SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 5-ADAMANTAN-1-YL-METHYL ANALOGUES OF TRIMETHOPRIM

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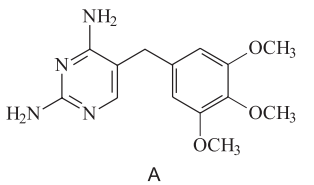
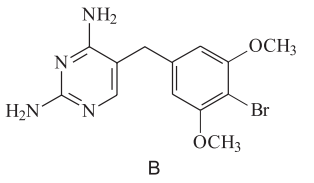
Abstract: A series of new trimethoprim [5-(3,4,5-trimethoxy-benzyl)-pyrimidine-2,4- diamine] analogues were prepared by condensation of adamantane-1-carbaldehyde with 3-methoxypropionitrile, followed by reaction of resulting mixture of 2-adamantan-1-ylmethyl-3-methoxy-acrylonitrile and 3-adamantan-1-yl-2-methoxymethyl-acrylonitrile with guanidine, acetamidine and thiourea, respectively. The activity of compounds obtained and sulfamethoxazole, alone and in combination, against several bacterial strains, as well as fungi was investigated.

Keywords: 5-adamantan-1-ylmethyl-substituted pyrimidines, antimicrobial activity

Certain benzylpyrimidines, such as trimethoprim (A), brodimoprim (B) (Figure 1) are potent and selective inhibitors of bacterial dihydrofolate reductase (DHFR), the enzyme which catalyzes the reduction of 7,8-dihydrofolate to 5,6,7,8-tetrahydrofolate with NADPH as a cofactor (1). DHFR plays an essential role in various biosynthetic reactions e.g. the DNA synthetic pathway and biosynthesis of certain amino acids (2, 3). Trimethoprim is a bacteriostatic agent used mainly in the prophylaxis and treatment of wide range of infections (4). It is commonly used in combination with sulfonamides e.g. sulfamethoxazole (Fig. 1, C), which results in a synergistic antibacterial effect by inhibiting successive steps in the folate synthesis.



A



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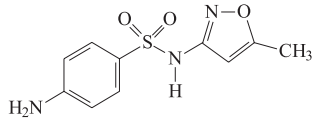

C

Figure 1. Stuctures of trimethoprim (A), brodimoprim (B), sulfamethoxazole (C).

On the other hand, compounds containing the adamantane subunit have long been of interest to medicinal chemistry and pharmacology. The aliphatic cage-like structure present in numerous agents and drugs improves their lipophilicity. Such compounds might be much better taken up by cells, and have enhanced blood–brain barrier penetration and increased accumulation in lipids. The most known of the clinically useful adamantane derivatives are antiviral drugs — amantadine (1-aminoadamantane), rimantidine and tromantadine (5). Another field where related amino-derivatives such as memantine are successfully employed is the treatment of certain neurological disorders: Parkinson's disease [6] and certain dementias, particularly Alzheimer's disease (7).

In 1971 Jonak et al. designed a novel growth inhibitor of mouse mammary adenocarcinoma cells – 2,4-diamino-5-adamantyl-6-methyl pyrimidine (DAMP) and found it to be a potent, lipid-soluble antifolate (8). Later, the ethyl sulfonate salt of DAMP has proven satisfactory over phase I clinical trial and has been recommended for phase II studies as an antitumor agent (9).

Our previous investigation revealed that the combination of an adamantyl moiety with pyrimidine ring leads to compounds of distinct antimicrobial properties (10, 11). It prompted us to synthesize some new adamantane analogues of trimethoprim.

EXPERIMENTAL

All commercial reagents and solvents were used without further purification. Reactions were monitored

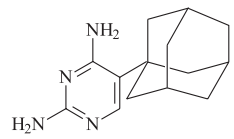

Figure 2. Structure of 2,4-diamino-5-adamantyl-6-methyl pyrimidine (DAMP).

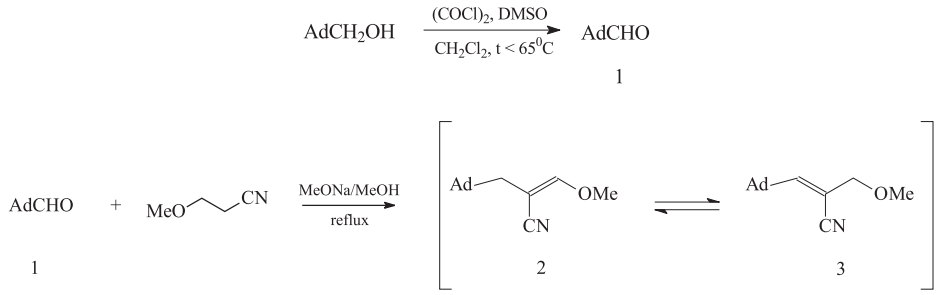

Figure 3. Synthesis of compounds **1-3**.

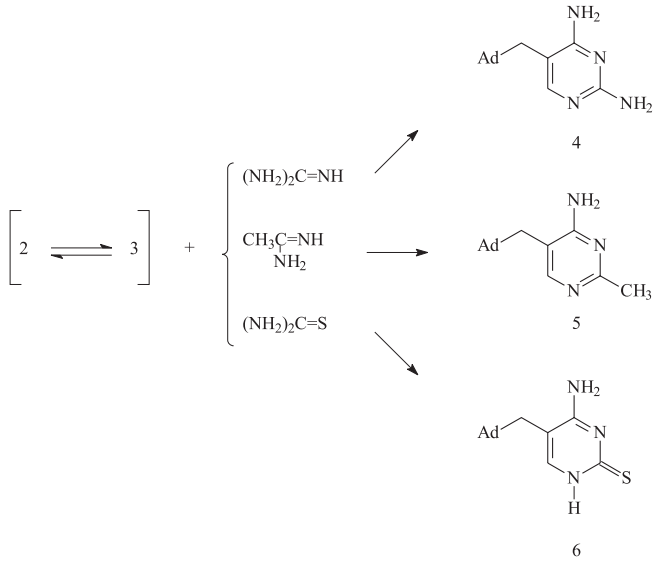

Figure 4. Synthesis of 5-adamantan-1-ylmethyl-substituted pyrimidines **4-6**.

by GC and TLC. Analytical thin-layer chromatography was performed on Kieselgel 60F_{254} plates (Merck); the spots were located by UV (254 nm) or iodine, R_f values are given for guidance. Preparative flash column chromatography was performed using silica gel (Merck) 200-400 mesh. Melting points (uncorr.) were measured in open capillary tubes on a Gallenkamp-5 apparatus. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Mercury-400BB spectrometer in $CDCl_3$ (for **1-5**) or D_6(DMSO) (for **6**), chemical shifts (δ) were expressed in ppm.

SYNTHESIS

The general synthetic pathway is given in Figure 3 and 4.

Adamantane-1-carbaldehyde **(1)**

All operations should be carried out in a well-ventilated hood. Oxalyl chloride (4.12 mL, 48 mmol) was dissolved in dichloromethane (60 mL) and cooled to –70°C, using dry ice-acetone bath. Then DMSO (7.40 mL, 104 mmol) in dichloromethane (12 mL) was added dropwise, and the mixture was stirred for 30 min at the temperature below –65°C. The solution of adamantane-1-methanol (6.65 g, 40 mmol) in dichloromethane (60 mL) was added slowly. The resulting mixture was stirred for additional 60 min at –65°C. Triethylamine (27.8 mL, 200 mmol) was added and stirring was continued until reaction reached room temperature. The mixture was diluted with water (40 mL) stirred for 15 min, the phases were separated and the water layer was extracted with dichloromethane (2 × 40 mL). Combined organic layers were washed with water (5 × 100 mL) and dried over magnesium sulfate. The solvent was evaporated to give pale yellow solid. The product was crystallized from petroleum ether to provide 5.91 g (90%) of compound **1** as white crystals: mp 142°C, lit. mp 141°C (12).

2-adamantan-1-ylmethyl-3-methoxy-acrylonitrile (**2**) and 3-adamantan-1-yl-2-methoxymethyl-acrylonitrile (**3**).

To a solution of sodium methoxide in methanol, prepared from sodium (299 mg, 13 mmol) and 7.6 mL of methanol, adamantane-1-carbaldehyde (1.64 g, 10 mmol) and 3-methoxypropionitrile (850 mg, 10 mmol) were added. The mixture was refluxed for 30 h. After cooling, the solvent was evaporated, and the residue was dissolved in ether. The solution was washed with water (2×50 mL) and dried over magnesium sulfate. Then the ether was removed under reduced pressure and the remaining oil was purified by column chromatography, using hexane/ethyl acetate (5:1) as an eluent (see Table 1).

5-adamantan-1-ylmethyl-pyrimidine-2,4-diamine (**4**), 5-adamantan-1-ylmethyl-2-methyl-pyrimidin-4-ylamine (**5**) and 5-adamantan-1-ylmethyl-4-amino-1H-pyrimidine-2-thione (**6**).

Solutions of sodium methoxide in methanol were prepared from sodium (207 mg, 9 mmol) and 6.5 mL of methanol for compound **4** and from (414 mg, 18 mmol) of sodium and 11 mL of methanol for **5-6**. Then for the synthesis of **4** and **5** a solution of guanidine hydrochloride (860 mg, 9 mmol) or acetamidine hydrochloride (851 mg, 9 mmol) in 2.5 mL of methanol was added. To obtain **6**, thiourea (685 mg, 9 mmol) in 2.5 mL of methanol was used. In the case of **4** and **5**, the precipitate of sodium chloride was filtered off after 10 min.

Table 1. Physico-chemical properties of compounds **1-6**.

Comp. No.	Formula	Yield (%)		M.p(°C)	R_f	% Found (required)		
						Carbon	Hydrogen	Nitrogen
1	$C_{11}H_{16}O$	96		142	0.73[a]	-	-	-
2	$C_{15}H_{21}NO$	56	10[c]	96-97	0.64[a]	77.93 (77.88)	9.15 (9.15)	6.00 (6.05)
3	$C_{15}H_{21}NO$		23[c]	46-47	0.56[a]	77.77 (77.88)	9.10 (9.15)	6.10 (6.05)
4	$C_{15}H_{22}N_4$	47		279 decomp.	0.32[b]	69.70 (69.73)	8.70 (8.58)	21.56 (21.68)
5	$C_{16}H_{23}N_3$	43		258 decomp.	0.52[b]	74.56 (74.67)	8.72 (9.01)	15.99 (16.33)
6	$C_{15}H_{21}N_3S$	27		295 decomp.	0.47[b]	65.04 (65.42)	7.60 (7.69)	15.17 (15.26)

[a] Hex/EtOAc 5:1 (v/v)
[b] $CHCl_3$/MeOH 7:1 (v/v)
[c] yield of a single isomer after separation by flash-column chromatography

Table 2. Spectroscopic data for compounds **1-6**.

Comp. No.	^{1}H NMR δ (ppm)	^{13}C NMR δ (ppm)
1	1.67-2.06 (m, 15H); 9.31 (s, 1H-CHO).	not measured
2	1.52-1.99 (m, 15H); 3.28 (s, 3H-OCH_3); 6.18(s, 1H-CH=).	28.1 (3C^{III}-Ada); 36.9 (3C^{II}-Ada); 37.5 (C^{IV}-Ada); 38.1 (3C^{II}-Ada); 41.4 (CH_2), 57.8 (OCH_3); 90.4 (>C=); 118.3 (CN); 133.4 (=CH).
3	1.70-2.02 (m, 15H); 3.35 (s, 3H-OCH_3); 3.96 (d, *J*=1.2 Hz, 2H-CH_2O); 6.11. (s, 1H-CH=)	8.0 (3C^{III}-Ada); 36.3 (3C^{II}-Ada); 36.6 (C^{IV}-Ada); 41.1 (3C^{II}-Ada); 58.0 (OCH_3); 74.5 (CH_2); 106.9 (=C<); 117.4 (CN); 159.0 (CH=).
4	1.50-1.95 (m, 15H); 2.06 (s, 2H-CH_2); 4.68 (s, 4H-2´NH_2); 7.62 (s, 1H_{pyr}).	28.5 (3C^{III}-Ada); 34.7 (C^{IV}-Ada); 36.8 (C^{II}-Ada); 42.3 (CH_2); 42.5 (3C^{II}-Ada); 104.2 (C-5$_{pyr}$); 158.7 (C-6$_{pyr}$); 161.5 (C-2$_{pyr}$); 163.1 C-4$_{pyr}$).
5	1.52-1.96 (m, 15H); 2.15 (s, 2H-CH_2); 2.49 (s, 3H-CH_3); 4.88 (s, 2H-NH_2); 7.88 (s, 1H_{pyr}).	25.4 (CH_3); 28.5 (3C^{III}-Ada); 34.8 (C^{IV}-Ada); 36.7 (C^{II}-Ada); 42.5 (3C^{II}-Ada); 42.5 (CH_2); 110.3 (C-5$_{pyr}$); 157.7 (C-6$_{pyr}$); 162.3 (C-4$_{pyr}$); 165.5 (C-2$_{pyr}$).
6	1.43-1.89 (m, 15H); 2.09 (s, 2H-CH_2); 3.32 (SH); 7.08 (s, 1H_{pyr}); 7.71 (NH, NH_2); 12.00 (NH, NH_2).	27.9 (3C^{III}-Ada); 33.9 (C^{IV}-Ada); 36.4 (C^{II}-Ada); 41.0 (3C^{II}-Ada); 104.3 (C-5$_{pyr}$); 142.0 (C-6$_{pyr}$); 162.9 (C-4$_{pyr}$); 177.6 (C-2$_{pyr}$); (CH_2) – covered by DMSO signal.

Next, the isomers **2** and **3** (693 mg, 3 mmol) were added to the solutions and the mixtures were refluxed for 30-45 h. After cooling, the solvent was evaporated and the crude yellow solids were purified by column chromatography using $CHCl_3$/MeOH/NH_4OH (88:10:2, v/v/v) for **4**, or $CHCl_3$/MeOH (8:1, v/v) for **5-6** elution.

The yields, R_f values, melting points, elemental analysis and spectroscopic data of the newly obtained compounds are listed in Table 1 and 2.

Antimicrobial evaluations

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* ATCC 8196, *Klebsiella pneumoniae* ATCC 13883, *Psudomonas vulgaris* NCTC 4635, *Psudomonas aeruginosa* NCTC 27863, *Stenotrophomonas maltophilia* ATCC 13637, *Bacillus cepacia* ATCC 25416, *Acinetobacter baumanii* ATCC 19606, *Bacillus bronchiseptica* ATCC 4617, fungi: *Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019, *Candida tropicalis* IBA 171, *Candida krusei* IBA 161, and *Candida quillermondi* IBA 155. 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 under standard conditions using Mueller-Hinton II agar medium (Becton-Dickinson) according to guidelines established by the NCCLS. For determination of antifungal activity the medium RPMI-1640 (Angus Buffer and Biochemicals) with 2% glucose agar was used. Solutions of tested agents, depending on their structures, were prepared in different mixtures of EtOH and $CHCl_3$. For disc-diffusion assays, sterile filter paper discs (9 mm diameter, Whatman No. 3 chromatographic paper) were dripped with tested compound solutions to load 400 mg of a given compound per disc. Concentration of sulfamethoxazole was 23.75 mg per disc. The distance between discs containing sulfamethoxazole and a tested compound was 2.5 cm. Results of antimicrobial activity were read after 18 h incubation at 35°C.

RESULTS AND DISCUSSION

The starting reagent for synthesis of new trimethoprim analogues was adamantane-1-carbaldehyde **1**. The synthesis of **1** was already reported in the literature. It is

Table 3. Antibacterial properties of compounds **4-6.**

Strains	Diameter of growth inhibition area, mm		
	4	Sulfamethoxazole	4+Sulfamethoxazole
E. faecalis ATCC 29212	21	13	25
B. subtilis ATCC 6633	24	30	34
B. stearothermophilus ATCC 7953	25	29	33
E. coli ATCC 25922	resistant	14	21

usually obtained by oxidation of adamantan-1-yl methanol using pyridinium chlorochromate (PCC) (13) or by reaction of adamantyl bromide with lithium in DMF (14). We have decided to use Swern oxidation method (15).

Literature survey has shown that many different protocols have been developed for the synthesis of 5-benzylpyrimidines. Most commonly employed methods involve base-catalyzed condensation of 3-alkoxy (2-4) or 3-aminopropionitriles (16-18) with an appropriate aldehyde, followed by reaction with guanidine (2-4, 16, 17), acetamidine (18), urea (19) or *N*-methylthiourea (20).

The second step of synthesis was performed using the procedure of Selassie et al. with modifications (2), giving 2-adamantan-1-ylmethyl-3-methoxy-acrylonitrile **2** in tautomeric equilibrium with 3-adamantan-1-yl-2-methoxymethyl-acrylonitrile **3** with satisfactory yield (56%). The ratio of the isomers, evaluated by GC was 1:2.5. An attempt to separate these compounds by flash-column chromatography resulted in three fractions. The first one contained only the nitrile **2** (10% yield) and the last one – tautomer **3** (23% yield), while the second one remained as the mixture of both.

Finally, the mixture of both isomers (used without separation) was cyclized with guanidine, acetamidine or thiourea to the corresponding 5-adamantan-1-ylmethylpyrimidines **4-6**, according to the procedure described in (3).

Our recent studies have demonstrated that some 6-(adamant-1-yl)-pyrimidines and their *S*-alkylated derivatives posses significant antibacterial activity particularly against *S. aureus* strain and *B. stearothermophilus* (10, 11). For those series the diameters of growth area ranged between 11 and 24 mm, for Gram-positive bacteria.

The present paper reports the synthesis and evaluation of antibacterial and antifungal properties of the novel 5-adamantan-1-ylmethyl-pyrimidines.The results, expressed as the diameter of growth inhibition area (in mm) are given in Table 3.

As can be seen, only 5-adamantan-1-ylmethyl-pyrimidine-2,4-diamine (**4**) showed activity against some Gram-positive bacteria strains (21-25 mm). It inhibited the growth of *E. faecalis*, *B. subtilis* and *B. stearothermophilus*. It should be noticed that this compound exhibited also a significant synergic effect with sulfamethoxazole against these strains (25-34 mm). Particularly, it was observed for *E. faecalis* usually resistant in the presence of sulfamethoxazole. Additionally, an increase of diameter of growth inhibition area appeared around the disc with sulfamethoxazole, at the side of the disc with compound **4** for *E. coli* ATCC 25922, while that strain was resistant to **4** itself. The active 5-adamantan-1-ylmethyl pyrimidine didn't show mutagenic activity in the disc-diffusion test, using *B. subtilis* M45 and *B. subtilis* H17 strains.

Generally, the combination of 5-adamantan-1-ylmethyl-pyrimidine-2,4-diamine and sulfamethoxazole definitely appeared to be more effective than sulfonamide or **4** alone.

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