J. prakt. Chem. 335 (1993) 623–627

Journal für praktische Chemie
Chemiker-Zeitung

Derivatives of 1,10- and 4,7-Phenanthrolinaldehydes and Di(N,N-di-ethylamino)ethoxyphenanthrolines as Potential Antitumor Agents

Jacek Młochowski and Jerzy Palus

Wrocław/Poland, Institute of Organic and Physical Chemistry, Technical University

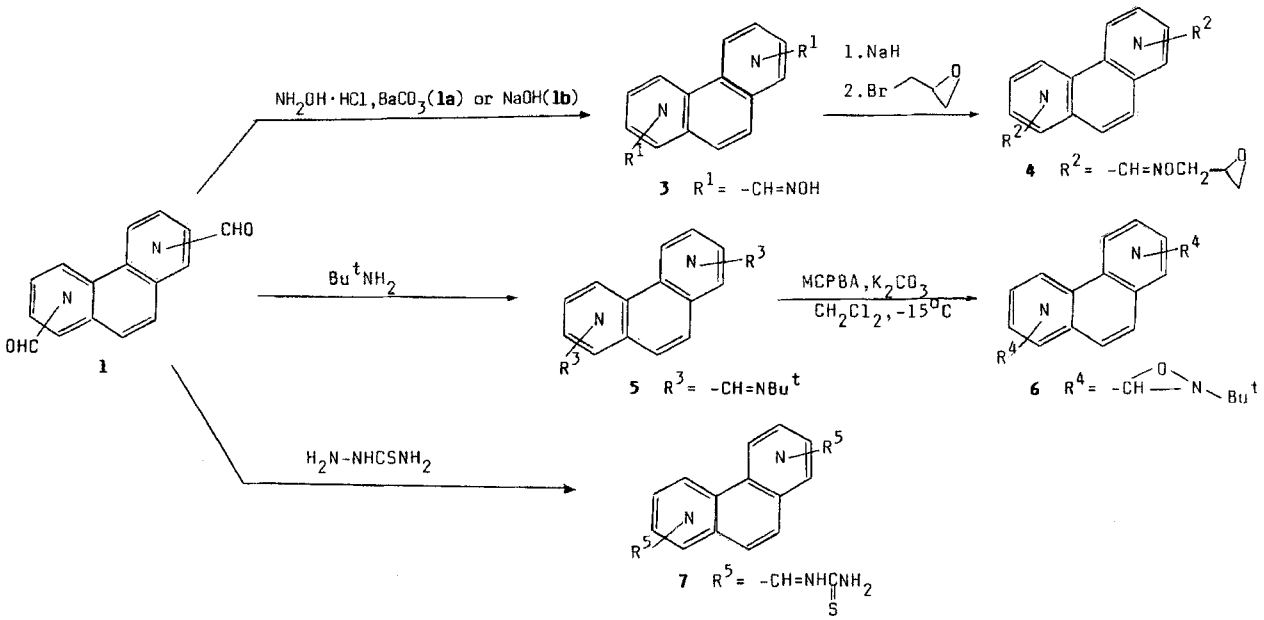
Received November 25th, 1992

Abstract. The syntheses of 1,10- and 4,7-phenanthroline derivatives having glycidyl, oxaziridinyl, N,N-diethylaminoethoxy and thiosemicarbazone groups, and related compounds are reported. Their activities against experimental human tumor cells A 549 in vitro in relation to the molecular structures are discussed.

It is known that planar tricyclic aromatic molecule, able to intercalate into the DNA double helix containing two reactive functions in the side chains should crosslink this polynucleotide, and transcriptional control of the cell proliferation takes place. For example, appreciate activity of bisglycidyl derivatives of anthraquinone and of 2,7-fluorenone diglycidyl ether and related compounds were reported [1,2]. The recently synthesized quinones and azines having the 2-alkyloxaziridinyl or imino groups also manifested high activity against experimental tumor cells [3,4].

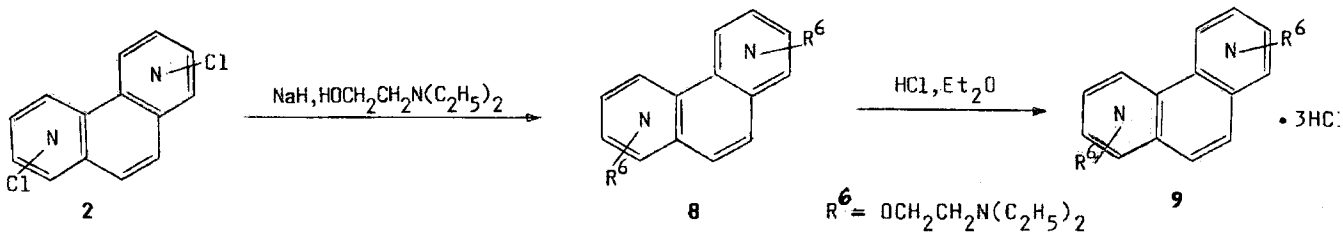
It was also reported that phenanthrolines having positively charged side chains interacted strongly with DNA on the mode of noncovalent binding this polynucleotide but no biological data for these compounds as antitumor agents were revealed [5].

Thiosemicarbazones derived from pyridine and isoquinolinealdehydes were also found as active compounds.



Compound 1, 3–7	Position: Nitrogen atoms	Position: Substituents
a	1,10	2,9
b	4,7	3,8

Scheme 1



Compound	Position	
2, 8, 9	Nitrogen atoms	Substituents
a	1,10	2,9
b	4,7	3,8

Scheme 2

Their biological action depends on the complexing of Fe(II) ions and then inhibition of ribonucleosidodiphosphorane reductase [6–12].

Taking into consideration the structural features mentioned above, we designed the derivatives of 1,10- and 4,7-phenanthroline having tricyclic azaaromatic system able to intercalate in DNA and bearing reactive functions able to form covalent bonds (the oxirane and oxaziridine ring or the azomethine group). Other compounds designed were able to interact elektrostatically (the N,N-diethylammonium group) or to chelate Fe(II) ions (thiosemicarbazones). These compounds were synthesized in the ways shown in the Schemes 1 and 2 and their characteristics are given in Table 1.

Derivatives of 1,10-phenanthroline were prepared from 1,10-phenanthroline-2,9-dicarboxaldehyde obtained by oxidation of 2,9-dimethyl-1,10-phenanthroline with selenium dioxide in dioxane [13]. The aldehyde **11a** was converted into oxime **3a** which etherified with 1-bromo-2,3-epoxypropane gave 2,9-di[N-(oxiranylmethyloxy)azomethino]-1,10-phenanthroline **4a**. The Schiff base **5a** derived from aldehyde **1a** was oxidized with 3-chloroperbenzoic acid (MCPBA) to 2,9-di (2-(tert-butyloxaziridinyl)-1,10-phenanthroline **6a**. It should be noted that under reaction conditions (temperature –15 °C) none ring nitrogen atom was oxidized although high reactivity of 1,10-phenanthroline towards N-oxidation is known [14]. Dithiosemicarbazone **7a** was prepared from dialdehyde **1a** in the usual way, and 2,9-di [2-(N,N-diethylamino) ethoxy]-1,10-phenanthroline (**8a**) was obtained from 2,9-dichloro-1,10-phenanthroline (**2a**) and 2-(N,N-diethyl-amino)ethanol and then converted into trihydrochloride **9a**.

In the similar way, the analogous derivatives of 4,7-phenanthroline: 3,8-di[N-(oxiranylmethyloxy)azomethino]-4,7-phenanthroline (**4b**), 3,8-di(2-tert-butyloxaziridinyl)-4,7-phenanthroline (**6b**) and dithiosemicarbazone (**7b**) were obtained from 4,7-phenanthroline-3,8-dicarboxaldehyde (**1b**). The aldehyde **1b** (previously unknown) was synthesized by oxidation of 3,8-dimethyl-4,7-phenanthroline (**1c**) with selenium dioxide in dioxane-water solution at elevated temperature. The starting compound **1c** was obtained by the reaction of 6-aminoquinaldine with crotonaldehyde. 3,8-Di[2-(N,N-diethylamino)ethoxy]-4,7-phenanthroline trihydrochloride was prepared from 3,8-dichloro-4,7-phenanthroline (**2b**) [5].

It should be mentioned that, due to chirality of the carbon atoms situated in small rings present in the glycidyl ethers **4** and oxaziridines **6**, the products obtained seem to be mixtures of enantiomers. Both aldimines (**5a,5b**) were pure diastereoisomers (most probably with trans configuration), and oxidized with MCPBA gave only trans isomers (**6a,6b**)(since two bulky substituents make cis form unfavorable). It was confirmed by analysis of the ^{1}H-NMR spectra because the singlet signal of proton bound to the oxaziridine ring was shifted to 5.03–5.27 ppm [3].

Disubstituted 1,10- and 4,7-phenanthroline derivatives (**3–7,9**) and monosubstituted ones: 2-[2-(N,N-diethylamino)ethoxy]-1,10-phenanthroline dihydrochloride (**10**), 1,10-phenanthroline-2-carboxaldehyde thiosemicarbazone (**11**) and 4,7-phenanthroline-3-carboxaldehyde thiosemicarbazone (**12**) were also tested for their cytotoxic activity. Moreover, three known active compounds of similar structure: 1,4-di(oxiranylmethyloxy)anthraquinone (**13**) [1], and thiosemicarbazones of quinoline-2-carboxaldehyde (**14**) and quinoline-8-carboxaldehyde (**15**) [14] were synthesized and tested.

All these sompounds were screened against human cancer cells A 549 (in vitro). Cytotoxicity was established and the CD_{50} values found are listed in Table 2. The result showed that among compounds under investigation most active was 2,9-bis[2-(N,N-diethylamino)-ethoxy]-1,10-phenanthroline trihydrochloride (**9a**). Its cytotoxicity was extremely high (CD_{50} = 4.4 × 10^{-7} μmol/ml). The 4,7-phenanthroline analog **9b** and monosubstituted 1,10-phenanthroline **10** were about hundred times less active. It seems possible that shape of the mo-

Table 1 Physical, analytical, and spectral data for derivatives of 1,10- and 4,7-phenanthroline

Compound	Formula	Yield (%)	m.p. (°C)	Found (required) [%] C	H	N	I. r. (KBr) cm^{-1}	1H-NMR ($CDCl_3$) δ_H (ppm)
1b	$C_{14}H_8N_2O_2$	23	263–266 (with decomp.)	71.03 (71.18)	3.28 (3.41)	11.80 (11.86)	[a]1705	[f]6.68 (d,2H,J=7Hz, H-2 and H-8); 6.85 (s,2H,H-5 and H-6); 7.75 (d,2H,J=7Hz,H-1 and H-10); 8.23 (s,2H,CHO)
1c	$C_{14}H_{12}N_2$	53	180–181	M.p. ref. [16] 180–181°C			–	3.10 (s,6H,-CH_3); 7.69 (d,2H, J=8Hz, H-3 and H-8); 8.47 (s,2H, H-5 and H-6); 8.89 (d,2H,J=8Hz, H-1 and H-7)
3b	$C_{14}H_{10}N_4O_2$	48	292–294 (with decomp.)	62.92 (63.10)	3.64 (3.79)	20.86 (21.04)	[b]3190	[g]6.70 (s,2H,H-5 and H-6); 7.03–7.48 (m,4H,H-1,2,9 and H-10); 7.80 (s,2H,-CH=N-)
4a	$C_{20}H_{18}N_4,O_4$	48	148–149	63.40 (63.51)	4.72 (4.76)	14.83 (14.81)	[c]1590	2.70–3.03 (m,4H,-CH(O)–**CH_2**); 3.28–3.50 (m,2H,-**CH**(O)–CH_2); 4.18-4.65 (m,4H,-OCH_2-); 7.84 (m,2H,H-3 and H-8) 8.26(m,4H,H-5 and H-6, -CH=N-); 8.69 (m,2H,H-4 and H-7)
4b	$C_{20}H_{18}N_4O_4$	65	176–178	63.42 (63.51)	4.71 (4.76)	14.90 (14.81)	[c]1590	2.68–2.98 (m,-CH(O)–**CH_2**); 3.23–3.53 (m,2H,-**CH**(O)–CH_2); 4.08–4.63 (m,4H,-OCH_2-); 8.10–8.91 (m,6H, -CH=NH-,H-2,H-9,H-1 and H-10); 8.45 (s,2H,H-5 and H-6)
5a	$C_{22}H_{26}N_4$	58	218–220	76.15 (76.31)	7.44 (7.51)	16.28 (16.18)	[c]1640	1.20 (s,18H,-CH_3); 7.82 (s, 2H,-CH=N-); 8.27 (d,2H,J=8Hz, H-3 and H-8); 8.55 (d,2H, J=8Hz,H-4 and H-7); 8.93 (s, 2H,H-5 and H-6)
5b	$C_{22}H_{26}N_4$	86	143–145	76.14 (76.31)	7.40 (7.51)	16.00 (16.18)	[c]1630	1.39 (s,18H,-CH_3); 8.28 (s, 2H,-CH=N-); 8.40 (d,2H,J=8Hz, H-2 and H-9); 8.69 (s,2H,H-5 and H-6); 8.95 (d,2H,J=8Hz, H-1 and H-10)
6a	$C_{22}H_{26}N_4O_2$	42	52– 53	70.10 (69.85)	6.99 (6.88)	14.94 (14.81)	[d]2970	1.13 (s,18H,-.CH_3); 5.27 (s, 2H,-**CH**(O)–N-); 7.56–7.64 (m, 4H,H-3,H-5,H-6 and H-8); 8.07 (d,2H,J=8Hz,H-4 and H-7)
6b	$C_{22}H_{26}N_4O_2$	56	103–105	69.73 (69.85)	6.64 (6.88)	14.76 (14.81)	[d]2940	1.17 (s,18H,-CH_3); 5.03 (s, 2H,-**CH**(O)–N-); 7.60 (d,2H,J=8Hz, H-2 and H-9); 8.13 (s,2H,H-5 and H-6); 8.80 (d,2H,J=8Hz, H-1 and H-10)
7a	$C_{16}H_{14}N_8S_2$	86	>360	50.38 (50.25)	3.82 (3.69)	29.41 (29.30)	[e]1268	[f,h]3.28 (m,2H,NH); 7.82-9.20 (m, 10H,ArH,NH_2); 12.08 (s,2H, -CH=N-)
7b	$C_{16}H_{14}N_8S_2$	88	>360	50.14 (50.25)	3.76 (3.69)	29.48 (29.30)	[e]1278	[f,h]3.63 (m,2H,NH); 8.22–9.13 (m, 8H,ArH,NH_2); 9.27-9.70 (m,2H, ArH); 11.93 (s,2H-CH=N-)

[a]$\tilde{\nu}_{C=O}$, [b]$\tilde{\nu}_{OH}$, [d]$\tilde{\nu}_{CH}$ in oxaziridine ring, [e]$\tilde{\nu}_{C=S}$, [f]Spectrum measured in DMSO. [g]Spectrum taken in D_2O for sodium salt obtained by treating oxime **3b** with NaH in ethanol under reflux. [h]HMDSO was used as a standard.

lecule and presence of two positively charged centers in the side chains interacting with polynucleotide phosphate anions play a crucial role in their biological action.

Other compounds also manifested appreciable activity. Among them, most active were di[N-(oxiranylmethyloxy)azomethino]phenanthrolines (**4**) and their precursors, oximes **3**. Dialdimines **5**, dioxaziridines **6** and dithiosemicarbazones **11, 12** also showed remarkable cyctotoxicity, similar to activity of 1,4-di(oxiranylmethyloxy)anthraquinone **13** used as the standard. The close similarity of the CD_{50} values for oxiranylmethyloxy derivates **4** and ongeneric oximes as well as for axaziridines **6** and aldimines **5**, being their precursors, were observed. The similar phenomenon was reported earlier for monooxaziridines and congeneric aldimines [3] and one can suppose that mechanism more complex than simple bioalkylation of DNA with small heterocyclic rings takes place.

The results found for thiosemicarbazones showed that the presence of the second thiosemicarbazone substituent (compounds **7**) does not enhance anticancer action in comparison with compounds having only one substituent, such as **11, 12, 14, 15**.

Table 2 Activity of 1,10- and 4,7-phenanthroline derivatives and related compounds against human lung adenocarcinoma cells A 549

Compound	CD_{50} (μg/ml)	(μmol/ml)	Compound	CD_{50} (μg/ml)	(μmol/ml)
3a	9.4×10^{-3}	3.5×10^{-5}	**7b**	4.1×10^{-2}	1.1×10^{-4}
3b[a)]	3.3×10^{-3}	1.1×10^{-5}	**9a**	2.3×10^{-4}	4.4×10^{-7}
4a	1.9×10^{-3}	5.0×10^{-6}	**9b**	1.7×10^{-2}	3.1×10^{-5}
4b	7.3×10^{-3}	1.9×10^{-5}	**10**	1.9×10^{-2}	5.1×10^{-5}
5a	5.1×10^{-2}	1.5×10^{-4}	**11**	2.3×10^{-2}	8.0×10^{-5}
5b	5.6×10^{-2}	1.6×10^{-4}	**12**	4.0×10^{-2}	1.4×10^{-4}
6a	4.7×10^{-2}	1.2×10^{-4}	**13**	4.7×10^{-2}	1.3×10^{-4}
6b	13.6×10^{-2}	3.6×10^{-4}	**14**	1.0×10^{-2}	4.3×10^{-5}
7a	12.2×10^{-2}	3.2×10^{-4}	**15**	5.0×10^{-3}	2.2×10^{-5}

[a)]Sodium salt of compound **3b** was tested

We wish to thank Prof. Dr. A. D. Inglot and Dr. E. Albin, Institute of Immunology and Experimental Therapy of Polish Academy of Sciences, Wrocław, for helpful guidance for the biological tests.

Experimental

All melting points were determined on a hot-stage microscope. I. R. spectra were recorded on an Perkin-Elmer 621 spectrometer. ^{1}H-NMR spectra were obtained on a Tesla 100 MHz spectrometer with TMS as an internal standard. The CD_{50} values for A 549 were established according to the procedure reported in ref. [15].

1,10-Phenanthroline-2,9-dicarboxaldehyde (**1a**) and its oxime (**3a**) were prepared according to ref. [13]. The mono- and di-[2-(N,N-diethylamino)ethoxy]phenanthroline hydrochlorides (**10,9a,9b**) were synthesized from mono- or dichlorophenanthrolines according to the method reported earlier [15]. Thiosemicarbazones **11, 12, 14, 15** were obtained from corresponding aldehydes [14] and 1,4-di(oxiranylmethyloxy) anthraquinone (**13**) was synthesized in the same way as reported in ref. [1].

4,7-Phenanthroline-3,8-dicarboxaldehyde (**1b**)

To a solution of selenium dioxide (3.36 g, 30 mmol) in water (6 ml), dioxane (60 ml) and 3,8-dimethyl-4,7-phenanthroline (**1c**) (3.0 g, 14.4 mmol) were added and the reaction mixture was refluxed for 6 h. After this period selenium was filtered off, the filtrate was concentrated in vacuo to the about 20 ml volume and treated with 15 % aqueous solution of sodium hydrocarbonate. Crude aldehyde **1b** precipitated and was filtered off, washed with water (3 × 50 ml) and chloroform (50 ml), and recrystallized from dioxane.

3,8-Dimethyl-4,7-phenanthroline (**1c**)

The suspension of arsenium pentoxide (45.3 g, 0.2 mole) and 6-amino-2-methylquinoline (52 g, 0.33 mole) in concentrated hydrochloric acid (600 ml) was heated to 90 °C under vigorous stirring and crotonaldehyde (26.6 g, 0.38 mole) was added dropwise during 1.5 h. The reaction was continued for additional 1 h, and then reaction mixture was allowed to stand for 24 h. After this period, the mixture was poured into water (1 l), neutralized with aqueous solution of sodium hydroxide until first precipitate occurred and filtered through Celite 505. The filtrate was alkalized with aqueous sodium hydroxide to pH about 12. The crude product precipitated and was filtered off, washed with water, dried in air and recrystallized from ethyl acetate giving pure compound **1c**.

Dioxime (**3b**)

The mixture of aldehyde **1b** (1.0 g, 4.2 mmol), hydroxylamine hydrochloride (2.94 g, 42.4 mmol), sodium hydroxide (1.69 g, 42.4 mmol) and dimethyl sulfoxide (50 ml) was refluxed for 5 h. After the reaction had finished, the mixture was poured into water (150 ml). The crude oxime **3b** was filtered off, washed with water (3 × 50 ml), dried at 120 °C, and recrystallized from dimethyl sulfoxide.

Di[N-(oxiranylmethyloxy)azomethino]phenanthrolines (**4a**) *and* (**4b**)

To oil-free sodium hydride (22 mmol), prepared from 50 % NaH in anhydrous dimethyl sulfoxide (35 ml), the oxime **3a** or **3b** (2.66 g, 10 mmol) was added and the reaction mixture was stirred at room temperature under moisture-free conditions until all sodium hydride had dissolved.

Then, 1-bromo-2,3-epoxypropane (4.11 g, 30 mmol) was added and the reaction was continued for 72 h. The reaction mixture was poured into water (150 ml) and the product was ex-

tracted with chloroform (3 × 50 ml) and dried over anhydrous $MgSO_4$. The solvent was evaporated in vacuo and crude product **4a** or **4b** was recrystallized from the mixture methylene chloride-hexane (1:5).

Dialdimines (**5a**) *and* (**5b**)

To the solution of corresponding aldehyde **1a** or **1b** (1.18 g, 5 mmol) in methylene chloride (100 ml), tert-butylamine (2.19 g, 30 mmol) and molecular sieves (Serva, 3A; 4 g) were added. The reaction mixture was allowed to stand at room temperature for 20 h, then it was filtered, the molecular sieves were washed with dry methylene chloride (15 ml), and the solvent and excess of amine were evaporated from filtrate in vacuo. The crude dialdimines were recrystallized from hexane (**5b**) or from the mixture methylene chloride-hexane 1:5 (**5a**).

Dioxaziridines (**6a**) *and* (**6b**)

To the cooled (ice/salt) solution of aldimine **5a** or **5b** (2.08 g, 28 mmol) in anhydrous methylene chloride (60 ml), anhydrous sodium carbonate (3.87 g, 28 mmol) was added, and then a solution of 50 % m-chloroperbenzoic acid (4.48 g, 13 mmol) in methylene chloride (30 ml) was added dropwise under vigorous stirring and the reaction was continued for 4 h. The mixture was filtered through basic alumina (15 g) to remove peroxides, and the filtrate was washed with 5 % aqueous sodium hydrogen carbonate (3 × 50 ml) and with water (3 × 50 ml) and dried with anhydrous magnesium sulfate. The solvent was removed in vacuo on the water bath and crude product was recrystallized from hexane.

Thiosemicarbazones (**7a** *and* **7b**)

To the solution of corresponding aldehyde **1a** or **1b** (0.1 g, 0.42 mmol) in glacial acetic acid (25 ml), the solution of thiosemicarbazide (0.078 g, 0.86 mmol), and acetic acid (0.1 ml) in water (2 ml) was added. The reaction mixture was refluxed for 2 h, then cooled, and the solid precipitated was filtered off, washed with 5 % aqueous sodium hydrogen carbonate (30 ml), water (50 ml) and dried in air. The crude product was recrystallized from N,N-dimethylformamide-water (2:1).

References

[1] U. Stachel, H. P. Kraemer, H. H. Sedlacek, Ger. Offen. DE 3.331.296 (1985); Chem. Abstr. **103** (1985) 195981w
[2] J. Młochowski, J. Palus, J. Prakt. Chem. **332** (1990) 5
[3] J. Młochowski, E.Kubicz, K. Koc, M. Mordarski, W. Peczynska, L. Syper, Liebigs Ann. Chem. **1988** , 455
[4] S. B. Said, J. Młochowski, J. Skarzewski, Liebigs Ann. Chem. **1990** , 461
[5] Z. Szulc, M. Fikus, J. Młochowski, J. Palus, Monatsh. Chem. **119** (1988) 263
[6] F. A. French, E. J. Blanz jr, Cancer Res. **25** (1965) 1454
[7] K. C. Agrawal, R. J. Cushley, W. J. Mc Murray, A. C. Sartorelli: J. Med. Chem. **13** (1970) 431
[8] F. A. French, E. J. Blanz jr, S. C. Shaddix, R. W. Brockman, J. Med. Chem **17** (1974) 172
[9] K. C. Agrawal, A. J. Lin, B. A. Booth, J. R. Wheaton, A. C. Sartorelli, J. Med. Chem. **17** (1974) 631
[10] P. D. Mooney, B. A. Booth, E. C. Moore, K. C. Agrawal, A. C. Sartorelli, J. Med. Chem. **17** (1974) 145
[11] K. C. Agrawal, B. A. Booth, S. M. De Nuzzo, A. C. Sartorelli, J. Med. Chem. **18** (1975) 368
[12] E. C. Moore, B. A. Booth, Cancer Res. **31** (1971) 235
[13] C. J. Chandler, L. W. Deady, J. A. Reiss, J. Heterocyclic Chem. **18** (1981) 559
[14] M. Jastrzebska-Glapa, J. Młochowski, W. Sliwa, J. Prakt. Chem. **319** (1977) 883
[15] A. D. Inglot, E. Wolna, Arch. Immun. Ther. Exp. **31** (1983) 593
[16] A. Funk, P. V. Divekar, Can. J. Microbiol. **5** (1959) 317

Address for correspondence:

Prof. Dr. Jacek Młochowski
Technical University of Wrocław, Institute fo Organic and Physical Chemistry
Wybrzeze Wyspianskiego 27
PL–50370 Wrocław, Poland