PHARMACEUTICAL SCIENCES

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STUDY OF ANALGESIC AND ANTI-EXUDATIVE ACTIVITY OF COMPOSITIONS N-(3,4-DIMETHOXYPHENYL)-2- [4-AMINO-5-(PYRIDIN-4-YL)-4H-1,2,4-TRIAZOLE-3-YLTHIO]ACETAMIDE IN EXPERIMENT

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Abstract 1,2,4-triazole synthetic derivatives have significant pharmacological potential and are widely studied by Ukrainian and foreign scientists. According to recent studies, the heterocyclic system of 1,2,4-triazole is a promising fragment for the synthesis of new biologically active substances with different types of pharmacological action. Therefore, our study of analgesic and anti-inflammatory activity of 1,2,4-triazole derivatives may be the basis for the development of new pharmacological compositions. Moreover, it is very beneficial to search for substances with improved pharmacological activity and increased safety.

In medical practice, combination pharmacotherapy is often used to increase the effectiveness of existing drugs. Combinations of drugs increase the therapeutic effect, reduce the duration of treatment and prevent complications. The possibility of

obtaining more significant pharmacological activity from the combination in comparison with each individual drug became the basis for the creation of a new medicine.

Our current work is focused on the study of the analgesic and anti-inflammatory effects of 1,2,4-triazole derivative compositions with adjuvant caffeine and immunostimulant glucosaminyl-muramyl dipeptide.

Key words: triazole, analgesic, anti-exudative, pharmacological activity.

Introduction Modern pharmacy aims to focus on creating new modern domestic drugs that would be more effective in terms of their activity and relatively non-toxic. Therefore, the search for new pharmacologically active drugs with a minimum number of side effects is a relevant objective of modern pharmacy [1-6].

The record shows that the heterocyclic system of 1,2,4-triazole is a promising fragment for the synthesis of new biologically active compounds (BAC) and are widely used in drag design. 1,2,3-triazole derivatives exhibit various types of biological activity with different types of pharmacological action, in particular, analgesic (AnA) and anti-exudative (AeA) actions. [7-9].

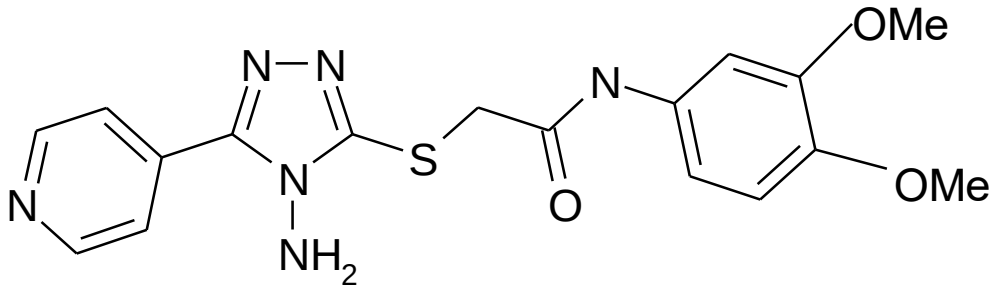
In medical practice, combination pharmacotherapy is often used to increase the efficacy of existing medicine using different fixed-combination of active pharmaceutical ingredients. Application of combination drugs increases the therapeutic effect, reduce the duration of treatment and prevent complications. As a rule, the main effects of combined drugs' use change in both pharmacokinetic and pharmacodynamic levels due to drug-drug interaction [10-17]. The possibility of obtaining more significant pharmacological activity from the combination in comparison with each individual drug became the basis for the creation of a new pharmaceutical compositions.

Combined anti-inflammatory and analgesic drugs often include adjuvant 1,3,7-thymethylxanthine (caffeine) as demonstrate the literature analysis and results of our previous studies [10-17], but pharmaceutical combinations with glucosaminyl-muramyl dipeptide (GMDP, licopid) are absent.

Therefore, our current study were focused on creating two pharmaceutical compositions: first (composition 1) consist of N-(3,4-dimethoxyphenyl)-2-[4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl-thio]acetamide (compound 1) and 1,3,7-thymethylxanthine; second (composition 2) include compound 1 and GMDP with subsequent investigation of AnA and AeA activity on rats under conditions of formalin-induced edema.

The goal of the investigation. Study of the effect of compound 1 on pain and exudation processes when it is mono-administered and in its compositions 1 and 2. In accordance with the set goal, we investigated the influence of 1,3,7-trimethylxanthine and GMDP on the AnA and AeA of compound 1.

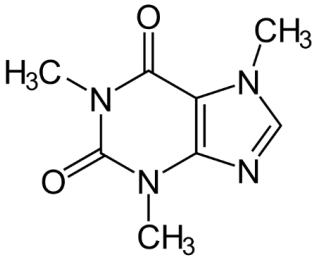
Materials And Methods The chemical structure of the synthesized compound 1 [18] is given below (pic. 1).



Pic. 1. N-(3,4-dimethoxyphenyl)-2-[4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl-thio]acetamide (compound 1).

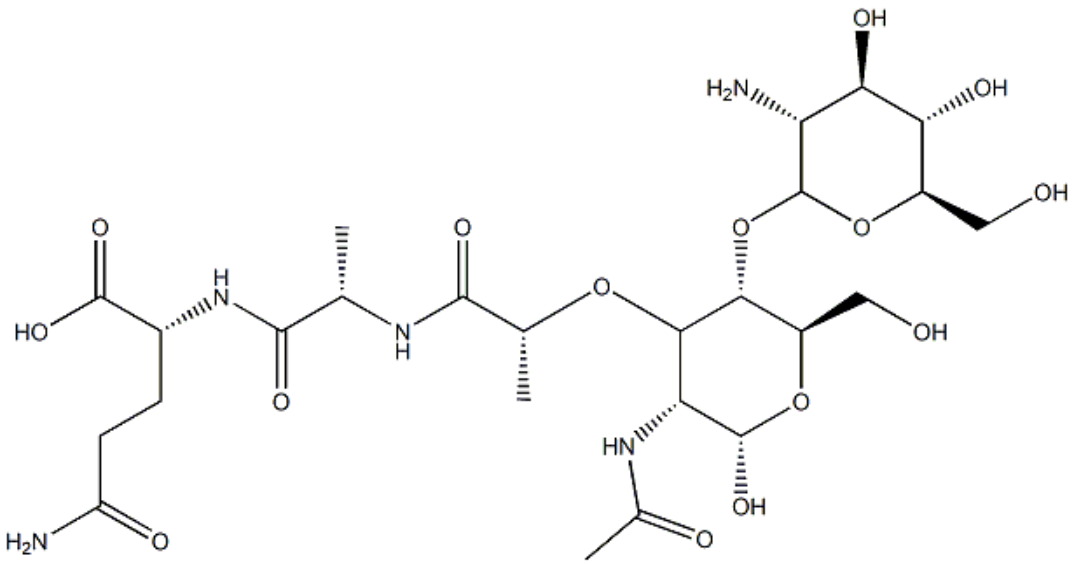
We studied AeA and its AnA [18].

In our research, we created two pharmaceutical compositions with potential adjuvants. Composition of pharmaceutical composition 1: compound 1 + 1,3,7-trimethylxanthine (Pic. 2)



Pic. 2. 1,3,7-trimethylxanthine

Components of the pharmaceutical composition 2: compound 1 + GMDP (Pic. 3)



Pic. 3. GMDP (glucosaminyl-muramyl dipeptide)

The conducted experiments and the doses of BAC were selected according to existing recommendations [19-20]. AnA and AeA of BAC and their pharmaceutical compositions were studied on white male rats using an experimental model of formalin-induced edema. The animals were divided into 7 groups of 6 animals in each group. In this experiment, aseptic-exudative inflammation was induced by subplantar injection of 0.1 ml of 2% formalin solution into the hind paw of animals. Animals received investigated compositions using simple technic of administration by oral gavage. The 1st control group were administered once 3% starch mucus (2 ml per 200 g of body weight of the animal) 1 hour before the development of maximum edema. The composition 1 with caffeine and the composition 2 with GMDP and a reference drug were introduced in a form of suspension on a starch mucus similarly. Animals of the 2nd - 4th groups received experimental drugs in 3% starch mucus: 2nd group - composition 1 at a dose of 10 mg per 1 kg of animal weight; 3rd group - 1,3,7-thymethylxanthine at the dose of 0.6 mg per 1 kg of animal weight; 4th group - GMDP at a dose of 0.6 mg per 1 kg of animal weight. Animals of the 5th group received the composition 1 (10 mg per 1 kg of animal weight) with 1,3,7-thymethylxanthine (0.6 mg per 1 kg of animal weight) and the 6th group - the composition 2 (10 mg per 1 kg of animal weight) with GMDP (0.6 mg per 1 kg of animal weight) respectively. Animals of the 7th group received reference drug diclofenac sodium at the dose of 8 mg per 1 kg of animals' weight.

An algesimeter (IITC Life Science, USA) that measures the threshold of tactile sensitivity were used for the evaluation of AnA. The Von Frey method is based on the measurement of tactile sensitivity to pain by applying the sharp filament of the sensor on in the central fold area of the hind paw of the animal. The withdrawal response was registered as a result to irritation. Measurements of the tactile pain sensitivity threshold were measured in grams before and 4 hours after induction of analgesia via subplantar administration of formalin. Investigated compositions were administered 1 hour before the maximum edema development. The obtained data were converted into the percentage of activity by the following formula:

$$AnA = \frac{\Delta Hc - \Delta Hex}{\Delta Hc} \cdot 100\%,$$

where AnA – analgesic activity (%);

ΔH_c – force applied on the paw in the control group (g);

ΔH_{ex} – force applied on the paw in the experimental group (g).

AeA (rat paw volume) was analyzed using a current-technology digital plethysmometer (IITC Life Science, USA) prior to drug administration and in 4 hours after modeling formalin injection in the maximum edema formation [6].

Relief of edema was expressed in milliliters. The percentage of inflammation suppression was calculated by the formula

$\frac{Vc-Vo}{Vc} \cdot 100\%$, where

Vo - the volume of the paw swelled in the experiment minus the initial paw volume in ml;

Vc - the volume of the paw swelled in the control minus the initial paw volume in ml.

Animals were kept in standard vivarium of the experimental biological clinic KhNMU in polypropylene cages on a standard diet with free access to water and food in compliance of storage, care and feeding norms specified in clinic's SOP. The temperature was kept at 19-24 °C, relative humidity did not exceed 60%, in compliance with sanitary and hygienic standards.

Experiment was performed according to the principles set out in the following

documents: Directive 210/63/EU of the European Parliament and of the EU Council "Protection of animals used for scientific purposes" (Brussels, 2010); «General ethical principles of animal experiments» (Kyiv, 2001); the Law of Ukraine «On protection of animals from of ill-treatment» №3477-IV of 21.02.2006 and Order of the Ministry of Education and Science of Youth and Sports of Ukraine“ On approval of the Procedure for conducting experiments, experiments on animals by scientific institutions” №249 of 01.03.2012 [4].

Statistics The obtained results were statistically controlled by methods of variation statistics using Student’s t-test and non-parametric methods of analysis (Mann-Whitney U Test) using computer programs STATISTICA 7.0, Stat Plus 2009 and MS Excel 2007.

Results And Discussions According to conducted experimental studies, the AnA and AeA of the compound 1 exceeded the reference drug by more than 1.6 – 1.7 times correspondingly. This effect can be explained by optimal molecular interactions of an anilide residue and two methoxy groups that present in the structure of N-(3,4-dimethoxyphenyl) -2-[4-amino-5-(pyridine-4-yl)-4H-1,2,4-triazol-3-ylthio]acetamide (compound 1) with the structure of a specific biological target. 1,3,7-trimethylxanthine showed the AnA value of 57.9% and AeA – 18.3%. GMDP appeared to be inactive regarding exudation and pain processes under a given set of experimental conditions.

Administration of composition 1 demonstrated that 1,3,7-trimethylxanthine potentiated both AnA and AeA. The obtained experimental data significantly exceeded the AnA and AeA of the reference drug, so we consider this composition 1 promising for further study (Table 1).

The addition of GMDP to compound 1 did not affect AnA and AeA of N-(3,4-dimethoxyphenyl)-2-[4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl-thio] acetamide under this experiment conditions (Table.1). Therefore, we consider it necessary to study AnA and AeA of composition 2 in other experimental conditions.

Table 1

Analgesic and anti-exudative activity of N-(3,4-dimethoxyphenyl)-2-[4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl-thio]acetamide compositions with potential adjuvants under conditions of formalin-induced edema in rats

№	Group	Analgesic activity, %	Anti-exudative activity, %
1.	Control	0	0
2.	Compound 1	72.9	75.9
3.	1,3,7-trimethylxanthine	57.9	18.3
4.	GMDP	0	0
5.	Composition 1	79.2	77.8
6.	Composition 2	72.9	75.9
7.	Diclofenac sodium	59.6	44.0

Conclusions

1. Two pharmaceutical two-component compositions with potential adjuvants were created based on the synthesized by us N-(3,4-dimethoxyphenyl)-2-[4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl-thio]acetamide (compound 1).

2. 1,3,7-trimethylxanthine (caffeine) and glucosaminyl-muramyl dipeptide (GMDP) were selected as adjuvant of analgesic and anti-exudative activity for compound 1: AnA of 1,3,7-trimethylxanthine was 57.9%, and AeA - 18.3%. The GMDP did not demonstrate AnA and AeA in these experimental conditions.

3. The pharmaceutical composition 1 (N-(3,4-dimethoxyphenyl)-2-[4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl-thio]acetamide with 1,3,7-trimethylxanthine) showed significant pharmacological activity compare to the reference drug. AnA comprised 72.9%, and AeA - 75.9%, which significantly exceeded the corresponding values of sodium diclofenac: 59.6% of AnA and 44% of AeA. Therefore, adding potential adjuvant 1,3,7-trimethylxanthine to the compound 1 is considered appropriate for AnA and AeA, and we consider that the pharmaceutical composition 1 is potential for advanced study.

4. The pharmaceutical composition 2 (N-(3,4-dimethoxyphenyl)-2-[4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl-thio]acetamide with GMPD) revealed AnA and

AeA similar to compound 1; the potential adjuvant GMDP turned out to be pharmacologically ineffective in relation to pain and exudation processes under the given conditions of the experiment. We continue to consider the study of AnA and AnA of pharmaceutical composition 2 in the other experimental conditions.

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