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Prediction of the Antiinflammatory Activity of New *S*-alkyl Derivatives of 1,2,4-triazol-3-thiones Using the PASS Computer Program and Molecular Docking

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Abstract

The strategy of rational approaches to the search for selective COX-2 inhibitors as potential antiinflammatory agents has been proposed and elaborated. It is based on the use of PASS-prediction and molecular docking. The choice of the basic structure of 4-amino-3-thio-1,2,4-triazole as a promising object of chemical modification has been substantiated. Using a modification of the primary molecule, a virtual library of *S*-derivatives of 5-substituted 4-amino(pyrrol)3-thio-4H-1,2,4-triazoles in the amount of 100 compounds (ten groups) has been obtained by introducing various pharmacophore fragments. Based on the analysis of the results of the PASS-prediction and molecular docking, six of the ten planned groups of compounds have been selected for the synthesis as promising selective COX-2 inhibitors. The reliability of the prediction results has already been confirmed for one of the promising group 4-amino-5-(pyridine-4-yl)-1,2,4-triazole (4H)-3-yl-thioacetamides.

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INTRODUCTION

The synthesis of new structures with the predicted activity is expedient to carry out in that class of chemical compounds where substances with a certain directed action have been already found (Oliveira *et al*., 2019). Scientists refer the heterocyclic system of 1,2,4-triazole to the privileged structure ("privileged scaffold"), since most of the derivatives of this heterocyclic synthesized exhibit some pharmacological activity, including the antiinflammatory (Zhuang *et al*., 2017). The combination of factors affecting biochemical processes, as well as the practical absence of toxic effects on the body, indicates the feasibility of further targeted search for new biologically active substances among derivatives of 1,2,4-triazole (Mioc *et al*., 2017). A careful study of the literature data on the spectrum of pharmacological properties of the heterocyclic system of 1,2,4-triazole allows us to confidently assert that the presence of this cycle in the structure of substances determines the manifestation of the antiinflammatory activity (Moise *et al*., 2009). Previous research by Sondhi *et al*. (2007) obtained a large group of derivatives of 1,2,4-triazole, pyridine, pyrrol,

and other related heterocyclic compounds, among them selective cyclooxygenase-2 (COX-2), 5-lipoxygenase (5-LOX), and COX-2/5-LOX double inhibitors were identified. Moreover, some studies suggest that the presence of the 1,2,4-triazole cycle causes selective inhibition of COX-2 (Cai *et al*., 2015; Jiang *et al*., 2010).

In addition, 1,2,4-triazole derivatives are low-toxic, rather simple in synthesis and highly reactive substances. It allows to introduce various pharmacophore fragments into their structure (Khanage *et al*., 2012). Our analysis of the scientific literature has shown that despite a large number of publications devoted to functional derivatives of 1,2,4-triazole the pharmacological potential of this class of compounds at the present stage is not exhausted.

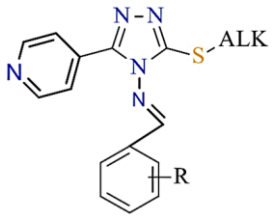
The compound of 4-amino-3-thio-1,2,4-triazole has a high synthetic potential, significant opportunities in terms of the introduction of pharmacophore fragments and, correspondingly, the expansion of the spectrum for searching the biological activity. The presence of a sulfur atom in the basic structure increases lipophilicity and, therefore, can improve absorption and bioavailability of the compounds synthesized on its basis (Jin *et al*., 2007). In addition, thio and amino groups are reaction centers for the introduction of additional pharmacophores into the molecule.

MATERIALS AND METHODS

The introduction of different pharmacophore fragments into the molecule by modifying the thio group and obtaining *S*-derivatives of 5-substituted 4-amino(pyrrol)3-thio-4H-1,2,4-triazoles (groups of compounds **I-X**). This allows increasing the chances of finding new effective compounds in this series, as presented in **Figure 1**. The following modification have been planned as follows:

1. Alkylation of 5-pyridine-4-amino(pyrrol)3-thio-1,2,4-triazole derivatives by haloalkanes (1-bromopropane, 1-bromohexane, 1-bromoheptan, 1-bromononane, 1-bromodecane) (**I**) in order to increase lipophilicity of the initial 5-pyridine-4-yl-4-amino-3-thio-4H-1,2,4-triazole.
2. Introduction of the arylidene aniline fragment (**II**) to alkyl derivatives of group (**I**), it will increase the number of unsaturated bonds in the molecule and may increase the activity.
3. Oxidation of 5-(pyridine-4)-4-amino-3-thio-1,2,4-triazole derivatives to alkyl sulfonyl derivatives (**III**) as in the structure of modern oxicams there is a sulfonyl group.
4. Introduction of the 5-(pyridine-4)-4-amino-3-thio-1,2,4-triazole fragment of alkyl urea (**IV**) into the basic structure since there are literature data concerning the effect of the acetamide residue the increase of the antiinflammatory activity.
5. Alkylation of 5-(pyridine-4(2,3))-4-amino-3-thio-1,2,4-triazoles (**V-VI**) by α-chloroacetamide's taking into account the literature data concerning the effect of the acetamide residue on the increase of the antiinflammatory activity.
6. Alkylation of 5-(furyl-2)-amino-3-thio-1,2,4-triazoles (**VII**) by α-chloroacetamide's.
7. Replacement of the amino group in *S*-alkyl derivatives of 5-(pyridine-2,4)-4-amino-3-thio-4H-1,2,4-triazoles (**VIII-IX**) and 5-(furyl-2)-4-amino-3-thio-4H-1,2,4-triazoles (**X**) on the pyrrol residue.

I



II

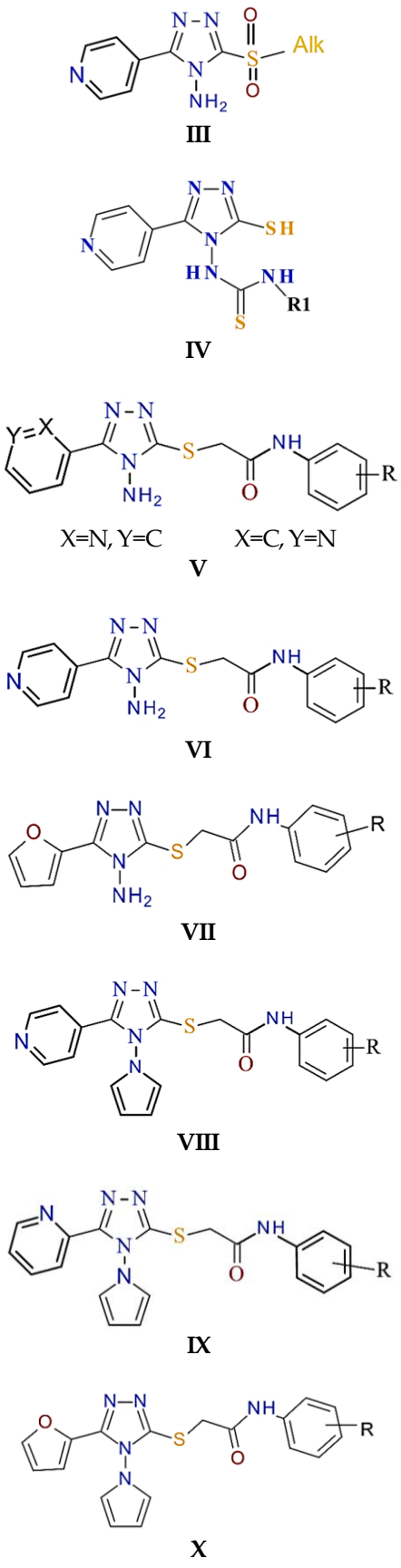


Figure 1. General formulas for the planned groups of new structures of 5-substituted 4-amino(pyrrol)3-thio-4H-1,2,4-triazoles from compounds **I** to **X**

A more real possibility of a comprehensive study of the biological activity of substances is the use of new computer prediction technologies *in silico* and their application to the assessment of the spectrum of activity of chemical compounds with subsequent testing of the substances studied according to the results of the prediction (Parasuraman, 2011). We have proposed a methodology for assessing the activity using the existing calculation programs.

The logical and structural assessment of the possible biological effect was performed using the Prediction of Activity Spectra for Substances (PASS) computer system online (http://www.pharmaexpert.ru/PassOnline). This system predicts 565 types of the biological activity by the structural formula of the chemical compound. These activities include the main and side pharmacological effects, mechanisms of their implementation and toxic manifestations, such as mutagenicity, carcinogenicity and teratogenicity (Lagunin *et al.*, 2000).

The prediction result is presented in the form of confidence "to be active" – Pa and "to be inactive" – Pi by the types of activity. It should be noted that the data obtained using the PASS program can serve only as an indicative characteristic when selecting promising molecules and help to conduct the primary sample of probable promising groups. It is known that the Pa value reflects, first of all, the similarity of the molecule with the most typical known drugs of the training sample (Assyl *et al.*, 2014).

In order to optimize the targeted search for COX-2 inhibitors as potential antiinflammatory agents and substantiate the feasibility of the experimental screening for the antiinflammatory activity the docking studies were also conducted (Laube *et al.*, 2016). Conducting the docking studies has allowed us to investigate the affinity of a definite group of compounds to this biological target, predict the ability of substances to inhibit the catalytic

activity of 1CX2 and 6COX, i.e. to identify their inhibitor, which is a key link in the pathogenesis of the disease. The use of *in silico* methods also makes it possible to save laboratory animals in the case of complete absence of affinity to potential biological targets (Leelananda & Lindert, 2016).

Method

Generation of the 3D-structure of the planned substances and their optimization using the method of molecular mechanics MM+ and semi-empirical quantum mechanical method PM3

Objects of this study were derivatives of 4-amino-5-(pyridine-2(3)-yl)-1,2,4-triazole(4H)-3-yl-thioacetamides; 4-amino-5-(pyridine-4-yl)-1,2,4-triazole(4H)-3-yl-thioacetamides; and their pyrrol derivatives of 2-((4-amino-5-(furan-2-yl)-1,2,4-triazole(4h)-3-yl)-sulfonyl)-*N*-acetamides and their pyrrol derivatives, as well as the known selective COX-2 inhibitors (celecoxib, natrium diclofenac) and non-selective inhibitor aspirin.

The choice of antiinflammatory biological targets and determination of their active sites

Crystallographic models of COX-2 was obtained from Protein Data Bank (www.rcsb.org), with PDB ID 6COX and PDB ID 1CX2 (Kurumbail *et al.*, 1997). Their difference lies in the different spatial groups of ligand binding. In both crystallographic models of COX-2 PDB ID 1CX2 (chains A, B, C, D) and PDB ID 6COX (chains A, B) the following ligands were isolated: selective COX-2 inhibitor SC-558, protoporphyrin IX (contains Fe), *N*-acetyl-D-glucosamine in accordance with the literature data that 1CX2 and 6COX crystallographic models represent such spatial groups as P 2_1 2_1 2 and I 2 2 2. (Kurumbail *et al.*, 1997). These data indicate the difference in protein conformations, and, therefore, the structural difference of the binding regions; it affects the protein-ligand interactions. Thus, we considered it expedient to take into account the data obtained as a result of the use of both crystallographic forms of COX-2 in the docking studies. After addition hydrogen atoms to 1CX2 and 6COX molecules, removal of water molecules, and isolation of chains B, C, D and B, respectively, the active sites of proteins 1CX2 were isolated as presented in **Figure 2**.

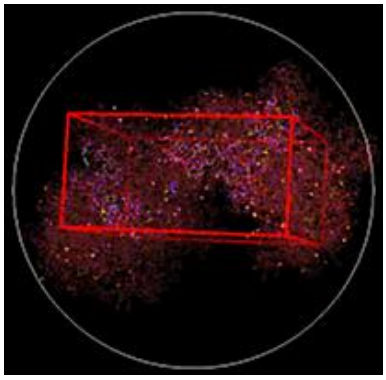


Figure 2. The active site of 1CX2 protein

Conducting the actual molecular docking

The docking studies of hypothetical compounds were conducted using a SCIGRESS software package (Fujitsu, Fukuoka, Japan with license 742F6852C191). The parameter used is binding free energy (kcal/mol), where the value of binding free energy of all test compounds is compared with comparative compounds such as celecoxib, aspirin, and natrium diclofenac. The genetic algorithm, an evolutionary search algorithm applied to solve optimization and modeling problems by sequential selection, combination and variation of the parameters studied was used for automatic docking. The use of the genetic algorithm makes it possible to effectively study the entire available space for the ligand (Torres *et al.*, 2019).

RESULTS AND DISCUSSION

According to the prediction results the probability of detection of the antiinflammatory activity in the group of *S*-alkyl derivatives of 5-(pyridine-4-yl)-3-thio-4-amino-4H-1,2,4-triazole (**I**) is insignificant, the average value of Pa does not exceed 0.39 for the antiinflammatory activity

and 0.32 for the analgesic activity. After replacing the amino group in position 4 of the 1,2,4-triazole cycle on the substituted benzylidene fragment an insignificant increase in the antiinflammatory and analgesic activity of compounds in group **II** is predicted. After this modification the highest probability of detection of the antiinflammatory activity is predicted for 4-methoxy, 4-nitro, and 4-butylphenyl derivatives; however, for these compounds, the Pa value does not exceed 0.4 for the antiinflammatory activity and 0.49 for the analgesic activity. The average value of Pa ≤ 0.500, hence, the group is not promising for the synthesis.

Despite the fact that the presence of a sulfonyl group can increase the antiinflammatory activity, which has been proven in modern COX inhibitors including piroxicam, tenoxicam, celecoxib, etc. (Cordero *et al*., 2001), the probability of its manifestation is insignificant according to the results of the computer prediction for sulfonyl derivatives of 5-(pyridine-4-yl)-3-thio-4-amino-4H-1,2,4-triazole (**III**). The Pa value does not exceed 0.35, in addition, there is a clear regularity: with an increase in the number of carbon atoms in alkyl radicals, the Pa value decreases and, therefore, there is the probability of detecting the antiinflammatory activity. Therefore, compounds **I** to **III** were not tested by molecular docking.

With a low degree of probability (Pa ≤ 0.3), the manifestation of the antiinflammatory activity for group **IV** of thiourea derivatives of 4-amino-3-thio-5-(pyridine-4-yl)-1,2,4-triazole(4H) is predicted. According to the data of the PASS computer program online, the antiinflammatory and analgesic activities are predicted for those groups of compounds that contain the pyridine cycle in position 5 (the activity indices are in the range from 0.51 to 0.66).

By the computer prediction data, 4-amino-5-(pyridine-2(3)-yl)-1,2,4-triazole(4H)-3-yl-thioacetamides (group **V**) are promising compounds. Further chemical modification by moving the nitrogen atom to the para-position of the pyridine cycle in 4-amino-5-(pyridine-4-yl)-1,2,4-triazole(4H)-3-yl-thioacetamides – the structure of general formula from group **VI**, and the introduction of the furyl fragment (group **VII**) were highly appropriate. For compounds in group **VII**, the indices of the antiinflammatory activity are the highest among all planned groups (0.63-0.65). But for compounds of these groups the indices of the analgesic activity do not significantly decrease and are in the range from 0.58 to 0.59.

Another variant of the expedient optimization was the chemical modification of the amino group in position 4. The analysis of the computer prediction data obtained for *S*-alkyl derivatives, in which the amino group in 5-(pyridine-2,4)-4-amino 3-thio-4H-1,2,4-triazoles (**VIII-IX**) and 5-(furyl-2)-4-amino 3-thio-4H-1,2,4-triazoles (**X**) was replaced by a pyrrol residue showed that the greatest probability of activity was also at the level of approximately 60%.

Thus, according to the results of the *in silico* studies among ten planned groups of *S*-derivatives of 5-(R)-4-amino-3-thio-4H-1,2,4-triazole (groups of compounds **I-X**), only six groups that are likely to be antagonists of prostaglandins, and can exhibit antiexudative and analgesic effects have been selected as promising compounds (Chalenko & Syrovaya, 2017).

The docking studies of the sample for derivatives of 4-amino-5-(pyridine-2(3)-yl)-1,2,4-triazole(4H)-3-yl-thioacetamides, 4-amino-5-(pyridine-4-yl)-1,2,4-triazole (4H)-3-yl-thioacetamides and their pyrrol derivatives, 2-((4-amino-5-(furan-2-yl)-1,2,4-triazole(4H)-3-yl)-sulfonyl)-*N*-acetamides and their pyrrol derivatives were conducted by the method of a flexible molecular docking into the active zone of the COX-2 enzyme (Mozziconacci *et al*., 2005). As a result of the docking studies the value of

Consensus scoring functions, which assess certain characteristics of the ligand-protein complex and indicate the possibility of their comparison, has been obtained. Consensus functions allow forming a rating of compounds according to the values of all scoring functions and analyzing data on the choice of potential agonists/antagonists of the biological target selected (Sliwoski *et al*., 2014). The average values of the calculated scoring functions of molecular docking for promising groups of compounds are given in **Table I**.

Table I. The values of scoring functions for promising groups of compounds and reference drugs obtained when conducting molecular docking

Compounds	Binding free energy (kcal/mol)	
	1CX2	6COX
Celecoxib	-99.81	-109.3
Aspirin	-77.7	-79.6
Natrium diclofenac	-70.3	-70.4
IV	-61.1 to -67.6	-61.4 to -66.5
V	-91.1 to -97.6	-91.1 to -97.6
VI	-108.1 to -122.7	-108.1 to -122.7
VII	-98.8 to -107.67	-98.8 to -107.67
VIII	-90.14 to -101.2	-90.14 to -101.2
IX	-93.17 to -100.7	-90.25 to -101.0
X	-87.14 to -91.2	-87.14 to -91.2

The values of scoring functions for almost all compounds in complexes with the COX-2 enzyme exceed the values of these functions for aspirin and natrium diclofenac. For group **VI** the average values of scoring functions for binding free energy exceed the values for celecoxib. Visualization of docking results for the most promising compound of group **VI** is presented in **Figure 3**.

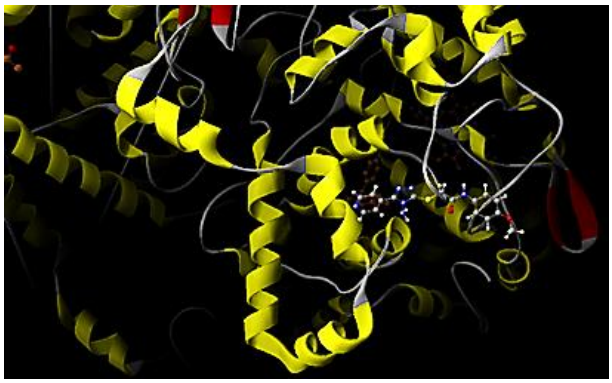


Figure 3. The promising compound in the protein active site (1CX2 crystallographic model)

The data obtained are an argument for studying the antiinflammatory activity for all six groups of compounds selected. Based on these data it has been determined that 4-amino-5-(pyridine-4-yl)-1,2,4-triazole(4H)-3-yl-thioacetamides and their pyrrol derivatives are characterized by the highest level of affinity calculated to all targets studied compared with other groups of compounds. Their affinity value is comparable to classical agonists, and it opens the opportunity of identifying new receptor agonists. The reliability of the prediction results has already been confirmed for one of the promising groups 4-amino-5-(pyridine-4-yl)-1,2,4-triazole(4H)-3-yl-thioacetamides.

The planned substances have been synthesized and tested for antiinflammatory activity, besides it has also been published scientifically (Chalenko *et al*., 2019).

CONCLUSION

In order to search for potential antiinflammatory agents 1,2,4-triazol-3-thiones have been selected as promising objects of chemical modification. The strategy of rational approaches to the search for selective COX-2 inhibitors as potential antiinflammatory agents has been proposed and elaborated. It is based on the use of the PASS-prediction and molecular docking. Based on the results of the PASS-prediction and molecular docking, six of the ten planned groups of compounds have been selected for the synthesis as promising selective COX-2 inhibitors.

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