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Novel adamantylated pyrimidines and their preliminary biological evaluations

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Abstract

The synthesis of adamantylated pyrimidines was based on the reaction of 3-(adamantan-1-yl)-3-oxopropionic acid ethyl ester with urea, thiourea, guanidine as well as acetamidine, respectively. Then the compounds obtained were converted into respective bromo-, thio- and *S*-alkyl derivatives. The molecular structures for some compounds were studied by X-ray methods. The significant anticancer and antimicrobial properties of [2-(6-adamantan-1-yl-2-methylpyrimidin-4-ylthio)ethyl]dimethylamine were found.
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Keywords: 6-(Adamantan-1-yl)pyrimidines; Anti-HIV; Anticancer and antimicrobial properties

1. Introduction

The adamantyl moiety present in numerous agents and drugs improves their lipophilicity due to aliphatic cage-like structure. Such compounds might be much better taken up by cells, and have enhanced blood–brain barrier penetration and increased accumulation in lipids. Therefore adamantane derivatives have received considerable attention because of their diverse biological activity. Many years ago, 1-aminoadamantane (amantadine) was shown to be an effective prophylactic agent against type influenza A [1]. Even today amantadine is tested in combination with other antiviral agents for treatment hepatitis C [2].

Some aminoadamantane derivatives show activity against HIV-1 and HIV-2 [3]. Amantadine is used also in treatment of Parkinson disease [4] and memantine is considered as a promising drug for the treatment of certain dementia, particularly Alzheimer's disease [5]. *N*-(Adamantan-1-yl)-maleimide exhibited selected inhibitory activity against different cancer cell lines [6,7]. We have reported previously that certain novel *N*-(adamantan-1-yl)phthalimides as well as adamantylamino-pyrimidines and –pyridines, enhance TNF-α [7–9]. The combination of an adamantyl moiety with imides, also gives compounds of significant antimicrobial properties [10].

The exocyclic aminoadamantylated pyrimidines were obtained by the attack of an adamantyl cation generated from 1-adamantanol in boiling trifluoroacetic acid on the respective aminopyrimidine. In the same conditions barbituric acid

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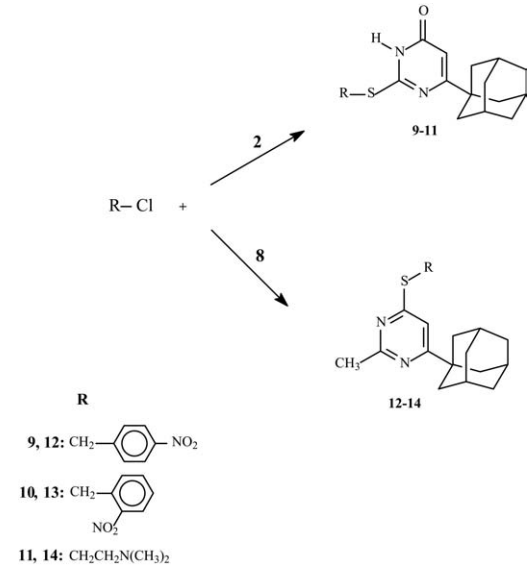
and 2-thiobarbituric acid gave C-5 adamantylated products [11,12]. Also 1,3-dimethyluracil and uridine were transformed into their 5-substituted adamantyl derivatives under such conditions. A ring closure procedure was used for the synthesis of 5-(1-adamantyl)uracil and its 2-thio congener, which were further transformed to respective α or β anomers of 2′-deoxyuridines [13]. In contrast to 5-adamantylated pyrimidines, their 6(4)-substituted analogues are practically unknown. Till now only one 6-adamantylated pyrimidine, namely 6-(adamantan-1-yl)-3-allyl-2-thiouracil was reported [14].

In this study we synthesized series of novel 6-adamantylpyrimidines. Also the crystallographic parameters and the preliminary biological evaluations on these novel modified heterocyclic compounds were shown.

2. Experimental

2.1. Synthesis

The syntheses were performed according to the Schemes 1 and 2. All chemicals used were analytical-grade commercial products (Aldrich) and were used without any further purification. The starting substrate 3-(adamantan-1-yl)-3-oxopropionic acid ethyl ester was obtained according to the procedure described previously [15]. Structures of novel compounds were confirmed by ^{1}H-NMR, UV, MS analysis and in two cases by X-ray crystallographic methods. Elemental analyses of new compounds were within ±0.4% of theoretical values. Their purity was over 95% and was controlled by TLC (SiO_2) and ^{1}H-NMR spectroscopy.

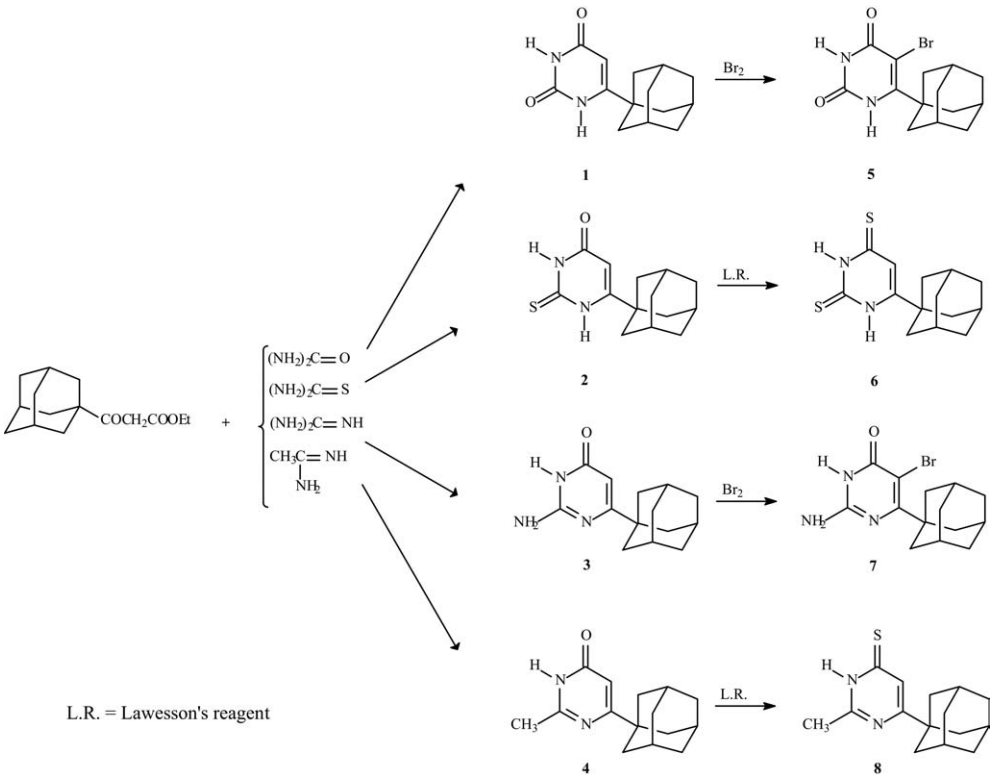


Scheme 2. Synthesis of compounds **9–14**.

2.1.1. Condensation procedure

Reactions were conducted by the Traube method and were similar to that given for 4-phenylisocytosine synthesis [16].

An equimolar amount of sodium (0.58 g, 25 mmol) was dissolved in anhydrous ethanol (20 ml). Then the solution was treated with 35 mmol of urea (for **1**) or thiourea (for **2**). For the synthesis of compounds **3** and **4**, hydrochloride salts



Scheme 1. Synthesis of compounds **1–8**.

of guanidine or acetamidine were used, respectively. In these cases, the amount of sodium was 1.16 g (50 mmol). After cooling, 12 g (12.5 mmol) of 3-(adamantan-1-yl)-3-oxopropionic acid ethyl ester was added dropwise to the mixture and refluxed for 12 h (for **1** 48 h). Then alcohol was evaporated and the residue dissolved in the least possible volume of cold water. The solution was then carefully acidified with dilute hydrochloric acid in order to precipitate the respective pyrimidine. The crude products were filtered off and crystallized from ethanol.

*2.1.1.1. 6-Adamantan-1-yl-1H-pyrimidine-2,4-dione (**1**).* Yield 24% (0.73 g); m.p. 325 °C (decomp.); TLC, chloroform–methanol, 6:1, R_f = 0.78; UV (H_2O/MeOH, 1:1) 263 (6500), (0,1 M KOH/MeOH, 1:1) 222 (7800), 264 (6300); *m/z* (EI) 246 (M^+, 100%), 189 (11), 135 (17); δ_H (200 MHz, DMSO-d_6) 1.68–2.00 (m, 15H, *H-Ada*), 5.21 (s, 1H, *H-5*), 10.48 (s, 1H, *N*(*3*)-*H*), 10.91 (s, 1H, *N*(1)-*H*).

*2.1.1.2. 6-Adamantan-1-yl-2-thioxo-2,3-dihydro-1H-pyrimidin-4-one (**2**).* Yield 60% (1.96 g); m.p. 285 °C (decomp.); TLC, chloroform–methanol, 6:1, R_f = 0.92; UV (H_2O/MeOH, 1:1) 275 (5200), (0,1 M KOH/MeOH, 1:1) 236 (10500), 259 (10500) 310 (6400). *m/z* (EI) 262 (M^+, 100%); δ_H (200 MHz, DMSO-d_6) 1.67–2.00 (m, 15H, *H-Ada*), 5.54 (s, 1H, *H-5*), 11.75 (s, 1H, *N*(3)-*H*), 12.30 (s, 1H, *N*(1)-*H*).

*2.1.1.3. 6-Adamantan-1-yl-2-amino-3H-pyrimidin-4-one (**3**).* Yield 60% (1.85 g); m.p. 340 °C (decomp.); TLC, chloroform–methanol, 6:1, R_f = 0.52; UV (H_2O/MeOH, 1:1) 262 (365), (0,1 M HCl/MeOH, 1:1) 259 (5800), (0,1 M KOH/MeOH, 1:1) 231 (7100), 274 (6500); *m/z* (EI) 245 (M^+, 100), 188 (17%); δ_H (200 MHz, DMSO-d_6) 1.68–2.00 (m, 15H, *H-Ada*), 5.35 (s, 1H, *H-5*), 6.32 (s, 2H, NH_2), 10.52 (s, 1H, *NH*).

*2.1.1.4. 6-Adamantan-1-yl-2-methyl-3H-pyrimidin-4-one (**4**).* Yield 75% (2.3 g); m.p. 246 °C (decomp.); TLC, chloroform–methanol, 6:1, R_f = 0.85; UV (H_2O/MeOH, 1:1) 223 (5000), 263 (3900), (0,1 M HCl/MeOH, 1:1) 229 (9500), (0,1 M KOH/MeOH, 1:1) 230 (8800), 264 (4100); *m/z* (EI) 244 (M^+, 100), 187(19%); δ_H (200 MHz, DMSO-d_6) 1.70 (s, 6H, *H-Ada*), 1.79 (s, 6H, *H-Ada*), 2.01 (s, 3H, *H-Ada*), 2.26 (s, 3H, CH_3), 5.89 (s, 1H, *H-5*), 12.00 (s, 1H, *NH*).

2.1.2. Bromination procedure

Compounds **5** and **7** were obtained by bromination of **1** and **3** in acetic acid, respectively [17]. A solution of bromine 0.39 g (2.46 mmol) in acetic acid (1 ml) was added dropwise to a hot solution of **1** or **3** (2.28 mmol) in acetic acid (13 ml). The mixture was heated up to 70 °C for 1°h. After cooling crude products deposited and were filtered off and crystallized from ethanol.

*2.1.2.1. 6-Adamantan-1-yl-5-bromo-1H-pyrimidine-2,4-dione (**5**).* Yield: 66% (0.49 g). m.p. 298 °C (decomp.); TLC, chloroform–methanol, 6:1, R_f = 0.84; UV (H_2O/MeOH, 1:1) 282 (5900), (0,1 M KOH/MeOH, 1:1) 227 (7200), 283 (5100); *m/z* (EI) 326 (M^+ 99), 325 (17), 324 (100), 246 (30), 135 (39), 79 (16%); δ_H (200 MHz, DMSO-d_6) 1.6–2.19 (m, 15H, *H-Ada*), 9.50 (s, 1H, *N*(*1*)-*H*), 11.57 (s, 1H, *N*(*3*)-*H*).

*2.1.2.2. 6-Adamantan-1-yl-2-amino-5-bromo-3H-pyrimidin-4-one (**7**).* Yield: 40% (0.3 g); m.p. 275 °C (decomp.); TLC, chloroform–methanol, 6:1, R_f = 0.61; UV (H_2O/MeOH, 1:1) 310 (3500), (0,1 M HCl/MeOH, 1:1) 228 (8200), 281 (6700), (0,1 M KOH/MeOH, 1:1) 235 (6400), 290(5600); *m/z* (EI) 325 (M^+, 71), 324 (19), 323 (73), 268 (18), 245 (45), 244 (100%); δ_H (200 MHz, DMSO-d_6) 1.68–2.18 (m, 15H, *H-Ada*), 6.83 (s, 2H, NH_2).

2.1.3. Thionation procedure

Compounds **6** and **8** were prepared similarly to the method described previously [18,19]. A mixture of **2** or **4** (5 mmol), Lawesson's reagent 1.21 g (3 mmol) and toluene (25 ml) was stirred and refluxed for 2 h. Compound **6** precipitated from the reaction mixture, while compound **8** remained in the solution. The crude products were purified by column chromatography and crystallization: **6** (SiO_2, hexane–EtOAc, 3:2, crystallized from EtOH) and **8** (chloroform–methanol, 15:1; crystallized from MeOH).

*2.1.3.1. 6-Adamantan-1-yl-1H-pyrimidine-2,4-dithione (**6**).* Yield: 41% (0.57 g); m.p. 300 °C (decomp.); TLC, chloroform–methanol, 20:1, R_f = 0.60; UV (H_2O/MeOH, 1:1) 290 (9900), 348 (8100), (0,1 M KOH/MeOH, 1:1) 221 (9100), 273 (16600), 370 (2600); *m/z* (EI) 278 (M^+, 80%). δ_H (200 MHz, DMSO-d_6) 1.69–2.02 (m, 15H, *H-Ada*), 6.29 (s, 1H, *H-5*), 12.36 (s, 1H, *N*(*1*)-*H*), 13.48 (s, 1H, *N*(*3*)-*H*).

*2.1.3.2. 6-Adamantan-1-yl-2-methyl-3H-pyrimidine-4-thione (**8**).* Yield: 68% (0.89 g); m.p. 266 °C; TLC, chloroform–methanol, 20:1, R_f = 0.64; UV (H_2O/MeOH, 1:1) 283 (5100), 332 (6000), (0,1 M HCl/MeOH, 1:1) 320 (14100), (0,1 M KOH/MeOH, 1:1) 229 (6300), 300 (9400); *m/z* (EI) 260 (M^+, 74), 203 (19%); δ_H (200 MHz, DMSO-d_6) 1.68-2.01 (m, 15H, *H-Ada*), 2.38 (s, 3H, CH_3), 6.85 (s, 1H, *H-5*), 13.68 (s,1H, *NH*).

2.1.4. Alkylation procedure (method I)

Compounds **9** and **10** were obtained from **2**, and the substrate for **12** and **13** was compound **8**. An equimolar mixture of **2** or **8** (1.6 mmol) with proper alkylating agent and 0.11 g (0.8 mmol) K_2CO_3 in ethanol (10 ml) was stirred at room temperature for 4 h. After the reaction completion the reaction mixtures were poured into water and neutralized with diluted hydrochloric acid. The crude products were crystallized from ethanol.

*2.1.4.1. 6-Adamantan-1-yl-2-[(4-nitrobenzyl)thio]-3H-pyrimidin-4-one (**9**).* Yield: 50% (0.32 g); mp 263 °C; TLC, chloroform–methanol, 20:1, R_f = 0.51; UV (H_2O/MeOH, 1:1) 286 (8500), (0,1 M HCl/MeOH, 1:1) 237 (9100), 284

(13500), (0,1 M KOH/MeOH, 1:1) 279 (12100); *m/z* (EI) 397 (M^+, 100), 262 (25), 230 (25%); δ_H (200 MHz, DMSO-d_6) 1.66–1.98 (m, 15H, *H-Ada*), 4.53 (s, 2H, CH_2), 5.89 (s, 1H, *H-5*), 7.65–7.76 (m, 2H, *ar.*), 8.12–8.24 (m, 2H, *ar.*).

*2.1.4.2. 6-Adamantan-1-yl-2-[(2-nitrobenzyl)thio]-3H-pyrimidin-4-one (**10**).* Yield: 72% (0.45 g); mp 268 °C; TLC, chloroform–methanol, 20:1, R_f = 0.49; UV (H_2O/MeOH, 1:1) 282 (7000), (0,1 M HCl/MeOH, 1:1) 280 (11300), (0,1 M KOH/MeOH, 1:1) 226 (11800), 273 (9100); *m/z* (EI) 397 (M^+, 1), 263 (48), 262 (100%); δ_H (200 MHz, DMSO-d_6) 1.68–2.00 (m, 15H, *H-Ada*), 4.71 (s, 2H, CH_2), 5.90 (s, 1H, *H-5*), 7.48–7.63 (m, 1H, *ar.*), 7.64–7.76 (m, 1H, *ar.*), 7.77–7.86 (m, 1H, *ar.*), 8.00–8.12 (m, 1H, *ar.*), 12.32 (s, 1H, *NH*).

*2.1.4.3. 4-Adamantan-1-yl-2-methyl-6-[(4-nitrobenzyl)thio]-pyrimidine (**12**).* Yield: 67% (0.42 g); m.p. 157 °C; TLC, chloroform–methanol, 20:1, R_f = 0.71; UV (H_2O/MeOH, 1:1) 280 (8900), (0,1 M HCl/MeOH, 1:1) 305 (17000); *m/z* (EI) 395 (M^+, 100), 362 (13), 260 (25), 71 (18%); δ_H (200 MHz, DMSO-d_6) 1.70–2.02 (m, 15H, *H-Ada*), 2.55 (s, 3H, CH_3), 4.58 (s, 2H, CH_2), 7.09 (s, 1H, *H-5*), 7.67–7.77 (m, 2H, *ar.*), 8.12–8.23 (m, 2H, *ar.*).

*2.1.4.4. 4-Adamantan-1-yl-2-methyl-6-[(2-nitrobenzyl)thio]-pyrimidine (**13**).* Yield: 60% (0.38 g); m.p. 132 °C; TLC, chloroform–methanol, 20:1, R_f = 0.72; UV (H_2O/MeOH, 1:1) 276 (9700), (0,1 M HCl/MeOH, 1:1) 303 (15300); *m/z* (EI) 395 (M^+, 4), 260 (100%); δ_H (200 MHz, DMSO-d_6) 1.70–2.00 (m, 15H, *H-Ada*), 2.55 (s, 3H, CH_3), 4.75 (s, 2H, CH_2), 7.07 (s, 1H, *H-5*), 7.47–7.60 (m, 1H, ar.), 7.65–7.75 (m, 1H, *ar.*), 7.77–7.86 (m, 1H, *ar.*), 7.97–8.08 (m, 1H, *ar.*).

2.1.5. Alkylation procedure (method II)

Compounds **11** and **14** were obtained from **2** and **8**, respectively. Equimolar mixtures of **2** or **8** (2.5 mmol) with 0.36 g (2.5 mmol) of 2-chloro–*N,N*-dimethyletylamine hydrochloride and 0.76 g DBU (5 mmol) in acetonitrile (20 ml) were stirred for 48 h at room temperature. The solvent was evaporated and the crude products were purified by column chromatography (SiO_2) using as an eluent chloroform–methanol mixture (for **11** 5:1 and for **14** 9:1).

*2.1.5.1. 6-Adamantan-1-yl-2-[(2-dimethylaminoethyl)thio]-3-H-pyrimidin-4-one (**11**).* Yield: 55% (0.46 g); m.p. 169 °C; TLC, chloroform–methanol, 6:1, R_f = 0.48; UV (H_2O/MeOH, 1:1) 279 (6000), (0,1 M HCl/MeOH, 1:1) 231 (11200), 276 (7000), (0,1 M KOH/MeOH, 1:1) 244 (4800), 277 (4000); *m/z* (EI) 333 (M^+, 1), 120 (28), 71 (100), 58 (49), 44 (31), 40 (56%); δ_H (200 MHz, DMSO-d_6) 1.68–2.00 (m, 15H, *H-Ada*), 2.21 (s, 6H, CH_3), 2.53 (t, 2H, *J* = 7.2 Hz, CH_2), 3.22 (t, 2H, *J* = 7.2 Hz, CH_2), 5.83 (s, 1H, *H-5*), 11.48 (s, 1H, *NH*).

*2.1.5.2. [2-(6-Adamantan-1-yl-2-methylpyrimidin-4-yl)thioethyl]dimethylamine (**14**).* Yield: 88% (0.73 g); m.p. 65 °C. TLC, chloroform–methanol, 6:1, R_f = 0.74; UV (H_2O/MeOH, 1:1) 278 (6900), (0,1 M HCl/MeOH, 1:1) 230 (6900), 297 (14700); *m/z* (EI) 331 (M^+, 0,5), 260 (10), 71 (100), 58 (47%); δ_H (200 MHz, DMSO-d_6) 1.67–2.05 (m, 15H, *H-Ada*), 2.20 (s, 9H, (CH_3), 2.51 (m, 2H, CH_2), 3.32 (m, 2H, CH_2), 7.03 (s, 1H, *H-5*).

2.2. X-ray measurements

Compounds **2** and **4** (10 mg) were dissolved in absolute ethanol (2 ml). Vials with respective solutions were placed in hermetic vessels with hexane. After 5–6 days, single crystals deposited on glass. Then they were carefully filtered-off and dried. Crystals of **2** and **4** having dimensions of 0.65 × 0.5 × 0.08 mm and 0.7 × 0.5 × 0.3 mm, respectively, were mounted consecutively on Kuma KM4 κ-axis diffractometer. After crystals centring the unit cells parameters were determined based on the least squares procedure performed for 31 strong reflections from a 2θ range of 11.7–21.9° and 12.0–24.0°, respectively, collected by using MoKα radiation (λ = 0.71073 Å). 4611 and 7400 reflections were collected for **2** and **4**, respectively. Data were corrected for Lorenz-polarization effects but not for absorption or extinction. Structures were solved using direct methods from SHELXS97 program and refined using SHELXL97 software [20,21].

2.2.1. Crystal and experimental data

(**2**): $C_{14}H_{18}N_2OS{\cdot}1/2C_6H_{14}$, Mr = 305.45; monoclinic space group C2/c, *a* =26.260(17), *b* = 10.561(4); *c* = 12.878(12) Å, *β* = 112.84(7)°; *V* = 3291(4) $Å^3$, *Z* = 8, *ρ* = 1.233 g cm^{-3}, F(000) = 1320, *μ*(MoKα) = 0.198 mm^{-1}. Final *R* and wR were 0.0463 and 0.1378, respectively, for 2124 observed data [with $I > 2\sigma(I)$].

(**4**): $C_{15}H_{20}N_2O$, Mr = 244.33; triclinic space group P–1, *a* = 6.532(5), *b* =13.296(11), *c* = 15.372(11) Å, *α* = 87.90(7), *β* = 83.41(6), *γ* = 84.82(7)°, *V* = 1320.3(18) $Å^3$, *Z* = 4, *ρ* = 1.229 g cm^{-3}; F(000) = 528, μ(MoKα) = 0.078 mm^{-1}. Final R and wR were 0.0580 and 0.1807, respectively, for 3452 observed data [with $I > 2\sigma(I)$].

Crystallographic data (excluding structure factors) for the structures **2** and **4** in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 229643 and 229644, respectively.

2.3. Biological evaluations

2.3.1. Antimicrobial studies

The following microorganisms were used: Gram-positive bacteria: *Staphylococcus aureus* ATCC 6538P, *Staphylococcus aureus* NCTC 4163, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6633, *Bacillus stearothermophilus* ATCC 7953; Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Escherichia coli* NCTC 8196, *Proteus vulgaris* NCTC 4635, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Psudomonas aeruginosa*

NCTC 6749, *Stenotrophomonas maltophilia* ATCC 13637, *Burkholderia cepacia* ATCC 25416, *Acinetobacter baumanii* ATCC 18606, *Bordetella bronchiseptica* ATCC 4617; fungi: *Candida albicans* ATCC 10231, *Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019. The microorganisms came from the State Institute of Hygiene (Warsaw, Poland), the Children Memorial Health Institute (Warsaw, Poland), and from the own collection of the Department of Pharmaceutical Microbiology, Medical University of Warsaw (Warsaw, Poland).

Antibacterial activity was examined by the disc-diffusion method and the MIC method under standard conditions using Mueller–Hinton II agar medium (Becton–Dickinson) according to guidelines established by the NCCLS [22,23]. Solutions of tested agents, depending on their structures, were prepared in different mixtures of EtOH, $CHCl_3$ and 2.5 M NaOH. For disc-diffusion assays, sterile filter paper discs (9 mm diameter, Whatman N°. 3 chromatographic paper) were dripped with tested compound solutions to load 400 μg of a given compound per disc. For MIC determinations, concentrations of tested compounds in solid medium ranged from 0.78 to 400 μg/ml, and agar plates were inoculated using 2 μl aliquots. The final inoculum of all studied organisms were 10^4 CFU/ml (colony forming units per ml), except the final inoculum of *E. faecalis* ATCC 29212 which was 10^5 CFU/mL. Results of antimicrobial activity were read after 18 h incubation at 35 °C.

2.3.2. Anticancer studies

The compounds tested were added to exponentially growing cell cultures (10^5/ml). After three days, the number of living cells was determined by using their capability to exclude the trypan blue dye. Following cell lines were used: HL-60 and KG-1 (acute myelogenous leukemia); CEM (acute T-cell leukemia); U-937 (acute monocytic leukemia); Jok-1 (hairy cell leukemia); BALL (acute B-cell leukemia), and Daudi (Burkitt's B-cell lymphoma). The cell lines were a generous gift from Professor Leif Andersson (Department of Pathology, Haartman Institute, University of Helsinki, Finland). Cell viability at the beginning of the tumor cultures was always >97%.

2.3.3. Antiviral studies

2.3.3.1. Cells. Human lymphocyte CEM cells were obtained from American Type Culture Collection and grown in *RPMI 1640* medium supplemented with 10% (*v/v*) inactivated fetal calf serum (Gibco), 2 mM L-glutamine (Flow Laboratories), and 0.075% (*v/v*) $NaHCO_3$. Cells were subcultured every 3 to 4 days.

2.3.3.2. Cytostatic activity of test compounds in cell culture. All assays were performed in 96-well microtiter plates (Falcoln 3072; Becton Dickinson, Paramus, NJ). To each well were added *ca*. 6×10^4 CEM cells (100 μl) and a given amount of the test compound (100 μl). The cells were allowed to proliferate for 96 h at 37 °C in a humidified CO_2-controlled atmosphere. At the end of the incubation period, the cells were counted in a Coulter counter (model ZB; Coulter Electronics Ltd., Harpenden, Hertfordshire, England). The 50% cytostatic concentration (CC_{50}) was defined as the concentration of compound that inhibited CEM cell proliferation by 50%.

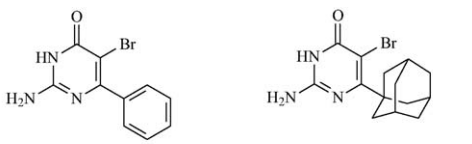
2.3.3.3. Viruses. HIV-1(III_B) was kindly provided by R.C. Gallo and M. Popovic (at that time at the National Cancer Institute, Bethesda, MD). HIV-2(ROD) was from L. Montagnier (at that time at the Pasteur Institute, Paris, France).

2.3.3.4. Antiviral activity of tested compounds in cell cultures. CEM cells were suspended at *ca*. 300,000 cells per ml of culture medium and infected with *ca*. 100 times the 50% cell-culture-infective doses of HIV-1(III_B) or HIV-2(ROD). Then, 100 μl of the infected cell suspensions were added to 200 μl microtiter plate wells containing 100 μl of appropriate serial (five-fold) dilutions of the tested compounds. The inhibitory effect of compounds on HIV-induced syncytium formation in CEM cells was examined microscopically on day 4 or 5 post infection. The EC_{50} was defined as the compound concentration that inhibits syncytium formation in the HIV-infected cell cultures by 50%.

3. Results and discussion

The known drug bropirimine i.e. 2-amino-5-bromo-6-phenyl-3*H*-pyrimidin-4-one, is an orally active immunomodulator that stimulates production of endogenous TNF-α and other cytokines and is now under phase II clinical trials for treatment of bladder carcinoma [21]. It is a low molecular weight compound acting as a ligand for TLR7 (Toll-like receptor) [24]. The inspiration for this study was the search for brompirimine analogues and expanding the list of 6(4)-adamantylated pyrimidines for pilot studies on their biological activity. The formulas of bropirimine and one of the compounds described below (6-adamantan-1-yl-2-amino-5-bromo-*3H*-pyrimidin-4-one) are presented in Fig. 1, for a comparison.

The synthesis of pyrimidines **1–4** similar to bropirimine was based on the Traube reaction of 3-(adamantan-1-yl)-3-oxopropionic acid ethyl ester with urea, thiourea, guanidine and acetamidine, respectively. In this case the adamantyl moiety was present in the starting reactant. Next,



Bropirimine **6-Adamantan-1-yl-2-amino-5-bromo-*3H*-pyrimidin-4-one**

Fig. 1. Structures of bropirimine and a related adamantyl derivative.

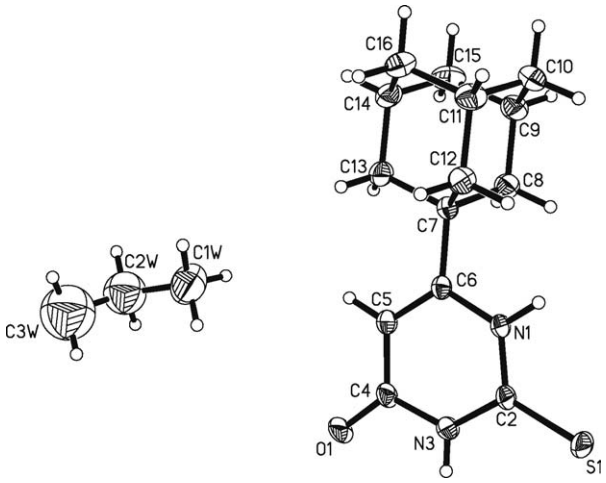


Fig. 2. Independent part of the unit cell. The non-hydrogen atoms are shown as 30% probability ellipsoids. Only the half of the solvent molecule is shown.

6-adamantan-1-yl-2-thioxo-2,3-dihydro-1*H*-pyrimidin-4-one (**2**) and 6-adamantan-1-yl-2-methyl-3*H*-pyrimidin-4-one (**4**) were treated with Lawesson's reagent to yield the respective 4-thioderivatives **6** and **8**. The compounds **1** and **3** were brominated with bromine to give the 5-substituted uracil **5** and isocytidine **7**, respectively. The alkylation of thioderivatives **2** and **8** with nitrobenzylchlorides and dimethylaminoethylchloride yielded the respective *S*-alkyl derivatives **9–11**. The general synthetic pathway is given in Schemes 1 and 2. Structures and purity of novel compounds were confirmed using chromatographic, spectroscopic and, in two cases, X-ray methods. Their full characterization is given in section 2.

As it has been mentioned, adamantyl derivatives are very interesting from a pharmacological point of view. For the better understanding of biological activity and agent–receptor interaction of such compounds, better knowledge of their molecular parameters is necessary. We have succeeded to obtain single crystals of **2** and **4** suitable for X-ray measurements. Structure of **2** unexpectedly contained disordered molecules of hexane which was used in preparation of monocrystals, while this compound obtained according to the procedure described in the Experimental part, was free from solvents and other impurities. The incorporation of hexane into space lattice during growth of crystals is a good illustration of the amphiphilic character of such molecules. This property is a very important factor in respect of biological evaluations. Fig. 2 shows the independent part of the unit cell, whereas Fig. 3 illustrates crystal packing of **2** shown along the c-axis.

The structure (**2**) is composed of molecules connected into infinite chains (perpendicular to the drawing and hence not visible) with N(1)–H(1)·O(1) hydrogen bonds between molecules related by the c-glide plane symmetry. The chains are interlinked by series of centrosymmetric N(3)–H(3)·S(1) hydrogen bonds between neighbouring molecules. The hydrophobic channels passing in the *c*-direction are filled out with disordered hexane molecules (shown with thin lines in the drawing).

Structure of **4** is triclinic with 2 independent molecules in an asymmetric part of the unit cell. Fig. 4 shows a molecular structure of one of them (Fig. 4).

The molecules are connected into centrosymmetic hydrogen bonded dimers (see Fig. 5) with N(3) – H(3) … O(1).

The planar fragments of molecules A and B are inclined by 58.98(8)° to each other. The molecule of **4** has two possible hydrogen bond acceptor sites: carbonyl oxygen and pyrimidine N^1 nitrogen; however, only the first one is active. The pyrimidine nitrogen N^1 is completely shielded by a methyl and an adamantyl moiety. The adamantyl fragment is a symmetric cage-like structure and one can assume that two

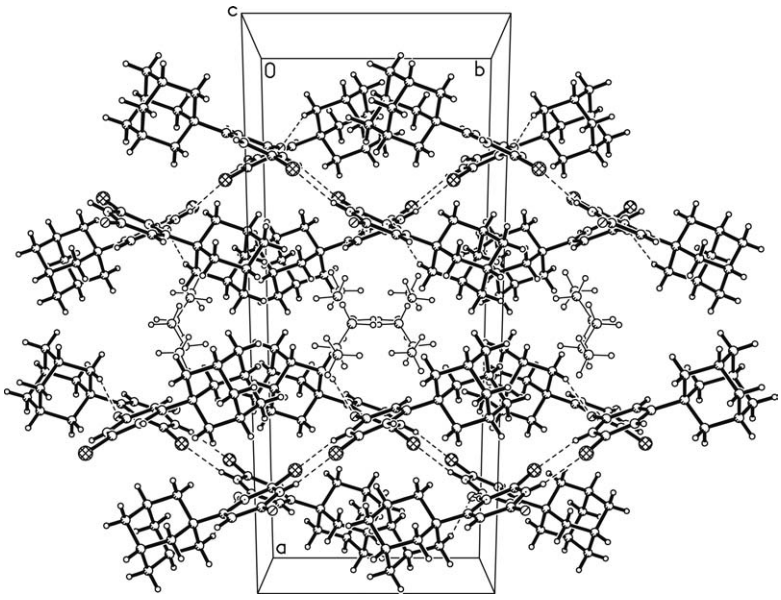


Fig. 3. The unit cell shown along the *c*-axis. The hydrogen bonds are shown as dashed lines. The disordered solvent molecules, drawn with thin lines, occupy hydrophobic channels passing in *c* direction.

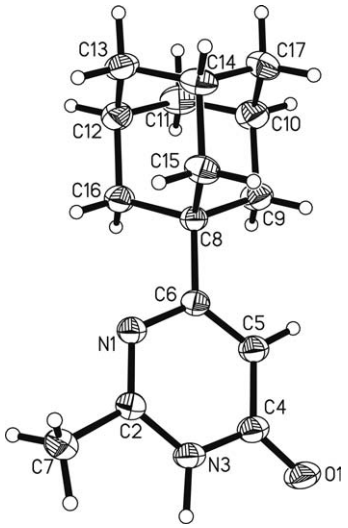


Fig. 4. Conformation of molecule A and numbering scheme.

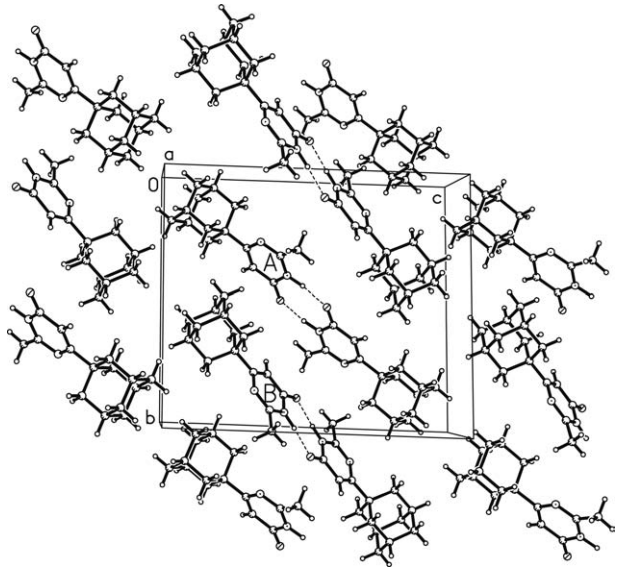


Fig. 5. Crystal packing shown along the a-axis. Two different molecules A and B are present in the asymmetric part of the unit cell. Molecules related by centre of symmetry are bonded in pairs with the N – H ... O intermolecular hydrogen bonds (shown as the dashed lines).

60° rotamers are energetically equivalent. Our ab initio quantum chemical calculations (RHF 6-31G**) showed, however, that the conformation present in a crystal is by 2.437 kcal/mol more stable than the alternative one.

As first biological examinations we performed antibacterial and antifungal studies. The activity in vitro of newly obtained derivatives of 6-(adamantan-1-yl)pyrimidines was tested against a wide range of bacteria and fungi including Gram-positive cocci and Gram-negative rods. The evaluation of the antimicrobial properties was carried out using the disc diffusion method. Compounds showing significant activity in the above test were next examined for their minimal inhibitory concentration (MIC). The results of tests are summarized in Table 1. Of all agents tested only two, **11** and **14**, shown modest antimicrobial activity. In comparison to **11**, compound **14** had from 5 to 8-fold higher activity against

Table 1
Antimicrobial activities of 6-(adamantan-1-yl)pyrimidines **11** and **14** against bacteria and fungi (the rest of compounds were completely inactive)

Bacteria strains	Diameter of the growth inhibition area [mm] and MIC[a] (in parenthesis)	
	11	**14**
Staphylococcus aureus ATCC 6538P	12 (>400)	18 (50)
Staphylococcus aureus NCTC 4163	13 (>400)	17 (100)
Enterococcus faecalis ATCC 29212	na[b]	19 (100)
Bacillus subtilis ATCC 6633	12 (>400)	23 (100)
Bacillus stearothermophilus ATCC 7953	15 (400)	21 (50)
Proteus vulgaris NCTC 4635	na	na
Escherichia coli ATCC 25922	na	na
Escherichia coli NCTC 819	na	11 (400)
Klebsiella pneumoniae ATCC 13883	na	na
Pseudomonas aeruginosa ATCC 27853	na	na
Psudomonas aeruginosa NCTC 6749	na	na
Stenotrophomonas maltophilia ATCC 13637	na	16(100)
Burkholderia cepacia ATCC 25416	na	na
Acinetobacter baumannii ATCC 18606	na	na
Bordetella bronchiseptica ATCC 4617	na	na
Candida albicans ATCC 10231	na	17 (100)
Candida albicans ATCC 90028	na	17 (100)
Candida parapsilosis ATCC 22019	na	20 (100)

[a] Minimal inhibitory concentration , μg/ml.
[b] No activity.

Gram-positive bacteria. Moreover, some of the Gram-negative bacteria, as *Bordetella bronchiseptica* ATCC 4617 and *Stenotrophomonas maltophilia* ATCC 13637, and all tested fungi were sensitive to **14**.

Then, compounds **1–14** were examined as anticancer agents. The pyrimidines tested were added to cultures of growing tumor cells. Following cell lines were used: HL-60 and KG-1 (acute myelogenous leukemia); CEM (acute T-cell leukemia); U-937 (acute monocytic leukemia); Jok-1 (hairy cell leukemia); BALL (acute B-cell leukemia, and Daudi (Burkitt's B-cell lymphoma). In the initial screening we tested each compound in 25 μg/ml concentration against two different cell lines (BALL and HL-60). As shown in Table 2, only compound **14** significantly inhibited the cell growth of BALL or HL-60 cells at 25 μg/ml. This compound was, therefore selected for further testing at several doses and

Table 2
Inhibition of the growth of two human cancer cell lines in vitro by 6-(adamantan-1-yl)pyrimidines. The test concentration was 25 μg/ml

Compounds	Cell number (% of untreated control cultures)	
	BALL	HL-60
1	81	98
2	67	76
3	96	77
4	96	67
5	104	85
6	68	32
7	89	83
8	31	43
9	101	78
10	70	50
11	47	41
12	75	81
13	29	35
14	<1	<1

Table 3
Results of inhibition (ID_{80} in μg/ml; in brackets in μM) of cell growth by compound **14**

Cancer cell lines	ID_{80}
BALL	17.3 [52.3]
CEM	17.5 [52.8]
Daudi	20.0 [60.4]
HL-60	16.4 [49.5]
Jok-1	7.0 [21.2]
KG-1	18.4 [57.1]
U-937	4.8 [014.5]

against seven different tumor cell lines. Dose–response curves for each target cell line were calculated (drawn) using four doses of the tested compound. Compound concentrations inhibiting the cell growth by 80% (ID_{80}) were determined from dose–response curves. The comparison of the sensitivities of seven different human cancer cell lines to this compound was performed by calculating the ID_{80} values. The ID_{80} values are shown in Table 3. As can be seen monocytic leukemia (U-937) and hairy cell leukemia (Jok-1) are the most, and Burkitt's lymphoma (Daudi) the least sensitive to **14**.

Next the compounds were evaluated for their inhibitory activity against HIV-1(III_B) and HIV-2(ROD)-induced cytopathicity in CEM cell cultures. As shown in Table 4 none of the compounds were inhibitors against these viruses in CEM cell cultures at subtoxic concentrations. Compounds **6** and **14** proved most cytotoxic (CC_{50} < 10 μg/ml).

4. Conclusion

Linking of the adamantyl moiety to pyrimidine rings leads to compounds of amphiphilic character. The crystallographic analysis of **2** revealed the high affinity of non-polar solvent to aliphatic part of this molecule, while the heterocyclic fragment exhibited significant polar properties. As it has been

Table 4
Anti-HIV-1 and HIV-2 activity and cytotoxic properties of 6-(adamantan-1-yl)pyrimidines expressed as EC_{50} and CC_{50} (in μg/ml; in brackets in μM), respectively

Compound	EC_{50}[a]		CC_{50}[b]
	HIV-1	HIV-2	
1	>100 [406]	>100 [406]	>100 [406]
2	>20 [76]	>20 [76]	75.2 [287]
3	>100 [382]	>100 [382]	100 [382]
4	>20 [82]	>20 [82]	41.8 [171]
5	>100 [307]	>100 [307]	>100 [307]
6	>4 [12]	>4 [12]	4.6 [14]
7	>20 [72]	>4 [14]	12.1 [43]
8	>20 [77]	>20 [77]	49.3 [189]
9	>4 [10]	>4 [10]	21.7 [55]
10	>4 [10]	>4 [10]	10.2 [27]
11	>20 [51]	>20 [51]	44.1 [111]
12	>100 [253]	>100 [253]	>100 [253]
13	>4 [12]	>4 [12]	18.7 [56]
14	>4 [12]	>4 [12]	6.2 [18]

[a] 50% Effective concentration or compound concentration, required to protect CEM cell cultures against the cytopathogenicity of HIV by 50%.
[b] 50% Cytotoxic concentration or compound concentration, required to reduce CEM cell proliferation by 50%.

mentioned in the Introduction such molecular architecture is very promising for medicinal applications. For this reason we performed several preliminary biological studies. Although the studied compounds had no antiviral activity, during the tests we observed pronounced cytotoxicity for same of the compounds. Therefore we studied activity of these compounds against several neoplastic cell lines. Anticancer evaluations indicate that only [2-(6-adamantan-1-yl)-2-methylpyrimidin-4-ylthioethyl]-dimethylamine (**14**) has significant activity against different human cancer cell lines, particularly against monocytic leukemia (U-937). The same compound **14** is also the most potent as antimicrobial agent. This observation indicates a direction for future syntheses and studies. It appears that the most promising compounds might be derivatives of 6-adamantan-1-yl-2-methyl-*3H*-pyrimidine-4-thione (**8**) with diverse 4-*S*- polar substituents as pharmacophoric groups.

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