Polish Journal of Microbiology
2014, Vol. 63, No 3, 335–339

ORIGINAL PAPER

Antimicrobial Properties of Substituted Quino[3,2-b]benzo[1,4]thiazines

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Submitted 22 November 2013, revised 24 June 2014, accepted 30 July 2014

Abstract

Our previous studies demonstrated that among phenothiazines several derivatives could be found showing strong antiproliferative actions and the property of inhibiting inducible tumor necrosis factor alpha (TNF α) production in human blood cultures. The aim of this investigation was to determine potential antimicrobial actions of forty four new phenothiazine derivatives with the quinobenzothiazine structure. The compounds showed differential antibacterial and antifungal activities against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* depending on the compound structures, concentrations and bacterial strains. More specifically, 6-(1-methyl-2-piperidylethyl) quinobenzothiazine displayed strongest actions against *S. aureus* and *E. coli* whereas 6-methanesulfonylaminobutyl-9-methylthioquinobenzothiazine exhibited the most universal antimicrobial properties. The correlation between antimicrobial activity and the chemical structure of quinobenzothiazines was discussed.

K e y w o r d s: phenothiazines, azaphenothiazines, antibacterial activity, antifungal activity, structure-activity relationship

Introduction

Phenothiazines exhibit wide spectrum of significant biological activities and belong to recognized antipsychotic, antihistaminic, antitussive and antiemetic drugs (Gupta and Kumar, 1988). The introduction of new substituents into the phenothiazine skeleton as well as the modification of the tricyclic ring system alter biological activities. Both classical and new modified phenothiazines were recently reported to have promising: anticancer, antibacterial, antiviral and anti-inflammatory activities (Gaye-Seye *et al.*, 2006; Motohashi *et al.*, 2006; Dasgupta *et al.*, 2008; Aaron *et al.*, 2009; Pluta *et al.*, 2011; Jaszczyszyn *et al.*, 2012). They are able to modify multidrug resistance and may be potentially used in the treatment of Alzheimer's, Creutzfeldt-Jakob's and AIDS diseases (Amaral and Kristiansen, 2001; Pluta *et al.*, 2011; Jaszczyszyn *et al.*, 2012). The antimicrobial activity was first observed for the phenothiazine compound, chlorpromazine, against *Bacillus anthracis* in 1976 (Molnar *et al.*, 1976). During over three decades prominent classical phenothiazines such as: chlorpromazine, promethazine, trifluoperazine, fluphenazine, levomepromazine, diethazine, trimeprazine and thioridazine were broadly examined for their antimicrobial activities. These results were collected in an excellent chapter written by Dasgupta *et al.* (2008). Antimicrobial potencies were reported for all examined phenothiazines. They were active both *in vitro* and *in vivo* and some of them could act in synergy with antibiotics against many genera, including enterobacteria, staphylococci and mycobacteria. Newly synthesized phenothiazines, possessing tri-, tetra- and pentacyclic ring system with various substituents (acyclic or cyclic) at the thiazine and/or carbon atoms, exhibited antibacterial and antifungal activities, in some cases equivalent or even more active than the reference drugs such as: ciprofloxacin, streptomycin, gentamycin, ampicillin, fluconazole and other (Singh *et al.*, 2003; Raval and Desai, 2005; Srivastava and Kohli, 2007; Swarnkar *et al.*, 2007; Bansode *et al.*, 2009a and 2009b; Dixit *et al.*, 2009; Salvi *et al.*, 2009).

We modified the phenothiazine structure by introduction of the pyridine and quinoline rings, instead of the benzene ones, to form tricyclic, tetracyclic and pentacyclic azaphenothiazines (Pluta *et al.*, 2000; Morak and Pluta, 2007; Nowak *et al.*, 2007; Jeleń and Pluta, 2008; 2009). Some of diquinothiazines and quinobenzothiazines

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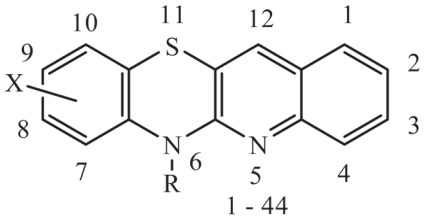


Fig. 1. The structure of quinobenzothiazines

exhibited significant anticancer activities against human tumor cell lines deriving from: colon, breast, kidney, ovary, prostate, central nervous system, melanoma and leukemia (Pluta *et al.*, 2010; Jeleń *et al.*, 2013). They also inhibited lipopolysaccharide-induced production of tumor necrosis factor alpha (TNF α) (Zimecki *et al.*, 2009; Jeleń *et al.*, 2013). Another azaphenothiazine, 10*H*-dipyridothiazine, was found to be an universal, low-toxic immunosuppressant, inhibiting both humoral and cellular immune response (Zimecki *et al.*, 2009). Recently obtained, angularly fused quinobenzothiazinones and quinobenzothiazinium salts, exhibited antioxidant, antiproliferative and antimicrobial activities (Kumar *et al.*, 2010; Zięba *et al.*, 2010; 2012; 2013).

Promising antimicrobial activities of recently synthesized phenothiazines prompted us to test forty four linearly fused quino[3,2-b]benzo[1,4]thiazines with various substituents for their potential antibacterial and antifungal activities (Fig. 1. and Table I).

Experimental

Materials and Methods

In the studies the following compound concentrations were used: 500 µg/ml, 250 µg/ml and 125 µg/ml. The compounds were initially dissolved in DMSO (dimethyl sulfoxide) at 1 mg/ml concentration, subsequent dilutions were prepared in water. The experiments were conducted on strains derived from the Polish Collection of Microorganisms (PCM), IITD, PAN, Wrocław or from ATCC – (The American Type Culture Collection): *Escherichia coli* PCM 2057, *Pseudomonas aeruginosa* PCM 2058, *Staphylococcus aureus* PCM 2054, *Candida albicans* ATCC 90028. The following culture media were used: nutrient broth, nutrient

Table I
The substituents of the quinobenzothiazines shown in Fig. 1

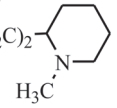
No	X	R	No	X	R
1	H	H	24	H	$(CH_2)_4NHCOOC_2H_5$
2	8-Cl	H	25	9-Cl	
3	8-Br	H	26	9-SCH_3	
4	8-CF_3	H	27	H	$(CH_2)_3NHCONHCH_2CH_2Cl$
5	9-CH_3	H	28	9-Cl	
6	9-Cl	H	29	9-SCH_3	
7	9-Br	H	30	H	$(CH_2)_4NHCONHCH_2CH_2Cl$
8	9-CF_3	H	31	9-Cl	
9	9-SCH_3	H	32	9-SCH_3	
10	10-Cl	H	33	H	$(CH_2)_3NHSO_2CH_3$
11	10-Br	H	34	9-Cl	
12	10-CF_3	H	35	9-SCH_3	
13	H	$(CH_2)_3N(C_2H_5)_2$	36	H	$(CH_2)_4NHSO_2CH_3$
14	H		37	9-Cl	
			38	9-SCH_3	
15	H	$(CH_2)_3NHCOCH_3$	39	H	$(CH_2)_3NHSO_2C_6H_4CH_3$
16	9-Cl		40	9-Cl	
17	9-SCH_3		41	9-SCH_3	
18	H	$(CH_2)_4NHCOCH_3$	42	H	$(CH_2)_4NHSO_2C_6H_4CH_3$
19	9-Cl		43	9-Cl	
20	9-SCH_3		44	9-SCH_3	
21	H	$(CH_2)_3NHCOOC_2H_5$			
22	9-Cl				
23	9-SCH_3				

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Table II
Summary of the antibacterial and antifungal effects by selected compounds (concentrations in μg/ml)

Nr	*E. coli*			*P. aeruginosa*			*S. aureus*			*C. albicans*		
	125	250	500	125	250	500	125	250	500	125	250	500
14	–	++	++	–	–	+	–	++	++	–	–	++
15	–	–	–	–	–	–	++	++	++	–	–	++
18	–	–	–	–	–	++	++	++	++	–	–	–
19	–	–	–	–	–	+	++	++	++	–	–	–
28	–	+	++	–	–	–	++	++	++	–	–	–
31	–	–	++	–	–	–	++	++	++	–	–	–
32	–	–	–	–	–	–	–	+	++	–	–	–
36	–	–	–	–	–	–	+	+	+		–	–
38	–	–	+	–	+	++	+	++	++	–	++	++

Degree of antibacterial and antifungal actions: – inactive, + weak active (opaque zone), ++ active (clear zone of growth inhibition)

agar, Sabouraud broth, Sabouraud agar and 0.9% NaCl. The media were from BTL, Łódź, Poland.

Preliminary studies were aimed at selection of the most active compounds. Precultures of tested strains of *E. coli*, *P. aeruginosa* and *S. aureus* in broth were incubated at 37°C, and *C. albicans* in Sabouraud's broth at 28°C. After an overnight incubation the bacterial cultures were diluted 10 × with broth and the yeast cultures with Sabouraud's broth. The suspensions (100 μl) of the microorganisms were plated onto nutrient agar or Sabouraud's agar. The plates were dried and 20 μl of the studied compound solutions at concentrations of: 500 μg/ml, 250 μg/ml and 125 μg/ml were applied. Control plates were treated with appropriate dilutions of DMSO. The plates were incubated for 24 h and the growth of bacterial or *Candida* colonies was evaluated. When the sample solution contained antimicrobial activity, a clear zone of growth inhibition on the agar was observed. Each test was performed in triplicate. The results are shown in Table II.

For further tests the most active compounds were chosen and the method of dilutions was applied. Precultures of *E. coli*, *P. aeruginosa* and *S. aureus* strains in the culture broth were incubated for 18–20 h at 37°C.

Table III
The antibacterial action of selected phenothiazines on growth of *E. coli* in culture

Compounds	Number of CFU × 10^7/ml Concentration (μg/ml)		
	500	250	125
14	1.2	3.01	12
31	4.2	8.9	10.1
38	5.7	9.5	9.9
Chlorpromazine	0	0.025	3.8

CFU – colony forming units; T_0 control – 4.2×10^7; T_{24} control – 18×10^7; 50% DMSO solution in water – 4.6×10^7; 25% DMSO solution in water – 9.8×10^7; 12.5% DMSO solution in water – 13×10^7.

Into wells of 24-well culture plates 1 ml of the bacterial culture liquid was applied, followed by addition of the tested compounds at appropriate concentrations. After 24 h incubation 100 μl of the bacteria culture was aspirated, diluted with 0.9% NaCl and seeded (100 μl) onto agar plates. After an overnight incubation at 37°C the numbers of colonies (colony forming units) were enumerated. The control cultures included: 1) no tested compounds, bacteria seeded at time „0 h", 2) no tested compounds, bacteria seeded at time "24 h",

Table IV
Antibacterial actions of selected phenothiazines on growth of *P. aeruginosa*

Compounds	Number of CFU × 10^7/ml Concentration (μg/ml)		
	500	250	125
18	7.8	8.4	9.5
19	7.5	8.5	9.1
38	3.2	4.6	8.1
Chlorpromazine	0.09	1.1	1.2

CFU – colony forming units; T_0 control – 6.2×10^7; T_{24} control – 13×10^7; 50% DMSO solution in water – 7.2×10^7; 25% DMSO solution in water – 7.9×10^7; 12.5% DMSO solution in water – 8.9×10^7.

Table V
The antibacterial action of selected phenothiazines on *S. aureus*

Compounds	Number of CFU × 10^7/ml Concentration (μg/ml)		
	500	250	125
14	1.2	3.01	12
31	4.22	8.9	10.1
38	5.7	9.5	9.9
Chlorpromazine	0	0.025	3.8

CFU – colony forming units; T_0 control – 4.2×10^7; T_{24} control – 18×10^7; 50% DMSO solution in water – 4.6×10^7; 25% DMSO solution in water – 9.8×10^7; 12.5% DMSO solution in water – 13×10^7.

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3) containing appropriate DMSO dilutions (50, 25 and 12.5%) incubated for 24 h, 4) containing chlorpromazine chloride at 500, 250 and 125 μg/ml (a reference drug). The results are presented in Tables III–V.

Results

The compounds investigated in this report represented three types of substituted azaphenothiazines: 8–10-substituted 6*H*-quinobenzothiazines (**1–12**), 6-substituted quinobenzothiazines (**13–15, 18, 21, 24, 27, 30, 33, 36, 39, 42**) and 6,9-disubstituted quinobenzothiazines (**16, 17, 19, 20, 22, 23, 25, 26, 28, 29, 31, 32, 34, 35, 37, 38, 40, 41, 43**, **44**) (Fig. 1. and Table I). The compounds were screened for antibacterial activity against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*, *P. aeruginosa*) strains as well as antifungal activity against *C. albicans*.

Table II contains results for only the most active (nine) antimicrobial compounds at concentrations of 500, 250 and 125 μg/ml. None of the 6*H*-quinobenzothiazines (**1–12**) exhibited antimicrobial activity (data not shown). *S. aureus* was the most sensitive strain and *C. albicans* the most resistant one for quinobenzothiazines (Table II). The antibacterial activities of selected compounds (**14, 31, 38**) against *E. coli*, *P. aeruginosa* and *S. aureus* are presented in Tables III–V, respectively. The growth of *E. coli* (Table III) was markedly inhibited by compound **14** at 250 and 500 μg/ml concentrations, whereas the activities of compounds **31** and **38** were rather week. In turn, the growth of *P. aeruginosa* (Table IV) was more clearly inhibited by **38** (250 and 500 μg/ml) in contrast to inactive **18** and **19** which were initially found antibacterial for *S. aureus*. Lastly, compound **14** was found active with regard to *S. aureus* at 250 and 500 μg/ml (Table V). In addition, **31** and **38** showed some activity at lower concentration (125 μg/ml).

Discussion

New quinobenzothiazines (**1–44**), shown in Fig. 1. and Table I, were recently synthesized in reactions of dichlorodiquinolinyl disulfide or diquinodithiin with substituted anilines and their hydrochlorides, followed by N-alkylation (Jeleń and Pluta, 2009; Jeleń *et al.*, 2013). They contain various substituents at the thiazine nitrogen atom: the hydrogen atom and pharmacophoric groups such as dialkylaminoalkyl diethylaminopropyl and 1-methyl-2-piperidinylethyl), acetylaminoalkyl, ethoxycarbonylaminoalkyl, chloroethylureidoalkyl, methanesulfonylaminoalkyl and *p*-toluenesulfonylaminoalkyl ones (where alkyl = propyl and butyl) and a simple substituent in the benzene ring: H, Cl, Br, CF_3 and SCH_3. These quinobenzothiazines were found to be antiproliferative, generally low cytotoxic and inhibitory with regard to TNF α production. The selected compounds exhibited anticancer activities against leukemia L-1210 cells, colon cancer SV-948 cells and epidermal carcinoma A-341 cells with a potency similar to cisplatin (Jeleń *et al.*, 2013).

The results of this investigation showed (Table II) that active compounds possess nitrogen group substituents at the thiazine nitrogen atom: chloroethylureidoalkyl, acetylaminoalkyl, ethoxycarbonylaminoalkyl and methanesulfonylaminoalkyl ones. The two former substituents were also present in the most potent antiproliferative and anticancer 6-substituted quinobenzothiazines (Jeleń *et al.*, 2013). The additional substituent (Cl, SCH_3) in position 9 did not significantly influence the tested activity. As the most active compounds show similar lipophilicity to 8–10-substituted 6*H*-quinobenzothiazines (**1–12**) (logP = 4.01–5.41) (Jeleń *et al.*, 2014), the lipophilic character does not play an important role in antimicrobial activity.

In summary, although compound **38** (6-methanesulfonylaminobutyl-9-methylthioquinobenzothiazine) turned out to be the most universal antimicrobial quinobenzothiazine as assessed by analysis of Table II, compound **14** (6-(1-methyl-2-piperidylethyl)quinobenzothiazine) showed stronger actions against *S. aureus* and *E. coli.* Although the studied quinobenzothiazines were less active than the reference drug chlorpromazine, compounds **38** and **14** were non-toxic with regard to human peripheral blood mononuclear cells as demonstrated elsewhere (Jeleń *et al.,* 2013).

Conclusion. Structure-activity relationship revealed that linearly fused quinobenzothiazines exhibit modest antimicrobial activity, weaker than angularly fused quinobenzothiazines. The nitrogen substituent at the thiazine nitrogen atom is necessary for their antibacterial activity. Although the antimicrobial activities of the studied compounds are considerably weaker than chlorpromazine, they surpass chlorpromazine by much lower cell toxicity. Although the compounds were primarily designed as potential anti-inflammatory drugs, the accompanying antibacterial properties increase attractiveness of the compounds as potential therapeutics.

Literature

Aaron J.J., M.D. Gaye Seye, S. Trajkovska and N. Motohashi. 2009. Bioactive Phenothiazines and Benzo[a]phenothiazines: spectroscopic studies and biological and biomedical properties and applications. Top Heterocycl. Chem., Springer-Verlag, Berlin, 16: 153–231.

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Amaral L. and J.E. Kristiansen. 2001. Phenothiazines: potential management of Creutzfeldt-Jacob disease and its variants. *Int. J. Antimicrob. Agents* 18: 411–417.

Bansode T.N., J.V. Shelke and P.M. Dongre. 2009a. Synthesis and antimicrobial activity of some new N-acyl substituted phenothiazines. *Eur. J. Med. Chem*. 44: 5094–5098.

Bansode T.N., P.M. Dongre and V.G. Dongre. 2009b. Synthesis, antimicrobial and antifungal activity of 1,3-di(2-substituted 10H-phenothiazin-10-yl)propan-1-one. *Pharm. Chem. J.* 43: 311–314.

Dasgupta A., S.G. Dastridar, Y. Shirataki and N. Motohashi. 2008. Antibacterial activity of artificial phenothiazines and isoflavones from plants. Top Heterocycl. Chem., Springer-Verlag, Berlin, 15: 67–132.

Dixit Y., R. Dixit, N. Gautam and D.C. Gautam. 2009. Synthesis and antimicrobial activities of novel biologically active heterocycles: 10*H*-phenothiazines, their ribofuranosides and sulfone derivatives. *Nucleosides, Nucleotides Nucleic Acids* 28: 998–1006.

Gaye-Seye M.D., J.J. Aaron, C. Parkanyi and N. Motohashi. 2006. Luminescence and photophysical properties of benzo[a]phenothiazines-therapeutic, physico-chemical, and analytical applications. *Curr. Drug Targets* 7: 1083–1093.

Gupta R.R. and M. Kumar. 1988. Synthesis, properties and reactions of phenothiazines. In: R.R. Gupta (Eds.) Phenothiazines and 1,4-Benzothiazines – Chemical and Biological Aspects. Elsevier, pp. 1–161.

Jaszczyszyn A., K. Gąsiorowski, P. Świątek, W. Malinka, K. Cieslik-Boczula J. Petrus and B. Matusewicz-Czarnik. 2012. Chemical structure of phenothiazines and their biological activity. *Pharmacol. Rep*. 64: 16–23.

Jeleń M. and K. Pluta. 2008. Synthesis of 6-aminoalkyldiquino-1,4-thiazines and their acyl and sulfonyl derivatives. *Heterocycles* 75: 859–870.

Jeleń M. and K. Pluta. 2009. Synthesis of quinobenzo-1,4-thiazines from diquino-1,4-dithiin and 2,2'-dichloro-3,3'-diquinolinyl disulfide. *Heterocycles* 78: 2325–2336.

Jeleń M., K. Pluta and B. Morak-Młodawska. 2014. Determination of the lipophilicity parameters of new antiproliferative 8–10-substituted quinobenzothiazines by computational methods and RP TLC. *J. Liq. Chromatogr.* & *Rel. Technol.* 73: 1373–1382.

Jeleń M., K. Pluta, M. Zimecki, B. Morak-Młodawska, J. Artym and M. Kocięba. 2013. Synthesis and selected immunological properties of substituted quino[3,2-b]benzo[1,4]thiazines. *Eur. J. Med. Chem.* 63: 444–456.

Kumar M., K. Sharma, R.M. Samarth and A. Kumar. 2010. Synthesis and antioxidant activity of quinolinobenzothiazinones. *Eur. J. Med. Chem.* 45: 4467–4472.

Molnar J., Y. Mandi and J. Kiraly. 1976. Antibacterial effect of some phenothiazine compounds and R-factor elimination by chlorpromazine. *Acta Microbiol. Acad. Sci. Hung*. 23: 45–54.

Morak-Młodawska B. and K. Pluta. 2007. Synthesis of novel dipyrido-1,4-thiazines. *Heterocycles* 71: 1347–1361.

Motohashi N., M. Kawase, K. Satoh and H. Sakagami. 2006. Cytotoxic potential of phenothiazines. *Curr. Drug Targets* 7: 1055–1066.

Nowak M., K. Pluta, K. Suwińska and L. Straver. 2007. Synthesis of new pentacyclic diquinothiazines. *J. Heterocycl. Chem*. 44: 543–550.

Pluta K., M. Jeleń, B. Morak-Młodawska, M. Zimecki, J. Artym and M. Kocięba. 2010. Anticancer activity of newly synthesized azaphenothiazines in NCI's anticancer screening bank. *Pharmacol. Rep.* 62: 319–332.

Pluta K., A. Maślankiewicz and M. Szmielew. 2000. N,N-Dialkylaminoalkyl substituted quinobenzo[1,4]thiazines and diquino[1,4] thiazines. *Phosphorus Sulfur Silicon Relat. Elem.* 159: 79–86.

Pluta K., B. Morak-Młodawska and M. Jeleń. 2011. Recent progress in biological activities of synthesized phenothiazines. *Eur. J. Med. Chem*. 46: 3179–3189.

Raval J.P. and K.R. Desai. 2005. Synthesis and antimicrobial activity of new tetrazolopyridinyl phenothiazines. *ARKIVOC* 13: 21–28.

Salvi V.K., S. Sharma, Ch. Sharma, D. Bhambi and G.L. Talesara. 2009. Synthesis of phtalimido or succinimido-[2-aryl-4-oxo-3-[{2-oxo-2-(phenothiazin-10-yl)ethyl}amino]-1,3-thiazolidin-5-yl] ethanoate and their antimicrobial activities. *Indian J. Chem*. 48B: 1583–1589.

Singh G., N. Kumar, A.K. Yadav and A.K. Mishra. 2003. Potential antimicrobial agents trifluoromethyl-10H-phenothiazines and ribofuranosides. *Heteroatom Chem*. 14: 481–486.

Srivastava S.D. and P. Kohli. 2007. Synthesis of 5-((10N-phenothiazinyl)methyl)-4-(benzylideneamino-3-mercaptotriazoles as antibacterial and antifungal agents. *Proc. Nat. Acad. Sci. India* 77A: 199–204.

Swarnkar P.K., P. Kriplani, G.N. Gupta and K.G. Ojha. 2007. Synthesis and antibacterial activity of some new phenothiazine derivatives. *E-J. Chem*. 4: 14–20.

Zięba A., Z. P. Czuba and W. Król. 2012. In vitro antimicrobial of novel azaphenothiazines derivatives. *Acta Pol. Pharm. Drug Res*. 69; 1149–1152.

Zięba A., M. Latocha and A. Sochanik. 2013. Synthesis and *in vitro* antiproliferative activity of novel 12(H)-quino[3,4-b][1,4]benzothiazine derivatives. *Med. Chem. Res*. 22: 4158–4163.

Zięba A., A. Sochanik, A. Szurko, M. Rams, A. Mrozek and P. Cmoch. 2010. Synthesis and *in vitro* antiproliferative activity of 5-alkyl-12(H)-quino[3,4-b] [1,4] benzothiazinium salts. *Eur. J. Med. Chem.* 45: 4733–4739.

Zimecki M., J. Artym, M. Kocięba, K. Pluta, B. Morak-Młodawska and M. Jeleń. 2009. The immunosupressive activities of newly synthesized azaphenothiazines in human and mouse models. *Cell. Mol. Biol. Lett*. 14: 622–635.

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