Preparation, Characterization, and Antimicrobial Activity of Some 5-Alkyl-2,4,6-substituted Pyrimidines

M. KOÓŠ and Z. NOVOTNÁ

Institute of Chemistry, Slovak Academy of Sciences, SK-842 38 Bratislava

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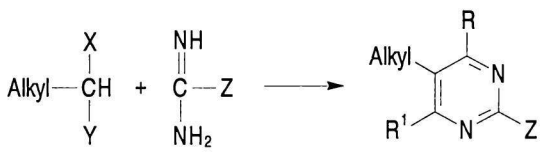
Fifteen new 5-alkyl-2,4,6-substituted pyrimidines were prepared by the cyclization reactions from corresponding alkylmalononitriles, ethyl 2-cyanoalkanoates or 2-alkyl-3-oxobutyrates where alkyl represented hexyl, heptyl or octyl. Spectral data as well as values of minimum inhibitory concentration against selected microorganisms are given. No significant antimicrobial efficiency was found in this respect.

It is known that some pyrimidine derivatives exhibit biological activity. Among them, bactericides [1—3], fungicides [4, 5], and antimycotics [6, 7] can be mentioned. Several pyrimidine pesticides are also produced industrially (Pirimor, Actellic, Basudin, Milcurb). Our previous findings [8, 9] that some five-membered nitrogen-containing heterocycles having hexyl, heptyl or octyl substituents in their molecules exhibit remarkable antimicrobial effects stimulated us to investigate also analogous alkyl derivatives of some substituted pyrimidines in this respect.

By the reaction of alkylmalononitriles (alkyl = hexyl, heptyl, octyl) with guanidine or thiourea, corresponding 5-alkyl-2,4,6-triaminopyrimidines *I*—*III* or 5-alkyl-4,6-diamino-2-mercaptopyrimidines *IV*—*VI* were prepared (Scheme 1, Series A and Series B). New starting alkylmalononitriles were obtained by dehydration of corresponding 2-alkyl-2-cyanoacetamides with phosphorus oxychloride according to the procedure given for malononitrile [10]. The use of thionyl chloride as a dehydrating agent led to the formation of highly polymeric material exclusively. Among the starting 2-alkyl-2-cyanoacetamides, only heptyl derivative was described in the literature [11]. Cyclization of ethyl 2-cyanoalkanoates with guanidine or urea afforded the corresponding 5-alkyl-2,4-diamino-6-hydroxypyrimidines *VII*—*IX* (Series C) or 5-alkyl-4-amino-2,6-dihydroxypyrimidines *X*—*XII* (Series D). Similarly, 5-alkyl-6-hydroxy-2-mercapto-4-methylpyrimidines *XIII*—*XV* (Series E) were obtained from ethyl 2-alkyl-3-oxobutyrates and thiourea. In accordance with an analogy from the literature [12—16], all these cyclizations proceeded in good yields (70—80 %). The survey of the prepared compounds and their characteristics are presented in Table 1.

In the IR spectra of the prepared pyrimidine compounds, characteristic bands at $\tilde{\nu}$ = 1606—1609 cm^{-1}, $\tilde{\nu}$ = 1558—1579 cm^{-1}, and $\tilde{\nu}$ = 1060—1080 cm^{-1} corresponding to the vibration of pyrimidine skeleton were observed. Mass spectra of all these compounds exhibited the peaks of molecular ions $[M]^+$ (I_r = 15—85 %). The base peaks (I_r = 100 %) in the



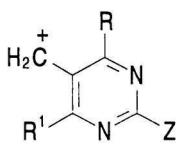
Series A:	X = Y = CN, Z = NH_2	*I*—*III*	R = R^1 = NH_2, Z = NH_2
Series B:	X = Y = CN, Z = SH	*IV*—*VI*	R = R^1 = NH_2, Z = SH
Series C:	X = CN, Y = COOEt, Z = NH_2	*VII*—*IX*	R = Z = NH_2, R^1 = OH
Series D:	X = CN, Y = COOEt, Z = OH	*X*—*XII*	R = NH_2, R^1 = Z = OH
Series E:	X = $COCH_3$, Y = COOEt, Z = SH	*XIII*—*XV*	R = CH_3, R^1 = OH, Z = SH

Scheme 1

Table 1. Characterization of the Prepared Compounds

Compound	Alkyl	R	R^1	Z	Formula	M_r	Yield/%	M.p./°C
I	Hexyl	NH_2	NH_2	NH_2	$C_{10}H_{19}N_5$	209.30	81	123—124
II	Heptyl	NH_2	NH_2	NH_2	$C_{11}H_{21}N_5$	213.32	79	136—137
III	Octyl	NH_2	NH_2	NH_2	$C_{12}H_{23}N_5$	227.34	80	129—130
IV	Hexyl	NH_2	NH_2	SH	$C_{10}H_{18}N_4S$	226.34	74	240—241
V	Heptyl	NH_2	NH_2	SH	$C_{11}H_{20}N_4S$	240.37	73	240—241
VI	Octyl	NH_2	NH_2	SH	$C_{12}H_{22}N_4S$	254.38	76	243—246
VII	Hexyl	NH_2	OH	NH_2	$C_{10}H_{18}N_4O$	210.26	72	145—146
VIII	Heptyl	NH_2	OH	NH_2	$C_{11}H_{20}N_4O$	224.30	70	143—144
IX	Octyl	NH_2	OH	NH_2	$C_{12}H_{22}N_4O$	238.32	69	145—146
X	Hexyl	NH_2	OH	OH	$C_{10}H_{17}N_3O_2$	211.29	71	236—237
XI	Heptyl	NH_2	OH	OH	$C_{11}H_{19}N_3O_2$	225.31	70	235—236
XII	Octyl	NH_2	OH	OH	$C_{12}H_{21}N_3O_2$	239.32	71	237—238
XIII	Hexyl	CH_3	OH	SH	$C_{11}H_{18}N_2OS$	226.32	75	186—187
XIV	Heptyl	CH_3	OH	SH	$C_{12}H_{20}N_2OS$	240.35	74	183—184
XV	Octyl	CH_3	OH	SH	$C_{13}H_{22}N_2OS$	254.37	72	184—185

spectra corresponded to the fragments resulting from α-cleavage (Formula 1). This fragmentation was con-



R	R^1	Z	*m/z*
NH_2	NH_2	NH_2	138
NH_2	NH_2	SH	155
NH_2	OH	NH_2	139
NH_2	OH	OH	140
CH_3	OH	SH	155

Formula 1

firmed by the presence of peaks of corresponding metastable ions.

^1H and ^{13}C NMR spectral data of the representative compounds are given in Experimental. These data reflect the possibility of different structural features of the objective pyrimidine derivatives (oxo-enol, amino-imino, thio-thioxo tautomerism).

The results of antimicrobial activity testing are summarized in Table 2. As a standard for determination of values of minimum inhibitory concentration (MIC) we used [1-(ethoxycarbonyl)pentadecyl]trimethylammonium bromide (Septonex), an antiseptic agent usually applied in practice. As can be seen, discussed 5-alkyl-2,4,6-substituted pyrimidines *I*—*XV* exhibit only low (MIC = 1000 ppm) or moderate (MIC = 10—100 ppm) antimicrobial activity, especially against some gram-positive bacteria.

EXPERIMENTAL

Starting ethyl 2-cyanoalkanoates and ethyl 2-alkyl-3-oxobutyrates were prepared according to the known procedures [17—19]. The other used chemi-

Table 2. Antimicrobial Activity (MIC/(μg cm^{-3})) of the Prepared Compounds

Compound	*Staphylococcus aureus*	*Bacillus subtilis*	*Streptococcus faecalis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella typhimurium*	*Candida albicans*
I	100	100	1000	1000	1000	1000	1000
II	10	10	<1000	<1000	100	100	<1000
III	10	10	100	100	100	100	100
IV	100	100	<1000	1000	<1000	1000	<1000
V	<100	10	<1000	<1000	<1000	<1000	1000
VI	100	100	<1000	1000	<1000	100	1000
VII	100	100	100	1000	1000	1000	<1000
VIII	<100	100	<1000	1000	1000	<1000	100
IX	100	<1000	<1000	1000	1000	1000	<1000
X	100	100	1000	1000	1000	<1000	1000
XI	100	100	1000	1000	1000	1000	<1000
XII	<1000	<1000	1000	1000	1000	<1000	1000
XIII	1000	1000	1000	1000	1000	1000	1000
XIV	<1000	1000	<1000	1000	1000	1000	1000
XV	<1000	<1000	<1000	1000	1000	1000	1000
Septonex	0.1	0.1	1	<1000	100	10	100

cals were commercially available products (Fluka, Buchs; Merck, Darmstadt; Lachema, Brno).

Melting points were determined on a Boetius PHMK 05 microscope. IR spectra (KBr pellets) were obtained on a Perkin—Elmer G-983 instrument. Mass spectra (70 eV) were measured on a Jeol JMS-100D spectrometer at an emission current of 300 μA, applying direct sample-introduction technique. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AM-300 spectrometer operating at 300.13 MHz or 75.46 MHz working frequencies in DMSO-d_6 or $CDCl_3$ solutions with TMS as an internal standard. For the assignment of signals in ^{13}C NMR spectra, DEPT and semiselective INEPT techniques were used. (*Note*: A prime index refers to the positions of corresponding alkyl at C-5.) Elemental analyses (with deviations from calculated values: ± 0.06 % C; ± 0.04 % H; ± 0.03 % N; ± 0.03 % S) were performed on a Perkin—Elmer 240 analyzer.

MIC was determined by using the suspension method on solid cultivation media [8].

Alkylmalononitriles

A mixture of corresponding ethyl 2-cyanoalkanoate (0.2 mol) and ammonium hydroxide (28 %, 50 cm^3) was stirred at room temperature for 1 h and then it was left to stand in refrigerator for 4 d. After addition of cold ethanol (25 cm^3), the solid product was filtered with suction, washed twice with cold ethanol (10 cm^3), and dried in vacuum desiccator to give corresponding 2-alkyl-2-cyanoacetamide (about 85 % yield; for hexyl and octyl derivative m.p. = 131—132 °C). To a solution of the above 2-alkyl-2-cyanoacetamide (0.15 mol) in 1,2-dichloroethane (150 cm^3), sodium chloride (10 g) and phosphorus oxychloride (8 cm^3, 31 mmol) were added at room temperature. Reaction mixture was stirred and heated under reflux for 8 h. After cooling, decantation of insoluble material and washing with 1,2-dichloroethane (10 cm^3), the solvent was distilled off and the residue was fractionated at reduced pressure giving corresponding alkylmalononitrile (about 83 % yields); hexylmalononitrile: b.p. = 96—98 °C (105 Pa); heptylmalononitrile: b.p. = 107—109 °C (105 Pa); octylmalononitrile: b.p. = 118—120 °C (105 Pa).

5-Alkyl-2,4,6-triaminopyrimidines *I—III*

To a solution of guanidine (10 mmol) (prepared from 10 mmol of guanidine carbonate and 28 mmol of sodium ethoxide in 25 cm^3 of dry ethanol and filtration to remove sodium carbonate), corresponding alkylmalononitrile (22 mmol) in dry ethanol (10 cm^3) was added. The mixture was heated under reflux for 6 h and left to stand overnight at room temperature. After decolourizing (with charcoal) and filtration of the hot solution, white product crystallized on cooling. Recrystallization from ethanol gave pure product in about 80 % yield.

Compound *I*: ^{1}H NMR spectrum ($CDCl_3$), δ: 4.56 (bs, 6H, 3 × NH_2), 2.20 (t, 2H, the first CH_2 in hexyl, J = 8.1 Hz), 1.46 (m, 2H, the second CH_2), 1.29 (m, 6H, the other CH_2), 0.89 (t, 3H, CH_3, J = 6.8 Hz). ^{13}C NMR spectrum ($CDCl_3$), δ: 161.9 (C-4, C-6), 160.5 (C-2), 88.2 (C-5), 31.8 (C-1′), 29.5 (C-2′), 27.9 (C-3′), 24.4 (C-4′), 22.6 (C-5′), 14.1 (CH_3).

5-Alkyl-4,6-diamino-2-mercaptopyrimidines *IV—VI*

A mixture of thiourea (20 mmol), sodium ethoxide (20 mmol), and corresponding alkylmalononitrile (20 mmol) in dry ethanol (30 cm^3) was heated under reflux for 3 h. Then, hot water (100 cm^3) was added followed by an addition of acetic acid (1.3 cm^3). After cooling, the separated product was filtered off and crystallized (after decolourizing with charcoal) from ethanol affording about 75 % yield of product.

Compound *V*: ^{1}H NMR spectrum (DMSO), δ: 6.58 (bs, 1H, SH), 3.57 (bs, 4H, 2 × NH_2), 2.28 (bs, 2H, the first CH_2 in heptyl), 1.36 (m, 10H, the other CH_2), 0.94 (t, 3H, CH_3, J = 6.5 Hz). ^{13}C NMR spectrum (DMSO), δ: 174.3 (C-4, C-6), 153.4 (C-2), 85.8 (C-5), 31.5 (C-1′), 29.0 (C-2′), 28.7 (C-3′), 27.5 (C-4′), 22.2 (C-5′), 22.0 (C-6′), 14.0 (CH_3).

5-Alkyl-2,4-diamino-6-hydroxypyrimidines *VII—IX*

To a cold solution of sodium ethoxide (20 mmol) in dry ethanol (20 cm^3), corresponding ethyl 2-cyanoalkanoate (20 mmol) in dry ethanol (10 cm^3) was added. After 10 min, ethanolic solution of guanidine (20 mmol; prepared as above) was added and the reaction mixture was heated under reflux for 2 h. Then, the solvent was evaporated, the solid product was dissolved in hot (80 °C) water and acidified with acetic acid (1.3 cm^3). Separated product was filtered off and recrystallized from ethanol—water. The yield was about 70 %.

Compound *VII*: ^{1}H NMR spectrum (DMSO), δ: 7.65 (bs, 1H, NH_{oxo}), 6.48 (bs, 1H, OH), 4.03 (bs, 8H, 4 × NH_2 (both tautomers)), 2.25 (bs, 2H, the first CH_2 in hexyl), 1.34 (m, 8H, the other CH_2), 0.93 (t, 3H, CH_3, J = 6.4 Hz). ^{13}C NMR spectrum (DMSO), δ: 168.8 (C-4 and C-4_{oxo}), 162.7 (C-2 and C-2_{oxo}), 160.9 (C-6_{oxo}), 153.0 (C-6), 120.2 (C-5_{oxo}), 87.9 (C-5), 31.7 (C-1′), 29.8 (C-2′), 28.2 (C-3′), 26.7 (C-4′), 22.2 (C-5′), 14.0 (CH_3).

5-Alkyl-4-amino-2,6-dihydroxypyrimidines *X—XII*

A mixture of sodium ethoxide (40 mmol) in dry ethanol (25 cm^3), corresponding ethyl 2-cyanoalkanoate (20 mmol), and urea (20 mmol) was

heated under reflux with stirring for 4 h. Then hot (80 °C) water (25 cm^3) was added and the reaction mixture was stirred for 10 min at 80 °C followed by neutralization with acetic acid (2.6 cm^3). After cooling, the separated product was filtered off and recrystallized from ethanol to yield about 70 % of product.

Compound *XI*: ^{1}H NMR spectrum (DMSO), δ: 7.88 (bs, 1H, NH), 3.67 (bs, 2H, NH_2), 2.24 (t, 2H, the first CH_2 in heptyl, J = 7.8 Hz), 1.28 (bs, 10H, the other CH_2), 0.92 (t, 3H, CH_3, J = 6.5 Hz). ^{13}C NMR spectrum (DMSO), δ: 162.8 (C-6), 158.1 (C-4), 153.3 (C-2), 88.5 (C-5), 31.4 (C-1′), 29.2 (C-2′), 28.4 (C-3′), 27.3 (C-4′), 22.4 (C-5′), 22.1 (C-6′), 14.2 (CH_3).

5-Alkyl-6-hydroxy-2-mercapto-4-methylpyrimidines *XIII—XV*

A mixture of sodium methoxide (44 mmol) in dry methanol (30 cm^3), thiourea (20 mmol), and corresponding ethyl 2-alkyl-3-oxobutyrate (20 mmol) was heated under reflux for 6 h and then it was allowed to stand overnight at room temperature. The solvent was evaporated, water (20 cm^3) was added and the product was precipitated by the addition of acetic acid (3.0 cm^3). Crude product was recrystallized (after decolourizing with charcoal) from ethanol giving about 75 % yield of pure product.

Compound *XV*: ^{1}H NMR spectrum (DMSO), δ: 2.31 (t, 2H, the first CH_2 in octyl, J = 6.9 Hz), 2.18 (s, 3H, CH_3 at C-4), 1.31 (bs, 12H, the other CH_2), 0.93 (t, 3H, CH_3, J = 6.4 Hz). ^{13}C NMR spectrum (DMSO), δ: 173.6 (C-2), 161.3 (C-6), 148.1 (C-4), 115.0 (C-5), 31.4 (C-1′), 29.0 (C-2′, C-3′), 28.8 (C-4′), 28.1 (C-5′), 24.1 (C-6′), 22.2 (CH_3 at C-4), 15.7 (C-7′), 14.0 (CH_3).

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