Pharmaceutical Chemistry Journal, Vol. 28, No. 3, 1994

SYNTHESIS AND BIOLOGICAL ACTIVITY OF 8-ALKYL(ARYL)-6-CYANOPYRIDO[2,3-d]-PYRIMIDINE-2,4,5-TRIONES

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UDC 547.859.1:542.451.8

It was previously shown [1-4] that the heterocyclization of the 1,3-dimethyl-5-cyanacetyl-6-aminouracils (I)-(VI) in an alkaline or acidic medium leads to the 7-amino- or 7-hydroxypyrido[2,3-d]pyrimidines correspondingly. With the object of isolating compounds with potential antibacterial activity, we continued the study of the chemical properties of the compounds (I)-(IV) and realized their reaction with the diethylacetals of DMF and DMA using an excess of the reagent. The 6-cyano-8-alkyl(benzyl, phenyl)pyrido[2,3-d]pyrimidine-2,4,5-triones (V)-(XII) were thereby obtained.

The PMR spectra of the compounds (V)-(VIII), obtained by the reaction of the uracils (I)-(IV) with the diethylacetal of DMF contain the signal of the C(7)-H protons in the region of 8.48-8.60 ppm. The PMR spectra of the pyrido[2,3-d]pyrimidines (IX)-(XII), synthesized with the participation of the diethylacetal of DNA and containing the methyl group at the position 7, contain the signal of the C(7)$-CH_3$ protons in the region of 2.30-2.76 ppm.

With the object of synthesizing compounds containing the 6-pyridinecarboxylic acid fragment of the antibacterial preparations pipemidic and pyromidic acids [5, 6], the conditions of the hydrolysis of the 6-cyano derivatives (V)-(XII) in alkaline and acidic media were studied. It was found that the heating of the compounds in 20% NaOH leads to the formation of a complex mixture of products, from which we could not isolate individual substances. The acid hydrolysis of the compounds was accomplished in concentrated sulfuric acid. The amides (XIV) and (XV) and carboxylic acid (XVI) and (XVII) were thereby obtained from the 6-cyano derivatives (VII) and (XI) depending on the conditions of the process.

TABLE 1. Synthesis and Physicochemical Characteristics of the Compounds (V)-(XVII)

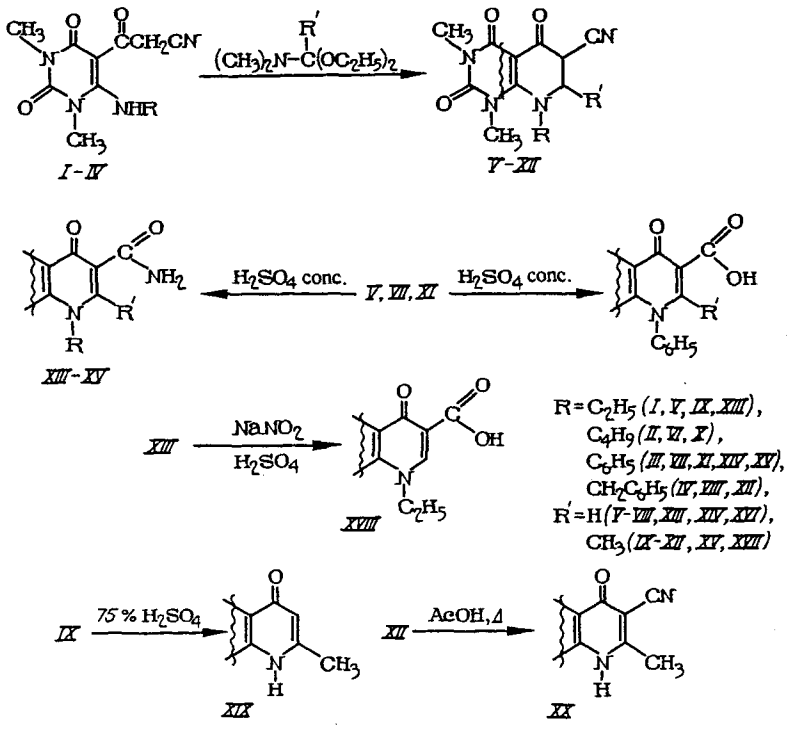
Compound	Reaction conditions			Yield, %	mp, °C	Mass spectrum, m/z (I/I_{max}, %)	Empirical formula
	reagent	reaction time, h	temperature, °C				
V	A	0,5	20	100	263—4	$M^{+\cdot}$ 260 (100)	$C_{12}H_{12}N_4O_3$
VI	A	0,5	40	42	289	$M^{+\cdot}$ 288 (72)	$C_{14}H_{16}N_4O_3$
VII	A	0,5	20	80	>300	...	$C_{16}H_{12}N_4O_3$
VIII	A	0,5	40	92	240—1	$M^{+\cdot}$ 322 (37)	$C_{17}H_{14}N_4O_3$
IX	B	0,5	20	82	268,5	$M^{+\cdot}$ 274 (100)	$C_{13}H_{14}N_4O_3$
X	B	0,5	70	42	245—6	$M^{+\cdot}$ 302 (89)	$C_{15}H_{18}N_4O_3$
XI	B	0,5	20	97	>300	...	$C_{17}H_{14}N_4O_3$
XII	B	0,5	70	94	142—3	$M^{+\cdot}$ 336 (26)	$C_{18}H_{16}N_4O_3$
XIII	C	0,5	50	53	302	...	$C_{12}H_{14}N_4O_3$
XIV	C	4,0	150	80	278	$M^{+\cdot}$ 326 (29)	$C_{16}H_{14}N_4O_3$
XV	C	4,0	120	36	289	$M^{+\cdot}$ 340 (2)	$C_{17}H_{16}N_4O_3$
XVI	C	7,0	190	46	272,5	...	$C_{16}H_{13}N_3O_5$
XVII	C	7,0	150	91	283—4	$M^{+\cdot}$ 341 (2)	$C_{17}H_{15}N_3O_5$

Notes. A) N,N-dimethylformamide diethylacetal; B) N,N-dimethylacetamide diethylacetal; C) concentrated H_2SO_4. All substances besides (X) were recrystallized from DMF; (X) was recrystallized from i-PrOH.

Novokuznetsk Chemico-Pharmaceutical Scientific-Research Institute. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 28, No. 3, pp. 39-42, March, 1994. Original article submitted May 25, 1993.

0091-150X/94/2803-0185$12.50

The hydrolysis of the nitrile group of the 8-alkyl(benzyl) derivatives (VIII), (IX), and (XII) in an acidic medium is complicated by the cleavage of the alkyl (benzyl) groups at the N(8) and, in individual cases, the decarboxylation of the intermediate carboxylic acids. Only the amide (XIII) was obtained from the 8-ethyl derivative (V) when it was heated in concentrated sulfuric acid. The treatment of the resulting amide (XIII) with sodium nitrite leads to the 6-carboxy-8-was ethylpyrido[2,3-d]pyrimidine (XVIII) with the yield of 68% calculated on the basis of the 6-cyanopyridopyrimidine (V). The heating of compound (IX) in sulfuric acid at 100°C gives the 1,3,7-trimethylpyrido[2,3-d]pyrimidine-2,4,5-trione (XIX), which previously described [7]. The attempt to recrystallize the compound (XII) in glacial AcOH ended in the formation of the derivative (XX), unsubstituted at the position 8.



EXPERIMENTAL (CHEMICAL)

The IR spectra were recorded on the "Specord IR-75" instrument using KBr tablets. The PMR spectra were recorded on the "Tesla BS-497" instrument (100 MHz) with HMDS as the internal standard. The mass spectra were obtained on the "Varian MAT-311" instrument with the direct input of the sample at the ion source.

The data of the elemental analysis of the compounds synthesized for C, H, and N correspond with the calculated values. The main characteristics of the compounds are presented in the Tables 1 and 2.

1,3-Dimethyl-8-ethyl-6-cyano-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidine-2,4,5-trione (V). To 0.5 g (2.0 mmole) of compound (I) are added 1.7 ml (5.0 mmole) of dimethylformamide diethylacetal, and the mixture is stirred for 0.5 h at 20°C. Diethyl ether (30 ml) is added, and the mixture is stirred; the residue is filtered off, washed with 3 ml of acetone and 5 ml of water, and dried. Compound (V) is obtained with the yield of 0.52 g (100%). The compounds (VI)-(XII) are obtained analogously.

1,3-Dimethyl-8-ethyl-6-carboxamido-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidine-2,4,5-trione (XIII). To 1.1 g (4.0 mmole) of compound (V) are added 5.5 ml of concentrated H_2SO_4; the mixture is heated to 50°C and stirred for 2 h. The mixture is cooled to 50°C and stirred for 2 h. The mixture is cooled to 5°C and poured onto ince with stirring; the 20% aqueous solution of NaOH is added dropwise until the pH 3.0 is reached. The precipitated residue is filtered off and washed with water to the pH 7.0. Compound (XIII) is obtained with the yield of 0.44 g (38%). The compounds (XIV) and (XV) are obtained analogously.

1,3-Dimethyl-8-ethyl-6-carboxy-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidine-2,4,5-trione (XVIII). The solution of 5.0 g (19.0 mmole) of compound (V) in 10 ml of concentrated H_2SO_4 is heated to 50°C and stirred for 30 min. The mixture is cooled to 0°C prior to the dropwise addition of 7 ml of water and 6.0 g (71 mmole) of $NaNO_2$ in portions at 0-5°C. The

TABLE 2. IR and PMR Spectra of the Compounds (V)-(XVIII) and (XX)

Compound	IR spectrum, ν_{max}, cm^{-1}	PMR spectrum (in CF_3COOD), δ, ppm (SSCC, J, Hz)
V	1680, 1693, 1720 (CO); 2200 (CN)	1,32 (3H, t, J=6), 3,14 (3H, s), 3,50 (3H, s), 4,31 (2H, q, J=6), 8,60 (1H, s)
VI	1680, 1693, 1727 (CO); 2227 (CN)	0,50 (3H, t, J=6), 0,85—1,25 (2H, m), 1,50—1,70 (2H, m), 3,15 (3H, s), 3,51 (3H, s), 4,26 (2H, t, J=6), 8,59 (1H, s)
VII	1633, 1673, 1726 (CO); 2220 (CN)	2,74 (3H, s), 3,17 (3H, s), 7,20—7,40 (5H, m), 8,58 (1H, s)
VIII	1680, 1706, 1733 (CO); 2227 (CN)	3,12 (3H, s), 3,40 (3H, s), 4,95 (2H, s), 6,93 (5H, s), 8,48 (1H, s)
IX	1667, 1693, 1720 (CO); 2227 (CN)	1,12 (3H, t, J=6), 2,76 (3H, s), 3,13 (3H, s), 3,46 (3H, s) 4,37 (2H, q, J=6)
X	1673, 1687, 1733 (CO); 2220 (CN)	0,52 (3H, t, J=6), 0,80—1,04 (2H, m), 1,18—1,42 (2H, m), 2,75 (3H, s), 3,14 (3H, s), 3,46 (3H, s), 4,32 (2H, t, J=6)
XI	1667, 1687, 1733 (CO); 2213 (CN)	2,30 (3H, s), 2,64 (3H, s), 3,13 (3H, s), 7,12—7,42 (5H, m)
XII	1680, 1693 1727 (CO); 2220 (CN)	2,32 (3H, s), 3,12 (3H, s) 3,48 (3H, s), 5,32 (2H, s), 6,94—7,34 (5H, m)
XIII	1653, 1680, 1720 (CO); 3125, 3253 (NH_2)	1,34 (3H, t, J=7), 3,17 (3H, s), 3,53 (3H, s), 4,36 (2H, q, J=7); 8,91 (1H, s)
XIV	1667, 1680, 1720 (CO)	2,70 (3H, s), 3,18 (3H, s), 7,23—7,36 (5H, m), 8,81 (1H, s)
XV	1667, 1680, 1713 (CO); 3040, 3172, 3367 (NH_2)	1,97 (3H, s), 2,61 (3H, s), 3,10 (3H, s), 7,55 (5H, s)
XVIII	1660, 1700, 1713 (CO)	1,33 (3H, t, J=7), 3,13 (3H, s), 3,47 (3H, s), 4,26 (2H, q, J=7), 8,60 (1H, s)
XVI	1660, 1720, 1733 (CO)	2,67 (3H, s), 3,17 (3H, s), 7,54—7,80 (5H, m), 8,19 (1H, s)
XVII	1692, 1707, 1713, 1733 (CO)	2,23 (3H, C), 2,59 (3H, s), 3,11 (3H, s), 7,50—7,70 (5H, m)
XX	1653, 1667, 1733 (CO); 227 (CN)	2,56 (3H, s), 3,15 (3H, s), 3,46 (3H, s)

Note. The OR spectra were recorded in mineral oil.

sodium sulfate is filtered off. The filtrate is diluted with 20 ml of water, and the precipitated residue is filtered off and washed with water until the pH 7.0 is reached. Compound (XVIII) is obtained with the yield of 3.8 g (68%).

1,3-Dimethyl-8-phenyl-6-carboxy-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidine-2,4,5-trione (XIX). The solution of 0.5 g (1.8 mmole) of compound (IX) in 5 ml of 75% H_2SO_4 is heated to 100°C and stirred at this temperature for 2 h. After the cooling to 20°C, the mixture is combined with 20 ml of water and cooled to 5°C. The precipitated residue is filtered off, washed with water to the pH 7.0, and dried. Compound (XIX) is obtained with the yield of 0.15 g (37%).

1,3,7-Trimethyl-6-cyano-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidine-2,4,5-trione (XX). The suspension of 2.0 g (5.9 mmole) of compound (XII) in 10 ml of glacial AcOH is heated until the complete solution of the residue is achieved at 118°C in the course of 0.5 h. The mixture is cooled to 20°C, and the precipitated residue is filtered off, washed with water to the pH 7.0, and dried. Compound (XX) is obtained with the yield of 0.8 g (55%).

EXPERIMENTAL (BIOLOGICAL)

The antibacterial and antituberculosis activity of the compounds synthesized was studied in relation to a series of microorganisms (Table 3). Test cultures utilized were 16 species of aerobic and facultative-anaerobic pathogenic and conditionally pathogenic microorganisms, 3 species of spore-forming aerobes and anaerobes (*Clostridium perfringens*), and 1 strain of *Mycobacterium tuberculosis* of the human type H37Rv.

The antimicrobial activity was determined by the method of serial dilutions with the utilization of the corresponding nutrient media: thioglycollate medium for the study of the sensitivity of anaerobes, Löwenstein–Jensen medium for *Mycobacterium tuberculosis*, and beef–peptone agar for the remaining species of microorganisms. The degree of antibacterial activity was evaluated from the MIC value. For the better differentiation of the preparations according to their antimicrobial action, the study of the sensitivity of the cultures was commenced from the concentration of 0.4% (4 mg/ml).

The results of the study showed that, on the whole, higher antimicrobial activity is possessed by those derivatives of 1,3-dimethylpyrido[2,3-d]pyrimidine which have the ethyl group at the position 8 [(V), (XIII), (XVII)]. No clear dependence of the activity on the presence of carboxy or carboxamide groups at the position 6 was shown, but it was established that the combination of the carboxamide group with the ethyl group (XIII) significantly increases the antibacterial activity [the mean MIC for (XIII) in relation to the test organisms studied is 1.34 mg/ml] in comparison with the 8-phenyl derivatives (VII) and (XI) (the mean MICs are 4.83 and 4.07 mg/ml correspondingly). Such a correlation is not observed when the carboxy group is introduced into the compounds having the phenyl or ethyl radical in their structure [(XVI)-(XVIII)]. Their antibacterial activity is practically the same: the mean MIC comprises 2.1-2.7 mg/ml. According to their action on microorganisms, all the compounds studied can be placed in the following order: (XIII) > (XVII) > (V) > (XVII) > (XVIII) > (VII) > (XV). Therefore, the most marked antibacterial properties (on the level of antiseptics) are possessed by 1,3-dimethyl-8-ethyl-6-carboxamido-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidine-2,4,5-trione (XIII), the mean MIC of which is 1.34 mg/ml.

TABLE 3. Antibacterial Activity of Derivatives of Hexahydropyrido[2,3-d]pyrimidine

Species of microorganisms	Minimal inhibitory concentration (MIC), mg/ml						
	XV	XVII	IX	XVI	XIII	XVIII	VII
St. aureus 209P	4,0	3,0	1,0	3,0	4,0	2,0	4,0
Streptococcus durans	4,0	3,0	4,0	3,0	4,0	2,0	2,0
Escherichia coli 675	4,0	4,0	2,0	3,0	2,0	2,0	4,0
Escherichia coli 0111	4,0	3,0	2,0	4,0	0,2	2,0	4,0
Citrobacter	4,0	1,5	4,0	2,0	2,0	2,0	4,0
Hafnia	2,0	...	4,0	1,5	0,8	2,0	4,0
Serrafia	4,0	...	4,0	3,0	2,0	2,0	4,0
Klebsiella	4,0	4,0	0,4	2,0	0,9	2,0	2,0
Proteus rettgeri	4,0	1,5	1,0	0,2	0,2	0,2	2,0
Pseudomonas aeruginosa 165	4,0	3,0	2,0	2,0	4,0	2,0	4,0
Salmonella paratyphi A	4,0	3,0	1,0	3,0	0,8	2,0	4,0
Salmonella paratyphi B	4,0	4,0	1,0	4,0	1,0	2,0	4,0
Salmonella tuphimurium	4,0	3,0	1,0	3,0	0,8	2,0	4,0
Salmonella typhi	4,0	1,2	0,8	1,0	0,8	0,8	4,0
Shigella flexheri 2a	...	...	1,0	2,0	0,2	1,5	4,0
Shigella sonnae	4,0	3,0	2,0	1,5	0,1	2,0	4,0
Yersinia enterocolitica	4,0	0,8	1,0	0,1	0,2	1,5	2,0
Yersinia preudotuberculosis	...	0,15	0,5	0,05	0,08	0,2	2,5
Bacillus cereus	4,0	0,8	4,0	0,8	0,8	0,2	3,0
Bacillus anthracoides 1312	4,0	0,8	4,0	0,8	0,8	0,4	3,0
Clostridium perfringens	0,5	1,2	1,0	1,0	0,5	0,5	1,5
Mycobacterium tuberculosis H37RV	1,0	1,0	1,0	0,5	1,0	1,0	0,5

The most sensitive to the compounds studied are Yersinia accompanying anaerobes (*Clostridium) Proteus*, and *Shigella flexneri*. The growth of *Mycobacterium tuberculosis* was inhibited at concentrations of 0.5-31.0 mg/ml.

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