for 8–9 hours and cooled. The formed precipitate was filtered, transferred into an aqueous solution of sodium bicarbonate, mix thoroughly. The precipitate is filtered, dried.

The procedure for preparation of N-phenyl-(2-(4'-methylyphenyl)-6-methylthio-7-(4'-methoxyphenyl)sulfonyl-1H-imidazo[1,2-b]pyrazole-1-yl)acetamide 5c were obtained according to the methods previously reported [13].

6. 2. Results and discussion

In previous studies, we described the interaction of 5-amino-4-arylsulfonyl-3-alkylthiopyrazoles with (N-aryl)chloroacetamides, which leads to the formation only N'-alkylation products [11]. It should be noted that during the reaction, we did not observe the impurities of the regionisomeric product or products of NH2-alkylation. This is evidenced by the results of TLS LCMS and by the data of 1H NMR spectroscopy (the absence of a double set of signals, signals of a free amino group, as well as the successful course of the reaction of its acylation) [13].

In this paper, we examined the interaction of 5-amino-4-arylsulfonyl-3-methylthiopyrazoles with chloroacetone, phenacyl bromides and a-chlorocylohexa-none (Fig. 1).

![Diagram](image)

**Fig. 1. Preparation of substituted 1H-imidazo[1,2-b]pyrazoles**

The use of the second method, the boiling of the starting reagents in propanol-1, allowed to slightly increase the yields of the final products and to exclude the stage of work with the oily product, although sometimes
the reaction did not reach the end and it was difficult to get rid of the original aminopyrazole.

Characteristic signals of the CH-proton of the pyrazole cycle in the spectra of products 4a-c are found at 8.19–8.32 ppm and NH-proton of imidazole fragment (δ 12.61–12.75 ppm), and a corresponding set of aromatic protons and substituents signals. Free cyclic amino group is subject to alkylation. Thus, using a standard synthetic procedure, in reaction 4c with (N-phenyl)chloroacetamide, compound 5c was received. 5-Amino-4-phenylsulfonyl-3-methylthiopyrazole 1a. Yield 87 %; M. p. 188 °C; 1H NMR δ: 2.43 (s, 1H, SCh), 6.09 (s, 2H, NH). 7.56 (m, 3H, Ar-H), 7.89 (d, 2H, Ar-H), 11.97 (br.s, 1H, NH).

5-Amino-4-(4'-methoxyphenyl)sulfonyl-3-methylthiopyrazole 1b. Yield 91%; M. p. 21 3 – 15 °C; 1H NMR δ: 2.47 (s, 1H, SCh), 3.75 (s, 3H, OCH3), 6.12 (s, 2H, NH), 7.36 (d, 2H, Ar-H), 7.93 (d, 2H, Ar-H), 12.07 (br.s, 1H, NH).

2-Methyl-6-methylthio-7-phenylsulfonyl-1H-imidazo[1,2-b]pyrazole 4a. Yield 26% (Method A), 34% (Method B); M. p. 214 – 16 °C; 1H NMR δ: 2.08 (s, 3H, CH3), 2.28 (s, 3H, SCh), 7.60 (m, 3H, Ar-H), 7.90 (dd, 2H, Ar-H), 8.03 (s, 1H, CH), 12.27 (br.s, 1H, NH).

2-(4-Chlorophenyl)-6-methylthio-7-phenylsulfonyl-1H-imidazo[1,2-b]pyrazole 4b. Yield 45% (Method B); M. p. 187 – 89 °C; 1H NMR δ: 2.49 (s, 3H, SCh), 7.54 (m, 5H, Ar-H), 7.86 (d, 2H, Ar-H), 8.02 (m, 2H, Ar-H), 8.32 (s, 1H, NH), 12.75 (s, 1H, NH).

7. Findings from the research and prospects of further development of this area
1. The reaction of substituted 5-aminopyrazoles with α-haloketones to form annealed 2,6,7-trisubstituted 1H-imidazo[1,2-b]pyrazoles was optimized.
2. The structure of synthesized substances was confirmed by the complex of modern spectral analysis methods.
3. The obtained 2-substituted 6-methylthio-7-phenylsulfonyl-1H-imidazo[1,2-b]pyrazoles are promising enough for the further biological research.

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Pavlo Tkachenko, Postgraduate student, Department of Drug and Analytical Toxicology, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine 61002, E-mail: toxchem@nuph.edu.ua

Olena Tkachenko, PhD, Associate Professor, Department of Quality Management, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine 61002 E-mail: elena_tkachenko75@ukr.net

Krystyna Netosova, PhD, Assistant, Department of Drug and Analytical Toxicology, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine 61002, E-mail: kulikovskaja.k@gmail.com

Oleksandr Borisov, PhD, Head of Laboratory, LLC “RPE “Enamine”, Chervonotkatska str., 78, Kyiv, Ukraine, 02660 E-mail: boav.79@gmail.com

Iryna Zhuravel, Doctor of Chemical Sciences, Professor, Department of Clinical Biochemistry, Forensic Toxicology and Pharmacy, Kharkiv Medical Academy of Post-graduate Education, Amosova str., 58, Kharkiv, Ukraine, 61176 E-mail: irina.tox@gmail.com

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QUALITY ASSESSMENT AND STABILITY STUDY OF COMPOUNDED FUROSEMIDE SYRUP

© D. Alfred-Ubbenbo, O. A. Zdoryk, V. A. Georgiyants

Лікарські засоби виготовлені в аптеках для перорального застосування, приготовлені з використанням готових лікарських засобів у якості джерела активних фармацевтичних інгредієнтів, характеризуються короткими термінами придатності через їх нестабільність.

Метою даного дослідження є вивчення фізичної, хімічної та мікробіологічної стабільності сиропів фуросеміду аптечного виготовлення впродовж 30 днів.

Матеріали і методи: Серії сиропів аптечного виготовлення 5 мг/мл, виготовлених з субстанції фуросеміду і таблеток (двох виробників) в якості джерел активних фармацевтичних інгредієнтів, зберігали в темному місці при температурі 5±3 °С та 23±2 °С для яких досліджували на 0, 7, 15, 23 та 30 дні зміни фізичної (рН, колір, запах, виділення газу, в’язкість), хімічної та мікробної стабільності. Було проведено стрес-тест, щоб відрізнити ознаки хімічної нестабільності, використовуючи метод тонкошарової хроматографії. Досліджувані зразки протягом терміну зберігання досліджували на мікробіологічну стабільність визначаючи загальну кількість аеробних мікроорганізмів (ТАМС <100), загальну кількість комбінованих дріжджів/цифрових гнізд (ТУМС <10) та відсутність Escherichia coli.

Результати: Протягом терміну зберігання для досліджуваних сиропів було не виявлено додаткових плям на хроматограмах, суттєвих змін рН, коліру, запаху, утворення газу, в’язкості. На 30-й день у досліджуваних зразках вміст фуросеміду складав (±99,3 %), загальний обсяг аеробних мікроорганізмів (<10), загальний сумарний вміст дріжджів/цифрових гнізд (<10), що є у межах допустимих значень. Зразки для яких перевіряли вплив стрес факторів на ТІШ хроматограми виявили наявність додаткових плям та зміни їх інтенсивності.

Висновки: Досліджено, що екстемпоральні сиропи фуросеміду, виготовлені як із субстанції так і таблеток, які зберігаються в скляних флаконах у темному місці при 5±3 °С та 23±2 °С, фізично, хімічно та мікробіологічно стабільні принаймні протягом 30 днів.

Ключові слова: фуросемід, сироп, лікарські засоби виготовлені в аптеках, внутрішньоаптечний контроль якості, стабільність, тонкошарова хроматографія.

1. Introduction

The need for compounded preparations as a panacea for patient-specific dosing, logistical and therapeutical quagmires in pharmacotherapy is usually faced with the challenge of affirming their stability. Dispersed solid dosage forms are often used as active pharmaceutical