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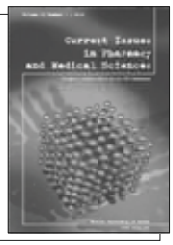

Synthesis and antimicrobial activity of 1-allyloxymethylpyrimidine derivatives

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ABSTRACT

The ways of obtaining a series of novel pyrimidine derivatives containing allyloxymethyl substituent is presented in position 1. The characterized compounds were tested for antimicrobial activity.

Keywords: 1-allyloxymethylpyrimidine derivatives, synthesis, antimicrobial activity

INTRODUCTION

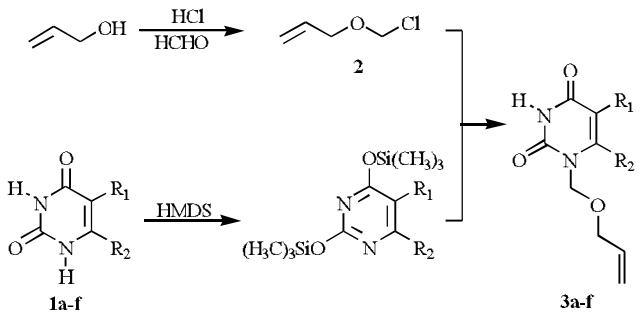
Pyrimidines are reported to have a broad spectrum of biological activities. Some are endowed with antitumor, antiviral, antimicrobial and antifungal properties [1, 2, 4, 8]. Of these, N1- or N1, N3-substituted 5-iodouracil and 5-hydroxymethyluracil (pyrimidine analogs) were reported to exhibit antibacterial activity, displaying inhibition against *Branhamella catarrhalis*, *Neisseria mucosa*, *Streptococcus pyogenes*. No antifungal activity however, was observed for all tested compounds [14].

Effective antitumor and antiviral therapeutic agents are substances which are enzyme inhibitors. Of these inhibitors, so far, both uridine (EC 2.4.2.3) and thymidine (EC 2.4.2.4) phosphorylase inhibitors have been examined. Uridine phosphorylase is responsible for the activation and deactivation of nucleosides, and their analogs are used in antitumor and antiviral treatment [4, 7]. Moreover, during treatments with the use of 5-fluorouracil (5-FU), in order to extend the time of exposure, the giving of phosphorylase inhibitors seems to be necessary. In addition, to decrease 5-FU toxicity, uridine is used. Because the plasma half-life of uridine is only 2 minutes, it is necessary to use uridine phosphorylase inhibitors. As a result of uridine phosphorolysis inhibition, the time of exposure and protective uridine activity in 5-FU presence is lengthened [16]. Using phosphorylase inhibitors undeniably protects against the 5-FU toxic activity, and at the same time, does not decrease its antitumor activity [13].

Because of this, researchers have been searching for substances which are potential uridine and thymidine phosphorylase inhibitors. Among the examined pyrimidine nucleoside analogs were: 5-bromoacyclouridine [16, 13, 10], 5-(phenylthio)acyclouridine [4, 1], 5-(phenylselenyl)acyclouridine [2], 5-(3-phenoxybenzyl)acyclouridine [11]. All of these uridine derivatives contain in position 1 2-hydroxyethoxymethyl group. In regard to the thymidine phosphorylase inhibitors, researchers have examined 5- and 6-substituent uracil derivatives [6].

EXPERIMENTAL DESIGN

The synthesis of new 1-allyloxymethyluracil derivatives was carried out according to the scheme shown as Fig. 1.



	a	b	c	d	e	f
R_1	$-NO_2$	-Br	-Me	$-(CH_2)_3-$	$-(CH_2)_4-$	-H
R_2	-H	-H	-H			-NHAc

Fig. 1. Scheme of synthesis of 1-allyloxymethyluracil derivatives

Moreover, allyloxymethylation of 4,6-dihydroxy-5-nitropyrimidine **4** was carried out and 1-allyloxymethyl-4,6-dihydroxy-5-nitropyrimidine **5** was obtained. Beyond this, allyl chloromethyl ether **2** was obtained as a result of passing

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gaseous hydrogen chloride through the mixture of allyl alcohol and paraformaldehyde according to [5]

The general method of obtaining 1-allyloxymethylpyrimidine derivatives **3a-f**, **5** was by: 0.01 mol compound **1a-f** or **4** being heated under reflux with 60 ml heksamethyldisilazane HMDS, and 125 mg of anhydrous ammonium sulphate being added in order to obtain the clear solution [according to 10]. The HMDS excess was then distilled under reduced pressure, and 0.02 mol allyl chloromethyl ether was added to the remains. Following this, the mixture was kept at room temperature for 24 h. After the unreacted ether filtration was decanted, the product was purified by crystallization.

The UV spectra were taken on a spectrophotometer Aquarius7250 (Cecil Instruments), while the ^{1}H NMR spectra were taken on either a Bruker Avance 200 spectrometer at 200MHz, or a Bruker DRX500 spectrometer at 500 MHz in DMSO-d_6 with TMS as the internal standard. The mass spectra were taken on a Finnigan MAT 95 mass spectrometer.

4,6-dihydroxy-5-nitropyrimidine (4) was obtained by nitration of 4,6-dihydroxypyrimidine (Fluka) with 65% nitric acid in acetic anhydride, according to [3]. The yield was 50%.

6-acetyloaminouracil (1f) was obtained by acetylation of 6-aminouracil (Aldrich), according to the procedure published in [15]. Yield 70%.

1-allyloxymethyl-5-nitrouracil (3a) was obtained in 22%; mp 114-117°C; R_f 0.59 (Alugram SIL G/UV-254, chloroform–EtOH, 92:8). UV spectrum (H_2O + 1% EtOH), λ_{max} (ε): 235.5 nm (8700), 300 nm (10800). ^{1}H NMR spectrum (200 MHz, DMSO-d_6), δ, ppm: 5.13-5.18 (1H, d4, H_A see Fig. 2), 5.22-5.31 (1H, d4, H_B), 5.88 (1H, m, H_C), 4.10-4.13 (2H, dt, $H_{D,E}$), 5.26 (2H, s, $H_{F,G}$), 9.29 (1H, s, H-6), 12.09 (1H, s, H-3). Mass spectrum (EI, 70 eV), *m/z* (I_{rel} ,%): 227 $[M]^+$ (53), 197 (100).

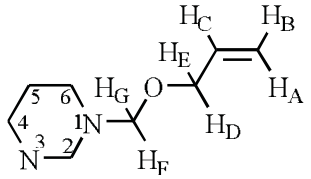

Fig. 2. Labeling of protons of allyloxymethyl group.

1-allyloxymethyl-5-bromouracil (3b) was obtained in 30%; mp 98-100°C; R_f 0.62 (Alugram SIL G/UV-254, chloroform–EtOH, 92:8). UV spectrum (H_2O + 1% EtOH), λ_{max} (ε): 213 nm (11000), 277 nm (9700). ^{1}H NMR spectrum (200 MHz, DMSO-d_6), δ, ppm: 5.11-5.18 (1H, d4, H_A), 5.21-5.29 (1H, d4, H_B), 5.86 (1H, m, H_C), 4.03-4.06 (2H, dt, $H_{D,E}$), 5.09 (2H, s, $H_{F,G}$), 8.28 (1H, s, H-6), 11.84 (1H, s, H-3). Mass spectrum (EI, 70 eV), *m/z* (I_{rel} ,%): 260, 262 $[M]^+$ (6.22, 6.16), 41 (100).

1-allyloxymethylthymine (3c) was obtained in 18%; mp 106-109°C; R_f 0.63 (Alugram SIL G/UV-254, chloroform–EtOH, 92:8). UV spectrum (H_2O + 1% EtOH), λ_{max} (ε):208.5 nm (7900), 265 nm (7000). ^{1}H NMR spectrum (200 MHz, DMSO-d_6), δ, ppm: 5.12-5.16 (1H, d4, H_A), 5.21-5.30 (1H, d4, H_B), 5.86 (1H, m, H_C), 4.00-4.03 (2H, dt, $H_{D,E}$), 5.06 (2H, s, $H_{F,G}$), 7.58 (1H, s, H-6), 11.33 (1H, s, H-3), 1.76 (3H, d, 5-CH_3). Mass spectrum (EI, 70 eV), *m/z* (I_{rel} ,%): 196 $[M]^+$ (5), 41 (100).

1-allyloxymethyl-5,6-trimethyleneuracil (3d) was obtained in 42%; mp 107-110°C; R_f 0.64 (Alugram SIL G/UV-254, chloroform–EtOH, 92:8). UV spectrum (H_2O + 1% EtOH), λ_{max} (ε): 205 nm (8600), 271.5 nm (10000). ^{1}H NMR spectrum (200 MHz, DMSO-d_6), δ, ppm: 5.12-5.17 (1H, d4, H_A), 5.20-5.28 (1H, d4, H_B), 5.86 (1H, m, H_C), 4.01-4.03 (2H, dt, $H_{D,E}$), 5.12 (2H, s, $H_{F,G}$), 11.14 (1H, s, H-3), (-$(CH_2)_3$-, 2.00(2H, m), 2.50 (2H, m), 2.63 (2H, m)). Mass spectrum (EI, 70 eV), *m/z*(I_{rel} ,%): 222 $[M]^+$ (29), 165 (100).

1-allyloxymethyl-5,6-tetramethyleneuracil (3e) was obtained in 35%; mp 124-126°C; R_f 0.66 (Alugram SIL G/UV-254, chloroform–EtOH, 92:8). UV spectrum (H_2O + 1% EtOH), λ_{max} (ε): 208.4 nm (14500), 268.7 nm (12600). ^{1}H NMR spectrum (200 MHz, DMSO-d_6), δ, ppm: 5.11-5.17 (1H, d4, H_A), 5.20-5.28 (1H, d4, H_B), 5.86 (1H, m, H_C), 4.01-4.04 (2H, dt, $H_{D,E}$), 5.25 (2H, s, $H_{F,G}$), 11.28 (1H, s, H-3), (-$(CH_2)_4$-, 1.56 (2H, m), 1.67 (2H, m), 2.21 (2H, t), 2.63 (2H, t)). Mass spectrum (EI, 70 eV), *m/z* (I_{rel} ,%): 236 $[M]^+$ (22), 179 (100).

1-allyloxymethyl-6-acetaminouracil (3f) was obtained in 16%; mp > 350°C; R_f 0.60 (Alugram SIL G/UV-254, chloroform–EtOH, 92:8). UV spectrum (H_2O + 1% EtOH), λl_{max} (ε): 210.5 nm (13200), 273.5nm (8700). ^{1}H NMR spectrum (500 MHz, DMSO-d_6), δ, ppm: 5.13-5.15 (1H, d4, H_A), 5.22-5.25 (1H, d4, H_B), 5.84 (1H, m, H_C), 4.01-4.03 (2H, dt, $H_{D,E}$), 5.33 (2H, s, $H_{F,G}$), 5.89 (1H, s, H-5), 11.10 (1H, s, H-3), 2.07 (3H, s, $COCH_3$). Mass spectrum (EI, 70 eV), *m/z* (I_{rel} ,%): 239 $[M]^+$ (43), 167 (100)

1-allyloxymethyl-4-hydroxy-5-nitro-6-oxopyrimidine (5) (Fig.3) was obtained in 21%; mp 175$_d$°C; R_f 0.49 (Alugram SIL G/UV-254, AcOH–BuOH–H_2O, 2:5:1). UV spectrum (H_2O + 1% EtOH), δl_{max} (ε): 204.5 nm (27600), 333 nm (5100). ^{1}H NMR spectrum (200 MHz, DMSO-d_6), δ, ppm: 5.14-5.19 (1H, d4, H_A), 5.23-5.31 (1H, d4, H_B), 5.87 (1H, m, H_C), 4.12-4.15 (2H, dt, $H_{D,E}$), 5.27 (2H, s, $H_{F,G}$), 9.20 (1H, s, H-2). MS (CI – isobutane) *m/z* (I_{rel},%): 228 $[M^+ + 1]$ (100).

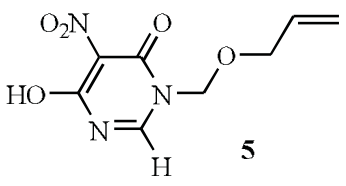

Fig. 3. 1-allyloxymethyl-4-hydroxy-5-nitro-6-oxopyrimidine **5**

MICROBIOLOGY

The antibacterial activities of compounds **3a-f**, **5** were tested by the disc-diffusion method, under standard conditions, using Mueller-Hinton agar medium, as described by

CLSI [12]. All chemical and solvents (DMSO) were purchased from Sigma. The results were read following 24 h incubation at 35°C for bacteria, 48 h at 35°C for fungi.

The antibacterial activity is expressed as Minimum Inhibitory Concentration (MIC) values in g/ml. The bacteria that were used are as follows: *Staphylococcus aureus* multidrugsensitive (2 strains), *Staphylococcus aureus* 209.P, *Staphylococcus aureus* multidrugresistance (2 strains), *Staphylococcus epidermidis* PCM 2118, *Staphylococcus hominis* PCM 2122, *Staphylococcus haemolyticus* PCM 2113, *Enterococcus faecalis* (3 strains), *Enterococcus faecalis* ATCC 51299, *Klebsiella pneumoniae* multidrugresistance (2 strains), *Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* multidrugresistance (3 strains), *Escherichia coli* ATCC 35218, *Acinetobacter baumannii* multidrugresistance (2 strains), *Acinetobacter juni* multidrugsensitive, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa* multidrugresistance (2 strains), *Candida albicans* (2 strains), *Candida famata* (2 strains). Clinical strains of bacteria and fungi were isolated from patients in the Department of Microbiology of A. Jurasz University Hospital No 1, in Bydgoszcz, Nicolaus Copernicus University in Toruń, Poland.

RESULTS AND DISCUSSION

Pyrimidines are an important group of compounds reported to have different biological activities. Hence, the present studies were undertaken in order to synthesize new derivatives and to investigate these for their antibacterial and antifungal activity. In summary, we have described the synthesis for the preparation of new 1-allyloxymethylpyrimidine derivatives. The obtained compounds were then subjected to the examination of their usage in the modulation of thymidine phosphorylase activity in human endometrium cancer [17].

These compounds were evaluated in vitro for their antibacterial and antifungal activities. As far as we know, this is the first time that the use of these new compounds has been trialed against bacteria and fungi. All examined strains displayed full growth up to the value 512 g/ml. No activity was shown against all tested fungi, Both Gram-positive and Gram-negative bacteria in the presence of compounds **3a-f**, **5** were also observed.

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