Acta Poloniae Pharmaceutica – Drug Research, Vol. 65 No. 4 pp. 427–434, 2008 ISSN 0001-6837
Polish Pharmaceutical Society

DRUG SYNTHESIS

SYNTHESIS AND ANTIBACTERIAL PROPERTIES OF 1,2,3-ARYL-1,2,3,4-TETRAHYDROPYRIMIDO[4,5-D]PYRIMIDINE DERIVATIVES

JERZY CIEPLIK[1*], MAŁGORZATA RAGINIA[1], JANUSZ PLUTA[2], OLAF GUBRYNOWICZ[2], IWONA BRYNDAL[3], and TADEUSZ LIS[3]

[1] Department of Organic Chemistry, Medical Academy, 9 Grodzka Street, 50-137 Wrocław, Poland
[2] Department of Applied Pharmacy, Medical Academy, 38 Szewska Street, 50-137 Wrocław, Poland
[3] Faculty of Chemistry, University of Wrocław, 14 Joliot-Curie Street, 50-383 Wrocław, Poland

Abstract: The 1,2,3-aryl-1,2,3,4-tetrahydropyrimido[4,5-d]pyrimidine derivatives have been synthesized and evaluated as antibacterial agents. The X-ray crystallography study has been undertaken to confirm structure of 2-(4-chlorophenyl)-1,3-di-(4-methoxyphenyl)-5-methyl-7-phenyl-1,2,3,4-tetrahydropyrimido[4,5-d]pyrimidine. The antibacterial properties were tested using six selected strains by comparing changes of chemical structures with growth of biological activity. Most of the synthesized compounds exhibited antibacterial activity, and their activity can be connected with the cyclization to the pyrimido[4,5-d]pyrimidine system and nature of the substituent attached to phenyl rings.

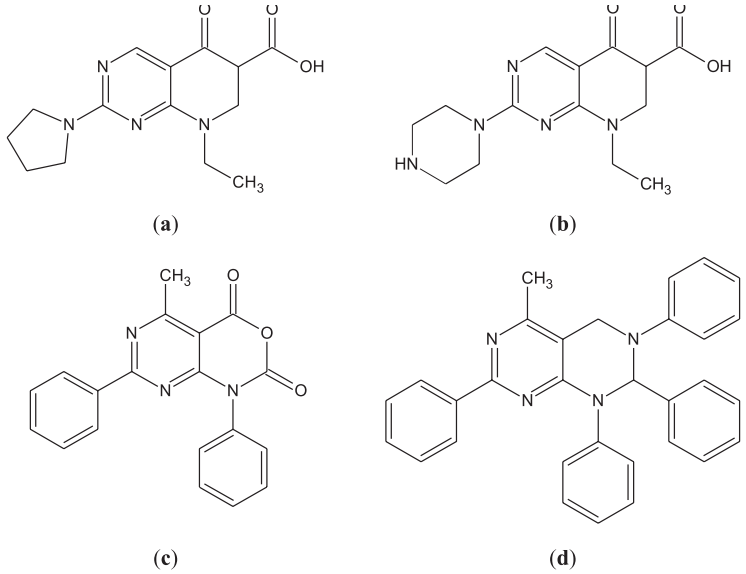
Keywords: pyrimido[4,5-d]pyrimidines, synthesis, antibacterial effect

Our earlier studies on series of pyrimidine derivatives have shown that the pyrimidine ring system reveals an extremely potent biological activity. The pyrimidine derivatives have been found to exhibit cytostatic (1, 2), immunomodulating (3, 4) and antibacterial activity properties (5-8). In particular, the studies concerning syntheses of pyrimido[4,5-d][1,3]oxazines (5) showing considerable similarity to quinoline chemotherapeutics agents being already in the use, e.g. piromidic and pipemidic acids, with an antibacterial effect encouraged us to further work leading to replacement of the pyrimido[4,5-d][1,3]oxazine system with pyrimido[4,5-d]pyrimidine system (Scheme 1). In continuation of our search for more potent antibacterial agents, herein we report the synthesis, antibacterial properties of 4-arylamine-6-methyl-2-phenyl-5-aminomethylpyrimidine and 1,2,3,4-tetrahydropyrimido[4,5-d]pyrimidine derivatives and the crystal structure of 2-(4'-chlorophenyl)-1,3-di-(4'-methoxyphenyl)-5-methyl-7-phenyl-1,2,3,4-tetrahydropyrimido[4,5-d]pyrimidine.

RESULTS and DISCUSSION

The target compounds **4-5** were prepared by synthetic route reported in Scheme 2. The starting substrates in our investigations were 4-arylamino-6-methyl-2-phenyl-5-pyrimidinecarboxylic acids **1** (5), which were reduced with $LiAlH_4$ (3). The obtained in this way 4-arylamino-6-methyl-2-phenyl-5-hydroxymethylpyrimidines **2** reacted with $SOCl_2$ to give 2-arylamino-6-methyl-2-phenyl-5-chloromethylpyrimidines **3**. The reaction of chloropyrimidine derivatives **3** with various aromatic amines yielded 4-arylamino-6-methyl-2-phenyl-5-aminomethylpyrimidines **4** (8). The obtained 5-aminomethylpyrimidines **4** were cyclized in Mannich's reaction to give 1,2,3,4-tetrahydropyrimido[4,5-d]pyrimidines **5**. Physical properties of these compounds are shown in Tables 1 and 2. The X-ray crystal structure of compound **5j** confirms the cyclization reaction. The molecular structure of (**5j**) with atom-labelling scheme is shown in Figure 1.
The newly obtained derivatives of 4-arylamino-6-methyl-2-phenyl-5-aminomethylpyrimidines (**4a**-**d**) and 1,2,3,4-tetrahydropyrimido[4,5-d]pyrimidine (**5a**-**5j**) were evaluated for their antibacterial and antifungal activity. The microbiological investigations were based on M-7 and A-5 standards (MIC Testing), using one mycotic and five bacterial strains: *Bacillus subtilis, Klebsiella pneumoniae, Enterococcus Faecalis, Staphylococcus epidermidis, Staphylococcus aureus* and *Candida albicans*. The

* Corresponding author: phone: +48 71 7840343; e-mail: jerzy@chorg.am.wroc.pl



Scheme 1. Chemical structures of (a) piromidic acid, (b) pipemidic acid, (c) pyrimido[4,5-d][1,3]oxazine system and (d) pyrimido[4,5-d]pyrimidine system.

MIC (minimum inhibitory concentration) values were determined and compared to erythromycin as reference drug. The most significant results are presented in Tables 3-4. The results indicate that compounds **4** (with exception of **4d**) exhibited significant antibacterial activity. Their antifungal activity is also satisfactory (Table 3). The cyclization of 5-aminomethylpyrimidines **4** to 1,2,3,4-tetrahydropyrimido[4,5-d]pyrimidines **5** caused considerable growth of antibacterial activity, but significantly increased antifungal activity. Placement of an electron-withdrawing group at N-1, C-2 and N-3 aryl rings leads to a decrease of activity. Compounds possessing three strongly electronegative substituents attached to phenyl rings, such as *e.g.*: chlorine atom (compounds **5h** and **5i**), emerged as the potent active antibacterial agents (Table 4).

The pyrimido[4,5-d]pyrimidines **5** show considerable structural similarity to quinoline chemotherapeutics already used in medical treatment: e.g. piromidic acid which is classified as the 1st generation quinolones and pipemidic acid – 2nd generation chemotherapeutic. The mechanism of quinoline chemotherapeutic activity consists in suppressing gyrase, the enzyme taking part in preparation of DNA for transcription, as well as suppressing DNA replication (9).

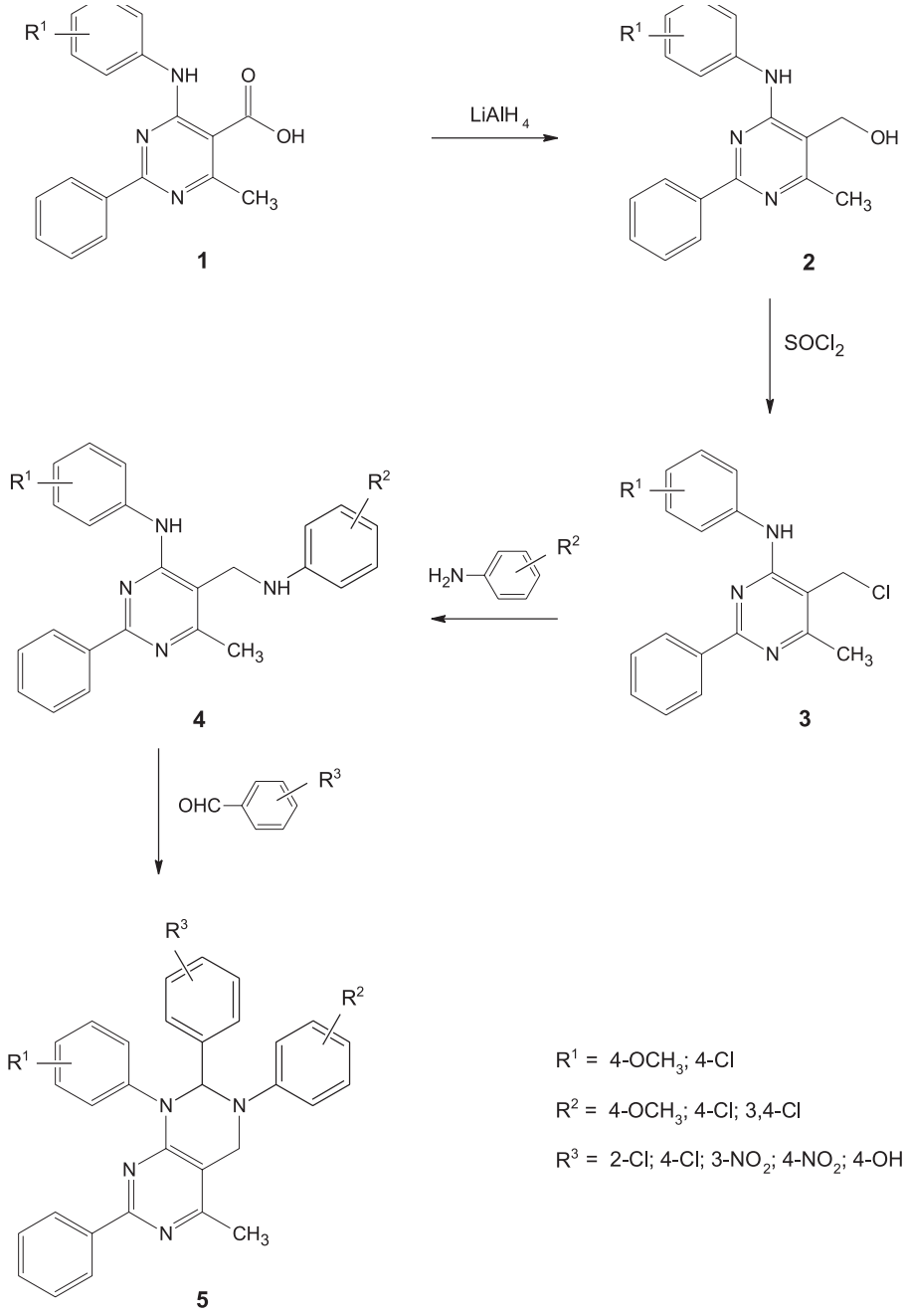
EXPERIMENTAL

Chemistry

Melting points were determined in a Köfler apparatus. ^{1}H NMR spectra were recorded on BS-487-C-80 MHz Tesla spectrometer. Infra-red (IR) spectra were recorded in nujol with a Specord spectrophotometer, at Analytical Laboratory of Medical Academy in Wrocław. Elemental analyses indicated by the symbols were within ± 0.4% of the theoretical values.

4-(4-methoxyphenyl)amino-5-[(4-methoxyphenyl) amino]methyl-6-methyl-2-phenylpyrimidine **4a**

The 5-hydroxymethyl-6-methyl-4-(4-ethoxyphenyl)amino-2-phenylpyrimidine **3a** (4 g, 0.00938 mol) was dissolved in chloroform (50 mL) and 4-methoxyaniline [*p*-anisidine] (2 g) was added. The reaction mixture was refluxed for 6 h with vigorous stirring, then was cooled and poured into water (300 mL). The aqueous solution was extracted three times with chloroform (50 mL). The combined chloroform phases were dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was crystallized from methanol to give compound **4a**. Yield: 82.5% (4.16 g), m.p. 156-158 ^{o}C; IR (KBr) ν [cm^{-1}]: 3440 (NH), 948 (NH); ^{1}H-NMR (300 MHz, $CDCl_3$)



Scheme 2. Synthesis of 1,2,3-aryl-1,2,3,4-tetrahydropyrimido[4,5-d]pyrimidine derivatives.

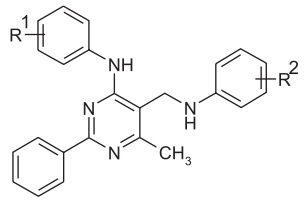
δ [ppm]: 1.50 (s, 3H, OCH_3), 1.52 (s, 3H, OCH_3), 1.85 (s, 3H, CH_3), 2.20 (t, 1H, NH), 2.85 (d, 2H, CH_2), 5.12 (s, 1H, NH), 7.20-8.35 (m, 13H, arom.). Compounds **4b**, **4c** and **4d** were obtained by adopting the same procedure.

4b: ^{1}H-NMR (300 MHz, $CDCl_3$) δ [ppm]: 1.40 (s, 3H, CH_3), 1.55 (s, 3H, OCH_3), 2.20 (t, 1H, alkyl-NH-aryl), 2.85 (d, 2H, CH_2), 5.12 (s, 1H, $aryl_2$-NH), 7.20-8.35 (m, 13H, arom.).

4c: ^{1}H-NMR (300 MHz, $CDCl_3$) δ [ppm]: 1.35 (s, 3H, CH_3), 1.60 (s, 3H, OCH_3), 2.25 (t, 1H, alkyl-NH-aryl), 2.85 (d, 2H, CH_2), 5.20 (s, 1H, $aryl_2$-NH), 7.30-8.40 (m, 13H, arom.).

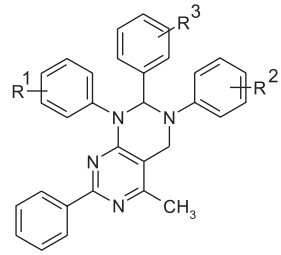
4d: ^{1}H-NMR (300 MHz, $CDCl_3$) δ [ppm]: 1.30 (s, 3H, CH_3), 1.55 (s, 3H, OCH_3), 2.20 (t, 1H, alkyl-NH-aryl), 2.80 (d, 2H, CH_2), 5.25 (s, 1H, $aryl_2$-NH), 7.20-8.35 (m, 12H, arom.).

Table 1. Physical data of compounds **4a**-**4d**.



Comp. No.	R^1	R^2	Formula	M.P.	Yield	ANALYSIS calc. / found			
				(°C)	(%)	C	H	N	Cl
4a	4-OCH_3	4-OCH_3	$C_{26}H_{26}N_4O_2$ (426.52)	156-158	82.5	73.22 73.46	6.14 6.44	13.14 13.33	
4b	4-OCH_3	4-Cl	$C_{25}H_{23}ClN_4O$ (430.94)	162-164	86.2	69.68 69.44	5.38 5.56	13.00 13.42	8.23 8.54
4c	4-Cl	4-OCH_3	$C_{25}H_{23}ClN_4O$ (430.94)	160-162	77.3	5.38 5.46	5.38 5.26	13.00 13.22	8.23 8.44
4d	4-OCH_3	3,4-Cl_2	$C_{25}H_{23}Cl_2N_4O$ (466.31)	168-170	83.4	64.38 64.49	4.97 4.52	12.01 12.29	15.20 15.32

Table 2. Physical data of compounds **5a**-**5j**.



Comp. No.	R^1	R^2	R^3	Formula (m.w.)	M.P.	Yield	ANALYSIS calc. / found			
					(°C)	(%)	C	H	N	Cl
5a	4-OCH_3	4-OCH_3	4-Cl	$C_{33}H_{29}ClN_4O_2$ (549.07)	215-217	84.5	72.19 72.24	5.32 5.54	10.20 6.50	6.46 6.50
5b	4-OCH_3	4-OCH_3	4-NO_2	$C_{33}H_{29}N_5O_4$ (559.62)	212-214	81.3	70.83 70.92	5.22 5.36	12.51 12.62	
5c	4-OCH_3	4-Cl	2-Cl	$C_{32}H_{26}Cl_2N_4O$ (553.49)	224-226	78.3	69.44 69.43	4.73 4.66	10.12 10.24	12.11 12.42
5d	4-OCH_3	3,4-Cl_2	4-OH	$C_{32}H_{26}Cl_2N_4O_2$ (569.49)	170-172	76.2	67.49 67.59	4.60 4.32	9.84 9.59	12.45 12.52
5e	4-Cl	4-OCH_3	2-Cl	$C_{32}H_{26}Cl_2N_4O$ (553.49)	211-213	77.2	69.44 69.53	4.73 4.86	10.12 10.44	12.11 12.52
5f	4-Cl	4-OCH_3	3-NO_2	$C_{32}H_{26}ClN_5O_3$ (564.04)	225-227	79.4	68.14 68.59	4.64 4.32	12.42 12.29	6.28 6.52
5g	4-Cl	4-OCH_3	4-OH	$C_{32}H_{27}ClN_4O_2$ (535.04)	171-173	74.6	71.84 71.94	5.09 5.23	10.47 10.33	6.63 7.01
5h	4-Cl	4-Cl	4-Cl	$C_{31}H_{23}Cl_3N_4$ (557.91)	207-209	72.5	66.74 66.71	4.15 4.11	10.04 10.31	19.06 19.08
5i	4-Cl	4-Cl	2-Cl	$C_{31}H_{23}Cl_3N_4$ (557.91)	222-224	71.3	66.74 66.81	4.15 4.21	10.04 10.41	19.06 19.18
5j	4-OCH_3	4-OCH_3	2-Cl	$C_{33}H_{29}ClN_4O_2$ (549.07)	217-219	82	72.19 72.31	5.32 5.14	10.20 10.23	6.46 6.22

Table 3. Minimal inhibitory concentrations (MIC) (μg/mL) Testing M-7, A-5 of compounds **4a-4c.**

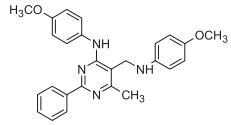
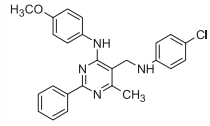
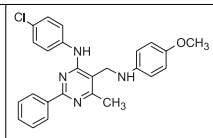
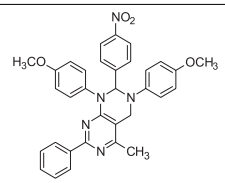
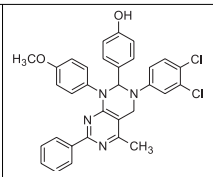
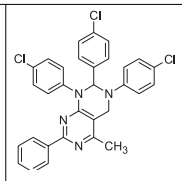
				Erythromycin
	4a	**4b**	**4c**	
Bacillus subtilis *ATCC – 6633*	64	32	32	2
Klebsiella pneumoniae *ATCC – 13886*	128	64	64	4
Enterococcus faecalis *ATCC – 27853*	128	128	128	8
Staphylococcus epidermidis *ATCC – 14990*	64	64	128	32
Staphylococcus aureus *ATCC – 6538P*	128	64	32	8
Candida albicans *ATCC – 10231*	128	32	32	128

Table 4. Minimal inhibitory concentrations (MIC) (μg/mL) Testing M-7, A-5 of compounds **5b, 5d and 5h**

				Erythromycin
	5b	**5d**	**5h**	
Bacillus subtilis *ATCC – 6633*	32	16	8	2
Klebsiella pneumoniae *ATCC – 13886*	32	32	16	4
Enterococcus faecalis *ATCC – 27853*	128	64	32	8
Staphylococcus epidermidis64 *ATCC – 14990*	32	32	32	
Staphylococcus aureus *ATCC – 6538P*	64	32	32	8
Candida albicans *ATCC – 10231*	128	128	64	128

2-(4-Chlorophenyl)-1,3-di(4-methoxyphenyl)-5-methyl-7-phenyl-1,2,3,4-tetrahydropyrimido[4,5-d]pyrimidine **5a**

4-(4-Methoxyphenyl)amino-5-[(4-methoxyphenyl)amino]methyl-6-methyl-2-phenylpyrimidine **4a** (4 g, 0.00728 mol) was dissolved in THF (50 mL), 36% HCl (1 mL) and 4-chlorobenzaldehyde (1 g) were added. The reaction mixture was refluxed for 18 h, then was cooled down and poured into water (300 mL). The solution was neutralized using 25% ammonia solution and extracted three times with chloroform (50 mL). The combined chloroform phases were dried over $MgSO_4$, filtered and concentrated under vacuum. The oily residue was purified by column chromatography on silica gel (200-400 mesh) using chloroform as eluent and crystallization from methanol to give **5a**. Yield: 84.5% (4.35 g), m.p. 215-217şC; IR (KBr) ν [cm $^{-1}$]: 1172 (N); ^{1}H-NMR (300 MHz, $CDCl_3$) δ [ppm]: 1.37 (s, 3H, CH_3), 1.56 (s, 3H, OCH_3), 1.58 (s, 3H, OCH_3), 2.80 (s, 2H, CH_2), 3.20 (s, 1H, CH), 7.50-8.40 (m, 17H, arom.).

Compounds **5b** and **5j** were obtained by adopting the same procedure.

5b: ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] 1.38 (s, 3H, CH_3), 1.57 (s, 4H, OCH_3), 1.60 (s, 4H, OCH_3), 2.83 (d, 2H, CH_2), 3.22 (s, 1H, CH), 7.50-8.40 (m, 17H, arom.).

5j: ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] 1.37 (s, 3H, CH_3), 1.55 (s, 4H, OCH_3), 1.58 (s, 4H, OCH_3), 2.80 (d, 2H, CH_2), 3.20 (s, 1H, CH), 7.50-8.40 (m, 17H, arom.).

2,3-Di(4-chlorophenyl)-1-(4-methoxyphenyl)-5-methyl-7-phenyl-1,2,3,4-tetrahydropyrimido[4,5-d]pyrimidine **5c**

4-(4-Methoxyphenyl)amino-5-[(4-chlorophenyl)amino]methyl-6-methyl-2-phenylpyrimidine **4b** (4 g, 0.00723 mol) was dissolved in THF (50 mL), 36% HCl (1 mL) and 4-chlorobenzaldehyde (1 g) were added. The reaction mixture was refluxed for 18 h, then was cooled down and poured into water (300 mL). The solution was neutralized using 25% ammonia solution and extracted three times with chloroform (50 mL). The combined chloroform phases were dried over $MgSO_4$, filtered and concentrated under vacuum. The oily residue was purified by column chromatography on silica gel (200-400 mesh) using chloroform as eluent and crystallization from methanol to give **5c**. Yield: 76.5% (3.93 g), mp 224-226^{o}C; IR (KBr) ν [cm $^{-1}$]: 1164 (N); ^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm]: 1.40 (s, 3H, CH_3), 1.56

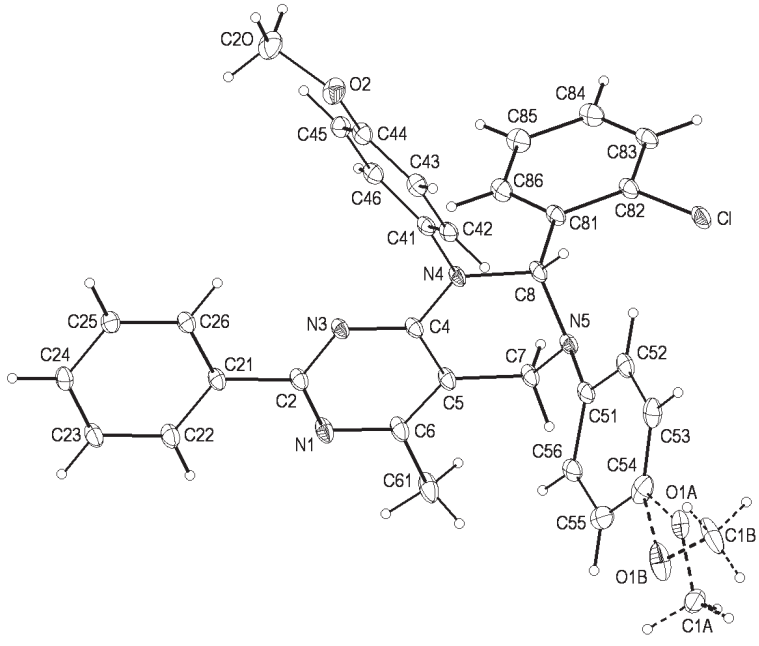


Figure 1. The molecular structure of **5j**, showing the atom-labelling scheme. Displacement ellipsoids are drawn at the 10% probability level and H atoms are shown as small spheres of arbitrary radii. The dashed lines indicate bonds between atoms from the disordered methoxy group.

(s, 3H, OCH_3), 2.80 (s, 2H, CH_2), 3.20 (s, 1H, CH), 7.50-8.40 (m, 17H, arom.).

Compounds **5d**, **5e**, **5f** and **5g** were obtained by adopting the same procedure.

5d: ^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm]: 1.35 (s, 3H, CH_3), 1.56 (s, 3H, OCH_3), 2.87 (s, 2H, CH_2), 3.22 (s, 1H, CH), 5.28 (s, 1H, aryl-OH), 7.50-8.40 (m, 16H, arom.).

5e: ^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm]: 1.37 (s, 3H, CH_3), 1.58 (s, 3H, OCH_3), 2.75 (s, 2H, CH_2), 3.20 (s, 1H, CH), 7.50-8.40 (m, 17H, arom.).

5f: ^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm]: 1.37 (s, 3H, CH_3), 1.58 (s, 3H, OCH_3), 2.87 (s, 2H, CH_2), 3.25 (s, 1H, CH), 7.50-8.40 (m, 17 H, arom.).

5g: ^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm]: 1.36 (s, 3H, CH_3), 1.58 (s, 3H, OCH_3), 2.85 (s, 2H, CH_2), 3.23 (s, 1H, CH), 5.27 (s, 1H, aryl-OH), 7.50-8.40 (m, 16H, arom.).

1,2,3-Tri(4-chlorophenyl)-5-methyl-7-phenyl-1,2,3,4-tetrahydropyrimido[4,5-d] pyrimidine **5h**

4-(4'-Chlorophenyl)amino-5-[(4'-chlorophenyl)amino]methyl-6-methyl-2-phenylpyrimidine **4h** (4 g, 0.00918 mol) was dissolved in THF (50 mL), 36% HCl (1 mL) and *p*-chlorobenzoic aldehyde (1 g) were added. The reaction mixture was refluxed for 18 h, then was cooled down and poured into water (300 mL). The solution was neutralized using 25% ammonia solution and extracted three times with chloroform (50 mL). The combined chloroform phases were dried over $MgSO_4$, filtered and concentrated under vacuum. The oily residue was purified by column chromatography on silica gel (200-400 mesh) using chloroform as eluent and crystallization from methanol to give **5h**. Yield: 74.4% (3.80 g), mp 207-209°C; IR (KBr) ν [cm $^{-1}$]: 3250 (N), 1560 (N); ^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm]: 1.47 (s, 3H, CH_3), 2.70 (d, 2H, CH_2), 3.30 (s, 1H, CH), 7.40-8.50 (m, 17H, arom.).

Compound **5i** was obtained by adopting the same procedure.

5i: ^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm]: 1.37 (s, 3H, CH_3), 2.85 (s, 2H, CH_2), 3.25 (s, 1H, CH), 7.25-8.45 (m, 17H, arom.).

X-ray crystallography for **5j**

Slow evaporation of a solution of **5j** in an acetone-methanol mixture (1:3, v/v) resulted in the formation of plates, that were submitted for X-ray analysis. X-ray data were collected from a single crystal, 0.25 × 0.3 × 0.6 mm, at 100 K using graphite-monochromated Cu-K_α (l = 1.5418 Å) radiation on a Xcalibur PX diffractometer (ω- and ϕ-scans). The instrument was equipped with Oxford Cryosystems low-temperature devices. The data were numerically corrected for absorption with the use of CRYSALIS RED 1.171, the Xcalibur PX Software (10). The structure was solved by direct methods using the SHELXS-97 program (11) and refined by full-matrix least-squares calculations on F^2 with SHELXL-97 (12). Non-hydrogen atoms were refined with anisotropically thermal parameters. The H atoms bound to C atoms were included in geometrically calculated positions, with the C-H distances in the range 0.95-1.00 Å, and refined using riding model, with $U_{iso}(H) = 1.5U_{eq}(C)$ for methyl and $1.2U_{eq}(C)$ for the remainder. The methoxy group bonded to the aromatic C54 atom was found to be disordered over two sites (denotes as A and B). The atoms from the methoxy group were refined with the occupancy factors of 0.5 and restraints on the C-O distances by the use of SADI instruction in SHELXL-97 (12). The asymmetric unit of the crystal consists of one compound (**5j**) molecule: $C_{33}H_{29}ClN_4O_2$, M_r = 549.05, monoclinic, space group $P2_1/c$, a = 8.975(3), b = 10.552(4), c = 29.296(12) Å, β = 91.15(4)°, V = 2774(2) Å^3, d_{calc} = 1.315, $F(000)$ = 1152, μ = 1.518 mm^{-1}, T_{min} = 0.527, T_{max} = 0.712, Z = 4, 18064 measured reflections ($-5 \le h \le 11$, $-13 \le k \le 12$, $-34 \le l \le 34$), 4667 independent and 3597 observed reflections with $I > 2\sigma(I)$ (R_{int} = 0.0245), 382 parameters, R = 0.0551, wR_2 = 0.116 for all reflections, $w = 1/[\sigma^2(F^2) + (0.0636P)^2 + 0.4246P]$ where $P = (F_o^2 + 2F_c^2)/3$.

CCDC 643168 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223 336033; e-mail: deposit@ccdc.cam.uk).

Microbiology

The obtained chemical compounds were investigated microbiologically on selected strains, in order to evaluate their bioactivity. The investigation was based on M- 7, A – 5 standards (MIC Testing). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard – Fifth Edition NCCLS (9). The fungal strains also were cultivated on this standard recommended broth – Mueller Hinton Broth II.

Sample bacterial cultures were suspended in 3 mL of a sterile solution of PBS according to 0.5 Mc Farland's standard (corresponding to 1 to 2 × 10^8 CFU/mL), and next were diluted with a sterile 1:10 PBS solution (giving 1 × 10^7 CFU/mL).

0.01 mL of obtained inoculum was added to 0.2 mL of sterile final dilutions of the investigated substances according to Tables 3 and 4, yielding 5×10^4 concentration of bacteria in the investigated samples. Six trials were carried out for every dilution of the investigated substance – one control without the inoculum.

REFERENCES

1. Machoń Z., Cieplik J.: Synthesis 2, 142 (1986).
2. Machoń Z., Cieplik J.: Pol. J. Pharmacol. Pharm. 40, 201 (1988) .
3. Cieplik J., Machoń Z., Zimecki M., Wieczorek Z.: Arch. Immunol.Ther. Exp. 41, 11 (1993).
4. Cieplik J., Machoń Z., Zimecki M., Wieczorek Z.: Farmaco 50, 131 (1995).
5. Machoń Z., Cieplik J.: Eur. J. Med. Chem-Chim. Ther. 19, 359 (1984).
6. Pluta J., Flendrich M., Cieplik J.: Boll. Chim. Farm. 135, 459 (1996).
7. Cieplik J., Pluta J., Meler A.: Arch. Pharm. (Weinheim) 330, 237 (1997).
8. Cieplik J., Pluta J., Gubrynowicz O.: Sci. Pharm. 68, 333 (2000).
9. Koga H., Itah A., Murayama S., Suzue S., Irikura T.: J. Med. Chem. 23**,** 1358 (1980).
10. Oxford Diffraction Ltd. (1995-2003). Xcalibur PX Software – CrysAlis CCD and CrysAlis RED Version 1.171. Oxford Diffraction Poland, Wrocław, Poland.
11. Sheldrick, G. M.: SHELXS-97. Program for the solution of crystal structures. University of Göttingen, Germany (1997). .
12. Sheldrick, G. M.: SHELXL-97. Program for the refinement of crystal structures. University of Göttingen, Germany (1997).
13. NCCLS Vol. 20 , No. 2, January 2002.

Received: 12. 12. 2007