Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry 14 (2006) 2985–2993
Bioorganic & Medicinal Chemistry

Synthesis, antitumor and anti-HIV activities of benzodithiazine-dioxides

Zdziasław Brzozowski,[a] Franciszek Sączewski[a,*] and Nouri Neamati[b]

[a]*Department of Chemical Technology of Drugs, Medical University of Gdańsk, 80-416 Gdańsk, Poland*
[b]*Department of Pharmaceutical Sciences, School of Pharmacy, University of Southern California, 1985 Zonal Avenue, PSC 304, Los Angeles, CA 90089, USA*

Received 18 October 2005; revised 6 December 2005; accepted 9 December 2005
Available online 6 January 2006

Abstract—Several 8-chloro-7-R^1-6-R^2-3- R^3-imidazo[1,2-*b*][1,4,2]benzodithiazine 5,5-dioxide derivatives (**9**–**11**, **16**–**19**, and **21**–**24**) have been synthesized as potential antitumor or anti-HIV agents. The in vitro antitumor and anti-HIV-1 activities of the compounds were determined in a panel of cell lines. The benzodithiazine-dioxide **10** showed 50% growth inhibitory activity in low micromolar against most cells. It was particularly effective in leukemia, lung, melanoma, ovarian, and renal cancer cells with GI_{50} values of 1–2 μM. Interestingly, benzodithiazine-dioxide **16** showed remarkable anti-HIV-1 activity with 50% effective concentration EC_{50} value of 0.94 μM and no significant cytotoxicity at 200.0 μM.

1. Introduction

Identification of a lead compound with desirable pharmacological property is of paramount importance. Less than 1% of compounds screened in a typical high-throughput screening against a desired target show promising activity to be considered as lead. A lead is generally considered as a compound that shows inhibition of a target with a 50% inhibitory concentration (IC_{50}) value of <10 μM.[1] Lead identification is very important and relatively easy in the early stages of drug discovery. However, a great number of leads never make it for further pharmacological studies. Therefore, prior to lead optimization it is important to pursue a lead molecule that is pharmacologically relevant. We routinely perform highly robust computational simulations of our lead compounds for desirable physicochemical and pharmacokinetic properties prior to their structure optimization.[2] Recently, we have been working on the synthesis and biological properties of novel sulfonamides.

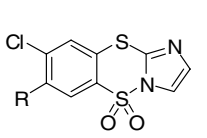
Sulfonamides are among a growing list of compounds with desirable anticancer and anti-HIV activities.[3–6] Previously, we have identified different cyclic sulfonamides with anticancer activities in a panel of cell lines[7–14] and anti-HIV activities in cell-based assays.[8,14] Some of the compounds were described as novel HIV-1 integrase inhibitors.[14,15] Recently, we have also reported on the syntheses of dithiazine-carboxylic acid derivatives with pronounced anticancer activity (Fig. 1, structures **I**, **II**, and **III**).[10] To better understand the nature of their anticancer versus their antiviral property, we extended our studies to the synthesis of new series of 8-chloro-5,5-dioxoimidazo[1,2-*b*][1,4,2]benzodithiazines (Fig. 1, structure **IV**). In this study, we describe the synthesis, anticancer and antiviral activities of a series of substituted benzodithiazines.

2. Results and discussion

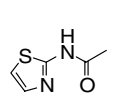
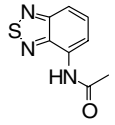
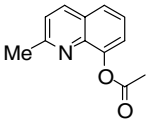
The previously described methods were employed for the synthesis of compounds **1**–**3**,[16] **4**,[17] **5**,[18] **6**, **7**,[8] **9** and **10**.[8] Similarly, we prepared novel *N*-(6-chloro-8-methyl-1,1-dioxo-1,4,2-benzodithiazin-3-yl)aminoacetaldehyde dimethyl acetal **8** and 8-chloro-6-methylimidazo[1,2-*b*][1,4,2]benzodithiazine 5,5-dioxide **11** (Scheme 1). The syntheses of the target compounds **16**–**19** were achieved by a convenient two-step procedure starting from 3-methylthio-1,4-2-benzodithiazines **1**, **3**, **4**, and **5** as shown in Scheme 2. First, the reaction of **1**, **3**–**5** with 2-propynylamine carried out in benzene or methanol (**5**) at

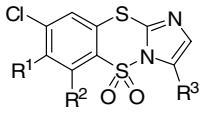
Keywords: 8-Chloro-7-R^1-6-R^2-3-R^3-imidazo[1,2-*b*][1,4,2]benzodithiazines; Synthesis; Antitumor activity; Anti-HIV activity.
* Corresponding author. Tel.: +48 58 349 3250; fax: +48 58 349 3257; e-mail: saczew@amg.gda.pl

0968-0896/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.12.024



I - III (see Table 1)

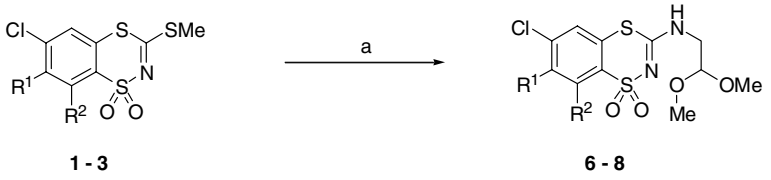
compd	I	II	III
R			



IV

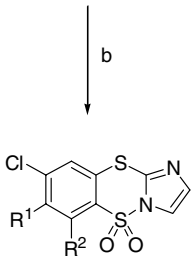
R^1 = H, Me, C≡N, COOH, alkylcarbamoyl, arylcarbamoyl or heteroarylcarbamoyl
R^2 = H or Me
R^3 = H or Me

Figure 1.



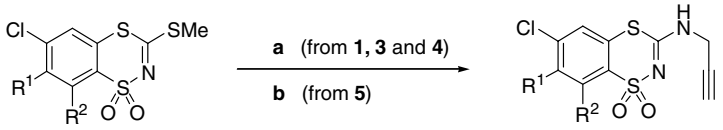
1 - 3 **6 - 8**

Compounds	R^1	R^2
1, 6, 9 [19]	Me	H
2, 7, 10 [19]	N≡C	H
3, 8, 11	H	Me



9 - 11

Scheme 1. Synthesis of 8-chloroimidazo[1,2-*b*][1,4,2]benzodithiazine 5,5-dioxides (**9**–**11**). Reagents, conditions, and yields: (a) $H_2NCH_2CH(OMe)_2$ (1.08 molar equiv), benzene, reflux, 15–24 h, 82–91%; (b) 98% H_2SO_4, 20–37 °C, 8–10 h, 81–96%.



1, 3-5 **12 - 15**

Compounds	R^1	R^2
1, 12, 16	Me	H
3, 13, 17	H	Me
4, 14, 18	4-Cl-PhNHCO	H
5, 15, 19	HO-CO	H

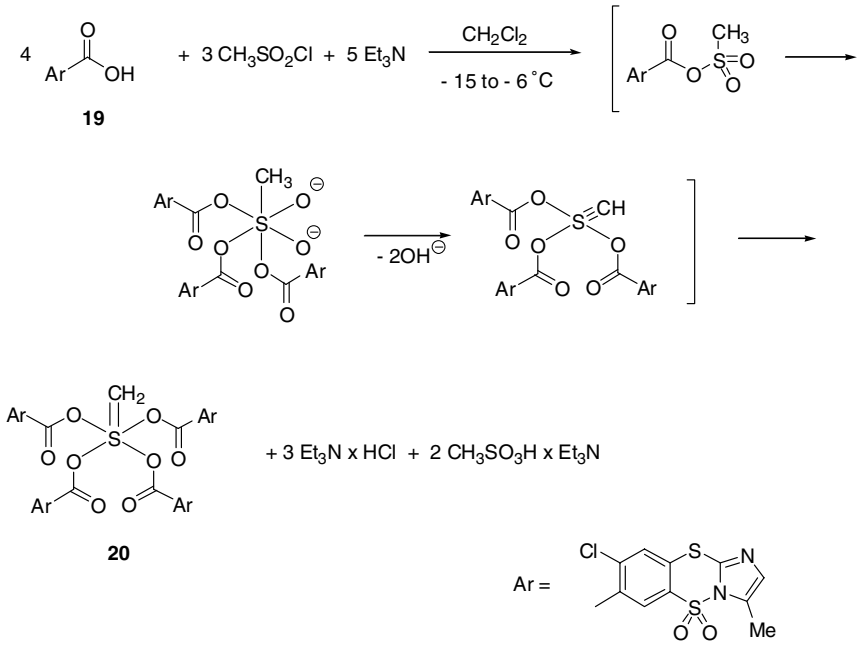
16 - 19

Scheme 2. Synthesis of 8-chloro-3-methylimidazo[1,2-*b*][1,4,2]benzodithiazine 5,5-dioxides (**16**–**19**). Reagents, conditions, and yields: (a) HC≡C–CH_2NH_2 (1.0 molar equiv), dry benzene, 20 °C, 3 h and then reflux for 40–50 h, 74–96%; (b) HC≡C–CH_2NH_2 (2.0 molar equiv), anhydrous methanol, 0–5 °C, 1 h and reflux for 22–25 h, 91%; (c) 95% sulfuric acid, room temperature, 90–94 h, 50–55 °C, 3 h 74–91%.

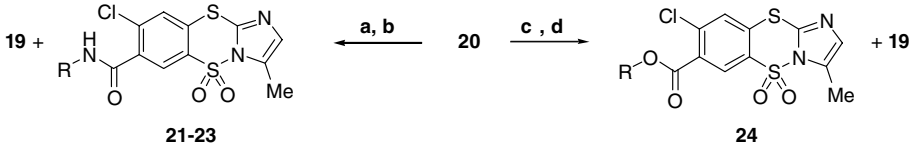
elevated temperature led to the formation of 3-(2-propynylamino)-1,4,2-benzodithaizines **12**–**15** in good yields (74–96%). Then, upon treatment of **12**–**15** with an excess of 95% sulfuric acid the desired 8-chloro-3-methylimidazo[1,2-*b*][1,4,2]benzodithiazine 5,5-dioxides **16**–**19** were obtained in 74–91% yield.

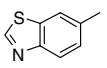
The reaction of a carboxylic acid with methanesulfonyl chloride in the presence of base usually gives rise to the corresponding mixed sulfonic-carboxylic anhydride.[19] However, when we treated carboxylic acid **19** (1 equiv) with methanesulfonyl chloride (0.75 equiv) in methylene chloride at −15 °C in the presence of triethylamine (1.25 equiv), a crystalline product of the methyleneorthosulfonictetrakis (8-chloro-3-methyl-5,5-dioxoimidazo-[1,2-*b*][1,4,2]benzodithiazine-7-carboxylic) anhydride **20** was obtained (Scheme 3) as a close analog to the previously described methyleneorthosulfonictetrakis (8-chloro-5,5-dioxoimidazo[1,2-*b*][1,4,2]benzodithiazine-7-carboxylic) anhydride.[10]

As shown in Scheme 4, the desired 3-methylimidazo[1,2-*b*][1,4,2]benzodithiazinecarboxamides **21**–**23** and 8-quinolyl 3-methylimidazo[1,2-*b*][1,4,2]benzodithiazinecarboxylate **24** were obtained by reacting the anhydride **20** with corresponding amines or 8-hydroquinoline, respectively. It is important to note that only two of four acyl groups of **20** were available for the formation of the



Scheme 3. Proposed mechanism of the formation of the mixed anhydride **20**.



compd	R
21	$(CH_3)_2CHCH_2$-
22	
23	
24	

Scheme 4. Synthesis of the amides **21**–**23** and ester **24** from mixed anhydride **20**. Reagents, conditions, and yields: (a) amine RNH_2 (2 molar equiv), Et_3N (3 molar equiv), *p*-dioxane, 20 °C, 1 h and then reflux, 12 h; (b) K_2CO_3, H_2O, 20 °C, 1 h, 78–83%; (c) 8-hydroxyquinoline (2.5 molar equiv), *p*-dioxane, reflux, 8 h; (d) K_2CO_3, H_2O, 20 °C, 2 h, 79%.

Table 1. Overview of the results of the in vitro antitumor screening for compounds **10**, **16**–**19**, **22**–**24**, and reference imidazobenzodithiazines **I**, **II**, and **III**[a]

Compound	No. of cell lines investigated	No. of cell lines giving positive $\log GI_{50}$, $\log TGI$, and $\log LC_{50}$						MG_MID[e]		Most sensible cell lines
		$\log GI_{50}$[b] [M]		$\log TGI$[c] [M]		$\log LC_{50}$[d] [M]		$\log GI_{50}$	$\log TGI$	
		No.	Range	No.	Range	No.	Range			
10	57	57	−6.69 to −4.86	57	−6.39 to −4.52	50	−5.42 to −4.11	−5.62	−5.17	Melanoma: M14; Lung: NCI-H522
22	57	55	−4.94 to −4.27	39	−4.58 to −4.01	22	−4.28 to −4.04	−4.59	−4.24	Breast: T-47D and MDA-MB-231/ATCC
I[10]	60	60	−5.91 to −4.55	60	−5.55 to −4.04	50	−5.18 to −4.01	−4.83	−4.48	Lung: NCI-522
II[10]	60	60	−5.77 to −4.34	57	−5.49 to −4.31	46	−5.24 to −4.02	−5.23	−4.75	Lung: HOP-62; Melanoma: UACC-257
III[10]	60	60	<−8.00 to −4.01	57	<−8.00 to −4.01	39	−5.24 to −4.04	−5.10	−4.54	Leukemia: HL-60 (TB); Renal: TK-10

[a] Data obtained from the NCI's in vitro disease-oriented human tumor cell screen (see Table 2 and Refs. 8,19,20 for details). Compounds **16**–**19**, **23**, and **24** were inactive.
[b] The log of the molar concentration that inhibits 50% net cell growth.
[c] The log of the molar concentration giving total growth inhibition.
[d] The log of the molar concentration leading to 50% net cell death.
[e] MG_MID = mean graph midpoint = arithmetical mean value for all tested cancer cell lines. If the indicated effect was not attainable within the used concentration interval, the highest tested concentration was used for the calculation.

desired products. The two remaining acyl groups could be recovered in the form of carboxylic acid **19** by quenching the reaction mixture with water (pH ~9).

Compounds **10**, **16**–**19**, and **22**–**24** were tested at National Cancer Institute (Bethesda, USA) against a panel of approximately 60 human tumor cell lines (NCI60). In the NCI60, compounds **16**–**19**, **23**, and **24** were inactive ($\log GI_{50} > -4.0$), whereas the compounds **10** and **22** exhibited reasonable activity against one or more human tumor cell lines (for overview of the results, see Table 1). In general, the highest anticancer activity was shown for derivatives bearing an electron-withdrawing substituent at position 7 of heterocyclic ring [Table 1: compd **10** (R^1 = CN); **I** (R^1 = thiazol-2-ylcarbamoyl); **II** (R^1 = benzo-2,1,3-thiadiazol-4-ylcarbamoyl); **III** (R^1 = 2-methyl-8-quinoloxycarbonyl)]. Compounds featuring the 3-methyl substitution were either significantly less active than unsubstituted congeners (compound **22** vs **II**) or inactive (compounds **16**–**19**, **23**, and **24**).

As presented in Table 2, the most active compound **10** showed log GI_{50} range of −6.69 to −6.08 and log TGI range of −6.39 to −5.60. Compound **10** was very potent in leukemia (CCRF-CEM and MOLT-4), lung (NCI-H522), melanoma (M14), colon (HT29), ovarian (OVCAR-3), and renal cancer (CAKI-1) cells.

The imidazo[1,2-*b*][1,4,2]benzodithiazine derivatives **9**–**11**, **16**, **17**, **19**, and **21** were further tested for their anti-HIV activity. As presented in Table 3, the data obtained from NCI's in vitro anti-HIV primary screen[23] indicate that the compound **16** bearing two electron-donating methyl groups at positions 3 and 7 showed the highest anti-HIV activity (EC_{50} = 0.9 μM) and cytotoxicity well above 200 μM (IC_{50} > 200 μM; therapeutic index TI > 212.7). The 3,8-dimethyl analogue **17** was less active (EC_{50} = 9.0 μM) and further loss of activity was observed for monosubstituted compound **9** (EC_{50} = 44.0 μM).

The anticancer versus anti-HIV activity in this group of compounds was studied by comparison of the structures **10** (most active anticancer agent) and **16** (most active anti-HIV agent). The comparison between 3D electrostatic potential maps[24] of **10** and **16** (Fig. 2) shows that three superimposable negative wells appear around the two oxygen atoms of sulfonyl group and N-4 nitrogen atom of the imidazo[1,2-*b*][1,4,2]benzodithiazine 1,1-dioxide ring. Moreover, a separated electrostatic region is positioned around the nitrogen atom of nitrile group of **10** with anticancer activity. Therefore, the exchange of the methyl group at position 7 by nitrile one seems to be the main factor affecting selectivity of the action.

3. 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 spectrophotometer; (^1H and ^{13}C NMR spectra) Varian Gemini 200 apparatus at 200 and 50 MHz, respectively

Table 2. Inhibition of in vitro cancer cell lines by imidazo[1,2-*b*][1,4,2]benzodithiazine **10**[a]

Panel cell line	$\log GI_{50}$[b] [M]	$\log TGI$[c] [M]	$\log LC_{50}$[d] [M]
Leukemia			
CCRF-CEM	−6.39	−5.71	−5.03
HL-60 (TB)	−5.72	−4.56	[e]
K-562	−5.68	−4.97	[e]
MOLT-4	−6.49	−5.84	−5.20
RPMI-8226	−5.56	−4.76	[e]
SR	−6.00	−5.45	[e]
Non-small lung cancer			
A549/ATCC	−4.91	−4.56	−4.21
EKVX	−4.91	−4.52	−4.13
HOP-62	−5.55	−5.09	−4.51
HOP-92	−5.78	−5.22	[e]
NCI-H226	−5.48	−4.96	−4.45
NCI-H23-	−5.83	−5.52	−5.20
NCI-H332M	−5.70	−5.41	−5.11
NCI-H460	−5.18	−4.66	−4.23
NCI-H522	−6.41	−6.02	−5.31
Colon cancer			
COLO 205	−4.97	−4.53	−4.11
HCT-116	−5.80	−5.52	−5.23
HCT-15	−5.97	−5.50	−5.16
HT29	−6.44	−5.70	−5.25
KM12	−5.52	−4.82	−4.37
SW-620	−5.46	−4.90	−4.42
CNS cancer			
SF-268	−5.49	−5.00	−4.39
SF-295	−5.42	−5.15	−4.66
SF-539	−5.58	−5.08	−4.47
SNB-19	−5.12	5.06	−4.31
SNB-75	−5.12	−5.06	4.33
U251	−5.82	−5.51	−5.25
Prostate cancer			
PC-3	−5.85	−5.52	−5.19
DU-145	−5.72	−5.33	−4.83
Melanoma			
LOX IMVI	−5.83	−5.50	−5.22
MALME-3M	−5.38	−4.77	−4.26
M14	−6.69	−6.39	−5.07
SK-MEL-2	−5.60	−5.14	−4.52
SK-MEL-28	−5.83	−5.40	−4.91
SK-MEL-5	−5.05	−4.66	−4.32
UACC-257	−5.79	−5.34	−4.73
UACC-62	−5.71	−5.44	−5.16
Ovarian cancer			
IGROVI	−5.63	−5.30	−4.89
OVCAR-3	−6.49	−5.89	−5.42
OVCAR-4	−5.44	−4.65	−4.32
OVCAR-5	−4.86	−4.57	−4.28
OVCAR-8	−5.58	−5.16	−4.34
SK-OV-3	−5.36	−4.81	−4.40
Renal cancer			
786-O	−4.86	−4.56	−4.26
ACHN	−5.70	−5.38	−5.06
CAKI-1	−6.08	−5.60	−5.15
RXF-393	−5.77	−5.26	−4.83
SN12C	−5.29	−4.76	−4.31
TK-1O	−4.88	−4.55	−4.25
UO-31	−5.56	−5.34	−5.12
Breast cancer			
MCF7	−5.58	−5.26	−4.65
NCI/ADR-RES	−5.83	−5.46	−5.09
MDA-MB-231/ATCC	−5.46	−5.00	−4.50
HS-578T	−5.57	−5.13	[e]
MDA-MB-435	−5.35	−4.70	−4.37
BT.-5.49	−5.67	−5.13	−4.42
T-47D	−5.91	−5.26	[e]

[a] Data obtained from the NCI's in vitro disease-oriented human tumor cell screen.
[b] The log of the molar concentration that inhibits 50% net cell growth.
[c] The log of the molar concentration giving total growth inhibition.
[d] The log of the molar concentration leading to 50% net cell death.
[e] The values of $\log LC_{50} > -4.00$.

Table 3. In vitro anti-HIV-1 drug screening results for imidazo[1,2-*b*][1,4,2]benzodithiazines **9**–**11**, **16**, **17**, **19**, and **21**[a]

Compound	EC_{50} (μM)[b]	CC_{50}[c] (μM)	TI_{50}[d]	Comments[e]
9	44.0	79.3	1.8	M
10	>200.0	1.1	—	I
11	>200.0	>200.0	—	I
16	0.94	>200.0	>212.7	A
17	9.0	>200.0	>22.2	A
19	>200.0	>200.0	—	I
21	>200.0	>200.0	—	I

[a] Data obtained from the NCI's in vitro anti-HIV primary screen.
[b] Effective concentration 50% (protection of HIV-1 infected CEM cells).
[c] Cytotoxic concentration 50% (toxicity to uninfected CEM cells).
[d] Therapeutic index = CC_{50}/EC_{50}.
[e] NCI designated activity: A (confirmed active); M (confirmed moderate); I (confirmed inactive).

(chemical shifts are expressed as δ values relative to Me_4Si as standard).

3.1. *N*-(6-Chloro-8-methyl-5,5-dioxo-1,4,2-benzodithiazin-3-yl)aminoacetaldehyde dimethyl acetal (8)

A solution of aminoacetaldehyde dimethyl acetal (2.84 g, 0.027 mol) and 3-methylbenzodithiazine **3** (7.34 g, 0.025 mol) in 100 mL of dry benzene was refluxed until the evolution had ceased (24 h), (caution: due to a high toxicity, MeSH should be trapped into an aqueous NAOH solution) and then left to stand overnight. The title compound thus obtained was collected by filtration, washed successively with benzene (3× 2 mL) and methanol (2× 2 mL), and dried (7.4 g, 84%): mp 116–117 °C; IR (KBr) 3210 (NH), 1575, 1555, 1540 (benzodithiazine ring), 1315, 1160, 1140 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.78 (s, 3H, CH_3-8) 3.43 (s, 6H, CH_3O–C–OCH_3), 3.67 (t, $J = 4.8$ Hz, 2H, CH_2), 4.53 (t,$J = 4.8$, 1H, CHO), 6.24 (s, 1H, NH), 7.18 (s, 1H, H-7), 7.27 (s, 1H, H-5) ppm. Anal: ($C_{12}H_{15}ClN_2O_4S_2$): C, 41.08; H, 4.31; N, 7.98. Found: C, 41.20; H, 4.43; N, 7.85.

3.2. 8-Chloro-6-methylimidazo[1,2-*b*][1,4,2]benzodithiazine 5,5-dioxide (11)

The *N*-(benzodi-thiazynyl)aminoacetaldehyde dimethyl acetal **8** (5.26 g, 0.015 mol) was added portionwise to

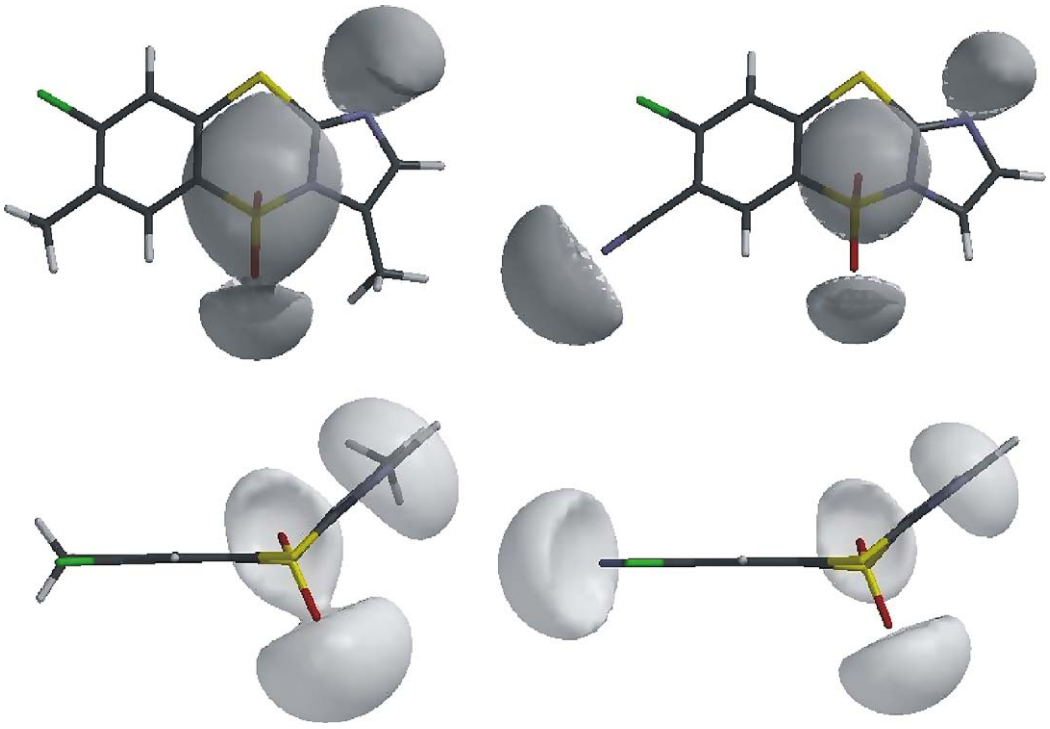


Figure 2. Comparison of the electrostatic potential maps of **10** (right) and **16** (left) isocontoured at −20 kcal/mol.[24]

98% sulfuric acid (23 mL). After an exothermic reaction was complete, the reaction mixture was kept at room temperature for 10 h. The solution obtained was poured into water-crushed ice mixture (700 g, 0–3 °C) and stirred at room temperature for 3 h. The precipitate was collected by filtration, washed thoroughly with water and ethanol (3× 2 mL), dried, and recrystallized from DMF (6 mL) to give **11** (3.5 g, 81%): mp 180–181 °C; IR (KBr) 1640, 1560, 1550, 1505 (imidazobenzodithiazine ring), 1360, 1185, 1175 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.73 (s, 3H, CH_3-6), 7.32 (s, 1H, H-7), 7.40 (s, 1H, H-9), 8.06 (d, J = 8.3 Hz, 2H, H-2 and H-3) ppm. Anal. Calcd for $C_{10}H_7ClN_2O_2S_2$: C, 41.89; H, 2.46; N, 9.77. Found: C, 41.93; H, 2.52; N, 9.90.

3.3. General procedure for the preparation of 6-chloro-3-(2-propynylamino)-1,4,2-benzodithiazine 1,1-dioxides (12–14)

A solution of the corresponding methylthiobenzodithiazine (**1**, **3** or **4**) (0.02 mol) and 1.1 g (0.02 mol) of 2-propynylamine in dry benzene (120–180 mL) was stirred at room temperature for 3 h. The suspension obtained was refluxed until the evolution of CH_3SH had ceased (40–50 h). The precipitate was filtered off and washed successively with benzene (3× 5 mL) and methanol (4× 5 mL).

In this manner, the following products were obtained.

3.4. 6-Chloro-7-methyl-3-(2-propynylamino)-1,4,2-benzodithiazine 1,1-dioxide (12)

Starting from methylthiobenzodithiazine **1** (5.87 g), the title compound **12** was obtained (5.8 g, 96%): mp 213–215 °C; IR (KBr) 3285 (NH), 2125 (C≡C), 1560 (C=N), 1345, 1150, (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.41 (s, 3H, CH_3), 3.16 (t, J = 2.5 Hz, 1H, C≡CH), 4.18 (d, J = 2.5 Hz, 2H, CH_2), 7.89 (s, 1H, H-5), 7.98 (s, 1H, H-8), 10.13, (br s, 1H, NH) ppm: ^{13}C NMR (DMSO-d_6) δ 19.33, 32.21, 75.00, 78.62, 126.47, 127.29, 128.09, 131.10, 137.14, 137.36, 162.50 ppm. Anal. Calcd for $C_{11}H_9ClN_2O_2S_2$: C, 43.92; H, 3.01; N, 9.31. Found: C, 43.90; H, 3.19; N, 9.48.

3.5. 6-Chloro-8-methyl-3-(2-propynylamino)-1,4,2-benzodithiazine 1,1-dioxide (13)

Starting from methylthiobenzodithiazine **3** (5.87 g), the title compound **13** was obtained (4.5 g, 74%): mp 185–187 °C; IR (KBr) 3245 (NH), 2125 (C≡C), 1565 (C=N), 1350, 1155, (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.63 (s, 3H, CH_3), 3.20 (t, J = 2.4 Hz, 1H, C≡CH), 4.17 (d, J = 2.4 Hz, 2H, CH_2), 7.55 (d, J = 1.5 Hz, 1H, H-7), 7.72 (d, J = 1.5 Hz, 1H, H-5), 10.04 (br s, 1H, NH) ppm. Anal. Calcd for $C_{11}H_9ClN_2O_2S_2$: C, 43.92; H, 3.01; N, 9.31. Found: C, 43.93; H, 3.14; N, 9.40.

3.6. *N*-(4-Chlorophenyl)-6-chloro-3-(2-propynylamino)-1,1-dioxo-1,4,2-benzodithiazine-7-carboxamide (14)

Starting from methylthiobenzodithiazine **4** (8.66 g), the title compound **14** was obtained (7.2 g, 83%): mp 146–148 °C; IR (KBr) 3290, 3195 (NH), 2120 (C≡C), 1660 (C=O), 1560 (C=N), 1310, 1160 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.25 (t, J = 2.4 Hz, 1H, C≡CH), 4.22 (d, J = 2.4 Hz, 2H, CH_2), 7.43 (d, J = 8.6 Hz, 2H, 4-ClPh), 7.72 (d, J = 8.6 Hz, 2H, 4-ClPh), 8.13 (s, 1H, H-5), 8.15 (s, 1H, H-8), 10.29 (s, 1H, NH), 10.83

(s, 1H, NH) ppm. Anal. Calcd for $C_{17}H_{11}Cl_2N_3O_3S_2$: C, 46.37; H, 2.52; N, 9.54. Found: C, 46.30; H, 2.66; N, 9.51.

3.7. Preparation of 6-chloro-3-(3-propynylamino)-1,1-dioxo-1,4,2-benzodithiazine-7- carboxylic acid (15)

To an ice-cold suspension of 6-chloro-3-methythio-1,1-dioxo-1,4,2-benzodithiazine-7-carboxylic acid **5** (12.95 g, 0.04 mol) in dry methanol (90 mL) was added with stirring 2-propynylamine (4.0 g, 0.08 mol). After 1 h, the ice bath was removed and the reaction mixture was refluxed until the evolution of MeSH had ceased (22–25 h). The solvent was evaporated under reduced pressure. The residue was dissolved in an aqueous solution of NaOH (1.6 g of NaOH in 250 mL water) and adjusted to pH 6.5 with 1% hydrochloric acid. After 0.5 h of stirring, a small amount of insoluble side products was filtered out together with charcoal added and the filtrate was slowly acidified to pH 1.5 with 0.5% hydrochloric acid. The title product, which precipitated, was immediately collected by filtration, washed thoroughly with water, and dried at temperatures gradually increasing to 100 °C. Yield 12.1 g (91%); mp 229–230 °C dec.; IR (KBr) 3320, 3215 (OH, NH), 2120 (C≡C), 1705, 1680 (C=O), 1565 (C=N), 1340, 1145 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.37 (d, J = 2.4 Hz, 1H, C≡CH), 4.21 (m, 3H, CH_2 and NH), 8.08 (s, 1H, H-5), 8.30 (s, 1H, H-8) ppm. Anal. Calcd for $C_{11}H_7ClN_2O_4S_2$: C, 39.94; H, 2.13, N, 8.47. Found: C, 39.84; H, 2.31; N, 8.64.

3.8. General procedure for the preparation of 8-chloro-3-methylimidazo[1,2-*b*][1,4,2]benzodithiazine 5,5-dioxides (16–19)

The corresponding benzodithiazine **12**, **13**, **14**, or **15** (0.015 mol) was added portionwise at room temperature to 95% sulfuric acid (35 mL). After the exothermic reaction was complete (1 h, 28–34 °C), the reaction mixture was kept at room temperature for 90–94 h, followed at 50–55 °C for 3 h. The solution obtained was poured into a water-crushed ice mixture (400–450 g, 0–4 °C) and stirred at room temperature for 2 h. The precipitated solid was collected by filtration, washed thoroughly with water and 2-propanol (4× 2 mL), and dried at temperatures gradually increasing to 90 °C.

In this manner, the following products were obtained.

3.9. 8-Chloro-3,7-dimethylimidazo[1,2-*b*][1,4,2]benzodithiazine 5,5-dioxide (16)

Starting from benzodithiazine **12** (4.51 g), the title compound **16** was obtained (3.6 g, 82%): mp 135–136 °C; IR (KBr) 2925 (CH_3), 1565 (C=N), 1370, 1185 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.48 (s, 3H, CH_3-7), 2.54 (s, 3H, CH_3-3), 6.79 (s, 1H, H-2), 7.63 (s, 1H, H-9), 7.97 (s, 1H, H-6) ppm; ^{13}C NMR ($CDCl_3$) δ 11.97, 20.54, 127.92, 128.44, 129.67, 130.65, 131.38, 133.44, 137.39, 139.27, 140.82 ppm. Anal. Calcd for $C_{11}H_9ClN_2O_2S_2$: C, 43.92; H, 3.01; N, 9.31. Found: C, 43.87; H, 3.17; N, 9.40.

3.10. 8-Chloro-3,6-dimethylimidazo[1,2-*b*][1,4,2]benzodithiazine 5.5-dioxide (17)

Starting from benzodithiazine **13** (4.51 g), the title compound **17** was obtained (3.4 g, 75%): mp 168–169 °C; IR (KBr) 2930 (CH_3), 1565 (C=N), 1365, 1180 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.54 (s, 3H, CH_3-3), 2.78 (s, 3H, CH_3-6), 6.81 (s, 1H, H-2), 7.28 (s, 1H, H-7), 7.47 (s, 1H, H-9) ppm; ^{13}C NMR ($CDCl_3$) δ 12.29, 22.30, 127.32, 128.53, 131.49, 132.21, 133.25, 134.72, 139.03, 139.32, 140.95 ppm. Anal. Calcd for $C_{11}H_9ClN_2O_2S_2$: C, 43.92; H, 3.01; N, 9.31. Found : 43.80; H, 3.12; N. 9.48.

3.11. *N*-(4-Chlorophenyl)-8-chloro-3-methyl-5,5-dioxoimidazo[1,2-*b*][1,4,2]benzodithiazine-7-carboxamide (18)

Starting from benzodithiazine **14** (6.6 g), the title compound **18** was obtained (4.9 g, 74%): mp 224–225 °C; IR (KBr) 3285 (NH), 2925 (CH_3), 1160 (C=O), 1575 (C=N), 1375, 1365, 1185 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.49 (s, 3H, CH_3-3), 7.03 (s, 1H, H-2), 7.43 (d, J = 8.8 Hz, 2H, 4-ClPh), 7.71 (d, J = 8.8 Hz, 2H, 4-ClPh), 8.36 (s, 1H, H-9), 8.50 (s, 1H, H-6), 10.83 (s, 1H, NH) ppm. Anal. Calcd for $C_{17}H_{11}Cl_2N_3O_3S_2$: C, 46.37; H, 2.52; N, 9.54. Found C, 46.49; H, 2.68; N, 9.41.

3.12. 8-Chloro-3-methyl-5,5-dioxoimidazo[1,2-*b*][1,4,2]benzodithiazine-7-carboxylic acid (19)

Starting from benzodithiazine **15** (4.96 g), the title compound **19** was obtained (4.5 g, 91%): mp 322–324 °C dec.; IR (KBr) 2920, 2765, 2520, 2460, 1715 (COOH), 1575 (C=N), 1370, 1190, (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.49 (s, 3H, CH_3-3), 7.03 (s, 1H, H-2), 8.32 (s, 1H, H-9), 8.50 (s, 1H, H-6) ppm. Anal. Calcd for $C_{11}H_7ClN_2O_4S_2$: C, 39.94; H, 2.13; N, 8.47. Found: C, 40.10; H, 2.15; N, 8.59.

3.13. Synthesis of methyleneorthosulfonictetrakis (8-chloro-3-methyl-5,5-dioxoimidazo[1,2-*b*][1,4,2]benzodithiazine-7-carboxylic) anhydride (20)

To a suspension of carboxylic acid **19** (16.54 g, 0.05 mol) in dry CH_2Cl_2 (40 mL) was added with stirring triethylamine (6.37 g, 0.063 mol). The solution obtained was cooled to −15 °C in an ice–NaCl bath, and to this was added dropwise over 45 min a solution of methanesulfonyl chloride (4.35 g, 0.038 mol) in anhydrous CH_2Cl_2 (25 mL). The reaction mixture was stirred for additional 3 h at −12 to −6 °C. The cooling bath was removed, and the reaction mixture allowed to warm to 18 °C. The solid that precipitated was collected by filtration and washed successively with CH_2Cl_2 (5× 5 mL), cold water (6× 10 mL), acetone (4× 5 mL), and CH_2Cl_2 (2× 5 mL). Drying under vacuum gave anhydride **20** (13.7 g, 80%): mp 211–213 °C; IR (KBr) 1805, 1740 (C=O, anhydride), 1375, 1195, 1175, 1140 (SO_2) and other characteristics at 1580, 1530, 1445, 1295, 1270, 1090, 1005, 905, 875, 765, 680 and 590 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.53 (s, 12H, 4× CH_3-3), 5.31 (s, 2H, S=CH_2), .6.88 (s, 4H, 4× H-2), 7.87 (s, 4H, 4× H-9), 8.75 (s, 4H, 4× H-6) ppm; ^{13}C NMR (DMSO-d_6) δ 10.96 (CH_3), 54.30

(S=CH_2), 164.20 (C=O), 127.68, 128.25, 130.45, 130.57, 131.68, 134.76, 137.61, 137.80 ppm. Anal. Calcd for $C_{45}H_{26}Cl_4N_8O_{16}S_9$: C, 39.59; H, 1.92; N, 8.21. Found: C, 39.68; H, 2.07; N, 8.36.

3.14. General procedure for the preparation of 8-chloro-3-methyl-5,5-dioxoimidazo[1,2-*b*][1,4,2]benzodithiazine-7-carboxyamides (21–23)

To a suspension of mixed anhydride **20** (3.41 g, 0.0025 mol) and the appropriate amine (0.005 mol) in dry *p*-dioxane (70 mL) was added triethylamine (0.75 g, 0.0075 mol). The reaction mixture was stirred at room temperature for 1 h, followed by reflux for 12 h. The solvent was evaporated under reduced pressure. To the residue, a solution of K_2CO_3 (1 g) in water (150 mL) was added, and this was stirred at room temperature for 1 h. The precipitate of the adequate carboxamide obtained was filtered, washed successively with water (6× 5 mL) and ethanol (5× 4 mL), and dried. The water-filtrates (pH 9–10) mixture was acidified with 1% hydrochloric acid to pH 1.5. Carboxylic acid **19** thus obtained as a side product was filtered out, washed with water, and dried (yields: 1.1–1.2 g, 66–72%).

In this manner, the following carboxamides were obtained.

3.15. *N*-(Isobutyl)-8-chloro-3-methyl-5,5-dioxoimidazo[1,2-*b*][1,4,2]benzodithiazine-7-carboxamide (21)

Starting from isobutylamine (0.55 g), the title compound was obtained (1.6 g, 83%): mp 174–175 °C; IR (KBr) 3255 (NH), 1640 (C=O), 1560 (C=N), 1380, 1190, 1170 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.91 (d, J = 6.3 Hz, 6H, CH_3-isobutyl), 1.80–1.83 (m, 1H, CH-isobutyl), 2.49 (s, 3H, CH_3-3), 3.08 (dd, J_{CH-CH} = 6.3 Hz, J_{CH-NH} = 5.3 Hz, 2H, CH_2-isobutyl), 7.03 (s, 1H, H-2), 8.17 (s, 1H, H-9), 8.29 (s, 1H, H-6), 8.70 (t, J = 5.3 Hz, 1H, NH) ppm. Anal. Calcd for $C_{15}H_{16}ClN_3O_3S_2$: C, 46.68; H, 4.18, N, 10.89. Found: C, 46.60; H, 4.32; N, 10.91.

3.16. *N*-(Benzo-2,1,3-thiadiazol-4-yl)-8-chloro-3-methyl-1,1-dioxoimidazo[1,2- *b*][1,4,2]benzodithiazine-7-carboxamide (22)

Starting from 4-aminobenzo-2,1,3-thiadiazole (0.76), the title compound was obtained (1.9 g, 82%); mp 227–228 °C; IR (KBr) 3340 (NH), 1673 (C=O), 1615, 1550 (C=N), 1375, 1190 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.57 (s, 3H, CH_3-3), 7.05 (s, 1H, H-2), 7.78 (dd, J = 8.4 Hz, J = 7.2 Hz, 1H) 7.89 (d, J = 8.4 Hz, 1H) 8.36 (s, 1H, H-9), 8.45 (d, J = 7.2 Hz, 1H), 8.51 (s, 1H, H-6), 11.63 (s, 1H, NH) ppm. Anal. Calcd for $C_{17}H_{10}ClN_5O_3S_3$: C, 44.01; H, 2.17; N, 15.09. Found: 43.96; H, 2.28; N, 15.27.

3.17. *N*-(Benzothiazol-6-yl)-8-chloro-3-methyl-5,5-dioxoimidazo[1,2-*b*][1,4,2]benzodithiazine-7-carboxamide (23)

Starting from 6-aminobenzothiazole (0.75 g), the title compound **23** was obtained (1.8 g, 78%): mp 252–253 °C; IR (KBr) 3260 NH), 1650 (C=O), 1570 (C=N) 1370, 1185 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.52 (s, 3H, CH_3-3), 7.06 (s, 1H, H-2), 7.70 (d, J = 8.2 Hz, 1H), 8.10 (d, J = 8.2 Hz, 1H), 8.39 (s, 1H), 8.55 (s, 1H), 8.66 (s, 1H), 9.34 (s, 1H, H-6), 11.00 (s, 1H, NH) ppm. Anal. Calcd for $C_{18}H_{11}ClN_4O_3S_3$: C, 46.69; H, 2.39; N, 12.10. Found: C, 46.78; H, 2.48; N, 12.03.

3.18. 8-Quinolyl 8-chloro-3-methyl-5,5-dioxoimidazo[1,2-*b*][1,2,4]benzodithiazine-7-carboxylate 24

A stirred mixture of mixed anhydride **20** (2.73 g, 0.002 mol), 8-hydroxyquinoline (0.73 g, 0.005 mol) and *p*-dioxane (30 mL) was refluxed for 8 h. The solvent was evaporated under reduced pressure. To the residue a solution of K_2CO_3 (1 g) in water (100 mL) was added, and this was stirred at room temperature for 2 h. The title compound **24** thus obtained was collected by filtration, washed with water, dried, and recrystallized from acetone (1.45 g, 79%): mp 231–232 °C; IR (KBr) 2925 (CH_3), 1745 (C=O), 1375, 1185 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.58 (s, 3H, CH_3), 6.87 (s, 1H, H-2), 7.48–7.55 (dd, $J_{3'4'}$ = 8.4 Hz, $J_{3'2'}$ = 4.2 Hz, 1H, H3′), 7.62–7.69 (m, 2H), 7.79–7.86 (m, 2H), 7.79–7.86 (m, 2H), 8.3 (dd, $J_{4'3'}$ = 8.4 Hz, $J_{4'2'}$ = 1.4 Hz, 1H, H-4′), 8.95 (dd, $J_{2'3'}$ = 4.2 Hz, $J_{2'4'}$ = 1.4 Hz, 1H, H-2′), 9.15 (s, 1H, H-6) ppm. Anal. Calcd for $C_{20}H_{12}ClN_3O_4S_2$: C, 52.46; H, 2.64; N, 9.17. Found: C, 52.38; H, 2.80; N, 9.15.

The water-filtrates (pH 8–8.5) mixture was acidified with 1% hydrochloric acid to pH 1. The precipitate thus obtained was filtered out, washed with water, and dried, giving 0.9 g (68%) of carboxylic acid **19** formed as a by-product.

3.19. Cytotoxicity assays

Compounds were tested against a panel of approximately 60 human tumor cell lines (NCI60). Details of this test system and the information, which is encoded by the activity pattern over all cell lines, have previously been published.[20–22] The antitumor activity of a test compound is given by three parameters for each cell line: log GI_{50} value (GI_{50} = molar concentration of the compound that inhibits 50% net cell growth), log TGI value (TGI = molar concentration of the compound leading to total inhibition of net cell growth), and log LC_{50} (LC_{50} = molar concentration of the compound leading to 50% net cell death). Furthermore, a mean graph midpoint (MG_MID) is calculated for each of the mentioned parameters, giving an averaged activity parameter over cell lines. Selectivity of a compound with respect to one or more cell lines of the screen is characterized by a high deviation of the particular cell line parameter compared to the MG_MID value.

3.20. Anti-HIV assays in cultured cell lines

The anti-HIV drug testing performed at NCI is based on a protocol described by Weislow et al.[23] In brief, all compounds were dissolved in DMSO and diluted in 1:100 in cell culture medium. Exponentially growing

T4 lymphocytes (CEM cell line) were added at 5000 cells per well. Frozen virus stock solutions were thawed immediately before use, suspended in complete medium to yield the desired multiplicity of infection (≈0.1) and added to the microtiter wells, resulting in a 1:200 final dilution of the compound. Uninfected cells with the compound serve as a toxicity control, and infected and uninfected cells without the compound serve as basic controls. Cultures were incubated at 37 °C in a 5% CO_2 atmosphere for 6 days. The tetrazolium salt, XTT [2,3-bis (2-methoxy-4-nitro-5-sulfenyl)-2*H*-tetrazolium-5-carboxamide], was added to all wells, and cultures were incubated to allow formazan color development by viable cells. Individual wells were analyzed spectrophotometrically to quantitate formazan production and in addition are viewed microscopically for detection of viable cells and confirmation of protective activity.

Acknowledgment

Authors thank the Ministry of Science and Informatization, Poland, for financial support (Grant No. 2 PO5F 03527).

References and notes

1. Neamati, N.; Barchi, J. J. *Curr. Top. Med. Chem.* **2002**, *2*, 211–227.
2. Plasencia, C.; Dayam, R.; Wang, Q.; Pinski, J.; Burke, T. R., Jr.; Quinn, D. I.; Neamati, N. *Mol. Cancer Ther.* **2005**, *4*, 1105–1113.
3. Casini, A.; Scozzafava, A.; Mastrolorenco, A.; Supuran, C. T. *Curr. Cancer Drug Targets* **2002**, *2*, 55–75.
4. Scozzofava, A.; Owa, T.; Mastrolorenzo, A.; Supuran, C. T. *Curr. Med. Chem* **2003**, *10*, 925–953.
5. Scozzafava, A.; Casini, A.; Supuran, C. T. *Curr. Med. Chem* **2002**, *9*, 1167–1185.
6. Supuran, C. T.; Casini, A.; Scozzafava, A. *Med. Res. Rev.* **2003**, *23*, 531–558.
7. Brzozowski, Z. *Acta Pol. Pharm. Drug Res.* **1997**, *54*, 49–55.
8. Brzozowski, Z. *Acta Pol. Pharm. Drug Res.* **1997**, *54*, 293–298.
9. Pomarnacka, E.; Kornicka, A.; Sączewski, F. *Heterocycles* **2001**, *55*, 753–762.
10. Brzozowski, Z.; Sączewski, F. *J. Med. Chem.* **2002**, *45*, 430–437.
11. Pomarnacka, E.; Gdaniec, M. *Bioorg. Med. Chem.* **2003**, *11*, 1259–1267.
12. Brzozowski, Z.; Sączewski, F.; Gdaniec, M. *Bioorg. Med. Chem.* **2003**, *11*, 3673–3681.
13. Brzozowski, Z.; Sączewski, F.; Gdaniec, M. *Eur. J. Med. Chem.* **2003**, *38*, 991–999.
14. Brzozowski, Z.; Sączewski, F.; Sanchez, T.; Kuo, C. L.; Gdaniec, M.; Neamati, N. *Bioorg. Med. Chem.* **2004**, *12*, 3663–3672.
15. Neamati, N.; Mazumder, A.; Sunder, S.; Owen, J. M.; Schultz, R. J.; Pommier, Y. *Antiviral Chem. Chemother.* **1997**, *8*, 485–495.
16. Brzozowski, Z.; Sławiński, J. *Acta. Pol. Pharm.* **1984**, *41*, 133–139.
17. Brzozowski, Z.; Gajewski, F.; Sławiński, J.; Pomarnacka, E. *Acta Pol. Pharm. Drug Res.* **1993**, *50*, 199–203.
18. Brzozowski, Z.; Sławiński, J. *Acta Pol. Pharm.* **1984**, *41*, 5–13.
19. Boehme, H.; Meyer-Dulheuer, K. H. *Liebigs Ann. Chem.* **1965**, *688*, 78–93.
20. Boyd, M. R. *Am. Assoc. Cancer Res.* **1989**, *30*, 652–663.
21. Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Poull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolf, A.; Gray-Goodrich, M.; Cambell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757–766.
22. Weinsten, J. N.; Myers, T. C.; O'Connor, P. M.; Friend, S. H., ; Fornance, A. J., Jr.; Kohn, K. W.; Fojo, T.; Bates, S. E.; Rubinstein, L. V.; Anderson, N. L.; Buolamwini, J. K.; van Osdol, W. W.; Monks, A. P.; Scudiero, D. A.; Sansiville, E. A.; Zaharewitz, D. W.; Bunow, B.; Viswanadhan, V. N.; Johnson, G. S.; Wittes, R. E.; Paull, K. D. *Science* **1997**, *275*, 343–395.
23. Wieslow, O. W.; Kiser, R.; Fine, D.; Bader, J.; Shoemaker, R. H.; Boyd, M. R. *J. Natl. Cancer Inst.* **1989**, *81*, 577–586.
24. Molecular modeling studies were performed using HF/6-31G** ab initio model as implemented into Spartan program, version 5.0, Wavefunction Inc., and installed on a Silicon Graphics *O2* workstation.