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Original article

Synthesis and selected immunological properties of substituted quino[3,2-b]benzo[1,4]thiazines☆

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ABSTRACT

A new type of azaphenothiazines – tetracyclic quino[3,2-b]benzo[1,4]thiazines, possessing common substituents (H, CH_3, Cl, Br, F, CF_3, SCH_3) in positions 8–10 and pharmacophoric aminoalkyl substituents in position 6, were obtained from diquinodithiin and 2,2′-dichloro-3,3′-diquinolinyl disulfide in several-step syntheses. Sixty one compounds, grouped as the 6*H*, 6-dialkylaminoalkyl, 6-acylaminoalkyl and sulfonylaminoalkyl derivatives, were tested for cytotoxicity, their effects on phytohemagglutin A (PHA)-induced proliferative response of human peripheral blood mononuclear cells (PBMC) and lipopolysaccharide (LPS)-induced tumor necrosis factor alpha (TNF-α) production by these cells. The compounds exhibited differential inhibitory activities in these tests and significantly varied in terms of cytotoxicity. The most promising compounds were tested for growth inhibition of leukemia L-1210 cells, colon cancer SV-948 cells and epidermal carcinoma A-341 cells. The most active compounds exhibited anticancer activity against these cell lines comparable to that of cisplatin. The structure–activity relationship of the compounds were discussed.

1. Introduction

Tricyclic phenothiazines with aminoalkyl substituents at the nitrogen atom are an important class of drugs exhibiting neuroleptic, antihistaminic, antitussive and antiemetic activities [1]. Recent reports describe very promising anticancer and antibacterial activities, reversal of multidrug resistance and possibility of treatment of Alzheimer's, Creutzfeldt–Jakob's and AIDS diseases, not only for classical but also for newly synthesized phenothiazines [2–6]. The modification strategies of the parent phenothiazine structures include two approaches: a) introduction of a new substituent, mainly at the thiazine nitrogen atom at position 10, and rarely at the carbon atom in the benzene ring, and b) substitution of one or two benzene rings with homoaromatic and heteroaromatic rings. The second way enables formation of new heterocyclic ring systems: tri-, tetra- and pentacyclic azaphenothiazines containing one, two, three and even four nitrogen atoms in the aromatic rings [2,3,7–10]. Such phenothiazine structure modifications can change potency and directions of activities of the basic structures.

In continuation of our search for pharmaceutically active azaphenothiazines, we modified the phenothiazine structure with the pyridine and quinoline rings to form dipyridothiazines, quinobenzothiazines and diquinothiazines [11–15]. Some diquinothiazines exhibited promising anticancer activities against human tumor cell lines deriving from colon, breast, kidney, lung, ovary, prostate, central nervous system, melanoma and leukemia [16]. In addition, 10*H*-dipyridothiazine (10*H*-2,7-diazaphenothiazine) was found to be a universal, low-toxic immunosuppressant, inhibiting both humoral and cellular immune responses [17].

Angularly fused tetracyclic azaphenothiazines with the quinoline moiety, such as substituted quino[3,4-b]benzothiazines and 5-alkylquino[3,4-b]benzothiazinium salts, were obtained in an original way from pentacyclic diquinodithiin (thioquinanthrene) and its ammonium salt *via* the dithiin ring opening – the thiazine ring closure reactions or *via* intramolecular cyclization of 1-methyl-4-(arylamino)quinolinium-3-thiolates and (o-phenylthio)enaminoquinolones. Some of these compounds exhibited anticancer and antioxidant activities [11,18–20]. Recently, we elaborated a convenient synthesis of a new type of azaphenothiazines with the quinoline ring, linearly fused tetracyclic 8–10-substituted 6*H*-quino[3,2-b]benzo[1,4]thiazines, in the reactions of isomeric pentacyclic diquinodithiin **1** or 2,2′-dichloro-3,3′-diquinolinyl disulfide

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(**2**) with substituted anilines *via* the dithiin ring opening in compound **1** and the disulfide cleavage in compound **2** followed by the thiazine ring closure processes [21].

In this study, we describe and discuss the synthesis and the structure of new 6-substituted 9-X-quino[3,2-b]benzo[1,4]thiazines (X = H, Cl and SCH_3) with three main types of pharmacophoric groups: dialkiloaminoalkyl, acylaminoalkyl and sulfonylaminoalkyl. First we evaluated cytotoxicity of these compounds against human peripheral blood mononuclear cells (PBMC). Next we investigated inhibition of phytohemagglutinin A (PHA)-induced proliferation of PBMC and regulation of lipopolysaccharide (LPS)-induced tumor necrosis factor alpha (TNF-α) production in human whole blood cell cultures. Selected compounds were also used for evaluation of anticancer activities against several tumor cell lines *in vitro*. The same biological activities were also studied for 6-unsubstituted 6*H*-quinobenzothiazines, possessing additional substituents in the benzene ring in positions 8–10. Altogether sixty one variously substituted quinobenzothiazines were tested.

2. Results and discussion

2.1. Chemistry

New azaphenothiazines, 6*H*-quino[3,2-b]benzo[1,4]thiazines, were obtained in a few-step syntheses. The substrates for these syntheses, pentacyclic diquinodithiin **1** and 2,2′-dichloro-3,3′-diquinolinyl disulfide (**2**), were obtained from 3,4-dihydro-2(1*H*)-quinolone with thionyl chloride in DMF [22,23]. The reactions of compounds **1** and **2** with substituted anilines and their hydrochlorides without a solvent or in monomethyl ether of diethylene glycol led to thirteen 8-, 9- and 10-substituted 6*H*-quinobenzothiazines (**3**–**5**) with the hydrogen, chlorine, bromine and fluorine atoms and the methyl, methylthio and trifluoromethyl groups (Scheme 1) [21].

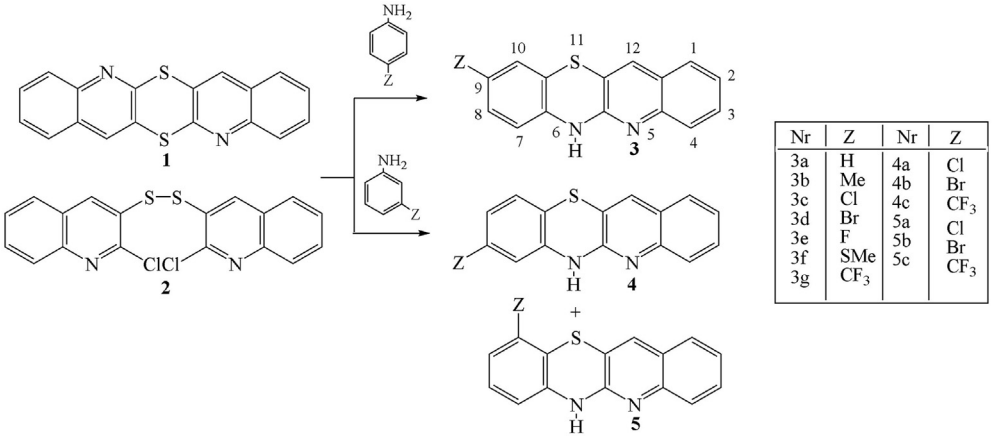
Next we introduced the pharmacophoric substituents in the quinobenzothiazine system at the thiazine nitrogen atom in position 6. We selected diverse *N*,*N*-dialkylaminoalkyl, *N*-acylaminoalkyl and *N*-sulfonylaminoalkyl groups, found in pharmacoactive compounds and anticancer phenothiazines [2,3,16]. As the pattern quinobenzothiazine substrates, we chose 6*H*-quinobenzothiazines **3a**, **3c** and **3f** with the hydrogen and chlorine atoms, and the methylthio group in position 9. The *N*,*N*-dialkylaminoalkyl substituents were introduced in the *N*-alkylation reactions with hydrochlorides of acyclic and cyclic dialkylaminoalkyl chlorides in boiling dioxane in the presence of sodium hydroxide. In this way, eighteen different 6-dialkylaminoalkyl derivatives **6**–**11** were obtained in 73–83% yield (Scheme 2).

Whereas the *N*,*N*-dialkylaminoalkyl substituents were introduced at the nitrogen atom in one step *N*-alkylation, the introduction of two other pharmacophoric substituents required three-step synthesis. 6*H*-quinobenzothiazines **3a**, **3c** and **3f** were alkylated with phthalimidopropyl and phthalimidobutyl bromides in dry toluene in the presence of sodium hydride into the phthalimidoalkyl derivatives **12a**–**f**. Next, these compounds underwent reactions with hydrazine in aqueous ethanol to give six aminoalkyl derivatives **13a**–**f** (alkyl = propyl and butyl) in 70–85% yield (Scheme 3).

Aminoalkylquinobenzothiazines **13a**–**f** were transformed into the *N*-acyl derivatives. The reactions with acetic anhydride, ethyl chloroformate and 2-chloroethyl isocyanate gave six 6-acetylaminoalkylquinobenzothiazines **14a**–**f**, six 6-ethoxycarbonylaminoalkylquinobenzothiazines **15a**–**f** and six 6-chloroethylureidoalkylquinobenzothiazines **16a**–**f** (possessing a half-mustard unit) in 63–91% yield. Aminoalkylquinobenzothiazines **13** were also transformed into the *N*-sulfonyl derivatives. The reactions with methanesulfonyl and *p*-toluenesulfonyl chlorides led to the sulfonamide derivatives: six 6-methanesulfonylaminoalkyl- and six 6-*p*-toluenesulfonylaminoalkylquino-benzothiazines **17a**–**f** and **18a**–**f** in 68–81% yield (Scheme 4).

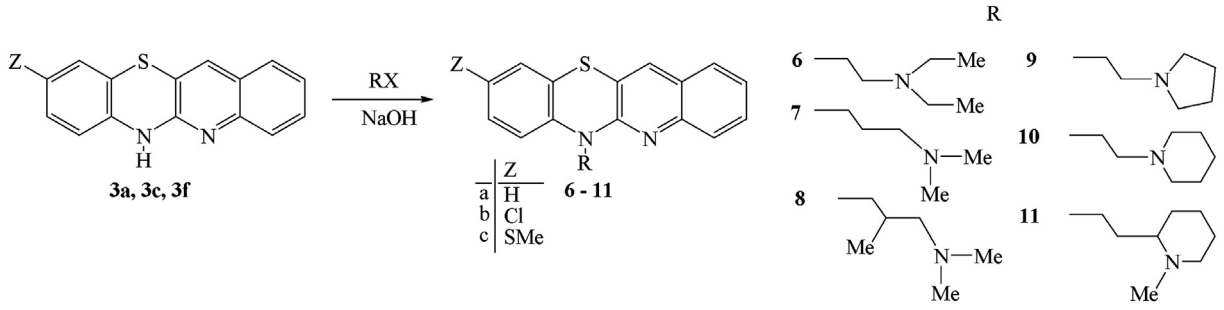
The analysis of the quinobenzothiazine structures was a crucial problem, as the synthesis may run with the Smiles rearrangement or by other unknown processes and may form also 5*H*-tautomers and 5-substituted products. In the reaction with *p*-anilines, other possible isomeric product structures **19**–**23** were depicted in Scheme 5.

The kind of fusion of the quinoline ring with the thiazine ring, [3,2-b] or [2,3-b], was initially solved using the homonuclear NOE experiment for the *N*-methyl derivative. Irradiation of the methyl protons gave an enhancement of the only one proton signal (the H7 proton) what pointed at the [3,2-b] ring fusion. A discrimination of the isomeric products of the reactions with *m*-anilines as 8- and 10-substituted quinobenzothiazines were also initially assigned basing on the coupling constants J_{ortho} and J_{meta} [21]. However, the ^{1}H NMR analysis did not exclude 7- and 9-substituted quinobenzothiazines as the result of the reverse Smiles rearrangement. Our supposition of the 8- and 10-substituted structures was recently confirmed by X-ray analysis of compound **4c** and the benzyl derivative of **5c** [24]. The structural problems of the products of the reactions with *p*-anilines were also solved using X-ray analysis of the product **14e** (the reaction with *p*-chloroaniline), possessing strong biological activities (vide infra). X-ray study confirmed the fused quinoline and thiazine system as [3,2-b], the chlorine atom in position 9 and the acetylaminobutyl substituent



Nr	Z	Nr	Z
3a	H	4a	Cl
3b	Me	4b	Br
3c	Cl	4c	CF_3
3d	Br	5a	Cl
3e	F	5b	Br
3f	SMe	5c	CF_3
3g	CF_3		

Scheme 1. Synthesis if 6*H*-quinobenzothiazines **3**–**5** from diquinodithiin **1** and 2,2′-dichloro-3,3′-diquinolinyl disulfide (**2**).



Scheme 2. Synthesis of 6-dialkylaminoalkylquinobenzothiazines **6**–**11**.

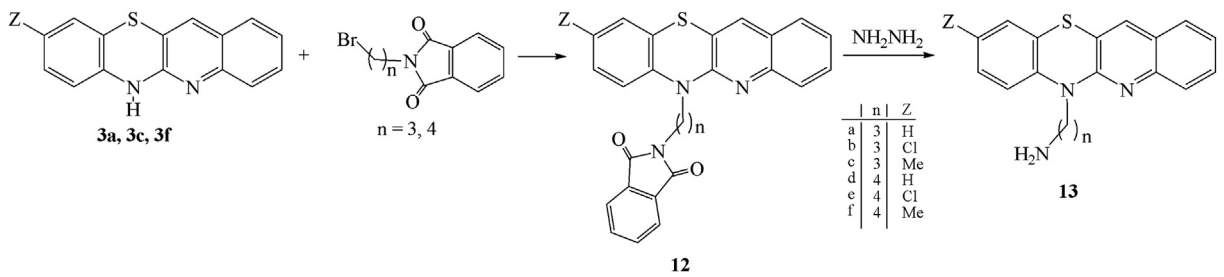
at the thiazine nitrogen atom in position 6 [25]. X-ray analysis excluded definitely structures **19**–**23** and confirmed that the *N*-alkylation reactions of 6*H*-quinobenzothiazines (**3a**, **3c** and **3f**) with dialkylaminoalkyl and phthalimidoalkyl chlorides occurred at the thiazine nitrogen atom.

2.2. Biological activities

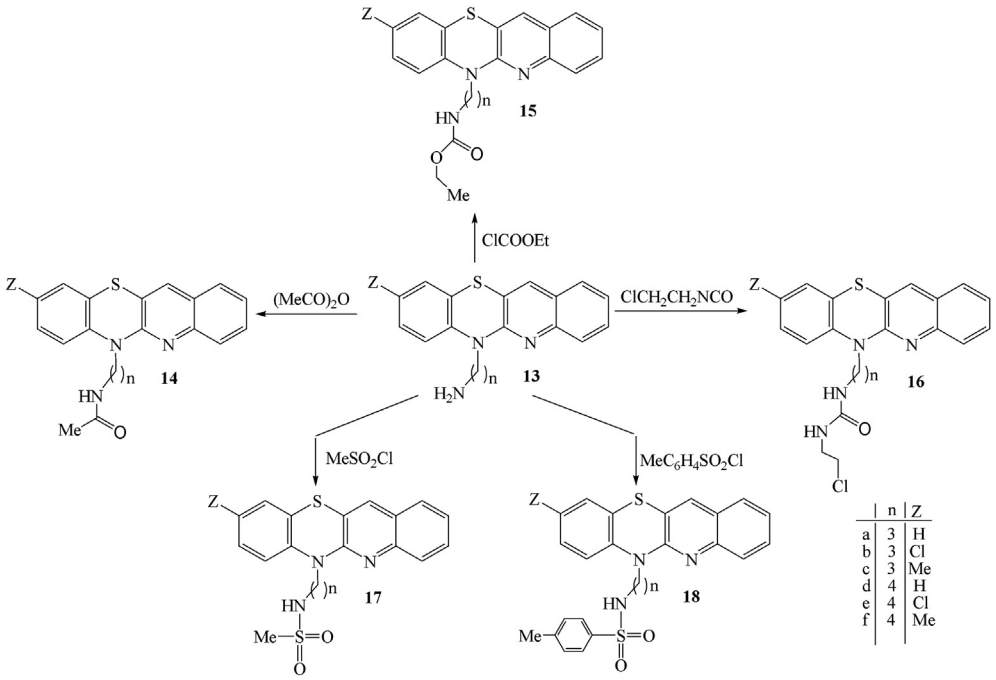
For the biological tests, sixty one quinobenzothiazines were selected being 8–10-substituted 6*H*-quinobenzothiazines **3**–**5** and 9-substituted quinobenzothiazines **6**–**18** with the *N*,*N*-dialkylaminoalkyl, *N*-acetylaminoalkyl, *N*-etoxycarbonylalkyl, chloroethylureidoalkyl, *N*-methanesulfonylaminoalkyl and *N*-*p*-toluenesulfonylaminoalkyl groups in position 6. The tests included the proliferative response of human peripheral blood mononuclear cells induced by phytohemagglutinin A, the cytotoxic effect on human peripheral blood mononuclear cells and lipopolysaccharide-induced production of tumor necrosis factor α. The combined results of the tests are presented in Table 1, which lists only compounds exhibiting low cytotoxicity (less than 20% of toxicity) at 10 μg/mL. Three most promising compounds, selected on the basis of their strong antiproliferative effects, were tested for anticancer activities against leukemia L-1210 cells, epidermal carcinoma A-431 cells and colon carcinoma SV-948 cells (the results shown in Fig. 1).

The proliferation test was performed at the concentrations of 10 and 50 μg/mL for all compounds (initially at 1 μg/mL only for selected compounds). Only few compounds were inactive and about twenty compounds were weakly inhibitory. Thirty four compounds exhibited distinct inhibitory activities up to 50% in comparison with the control test, most of them at the concentration of 10 μg/mL. Out of compounds **3**–**5**, possessing the hydrogen atom at the thiazine nitrogen atom and selected groups in the benzene ring, compound **3e**, with the fluorine atom in position 9, was found extremely active (100% inhibition at 10 μg/mL). However, this compound was not included in Table 1 because of its cytotoxicity (see below). Other compounds with the hydrogen, chlorine, bromine and fluorine atoms, and the methyl, methylthio and trifluoromethyl groups exhibited lower activities with at most 25% inhibition at 10 μg/mL. The nature of the substituents and their location in the benzene ring (8–10) did not show strong direct influence on the activity. Compounds **6**–**11** with the dialkylaminoalkyl groups exhibited extremely strong activities at the concentration of 10 μg/mL, with exception of compound **11a**. There were no differences in the activities between the propylene and butylene linkages and acyclic and cyclic amino groups in compounds **6**–**11**. *N*-Acylaminoalkyl- and *N*-sulfonylaminoalkyl derivatives **14**–**17** exhibited also strong activities but generally lesser than dialkylaminoalkyl derivatives **6**–**11**. The exceptions were chloroethylureidopropyl and chloroethylureidobutyl derivatives **16b**, **16c**, **16e** and **16f** which were as strongly active as dialkyaminoalkyl derivatives **6**–**11**. It is of interest that compounds **16a** and **16d** with the same chloroethylureidoalkyl groups were much less active what is probably attributed to the weak influence of the hydrogen atom in position 9 in comparison with the chlorine atom or the methylthio group. The kind of the alkyl group (propyl or butyl) had no significant influence on the antiproliferative activity. Only compounds **18a**–**f** with the *p*-toluenesulfonylaminoalkyl groups were less active or inactive even at 50 μg/mL.

As the inhibitory effects of the compounds could be caused by their high cytotoxicity, all the compounds were tested for their effects on peripheral blood mononuclear cells. Generally, inactive and less active compounds were non-toxic. The very strongly antiproliferative compound **3e** turned out to be moderately cytotoxic (50% inhibition at 10 μg/mL). The most strongly antiproliferative dialkylaminoalkyl compounds **6**–**11** with over 93% inhibition at 10 μg/mL showed also strong cytotoxicity (over 82% inhibition, respectively). Only two compounds **10b** and **10c** exhibited low cytotoxicity at 10 μg/mL. Similarly, very strongly antiproliferative chloroethylureidoalkyl compounds (**16b**, **16c**, **16e** and **16f**) and significantly antiproliferative *N*-acetylaminoalkyl **14**, *N*-etoxycarbonylaminoalkyl **15** and *N*-methanesulfonylaminoalkyl **17** compounds did not exhibit toxic effects at the concentration of 10 μg/mL. It is worth noting that some of these compounds (**14b**, **14c**, **14e**, **14f**, **15a**, **15d**, **17b**, **17c**, **17e** and **17f**) were non-toxic even at the concentration of 50 μg/mL. Some of the compounds, designated as NT, even enhanced survival of the tested PBMC.



Scheme 3. Synthesis of 6-aminoalkylquinobenzothiazines **13**.

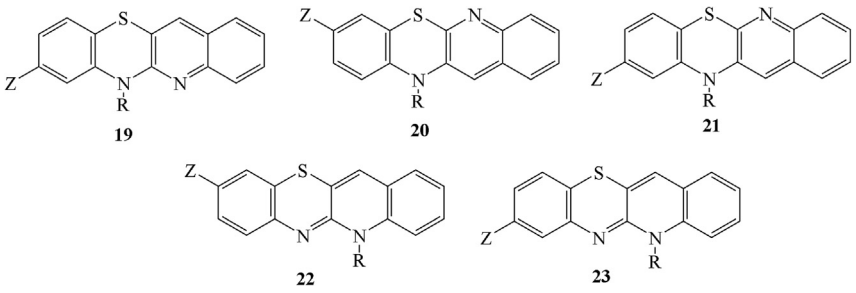


Scheme 4. Transformations of 6-aminoalkylquinobenzothiazines **13** into *N*-acyl **14**–**16** and *N*-sulfonyl **17**–**18** derivatives.

The inhibitory effects of the compounds on LPS-induced TNF-α production were tested at the concentration of 5 and 25 μg/mL. Twenty quinobenzothiazines of all types exhibited significant inhibition of at least 50% at 5 μg/mL. The strong suppressive effect (at least 70% inhibition) was observed for nine compounds.

The most promising compounds **14c**, **16b** and **16f** (being strongly antiproliferative and relatively low cytotoxic) were selected for evaluation of anticancer activities against three cancer cell lines. The anticancer activity was tested at the concentrations of 0.1–50 μg/mL using cisplatin as the reference drug (Fig. 1). All these compounds exhibited the anticancer activities similar to the cisplatin activity at the concentration of 10–50 μg/mL, and in some cases quinobenzothiazines were found even more active (91.8–97.0% inhibition at 10 μg/mL). Compounds **16b** and **16f** were also more active (96% inhibition) against colon carcinoma SV-948 cells at the concentration of 5 μg/mL. When evaluating the anticancer effects of the compounds *in vitro* one has to consider high toxicity [26] and side-effects [27] of cisplatin, the reference drug, used in this study. Cisplatin showed, for example, LD50 (killing of 50% of granulocyte/macrophage progenitor cells) already at 0.9 μg/mL after 1 h of culture and that value was as low as 0.12 μg/mL for a continuous cell culture [26].

In general, several activity patterns among the studied compounds can be described: i/ **14e**, **16b** which are strongly antiproliferative already at low dose but little inhibitory with regard to TNF-α production, ii/ **16f** with exceptional antiproliferative action at low dose and strong TNF-α inhibition and iii/ **3b**, **4c**, **5c** showing no or little inhibition of proliferation associated with strong inhibition of inducible TNF-α production. It seems, therefore, that the mechanisms of action among the studied derivatives are differential. Motohashi and co-workers found that new anticancer phenothiazines with the chloroethylureidoalkyl and acylaminoalkyl substituents and tetracyclic benzophenothiazines effectively inhibited the Con-A-induced T cell proliferation, slightly but selectively inhibited the antibody-dependent cellular cytotoxicity (ADCC) activity and enhanced the NK cell activity in non-transformed cells. The effects of these compounds on cancer cell growth and differentiation varied depending on the cell growth and differentiation stages. Only a few benzophenothiazines induced nucleosome-size DNA fragmentation. Other benzophenothiazines, classical neuroleptic phenothiazines and chloroethylureidoalkylphenothiazines did not act in this way. The last compounds were supposed to act on the cancer cells *via* alkylurea induced alkylation of proteins or on DNA by a particular intercalation [2,28–30]. As our quinobenzothiazines have structures close to the Motohashi's phenothiazines their cytotoxic activities probably are induced by the same mechanisms. The X-ray study of compound **14e** revealed unexpectedly planar tetracyclic ring system [25], what may suggest its mechanism of the antiproliferative effect by DNA intercalation for this type of phenothiazines.



Scheme 5. Other possible structures of quinobenzothiazines.

Table 1
Activities of the compounds in selected immunological assays. The table shows: the degree of cytotoxicity against PBMC of the selected compounds – exhibiting low cytotoxicity (less than 20% of toxicity) at 10 μg/mL or being non toxic (NT), effects on phytohemagglutin A (PHA)-induced proliferative response of human peripheral blood mononuclear cells and lipopolysaccharide-induced TNF-alpha production by these cells. The results are given in percentage inhibition as compared with appropriate DMSO controls. Positive values denote inhibition, negative stimulation.

No.	Cytotoxicity		Antiproliferative activity		TNF-α		No.	Cytotoxicity		Antiproliferative activity		TNF-α	
	10 μg/mL	50 μg/mL	10 μg/mL	50 μg/mL	5 μg/mL	25 μg/mL		10 μg/mL	50 μg/mL	10 μg/mL	50 μg/mL	5 μg/mL	25 μg/mL
3a	NT	NT	7.1	14.4	25.6	16.1	**15c**	NT	NT	45.4	38.8	74.9	70.1
3b	2.4	15.4	−17.9	−6.2	54.1	66.6	**15d**	NT	NT	73.9	83.7	0.2	12.3
3c	NT	NT	−0.3	5.7	2.0	9.6	**15e**	NT	NT	−0.7	−12.0	13.2	8.41
3d	NT	NT	−9.7	19.0	16.2	10.1	**15f**	NT	NT	44.3	27.8	50.9	33.1
3f	NT	NT	0.0	2.9	22.5	19.2	**16a**	8.5	NT	11.2	77.9	14.6	5.0
3g	NT	NT	24.9	61.0	51.1	−5.6	**16b**	15.9	89.5	98.6	100.0	9.2	72.1
4a	NT	NT	16.3	27.1	−1.2	19.2	**16c**	NT	92.7	93.0	93.7	50.7	1.0
4b	NT	NT	4.3	20.6	11.2	−25.0	**16d**	7.9	NT	16.6	88.7	−1.8	4.3
4c	NT	21.5	21.7	0.3	72.3	48.8	**16e**	19.2	86.3	98.4	94.6	54.8	56.0
5a	NT	13.0	10.7	10.8	17.0	4.6	**16f**	4.2	95.1	94.8	92.7	73.3	47.5
5b	NT	NT	0.1	16.4	13.6	8.4	**17a**	NT	NT	36.6	74.6	8.1	60.6
5c	5.0	NT	2.3	13.6	74.9	−2.6	**17b**	NT	NT	73.4	74.4	1.2	30.5
10b	19.2	83.8	96.3	98.2	−70.5	56.7	**17c**	NT	NT	57.3	65.4	73.7	47.5
10c	18	91.7	93.8	92	49.2	34.6	**17d**	NT	NT	42.3	94.3	14.0	42.3
11a	NT	4.5	−23.5	39.8	49.1	62.4	**17e**	NT	NT	59.4	43.9	−7.5	24.5
14a	NT	91.9	68.7	92.4	−0.4	32.2	**17f**	NT	NT	53.3	62.7	72.8	13.2
14b	NT	NT	50.8	58.3	11.4	60.6	**18a**	NT	NT	6.6	−0.8	3.8	15.6
14c	NT	NT	72.5	68.8	71.7	64.5	**18b**	NT	NT	12.9	22.7	5.9	20.9
14d	NT	NT	72.7	94.3	0.0	20.2	**18c**	NT	NT	−5.3	−46.4	72.1	20.8
14e	NT	NT	71.2	92.6	13.2	53.4	**18d**	NT	NT	3.3	2.4	15.8	12.5
14f	NT	7.9	54.7	42.0	46.8	60.4	**18e**	NT	NT	13.2	19.8	20.7	−12.7
15a	NT	NT	69.7	88.4	7.7	14.1	**18f**	NT	NT	−5.0	−34.7	70.4	37.5
15b	NT	NT	45.0	60.4	10.3	31.7							

3. Conclusion

We report here a few step synthesis of sixty novel tetracyclic 9-substituted 6-aminoalkylquinobenzothiazines. The kind of the aminoalkyl substituent at the thiazine nitrogen atom such as dialkylaminoalkyl, acylaminoalkyl, sulfonylaminoalkyl, played a crucial role in the antiproliferative and anticancer activities. The additional substituent in the benzene ring (for example the chlorine and fluorine atoms and the methylthio group) enhanced the activities in comparison with the hydrogen atom. The 9-fluoroquinobenzothiazine ring system was a significant pharmacophoric unit, as compound **3e** without any aminoalkyl groups at the thiazine nitrogen atom, exhibited strong antiproliferative activity. The most active compounds **14e**, **16b** and **16f** possessed the acetylaminobutyl, chlororoethylureidopropyl and chlororoethylureidobutyl groups and exhibited activities comparable to that of cisplatin. A combination of the quinobenzothiazine system and two kinds of substituents (the aminoalkyl at the thiazine nitrogen atom and some simple groups in the benzene ring) is a useful starting point for further study to found more potent anticancer agents and their mechanism of action.

4. Experimental

4.1. General techniques

Melting points were determined in open capillary tubes on a Boetius melting point apparatus and were uncorrected. The NMR spectra were recorded on Bruker DRX and Bruker Fourier 300 spectrometers (^{1}H at 500 MHz, ^{13}C at 125 and 75 MHz) in $CDCl_3$ or DMSO-d_6. Fast Atom Bombardment (FAB MS) mass spectra were run on a Finnigan MAT 95 spectrometer at 70 eV.

4.2. Chemistry

4.2.1. Synthesis of substrates *1*, *2* and 6*H*-quinobenzothiazines *3*–*5*

Diquino-1,4-dithiin **1** and 2,2′-dichloro-3,3′-diquinolinyl disulfide (**2**) were obtained according to the described procedures. 8–10-Substituted 6*H*-quinobenzothiazines **3a**–**g**, **4a**–**c** and **5a**–**c** were obtained as in the described synthesis from dithiin **1** and disulfide **2** with *p*- and *m*-substituted anilines [21].

4.2.2. Synthesis of 9-substituted 6-dialkylaminoalkylquinobenzothiazines *6*–*11*

A mixture of 6*H*-quinobenzothiazine **3a** (0.13 g, 0.5 mmol) [or 9-chloro-6*H*-quinobenzothiazine **3c** (0.14 g, 0.5 mmol) or 9-methylthio-6*H*-quinobenzothiazine **3f** (0.15 g, 0.5 mmol)], sodium hydroxide (0.30 g, 7.5 mmol) and hydrochloride of dialkylaminoalkyl chloride (1.5 mmol, 2-diethylaminoethyl – 0.26 g, 3-dimethylaminopropyl – 0.24 g, 3-dimethylamino-2-methylpropyl – 0.26 g, 2-(1-pyrrolidyl)ethyl – 0.26 g, 2-(1-piperidyl)ethyl – 0.28 g, 2-(1-methyl-2-piperydinyl)ethyl – 0.30 g) in dry dioxane (5 mL) was refluxed for 3 h. After cooling the reaction mixture was poured into water (25 mL) and extracted with chloroform (3 × 10 mL). The combined extracts were washed with water to pH = 7 and dried over Na_2SO_4. Chloroform was evaporated *in vacuo* and the residue was purified by column chromatography (Al_2O_3, $CHCl_3$) to give compounds **6**–**11**.

*4.2.2.1. 6-(2-Diethylaminoethyl)quinobenzothiazine (**6a**).* (0.13 g, 74%), an oil. ^{1}H NMR ($CDCl_3$) δ: 1.17 (t, 6H, $2CH_3$), 2.73 (m, 4H, $2CH_2$), 2.95 (t, 2H, CH_2), 4.23 (m, 2H, NCH_2), 6.89 (t, 1H, H-9), 7.03 (2d, 2H, H-7, H-10), 7.14 (t, 1H, H-8), 7.23 (t, 1H, H-2), 7.46 (m, 2H, H-1, H-3), 7.54 (s, 1H, H-12), 7.67 (d, 1H, H-4). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 12.14, 44.60, 47.82, 48.48, 112.09, 115.56, 118.32, 119.86, 122.49, 124.07, 126.09, 126.67, 127.25, 127.68, 128.98, 131.29, 141.44, 145.78, 151.77. FAB MS *m*/*z*: 350 (M + 1, 100), 277 [M +1 – $NH(C_2H_5)_2$, 58], 250 [M + 1 – $C_2H_4N(C_2H_2)_2$, 50]. Anal. calcd. for $C_{21}H_{23}N_3S$: C 72.17, H 6.63, N 12.02, S 9.17. Found: C 72.01, H 6.75, N 11.87, S 9.01.

*4.2.2.2. 6-(2-Diethylaminoethyl)-9-chloroquinobenzothiazine (**6b**).* (0.15 g, 78%), m.p. 74–75 °C. ^{1}H NMR ($CDCl_3$) δ: 1.17 (t, 6H, $2CH_3$), 2.74 (m, 4H, $2CH_2$), 2.94 (t, 2H, CH_2), 4.31 (m, 2H, NCH_2), 6.97 (d, 1H, H-7), 7.03 (d, 1H, H-10), 7.10 (dd, 1H, H-8), 7.25 (t, 1H, H-2), 7.48 (t, 1H, H-3), 7.49 (d, 1H, H-1), 7.57 (s, 1H, H-12), 7.67 (d, 1H, H-4). FAB

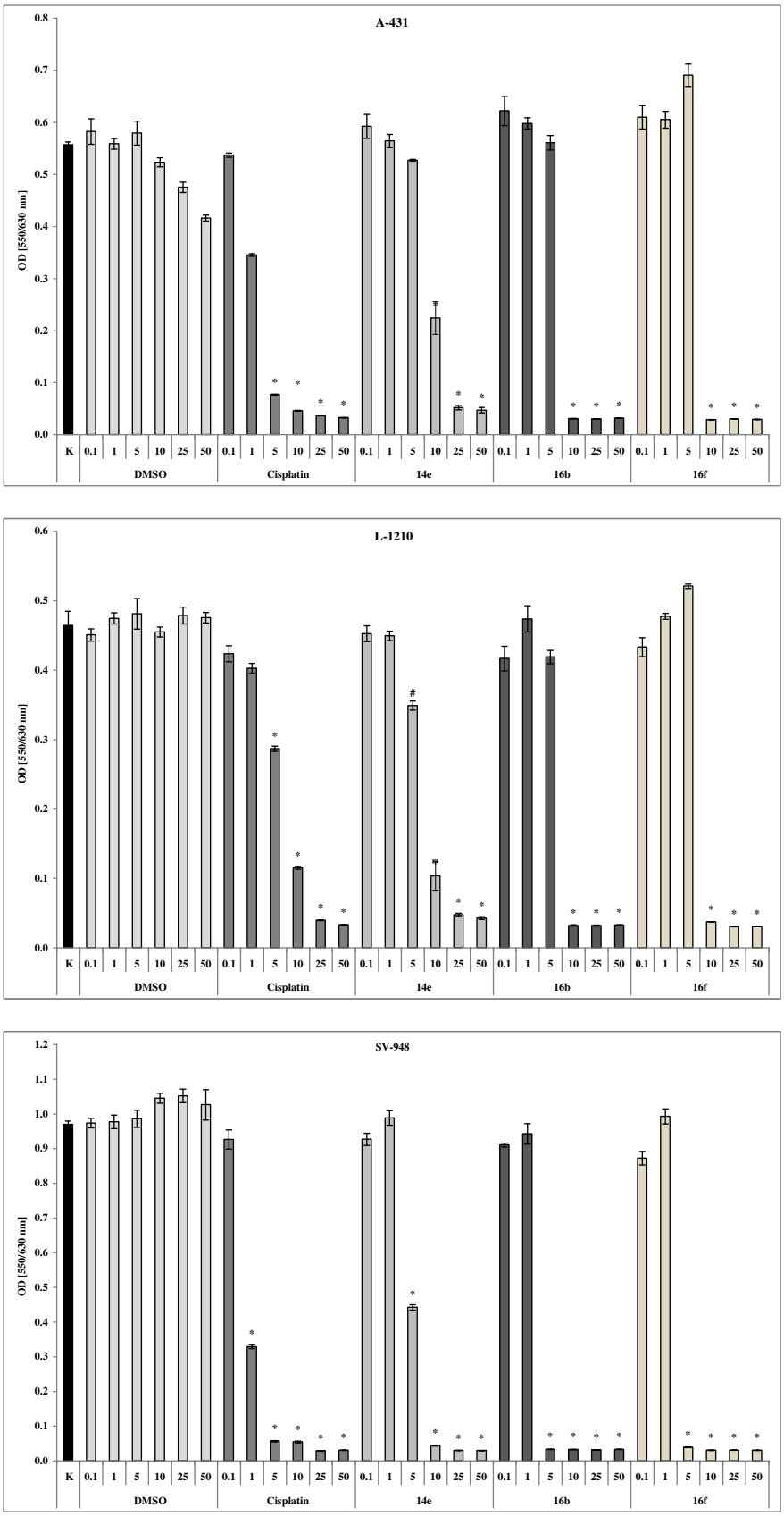


Fig. 1. The anticancer activities of selected compounds at concentrations of 0.1–50 μg/mL. A-431, L-1210 and SW-948 cell lines were used in the study. The results are presented as the mean optical density ±SE (*$p < 0.001$, #$p < 0.05$).

MS *m/z*: 384 (M + 1, 82), 311 [M + 1 − $NH(C_2H_5)_2$, 100], 284 [(M + 1 − $C_2H_4N(C_2H_2)_2$, 30]. Anal. calcd. for $C_{21}H_{22}ClN_3S$: C 65.70, H 5.78, N 10.94. Found: C 65.48, H 5.71, N 10.78.

*4.2.2.3. 6-(2-Diethylaminoethyl)-9-methylthioquinobenzothiazine (**6c**).* (0.16 g, 81%), m.p. 48–49 °C. ^{1}H NMR ($CDCl_3$) *δ*: 1.18 (t, 6H, $2CH_3$), 2.44 (s, 3H, CH_3), 2.75 (m, 4H, $2CH_2$), 2.95 (m, 2H, CH_2), 4.32 (m, 2H, NCH_2), 6.98 (m, 2H, H-7, H-10), 7.07 (d, 1H, H-8), 7.23 (t, 1H, H-2), 7.46 (m, 2H, H-1, H-3), 7.55 (s, 1H, H-12), 7.66 (d, 1H, H-4). ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 12.13, 17.10, 44.64, 47.81, 48.55, 115.95, 117.86, 120.81, 124.11, 125.76, 125.97, 126.10, 127.22, 127.24, 129.09, 131.45, 131.55, 139.38, 145.78, 151.55. FAB MS *m/z*: 396 (M + 1, 100), 380 (M + 1 − CH_3), 323 (M + 1 − $N(C_2H_5)_2$, 90), 310 [M + 1 − $CH_2N(C_2H_5)_2$, 15], 296 [M + 1 − $C_2H_4N(C_2H_5)_2$, 30]. Anal. calcd. for $C_{22}H_{25}N_3S_2$: C 66.80, H 6.37, N 10.62. Found: C 66.62, H 6.39, N 10.38.

*4.2.2.4. 6-(3-Dimethylaminopropyl)quinobenzothiazine (**7a**).* (0.13 g, 78%), m.p. 65–66 °C. ^{1}H NMR ($CDCl_3$) *δ*: 2.08 (m, 2H, CH_2), 2.30 (s, 6H, $2CH_3$), 2.49 (m, 2H, CH_2), 4.26 (t, 2H, NCH_2), 6.96 (t, 1H, H-9), 6.89 (d, 1H, H-7), 7.07 (d, 1H, H-10), 7.15 (t, 1H, H-8), 7.24 (t, 1H, H-2), 7.48 (t, 1H, H-3), 7.49 (d, 1H, H-1), 7.59 (s, 1H, H-12) 7.69 (d, 1H, H-4). ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 24.38, 43.93, 45.51, 57.43, 115.77, 118.70, 120.36, 122.56, 124.09, 126.03, 126.14, 126.81, 127.33, 127.69, 129.01, 131.58, 141.50, 145.79, 152.38. FAB MS *m/z*: 336 (M + 1, 100), 291 [M + 1 − $NH(CH_3)_2$, 52], 250 [M + 1 − $C_3H_6NH(CH_3)_2$, 28]. Anal. calcd. for $C_{20}H_{21}N_3S$: C 71.61, H 6.61, N 12.53. Found: C 71.40, H 6.69, N 12.28.

*4.2.2.5. 6-(3-Dimethylaminopropyl)-9-chloroquinobenzothiazine (**7b**).* (0.15 g, 81%), an oil. ^{1}H NMR ($CDCl_3$) *δ*: 2.09 (m, 2H, CH_2), 2.36 (s, 6H, $2CH_3$), 2.57 (m, 2H, CH_2), 4.25 (t, 2H, CH_2), 6.82 (d, 1H, H-7), 7.06 (d, 1H, H-10), 7.11 (dd, 1H, H-8), 7.27 (t, 1H, H-2), 7.50 (t, 1H, H-3), 7.51 (s, 1H, H-1), 7.61 (s, 1H, H-12), 7.69 (d, 1H, H-4). FAB MS *m/z*: 370 (M + 1, 100), 325 [M + 1 − $NH(CH_3)_2$, 51], 298 [M + 1 − $C_2H_4NH(CH_3)_2$, 20], 284 [M + 1 − $C_3H_6NH(CH_3)_2$, 18]. Anal. calcd. for $C_{20}H_{20}ClN_3S$: C 64.94, H 5.45, N 11.36. Found: C 64.81, H 5.40, N 11.08.

*4.2.2.6. 6-(3-Dimethylaminopropyl)-9-methylthioquinobenzothiazine (**7c**).* (0.15 g, 79%), m.p. 55–56 °C. ^{1}H NMR ($CDCl_3$) *δ*: 2.18 (m, 2H, CH_2), 2.45 (s, 9H, $3CH_3$), 2.72 (m, 2H, CH_2), 4.27 (m, 2H, NCH_2), 6.90 (d, 1H, H-7), 7.02 (d, 1H, H-10), 7.08 (d, 1H, H-8), 7.25 (t, 1H, H-2), 7.49 (t, 1H, H-3), 7.50 (s, 1H, H-1), 7.61 (s, 1H, H-12), 7.67 (d, 1H, H-4). FAB MS *m/z*: 382 (M + 1, 100), 337 [M − $N(CH_3)_2$, 95], 309 [M − $C_2H_4N(CH_3)_2$, 35], 296 [M + 1 − $C_3H_6N(CH_3)_2$, 35]. Anal. calcd. for $C_{21}H_{23}N_3S_2$: C 66.11, H 6.08, N 11.01. Found: C 66.00, H 6.011, N 10.87.

*4.2.2.7. 6-(3-Dimethylamino-2-methylpropyl)quinobenzothiazine (**8a**).* (0.13 g, 74%), an oil. ^{1}H NMR ($CDCl_3$) *δ*: 0.99 (t, 3H, CH_3), 2.20 (m, 1H, CH_2), 2.26 (s, 6H, $2CH_3$), 2.27 (m, 1H, CH), 2.35 (m, 1H, CH_2), 4.35 (m, 2H, NCH_2), 6.95 (t, 1H, H-9), 7.07 (d, 1H, H-7), 7.14 (d, 1H, H-10), 7.19 (t, 1H, H-8), 7.27 (t, 1H, H-2), 7.50 (t, 1H, H-3), 7.53 (d, 1H, H-1), 7.68 (s, 1H, H-12), 7.73 (d, 1H, H-4). FAB MS *m/z*: 350 (M + 1, 100), 305 [M + 1 − $NH(CH_3)_2$, 62], 363 [M + 1 − $C_3H_7N(CH_3)_2$, 36], 250 [M + 1 − $C_4H_8N(CH_3)_2$, 24]. Anal. calcd. for $C_{20}H_{21}N_3S$: C 71.61, H 6.61, N 12.53. Found: C 71.35, H 6.71, N 12.31.

*4.2.2.8. 6-(3-Dimethylamino-2-methylpropyl)-9-chloroquinobenzothiazine (**8b**).* (0.15 g, 78%), an oil. ^{1}H NMR ($CDCl_3$) *δ*: 0.98 (d, 3H, CH_3), 2.20 (m, 1H, CH_2), 2.26 (s, 6H, $2CH_3$), 2.28 (m, 1H, CH), 2.33 (m, 1H, CH_2), 4.35 (m, 2H, NCH_2), 6.98 (d, 1H, H-7), 7.13 (d, 1H, H-10), 7.14 (dd, 1H, H-8), 7.26 (t, 1H, H-2), 7.52 (t, 1H, H-3), 7.54 (d, 1H, H-1), 7.68 (s, 1H, H-12), 7.72 (d, 1H, H-4). FAB MS *m/z*: 384 (M + 1, 100), 339 [M + 1 − $NH(CH_3)_2$, 78], 297 [M + 1 − $C_3H_7N(CH_3)_2$, 35], 284 [M + 1 − $C_4H_8N(CH_3)_2$, 22]. Anal. calcd. for $C_{21}H_{22}ClN_3S$: C 65.70, H 5.78, N 10.94. Found: C 65.41, H 5.70, N 10.71.

*4.2.2.9. 6-(3-Dimethylamino-2-methylpropyl)-9-methylthioquinobenzothiazine (**8c**).* (0.15 g, 76%), an oil. ^{1}H NMR ($CDCl_3$) *δ*: 1.02 (d, 3H, CH_3), 2.30 (s, 6H, $2CH_3$), 2.46 (s, 3H, CH_3), 4.31 (m, 5H, $2CH_2$, CH), 7.04 (d, 1H, H-7), 7.07 (d, 1H, H-10), 7.10 (d, 1H, H-8), 7.26 (t, 1H, H-2), 7.52 (t, 1H, H-3), 7.53 (d, 1H, H-1), 7.68 (s, 1H, H-12), 7.70 (d, 1H, H-4). FAB MS *m/z*: 396 (M + 1, 95), 351 (M − $N(CH_3)_2$, 100), 309 [M − $C_3H_7N(CH_3)_2$, 60], 296 [M + 1 − $C_4H_8N(CH_3)_2$, 30]. Anal. calcd. for $C_{22}H_{25}N_3S_2$: C 66.80, H 6.37, N 10.62. Found: C 66.65, H 6.32, N 10.35.

*4.2.2.10. 6-(1-Pyrrolidylethyl)quinobenzothiazine (**9a**).* (0.14 g, 82%), m.p. 68–69 °C. ^{1}H NMR ($CDCl_3$) *δ*: 1.88 (m, 4H, $2CH_2$), 2.80 (m, 4H, $2CH_2$), 3.04 (m, 2H, CH_2), 4.46 (m, 2H, NCH_2), 6.90 (t, 1H, H-9), 7.06 (2d, 2H, H-7, H-10), 7.15 (t, 1H, H-8), 7.24 (t, 1H, H-2), 7.47 (m, 2H, H-1, H-3), 7.57 (s, 1H, H-12), 7.68 (m, 1H, H-4). ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 23.55, 44.62, 52.12, 54.50, 115.71, 118.43, 120.00, 122.58, 124.14, 126.08, 126.73, 127.31, 127.73, 129.00, 131.47, 141.35, 145.71, 151.93. FAB MS *m/z*: 348 (M + 1, 52), 277 (M + 1 − C_4H_9N, 100), 250 (M + 1 − $C_2H_4NC_4H_8$, 28). Anal. calcd. for $C_{21}H_{21}N_3S$: C 72.59, H 6.09, N 12.09. Found: C 72.41, H 6.14, N 11.89.

*4.2.2.11. 6-(1-Pyrrolidylethyl)-9-chloroquinobenzothiazine (**9b**).* (0.15 g, 79%), m.p. 68–69 °C. ^{1}H NMR ($CDCl_3$) *δ*: 1.96 (m, 4H, $2CH_2$), 2.95 (m, 4H, $2CH_2$), 3.15 (m, 2H, CH_2), 4.51 (m, 2H, NCH_2), 6.04 (m, 2H, H-7, H-10), 7.13 (d, 1H, H-8), 7.28 (t, 1H, H-2), 7.50 (t, 1H, H-3), 7.51 (d, 1H, H-1), 7.61 (s, 1H, H-12), 7.67 (d, 1H, H-4). FAB MS *m/z*: 382 (M + 1, 76), 311 (M + 1 − HNC_4H_8, 100), 284 (M + 1 − $C_2H_4NC_5H_{10}$, 15). Anal. calcd. for $C_{21}H_{20}ClN_3S$: C 66.04, H 5.28, N 11.00. Found: C 65.88, H 5.31, N 10.81.

*4.2.2.12. 6-(1-Pyrrolidylethyl)-methylthioquinobenzothiazine (**9c**).* (0.15 g, 76%), m.p. 90–91 °C. ^{1}H NMR ($CDCl_3$) *δ*: 1.93 (m, 4H, $2CH_2$), 2.45 (s, 3H, SCH_3), 1.74 (m, 4H, $2CH_2$), 2.88 (m, 4H, $2CH_2$), 3.09 (m, 2H, CH_2), 4.49 (m, 2H, NCH_2), 7.00 (d, 1H, H-7), 7.05 (d, 1H, H-10), 7.09 (d, 1H, H-8), 7.26 (t, 1H, H-2), 7.49 (t, 1H, H-3), 7.50 (d, 1H, H-1), 7.59 (s, 1H, H-12), 7.68 (d, 1H, H-4). FAB MS *m/z*: 394 (M + 1, 100), 323 (M − NC_4H_8, 90), 309 [M − $CH_2N(CH_2)_4$, 10], 296 [M + 1 − $C_2H_4N(CH_2)_4$, 30]. Anal. calcd. for $C_{22}H_{23}N_3S_2$: C 67.14, H 5.89, N 10.68. Found: C 66.95, H 5.91, N 10.42.

*4.2.2.13. 6-(1-Piperidylethyl)quinobenzothiazine (**10a**).* (0.15 g, 83%), m.p. 71–72 °C. ^{1}H NMR ($CDCl_3$) *δ*: 1.58 (m, 2H, CH_2), 1.87 (m, 4H, $2CH_2$), 2.90 (m, 4H, $2CH_2$), 3.13 (m, 2H, CH_2), 4.60 (m, 2H, NCH_2), 6.92 (t, 1H, H-9), 7.07 (d, 1H, H-7), 7.21 (d + t, 2H, H-8, H-10), 7.26 (t, 1H, H-2), 7.46 (m, 2H, H-1, H-3), 7.59 (s, 1H, H-12), 7.67 (d, 1H, H-4). FAB MS *m/z*: 362 (M + 1, 100), 277 (M + 1 − $C_5H_{11}N$, 55), 250 (M + 1 − $C_2H_4NC_5H_{10}$, 21). Anal. calcd. for $C_{22}H_{23}N_3S$: C 73.10, H 6.41, N 11.62. Found: C 73.02, H 6.29, N 11.37.

*4.2.2.14. 6-(1-Piperidylethyl)-9-chloroquinobenzothiazine (**10b**).* (0.16 g, 81%), m.p. 93–94 °C. ^{1}H NMR ($CDCl_3$) *δ*: 1.53 (m, 2H, CH_2), 1.74 (m, 4H, $2CH_2$), 2.67 (m, 4H, $2CH_2$), 2.92 (m, 2H, CH_2), 4.45 (m, 2H, NCH_2), 7.04 (d, 1H, H-7), 7.12 (m, 2H, H-8, H-10), 7.27 (t, 1H, H-2), 7.49 (t, 1H, H-3), 7.50 (d, 1H, H-1), 7.59 (s, 1H, H-12), 7.67 (d, 1H, H-4). FAB MS *m/z*: 396 (M + 1, 32), 311 (M + 1 − HNC_5H_{10}, 100), 284 (M + 1 − $C_2H_4NC_5H_{10}$, 21). Anal. calcd. for $C_{22}H_{22}ClN_3S$: C 66.74, H 5.60, N 10.61. Found: C 66.49, H 5.64, N 10.36.

*4.2.2.15. 6-(1-Piperidylethyl)-9-methylthioquinobenzothiazine (**10c**).* (0.16 g, 79%), m.p. 59–60 °C. ^{1}H NMR ($CDCl_3$) *δ*: 1.66 (m, 2H, CH_2), 1.70 (m, 6H, $3CH_2$), 2.44 (s, 3H, CH_3), 2.59 (m, 2H, CH_2), 2.88 (m, 2H,

CH_2), 4.41 (m, 2H, NCH_2), 6.99 (d, 1H, H-7), 7.03 (d, 1H, H-10), 7.08 (dd, 1H, H-8), 7.24 (t, 1H, H-2), 7.48 (t, 1H, H-3), 7.49 (d, 1H, H-1), 7.57 (s, 1H, H-12), 7.67 (d, 1H, H-4). FAB MS *m/z*: 408 (M + 1, 100), 323 (M + 1 − HNC_5H_{10}, 10), 284 (M + 1 − $C_2H_4NC_5H_{10}$, 20). Anal. calcd. for $C_{23}H_{25}N_3S_2$: C 67.78, H 6.18, N 10.31. Found: C 67.40, H 6.21, N 10.09.

*4.2.2.16. 6-(1-Methyl-2-piperidylethyl)quinobenzothiazine (**11a**).* (0.15 g, 80%), m.p. 109–110 °C. ^{1}H NMR ($CDCl_3$) δ: 1.43 (m, 4H, $2CH_2$), 1.83 (m, 2H, CH_2), 2.21 (m, 4H, $2CH_2$), 2.44 (s, 3H, CH_3), 2.88 (m, 1H, CH), 4.18 (m, 1H, NCH_2), 4.30 (m, 1H, NCH_2), 6.89 (t, 1H, H-9), 6.93 (d, 1H, H-7), 7.05 (d, 1H, H-10), 7.14 (t, 1H, H-8), 7.23 (t, 1H, H-2), 7.47 (m, 2H, H-1, H-3), 7.57 (s, 1H, H-12), 7.66 (m, 1H, H-4). FAB MS *m/z*: 376 (M + 1, 100), 277 (M + 1 − $C_5H_{10}NCH_3$, 19), 263 (M + 1 − $C_6H_{12}NCH_3$, 48), 250 (M + 1 − $C_2H_4NC_5H_9CH_3$, 37). Anal. calcd. for $C_{23}H_{25}N_3S$: C 73.56, H 6.71, N 11.19. Found: C 73.44, H 6.70, N 11.02.

*4.2.2.17. 6-(1-Methyl-2-piperidylethyl)-9-chloroquinobenzothiazine (**11b**).* (0.15 g, 73%), m.p. 139–140 °C. ^{1}H NMR ($CDCl_3$) δ: 1.51 (m, 2H, CH_2), 1.92 (m, 8H, $4CH_2$), 2.66 (s, 3H, CH_3), 3.16 (m, 1H, CH), 4.31 (m, 2H, NCH_2), 6.93 (d, 1H, H-7), 7.12 (d, 1H, H-10), 7.18 (d, 1H, H-8), 7.33 (t, 1H, H-2), 7.55 (t, 1H, H-3), 7.57 (d, 1H, H-1), 7.67(s, 1H, H-12), 7.69 (d, 1H, H-4). FAB MS *m/z*: 410 (M + 1, 100), 311 (M + 1 − $C_5H_{10}NCH_3$, 26), 297 (M + 1 − $C_6H_{12}NCH_3$, 60), 284 (M + 1 − $C_2H_4C_5H_9NCH_3$, 49). Anal. calcd. for $C_{23}H_{24}ClN_3S$: C 67.38, H 5.90, N 10.25. Found: C 67.19, H 5.88, N 10.02.

*4.2.2.18. 6-(1-Methyl-2-piperidylethyl)-9-methylthioquinobenzothiazine (**11c**).* (0.17 g, 81%), m.p. 129–130 °C. ^{1}H NMR ($CDCl_3$) δ: 1.40 (m, 2H, CH_2), 1.72 (m, 4H, $2CH_2$), 1.84 (m, 2H, CH_2), 2.17 (m, 2H, CH_2), 2.41 (s, 3H, SCH_3), 2.53 (s, 3H, NCH_3), 3.02 (m, 1H, CH), 4.24 (m, 2H, NCH_2), 6.87 (d, 1H, H-7), 7.00 (d, 1H, H-10), 7.09 (d, 1H, H-8), 7.25 (t, 1H, H-2), 7.48 (t, 1H, H-3), 7.49 (d, 1H, H-1), 7.59 (s, 1H, H-12), 7.64 (d, 1H, H-4). FAB MS *m/z*: 422 (M + 1, 100), 309 [M + 1 − CHN(CH_3) C_5H_9, 15], 296 [M + 1 − $(CH_2)_2NCH_3C_5H_9$, 15]. Anal. calcd. for $C_{24}H_{27}N_3S_2$: C 68.37, H 6.45, N 9.97. Found: C 68.14, H 6.47, N 9.73.

*4.2.3. Synthesis of 9-substituted 6-phthalimidoalkylquinobenzothiazines **12***

To a stirred solution of 6*H*-quinobenzothiazine **3a** (0.25 g, 1 mmol) [or 9-chloro-6*H*-quinobenzothiazine **3c** (0.28 g, 1 mmol) or 9-methylthio-6*H*-quinobenzothiazine **3f** (0.30 g, 1 mmol)] in dry toluene (10 mL) NaH (0.24 g, 10 mmol, washed out with hexane) was added. The mixture was refluxed for 30 min and a solution of *N*-(bromoalkyl)phthalimide [2.2 mmol, *N*-(3-bromopropyl)phthalimide − 0.60 g, *N*-(4-bromobutyl)phthalimide − 0.62 g] in dry toluene (5 mL) was added. The mixture was refluxed for 24 h. Next toluene was evaporated *in vacuo* and the residue was extracted with $CHCl_3$ (2 × 5 mL). The extract was concentrated and purified by column chromatography (silica gel, $CHCl_3$) to give compounds **12**.

*4.2.3.1. 6-Phthalimidopropylquinobenzothiazine (**12a**).* (0.39 g, 89%), m.p. 104–105 °C. ^{1}H NMR ($CDCl_3$) δ: 2.34 (m, 2H, CH_2), 3.94 (t, 2H, NCH_2), 4.32 (t, 2H, NCH_2), 6.90 (m, 1H, H-7), 6.92 (m, 1H, H-9), 7.05 (d, 1H, H-10), 7.14 (t, 1H, H-8), 7.22 (t, 1H, H-2), 7.42 (t, 1H, H-3), 7.46 (d, 1H, H-1), 7.55 (m, 2H, H-4, H-12), 7.66 (m, 2H, $2H_{phthal.}$), 7.77 (m, 2H, $2H_{phthal.}$). FAB MS *m/z*: 438 (M + 1, 100), 437 (M^+, 65), 250 (M − $C_{11}H_9O_2N$, 30). Anal. calcd. for $C_{26}H_{19}N_3O_2S$: C 71.38, H 4.38, N 9.60. Found: C 71.23, H 4.35, N 9.43.

*4.2.3.2. 9-Chloro-6-phthalimidopropylquinobenzothiazine (**12b**).* (0.40 g, 85%), m.p. 161–162 °C. ^{1}H NMR ($CDCl_3$) δ: 2.30 (m, 2H, CH_2), 3.91 (t, 2H, NCH_2), 4.35 (t, 2H, NCH_2), 6.84 (m, 1H, H-7), 7.05 (d, 1H, H-10), 7.12 (dd, 1H, H-8), 7.28 (t, 1H, H-2), 7.47 (t, 1H, H-3), 7.50 (d, 1H, H-1), 7.62 (s, 1H, H-12), 7.67 (m, 3H, H-4, $2H_{phthal.}$), 7.75 (m, 2H, $2H_{phthal.}$). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 25.34, 35.97, 42.81, 116.28, 117.79, 122.49, 123.13, 124.34, 125.90, 126.12, 126.33, 127.38, 127.68, 129.22, 131.92, 132.04, 133.87, 139.98, 145.54, 151.73, 168.33. FAB MS *m/z*: 472 (M + 1, 100), 311 (M − $C_9H_6O_2N$, 20), 297 (M − $C_{10}H_8O_2N$, 20), 284 (M + 1 − $C_{11}H_{10}O_2N$, 30). Anal. calcd. for $C_{26}H_{18}ClN_3O_2S$: C 66.17, H 3.84, N 8.90. Found: C 66.01, H 3.88, N 8.69.

*4.2.3.3. 9-Methylthio-6-phthalimidopropylquinobenzothiazine (**12c**).* (0.42 g, 87%), m.p. 141–142 °C. ^{1}H NMR ($CDCl_3$) δ: 2.27 (m, 2H, CH_2), 2.46 (s, 3H, CH_3), 3.85 (t, 2H, NCH_2), 4.56 (m, 2H, NCH_2), 6.97 (d, 1H, H-7), 7.02 (d, 1H, H-10), 7.12 (dd, 1H, H-8), 7.38 (t, 1H, H-2), 7.57 (t, 1H, H-3), 7.58 (d, 1H, H-1), 7.63 (m, 2H, $2H_{phthal}$), 7.69 (m, 3H, H-12, $2H_{phthal.}$), 7.80 (d, 1H, H-4). FAB MS *m/z*: 484 (M + 1, 100), 296 (M − $C_3H_6C_8H_4O_2$ N, 10). Anal. calcd. for $C_{27}H_{21}N_3O_2S_2$: C 67.06, H 4.38, N 8.69. Found: C 66.91, H 4.36, N 8.45.

*4.2.3.4. 6-Phthalimidobutylquinobenzothiazine (**12d**).* (0.40 g, 89%), m.p. 111–112 °C. ^{1}H NMR ($CDCl_3$) δ: 1.92 (m, 4H, $2CH_2$), 3.78 (t, 2H, CH_2), 4.28 (t, 2H, NCH_2), 6.88 (t, 1H, H-9), 6.90 (d, 1H, H-7), 7.05 (d, 1H, H-10), 7.13 (t, 1H, H-8), 7.22 (t, 1H, H-2), 7.44 (t, 1H, H-3), 7.47 (d, 1H, H-1), 7.58 (s, 1H, H-12), 7.63 (d, 1H, H-4), 7.69 (m, 2H, $2H_{phthal.}$), 7.81 (m, 2H, $2H_{phthal.}$). FAB MS *m/z*: 452 (M + 1, 100), 263 (M + 1 − $C_3H_6NO_2C_8H_4$, 20), 250 (M − $C_{12}H_{12}O_2N$, 50). Anal. calcd. for $C_{27}H_{21}N_3O_2S$: C 71.82, H 4.69, N 9.31. Found: C 71.60, H 4.67, N 9.08.

*4.2.3.5. 9-Chloro-6-phthalimidobutylquinobenzothiazine (**12e**).* (0.42 g, 88%), m.p. 139–140 °C. ^{1}H NMR ($CDCl_3$) δ: 1.90 (m, 4H, $2CH_2$), 3.77 (m, 2H, NCH_2), 4.28 (m, 2H, NCH_2), 6.80 (d, 1H, H-7), 7.01 (d, 1H, H-10), 7.08 (dd, 1H, H-8), 7.25 (m, 1H, H-2), 7.46 (d, 1H, H-1), 7.48 (t, 1H, H-3), 7.61 (s, 1H, H-12), 7.70 (m, 3H, H-4, $2H_{phthal.}$), 7.81 (m, 2H, $2H_{phthal.}$). FAB MS *m/z*: 486 (M + 1, 100), 284 (M − $C_{12}H_{12}O_2N$, 30). Anal. calcd. for $C_{27}H_{20}ClN_3O_2S$: C 66.73, H 4.15, N 8.65. Found: C 66.59, H 4.17, N 8.51.

*4.2.3.6. 9-Methylthio-6-phthalimidobutylquinobenzothiazine (**12f**).* (0.43 g, 87%), m.p. 118–119 °C. ^{1}H NMR ($CDCl_3$) δ: 2.27 (m, 2H, CH_2), 2.46 (s, 3H, CH_3), 3.85 (t, 2H, NCH_2), 4.56 (m, 2H, NCH_2), 6.97 (d, 1H, H-7), 7.02 (d, 1H, H-10), 7.12 (dd, 1H, H-8), 7.38 (t, 1H, H-2), 7.57 (t, 1H, H-3), 7.58 (d, 1H, H-1), 7.63 (m, 2H, $2H_{phthal.}$), 7.69 (m, 3H, H-12, $2H_{phthal.}$), 7.80 (d, 1H, H-4). FAB MS *m/z*: 484 (M + 1, 100), 296 (M − $C_3H_6C_8H_4O_2$ N, 10). Anal. calcd. for $C_{27}H_{21}N_3O_2S$: C 67.58, H 4.66, N 8.44. Found: C 67.51, H 4.63, N 8.29.

*4.2.4. Synthesis of 9-substituted 6-aminoalkylquinobenzothiazines **13***

To a boiling solution of 6-phthalimidoalkylquinobenzothiazines **12a–f** (1 mmol) in EtOH (25 mL) 80% aqueous solution of hydrazine (0.2 mL, 5 mmol) was added. The mixture was refluxed for 2 h. After cooling the reaction mixture was acidified to pH = 2 with conc. hydrochloric acid and evaporated. Water (10 mL) was added to the residue, the resulting solid was filtered off and washed with 10% hydrochloric acid. Combined filtrates were alkalized to pH = 10 and the resulted solid was filtered off, washed with water, dried and purified by column chromatography (SiO_2, $CHCl_3$–EtOH 10:1) to give compounds **13**.

*4.2.4.1. 6-Aminopropylquinobenzothiazine (**13a**).* (0.25 g, 81%), m.p. 142–143 °C. ^{1}H NMR ($CDCl_3$) δ: 2.16 (m, 2H, CH_2), 3.03 (t, 2H, NCH_2), 4.28 (t, 2H, NCH_2), 6.88 (m, 1H, H-7), 6.90 (m, 1H, H-9), 7.03 (d, 1H, H-10), 7.12 (t, 1H, H-8), 7.18 (t, 1H, H-2), 7.43 (m, 2H, H-1, H-3), 7.52 (s, 1H, H-12), 7.67 (d, 1H, H-4). FAB MS *m/z*: 308 (M + 1, 100), 291 (M + 1 − NH_3, 50), 263 (M + 1 − $C_2H_5NH_2$, 40), 250

[(M + 1 − $C_3H_6NH_2$), 50]. Anal. calcd. for $C_{18}H_{17}N_3S$: C 70.33, H 5.57, N 13.67. Found: C 70.21, H 5.59, N 13.48.

*4.2.4.2. 6-Aminopropyl-9-chloroquinobenzothiazine (**13b**).* (0.26 g, 76%), m.p. 84–85 °C. ^{1}H NMR (DMSO-d_6) δ: 2.08 (m, 2H, CH_2), 2.97 (t, 2H, NCH_2), 4.24 (m, 2H, NCH_2), 7.14 (d, 1H, H-7), 7.25 (dd, 1H, H-8), 7.34 (m, 2H, H-2, H-10), 7.56 (t, 1H, H-3), 7.68 (m, 2H, H-1, H-4), 8.01 (s, 1H, H-12). FAB MS *m*/*z*: 342 (M + 1, 100), 325 (M + 1 − NH_3, 40), 295 (M + 1 − C_4H_6N, 20), 284 (M + 1 − C_3H_8N, 35). Anal. calcd. for $C_{18}H_{16}ClN_3S$: C 63.24, H 4.72, N 12.29. Found: C 63.10, H 4.75, N 12.03.

*4.2.4.3. 6-Aminopropyl-9-methylthioquinobenzothiazine (**13c**).* (0.30 g, 85%), m.p. 94–95 °C. ^{1}H NMR (DMSO-d_6) δ: 2.40 (m, 2H, CH_2), 2.49 (s, 3H, CH_3), 2.73 (m, 2H, CH_2), 4.23 (m, 2H, NCH_2), 7.10 (d, 1H, H-7), 7.11 (d, 1H, H-8), 7.29 (t, 1H, H-2), 7.45 (d, 1H, H-10), 7.52 (t, 1H, H-3), 7.63 (m, 2H, H-1, H-4), 7.94 (s, 1H, H-12). FAB MS *m*/*z*: 354 (M + 1, 100), 337 (M + 1 − NH_3, 40), 296 (M + 1 − $C_3H_6NH_2$, 20). Anal. calcd. for $C_{19}H_{19}N_3S_2$: C 64.56, H 5.42, N 11.89. Found: C 64.46, H 5.45, N 11.61.

*4.2.4.4. 6-Aminobutylquinobenzothiazine (**13d**).* (0.27 g, 84%), m.p. 158–159 °C. ^{1}H NMR ($CDCl_3$) δ: 1.66 (m, 2H, CH_2), 1.92 (m, 2H, CH_2), 2.28 (t, 2H, NCH_2), 4.24 (t, 2H, NCH_2), 6.90 (t, 1H, H-9), 6.91 (d, 1H, H-7), 7.06 (d, 1H, H-10), 7.15 (t, 1H, H-8), 7.23 (t, 1H, H-2), 7.47 (t, 1H, H-3), 7.48 (d, 1H, H-1), 7.58 (s, 1H, H-12), 7.69 (d, 1H, H-4). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 27.96, 29.72, 38.95, 42.75, 115.75, 118.41, 120.09, 122.77, 124.25, 125.94, 126.09, 126.71, 126.93, 127.72, 129.22, 131.64, 140.91, 145.35, 152.07. FAB MS *m*/*z*: 322 (M + 1, 100), 263 (M + 1 − $C_3H_7NH_2$, 30), 250 (M + 1 − $C_4H_8NH_2$, 75). Anal. calcd. for $C_{19}H_{19}N_3S$: C 71.00, H 5.96, N 13.07. Found: C 7.89, H 5.91, N 12.88.

*4.2.4.5. 6-Aminobutyl-9-chloroquinobenzothiazine (**13e**).* (0.25 g, 70%), m.p. 95–96 °C. ^{1}H NMR (DMSO-d_6) δ: 1.68 (m, 2H, CH_2), 1.77 (m, 2H, CH_2), 2.83 (m, 2H, NCH_2), 4.19 (m, 2H, NCH_2), 7.10 (d, 2H, H-7), 7.22 (d, 1H, H-10), 7.30 (m, 2H, H-8, H-2), 7.53 (t, 1H, H-3), 7.64 (m, 2H, H-1, H-4), 7.97 (s, 1H, H-12). FAB MS *m*/*z*: 356 (M + 1, 100), 284 (M + 1 − $C_4H_8NH_2$, 35). Anal. calcd. for $C_{19}H_{18}ClN_3S$: C 64.12, H 5.10, N 11.81. Found: C 64.00, H 5.11, N 11.59.

*4.2.4.6. 6-Aminobutyl-9-methylthioquinobenzothiazine (**13f**).* (0.30 g, 83%), m.p. 102–103 °C. ^{1}H NMR (DMSO-d_6) δ: 1.50 (m, 2H, CH_2), 1.73 (m, 2H, CH_2), 2.46 (s, 3H, SCH_3), 3.29 (m, 2H, CH_2), 4.16 (m, 2H, NCH_2), 7.03 (d, 2H, H-7), 7.07 (m, 2H, H-9, H-10), 7.27 (t, 1H, H-2) 7.50 (t, 1H, H-3), 7.58 (d, 1H, H-1), 7.26 (d, 1H, H-4), 7.92 (s, 1H, H-12). FAB MS *m*/*z*: 368 (M + 1, 100), 351 (M + 1 − NH_3, 50), 337 (M − CH_2NH_2, 30), 309 (M − $C_3H_6NH_2$, 50), 296 (M + 1 − $C_4H_8NH_2$, 90). Anal. calcd. for $C_{20}H_{21}N_3S_2$: C 65.36, H 5.76, N 11.43. Found: C 65.16, H 5.71, N 11.28.

*4.2.5. Synthesis of 9-substituted 6-acetylaminoalkylquinobenzothiazines **14***

To a suspension of aminoalkylquinothiazines **13a**–**f** (1 mmol) in pyridine (5 mL) acetic anhydride (6 mL, 64 mmol) was added and the mixture was stirred at rt for 24 h. The reaction mixture was poured into water (20 mL) and the resulting solid was filtered off, washed with water, air-dried and purified by column chromatography (Al_2O_3, $CHCl_3$) to give compounds **14**.

*4.2.5.1. 6-Acetylaminopropylquinobenzothiazine (**14a**).* (0.31 g, 89%), m.p. 114–115 °C. ^{1}H NMR ($CDCl_3$) δ: 1.99 (s, 3H, CH_3), 2.09 (m, 2H, CH_2), 3.43 (m, 2H, NCH_2), 4.39 (m, 2H, NCH_2), 6.28 (s, 1H, NH), 6.96 (m, 2H, H-7, H-9), 7.09 (d, 1H, H-10), 7.16 (t, 1H, H-8), 7.28 (t, 1H, H-2), 7.58 (m, 2H, H-1, H-3), 7.64 (s, 1H, H-12), 7.72 (m, 1H, H-4). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 23.41, 26.44, 37.54, 42.34, 115.82, 118.61, 120.42, 122.97, 124.37, 126.11, 126.32, 126.79, 126.94, 127.81, 129.31, 131.96, 141.00, 145.50, 152.58, 170.12. FAB MS *m*/*z*: 350 (M + 1, 100), 263 (M + 1 − $C_2H_5NHCOCH_3$, 10), 250 (M + 1 − $C_3H_6NHCOCH_3$, 25). Anal. calcd. for $C_{20}H_{19}N_3OS$: C 68.74, H 5.48, N 12.02. Found: C 68.51, H 5.50, N 11.79.

*4.2.5.2. 6-Acetylaminopropyl-9-chloroquinobenzothiazine (**14b**).* (0.32 g, 84%), m.p. 172–173 °C. ^{1}H NMR ($CDCl_3$) δ: 2.00 (s, 3H, CH_3), 2.08 (m, 2H, CH_2), 3.43 (m, 2H, NCH_2), 4.50 (m, 2H, NCH_2), 6.92 (d, 1H, H-7), 7.12 (d, 1H, H-10), 7.16 (d, 1H, H-8), 7.36 (t, 1H, H-2), 7.58 (m, 2H, H-1, H-3), 7.74 (s, 1H, H-12), 8.05 (m, 1H, H-4). FAB MS *m*/*z*: 384 (M + 1, 100), 311 (M + 1 − $CH_3NHCOCH_3$, 40), 297 (M + 1 − $C_2H_5NHCOCH_3$, 45), 284 (M + 1 − $C_3H_6NHCOCH_3$, 65). Anal. calcd. for $C_{20}H_{18}ClN_3OS$: C 62.57, H 4.73, N 10.95. Found: C 62.29, H 4.75, N 10.77.

*4.2.5.3. 6-Acetylaminopropyl-9-methylthioquinobenzothiazine (**14c**).* (0.34 g, 86%), m.p. 138–139 °C. ^{1}H NMR ($CDCl_3$) δ: 1.26 (t, 3H, CH_3), 2.07 (m, 2H, CH_2), 2.45 (s, 3H, SCH_3), 3.36 (m, 2H, CH_2), 4.10 (m, 2H, CH_2), 4.34 (m, 2H, NCH_2), 6.88 (d, 1H, H-7), 6.99 (d, 1H, H-10), 7.08 (dd, 1H, H-8), 7.28 (t, 1H, H-2), 7.51 (m, 2H, H-1, H-3), 7.63 (s, 1H, H-12), 7.93 (m, 1H, H-4). FAB MS *m*/*z*: 426 (M + 1, 100), 296 (M + 1 − $C_3H_6NHCO_2C_2H_5$, 40). Anal. calcd. for $C_{21}H_{21}N_3OS_2$: C 63.77, H 5.35, N 10.62. Found: C 63.55, H 5.37, N 10.47.

*4.2.5.4. 6-Acetylaminobutylquinobenzothiazine (**14d**).* (0.33 g, 91%), m.p. 132–133 °C. ^{1}H NMR ($CDCl_3$) δ: 1.70 (m, 2H, CH_2), 1.91 (m, 2H, CH_2), 1.93 (s, 3H, CH_3), 3.36 (m, 2H, NCH_2), 4.29 (m, 2H, NCH_2), 6.93 (m, 2H, H-7, H-9), 7.09 (d, 1H, H-10), 7.17 (t, 1H, H-8), 7.25 (t, 1H, H-2), 7.50 (t, 1H, H-3), 7.51 (d, 1H, H-1), 7.63 (s, 1H, H-12), 7.75 (m, 1H, H-4). FAB MS *m*/*z*: 364 (M + 1, 100), 277 (M + 1 − $C_2H_5NHCOCH_3$, 15), 263 (M + 1 − $C_3H_7NHCOCH_3$, 20), 250 (M + 1 − $C_4H_8NHCOCH_3$, 50). Anal. calcd. for $C_{21}H_{21}N_3OS$: C 69.39, H 5.82, N 11.56. Found: C 69.22, H 5.85, N 11.43.

*4.2.5.5. 6-Acetylaminobutyl-9-chloroquinobenzothiazine (**14e**).* (0.34 g, 86%), m.p. 144–145 °C. ^{1}H NMR ($CDCl_3$) δ: 1.72 (m, 2H, CH_2), 1.91 (m, 2H, CH_2), 1.96 (s, 3H, CH_3), 3.34 (m, 2H, NCH_2), 4.40 (m, 2H, NCH_2), 6.90 (d, 1H, H-7), 7.10 (m, 1H, H-8), 7.16 (d, 1H, H-10), 7.25 (m, 1H, H-2), 7.34 (t, 1H, H-3), 7.56 (m, 2H, H-1, H-4), 7.73 (s, 1H, H12). FAB MS *m*/*z*: 398 (M + 1, 100), 297 (M + 1 − $C_3H_7NHCOCH_3$, 35), 284 (M + 1 − $C_4H_8NHCOCH_3$, 80). Anal. calcd. for $C_{21}H_{20}ClN_3OS$: C 63.39, H 5.07, N 10.56. Found: C 63.26 H 5.05, N 10.38.

*4.2.5.6. 6-Acetylaminobutyl-9-methylthioquinobenzothiazine (**14f**).* (0.345 g, 86%), m.p. 135–136 °C. ^{1}H NMR ($CDCl_3$) δ: 1.75 (t, 2H, CH_2), 1.96 (t, 2H, CH_2), 1.98 (s, 3H, CH_3), 2.47 (s, 3H, SCH_3), 3.34 (m, 2H, CH_2), 4.54 (m, 2H, NCH_2), 6.97 (d, 1H, H-7), 7.03 (d, 1H, H-10), 7.12 (d, 1H, H-8), 7.40 (t, 1H, H-2), 7.58 (d, 1H, H-1), 7.63 (t, 1H, H-3), 7.80 (s, 1H, H-1, H-12), 8.49 (d, 1H, H-4). FAB MS *m*/*z*: 410 (M + 1, 100), 296 (M + 1 − $C_4H_8NHCOCH_3$, 45). Anal. calcd. for $C_{22}H_{23}N_3OS_2$: C 64.52, H 5.66, N 10.26. Found: C 64.38, H 5.63, N 10.05.

*4.2.6. Synthesis of 9-substituted 6-etoxycarbonylaminoalkylquinobenzothiazines **15***

To a stirred solution of aminoalkylquinobenzothiazines **13a**–**f** (1 mmol) in a mixture of CH_2Cl_2 (5 mL) and 10% Na_2CO_3 solution (5 mL), a solution of ethyl chloroformate (1.3 mL, 1.3 mmol) in CH_2Cl_2 (3 mL) was added. The mixture was stirred at rt for 24 h. The organic phase was separated and aqueous phase was extracted with CH_2Cl_2 (2 × 5 mL). The combined extracts were washed with water (2 × 10 mL) and dried over Na_2SO_4. The drying agent was filtered off and filtrate was evaporated. The resulting residue was purified by column chromatography (Al_2O_3, $CHCl_3$) to give compounds **15**:

*4.2.6.1. 6-Ethoxycarbonylaminopropylquinobenzothiazine (**15a**).* (0.30 g, 79%), m.p. 82–83 °C. ^{1}H NMR ($CDCl_3$) δ: 1.28 (t, 3H, CH_3), 2.10 (m, 2H, CH_2), 3.38 (m, 2H, NCH_2), 4.13 (m, 2H, CH_2), 4.32 (m, 2H, NCH_2), 6.92 (t, 1H, H-9), 6.94 (d, 1H, H-7), 7.06 (d, 1H, H-10), 7.15 (t, 1H, H-8), 7.27 (t, 1H, H-2), 7.50 (m, 2H, H-1, H-3), 7.60 (s, 1H, H-12), 7.85 (m, 1H, H-4). FAB MS *m/z*: 380 (M + 1, 100), 277 (M + 1 − $CH_2NHCO_2C_2H_5$, 15), 263 (M + 1 − $C_2H_5NHCO_2C_2H_5$, 20), 250 (M + 1 − $C_3H_6NCO_2C_2H_5$, 35). Anal. calcd. for $C_{21}H_{21}N_3O_2S$: C 66.47, H 5.58, N 11.07. Found: C 66.40, H 5.51, N 10.88.

*4.2.6.2. 9-Chloro-6-ethoxycarbonylaminopropylquinobenzothiazine (**15b**).* (0.32 g, 77%), m.p. 121–122 °C. ^{1}H NMR ($CDCl_3$) δ: 1.24 (t, 3H, CH_3), 2.06 (m, 2H, CH_2), 3.37 (m, 2H, NCH_2), 4.07 (m, 2H, NCH_2), 4.39 (t, 2H, NCH_2), 6.89 (d, 1H, H-7), 7.08 (d, 1H, H-10), 7.13 (dd, 1H, H-8), 7.32 (t, 1H, H-2), 7.53 (m, 2H, H-1, H-3), 7.68 (s, 1H, H-12), 8.05 (m, 1H, H-4). FAB MS *m/z*: 414 (M + 1, 100), 311 (M + 1 − $CH_3NHCO_2C_2H_5$, 40), 297 (M + 1 − $C_2H_5NHCO_2C_2H_5$, 45), 284 (M + 1-$C_3H_6NHCO_2C_2H_5$, 65). Anal. calcd. for $C_{21}H_{20}ClN_3O_2S$: C 60.94, H 4.87, N 10.15. Found: C 60.73, H 4.875 N 10.01.

*4.2.6.3. 6-Ethoxycarbonylaminopropyl-9-methylthioquinobenzothiazine (**15c**).* (0.36 g, 85%), m.p. 125–126 °C. ^{1}H NMR ($CDCl_3$) δ: 1.26 (t, 3H, CH_3), 2.07 (m, 2H, CH_2), 2.45 (s, 3H, SCH_3), 3.36 (m, 2H, CH_2), 4.10 (m, 2H, CH_2), 4.34 (m, 2H, NCH_2), 6.88 (d, 1H, H-7), 6.99 (d, 1H, H-10), 7.08 (dd, 1H, H-8), 7.28 (t, 1H, H-2), 7.51 (m, 2H, H-1, H-3), 7.63 (s, 1H, H-12), 7.93 (m, 1H, H-4). FAB MS *m/z*: 426 (M + 1, 100), 296 (M + 1 − $C_3H_6NHCO_2C_2H_5$, 40). Anal. calcd. for $C_{22}H_{23}N_3O_2S_2$: C 62.09, H 5.45, N 9.87. Found: C 61.87, H 5.46, N 9.63.

*4.2.6.4. 6-Ethoxycarbonylaminobutylquinobenzothiazine (**15d**).* (0.35 g, 89%), m.p. 90–91 °C. ^{1}H NMR ($CDCl_3$) δ: 1.26 (t, 3H, CH_3), 1.70 (m, 2H, CH_2), 1.92 (m, 2H, CH_2), 3.29 (m, 2H, NCH_2), 4.10 (m, 2H, CH_2), 4.26 (m, 2H, NCH_2), 6.91 (m, 2H, H-7, H-9), 7.08 (d, 1H, H-10), 7.16 (t, 1H, H-8), 7.25 (t, 1H, H-2), 7.49 (t, 1H, H-3), 7.50 (d, 1H, H-1), 7.60 (s, 1H, H-12), 7.74 (m, 1H, H-4). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.68, 23.48, 27.36, 40.49, 44.80, 60.70, 115.74, 118.68, 120.48, 122.62, 124.13, 126.03, 126.13, 126.86, 127.27, 127.65, 129.06, 131.71, 141.34, 145.72, 152.45, 156.73. FAB MS *m/z*: 394 (M + 1, 100), 277 (M + 1 − $C_2H_5NHCO_2C_2H_5$, 20), 250 (M + 1 − $C_4H_8NCO_2C_2H_5$, 35). Anal. calcd. for $C_{22}H_{23}N_3O_2S$: C 67.15, H 5.89, N 10.68. Found: C 67.01, H 5.88, N 10.41.

*4.2.6.5. 9-Chloro-6-ethoxycarbonylaminobutylquinobenzothiazine (**15e**).* (0.34 g, 80%), m.p. 130–131 °C. ^{1}H NMR ($CDCl_3$) δ: 1.21 (m, 5H, CH_3, CH_2), 1.69 (m, 2H, CH_2), 1.89 (m, 2H, NCH_2), 3.25 (m, 2H, NCH_2), 4.06 (m, 2H, NCH_2), 6.98 (d, 1H, H-7), 7.15 (d, 1H, H-10), 7.20 (dd, 1H, H-8), 7.26 (t, 1H, H-2), 7.40 (t, 1H, H-3), 7.59 (d, 1H, H-1), 7.63 (d, 1H, H-4), 7.82 (s, 1H, H-12). FAB MS *m/z*: 428 (M + 1, 100), 297 (M + 1 − $C_3H_7NHCO_2C_2H_5$, 30), 284 (M + 1 − $C_4H_8NHCO_2C_2H_5$, 62). Anal. calcd. for $C_{22}H_{22}ClN_3O_2S$: C 61.75, H 5.18, N 9.82. Found: C 61.47, H 5.20, N 9.60.

*4.2.6.6. 6-Ethoxycarbonylaminobutyl-9-methylthioquinobenzothiazine (**15f**).* (0.37 g, 84%), m.p. 95–96 °C. ^{1}H NMR ($CDCl_3$) δ: 1.22 (t, 3H, CH_3), 1.69 (t, 2H, CH_2), 1.90 (t, 2H, CH_2), 2.46 (s, 3H, CH_3), 3.28 (m, 2H, CH_2), 4.09 (m, 2H, CH_2), 4.36 (n, 2H, NCH_2), 6.89 (d, 1H, H-7), 7.02 (d, 1H, H-10), 7.09 (d, 1H, H-8), 7.30 (t, 1H, H-2), 7.52 (d, 1H, H-1), 7.53 (t, 1H, H-3), 7.67 (s, 1H, H-12), 7.96 (d, 1H, H-4). FAB MS *m/z*: 440 (M + 1, 100), 296 (M + 1 − $C_4H_8NHCO_2C_2H_5$, 35). Anal. calcd. for $C_{23}H_{25}N_3O_2S_2$: C 62.84, H 5.73, N 9.56. Found: C 62.61, H 5.76, N 9.39.

*4.2.7. Synthesis of 9-substituted 6-chloroethylureidoalkylquinobenzothiazines **16***

To a stirred solution of 6-aminoalkyldiquinothiazines **13a–f** (1 mmol) in ethanol (25 mL) at 0 °C 2-chloroethyl isocyanate (0.16 mL, 2 mmol) was added. The mixture was stirred at 0 °C for 1 h and at rt for 24 h. After evaporation of EtOH *in vaccuo* the residue was purified by column chromatography (Al_2O_3, $CHCl_3$) to give compounds **16**.

*4.2.7.1. 6-Chloroethylureidopropylquinobenzothiazine (**16a**).* (0.27 g, 66%), m.p. 177–178 °C. ^{1}H NMR ($CDCl_3$) δ: 1.25 (m, 2H, CH2), 2.09 (m, 2H, CH_2), 3.49 (m, 2H, NCH_2), 3.50 (m, 2H, CH_2), 3.57 (m, 2H, NCH_2), 4.44 (broad s, 2H, 2NH), 6.99 (m, 2H, H-7, H-9), 7.12 (d, 1H, H-10), 7.20 (t, 1H, H-8), 7.31 (t, 1H, H-2), 7.54 (m, 2H, H-1, H-3), 7.69 (m, 1H, H-4) 7.93 (s, 1H, H-12). FAB MS *m/z*: 413 (M + 1, 40), 377 (M + 1 − HCl, 40), 277 (M + 1 − $CH_2NHCONHCH_2CH_2Cl$, 35), 263 (M + 1 − $C_2H_5NHCONHCH_2CH_2Cl$, 45), 250 (M − $C_3H_6NHCONHCH_2CH_2Cl$, 70). Anal. calcd. for $C_{21}H_{21}ClN_4OS$: C 61.08, H 5.13, N 13.57. Found: C 60.88, H 5.12, N 13.39.

*4.2.7.2. 6-Chloroethylureidopropyl-9-chloroquinobenzothiazine (**16b**).* (0.31 g, 69%), m.p. 151–152 °C. ^{1}H NMR ($CDCl_3$) δ: 1.25 (m, 2H, CH_2), 2.09 (m, 2H, CH_2), 3.43 (m, 2H, NCH_2), 3.52 (m, 2H, NCH_2), 3.59 (broad s, 2H, 2NH), 4.59 (t, 2H, NCH_2), 7.10 (d, 1H, H-7), 7.21 (d, 1H, H-10), 7.29 (d, 1H, H-8), 7.55 (t, 1H, H-2), 7.66 (d, 1H, H-1), 7.75 (m, 2H, H-3, H-4), 7.97 (s, 1H, H-12). FAB MS *m/z*: 448 (M + 1, 21) 411 (M + 1 − HCl, 100), 368 (M − $NH_2CH_2CH_2Cl$, 22), 325 (M − $NH_2CONHCH_2CH_2Cl$, 38), 284 (M + 1 − $C_3H_6NHCONHCH_2CH_2Cl$, 43). Anal. calcd. for $C_{21}H_{20}Cl_2N_4OS$: C 56.38, H 4.51, N 12.52. Found: C 56.19, H 4.53, N 12.37.

*4.2.7.3. 6-Chloroethylureidopropyl-9-methylthioquinobenzothiazine (**16c**).* (0.30 g, 66%), m.p. 167–168 °C. ^{1}H NMR ($CDCl_3$) δ: 1.25 (m, 2H, CH_2), 2.06 (m, 2H, CH_2), 2.46 (s, 3H, SCH_3), 3.48 (m, 2H, CH_2), 3.49 (m, 2H, CH_2), 3.55 (t, 2H, NCH_2), 4.50 (broad s, 2H, 2NH), 6.92 (d, 1H, H-7), 7.02 (d, 1H, H-10), 7.08 (d, 1H, H-8), 7.34 (t, 1H, H-2), 7.54 (d, 1H, H-1), 7.58 (t, 1H, H-3), 7.72 (s, 1H, H-12), 8.14 (m, 1H, H-4). FAB MS *m/z*: 458 (M^+, 100), 459 (M + 1, 90), 380 (M − NHC_2H_4Cl, 30), 323 (M − $CH_2NHCONHC_2H_4Cl$, 10), 310 (M + 1 − $C_2H_4NHCONHC_2H_4Cl$, 20), 296 (M + 1 − $C_3H_6NHCONHC_2H_4Cl$, 40). Anal. calcd. for $C_{22}H_{23}ClN_4OS_2$: C 57.57, H 5.05, N 12.21. Found: C 57.38, H 5.07, N 12.00.

*4.2.7.4. 6-Chloroethylureidobutylquinobenzothiazine (**16d**).* (0.28 g, 66%), m.p. 132–133 °C. ^{1}H NMR ($CDCl_3$) δ: 1.67 (m, 2H, CH_2), 1.89 (m, 2H, CH_2), 3.27 (m, 2H, CH_2), 3.47 (m, 2H, CH_2), 3.56 (m, 2H, NCH_2), 4.23 (m, 2H, NCH_2), 4.96 (broad s, 2H, 2NH), 6.91 (m, 2H, H-7, H-9), 7.06 (m, 1H, H-10), 7.15 (t, 1H, H-8), 7.24 (t, 1H, H-2), 7.47 (t, 1H, H-3), 7.48 (d, 1H, H-1), 7.58 (s, 1H, H-12), 7.69 (m, 1H, H-4). FAB MS *m/z*: 427 (M + 1, 100), 391 (M + 1 − HCl, 35), 263 (M + 1 − $C_3H_7NHCONHCH_2CH_2Cl$, 30), 250 (M + 1 − $C_4H_8NHCONHCH_2CH_2Cl$, 80). Anal. calcd. for $C_{22}H_{23}ClN_4OS$: C 61.89, H 5.43, N 13.12. Found: C 61.62, H 5.44, N 12.87.

*4.2.7.5. 6-Chloroethylureidobutyl-9-chloroquinobenzothiazine (**16e**).* (0.29 g, 63%), m.p. 164–165 °C. ^{1}H NMR ($CDCl_3$) δ: 1.25 (m, 2H, CH_2), 1.88 (m, 2H, CH_2), 1.99 (m, 4H, $2CH_2$), 3.50 (m, 2H, NCH_2), 3.71 (m, 2H, NCH_2), 4.75 (broad s, 2H, 2NH), 7.10 (d, 1H, H-7), 7.35 (dd, 1H, H-8), 7.59 (m, 2H, H-2, H-10), 7.71 (d, 1H, H-1), 7.82 (m, 2H, H-3, H-4), 8.06 (s, 1H, H-12). FAB MS *m/z*: 462 (M + 1, 3), 425 (M − HCl, 100), 382 (M − $NH_2CH_2CH_2Cl$, 6), 297 (M + 1 − $C_3H_7NHCONHCH_2CH_2Cl$, 16), 284 (M + 1 − $C_4H_8NHCONHCH_2CH_2Cl$, 38). Anal. calcd. for $C_{22}H_{22}Cl_2N_4OS$: C 57.27, H 4.81, N 12.14. Found: C 57.20, H 4.82, N 11.93.

*4.2.7.6. 6-Chloroethylureidobutyl-9-methylthioquinobenzothiazine (**16f**).* (0.32 g, 68%), m.p. 129–130 °C. ^{1}H NMR ($CDCl_3$) δ: 1.25 (m, 2H, CH_2), 1.71 (m, 2H, CH_2), 1.92 (m, 2H, CH_2), 2.46 (s, 3H, CH_3), 3.32 (m, 2H, NCH_2), 3.50 (m, 2H, CH_2), 3.58 (m, 2H, NCH_2), 4.40 (broad s, 2H, 2NH), 6.92 (d, 1H, H-7), 7.03 (d, 1H, H-10), 7.12 (d, 1H, H-8), 7.27

(t, 1H, H-2), 7.39 (t, 1H, H-3), 7.55 (m, 2H, H-1, H-4), 7.72 (s, 1H, H-12). FAB MS *m/z*: 473 (M + 1, 98), 472 (M^+, 100), 437 (M + 1 − HCl), 394 (M − $NHCH_2CH_2Cl$, 35), 309 (M + 1 − $C_3H_7NHCONHCH_2CH_2Cl$, 40), 296 (M + 1 − $C_4H_8NHCONHCH_2CH_2Cl$, 100). Anal. calcd. for $C_{23}H_{25}ClN_4OS_2$: C 58.40, H 5.33, N 11.84. Found: C 58.29, H 5.35, N 11.61.

4.2.8. Synthesis of 9-substituted 6-methanesulfonylaminoalkylquinobenzothiazines ***17***

To a stirred solution of aminoalkyldiquinothiazines **13a–f** (1 mmol) in a mixture of CH_2Cl_2 (10 mL) and 10% Na_2CO_3 solution (14 mL), a solution of methanesulfonyl chloride (0.12 mL, 1.5 mmol) was added. The mixture was stirred at rt for 24 h. The organic phase was separated and aqueous phase was extracted with CH_2Cl_2 (2 × 5 mL). The combined extracts were washed with water (2 × 10 mL) and dried over Na_2SO_4. The drying agent was filtered off and filtrate was evaporated. The resulting residue was purified by column chromatography (Al_2O_3, $CHCl_3$) to give compounds **17**.

*4.2.8.1. 6-Methanesulfonylaminopropylquinobenzothiazine (**17a**).* (0.28 g, 73%), m.p. 117–118 °C. ^{1}H NMR ($CDCl_3$) δ: 2.16 (m, 2H, CH_2), 2.86 (s, 3H, CH_3), 3.31 (m, 2H, NCH_2), 4.53 (m, 2H, NCH_2), 5.99 (s, 1H, NH), 6.97 (m, 2H, H-7, H-9), 7.07 (d, 1H, H-10), 7.17 (t, 1H, H-8), 7.30 (t, 1H, H-2), 7.51 (d, 1H, H-1), 7.55 (t, 1H, H-3), 7.63 (s, 1H, H-12), 7.92 (m, 1H, H-4). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 27.35, 39.97, 40.86, 41.82, 115.50, 118.06, 119.90, 123.06, 124.59, 126.08, 126.20, 126.53, 126.82, 127.76, 129.69, 131.88, 140.55, 145.22, 151.96. FAB MS *m/z*: 386 (M + 1, 100), 277 (M + 1 − $CH_2NHSO_2CH_3$, 35), 250 (M + 1 − $C_3H_6NHSO_2CH_3$, 60). Anal. calcd. for $C_{19}H_{19}N_3O_2S_2$: C 59.20, H 4.97, N 10.90. Found: C 59.03, H 4.92, N 10.67.

*4.2.8.2. 9-Chloro-6-methanesulfonylaminopropylquinobenzothiazine (**17b**).* (0.32 g, 76%), m.p. 129–130 °C. ^{1}H NMR ($CDCl_3$) δ: 2.14 (m, 2H, CH_2), 2.87 (s, 3H, CH_3), 3.30 (m, 2H, NCH_2), 4.44 (t, 2H, NCH_2), 6.88 (d, 1H, H-7), 7.06 (d, 1H, H-10), 7.11 (dd, 1H, H-8), 7.32 (t, 1H, H-2), 7.53 (d, 1H, H-1), 7.56 (t, 1H, H-3), 7.65 (s, 1H, H-12), 7.97 (m, 1H, H-4). FAB MS *m/z*: 420 (M + 1, 100), 311 (M + 1 − $CH_3NHSO_2CH_3$, 25), 297 (M + 1 − $C_2H_5NHSO_2CH_3$, 30), 284 (M + 1-$C_3H_6NHSO_2CH_3$, 50). Anal. calcd. for $C_{19}H_{18}ClN_3O_2S_2$: C 54.34, H 4.32, N 10.01. Found: C 54.09, H 4.34, N 9.82.

*4.2.8.3. 6-Methanesulfonylaminopropyl-9-methylthioquinobenzothiazine (**17c**).* (0.33 g, 77%), m.p. 100–101 °C. ^{1}H NMR ($CDCl_3$) δ: 2.13 (m, 2H, CH_2), 2.45 (s, 3H, CH_3), 2.86 (s, 3H, SCH_3), 3.29 (m, 2H, CH_2), 4.41 (m, 2H, NCH_2), 6.88 (d, 1H, H-7), 6.97 (d, 1H, H-10), 7.05 (d, 1H, H-8), 7.28 (t, 1H, H-2), 7.49 (d, 1H, H-1), 7.53 (t, 1H, H-3), 7.61 (s, 1H, H-12), 7.91 (m, 1H, H-4). FAB MS *m/z*: 432 (M + 1, 100), 353 (M + 1 − SO_2CH_3, 10), 323 (M − $CH_2NHSO_2CH_3$, 25), 309 (M − $C_2H_4NHSO_2CH_3$, 30), 296 (M + 1 − $C_3H_6NHSO_2CH_3$, 60). Anal. calcd. for $C_{20}H_{21}N_3O_2S_3$: C 55.66, H 4.90, N 9.74. Found: C 55.48, H 4.88, N 9.59.

*4.2.8.4. 6-Methanesulfonylaminobutylquinobenzothiazine (**17d**).* (0.27 g, 68%), m.p. 97–98 °C. ^{1}H NMR ($CDCl_3$) δ: 1.78 (m, 2H, CH_2), 1.96 (m, 2H, CH_2), 2.93 (s, 3H, CH_3), 3.25 (m, 2H, NCH_2), 4.34 (m, 2H, NCH_2), 4.76 (m, 1H, NH), 6.95 (m, 2H, H-7, H-9), 7.10 (d, 1H, H-10), 7.18 (t, 1H, H-8), 7.28 (t, 1H, H-2), 7.51 (m, 2H, H-1, H-3), 7.64 (s, 1H, H-12), 7.85 (m, 1H, H-4). FAB MS *m/z*: 400 (M + 1, 100), 263 (M − $C_3H_6NHSO_2CH_3$, 25), 250 (M + 1 − $C_4H_8NHSO_2CH_3$, 50). Anal. calcd. for $C_{20}H_{21}N_3O_2S_2$: C 60.13, H 5.30, N 10.52. Found: C 59.96, H 5.29, N 10.36.

*4.2.8.5. 9-Chloro-6-methanesulfonylaminobutylquinobenzothiazine (**17e**).* (0.32 g, 74%), m.p. 163–164 °C. ^{1}H NMR ($CDCl_3$) δ: 1.83 (m, 2H, CH_2), 2.10 (m, 2H, CH_2), 2.96 (s, 3H, CH_3), 3.27 (m, 2H, NCH_2), 4.70 (m, 2H, NCH_2), 7.06 (d, 1H, H-7), 7.18 (d, 1H, H-10), 7.47 (m, 2H, H-2, H-8), 7.62 (d, 1H, H-1), 7.70 (m, 2H, H-3, H-4), 7.90 (s, 1H, H-12). FAB MS *m/z*: 434 (M + 1, 100), 297 (M − $C_3H_6NHSO_2CH_3$, 19), 284 (M + 1 − $C_4H_8NHSO_2CH_3$, 43). Anal. calcd. for $C_{20}H_{20}ClN_3O_2S_2$: 2 55.35, H 4.65, N 9.68. Found: C 55.21, H 4.65, N 9.49.

*4.2.8.6. 6-Methanesulfonylaminobutyl-9-methylthioquinobenzothiazine (**17f**).* (0.34 g, 76%), m.p. 119–120 °C. ^{1}H NMR ($CDCl_3$) δ: 1.78 (m, 2H, CH_2), 1.94 (m, 2H, CH_2), 2.46 (s, 3H, SCH_3), 2.94 (s, 3H, CH_3), 3.25 (m, 2H, CH_2), 4.32 (m, 2H, NCH_2), 6.86 (d, 1H, H-7), 7.05 (d, 1H, H-10), 7.09 (d, 2H, H-8), 7.27 (t, 1H, H-2), 7.51 (m, 2H, H-1, H-3), 7.65 (s, 1H, H-12), 7.78 (m, 1H, H-4). FAB MS *m/z*: 446 (M + 1, 100), 309 (M − $C_3H_6NHSO_2CH_3$, 15), 296 (M + 1 − $C_4H_8NHSO_2CH_3$, 40). Anal. calcd. for $C_{21}H_{23}N_3O_2S_3$: C 56.60, H 5.20, N 9.43. Found: C 56.40, H 5.21, N 9.28.

4.2.9. Synthesis of 9-substituted 6-p-toluenesulfonylaminolkylquinobenzothiazines ***18***

To a stirred solution of aminoalkyldiquinothiazines **13a–f** (1 mmol) in a mixture of CH_2Cl_2 (6 mL) and 10% Na_2CO_3 solution (7 mL), a solution of *p*-toluenesulfonyl chloride (0.28 g, 1.5 mmol) in CH_2Cl_2 (6 mL) was added. The mixture was stirred at rt for 24 h. The organic phase was separated and aqueous phase was extracted with CH_2Cl_2 (2 × 5 mL). The combined extracts were washed with water (2 × 10 mL) and dried over Na_2SO_4. The drying agent was filtered off and filtrate was evaporated. The resulting residue was purified by column chromatography (Al_2O_3, $CHCl_3$) to give: compounds **18**.

*4.2.9.1. 6-p-Toluenesulfonylaminopropylquinobenzothiazine (**18a**).* (0.36 g, 78%), m.p. 68–69 °C. ^{1}H NMR ($CDCl_3$) δ: 2.02 (m, 2H, CH_2), 2.35 (s, 3H, CH_3), 3.11 (m, 2H, NCH_2), 4.24 (m, 2H, NCH_2), 6.86 (d, 1H, H-7), 6.91 (t, 1H, H-9), 7.02 (d, 1H, H-10), 7.10 (t, 1H, H-8), 7.14 (d, 2H, $2H_{benz.}$), 7.29 (t, 1H, H-2), 7.49 (d, 1H, H-1), 7.56 (m, 2H, H-3, H-12), 7.61 (d, 2H, $2H_{benz.}$), 7.92 (m, 1H, H-4). FAB MS *m/z*: 462 (M + 1, 100), 277 (M + 1 − $CH_2NHSO_2C_6H_4CH_3$, 60), 263 (M + 1 − $C_2H_5NHSO_2C_6H_4CH_3$, 70), 251 (M + 1 − $C_3H_5NHSO_2C_6H_4CH_3$, 96), 250 (M + 1 − $C_3H_6NHSO_2C_6H_4CH_3$, 90). Anal. calcd. for $C_{25}H_{23}N_3O_2S_2$: C 65.05, H 5.02, N 9.10. Found: C 64.81, H 5.00, N 8.95.

*4.2.9.2. 9-Chloro-6-p-toluenesulfonylaminopropylquinobenzothiazine (**18b**).* (0.38 g, 77%), m.p. 116–117 °C. ^{1}H NMR ($CDCl_3$) δ: 2.01 (m, 2H, CH_2), 2.36 (s, 3H, CH_3), 3.11 (m, 2H, NCH_2), 4.29 (t, 2H, NCH_2), 6.86 (d, 1H, H-7), 7.04 (d, 1H, H-10), 7.10 (dd, 1H, H-8), 7.16 (d, 2H, $2H_{benz.}$), 7.34 (t, 1H, H-2), 7.52 (d, 1H, H-1), 7.59 (t, 1H, H-3), 7.63 (m, 3H, H-12, $2H_{benz.}$), 8.03 (d, 1H, H-4). FAB MS *m/z*: 496 (M + 1, 100), 311 (M + 1 − $CH_3NHSO_2C_6H_4CH_3$, 35), 297 (M + 1 − $C_2H_5NHSO_2C_6H_4CH_3$, 30), 284 (M + 1 − $C_3H_6NHSO_2C_6H_4CH_3$, 45). Anal. calcd. for $C_{25}H_{22}ClN_3O_2S_2$: C 60.53, H 4.47, N 8.47. Found: C 60.45, H 4.42, N 8.33.

*4.2.9.3. 9-Methylthio-6-p-toluenesulfonylaminopropylquinobenzothiazine (**18c**).* (0.40 g, 79%), m.p. 70–71 °C. ^{1}H NMR ($CDCl_3$) δ: 2.00 (m, 2H, CH_2), 2.35 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 3.10 (m, 2H, CH_2), 4.29 (m, 2H, NCH_2), 6.79 (d, 1H, H-7), 6.94 (d, 1H, H-10), 7.01 (d, 1H, H-8), 7.14 (d, 2H, $2H_{benz.}$), 7.31 (t, 1H, H-2), 7.50 (d, 1H, H-1), 7.58 (m, 2H, H-3, H-12) 7.65 (d, 2H, $2H_{benz.}$), 7.93 (d, 1H, H-4). FAB MS *m/z*: 508 (M + 1, 100), 323 (M − $CH_2NHSO_2C_6H_4CH_3$, 25), 309 (M − $C_2H_4NHSO_2C_6H_4CH_3$, 30), 296 (M + 1 − $C_3H_6NHSO_2C_6H_4CH_3$, 40). Anal. calcd. for $C_{26}H_{25}N_3O_2S_3$: C 61.51, H 4.96, N 8.28. Found: C 61.38, H 4.94, N 8.09.

*4.2.9.4. 6-p-Toluenesulfonylaminobutylquinobenzothiazine (**18d**).* (0.35 g, 74%), m.p. 63–64 °C. ^{1}H NMR ($CDCl_3$) δ: 1.66 (m, 2H, CH_2),

2.39 (m, 2H, CH_2), 2.39 (s, 3H, CH_3), 3.07 (m, 2H, NCH_2), 4.19 (m, 2H, NCH_2), 4.65 (m, 1H, NH), 6.87 (d, 1H, H-7), 6.92 (t, 1H, H-9), 7.08 (d, 1H, H-10), 7.15 (t, 1H, H-8), 7.25 (m, 3H, H-2, $2H_{benz.}$), 7.49 (m, 2H, H-1, H-3), 7.61 (s, 1H, H-12), 7.71 (m, 3H, H-4, $2H_{benz.}$). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 21.53, 23.30, 26.96, 42.87, 44.48, 115.73, 118.62, 120.45, 122.69, 124.19, 126.01, 126.15, 126.86, 127.08, 127.22, 127.71, 129.14, 129.70, 131.74, 136.99, 141.20, 143.34, 145.64, 152.38. FAB MS *m/z*: 476 (M + 1, 100), 369 (M + 1 − $OC_6H_4CH_3$, 25), 277 (M + 1 − $C_2H_4NHSO_2C_6H_4CH_3$, 20), 250 (M + 1 − $C_4H_8NHSO_2C_6H_4CH_3$, 20). Anal. calcd. for $C_{26}H_{25}N_3O_2S_2$: C 65.66, H 5.30, N 8.83. Found: C 65.46, H 5.26, N 8.59.

*4.2.9.5. 9-Chloro-6-p-toluenesulfonylaminobutylquinobenzothiazine (**18e**).* (0.38 g, 75%), m.p. 140–141 °C. ^1H NMR ($CDCl_3$) δ: 1.68 (m, 2H, CH_2), 1.88 (m, 2H, CH_2), 2.40 (s, 3H, CH_3), 3.05 (m, 2H, NCH_2), 4.34 (m, 2H, NCH_2), 6.86 (d, 1H, H-7), 7.09 (d, 1H, H-10), 7.14 (d, 1H, H-8), 7.25 (m, 2H, $2H_{benz.}$), 7.34 (t, 1H, H-2), 7.55 (d, 1H, H-1), 7.56 (t, 1H, H-3), 7.72 (m, 3H, H-12, $2H_{benz.}$), 8.04 (d, 1H, H-4). FAB MS *m/z*: 510 (M + 1, 100), 297 (M + 1 − $C_3H_7NHSO_2C_6H_4CH_3$, 21), 284 (M + 1 − $C_4H_8NHSO_2C_6H_4CH_3$, 50). Anal. calcd. for $C_{26}H_{24}ClN_3O_2S_2$: C 61.22, H 4.74, N 8.24. Found: C 61.01, H 4.71, N 8.07.

*4.2.9.6. 9-Methylthio-6-p-toluenesulfonylaminobutylquinobenzothiazine (**18f**).* (0.42 g, 81%), m.p. 119–120 °C. ^1H NMR ($CDCl_3$) δ: 1.64 (m, 2H, CH_2), 1.83 (m, 2H, CH_2), 2.38 (s, 3H, CH_3), 2.44 (s, 3H, SCH_3), 3.05 (m, 2H, CH_2), 4.16 (m, 2H, NCH_2), 6.78 (d, 1H, H-7), 6.99 (d, 1H, H-10), 7.06 (d, 1H, H-8), 7.25 (m, 3H, H-2, $2H_{benz.}$), 7.49 (m, 1H, H-1, H-3), 7.59 (s, 1H, H-12), 7.72 (m, 5H, H-12, $2H_{benz.}$). FAB MS *m/z*: 522 (M + 1, 100), 521 (M^+, 55), 309 (M-$C_3H_7NHSO_2C_6H_4CH_3$, 40). Anal. calcd. for $C_{27}H_{27}N_3O_2S_3$: C 62.16, H 5.22, N 8.05. Found: C 62.01, H 5.19, N 7.81.

4.3. Biological assays

4.3.1. Preparation of the compounds for biological assays

The compounds were dissolved in DMSO (10 mg/mL) and subsequently diluted in RPMI-1640 cell culture medium (see below).

4.3.2. Isolation of the peripheral blood mononuclear cells (PBMC)

Venous blood from a single donor was withdrawn into heparinized syringes and diluted twice with PBS. PBMC were isolated by centrifugation on Ficoll-uropoline gradient (density 1.077 g/mL) and centrifuged at 800× *g* for 20 min at 4 °C. The interphase cells, consisting of lymphocytes (20%) and monocytes (80%) were then washed three times with Hanks' medium and re-suspended in a culture medium, referred to below as the culture medium, consisting of RPMI-1640, supplemented with 10% fetal calf serum, L-glutamine, sodium pyruvate, 2-mercaptoethanol and antibiotics, at density of 2×10^6 cells/mL.

4.3.3. PHA-induced proliferation of human blood mononuclear cells

The isolated PBMC were distributed into 96-well flat-bottom plates in 100 μL aliquots (2×10^5cells/well). PHA was added at a concentration of 5 μg/mL. The compounds were tested at doses of 10 and 50 μg/mL. DMSO at appropriate dilutions served as control. After a four-day incubation in a cell culture incubator, the proliferative response of the cells was determined by the colorimetric MTT method [31]. The results are given in percentage inhibition as compared with appropriate DMSO controls.

4.3.4. Cytotoxicity of the compounds against human blood mononuclear cells

PBMC at density of 2×10^5/well, re-suspended in the culture medium, were cultured for 24 h in a cell culture incubator with the preparations at indicated concentrations. Cell survival was determined by MTT colorimetric method [31]. The results are given in percentage inhibition as compared with appropriate DMSO controls.

4.3.5. Lipopolysaccharide-induced TNF-a production in whole blood cell culture

Venous blood from a single donor was diluted 10× with RPMI-1640 medium and distributed in 1 mL aliquots in 24-well culture plates. The cultures were stimulated by addition of 1 μg/mL of LPS from *Escherichia coli*, O111:B4. The compounds were added to the cultures at concentrations of 5 and 25 μg/mL. Higher concentrations of the compounds could not be used because of inhibitory effects on TNF-α production by corresponding DMSO (the solvent) dilutions. Appropriate dilutions of DMSO served as controls. After overnight incubation in a cell culture incubator, the supernatants were harvested and frozen at −20 °C until cytokine determination by a biological assay [32]. The results are given in percentage inhibition as compared with appropriate DMSO controls.

4.3.6. Growth inhibition of tumor cell lines

A-431 epidermal cell line, L-1210 lymphoma and SW-948 colon tumor cell lines derived from the Collection of Cell Lines of The Institute of Immunology and Experimental Therapy, Wrocław, Poland. The lines were re-suspended in the culture medium and distributed into 96-well flat-bottom plates. L-1210 was present at 1.5×10^4 cells/well while SW-948 and A-431 at 2.5×10^4 cells/well. The preparations were added to the wells at the concentration range of 0.1–50 μg/mL. Cisplatin was used as a reference drug in the same concentrations. After 3-day incubation in a cell culture incubator, the proliferation was determined using MTT colorimetric method. The data are presented as a mean OD value from quadruplicate wells ±SE.

4.3.7. Statistics

The results are presented as mean values ± standard error (SE) or percentage inhibition = [(control value − tested value)/control value]*100. Brown–Forsyth's test was used to determine the homogeneity of variance between groups. When the variance was homogenous, analysis of variance (One-way ANOVA) was applied, followed by *post hoc* comparisons with the Tukey's test to estimate the significance of the difference between groups. Nonparametric data were evaluated with the Kruskal–Wallis' analysis of variance. Significance was determined at $p < 0.05$. Statistical analysis was performed using STATISTICA 6.1 for Windows.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.02.023.

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