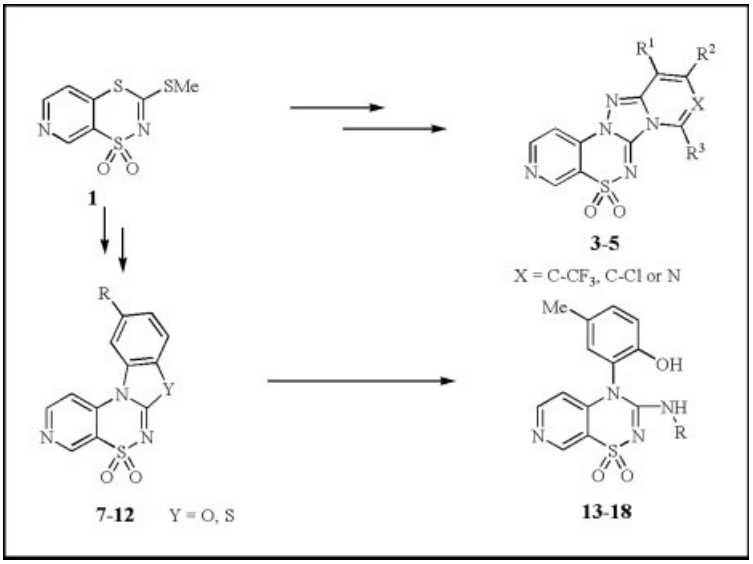
Application of 3-Methylthiopyrido[4,3-*e*]-1,4,2-dithiazine 1,1-Dioxide to the Synthesis of Novel Series of 4*H*-Pyrido[4,3-*e*]-1,2,4-thiadiazine Derivatives with Potential Biological Activity

Zdzisław Brzozowski,[a] Jarosław Sławiński,[b]* Anna Kędzia,[c] Ewa Kwapisz,[c] and Maria Gdaniec[d]

[a]Department of Chemical Technology of Drugs, Medical University of Gdańsk, 80-416 Gdańsk, Poland
[b]Department of Organic Chemistry, Medical University of Gdańsk, 80-416 Gdańsk, Poland
[c]Department of Oral Microbiology, Medical University of Gdańsk, 80-227 Gdańsk, Poland
[d]Department of Crystallography, Faculty of Chemistry, A. Mickiewicz University, 60-780 Poznań, Poland
*E-mail: jaroslaw@amg.gda.pl

Received July 7, 2009
DOI 10.1002/jhet.272
Published online 11 November 2009 in Wiley InterScience (www.interscience.wiley.com).



Two series of 4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine derivatives **3–5** and **7–12** were synthesized by the reactions of 3-methylthiopyrido[4,3-*e*]-1,4,2-dithiazine 1,1-dioxide **1** with 2-or 6-hydrazinoazines and 2-aminophenols or 2-aminothiophenol, respectively. Aminolysis of **8** (R = Me, Y = O) afforded the corresponding 3-(R-amino)-4-(2-hydroxy-5-methylphenyl)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides **13–18**. The structures of these compounds were confirmed on the basis of elemental analysis, spectral data, and X-ray crystallography. Compounds **3–5**, **7–10**, **12–15**, and **17–18** were screened *in vitro* for antibacterial activity. Moreover, preliminary *in vitro* anticancer assay was performed for compounds **3**, **7**, **10**, **11–13**, and **17–18** at the National Cancer Institute (Bethesda, MD) at a single dose (10 μ*M*) in the full NCI 60 cell panel.

J. Heterocyclic Chem., **46**, 1396 (2009).

INTRODUCTION

The aryl/heteroaryl sulfonamides constitute an important class of compounds with several types of biological activities and well-established safety profile [1]. Previously, as part of an extensive research program on the synthesis of compound containing 2-thiobenzenesulfonamide scaffold, several series of novel sulfonamides with remarkable antitumor activity [2–22], anti-HIV activity [2–6,16,22–28], or carbonic anhydrase inhibitors [29–31] were discovered in our laboratories. In the course of study on the synthesis of heterocyclic compounds bearing sulfonamide moiety, we developed a new synthetic method for preparation of 4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (**I–III**, Fig. 1) which involves reaction of the previously described [32] 3-methylthiopyrido[4,3-*e*]-1,4,2-thiadiazine 1,1-dioxide (**1**) with 2- or 6-hydrazinoazines, 2-aminophenols or 2-aminothiophenols as a key step. The previously described 4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides (**IV** and **V**, Fig. 1) as the potassium channel openers, were obtained starting from 4-(hydroxy-, amino-, or alkylamino) pyridine-3-sulfonamide [33,34].

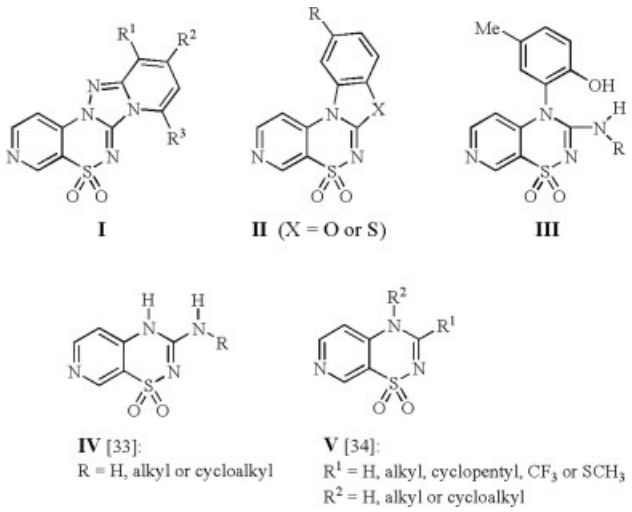


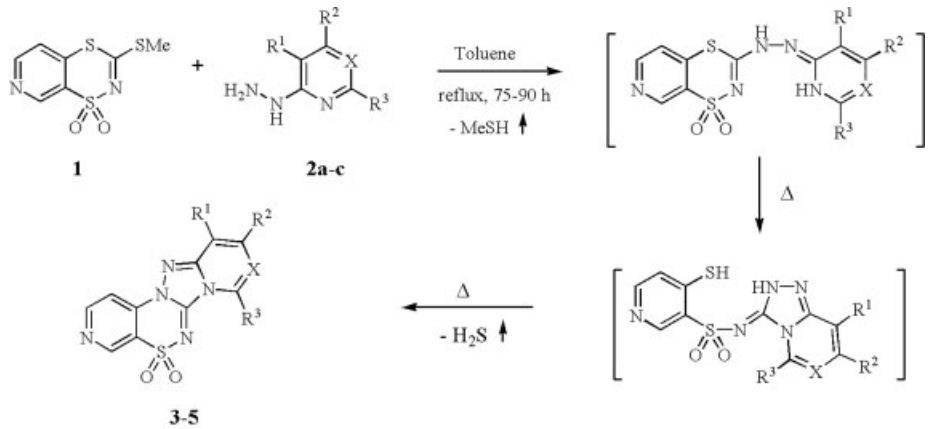
Figure 1. General structures of novel 4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides **I–III** and structures of known potassium channel openers **IV** and **V**.

RESULTS AND DISCUSSION

Synthesis of the target compounds **3–5** and **7–12** was achieved by a convenient one-pot procedure starting from methylthiopyridodithiazine **1** as shown in Schemes 1 and 2. Thus, the reaction of **1** with appropriate 2-hydrazinopyridine **2a–b** or 2,4-dimethyl-6-hydrazinopyrimidine **2c** carried out in boiling toluene led to the pyridothiadiazine derivatives **3–5**. An analogous reaction of 2-aminophenols **6a–e** or 2-aminothiophenol **6f** with **1** furnished 5-thia-3,6,11b-triaza-benzo[*c*]fluorene 5,5-dioxides **7–12**. We propose a reaction sequence for the transformations as shown in Scheme 2. The initial step is believed to be formation of 3-arylaminopyridodithiazine intermediate **A**, which may arise by nucleophilic displacement of the thiomethyl group, and subsequent transformation resulting in the formation of 4-mercaptopyridine intermediate **B**. In the final stage of the reaction, the nonisolable intermediate **B** undergoes an intramolecular ring closure *via* two-step addition–elimination (S_NAr) process leading to the formation of benzo[*c*]-fluorene derivatives **7–12** in 46–58% yields. The structures of the newly obtained compounds **3–5** and **7–12** were confirmed by elemental analyses (C, H, N) and spectroscopic data presented in the experimental section.

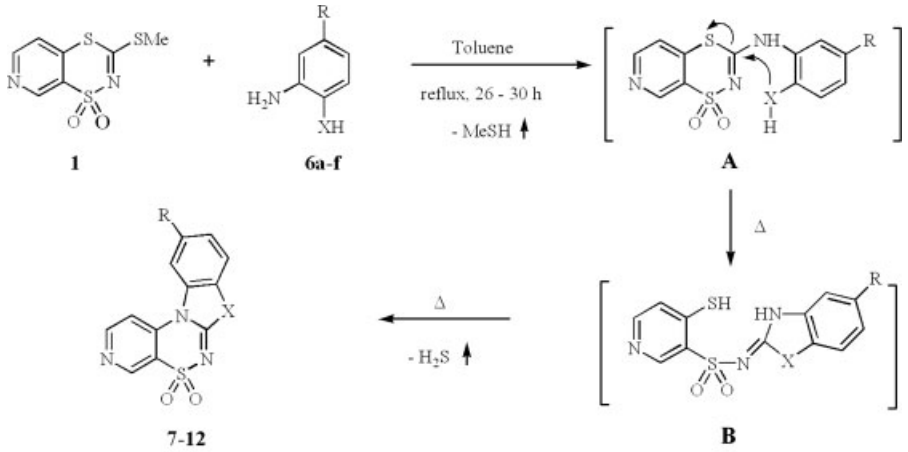
Further reactions of **8** with methylhydrazine or amines in boiling THF led to the formation of desired 3-(R-amino)-4-(2-hydroxy-5-methylphenyl)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides **13–18** in good yields (78–92%). The proposed mechanism leading to the compounds **13–18** is outlined in Scheme 3. Nucleophilic attack of amine at the carbon C-6b atom of benzo[*c*]-fluorene ring results in C—O bond cleavage, and formation of the 4-substituted-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine products **13–18** (structure **C**). It is worth noticing, however, that the spectroscopic data for the latter

Scheme 1. Synthesis and proposed mechanism of the formation of pyridothiadiazine derivatives **3–5**.



Compd	R^1	R^2	X	R^3	% yield
2a, 3	Cl	H	C-CF_3	H	68
2b, 4	CF_3	H	C-Cl	H	63
2c, 5	H	Me	N	Me	51

Scheme 2. Syntheses and proposed mechanism of the formation of 5-thia-3,6,11b-triaza-benzo[c]fluorene 5,5-dioxide derivatives **7–12**.



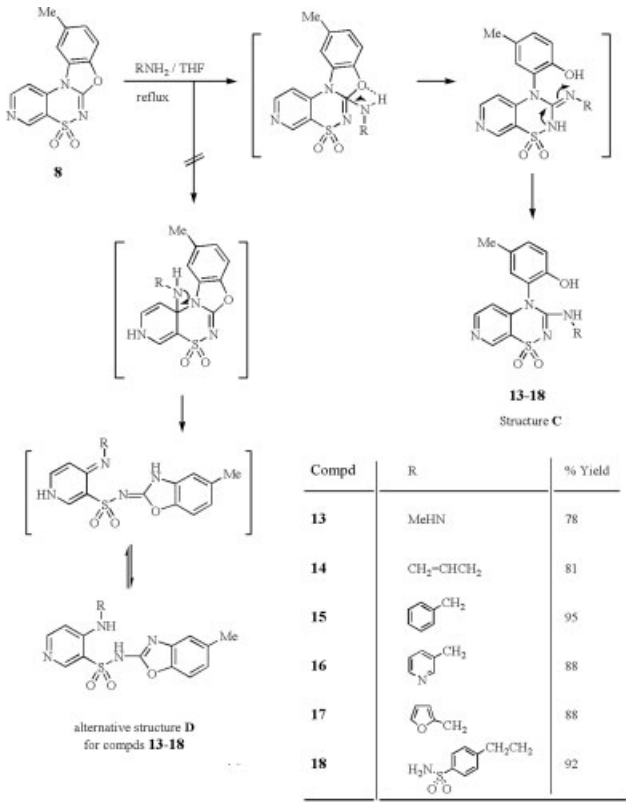
Compd	X	R	% yield
6a, 7	O	H	50
6b, 8	O	Me	57
6c, 9	O	Cl	49
6d, 10	O	NO_2	46
6e, 11	O	$EtSO_2$	58
6f, 12	S	H	49

compounds (Experimental section) did not allow straightforward discrimination between the actual pyrido[4,3-*e*]-1,2,4-thiadiazines **13–18** (structure **C**) and the alternative 4-aminopyridine-3-sulfonamides (structure **D**, Scheme 3). Therefore, X-ray crystallography was undertaken to confirm the proposed structure **C** on representative compounds **14** and **17**. Molecular structures of compounds **14** and **17** are shown in Figures 2 and 3, respectively. These compounds crystallize as pyridothiadiazine derivatives, *i.e.*, the 2-hydroxy-5-methylphenyl moiety is bound to the N-4 nitrogen atom of 1,2,4-thiadiazine ring.

Biological assay. Compounds **3–5**, **7–10**, **12–15**, and **17–18** were tested *in vitro* for antibacterial activity. The investigation was carried out on 26 strains of anaerobic bacteria isolated from the oral cavity, respiratory tract, and intestinal tract, as well as 11 standard strains. The anaerobes belonged to the following genera: *Finegoldia* (2 strains), *Micromonas* (3), *Actinomyces* (2), *Propionibacterium* (2), *Prevotella* (6), *Porphyromonas* (2), *Fusobacterium* (3), *Bacterioides* (6), and standard strains: *Bacteroides fragilis* ATCC 25285, *Fusobacterium nucleatum* ATCC 25586, *Peptostreptococcus anaerobius* ATCC 27337, *Peptostreptococcus magnus* ATCC 29328, and *Propionibacterium acnes* ATCC 11827. The susceptibility of the anaerobic bacteria was determined by means of the plate dilution technique in Brucella agar supplemented with 5% sheep blood [35–37]. The derivatives were dissolved in 1 mL of DMSO immediately before the experiment. Further dilutions were performed in sterile distilled water. The following concentrations of the compounds were used: 200, 100, 50, 25, 12.5, and 6.2 μg/mL. Metronidazole was used as a reference compound. The inoculum containing 10^6 CFU/spot was applied to the agar plates with Steers replicator. The inoculated agar plates and compound-free ones were incubated in anaerobic jars for 48 h at 37°C in 10% CO_2, 10% H_2 and 80% N_2 atmosphere with palladium catalyst and indicator of anaerobiosis.

Furthermore, 25 strains of aerobic bacteria isolated from the oral cavity, respiratory tract, and intestinal tract as well as six standard strains were tested. The aerobes were as follows: *Staphylococcus* (four strains), *Corynebacterium* (2), *Klebsiella* (3), *Acinetobacter* (2), *Escherichia* (6), *Pseudomonas* (8), as well as standard strains: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* ATCC 13883, *Acinetobacter baumannii* ATCC 19606, *Escherichia coli* ATCC 25922, *and Pseudomonas aeruginosa* ATCC 27853. Amikacin was used as a reference compound. The susceptibility of the aerobic bacteria was determined by means of agar dilution technique with Mueller-Hinton agar [35–37]. Further dilutions were performed in sterile distilled water. The following concentrations of the compounds were used: 200, 100, 50, 25, 12.5, and 6.2 μg/mL. The inoculum containing 10^6

Scheme 3. Syntheses and proposed mechanism of the formation of 3-(R-amino)-4-(2-hydroxy-5-methylphenyl)-4H-pyrido[4,3-e]-1,2,4-thiadiazine 1,1-dioxides **13–18**.



Compd	R	% Yield
13	MeHN	78
14	CH_2=$CHCH_2$	81
15		95
16		88
17		88
18		92

CFU/spot was applied to the agar plates with Steers replicator. The inoculated agar plates and the compound-free ones were incubated for 24 h at 37°C in aerobic conditions. The minimal inhibitory concentration (MIC) was defined as the lowest compound concentration, which inhibited growth of bacteria.

The susceptibility of anaerobic and aerobic bacteria toward compounds **3–5**, **7–10**, **12–15**, and **17–18** was shown in Table 1. The results have been compared with those obtained either for metronidazole (anaerobes) or amikacin (aerobes). Activity toward anaerobic bacteria exhibited 3 of 13 tested compounds (**3**, **4**, and **18**). The anaerobes were the most susceptible for compounds **4** and **18** which inhibited 7–8 (27–31%) strains at concentrations in the range from 25 to 100 μg/mL, whereas compound **3** inhibited growth of 2 (8%) strains in lower concentrations within limits 6.2–25 μg/mL) and other 3 (12%) strains at concentration of 100 μg/mL (Table 1). The compounds active toward anaerobic bacteria (**3**, **4**, and **18**) were effective to both Gram-positive and Gram-negative strains.

In general, aerobic bacteria were less susceptible to the tested compounds **3–5**, **7–10**, **12–15**, and **17–18**. Activity toward aerobes was shown only for compound

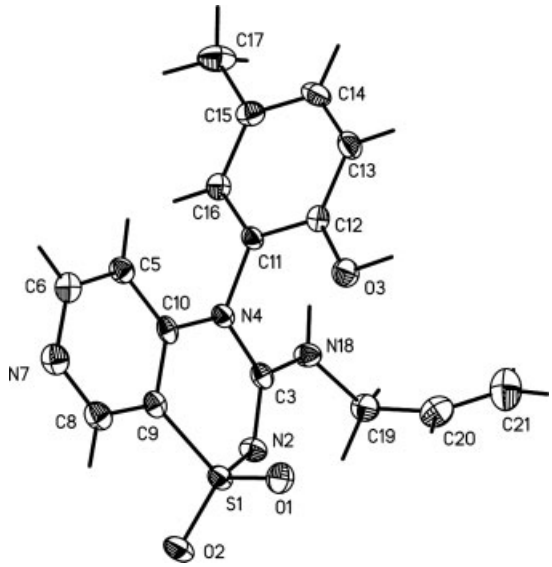


Figure 2. ORTEP drawing of compound **14** with the atom labeling scheme. Displacement ellipsoids are drawn at the 50% probability level.

13 which acted against 6 (24%) strains at concentrations ranged from 50 to 100 μg/mL and was more effective to Gram-negative strains. It is worthy of notice, however, that **13** was able to inhibit growth of four among six standard strains tested, such as *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* ATCC 13883, and *Escherichia*

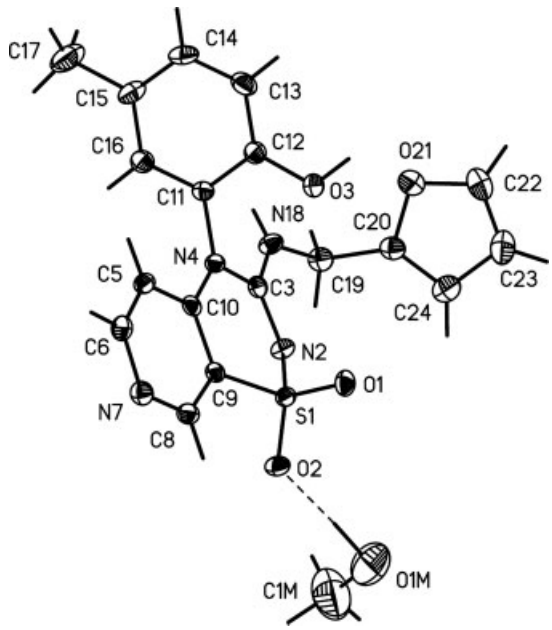


Figure 3. ORTEP drawing of compound **17** with the atom labeling scheme. Displacement ellipsoids are drawn at the 50% probability level.

Table 1

Antibacterial activity of compounds **3–5**, **7–10**, **12–15**, and **17–18**.

	MIC (μg/mL)													
Anaerobic bacteria	Metronidazole[a]	3	4	5	7	8	9	10	12	13	14	15	17	18
Gram-positive														
Finegoldia magna	≤0.4	–	50	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	50
Micromonas micros	≤0.4	≤6.2	50	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	25
Actinomyces israelii	1.0	≥200	25	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	50
Propionibacterium acnes	≥100	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200
Gram-negative														
Prevotella bivia	≤0.4	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200
Prevotella buccalis	≤0.4	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200
Prevotella intermedia	≤0.4	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200
Prevotella loescheii	≤0.4	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200
Porphyromonas asaccharolytica	≤0.4	100	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	50
Fusobacterium nucleatum	≤0.4	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	100
Fusobacterium necrophorum	1.6	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200
Bacteroides fragilis	≤0.4	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200
Bacteroides ureolyticus	12.5	25	100	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200
Aerobic bacteria	Amikacin[b]													
Gram-positive														
Staphylococcus aureus	≤6.2	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	100	≥200	≥200	≥200	≥200
Corynebacterium spp.	25	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	100	≥200	≥200	≥200	≥200
Gram-negative														
Klebsiella pneumoniae	≤6.2	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200
Acinetobacter baumannii	≤6.2	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200
Escherichia coli	≤6.2	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	100	≥200	≥200	≥200	≥200
Pseudomonas aeruginosa	≤6.2	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200
Pseudomonas stutzeri	12.5	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	50	≥200	≥200	≥200	≥200

[a] Metronidazole (Sigma).
[b] Amikacin sulfate salt (Sigma).

coli ATCC 25922 (MIC 100 μg/mL). The remaining compounds were active toward aerobic bacteria at the concentration equal or higher then 200 μg/mL.

The compounds **3**, **7**, **10**, **11–13**, and **17–18** were tested *in vitro* at the National Cancer Institute (Bethesda, MD) at a single dose (10 μ*M*) in the full NCI 60 cell panel. All tested compounds acted selectively and exhibited structure depended reasonable or high activity against one to two cell lines of leukemia, lung and CNS cancers, or renal cancer cells as shown in Table 2. Compounds **7** and **17** were the most potent of all derivatives tested. Relatively highest sensitivity to the compounds described here was found for cell lines of CNS cancer (SF-295), leukemia (SR, MOLT-4), and lung cancer (HOP-92) (Table 2). It is pertinent to note that further evaluations concerning biological activity of pyridosulfonamides of type **II** and **III** (Fig. 1) are still in progress.

EXPERIMENTAL

The following instruments and parameters were used: melting points Büchi 535 apparatus; ir spectra: KBr pellets, 400–4000 cm^{-1} Perkin Elmer 1600 FTIR spectrometer; ^1H and ^{13}C NMR: Varian Gemini 200 apparatus at 200 and 50 MHz, respectively; chemical shifts are expressed as δ values relative to Me_4Si as standard. The starting 3-methylthiopyrido[4,3-*e*]-1,4,2-dithiazine 1,1-dioxide **1** was prepared according to method described previously [32].

General procedure for the preparation of pyridothiadiazine derivatives (3–5). A mixture of 3-methylthiopyrido[4,3-*e*]-1,4,2-dithiazine 1,1-dioxide **1** (1.48 g, 6 mmol) and the corresponding 2-hydrazinopyridine **2a–b** or 2,4-dimethyl-6-hydrazinopyrimidine **2c** (6 mmol) in dry toluene (20 mL) was refluxed with stirring until the evolution of MeSH and H_2S had ceased (75–90 h). (CAUTION: because of high toxicity, MeSH and H_2S should be trapped in an aqueous NaOH solution). After cooling to room temperature, the precipitate was collected by filtration, washed successively with toluene (4 × 3 mL) and methanol (3 × 1 mL), dried and purified by crystallization from DMF. In this manner, the following compounds were obtained.

10-Chloro-8-trifluoromethyl-5-thia-3,6,6b,11,11a-pentaazabenzo[a]fluorene 5,5-dioxide (3). Starting from 3-chloro-5-trifluoromethyl-2-hydrazinopyridine **2a** (1.27 g) the title compound **3** was obtained (1.55 g, 68%): mp 347–348°C dec.; ir (KBr): 1645, 1580 (C=N, C=C) 1310, 1185, 1160 (SO_2) cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6): δ 8.05 (d, J = 5.6 Hz, 1H, H-1), 8.30 (s, 1H, H-9), 8.99 (s, 1H, H-7), 9.03 (d, J = 5.6 Hz, 1H, H-2), 9.26 (s, 1H, H-4) ppm; ^{13}C NMR (dimethyl sulfoxide-d_6): δ 109.95 (CF_3), 115.95, 116.65, 119.17,

Table 2

In vitro tumor growth inhibition data for compounds **3, 7, 10, 11–13**, and **17–18**.[a]

Compound	Tumor cell line	Growth inhibition %
3	Leukemia	
	MOLT-4	67.7
7	Leukemia	
	SR	89.8
	MOLT-4	82.2
10	CNS cancer	
	SF-295	61.1
11	Nonsmall cell lung cancer	
	HOP-92	80.7
12	Leukemia	
	CRF-CEM	65.6
	Nonsmall cell lung cancer	
	HOP-92	63.1
13	Renal cancer	
	RXF 393	60.1
	UO-31	51.5
17	CNS cancer	
	SF-295	99.1
	Nonsmall cell lung cancer	
	HOP-92	72.3
18	CNS cancer	
	SF-295	81.4

[a] Data obtained from the National Cancer Institute (Bethesda MD). Compounds were tested at a single dose (10 μ*M*) in the full NCI 60 cell panel.

121.15, 124.80, 128.56, 138.05, 143.33, 143.55, 146.56, 154.15 ppm. *Anal*. Calcd. for $C_{12}H_5ClF_3N_5O_2S$ (375.71): C, 38.36; H, 1.34; N, 18.64. Found: C, 38.38, H, 1.42, N, 18.76.

8-Chloro-10-trifluoromethyl-5-thia-3,6,6b,11,11a-pentaaza-benzo[a]fluorene 5,5-dioxide (4). Starting from 5-chloro-3-trifluoromethyl-2-hydrazinopyridine **2b** (1.27 g) the title **4** was obtained (1.43 g, 63%): mp 392–394°C dec.; ir (KBr): 1635, 1585, (C=N, C=C), 1335, 1310, 1170, 1145 (SO_2) cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6): δ 7.97 (d, J = 5.5 Hz, 1H, H-1), 8.31 (s, 1H, H-9), 8.96 (d, J = 5.5 Hz, 1H, H-2), 9.05 (s, 1H, H-7), 9.20 (s, 1H, H-4), ppm. *Anal*. Calcd. for $C_{12}H_5ClF_3N_5O_2S$ (375.71): C, 38.36; H, 1.34; N, 18.64. Found: C, 38.42, H, 1.39, N, 18.68.

7,9-Dimethyl-5-thia-3,6,6b,11,11a-heksaaza-benzo[a]fluorene 5,5-dioxide (5). Starting from 2,4-dimethyl-6-hydrazinopyrimidine **2c** (0.83 g) the title **5** was obtained (0.93 g, 51%): mp 406–408°C dec.; ir (KBr): 1640, 1595, 1570 (C=N, C=C), 1300, 1295, 1150 (SO_2) cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6): δ 2.72 (s, 3H, CH_3-9), 2.90 (s, 3H, CH_3-7), 8.26 (d, J = 5.8 Hz, 1H, H-1), 8.60 (s, 1H, H-10), 8.98 (d, J = 5.8 Hz, 1H, H-2), 9.14 (s, 1H, H-4) ppm. *Anal*. Calcd. for $C_{12}H_{10}N_6O_2S$ (302.21): C, 47.67; H, 3.33; N, 27.79. Found: C, 47.61, H, 3.50, N, 27.78.

General procedure for the preparation of 5-thia-3,6,11b-triaza-benzo[*c*]fluorene 5,5-dioxide derivatives (7–12). A solution of 3-methylthiopyrido[4,3-*e*]-1,4,2-dithiazine 1,1-dioxide **1** (1.73 g, 7 mmol) and appropriate 2-aminophenol **6a–e** or 2-aminothiophenol **6f** (7.2 mmol) in dry 1,4-dioxane (7 mL) was refluxed with stirring until the evolution of MeSH and H_2S had ceased (26–30 h). (CAUTION: because of high toxicity, MeSH and H_2S should be trapped in an aqueous NaOH solution). After cooling to room temperature, the reaction mixture was left overnight. The precipitate was collected by filtration, washed with 1,4-dioxane (2 × 0.5 mL), dried, and purified by crystallization from DMF. In this manner, the following products were obtained.

7-Oxa-5-thia-3,6,11b-triaza-benzo[c]fluorene 5,5-dioxide (7). Starting from 2-aminophenol **6a** (0.78 g) the title compound **7** was obtained (0.97 g, 50%): mp 352–353°C dec.; ir (KBr): 1635, 1580, 1560 (C=N, C=C), 1360, 1305, 1175, 1135 (SO_2) cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6): δ 7.47–7.58 (m, 2H, H-9, and H-10), 7.35–7.80 (m, 1H, H-11), 8.30–8.33 (m, 1H, H-8), 8.35 (d, J = 5.9 Hz, 1H, H-1), 8.96 (d, J = 5.9 Hz, 1H, H-2), 9.22 (s, 1H, H-4) ppm. *Anal*. Calcd. for $C_{12}H_7N_3O_3S$ (273.27): C, 52.74; H, 2.58; N, 15.37. Found: C, 52.72, H, 2.69, N, 15.49.

10-Methyl-7-oxa-5-thia-3,6,11b-triaza-benzo[c]fluorene 5,5-dioxide (8). Starting from 2-amino-4-methylphenol **6b** (0.89 g) the title compound **8** was obtained (1.15 g, 57%): mp 346–347°C; ir (KBr): 1660, 1625, 1575 (C=N, C=C), 1335, 1305, 1170 (SO_2) cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6): δ 3.30 (s, 3H, CH_3), 7.31 (d, J = 8.4 Hz, 1H, H-9), 7.63 (d, J = 8.4 Hz, 1H, H-8), 8.17 (s, 1H, H-11), 8.37 (d, J = 5.9 Hz, 1H, H-1), 8.96 (d, J = 5.9 Hz, 1H, H-2), 9.21 (s, 1H, H-4) ppm; ^{13}C NMR (dimethyl sulfoxide-d_6): δ 21.28, 110.91, 111.31, 118.91, 126.64, 126.89, 127.27, 135.91, 138.44, 141.94, 146.59, 154.00 ppm. *Anal*. Calcd. for $C_{13}H_9N_3O_3S$ (287.29): C, 54.35; H, 3.15; N, 14.62. Found: C, 54.39, H, 3.27, N, 14.64.

10-Chloro-7-oxa-5-thia-3,6,11b-triaza-benzo[c]fluorene 5,5-dioxide (9). Starting from 2-amino-4-chlorophenol **6c** (1.03 g) the title compound **9** was obtained (1.06 g, 49%): mp 357–358°C; ir (KBr): 1670, 1620, 1575 (C=N, C=C), 1385, 1315, 1170 (SO_2) cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6): δ 7.57 (d, J = 8.8 Hz, 1H, H-9), 7.97 (d, J = 8.8 Hz, 1H, H-8), 8.38 (d, J = 5.8 Hz, 1H, H-1), 8.46 (s, 1H, H-11), 8.94 (d, J = 5.8 Hz, 1H, H-2), 9.21 (s, 1H, H-4) ppm; ^{13}C NMR (dimethyl sulfoxide-d_6): δ 111.12, 113.04, 114.69, 118.81, 126.45, 128.44, 130.19, 137.97, 142.76, 146.63, 153.09, 154.15 ppm. *Anal*. Calcd. for $C_{12}H_6ClN_3O_3S$ (307.71): C, 46.84; H, 1.96; N, 13.65. Found: C, 46.81, H, 2.02, N, 13.67.

10-Nitro-7-oxa-5-thia-3,6,11b-triaza-benzo[c]fluorene 5,5-dioxide (10). Starting from 2-amino-4-nitrophenol **6d** (1.11 g) the title compound **10** was obtained (1.04 g, 46%): mp 393–396°C dec.; ir (KBr): 1680, 1630, 1570 (C=N, C=C), 1385, 1340, 1170, 1155 (SO_2) cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6): δ 8.03 (d, J = 8.7 Hz, 1H, H-8), 8.45 (d, J = 6.0 Hz, 2H, H-1, and H-2), 8.99 (s, 2H, H-10, and H-11), 9.27 (s, 1H, H-4) ppm. *Anal*. Calcd. for $C_{12}H_6N_4O_5S$ (318.27): C, 45.28; H, 1.90; N, 17.60. Found: C, 45.34, H, 2.00, N, 17.59.

10-(Ethylsulfonyl)-7-oxa-5-thia-3,6,11b-triaza-benzo[c]fluorene 5,5-dioxide (11). Starting from 2-amino-4-(ethylsulfonyl)phenol **6e** (1.45 g) the title compound **11** was obtained (1.49 g, 58%): mp 283–284°C; ir (KBr): 1665, 1620, 1580 (C=N, C=C), 1350, 1325, 1165, 1135 (SO_2) cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6): δ 1.15 (t, J = 5.2 Hz, 3H, CH_3), 3.52 (q, 2H, CH2), 8.03 (s, 2H, H-8, and H-9), 8.40 (d, J = 5.5 Hz, 1H, H-1), 8.62 (s, 1H, H-11), 9.04 (d, J = 5.5 Hz, 1H, H-2), 9.26 (s, 1H, H-4) ppm. *Anal*. Calcd. for $C_{14}H_{11}N_3O_5S_2$ (365.40): C, 46.02; H, 3.03; N, 11.49. Found: C, 46.11, H, 3.19, N, 11.52.

5,7-Dithia-3,6,11b-triaza-benzo[c]fluorene 5,5-dioxide (12). Starting from 2-aminothiophenol **6f** (0.9 g) the title compound **12** was obtained (1.0 g, 49%): mp 318–319°C; ir (KBr): 1685, 1580, 1565 (C=N, C=C), 1345, 1320, 1160 (SO_2) cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6): δ 7.56 (t, J = 7.6 Hz, 1H, H-9), 7.63 (t, J = 7.6 Hz, 1H, H-10), 8.09 (d, J = 7.6 Hz, 1H, H-8), 8.25 (d, J = 7.6 Hz, 1H, H-11), 8.27 (d, J = 5.9 Hz, 1H, H-1), 8.97 (d, J = 5.9 Hz, 1H, H-2), 9.21 (s, 1H, H-4) ppm; ^{13}C NMR (dimethyl sulfoxide-d_6): δ 112.52, 117.76, 119.69, 123.50, 124.94, 127.56, 128.26, 135.51, 141.32, 146.83, 154.25, 167.59 ppm. *Anal*. Calcd. for $C_{12}H_7N_3O_2S_2$ (289.34): C, 49.81; H, 2.44; N, 14.52. Found: C, 49.85, H, 2.52, N, 14.61.

General procedure for the preparation of 3-(R-amino)-4-(2-hydroxy-5-methylphenyl)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides (13–17). A solution of dioxide **8** (0.58 g, 2 mmol) and the appropriate amine RNH_2 (2.2 mmol) in tetrahydrofuran (8 mL) was stirred at reflux for 3 h. The solvent was evaporated under lowered pressure, and then dry residue was dissolved in hot methanol (5–10 mL). After cooling to room temperature and standing overnight, the precipitate of the adequate product was filtered off, washed with methanol, and dried at temperatures gradually increasing to 105°C.

4-(2-Hydroxy-5-methylphenyl)-3-(2-methylhydrazino)-4H-pyrido[4,3-e]-1,2,4-thiadiazine 1,1-dioxide (13). Starting from methylhydrazine (0.1 g), the title compound **13** was obtained (0.52 g, 78%): mp 187–189°C dec.; ir (KBr): 3425, 3315, 3195 (OH, NH), 1595, 1545 (C=N, C=C), 1345, 1175 (SO_2) cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 2.22 (s, 3H, CH_3 Ph), 3.38 (s, 3H, CH_3N), 6.19 (d, J = 5.6 Hz, 1H, H-5, pyridothiadiazine), 6.90–6.94 (m, 2H, arom.), 7.09–7.14 (m, 1H, arom.) 8.83 (d, J = 5.6 Hz, 1H, H-6, pyridothiadiazine), 8.69 (s, 1H, H-8, pyridothiadiazine), 9.30–9.80 (br.s, 3H, OH and 2 × NH) ppm. *Anal*. Calcd. for $C_{14}H_{15}N_5O_3S$ (333.37): C, 50.44; H, 4.53; N, 21.00. Found: C, 50.47; H, 4.62; N, 21.02.

3-Allylamino-4-(2-hydroxy-5-methylphenyl)-4H-pyrido [4,3-e]-1,2,4-thiadiazine 1,1-dioxide (14). Starting from allylamine (0.13 g), the title compound **14** was obtained (0.56 g, 81%): mp 247–248°C; ir (KBr): 3500, 3380 (OH), 3335 (NH), 1600, 1555 (C=N, C=C), 1345, 1175, 1160 (SO_2) cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 2.27 (s, 3H, CH_3), 3.81 (d, J = 4.2 Hz, 2H, CH_2), 5.04 (dd, J_{cis} = 10.3 Hz, J_{gem} = 1 Hz, 1H, NCH_2 CH_C=CH_A), 5.09 (dd, J_{trans} = 17.2 Hz, I_{gem} = 1.0 Hz, 1H, NCH_2 CH_C=CH_B), 5.75–5.86 (m, 1H, NCH_2CH_C=CH_2), 6.23 (d, J = 5.9 Hz, 1H, H-5, pyridothiadiazine), 7.03–7.08 (m, 1H, arom.), 7.13 (br.s, 1H, OH), 7.21–7.32 (m, 2H, arom.), 8.47 (d, J = 5.9 Hz, 1H, H-6, pyridothiadiazine), 8.84 (s, 1H, H-8, pyridothiadiazine), 10.20 (s, 1H, NH) ppm; ^{13}C NMR (dimethyl sulfoxide-d_6) δ 20.22, 43.71, 110.61, 115.44, 118.17, 119.92, 120.20, 130.45, 130.56, 133,22, 134.42, 144.48, 145.11, 150.40, 152.02, 152.67 ppm. *Anal*. Calcd for $C_{16}H_{16}N_4O_3$ S (344.40): C, 55.80; H, 4.68; N, 16.27, Found: C, 55.84; H, 4.77; N, 16.39.

3-Benzylamino-4-(2-hydroxy-5-methylphenyl)-4H-pyrido[4,3-e]-1, 2,4-thiadiazine 1,1-dioxide (15). Starting from benzylamine (0.24 g), the title compound **15** was obtained (0.75 g, 95%): mp 256–257°C; ir (KBr): 3660, 3485, 3410 (OH), 3290 (NH), 1600, 1560 (C=N, C=C), 1340, 1180, 1150 (SO_2)cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 2.27 (s, 3H, CH_3), 4.43 (d, J = 4.1 Hz, 2H, CH_2), 6.28 (d, J = 5.9 Hz, 1H, pyridothiadiazine), 7.06–7.3 (m, 8H, arom.), 7.64 (br.s, 1H, OH), 8.49 (d, J = 5.9 Hz, 1H, H-6, pyridothiadiazine), 8.58 (s, 1H, H-8, pyridothiadiazine), 10.30 (br.s, 1H, NH) ppm; ^{13}C NMR (dimethyl sulfoxide-d_6) δ 20.23, 44.76, 110.67, 118.19, 119.93, 120.24, 126.96, 128.44, 130.43, 130.59, 133.26, 138.81, 144.52, 145.12, 150.73, 152.12, 152.74 ppm. *Anal*. Calcd. for $C_{20}H_{18}N_4O_3S$ (394.45): C, 60.89; H, 4.59; N, 14.20. Found: C, 60.92; H, 4.67; N, 14.19.

4-(2-Hydroxy-5-methylphenyl)-3-(2-pyridylmethylamino)-4H-pyrido[4,3-e]-1,2,4-thiadiazine 1,1-dioxide (16). Starting from 2-pyridylmethylamine (0.24 g) the title compound **16** was obtained (0.7 g, 88%): mp 168–169°C dec.; ir (KBr): 3470 (OH), 3275 (NH), 1600, 1590, 1575 (C=N, C=C), 1345, 1150 (SO_2)cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 2.27 (s, 3H, CH_3), 3.14 (d, J = 5.1 Hz, 2H, CH_2), 6.27 (d, J = 5.9 Hz, 1H, H-5, pyridothiadiazine), 7.07 (d, J = 8.3 Hz, 1H, arom.), 7.20 (s, 1H, OH), 7.29–7.36 (m, 2H, arom.), 7.66–7.70 (m, 2H, arom.), 8.42–8.47 (m, 2H, arom.), 8.50 (d, J = 5.9 Hz, 1H, H-6, pyridothiadiazine), 8.85 (s, 1H, H-8, pyridothiadiazine), 10.27 (s, 1H, NH) ppm. *Anal*. Calcd. for $C_{19}H_{17}N_5O_3S$ (395.43): C, 57.71; H, 4.33; N, 17.71. Found: C, 57.78; H, 4.41; N, 17.89.

3-(Furfurylamino)-4-(2-hydroxy-5-methylphenyl)-4H-pyrido[4,3-e]-1,2,4-thiadiazine 1,1-dioxide (17). Starting from furfurylamine (0.22 g), the title compound **17** was obtained (0.68 g, 88%): mp 240–241°C dec.; ir (KBr): 3505 (OH), 3375 (NH), 1605, 1590, 1555 (C=N, C=C), 1340, 1175, 1150 (SO_2) cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 2.27 (s, 3H, CH_3), 4.37 (s, 2H, CH_2), 6.26 (d, J = 5.6 Hz, 2H, arom.), 6.38 (s, 1H, arom.), 7.05 (d, J = 8.3 Hz, 1H, arom.), 7.14 (s, 1H, OH), 7.30 (d, J = 8.3 Hz, 1H arom.), 7.45–7.60 (m, 2H, arom.), 8.48 (d, J = 5.9 Hz, 1H, H-6, pyridothiadiazine), 8.86 (s, 1H, H-8, pyridothiadiazine), 10.22 (s, 1H, NH) ppm. *Anal*. Calcd. for $C_{18}H_{16}N_4O_4S$ (384.42): C, 56.24; H, 4.19; N, 14.57. Found: C, 56.30; H, 4.27; N, 14.58.

Synthesis of 4-(2-hydroxy-5-methylphenyl)-3-[2-(4-sulfamoylphenyl)ethylamino]-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (18). A solution of dioxide **8** (0.58 g, 2 mmol) and 4-(2-aminoethyl)benzenesulfonamide (0.44 g, 2.2 mmol) in tetrahydrofuran (25 mL) was refluxed with stirring for 5 h. After cooling to room temperature, the suspension was left overnight. The precipitate was collected by filtration, washed successively with tetrahydrofuran (2 × 1.5 mL) and methanol (5 × 2 mL), and dried initially at room temperature and then at 105°C. Yield 0.9 g (92%): mp 276–277°C; ir (KBr): 3500 (OH), 3360, 3305, 3280 (NH and SO_2NH_2), 1600, 1555 (C=N, C=C), 1335, 1160, 1150 (SO_2)cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 2.24 (s, 3H, CH_3), 2.86 (t, J = 5.0 Hz, 2H, $NCH_2CH_2PhSO_2$), 3.39 (t, J = 5.0 Hz, 2H, $NCH_2CH_2PhSO_2$), 6.25 (d, J = 5.2 Hz, 1H, H-5, pyridothiadiazine), 6.95–7.19 (m, 3H, arom.), 7.27 (br.s, 3H, SO_2NH_2, and OH), 7.35 (d, J = 7.4 Hz, 2H, H-3 and H-5, Ph SO_2), 7.74 (d, J = 7.4 Hz, 2H, H-2 and H-6, Ph SO_2), 8.47 (d, J = 5.2 Hz, 1H, H-6, pyridothiadiazine), 8.88 (s, 1H, H-8, pyridothiadiazine), 10.08 (s, 1H, NH) ppm. *Anal*. Calcd. for $C_{21}H_{21}N_5O_5S_2$ (487.56): C, 51.73; H, 4.34; N, 14.36. Found: C, 51.70; H, 4.41; N, 14.45.

X-ray structure analysis. The diffraction data were collected with a KumaCCD diffractometer using graphite monochromated Mo K_α radiation. The intensity data were collected and processed using Oxford Diffraction CrysAlis Software [38]. The crystal structures were solved by direct methods

with the program SHELXS-97 [39] and refined by full-matrix least-squares method on F^2 with SHELXL-97 [40].

Crystal data for **14**: $C_{16}H_{16}N_4O_3S$, monoclinic, space group $P2_1/c$, $a = 9.2385(9)$, $b = 11.6851(10)$, $c = 15.0960(14)$ Å, $\beta = 95.274(8)^\circ$, $V = 1622.8(3)$ Å^3, $Z = 4$, $d_x = 1.410$ g cm^{-3}, $T = 130$ K. Data were collected for a crystal with dimensions $0.4 \times 0.4 \times 0.1$ mm. Final R indices for 2570 reflections with $I > 2\sigma(I)$ and 227 refined parameters are: $R_1 = 0.0367$, $wR_2 = 0.0942$ ($R_1 = 0.0502$, $wR_2 = 0.1009$ for all 3309 data).

Crystal data for **17**: $C_{18}H_{16}N_4O_4S{\cdot}CH_3OH$, monoclinic, space group $P2_1/c$, $a = 10.9766(10)$, $b = 11.4912(11)$, $c = 15.6294(15)$ Å, $\beta = 102.592(8)^\circ$, $V = 1924.0(3)$ Å^3, $Z = 4$, $d_x = 1.438$ g cm^{-3}, $T = 130$ K. Data were collected for a crystal with dimensions $0.4 \times 0.4 \times 0.1$ mm. Final R indices for 2798 reflections with $I > 2\sigma(I)$ and 271 refined parameters are as follows: $R_1 = 0.0435$, $wR_2 = 0.1131$ ($R_1 = 0.0640$, $wR_2 = 0.1235$ for all 3903 data).

Crystallographic data for compounds **14** and **17** have been deposited with Cambridge Crystallographic Data Centre (CCDC deposition numbers CCDC 684581 and 684582, respectively). Copies of the data can be obtained upon request from CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, quoting the deposition numbers.

Acknowledgments. The authors are very grateful to Dr. V.L. Narayanan, Chief Drug Synthesis, Chemistry Branch, National Cancer Institute (Bethesda, MD) for the *in vitro* anticancer screening.

REFERENCES AND NOTES

[1] Negwer, M. Organic-Chemical Drugs and Their Synonyms; Akademie Verlag: Berlin, 1994.

[2] Brzozowski, Z. Acta Polon Pharm Drug Res 1995, 52, 91.

[3] Brzozowski, Z. Acta Polon Pharm Drug Res 1995, 52, 287.

[4] Brzozowski, Z. Acta Polon Pharm Drug Res 1996, 53, 269.

[5] Brzozowski, Z. Acta Polon Pharm Drug Res 1997, 54, 293.

[6] Brzozowski, Z. Acta Polon Pharm Drug Res 1998, 55, 375.

[7] Brzozowski, Z.; Kornicka, A. Acta Polon Pharm Drug Res 1999, 56, 135.

[8] Brzozowski, Z.; Sączewski, F.; Gdaniec, M. Eur J Med Chem 2002, 37, 285.

[9] Brzozowski, Z.; Sączewski, F. J Med Chem 2002, 45, 430.

[10] Brzozowski, Z.; Sączewski, F.; Gdaniec, M. Bioorg Med Chem 2003, 11, 3673.

[11] Brzozowski, Z.; Sączewski, F.; Gdaniec, M. Eur J Med Chem 2003, 38, 991.

[12] Pomarnacka, E.; Gdaniec, M. Bioorg Med Chem 2003, 11, 1259.

[13] Sławiński, J.; Gdaniec, M. Eur J Med Chem 2005, 40, 377.

[14] Brzozowski, Z.; Sączewski, F. J Heterocycl Chem 2005, 42, 1297.

[15] Sławiński, J.; Brzozowski, Z. Eur J Med Chem 2006, 41, 1180.

[16] Brzozowski, Z.; Sączewski, F.; Neamati, N. Bioorg Med Chem 2006, 14, 2985.

[17] Brzozowski, Z.; Sławiński, J. Pol J Chem 2007, 81, 1419.

[18] Brzozowski, Z.; Sławiński, J. Pol J Chem 2007, 81, 1433.

[19] Brzozowski, Z.; Sączewski, F.; Sławiński, J. Pol J Chem 2007, 81, 2133.

[20] Brzozowski, Z.; Sączewski, F.; Sławiński, J. Eur J Med Chem 2007, 42, 1218.

[21] Brzozowski, Z.; Sączewski, F.; Sławiński, J.; Bednarski, P. J.; Grünert, R.; Gdaniec, M. Bioorg Med Chem 2007, 15, 2560.

[22] Brzozowski, Z.; Sławiński, J.; Sączewski, F.; Sanchez, T.; Neamati, N. Eur J Med Chem 2008, 43, 1188.

[23] Brzozowski, Z. Acta Polon Pharm Drug Res 1998, 55, 473.

[24] Kuo, Ch. L.; Assefa, H.; Brzozowski, Z.; Sławiński, J.; Sączewski, F.; Buolamwini, I. K.; Neamati, N. J Med Chem 2004, 47, 385.

[25] Brzozowski, Z.; Sączewski, F.; Sanchez, T.; Kuo, Ch. L.; Gdaniec, M.; Neamati, N. Bioorg Med Chem 2004, 12, 3663.

[26] Brzozowski, Z.; Sławiński, J. Pol J Chem 2006, 80, 1807.

[27] Brzozowski, Z.; Sączewski, F.; Sanchez, T.; Kuo, Ch. L.; Gdaniec, M.; Neamati, N. Bioorg Med Chem Lett 2006, 16, 5298.

[28] Brzozowski, Z.; Sączewski, F. J Heterocycl Chem 2007, 44, 261.

[29] Sączewski, F.; Innocenti, A.; Brzozowski, Z.; Sławiński, J.; Pomarnacka, E.; Kornicka, A.; Scozzafava, A.; Supuran, C. T. J Enzyme Inhib Med Chem 2006, 21, 563.

[30] Sączewski, F.; Sławiński, J.; Kornicka, A.; Brzozowski, Z.; Pomarnacka, E.; Innocenti, A.; Scozzafava, A.; Supuran, C. T. Bioorg Med Chem Lett 2006, 16, 4846.

[31] Sączewski, F.; Innocenti, A.; Sławiński, J.; Kornicka, A.; Brzozowski, Z.; Pomarnacka, E.; Scozzafava, A.; Temperini, C.; Supuran, C. T. Bioorg Med Chem 2008, 16, 3933.

[32] Brzozowski, Z.; Sączewski, F.; Sławiński, J. J Heterocycl Chem 2008, 45, 1407.

[33] Pirotte, B.; Tullio, P.; Lebrun, Ph.; Antoine, M.-H.; Fontaine, J.; Masereel, B.; Schynts, M.; Dupont, L.; Herchulez, A.; Delarge, J. J Med Chem 1993, 36, 3211.

[34] Tullio, P.; Pirotte, B.; Lebrun, Ph.; Fontaine, J.; Dupont, L.; Antoine, M.-H.; Ouedraogo, R.; Khelili, S.; Maggetto, C.; Masereel, B.; Diouf, O.; Podona, T.; Delarge, J. J Med Chem 1996, 39, 937.

[35] Aldridge, K. E.; Ashcraft, D.; Cambre, K.; Pierson, C. L.; Jenkins, S. G.; Rosenblatt, J. L. Antimicrob Agents Chemother 2001, 45, 1238.

[36] Balows, A.; Hausler, H. J.; Herrmann, K. L.; Isenberg, H. D.; Shadomy, H. J. Manual of Clinical Microbiology, 5th ed.; American Society for Microbiology: Washington, 1991.

[37] Forbes, B. A.; Sahn, D. F.; Weissfeld, A. S. Bailey and Scott's Diagnostics Microbiology, 12th ed.; Mosby Elsevier: St. Louis, 2007.

[38] Oxford Diffraction. Crysalis CCD and RED, ver. 1.171; Oxford Diffraction: Oxfordshire, UK, 2004.

[39] Sheldrick, G. M. SHELXS-97: Program for a Crystal Structure Solution; University of Göttingen: Germany, 1997.

[40] Sheldrick, G. M. SHELXL-97: Program for the Refinement of a Crystal Structure from Diffraction Data; University of Göttingen: Germany, 1997.