Pharmacological Reports
2010, 62, 319–332
ISSN 1734-1140

Anticancer activity of newly synthesized azaphenothiazines from NCI's anticancer screening bank#

Krystian Pluta[1], Małgorzata Jeleń[1], Beata Morak-Młodawska[1], Michał Zimecki[2], Jolanta Artym[2], Maja Kocięba[2]

[1]The Medical University of Silesia, Department of Organic Chemistry, Jagiellońska 4, PL 41-200 Sosnowiec, Poland

[2]Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Department of Experimental Therapy, R. Weigla 12, PL 53-114 Wrocław, Poland

Correspondence: Krystian Pluta, e-mail: pluta@sum.edu.pl

Abstract:
The activity of the newly synthesized azaphenothiazines: tricyclic 10-substituted dipyridothiazines **1**–**9**, pentacyclic 6-substituted diquinothiazines **10**–**22** and hexacyclic diquinothiazinium salt **23** was tested on 55–60 *in vitro* cell lines. The cell lines included nine types of cancer: leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer (National Cancer Institute, Bethesda, MD, USA). The features of the chemical substituent at the thiazine nitrogen atom confer the anticancer activity of diquinothiazines **10**–**23.** Unexpectedly, the most active of the dipyridothiazines **1-9** was the unsubstituted compound **1** (the substituent is a hydrogen atom). The most cytotoxic compound was the half-mustard derivative **18.** The GI_{50} value of this compound was –7.06 (corresponding to 40 ng/ml) when tested on the melanoma cell line SK-MEL-5 and –6.0 – –6.62 using cell lines from various cancers including: leukemia (CCRF-CEM), the MOLT-4 cell line, colon cancer (HCT-116), central nervous system cancer (SNB-75 and SF-295), prostate cancer (PC-3), non-small cell lung cancer (NCI-H460 and HOP-92), ovarian cancer (IGROV1 and OVCAR-4) and breast cancer (MDA-MB-460). The ethylene group in the aminoalkylazaphenothiazines is as a good linker and is similar to the propylene and butylene linkers in aminoalkylphenothiazines. To our knowledge, this is the first demonstration of significant azaphenothiazine anticancer activity.

Key words:
azaphenothiazines, dipyridothiazines, diquinothiazines, anticancer activity

Introduction

Phenothiazines are an important class of heterocyclic compounds possessing not only widely recognized neuroleptic activity, but also antihistaminic, antitussive and antiemetic activities [4, 16]. Recent reports have measured other activities of typical and newly synthesized phenothiazines including anticancer [3, 14–18], antiplasmid [8] and antibacterial activities [1, 15]. In addition, phenothiazines have been evaluated for the reversal of multi-drug resistance (MDR) [2, 7]

Part CXV in the series of Azinyl Sulfides

and treatment of Alzheimer's [12], Creutzfeldt-Jacob [1] and AIDS diseases [26]. Typical neuroleptic phenothiazines [3, 13, 15, 17, 22, 28] and new phenothiazine derivatives [3, 13–19, 24, 27] have anticancer activity; this activity is conferred by new pharmacophoric substituents (other than dialkylaminoalkyl groups) at the thiazine nitrogen atom and by substitution of the benzene ring with a naphthalene ring to form benzophenothiazines and dibenzothiazines.

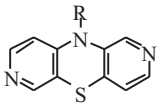
In an effort to develop active pyridine and quinoline derivatives, we modified the phenothiazine structure with either a pyridine or quinoline ring to form three new types of azaphenothiazines: 10-substituted 2,7-diazaphenothiazines (10-substituted dipyridothiazines) **1**–**9**, 6-substituted dibenzo-1,9-diazaphenothiazines (6-substituted diquinothiazines) **10**–**22** and 5,6-ethylenediquinothiazinium salt **23**. We selected alkyl, aryl, heteroaryl, dialkylaminoalkyl, *N*-acylaminoalkyl and *N*-sulfonylaminoalkyl groups as the substituents at the thiazine nitrogen atom. Some substituents were selected using computer-aided studies [23] and others were selected based on the pharmacophoric groups found in anticancer and antibacterial phenothiazines [15, 18, 27].

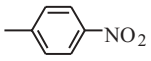
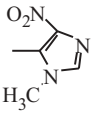
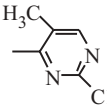
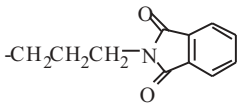
The anticancer activities of newly synthesized azaphenothiazines **1**–**23** were determined using cancer cell lines in order to gain insight into the structure-activity relationship and to identify effective anticancer drugs.

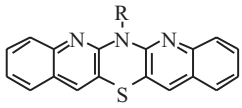
Materials and Methods

Chemicals

Azaphenothiazines **1**–**23** (Fig. 1) were synthesized as described previously [6, 11, 21].



R	NSC No.
1. -H	737217/1
2. -CH_3	743522/1
3. 	743518/1
4. 	743523/1
5. 	743524/1
6. -$CH_2CH_2N(C_2H_5)_2$	743521/1
7. -$CH_2CH(CH_3)CH_2N(CH_3)_2$	743519/1
8. 	743525/1
9. -$CH_2CH_2CH_2NHCOCH_3$	743520/1



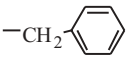
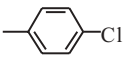
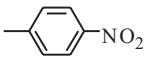
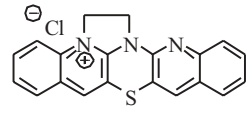
R	NSC No.
10. -$CH_2CH_2CH_2CH_3$	744651/1
11. 	744654/1
12. 	744652/1
13. 	744653/1
14. 	744655/1
15. -$CH_2CH_2NHCOCH_3$	744657/1
16. -$CH_2CH_2N(C_2H_5)_2$	744656/1
17. -$CH_2CH_2NHSO_2C_6H_4CH_3$	744658/1
18. -$CH_2CH_2NHCONHCH_2CH_2Cl$	744659/1
19. -$CH_2CH_2CH_2N(CH_3)_2$	744660/1
20. -$CH_2CH_2CH_2NHCOCH_3$	744661/1
21. -$CH_2CH_2CH_2NHCOOC_2H_5$	744663/1
22. -$CH_2CH_2CH_2NHSO_2C_6H_4CH_3$	744662/1
23. 	737216/1

Fig. 1. Structures of azaphenothiazines **1**–**23** with NCI code numbers

Assay for anticancer activity

The effects of azaphenothiazines **1**–**23** on the percentage of growth (PG), the 50% growth inhibitory concentration (GI_{50}), the total tumor growth inhibitory concentration (TGI) and the 50% lethal concentration (LC_{50}) were determined in 55–60 cell lines comprising nine types of human cancers (6 leukemia, 9 non-small cell lung cancer, 7 colon cancer, 6 central nervous system cancer, 8 melanoma, 6 ovarian cancer, 8 renal cancer, 2 prostate cancer and 8 breast cancer cell lines, at the National Cancer Institute (Bethesda, MD, USA) *In Vitro* Anticancer Drug Discovery Screen [25]. Azaphenothiazines were initially tested at 10^{-5} mol/l (PG). If the initial results were promising, further testing was performed at four concentrations: 10^{-4}, 10^{-6}, 10^{-7}, and 10^{-8} mol/l (PG, GI_{50}, TGI and LC_{50}). Azaphenothiazines that displayed activity at low concentrations were considered the most effective compounds. Compounds were denoted: very active, active, less active, or inactive.

Screening Data Report components

The calculated measurement of effect: percentage growth (PG)

The effect of a compound on a cell line was measured according to one of the two equations listed below:

If (Mean OD_{test} – Mean OD_{tzero}) ≥ 0, then
$PG = 100 \times (\text{Mean } OD_{test} - \text{Mean } OD_{tzero})/(\text{Mean } OD_{ctrl} - \text{Mean } OD_{tzero})$

If (Mean OD_{test} – Mean OD_{tzero}) < 0, then
$PG = 100 \times (\text{Mean } OD_{test} - \text{Mean } OD_{tzero})/\text{Mean } OD_{tzero}$

where: Mean OD_{tzero} = average optical density of the SRB-derived color measurements immediately before exposure of cells to the test compound. Mean OD_{test} = average optical density of the SRB-derived color measurements 48 hours after exposure of cells to the test compound. Mean OD_{ctrl} = average of optical density of the SRB-derived color measurements of cells unexposed to the test compound.

The data sheet

This page displays the experimental data that were collected for each cell line. The first two columns indicate the sub-panel (e.g., leukemia) and cell line (e.g., CCRF-CEM). The second two columns list the Mean OD_{tzero} and Mean OC_{ctrl}; this is followed by five columns that list the Mean OD_{test} for each drug concentration. The drug concentration is expressed as the $\log_{10}$ (molar or mg/ml). Finally, the last five columns list the calculated PGs for each concentration. The response parameters GI_{50}, TGI, and LC_{50} are interpolated values of the concentrations at which the PG is +50.0 and –50.0, respectively. Sometimes these response parameters cannot be obtained by interpolation. For instance, if all of the PGs in a given row exceed +50 then none of the parameters can be obtained by interpolation. In this case, the value given for each response parameter is the highest concentration tested and is preceded by a ">" sign. This rule is extended to the other possible situations where a response parameter cannot be obtained by interpolation.

Dose-response curves

The dose-response curve page was created by plotting the PGs against the $\log_{10}$ of the corresponding drug concentration for each cell line. The cell line curves are grouped by sub-panel. Horizontal lines are provided at the PG values of +50 and –50. The concentrations corresponding to the intersection of the curve with these PG values are the GI_{50}, TGI and LC_{50}, respectively.

The mean graphs

Mean graphs facilitate visual scanning of data for patterns of selectivity with respect to a selected response parameter for a particular cell line or for particular sub-panels. Differences in the selectivity patterns may occur for the same compound in the same cell lines when different parameters are compared. The mean graphs page of the data package displays the principal response parameters: GI_{50}, TGI and LC_{50}. The bars extending to the right represent the sensitivity of cell line to the test agent that is in excess of the average sensitivity of all tested cell lines. Since the bar scale is logarithmic, a bar that is two units to the right implies that the compound achieved the response parameter (e.g. GI_{50}) for the cell line at a concentration that is one-hundredth of the mean concentration required for all the cell lines; thus, the selected cell line is sensitive to that compound. Similarly, bars that extend to the left imply sensitivity lesser than the mean. If a given parameter for a particular drug and cell line combination was impossible to determine by interpolation, the

bar length shown is either based on the highest concentration tested (the listed $\log_{10}$ of the response parameter will be preceded by a ">") or the lowest concentration tested (the listed $\log_{10}$ will be preceded by a "<"). The values at either limit (> or <) are displayed in the mean graph. The mean used in the mean graph may not be the actual mean of the parameter tested. For this reason, we shall refer to this value as the MG-MID (for mean graph midpoint) [25].

The toxicity test

Mouse splenocytes were plated in culture medium in a 96-well flat-bottom plate at a concentration of 2×10^5/100 µl/well. Compounds were added at a final concentration range of 10^{-6} to 10^{-4} mol/l. After an overnight incubation, the cells were treated with 0.25 µl of the MTT reagent [5], and the cell viability was measured as the optical density of 550/630 nm. The results were presented as the mean optical density values calculated from quadruplicate wells ± standard error.

Results and Discussion

The most significant and persistent modifications of the phenothiazine structure were the pharmacophoric substituents at the thiazine nitrogen atom and the substitution of the benzene ring with an azine ring; the latter modification formed the azaphenothiazines. These diazaphenothiazines represent tricyclic (**1**–**9**), pentacyclic (**10**–**22**) and hexacyclic (**23**) ring systems. It is worth noting that the last compound, unlike the others, represents a type of phenothiazinium salt structure. Tables 1–9 contain all the data (GI_{50},. TGI and LC_{50}) of the effects of active and very active compounds (**1, 16**–**19** and **23**) on the cancer cell lines. Dipyridothiazines **1**–**9** were less active than diquinothiazines **10**–**23**. Of the nine dipyridothiazines tested, only compound **1,** in some cases, was similarly active (PG) to the diquinothiazines. In some cell lines (EKVX, HS578 and UO-31), compounds **2** and **3** were the same or more active in the pre-screen evaluation than compound **1**. Of the other diquinothiazines, compound **10** had remarkable PG values when tested on the breast cancer cell lines MCF-7 (7%) and T-47D (14%) and on the renal cancer line UO-31 (22%) and the non-small cell lung cancer line HOP-92 (28%). Similar activities in these cell lines were also observed for compounds **15** (19%), **21** (24%), **14** (27%) and **20** (29%). Compound **12** was less active against the MCF-7 breast cancer cell line and the UO-31 renal cancer cell line. In addition, compounds **13** and **22** were less active against the

Tab. 1. Anti-leukemia activity of azaphenothiazines **1**, **16**–**19** and **23**

Compd's number	CCRF-CEM			HL-60(TB)			K-562			MOLT-4		
	$\log_{10}$ of sample concentration (mol)											
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
1	–4.43	> –4	> –4	–4.69	–4.05	> –4	–4.26	> –4	> –4	–4.48	> –4	> –4
16	–5.70	–5.25	–4.52	–5.80	–5.47	–5.14	–5.84	–	–	–5.79	–5.42	–5.06
17	> –4	> –4	> –4	> –4	> –4	> –4	> –4	> –4	> –4	> –4	> –4	> –4
18	–6.62	–5.69	–4.75	–5.93	–5.54	–5.15	–5.80	–5.20	> –4	–6.01	–5.49	> –4
19	–5.95	–5.59	–5.23	–5.87	–5.55	–5.23	–6.19	> –4	> –4	–6.05	–5.64	–5.25
23	–4.56	> –4	> –4	–4.49	> –4	> –4	–4.85	> –4	> –4	–4.66	> –4	> –4

Compd's number	RPMT-8226			SR		
	$\log_{10}$ of sample concentration (mol)					
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
1	–4.44	> –4	> –4	–	–	–
16	–5.90	–5.48	–5.06	–	–	–
17	–	> –4	> –4	> –4	> –4	> –4
18	–5.45	–5.64	–5.08	–5.75	–5.27	> –4
19	–6.35	–5.68	–5.22	–	–	–
23	–4.81	> –4	> –4	–4.87	> –4	> –4

UO-31 renal cancer cell line. Compounds **14** and **21** were minimally active against the SK-MEL-2 melanoma cell line and the T-47D breast cancer cell line, respectively.

Anti-leukemia activity

Azaphenothiazines **18** and **19** had very potent anticancer activity. The GI_{50} values of compound **18** when tested on CCRF-CEM, MOLT-4 and HL-60 cells were –6.62, –6.01 and –5.93, respectively (Tab. 1). Similarly, the GI_{50} values for compound **19** when tested on the RPMT-8226, K-562 and CCRF-CEM cell lines were –6.35, –6.19 and –5.95, respectively. Compound **18** had a significant TGI value when CCRF-CEM, HL-60, MOLT-4 and RPMT-8226 cells were tested; the TGI values were –5.69, –5.54, –5.49 and –5.64, respectively. Similarly, compound **19** had a significant TGI value when tested on the same cells; these values were –5.59, –5.55, –5.64 and –5.68, respectively. The LC_{50} values for compound **19** ranged between –5.22 and –5.25 when tested on CCRF-CEM, HL-60, MOLT-4 and RPMT-8226 cells. The LC_{50} values of compound **18** were in the range of –5.08 – –5.15 when tested on HL-60 and RPMT-8226 cells.

Tab. 2. Anti-non-small cell lung cancer activity of azaphenothiazines **1**, **16**–**19** and **23**

Compd's number	A549/ATCC			EKVX			HOP-62			HOP-92		
	Log_{10} of sample concentration (mol)											
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
1	–4.48	> –4	> –4	–4.36	> –4	> –4	–5.82	–4.22	> –4	–5.08	–4.12	> –4
16	–5.44	–5.90	–4.26	–5.35	–4.76	–4.21	–5.80	–5.46	–5.13	–6.06	–5.49	–4.91
17	–	> –4	> –4	> –4	> –4	> –4	–4.79	–4.01	> –4	–	–	–
18	–5.19	–4.65	–4.20	–5.37	–4.71	–4.26	–5.36	–4.72	–4.32	–6.14	–5.13	–4.23
19	–5.69	–5.41	–	–5.82	–5.49	–5.16	–5.89	–5.59	–5.29	–6.48	–5.85	–5.32
23	–4.92	> –4	> –4	–4.68	> –4	> –4	–4.68	–4.11	> –4	–4.55	> –4	> –4

Compd's number	NCI-H226			NCI-H23			NCI-H322M			NCI-H460		
	Log_{10} of sample concentration (mol)											
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
1	–4.31	> –4	> –4	–4.36	> –4	> –4	–4.39	> –4	> –4		> –4	> –4
16	–5.48	–4.85	–4.24	–5.54	–4.90	–4.28	–5.35	–4.72	–4.31	–5.79	–5.50	–5.20
17	–	–	–	–4.83	> –4	> –4	> –4	> –4	> –4	> –4	> –4	> –4
18	–5.74	–4.72	–4.34	–5.43	–4.68	–4.19	–4.95	–4.61	–4.28	–6.31	–5.73	–5.34
19	–5.83	–5.39	–4.68	–5.85	–5.54	–5.23	–5.80	–5.52	–5.25	–5.80	–5.50	–5.21
23	–4.35	> –4	> –4	–4.66	> –4	> –4	–4.27	> –4	> –4	–4.69	–4.19	> –4

Compd's number	NCI-H522		
	Log_{10} of sample concentration (mol)		
	GI_{50}	TGI	LC_{50}
1	–4.39	> –4	> –4
16	–5.84	–5.51	–5.18
17	–4.07	> –4	> –4
18	–4.42	–4.55	> –4
19	–5.80	–5.48	–
23	–4.57	> –4	> –4

Anti-non-small cell lung cancer activity

Azaphenothiazines **1**, **16**, **18** and **19** had very potent anticancer activity (Tab. 2). The most active was compound **19** with a GI_{50} value of –6.48 when tested on HOP-92 cells; the GI_{50} value ranged between –5.80 and –5.89 when the EKVX, HOP-62, NCI-H226, NCI-H322M, NCI-H460 and NCI-H522 cell lines

Tab. 3. Anti-colon cancer activity of azaphenothiazines **1**, **16–19** and **23**

Compd's number	COLO 205			HCC-2998			HCT-116			HTC-15		
	Log_{10} of sample concentration (mol)											
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
1	–4.93	–4.23	> –4	–4.31	> –4	> –4	–4.75	–4.16	> –4	–4.43	> –4	> –4
16	–5.88	–5.55	–5.21	–5.77	–5.43	–5.09	–5.88	–5.56	–5.24	–5.78	–5.43	–5.09
17	> –4	> –4	> –4	–	–	–	–5.12	> –4	> –4	> –4	> –4	> –4
18	–5.87	–5.56	–5.25	–5.77	–5.29	–4.72	–6.08	–5.30	–4.62	–5.53	–4.80	–4.09
19	–5.90	–5.55	–5.19	–5.78	–5.49	–5.19	–5.96	–5.64	–5.31	–5.88	–5.54	–5.21
23	–5.45	–4.59	> –4	–4.73	–4.19	> –4	–4.51	> –4	> –4	> –4	> –4	> –4

Compd's number	HT-29			KM 12			SW 620		
	Log_{10} of sample concentration (mol)								
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
1	–4.24	> –4	> –4	–4.49	> –4	> –4	–4.45	> –4	> –4
16	–5.89	–5.42	–4.76	–5.72	–5.39	–5.06	–5.81	–5.53	–5.25
17	> –4	> –4	> –4	> –4	> –4	> –4	> –4	> –4	> –4
18	–5.92	–5.11	–4.41	–5.61	–4.96	–4.37	–5.92	–5.59	–5.27
19	–5.93	–5.29	> –4	–5.82	–5.54	–5.26	–5.83	–5.54	–5.25
23	–4.47	> –4	> –4	–4.56	–4.39	> –4	–4.49	> –4	> –4

were tested. Compound **18** had a GI_{50} value of –6.31 when tested on NCI-H460 cells, –6.14 on HOP-92 cells and –5.74 on NCI-H226 cells. Compound **16** had GI_{50} values of –6.06 when tested on HOP-92 cells and ranged between –5.79 and –5.84 when the HOP-62, NCI-460 and NCI-H522 cells lines were tested. Dipyridothiazine **1** had significant activity with GI_{50} values of –5.82 when HOP-62 cells were tested and –5.08 for the treatment of HOP-92 cells. The TGI values for compound **19** ranged between –5.41 and –5.59 in these cells with the exception of HOP-92 cells, which had a TGI value of –5.85. The TGI value for compound **16** was –5.90 when tested on A549/ATCC cells. Compound **18** had a TGI value of –5.73 when tested on NCI-H460 cells. Compounds **18**, **19** and **16** had the greatest LC_{50} values; these values were –5.34 (NCI-H460 cells), –5.16 – –5.29 (EKVX, HOP-62, HOP-92, NCI-H23, NCI-H322M and NCI-H460 cells) and –5.13 – –5.20 (HOP-62, NCI-H-460 and NCI-H522 cells).

Anti-colon cancer activity

Compounds **16–19** and **23** had very potent anticancer activity (Tab. 3). Compound **18** had the best GI_{50} value (–6.08) when administered to HCT-116 cells; GI_{50} values ranged between –5.53 and –5.92 when the compound was tested on the remaining six rest cell lines. Compounds **19** and **16** had GI_{50} value ranges of –5.78 – –5.93 and –5.72 – –5.89, respectively, when tested on a panel of colon cancer cell lines. Compound **23** had a GI_{50} value of –5.45 when tested on COLO205 cells and compound **17** had a GI_{50} value of –5.12 when HCT-116 cells were tested. Compounds **19** and **16** had TGI values in the range of –5.29 – –5.64 and –5.39 – –5.56, respectively, when the entire cell line panel was tested. Compound **18** had similar activity (TGI = –5.11 – –5.59) when COLO205, HCC-2998, HCT-116, HT29 and SW620 cells were tested. The LC_{50} values of compounds **16** and **19** were very similar: –5.06 – –5.25 and –5.19 – –5.31 when tested on COLO205, HCC-2998, HCT-116, HCT-15,

Tab. 4. Anti-CNS cancer activity of azaphenothiazines **1**, **16**–**19** and **23**

Compd's number	SF-268			SF-295			SF-539			SNB-19		
	Log_{10} of sample concentration (mol)											
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
1	–4.09	> –4	> –4	–4.28	> –4	> –4	–4.47	> –4	> –4	–4.47	> –4	> –4
16	–5.51	–4.94	–4.42	–5.77	–5.46	–5.15	–5.80	–5.53	–5.25	–5.80	–5.53	–5.25
17	–4.61	> –4	> –4	–5.42	–4.83	–4.07	–4.98	–4.31	> –4	–4.98	–4.31	> –4
18	–5.56	–4.77	–4.26	–6.10	–5.10	–4.34	–	–4.86	–4.41	–	–4.86	–4.41
19	–5.80	–5.52	–5.25	–5.86	–5.55	–5.25	–5.79	–5.53	–5.26	–5.79	–5.53	–5.26
23	–4.63	> –4	> –4	–4.18	> –4	> –4	–4.44	> –4	> –4	–4.44	> –4	> –4

Compd's number	SNB-75			U-251		
	Log_{10} of sample concentration (mol)					
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
1	–4.36	> –4	> –4	–4.36	> –4	> –4
16	–5.79	–5.22	–4.58	–5.70	–5.27	–4.73
17	–5.84	–4.86	–4.25	–5.24	> –4	> –4
18	–6.56	–5.22	–4.42	–5.51	–4.83	–4.40
19	–5.81	–5.48	–	–5.80	–5.51	–5.23
23	–4.57	> –4	> –4	–4.62	> –4	> –4

KM12 and SW620 cells. Compound **18** had LC_{50} values of –5.25 when tested on COLO205 cells and –5.27 when SW620 cells were tested.

Anti-CNS cancer activity

Compounds **16**–**19** exhibited very potent anticancer activity (Tab. 4). Compound **18** had the greatest GI_{50} values when tested on the SNB-75 and SF-295 cell lines (–6.56 and –6.10, respectively). For the SF-268, SNB-19 and U-251 cell lines, the GI_{50} values for compound **18** were significant (–5.24 – –5.56). Compounds **19** and **16** had similar activity on all cells lines (–5.77 – –5.86 and –5.51 – –5.80, respectively). Compound **17** had a GI_{50} value of –5.84 when tested on SNB-75 cells and –5.42 when SF-295 were tested. The TGI values for compound **19** for all cells lines had a range of –5.48 – –5.55. Compound **16** had TGI values of –5.53 and –5.46 when the SF-539 and SF-295 cell lines were tested, respectively. For the same cell lines, compound **18** had TGI values of –5.22 and –5.10, respectively. The LC_{50} values for compound **19** ranged between –5.23 and –5.26 when the SF-268, SF-295, SF-539, SNB-19 and U-251 cell lines were tested. Compound **16** had LC_{50} values of –5.25 and –5.15 for the SF-539 and SF-295 cell lines, respectively.

Anti-melanoma activity

Compounds **16**, **18**, **19** and **23** had very potent anticancer activity (Tab. 5). Compound **18** had an extremely high GI_{50} value of –7.06 when tested on SK-MEL-5 cells; this activity corresponds to 40 ng/ml. This is the most potent azaphenothiazine compound tested on all sixty of the cell lines. This compound had a high activity (GI_{50} = –5.63 – –5.82) when tested on the other lines, including M-14, MALME-3M, UACC-257 and UACC-62. Similarly, compound **19** had high GI_{50} values (–5.75 – –5.98) when the melanoma cell lines were tested. Compound **16** exhibited slightly less activity (–5.34 – –5.84) on the same cells. In addition, compound **23** had similar activity when tested on two cell lines, MALME-3M (–5.38) and SK-MEL-5 (–5.44). The best TGI value was from compound **18** (–5.92) when tested on SK-MEL-5 cells. Compounds **19** and **16** had high TGI values when tested on all the melanoma cell lines (–5.45 – –5.66 and –5.27 – –55, respectively). In contrast to compound **18,** which had low LC_{50} values, compounds **19** and **16** had high LC_{50} values (–5.15 – –5.33) when tested on the melanoma cell lines. Similarly, these compounds had a high LC_{50} value for and the other seven cancer cell lines.

Tab. 5. Anti-melanoma activity of azaphenothiazines **1**, **16**–**19** and **23**

Compd's number	LOX IMVI			MALME-3M			M-14			SK-MEL-2		
	Log_{10} of sample concentration (mol)											
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
1	–4.40	> –4	> –4	> –4	> –4	> –4	–4.49	> –4	> –4	–4.45	> –4	> –4
16	–5.80	–5.52	–5.53	–5.73	–5.44	–5.14	–5.76	–5.45	–5.15	–5.79	–5.27	–4.59
17	> –4	> –4	> –4	–4.55	> –4	> –4	> –4	> –4	> –4	–4.85	–4.19	> –4
18	–5.53	–4.78	–4.01	–5.71	–4.75	–4.25	–5.63	–4.89	–4.41	–5.53	–4.71	–4.30
19	–5.81	–5.50	–5.19	–5.88	–5.54	–5.20	–5.77	–5.49	–5.21	–5.89	–5.55	–5.21
23	–4.38	> –4	> –4	–5.38	–4.63	> –4	–4.79	–4.35	> –4	–4.75	–4.36	> –4

Compd's number	SK-MEL-28			SK-MEL-5			UACC-257			UACC-62		
	Log_{10} of sample concentration (mol)											
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
1	> –4	> –4	> –4	–4.44	> –4	> –4	–4.15	> –4	> –4	–4.38	> –4	> –4
16	–5.71	–5.43	–5.15	–5.84	–5.55	–5.25	–5.65	–5.34	–5.03	–5.74	–5.40	–5.06
17	–	> –4	> –4	–	> –4	> –4	–	> –4	> –4	–4.95	–4.15	> –4
18	–5.37	–4.81	–4.40	–7.06	–5.92	–4.46	–5.73	–4.99	–4.42	–5.82	–4.75	–4.38
19	–5.98	–5.66	–5.33	–5.80	–5.52	–5.25	–5.75	–5.45	–5.15	–5.84	–5.56	–5.27
23	–4.68	–4.08	> –4	–5.44	–4.70	–4.01	–4.94	–4.46	> –4	–4.87	–4.54	–4.21

Anti-ovarian cancer activity

Compounds **16**, **18** and **19** had very potent anticancer activity (Tab. 6). The best GI_{50} values were for compound **16** when tested on IGROV1 cells (–6.72) and OVCAR-3 and OVCAR-5 cells (–5.70 – –5.71). Compound **18** had remarkable GI_{50} values for IGROV1 cells (–6.38), OVCAR-4 cells (–6.35) and OVCAR-3 cells (–5.77). Compound **19** had remarkable GI_{50} values when tested on IGROV1 cells (–6.54) and the rest of the ovarian cancer lines (–5.70 – –5.82). The TGI values for compound **19** were –5.82 when tested on IGROV1 cells and ranged between –5.43 and –5.56 for the rest of the ovarian cancer lines. Compound **16** had high TGI values when IGROV1, OVCAR-3 and OVCAR-5 cells were tested (–5.68, –5.36 and –5.14, respectively). Compound **18** had a high TGI value of –5.26 when OVCAR-4 cells were tested. The best LC_{50} values were for compound **19** when tested on SK-OV-3, OVCAR-3 and OVCAR-5 cells (–5.24 – –5.25) and for compound **16** when tested on IGROV1, OVCAR-3 and OVCAR cells (–5.02 – –5.24).

Anti-renal cancer activity

Compounds **16**–**19** had very potent anticancer activity (Tab. 7). Compound **17** had the best GI_{50} value (–6.32) when tested on the 736-O cell line. High GI_{50} values (–5.71 – –5.90) were determined for compound **19** when tested on all the cell lines. In addition, compound **16** had a high GI_{50} values when tested on 736-O cells (–5.92) and on the other cell lines (–5.42 – –5.77). Compound **18** had high GI_{50} values when tested on ACHN, RXF393, SN12C and UO-31 cells (–5.19 – –5.36). The best GI_{50} values were for compound **19**; the GI_{50} values for this compound ranged between –5.42 and –5.60 for all the cell lines. Compound **16** had GI_{50} values between –5.00 and –5.27 when tested on CAKI-1, A498, TK-10, UO-31 and 736-O cells. Compound **19** had the best LC_{50} values; these ranged between –5.21 and –5.30 for the seven

Tab. 6. Anti-ovarian cancer activity of azaphenothiazines **1**, **16**–**19** and **23**

Compd's number	IGROV 1			OVCAR-3			OVCAR-4			OVCAR-5		
	Log_{10} of sample concentration (mol)											
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
1	–	–	–	–4.08	> –4	> –4	> –4	> –4	> –4	> –4	> –4	> –4
16	–6.72	–5.68	–5.18	–5.70	–5.36	–5.02	–5.44	–4.80	–4.32	–5.44	–4.80	–4.32
17	–5.17	> –4	> –4	> –4	> –4	> –4	–	> –4	> –4	–	> –4	> –4
18	–6.38	–4.51	> –4	–5.77	–4.92	–4.44	–6.35	–5.26	–4.45	–6.35	–5.26	–4.45
19	–6.54	–5.82	–	–5.82	–5.53	–5.26	–5.70	–5.56	–	–5.70	–5.56	–
23	–4.64	> –4	> –4	–4.67	> –4	> –4	–4.61	> –4	> –4	–4.61	> –4	> –4

Compd's number	OVCAR-8			SK-OV-3		
	Log_{10} of sample concentration (mol)					
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
1	–4.45	> –4	> –4		> –4	> –4
16	–5.39	–4.65	> –4	–5.38	–4.76	–4.37
17	–5.22	> –4	> –4	–5.02	–4.32	> –4
18	–5.47	–4.66	–4.07	–5.30	–4.71	–4.33
19	–5.77	–5.43	–	–5.80	–5.52	–5.25
23	–4.60	> –4	> –4	> –4	> –4	> –4

Tab. 7. Anti-renal cancer activity of azaphenothiazines 1, 16-19 and 23

Compd's number	736-0			A498			ACHN			CAKI-1		
	Log_{10} of sample concentration (mol)											
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
1	–4.63	–4.11	> –4	–4.71	–4.26	> –4	–4.57	> –4	> –4	> –4	> –4	> –4
16	–5.92	–5.27	–4.68	–5.45	–5.02	–4.49	–5.53	–4.99	–4.46	–5.60	–5.00	–4.33
17	–6.32	–4.68	> –4	–4.74	–4.14	> –4	–5.01	> –4	> –4	–4.28	> –4	> –4
18	–5.09	–4.65	–4.27	–5.09	–4.66	–4.30	–5.32	–4.68	–4.28	–4.97	–4.51	–4.06
19	–5.90	–5.60	–5.30	–5.80	–5.53	–5.25	–5.81	–5.53	–5.25	–5.84	–5.52	–5.21
23	–4.37	> –4	> –4	–4.47	> –4	> –4	> –4	> –4	> –4	–4.19	> –4	> –4

Compd's number	RXF 393			SN12C			TK-10			UO-31		
	Log_{10} of sample concentration (mol)											
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
1	–4.81	–4.37	> –4	–4.24	> –4	> –4	–4.37	> –4	> –4		> –4	> –4
16	–5.42	–4.74	> –4	–5.53	–4.85	–4.35	–5.45	–5.13	–4.46	–5.77	–5.14	–4.45
17	–	–	–	> –4	> –4	> –4	–4.57	> –4	> –4	> –4	> –4	> –4
18	–5.19	–4.55	–4.03	–5.36	–4.46	–4.18	–5.06	–4.64	–4.27	–5.29	–4.57	–4.02
19	–5.71	–5.42	–	–5.89	–5.59	–5.29	–5.78	–5.51	–5.24	–5.90	–5.57	–5.24
23	–4.86	–4.26	> –4	–4.67	> –4	> –4	–4.44	> –4	> –4	> –4	> –4	> –4

cell lines: 736-O, A498, ACHN, CAKI-1, SN12C, TK-10 and UO-3.

Anti-prostate cancer activity

Compounds **16**, **18** and **19** had very potent anticancer activity (Tab. 8). The best GI_{50} value (–6.14) was for compound **18** when tested on PC-3 cells. Compounds **16** and **19** had similar activity (GI_{50} = –5.51 – –5.87) when tested on two prostate cancer lines. Compound **19** exhibited significant TGI values ranging between –5.52 and –5.56 for the same cell lines. The TGI values for compounds **16** and **18** were –5.56 and –5.18 for PC-3 cells, respectively. The same significant LC_{50} value (–5.26) was determined for compound **19** for the two cell lines and for compound **16** when tested on PC-3 cells.

Anti-breast cancer activity

Compounds **16**, **18**, **19** and **23** had very potent anticancer activity (Tab. 9). The best GI_{50} value (–6.21) was for compound **18** when tested on MDA-MB-468 cells; GI_{50} values for MCF-7 and MDA-MB-435 were also high (–5.93 and –5.78, respectively). Compound **19** also had a high GI_{50} value (–6.07) for MCF-7 cells and for the other cell lines (–5.73 – –5.86). Compound **16** had a GI_{50} value of –5.98 when tested on MCF-7 cells and –5.65 – –5.75 for the other four lines. Compound **23** exhibited a GI_{50} value of –5.06 when tested on MCF-7 cells. The TGI values of compound **19** were –5.27 – –5.66 when tested on all the breast cancer lines tested. This compound had values of –5.60 when tested on MCF-7 cells and –5.20 – –5.35 for the four other lines. Compound **18** exhibited TGI values of –5.59 and –5.57 when tested on MDA-MB-468 and MCF-7 cells, respectively. The best LC_{50} value (–5.97) was for compound **16** when tested on MDA-MB-435 cells. Compounds **16**, **18** and **19** had similar activity against MCF-7 (TGI = –5.22, –5.25 and –5.30, respectively). Compound **19** also exhibited high TGI values ranging between –5.21 and –5.27 for the other four lines.

Six compounds of various structure (**1**, **16**–**19** and **23**) exhibited antiproliferative action that was dependent on the cancer type. Compound **1** was active against the non-small cell lung cancer cell lines (HOP-62 and HOP-92) but was completely inactive at the concentration of 10^{-4} M against a CNS cancer cell line (SF-268), the melanoma cell lines (MALME-3M, SK-MEL-28), the ovarian cancer cell lines (OVCAR-4 and two other lines), a renal cancer cell line (CAKI-1) and a breast cancer cell line (MDA-MB-231/ATCC). Compound **17** had high activity when tested on a colon cancer cell line (HCT-116), the CNS cancer cell lines (SNB-75, SF-295 and U-251), the ovarian cancer cell lines (IGROV1, OVCAR-8, SK-OV-3), a renal cancer cell line (736-O) and the breast cancer cell lines (HS 578T, T-47D, NCI/ADR-ES, MDA-MB-231/ATCC) but was completely inactive when tested on the leukemia cell lines, five non-small cell lung cancer cell lines, five colon cancer cell lines, five melanoma cell lines, three ovarian cancer cell lines, two renal cancer cell lines two prostate cancer cell lines and two breast cancer cell lines. Compound **23** was activate when tested on a colon cancer cell line (COLO205), the melanoma cell lines (MALME-3M, SK-MEL-5) and a breast cancer cell line (MCF-7). However, compound **23** was completely inactive on some colon (1), ovarian (2), renal (2) and breast (1) cancer cell lines. Compounds **16** and **19** have a similar structure (possessing the diethylaminoethyl and dimethylaminopropyl substituents at the thiazine nitrogen atom) and had nearly similar activity on each of the cell lines tested ($logGI_{50} < –5$). The most active was compound **18** when tested on the melanoma cell line SK-MEL-5 ($logGI_{50} < –7$). This compound had weak activity on two non-small cell lung cancer cell lines, one renal cancer cell line and one breast cancer cell line ($logGI_{50} > –5$).

Motohashi and co-workers determined that new phenothiazine derivatives had anticancer activity; these derivatives were termed "half-mustard type" phenothiazines (chloroethylureidoalkyl phenothiazi-

Tab. 8. Anti-prostate cancer activity of azaphenothiazines **1**, **16**–**19** and 23

Compd's number	PC-3			DU-145		
	Log_{10} of sample concentration (mol)					
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
1	–4.38	> –4	> –4	–4.35	> –4	> –4
16	–5.87	–5.56	–5.26	–5.51	–5.11	–4.57
17	–	> –4	> –4	> –4	> –4	> –4
18	–6.14	–5.18	–4.25	–5.60	–4.81	–4.40
19	–5.87	–5.56	–5.26	–5.78	–5.52	–5.26
23	–4.56	> –4	> –4	–4.27	> –4	> –4

Tab. 9. Anti-breast cancer activity of azaphenothiazines **1**, **16**–**19** and **23**

Compd's number	MCF-7			NCI/ADR-RES			MDA-MB-231/ATCC			HS 578T		
	Log_{10} of sample concentration (mol)											
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
1	–4.48	> –4	> –4	–4.43	> –4	> –4	–4.01	> –4	> –4	–4.53	> –4	> –4
16	–5.98	–5.60	–5.22	–5.42	–4.78	–4.29	–5.75	–5.31	–4.71	–5.70	–5.20	–4.57
17	> –4	> –4	> –4	–5.20	> –4	> –4	–5.31	> –4	> –4	–5.78	–5.10	> –4
18	–5.93	–5.57	–5.21	–5.41	–4.71	–4.29	–5.36	–4.66	–4.20	–5.42	–4.60	> –4
19	–6.07	–5.66	–5.30	–5.73	–5.27	–4.65	–5.85	–5.56	–5.27	–5.86	–5.54	–5.21
23	–5.06	> –4	> –4	> –4	> –4	> –4	–4.50	> –4	> –4	–4.74	–4.07	> –4

Compd's number	MDA-MB-435			BT-549			T-47D			MDA-MB-468		
	Log_{10} of sample concentration (mol)											
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
1	–4.10	> –4	> –4	–4.23	> –4	> –4	–4.46	> –4	> –4	–	–	–
16	–5.71	–5.35	–5.97	–5.38	–4.72	–4.21	–5.65	–4.65	–4.17	–	–	–
17	> –4	> –4	> –4	–4.80	> –4	> –4	–5.58	> –4	> –4	–4.52	> –4	> –4
18	–5.78	–4.93	> –4	–4.99	–4.62	–4.26	–5.49	–4.66	–4.08	–6.21	–5.59	–
19	–5.82	–5.53	–5.24	–5.85	–5.55	–5.25	–5.85	–5.49	–	–5.78	–5.35	> –4
23	–4.68	–4.18	> –4	–4.72	> –4	> –4	–4.82	–4.19	> –4	–	–	–

nes). Benzophenothiazines and acylaminoalkyl phenothiazines effectively inhibited Con-A-induced T cell proliferation, slightly but selectively inhibited antibody-dependent cellular cytotoxicity (ADCC) and enhanced NK cell activity in non-transformed cells [16].

The effects of the phenothiazine derivatives on cancer cell growth and differentiation vary depending on the stage of cell growth and differentiation. Only a few benzophenothiazines induced nucleosome-sized DNA fragmentation; other benzophenothiazines such as classical neuroleptic phenothiazines, and "half-mustard type" phenothiazines did not. The latter type of compounds may act on cancer cells *via* alkylurea-induced alkylation of proteins or by intercalation into specific DNA [16, 18, 19, 24]. Because diquinothiazines (**10**-**23**) have a similar structure to benzophenothiazines (additional benzene rings), compound **18** is a quinoline analog of the "half-mustard type" phenothiazines. These compounds may elicit cytotoxic activities through the same mechanisms. Further studies are required to test these possibilities.

Compounds **16** and **19** are quinoline analogs of diethazine and promazine. Therefore, their potent anticancer activity is hypothesized to be related to the azaphenothiazine ring system (i. e., diquinothiazine system). Compound **16** is much more active than compound **6**, which contains the same aminoalkyl group. We observed that diquinothiazines are much more lipophilic than dipyridothiazines and typical phenothiazines [9, 10, 20]. Higher lipophilicity makes these compounds more permeable to cell membranes [18].

Since potent anticancer compounds such as **1**, **16**–**19** and **23** may be highly toxic for normal cells, the effects of these compounds on the viability of mouse splenocytes were determined in an overnight culture. The compounds were administered at a range of concentrations (10^{-6}–10^{-4} mol/l). In addition, two reference compounds were tested; chlorpromazine is a typical phenothiazine drug and cyclosporine A is an antiproliferative agent. At the concentration range tested, compound **19** and, unexpectedly, chlorpromaz-

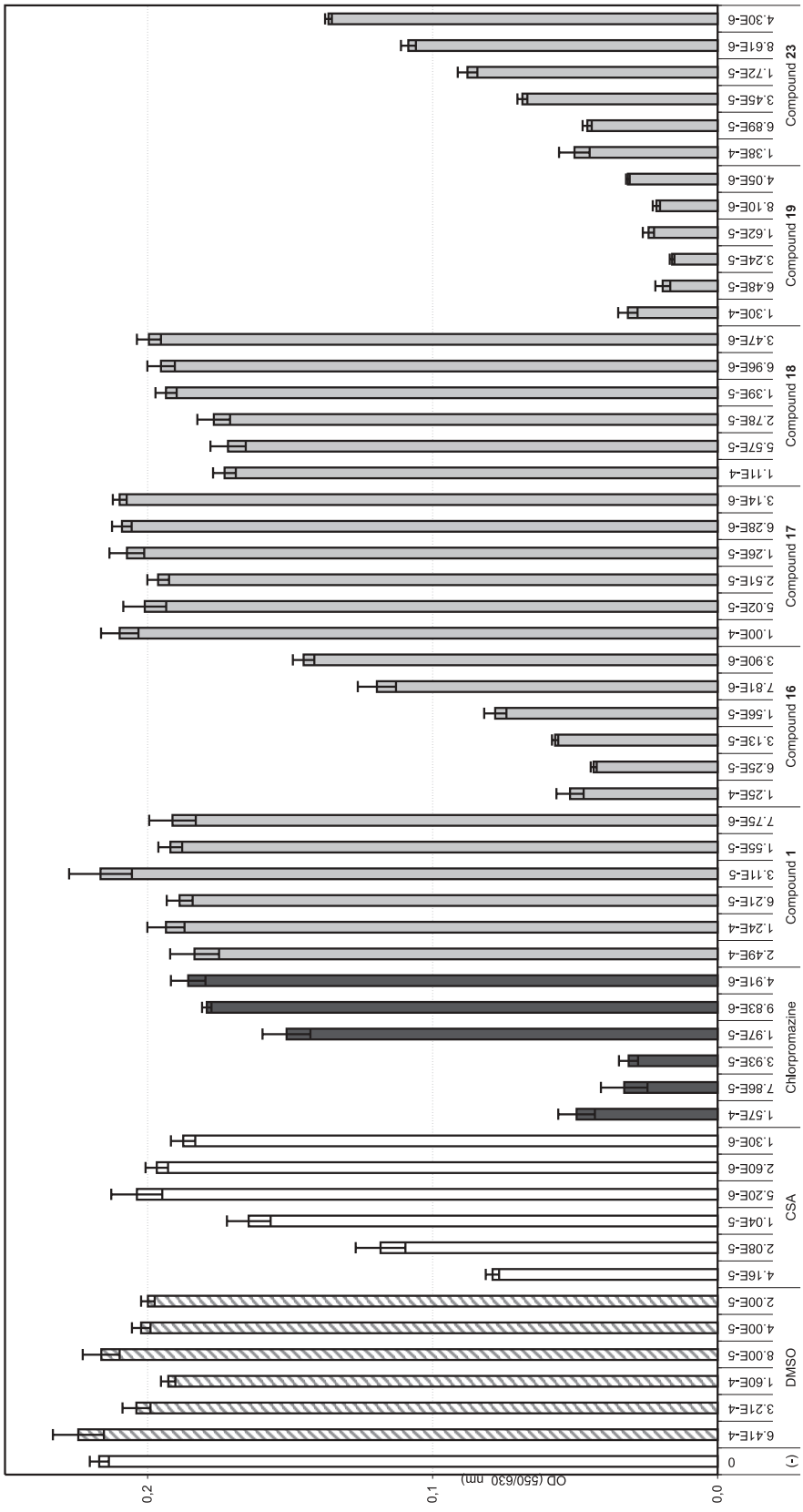


Fig. 2. The effects of azaphenothiazines **1**, **16**–**19**, **23**, cyclosporine A and chlorpromazine on the viability of mouse splenocytes (concentration in mol/l, for example 6.41E–4 = 6.41×10^{-4} mol/l)

antiproliferative agent. At the concentration range tested, compound **19** and, unexpectedly, chlorpromazine were highly toxic, whereas compounds **16** and **23** and cyclosporine A were less toxic (Fig. 2). In addition, compounds **1**, **17** and **18** were minimally toxic. It is worth noting that the most active compound, compound **18,** is slightly less active than compounds **16** and **17**; these compounds had potent anticancer activity at the lower concentrations of 10^{-7} M/l and 10^{-8} M/l, respectively (compound **18** against SK-MEL-5 line, $GI_{50} = 8.7 \times 10^{-8}$ M/l).

Conclusions

All sixty cancer lines were very sensitive to at least one or more azaphenothiazines. The most active compounds of the twenty three azaphenothiazines tested were 10*H*-dipyridothiazine **1**, 6-diethylaminoethyldiquinothiazine **16**, 6-*p*-toluenosulfonylaminoethyldiquinothiazine **17**, 6-chloroethylureidoethyldiquinothiazine **18**, 6-dimethylaminopropyldiquinothiazine **19** and 5,6-ethylenediquinothiazinium chloride **23**.

These compounds exhibited very potent anticancer activity, with the lowest GI_{50} value equaling –7.06 (compound **18** tested on the melanoma cell line SK-MMEL-5), the lowest TGI value equaling –5.92 (compound **18** tested on SK-MMEL-5 cells) and the lowest LC_{50} value equaling –5.97 (compound **16** tested on the breast cancer cell line MDA-MB-435). In addition to these compounds, compounds **1**, **17**, **19** and **23** had slightly lower anticancer activity when tested on some of the other cancer lines.

The present study suggests that the type of azaphenothiazine moiety is very important for anticancer activity. Diquinothiazines **10**–**23** were much more active against cancer cell lines than dipyridothiazines **1**–**9**. The nature of the substituent at the thiazine nitrogen atom also plays an important role in the anticancer activity of diquinothiazines **10**–**23.** However, in the case of dipyridothiazines **1**–**9**, the unsubstituted compound **1** unexpectedly was the most active. In general, diquinothiazines that contain aminoalkyl substituents (and their derivatives) were more active than those with the alkyl, aryl, or heteroaryl substituents. The most potent anticancer compound was the "half-mustard type" derivative **18**. The ethylene group in our aminoalkylazaphenothiazines is as good a linker as the propylene and butylene groups in the aminoalkylazaphenothiazines [18, 27]. Compounds **1**, **17** and **18** were practically non-toxic. This report is the first that demonstrates the very potent anticancer activity of azaphenothiazines. In addition, the anticancer action of some compounds appears to be selective, as evidenced by the lack of toxicity against normal mouse lymphocytes. These characteristics are highly desirable and support their potential therapeutic application.

Acknowledgment:
The authors wish to thank the Developmental Therapeutics Program (National Cancer Institute, Bethesda, MD, USA) for the anticancer screening of azaphenothiazines **1**–**23** and Professor Noburu Motohashi for his inspiring papers.

References:

1. Amaral L, Kristiansen JE: Phenothiazines: potential management of Creutzfeldt-Jacob disease and its variants. Int J Antimicrob Agents, 2001, 18, 411–417.
2. Amaral L, Martins M, Viveiros M: Enhanced killing of intracellular multidrug-resistant *Mycobacterium tuberculosis* by compounds that affect the activity of efflux pumps. J Antimicrob Chemother, 2007, 59, 1237–1246.
3. Barbieri F, Alama A, Tasso B, Boido V, Bruzdo C, Sparatore F: Quinolizidinyl derivatives of iminobenzyl and phenothiazine as multidrug resistance modulators in ovarian cancer cells. Invest New Drug, 2003, 21, 413–420.
4. Gupta RR, Kumar M: Synthesis, properties and reactions of phenothiazines. In*:* Phenothiazines and 1,4-Benzothiazines – Chemical and Biological Aspects. Ed. Gupt, RR, Elsevier, Amsterdam, 1988, 1–161.
5. Hansen MB, Nielsen SE and Berg K: Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. J Immunol Methods, 1989, 119, 203–210.
6. Jeleń M, Pluta K: Synthesis of 6-aminoalkyldiquinithiazines and their acyl and sulfonyl derivatives. Heterocycles, 2008, 75, 859–870.
7. Mayur YC, Jagadeesh S, Thimmaiah KN: Targeting calmodulin in reversing multi drug resistance in cancer cells. Mini-Rev Med Chem, 2006, 6, 1383–1389.
8. Molnár A, Amaral L, Molnár J: Antiplasmid effect of promethazine in mixed bacterial cultures. Int J Antimicrob Agents, 2003, 22, 217–222.
9. Morak B, Nowak M, Pluta K: Determination of the lipophilicity parameters R_{M0} and LogP of new azaphenothiazines by reversed-phase thin-layer chromatography. J Liq Chromatogr Rel Technol, 2007, 30, 1845–1854.
10. Morak B, Pluta K: RP TLC Determination of the lipophilicity of new 10-substituted 2,7-diazaphenothiazines. J Liq Chromatogr Rel Technol*,* 2008, 31, 611–618.
11. Morak-Młodawska B, Pluta K: Synthesis of novel dipyrido-1,4-thiazines. Heterocycles, 2007, 71, 1347–1361.

12. Mosnaim AD, Ranade VV, Wolf ME, Puente J, Valenzuela MA: Phenothiazine molecule provides the basic chemical structure for various classes of pharmacotherapeutic agents. Am J Ther, 2006, 13, 261–273.
13. Motohashi N: Antitumor activity of phenothiazines (phenothiazine Oncology). In: Phenothiazines and 1,4-Benzothiazines – Chemical and Biological Aspects. Ed. Gupta, RR, Elsevier, Amsterdam, 1988, 705–770.
14. Motohashi N, Kawase M, Saito M, Kurihara T, Hever A, Ocsocvszki I, Tanaka M, Molnár J: Synthesis and antitumor activity of 1-(2-chloroethyl)-3-(2-substituted-10*H*-phenothiazin-10-yl)alkyl-1-ureas as potent anticancer agents. Anticancer Res, 1996, 16, 2525–2532.
15. Motohashi N, Kawase M, Saito M, Kurihara T, Satoh K, Nakashima H, Premanathan M et al.: Synthesis and biological activity of N-acylphenothiazines. Int J Antimicrob Agents, 2000, 14, 203–207.
16. Motohashi N, Kawase M, Saito S, Sakagami H: Antitumor potential and possible targets of phenothiazine-related compounds. Curr Drug Targets, 2000, 237–245.
17. Motohashi N, Kawase M, Satoh K, Sakagami H: Cytotoxic potential of phenothiazines. Curr Drug Targets, 2006, 7, 1055–1066.
18. Motohashi N, Kurihara T, Sakagami H, Szabo D, Csuri K, Molnár J: Chemical structure and tumor type specificity of "half-mustard type" phenothiazines. Anticancer Res, 1999, 19, 1859–1864.
19. Nagy S, Argyelan G, Molnár J, Kawase M, Motohashi N: Antitumor activity of phenothiazine-related compounds. Anticancer Res, 1996, 16, 1915–1918.
20. Nowak M, Pluta K: Study of the lipophilicity of novel diquinothiazines J Planar Chromatogr, 2006, 19, 157–160.
21. Nowak M, Pluta K, Suwińska K, Straver L: Synthesis of new pentacyclic diquinothiazines. J Heterocycl Chem, 2007, 44, 543–550.
22. Okumura H, Nakazawa J, Tsuganezawa K, Usui T, Osada H, Matsumoto T, Tanaka A, Jokohama S: Phenothiazine and carbazole-related compounds inhibit mitotic kinesin Eg5 and trigger apoptosis in transformed culture cells. Toxicol Lett, 2006, 166, 44–52.
23. PASS (Prediction of Activity Spectra for Substance) http://ibmc.msk.ru/PASS.
24. Sakagami H, Takahashi H, Yoshida H, Yamamura M, Fukuchi K, Gomi K, Motohashi N, Takeda M: Induction of DNA fragmentation in human Myelogenous Leukaemic cell lines by phenothiazine-related compounds. Anticancer Res, 1995, 15, 2533–2540.
25. Shoemaker R, H: The NCI human tumor cell line anticancer drug screen. Nature Rev, 2006, 6, 813–823.
26. Viveiros M, Martins M, Couto I, Kristiansen JE, Molnár J, Amaral L: The *in vitro* activity of phenothiazines against *Mycobacterium avium*: potential of thioridazine for therapy of the co-infected AIDS patients. In Vivo, 2005, 19, 733–736.
27. Wuonola MA, Palfreyman MG, Motohashi N, Kawase M, Gabay S, Gupta RR, Molnár J: The primary *in vitro* anticancer activity of "half-mustard type" phenothiazines in NCI's revised anticancer screening paradigm. Anticancer Res, 1998, 18, 337–348.
28. Zhelev Z, Ohba H, Bakalova R, Hadjimitova V, Ishikawa M, Shinohara Y, Baba Y: Phenothiazines suppress proliferation and induce apoptosis in cultured leukemic cells without any influence on the viability of normal lymphocytes. Cancer Chemother Pharmacol, 2004, 53, 267–275.

Received:
April 21, 2009; in revised form: October 13, 2009.