and chromatographed in the same system, passing the solvents six times through the plate. From the lower zone, 0.1 g (7.2%) of 1-(2-desoxy-α-D-ribofuranosyl)-5-isopropoxy-methyluracil (X) was isolated. UV spectrum; $\lambda_{max}$ 266 nm, ε 10000. $C_{13}H_0N_2O_6 \cdot 3H_2O$.

## LITERATURE CITED


1. T. A. Bektemirov, É. V. Chekuniva, O. G. Andzhaparidze, et al., Vopr. Virul., No. 6, 603-606 (1979).
2. S. Ya. Mel'nik, A. A. Bakhmedova, I. V. Yartseva, et al., Bioorgan. Khim., 8, No. 8, 1102-1107 (1982).
3. S. Ya. Mel'nik, T. D. Miniker, I. V. Yartseva, et al., Bioorgan. Khim., 9, No. 10, 1395-1400 (1983).
4. S. Ya. Mel'nik, A. A. Bakhmedova, T. D. Miniker, et al., Bioorgan. Khim., 10, No. 12, 1645-1654 (1984).
5. S. Ya. Mel'nik, A. A. Bakhmedova, T. P. Nedorezova, et al., Bioorgan. Khim., 11, No. 9, 1248-1252 (1985).
6. S. Ya. Mel'nik, A. A. Bakhmedova, I. V. Yartseva, et al., Bioorgan. Khim., 13, No. 7, 934-940 (1982).

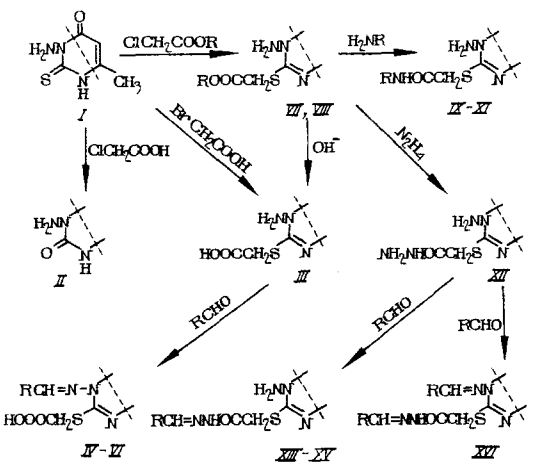

# ANTIVIRAL ACTIVITY OF PYRIMIDIN-2-YLTHIOACETIC ACID DERIVATIVES


T. G. Koksharova, N. V. Volkova,
L. N. Dianova, V. I. Il'chenko,
V. G. Platonov, and I. R. Shcherbakova



UDC 615.281.8:547.844.012.1


Pyrimidinylthioacetic acids have a wide spectrum of biological activity, including the antiviral activity [1, 2, 5, 6]. To study their anti-influenza activity, we prepared the derivatives of 1-amino-4-methyl-6-oxopyrimidin-2-ylthioacetic acid (III) — compounds IV-XVI (Table 1).



R=H(IX), $CH_3$(VII, X), $C_2H_5$(VIII), $CH_2CH_2OH$(XI), $C_6H_4NO_2$-*n*(IV, XIII), $C_6H_5$(V, XIV), $C_6H_4OH$-*o*(VI, XV, XVI).

One of the main methods of synthesis of heterylthioacetic acids is alkylation of mercapto derivatives with haloacetic acids. The starting 1-amino-4-methyl-6-oxo-2(3H)-pyrimidinethione (I) did not react with chloroacetic acid at room temperature in a 1 N KOH solution, while with increase in the temperature, the main reaction product was pyrimidinone


S. M. Kirov Ural' Polytechnical Institute, Sverdlovsk. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 26, No. 3, pp. 57-59, March, 1992. Original article submitted February 27, 1991.



0091-150X/92/2603-0255$12.50

TABLE 1. 1-Amino-4-methyl-6-oxopyrimidin-2-ylthioacetic Acid (III) and Its Derivatives IV-XVI

| Compound | Yield, % | mp., °C | $R_f$ | Empirical formula |
|---|---|---|---|---|
| III | 83 | 185 | 0,57(*Б*) | $C_7H_9N_3O_3S$ |
| IV | 65 | 219 | 0,65(*А*) | $C_{14}H_{12}N_4O_5S$ |
| V | 41 | 207 | 0,66(*А*) | $C_{14}H_{13}N_3O_3S$ |
| VI | 20 | 179 | 0,68(*А*) | $C_{14}H_{13}N_3O_4S$ |
| VII | 78 | 94 | 0,44(*Б*) | $C_8H_{11}N_3O_3S$ |
| VIII | 65 | 96 | 0,59(*Б*) | $C_9H_{13}N_3O_3S$ |
| IX | 68 | 190 | 0,62(*А*) | $C_7H_{10}N_4O_2S$ |
| X | 70 | 202 | 0,64(*А*) | $C_8H_{12}N_4O_2S$ |
| XI | 10 | 213 | 0,27(*Б*) | $C_9H_{14}N_4O_3S$ |
| XII | 85 | 189 | 0,48(*А*) | $C_7H_{11}N_5O_2S$ |
| XIII | 63 | 244 | 0,82(*А*) | $C_{14}H_{14}N_6O_4S$ |
| XIV | 71 | 184 | 0,87(*А*) | $C_{14}H_{15}N_5O_2S$ |
| XV | 82 | 210 | 0,8(*А*) | $C_{14}H_{15}N_5O_3S$ |
| XVI | 47 | 224 | 0,3(*Б*) | $C_{21}H_{19}N_5O_4S$ |

TABLE 2. Spectral Characteristics of Compounds III-XVI

| Compound | UV, $\lambda_{max}$ (log ε, EtOH) | IR, ν, $cm^{-1}$ | PMR, δ, ppm |
|---|---|---|---|
| III | 257(4,12) | 1740, 1670(CO) 3350, 3295(NH) | 2,1 (d, 3H, $CH_3$); 3,85 (s, 2H, $SCH_2$); 5,79 (s, 2H, $NH_2$); 6,1 (q, 1H, H-5); $JCH_3$, H-5 0,8 Hz |
| IV | 278(4,17) | 1730, 1695(CO) | 2,12 (s, 2H, $CH_3$); 3,86 (s, 2H, $SCH_2$); 6,1 (s, 1H, H-5); 7,85—8,3 (m, 4H, P); 11,2 (s, 1H, CH=N) |
| V | | 1730, 1600(CO) | 2,2 (s, 3H, $CH_3$); 3,9 (s, 2H, $SCH_2$); 6,22 (s, 1H, H-5); 7,48—8,05 (m, 5H, Ph); 9,22 (s, 1H, CH=N) |
| VI | 268(4,19) 325 (4,02) | 1740, 1700(CO) | 2,19 (s, 3H, $CH_3$); 3,9 (s, 2H, $SCH_2$); 6,22 (s, 1H, H-5); 6,9—7,98 (m, 5H, Ph, OH); 9,34 (s, 1H, CH=N) |
| VII | 220(3,93) 285(3,92) | 1730, 1690(CO) 3285, 3185(NH) | 2,15 (s, 3H, $CH_3$); 3,7 (s, 3H, $OCH_3$); 3,9 (s, 2H, $SCH_2$); 5,8 (s, 2H, $NH_2$); 6,15 (s, 1H, H-5) |
| VIII | 219(3,96) 285(3,96) | 1720, 1695(CO) 3280, 3170(NH) | 1,25 (t, $CH_3$, 3H); 2,15 (s, 3H, $CH_3$); 3,9 (s, 2H, $SCH_2$); 4,2 (q, 2H, $CH_2$); 5,8 (s, 2H, $NH_2$); 6,15 (s, 1H, H-5) |
| IX | 222(4,01) 286(3,10) | 1675, 1660(CO) 3375, 3300(NH) | 2,15 (s, 3H, $CH_3$); 3,7 (s, 2H, $SCH_2$); 5,72 (s, 2H, $NH_2$); 6,08 (s, 1H, H-5); 7,1 (s, 2H, $NH_2$) |
| X | 282(3,98) | 1690, 1665(CO) 3370, 3310(NH) | 2,14 (s, 3H, $CH_3$); 2,6 (s, 3H, $CH_3$); 3,7 (s, 2H, $SCH_2$); 5,71 (s, 2H, $NH_2$); 6,07 (s, 1H, H-5) |
| XI | | 1680(CO) 3190, 3080(NH) | 2,3(s, 3H, $CH_3$); 3,7 (s, 4H, $SCH_2$, $CH_2$); 4,41 (t, 2H, $CH_2$); 6,05 (s, 1H, H-5); 4,49 (s, 2H, $NH_2$); 11,29 (s, 1H, NH) |
| XII | 285(4,04) | 1685, 1650(CO) 3310, 3220(NH) | 2,2 (d, 3H, $CH_3$); 3,7 (s, 2H, $SCH_2$); 5,74 (s, 2H, $NH_2$); 6,1 (q, 1H, H—5); $JCH_3$, H-5 0,8 Hz |
| XIII | | 1700, 1665(CO) 3310, 3220(NH) | 2,06; 2,13 (3H, $CH_3$); 3,89; 4,33 (2H, $SCH_2$); 5,76 (s, 2H, $NH_2$); 6,06 (s, 1H, H-5); 7,9—8,3 (m, 4H, Ph); 11,8; 11,95 (1H, CH=N) |
| XIV | | 1690, 1670(CO) 3310, 3220(CO) | 2,2 (s, 3H, $CH_3$); 3,88; 4,33 (2H, $SCH_2$); 5,8 (s, 2H, $NH_2$); 6,13 (s, 1H, X-5); 7,3—7,8 (m, 5H, Ph); 8,1; 8,28 (1H, CH=N) |
| XV | 281(4,45) 290(4,43) 322(4,01) | 1705, 1675(CO) 3280, 3200(NH) | 2,2 (d, 3H, $CH_3$); 3,9; 4,33 (2H, $SCH_2$); 5,81 (s, 2H, $NH_2$); 6,15 (q, 1H, H-5); 6,6—7,7 (m, 4H, Ph); 8,4; 8,5 (1H, CH=N) $JCH_3$, H-50,9 Hz |
| XVI | | 1700, 1670(CO) | 2,24 (s, 3H, $CH_3$); 4,01—4—44 (m, 2H, $SCH_2$); 6,24 (s, 1H, H-5); 6,8—7,9 (m, 8H, Ph); 8,44; 8,50 (1H, CH=N); 9,42 (s, 1H, CH=N) |

II, When bromoacetic acid was used, the reaction proceeded at room temperature, and acid III was isolated in a high yield. Of the several possible paths of reaction, the S-alkylation is indicated by the spectral data (Table 2).

Reaction of acid III with aldehydes gave azomethines IV-VI, which readily undergo hydrolysis in solutions of acids and alkalies, which, according to the literature data, may account for the presence of a biological activity of these compounds [7].

With methyl and ethyl esters of chloroacetic acid in solutions of the corresponding alcoholates, compound I forms low-melting esters of pyrimidin-2-ylthioacetic acid VII, VIII. The two compounds rapidly hydrolyze into III in solutions of alkalies showing stability toward acid hydrolysis. Esters VII and VIII were converted by the action of concentrated ammonia solution into an amide of pyrimidinylthioacetic acid IX, but in a low yield, since the main reaction product was acid III. Compound IX was obtained in a high yield from I and chloroacetamide. Products X-XII were obtained by the reaction of VII and VIII with methylamine, ethanolamine and hydrazine hydrate; bands are observed in their IR spectra absorption corresponding to the vibrations of CO and NH groups. The chemical shifts of all the groups of protons in the PMR spectra are located in intervals confirming the structures of the compounds.

TABLE 3. Results of Testing Antiviral Activity

| Compound | Influenza virus type A | | Influenza virus type B | |
|---|---|---|---|---|
| | in ovo | in vivo | in ovo | in vivo |
| I | — | — | — | — |
| III | + | — | — | — |
| IV | — | ++ | — | ++ |
| VI | — | ++ | — | ++ |
| IX | + | — | — | — |
| XII | ++ | +++ | — | + |
| XIII | — | — | — | — |
| XV | + | — | — | — |
| Remantadine | ++++ | ++++ | — | — |
| Adapromine | | | ++++ | ++++ |

Note. Values of effectivity indexes: less than 30%; + 30-39%; ++ 40-59%; +++ 60-79%; ++++ more than 80%.

Hydrazones XIII-XV were obtained by the reaction of equimolar amounts of compounds XII and the corresponding aldehydes. The N-amino group underwent reaction with an excess of the aldehyde and product XVI separated out. The characteristic feature of the PMR spectra of the hydrazones consisting in splitting of signals of the S-$CH_2$ group protons and of a proton of the CH=N fragment, indicates that compounds XIII-XVI exist in the form of mixtures of two isomers. The ratio of the isomers is constant, is independent of the nature of the aldehyde residue and was determined as equal to 3:2. From the good agreement with the literature data, this type of isomerism can be characterized as syn-anti [4].

## EXPERIMENTAL (CHEMICAL)

The IR spectra were recorded on a Specord UR-752 spectrophotometer (KBr tablets), the PMR spectra, on a Perkin-Elmer spectrometer (60 MHz) in DMSO-$d_6$, using TMS as internal standard. The UV spectra were run on a Beckman Spectrophotometer UV-5270. The purity of the compounds was monitored by TLC on Silufol UV-254 plates using the systems of solvents A (propanol-ammonia, 3:1); and B (chloroform–ethanol, 9:1), with detection in UV light. The elemental analysis data correspond to the calculated values. The characteristics of the synthesized compounds are presented in Table 1.

1-Amino-4-methyl-6-oxo-2(3H)pyrimidinethione (I) was obtained according to [8].

1-Amino-4-methyl-6-oxopyrimidin-2-ylthioacetic Acid (III). A 0.2 g portion (13 mmoles) of pyrimidine I was dissolved in 5 ml of a 2 N solution of KOH and 0.24 g (17 mmoles) of bromoacetic acid was added. After stirring for 3 h at room temperature, the solution was neutralized with concentrated HCl. The precipitate was filtered off.

1-p-Nitro-benzylideneamino-4-methyl-6-oxopyrimidin-2-ylthioacetic Acid (IV). A 0.05 g portion (23 mmoles) of compound III (23 mmoles) of compound III was dissolved with heating in 10 ml of ethanol, 0.36 g (23 mmoles) of p-nitro-benzaldehyde was added and the mixture was boiled for 4 h, was then cooled and the precipitate was filtered off.

Compounds V and VI were obtained in a similar way.

Methyl ester of 1-Amino-4-methyl-6-oxopyrimidin-2-ylthioacetic Acid (VII). A 0.62 g portion (4 mmoles) of pyrimidine I was heated to boiling in 30 ml of anhydrous methanol, and then sodium methylate was added (prepared from 0.1 g of sodium and 2 ml of methanol). A solution of 0.7 ml (8 mmoles) methyl chloroacetate in methanol was added in portions, the mixture was boiled with stirring for 4 h, and NaCl was filtered off. The filtrate was evaporated to half its volume. The residue was diluted with water, and the product was filtered off. Compound VIII (R = $C_2H_5$) was obtained in a similar way.

1-Amino-4-methyl-6-oxopyrimidin-2-ylthioacetic Acid Amide (IX). A) A suspension of 1 mmole of VII or VIII was stirred for 5 min in 10 ml of a 25% $NH_4OH$ solution and the precipitate was quickly filtered off.

B) A 0.75 g portion (48 mmoles) of I was boiled in 60 ml of anhydrous ethanol for 30 min, and then a solution of sodium ethylate (prepared from 0.12 g of Na in 3 ml of anhydrous

ethanol) and 0.29 g (53 mmoles) of chloroacetamide were added. The mixture was boiled with stirring for 5 h, then was cooled, and the product was filtered off.

1-Amino-4-methyl-6-oxopyrimidin-2-ylthioacetic Acid Methylamide (X). A suspension of 0.2 g (10 mmoles) of VII was stirred for 5 min at room temperature in 10 ml of a 25% aqueous solution of methylamine. The precipitate was filtered off.

1-Amino-4-methyl-6-oxopyrimidin-2-ylthioacetic Acid Monoethanolamide (XI). A 0.2 g portion (1 mmole) of VII was dissolved in 20 ml of ethanol and 0.22 ml (4 mmoles) of monoethanolamine was added. The mixture was boiled for 8 h, then evaporated to dryness, and the residue was washed with chloroform.

1-Amino-4-methyl-6-oxopyrimidin-2-ylthioacetic Acid Hydrazide (XII). A 1 mmole portion of VII or VIII was dissolved with stirring in 20 ml of anhydrous ethanol and 0.5 ml of hydrazine hydrate was added. After 1 h the precipitate was filtered off.

Hydrazones XIII-XV. Equimolar amounts of compound XII and the corresponding aldehyde were boiled in an ethanolic solution to completion of the reaction (TLC). The solvent was evaporated to dryness, and the residue was washed with ether.

## EXPERIMENTAL (BIOLOGICAL)

The investigation of the anti-influenza activity was carried out on developing chicken embryos and white mice, infected with the influenza virus type A and B. Each experiment included three objects: the embryos (mice) to which the tested compound was introduced; embryos (mice), to which a compound active with respect to the virus studied was introduced – remantadine for the influenza virus type A and adapromine for virus type B; embryos (mice) to which a placebo was introduced (a physiological solution or distilled water). In the tests in vivo, the preparation was introduced five times: 24 and 1 h before the infection and 24, 48 and 72 h after infection, which presupposes the simultaneous manifestation of the prophylactic and therapeutic action. The activity was evaluated according to a scheme presented in instructions approved by the Ministry of Public Health of the USSR [3].

The data in Table 3 show that the derivatives of 1-amino-4-methyl-6-oxopyrimidin-2-ylthioacetic acid have an antiviral action mainly with respect to the type A influenza virus. In experiments on animals the effect is greater than on the chicken embryos. The most active is hydrazide XII, while on transition to hydrazones the activity disappears. At the same time azomethines IV and VI displayed a medium activity in vivo for both strains of the virus, which is clearly due to a biotransformation of the compounds in the organism.

## LITERATURE CITED


1. P. I. Vainilavichus, V. S. Lauchis, and M. S. Mushkauskaite, Zh. Vses. Khim. O-va. D. I. Mendeleeva, 22, 474-476 (1977).
2. P. I. Vainilavichus, M. M. Burbulene, and N. D. Lautsyuvene, Khim. farm. Zh., 16, 35-38 (1982).
3. V. I. Il'enko. Methods of Testing and Evaluation of Antiviral Activity of Chemical Compounds with Respect to Influenza Virus [in Russian], Leningrad (1977).
4. Yu. P. Kitaev and V. I. Buzykin, Hydrazones [in Russian], Nauka, Moscow (1974).
5. Czechoslovakian Patent 88060 (1958): Ref. Khim. Zh. 85959 (1960).
6. Z. Budesinsky and V. Letovsky, Czechosl. Farmac., 158, 432-437 (1966).
7. J. H. Billman and M. S. Khan, J. Pharmac. Sci., 57, 1817 (1968).
8. T. Tsuji and T. Ueda, Chem. Pharm. Bull., 19, 2530-2533 (1971).