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SYNTHESIS AND BIOLOGICAL ACTIVITY OF [7-AMINO-S-TRIAZOLE[1,5-C]PYRMIDIDYL-5]-THIOACETIC ACID DERIVATIVES

L. N. Dianova, T. G. Koksharova, N. V. Volkova, G. M. Anoshina, V. I. Il'enko, and G. G. Vatulina

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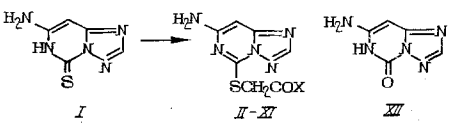
The s-triazole[1,5-c]pyrimidines constitute a limited and relatively little-studied class of compounds. Nevertheless, substances have been found among them that display a broad range of pharmacological activity, including bronchodilating [4], sedative, hypotensive [6], anti-inflammatory, and analgesic [5, 6] properties. This makes the synthesis and search for new biologically-active substances in the s-triazole[1,5-c]pyrimidine series particularly worthwhile.

The present work deals with the synthesis of (7-amino-s-triazole[1,5-c]pyrimidyl-5)-thioacetic acid (II) and its substituted derivatives (compounds III-XI) and cites the results obtained from investigating their antitumor, antiviral, and radiation-protective effects.

5(6H)Thio-7-amino-s-triazole[1,5-c]pyrimidine (I), which the authors have obtained in a previous investigation [2], was used as the starting reagent for the synthesis of compounds II-XI.

Acid II was synthesized by the reaction of compound I with chloro(bromo)acetic acid, the action of triazolepyrimidinethione with chloroacetic acid in 2 N NaOH at room temperature producing a low yield (~30%). When the reaction temperature was increased, a second product was formed (in addition to acid II), which was identified from analytical and spectral data as 5(6H)oxo-7-amino-s-triazole[1,5-c]pyrimidine (XII). Its IR spectrum contained an intensive carboxyl group absorption band at 1770 cm^{-1}. Proton signals for the pyrimidine and triazole rings and the amino group appeared in the PMR spectrum. Mass spectrometry revealed that the most intensive peak belongs to the M^+ (m/z 151) molecular ion, whose fragmentation occurs with the formation of the $M-HNCO^+$ (m/z 108) ion. When bromoacetic acid was used instead of chloroacetic, compound II was obtained with a yield of more than 70%.



I II-XI XII

X=OH (II), OMe (III), OEt (IV), NH_2 (V), NHMe (VI), NMe_2 (VII), $NHNH_2$ (VIII), $NHN=CHC_6H_4NO_2$-4 (IX), $NHN=CNC_6H_4OH$-2 (X), $NHN=CHCBr\cdot CHC_6H_5$ (XI)

S. M. Kirov Urals Polytechnical Institute, Sverdlovsk. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 26, No. 2, pp. 30-32, February, 1992. Original article submitted February 27, 1991.

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TABLE 1. Characteristics of s-Triazole[1,5-c]pyrmidines

Compound	Yield, %	mp, °C	Empirical formula	PMR spectra (DMSO-d_6), γ, ppm
II	74	250	$C_7H_7N_5O_2S$	8,13 (1H, s, 2-H); 6,18 (1H, s, 8-H); 4,14 (2H, s, S—CH_2); 6,56 (2H, NH_2)
III	75	144	$C_8H_9N_5O_2S$	8,15 (1H, s, 2-H); 6,18 (1H, s, 8-H); 4,22 (2H, s, S—CH_2); 3,70 (3H, s, O—CH_3); 1,15 (3H, s CH_3); 6,59 (2H, NH_2)
IV	77	86	$C_9H_{11}N_5O_2S$	8,15 (1H, s, 2-H); 6,15 (1H, s, 8-H); 4,15 (2H, q, O—CH_2); 6,52 (2H, NH_2)
V	97	203	$C_7H_8N_6OS$	8,15 (1H, s, 2-H); 6,15 (1H, s, 8-H); 3,93 (2H, s, S—CH_2); 6,56 (2H, NH_2); 7,25 (2H, NH_2)
VI	70	242	$C_8H_{10}N_6OS$	8,15 (1H, s, 2-H); 6,15 (1H, s, 8-H); 3,92 (2H, s, S—CH_2); 2,62 (3H, s CH_3); 6,58 (2H, NH_2); 7,98 (1H, NH)
VII	65	170	$C_9H_{12}N_6OS$	. . .
VIII	90	193	$C_7H_9N_7OS$	8,15 (1H, s, 2-H); 6,15 (1H, s, 8-H); 3,90 (2H, s, S—CH_2); 6,60 (2H, NH_2); 4,30 (2H, NH_2); 9,20 (1H, NH)
IX	73	255	$C_{14}H_{13}N_8O_3S$	8,23 (1H, s, 2-H); 6,19 (1H, s, 8-H); 7,95; 8,37 (1H, N=CH); 8,12 (4H, m Ph); 6,65 (2H, NH_2); 4,19; 4,66 (2H, NH_2); 12,00 (1H, NH)
X	76	198	$C_{14}H_{13}N_7O_2S$	8,20 (1H, s 2-H); 6,20 (1H, s, 8-H); 4,15; 4,57 (2H, s, S—CH_2), 8,37; 8,49 (1H, N=CH); 6,73—7,87 (4H, m, Ph); 6,62 (2H, NH_2); 10,1; 11,0 (OH)
XI	73	238	$C_{16}H_{14}BrN_7OS$	8,21 (1H, s, 2-H); 6,16 (1H, s, 8-H); 4,13; 4,55 (2H, s, S—CH_2); 7,30—8,05 (7H, m); 6,60 (2H, NH_2); 11,80; 11,87 (1H, NH)
XII	90	300	$C_5H_5N_5O$	8,15 (1H, s, 2-H); 5,92 (1H, s, 8-H); 6,50 (2H, NH_2)

Methyl and ethyl (7-amino-s-triazole[1,5-c]pyrimidyl-5)thioacetates (III, IV) were synthesized by reacting compound I with methyl or ethyl chloroacetate in solutions of the corresponding alcoholates. The substances obtained readily underwent ester interchange and were quickly hydrolyzed to acid II in acid and alkaline solutions.

The (7-amino-s-triazole[1,5-c]pyrimidyl-5)thioacetamides V-VII were obtained by reacting esters III and IV with ammonia, as well as with aqueous methylamine and dimethylamine solutions. When the reaction was carried out with a concentrated ammonia solution, the main reaction product was acid II, rather than amide V, which was formed in low yield. By passing gaseous ammonia into an alcoholic solution of the ester, the end product yield was raised to 50%. Amide V was obtained in high yield by boiling I with chloroacetamide in a sodium alcoholate solution. When the esters were reacted with aqueous methyl- and dimethylamine at room temperature, amides VI and VII, respectively, were obtained.

The reaction of compounds III and IV with hydrazine-hydrate in aqueous ethanol produced hydrazide VIII, which formed aralkylidenehydrazones IX-XI with p-nitrobenzaldehyde, and salicyl and α-bromocinnamic aldehydes. A double set of S—CH_2 proton signals and a CH=N fragment proton in the PMR spectrum indicates that they exist as a mixture of two stereoisomers.

The structure of the newly-obtained compounds was borne out by IR, PMR, MS, and elemental analysis (data from the latter was in accord with calculated values). Their physical properties are given in Table 1.

EXPERIMENTAL (CHEMICAL)

TLC on Silufol UV-254 plates (Czechoslovakia) was used to monitor the course of the reactions and verify the individuality of the compounds. IR spectra were taken on a UR-20 spectrometer (KBr). PMR spectra were recorded on a Perkin-Elmer R-12B instrument (60 MHz) in DMSO-d_6 solution, internal standard TMS. Mass spectra were registered on a MAT-311A (accelerating voltage 70 eV).

(7-Amino-s-triazole[1,5-c]pyrimidyl-5)Thioacetic Acid (II). A sample of 0.2 g (1.3 mmoles) of compound I was dissolved in 5 ml of 2 N KOH solution, to which was added 0.24 g (1.7 mmoles) of bromoacetic acid. After stirring for 1 h at room temperature the solution was neutralized with concentrated hydrochloric acid, the precipitate was filtered off and recrystallized from aqueous ethanol.

Methyl (7-Amino-s-triazole[1,5-c]pyrimidyl-5)Thioacetate (III). A suspension of 0.67 g (4 mmoles) of compound I in 30 ml of dry methanol was added to 0.24 g (4.5 mmoles) sodium methylate, then a solution of 0.7 ml (8 mmoles) of methyl chloroacetate in 10 ml of methanol was poured in small amounts. After boiling for 3 h the mixture was cooled and NaCl was filtered off. The filtrate was evaporated to dryness at reduced pressure and the residue was treated with water. The precipitate was filtered off and recrystallized from aqueous ethanol.

Ethyl (7-Amino-s-triazole[1,5-c]pyrimidyl-5)Thioacetate (IV). This was obtained similarly to compound III from 1 g (6 mmoles) of compound I in 30 ml of dry ethanol, 0.44 g (6.5 mmoles) of sodium ethylate and 1.3 ml (11 mmoles) of ethyl chloroacetate.

Amide of (7-Amino-s-triazole[1,5-c]pyrimidyl-5)Thioacetic Acid (V). A. To a suspension of 0.80 g (4.8 mmoles) of compound I in 50 ml of dry ethanol were added with boiling 0.28 g (5.2 mmoles) of sodium ethylate and 0.49 g (5.3 mmoles) of chloroacetamide. The mixture was boiled for 3 h with stirring and NaCl was filtered off. The filtrate was boiled down at reduced pressure to half its volume and the product was precipitated with water. The precipitate was filtered off and recrystallized from water.

B. Ammonia was passed through a solution of 0.3 g (1.3 mmoles) of compound III in 20 ml of dry ethanol for 2 h. The solvent was boiled down at reduced pressure to half its volume, then the product was precipitated with water, filtered, and recrystallized from water.

Methylamide of (7-Amino-s-triazole[1,5-c]pyrimidyl-5)Thioacetic Acid (VI). A suspension of 0.5 g of compound III (IV) in 10 ml of 25% aqueous methylamine solution was stirred at room temperature for 5 min. The precipitate was filtered off and recrystallized from aqueous ethanol.

Dimethylamide of (7-Amino-s-triazole[1,5-c]pyrimidyl-5) Thioacetic Acid (VII) was obtained similarly to compound VI from 0.5 g of compound III (IV) and a 33% aqueous dimethylamine solution.

Hydrazide of (7-Amino-s-triazole[1,5-c]pyrimidyl-5)Thioacetic Acid (VIII). To a solution of 0.3 g (1.2 mmoles) of compound IV in 30 ml of ethanol were added 1 ml (10 mmoles) of 50% hydrazine-hydrate and 10 ml of diethyl ester. The mixture was stirred at room temperature for 0.5 h, then the precipitate was filtered off and recrystallized from aqueous ethanol.

Aralkylidenehydrazides of (7-Amino-s-triazole[1,5-c]pyrimidyl-5)Thioacetic Acid (IX-XI). A sample of 0.2 g (8.4 mmoles) of compound VIII was dissolved in 10 ml of 50% aqueous ethanol and 8.4 mmoles of the corresponding aldehyde was added. After boiling for 2 h the reaction mass was cooled and the precipitate was filtered off. It was then washed with ether or acetone and crystallized.

EXPERIMENTAL (BIOLOGICAL)

The antitumor activity of the synthesized substances was tested on $C57Bl_6$ mice and outbred mice, using experimental tumor strains (mammary adenocarcinoma AK-755, sarcomas 37 and 180) obtained from the strain bank of the Soviet Academy of Medical Sciences' Cytology Department. Six animals were tested for each dose and 18 animals comprised the control group. Treatment was begun 48 hours after tumor transplantation. The substances were administered in starch suspensions by intraperitoneal injection five times every 24 hours. The animals were destroyed 14 days after the start of the experiment. Antitumor effects were assessed in terms of percentage inhibition of tumor growth. In addition, the median lethal dose, LD_{50}, of the substances was determined. The compounds were introduced in doses of 200, 400, 600, 800, and 1000 mg/kg by single intraperitoneal injection into 6 mice for each dosage. After 14 days the animals were destroyed. During the observation period the animals were seen to be in good general condition with smooth, shiny coats and a normal appetite. No deviations in terms of behavior or respiratory system were encountered. None of the animals died in this 14-day period.

It was found that the tested compounds were of low toxicity (LD_{50} of 1000 mg/kg) and some (compounds II, V, VIII-XI) had an effect on tumor growth. Acid II, for example, had a moderate antitumor effect on sarcoma 37 (49 ± 3.2% tumor growth inhibition) and a slightly stimulating effect on the growth of AK-755. Amide V, however, suppressed the growth of both sarcoma 37 and AK-755 by 35 ± 2.3%. Acid hydrazide VIII caused the growth of AK-755 to be inhibited by 35 ± 2.1%, while showing a slight stimulating effect towards sarcoma 37. A clearly-defined stimulating effect of between 58 and 200% on the growth of sarcoma 37 was observed for the hydrazide hydrazones IX-XI. None of the substances proved active with respect to sarcoma 180 in the tests.

The antiviral activity of the compounds was tested on white mice infected with type A and type B influenza viruses. At least two tests were carried out for each compound,

40 mice being used for each experiment. Each preparation was administered in five stages, at 24 hours and one hour before infection, and at 24, 48, and 72 hours after infection, in order to test both the prophylactic and therapeutic properties of the compounds. Each experiment included three subjects: mice given the test compound; mice given a compound that is active with respect to the tested virus (remantidin for influenza type A virus and adapromin for type B); mice given a placebo (usually a physiologic saline solution or water) instead of the test compound. Activity investigation and assessment was carried out using the system outlined in the methodology [3].

Tests showed that ethyl (7-amino-s-triazole[1,5-c]pyrimidyl-5)thioacetate (IV) is of the greatest interest, as it displayed antiviral activity with respect to type A and type B influenza viruses in the animal experiment (protection index was 61 ± 8.5% for type A and 63 ± 9.2% for type B).

Testing of radiation-protective properties was carried out on male C57B1/6 mice weighing 20-22 g. The preparations were administered by intraperitoneal injection in a Tween-water solution (1:19). The doses causing a definite probability of death in the test animals (LD_{16}, LD_{50}, and LD_{84}) were determined using the Litchfield and Wilcoxon modification probit analysis method [1]. The development of poisoning and death struggle symptoms were observed for a preparation dose of 1/3 LD_{50} at 15, 30, and 60 minutes before radiation. The animals were exposed to a gamma-ray source with dose rate of 1.1 crg/sec. Radiation dosage corresponded to the minimum absolute lethal dose (LD_{95-99}) of 8 gr. Radiation protection effects were evaluated in terms of 30-day survival and mean lifespan of dead animals.

Moderate radiation protection properties were displayed by compound IV, which increased the survival rate of the animals by 60% compared to the control group for a 7.5 gr radiation dose.

Thus, for the first time compounds have been identified in the s-triazole[1,5-c]pyrimidine series that display antitumor, antiviral, and radiation-protection activity, underlining the fact that it is advisable to continue the search for biologically-active substances in this series.

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