Acta Poloniae Pharmaceutica – Drug Research, Vol. 69 No. 6 pp. 1149–1152, 2012 ISSN 0001-6837
Polish Pharmaceutical Society

PHARMACOLOGY

IN VITRO ANTIMICROBIAL ACTIVITY OF NOVEL AZAPHENOTHIAZINE DERIVATIVES

ANDRZEJ ZIĘBA[1]*, ZENON PAWEŁ CZUBA[2] and WOJCIECH KRÓL[2]

[1] Department of Organic Chemistry, Medical University of Silesia, Jagiellońska 4, 41-200 Sosnowiec, Poland
[2] Department of Microbiology and Immunology, Medical University of Silesia, Jordana 19, 41-808 Zabrze, Poland

Abstract: Antimicrobial activity *in vitro* of a series of novel azaphenothiazine derivatives containing a quinoline moiety was investigated using Gram-positive (*Staphylococcus aureus, Enterococcus faecalis*) and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) strains as well as in *Candida albicans* yeast. The examined compounds showed the highest activity against *Enterococcus faecalis* and *Escherichia coli* whereas activity against *Pseudomonas aeruginosa* was the lowest. Compound **1d** demonstrates the highest activity against all tested bacterial strains. Compounds **1c, 1h** and **1k** with various substituents (CH_3, OH, NH_2) at C11 position of the quinobenzothiazine ring, did not exhibit activity against any tested bacterial strain. Only compounds **1m** and **1n** with long aliphatic chains at the quinoline nitrogen atom showed antifungal activity. Correlations between antimicrobial activity and chemical structure of the tested compounds were observed.

Keywords: minimal inhibitory concentration, antimicrobial activity, phenothiazine, azaphenothiazine

From medicinal chemistry perspective, phenothiazines are an important group of condensed three-ring heterocycles (1). Phenothiazine derivatives and their analogues containing 1,4-thiazine structural fragment show diverse biological activities, including antimalarial (2), antipsychotropic (3), antimicrobial (4), antitubercular (5, 6), antitumor (7) and anti-inflammatory (8). Phenothiazine derivatives that contain aminoalkyl substituents at the thiazine nitrogen atom are used as antipsychotropic and antihistamine drugs (9). Extensive search has been conducted regarding new methods of synthesizing potentially useful phenothiazine derivatives having pharmacological activity. In our earlier studies, we described synthesis, structure, physical and chemical properties and antiproliferative activity of novel tetracyclic azaphenothiazine derivatives, containing a quinoline structural fragment (10).

One of the most important issues in current medical practice are antibiotic-resistant bacterial infections (11–13). Their pervasiveness justifies the search for innovative antimicrobial agents featuring novel chemical structures and mechanisms of action, helpful in combating infections (14–17). In this report we describe novel tetracyclic azaphenothiazine derivatives and the results of antimicrobial tests based on their use.

MATERIALS AND METODS

Chemistry

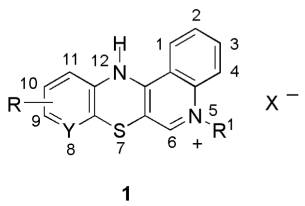
All of the analyzed azaphenothiazine derivatives were obtained with methods previously developed in our laboratory. 5-Alkyl-12(*H*)-quino[3,4-b][1,4]benzothiazinium chloride **1**(**a-l**) was obtained *via* cyclization of 1-alkyl-4-arylaminoquinolinium-3-thiolates (18, 19); derivatives **1**(**m, n**) were obtained by alkylating 12(*H*)-quino[3,4-b][1,4]benzothiazine using appropriate alkyl bromides (10).

Microbiology

The following strains, obtained from American Type Culture Collection (ATCC), were used to test antibacterial and antifungal activities of azaphenothi-

* Corresponding author: e-mail: zieba@sum.edu.pl

azine derivatives **1**(**a**–**n**) *in vitro*: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10231. Minimal inhibitory concentrations (MIC) were determined using a modified broth macrodilution method, as described by Clinical and Laboratory Standards Institute (20, 21). The final concentration of microorganisms in each broth macrodilution tube was approximately 5×10^5 CFU/mL of Mueller-Hinton broth (MHB). Aqueous solutions of compounds **1**(**a**–**n**) were added to the medium at varying final concentrations (1–512 μg/mL). The test was performed using 96-well microplates. The MIC was defined as the lowest concentration of compounds **1**(**a**–**n**) that resulted in no visible growth after 18-h incubation at 35°C in ambient air. The intensities of the bacterial and fun-



1

1a	R = H	Y = CH	R^1 = Me	X = Cl
1b	R = 9-Me	Y = CH	R^1 = Me	X = Cl
1c	R = 11-Me	Y = CH	R^1 = Me	X = Cl
1d	R = 9-Cl	Y = CH	R^1 = Me	X = Cl
1e	R = 9-F	Y = CH	R^1 = Me	X = Cl
1f	R = 9-OH	Y = CH	R^1 = Me	X = Cl
1g	R = 10-OH	Y = CH	R^1 = Me	X = Cl
1h	R = 11-OH	Y = CH	R^1 = Me	X = Cl
1i	R = 9-NH_2	Y = CH	R^1 = Me	X = Cl
1j	R = 10-NH_2	Y = CH	R^1 = Me	X = Cl
1k	R = 11-NH_2	Y = CH	R^1 = Me	X = Cl
1l	R = H	Y = N	R^1 = Me	X = Cl
1m	R = H	Y = CH	R^1 = Bu	X = Br
1n	R = H	Y = CH	R^1 = Dec	X = Br

Figure 1. Modifications of the structure of 5-alkyl-12(*H*)-quino[3,4-b][1,4]benzothiazinium salts **1**.

Table 1. Minimal inhibitory concentration (MIC, in ěg/mL) for 5-alkyl-12(*H*)-quino[3,4-b][1,4]benzothiazine salts **1(a–n),** ceftazidime and clotrimazole.

Compound	*E. coli*	*P. aeruginosa*	*S. aureus*	*E. faecalis*	*C. albicans*
1a	7	–	52	6	–
1b	24	–	31	11	–
1c	–	–	–	–	–
1d	6	180	35	4	–
1e	28	240	26	9	–
1f	14	–	34	108	–
1g	54	–	78	105	–
1h	–	–	–	–	–
1i	39	–	78	–	–
1j	78	–	79	–	–
1k	–	–	–	–	–
1l	25	–	52	31	–
1m	52	–	–	–	108
1n	285	–	–	–	42
Ceftazidime	1	8	8	2	–
Clotrimazole	–	–	–	–	4

gal growth were evaluated by measuring sample absorbance at 550 nm (Bio-Tek Instruments, Inc., Winooski, VT, USA) at the beginning and after 18 h of the culture. The studies were conducted in duplicate and were repeated at least once on a different day.

RESULTS AND DISCUSSION

Antimicrobial activity

The present report lists the initial results of antimicrobial activity tests conducted *in vitro* for the 5-alkyl-12(*H*)-quino[3,4-b][1,4]benzothiazinium salts **1**(**a**–**n**)**.** X-ray analysis of compounds **1a** (18) and **1l** (19) has shown that their structures are completely planar and that the greatest atomic deflection from the plane, determined by the ring-forming atoms, is 0.068 Å. It could be assumed that, due to the planar structure of tetracyclic quinobenzothiazine moiety present in the investigated compounds, their mechanism of antibacterial action involves DNA intercalation, as in the case of proflavine. The structure of the examined quinobenzothiazine salts **1** was modified by introducing substituents (CH_3, F, Cl, OH or NH_2) into various positions of the benzene ring **1**(**a**–**k**) and an additional nitrogen atom in the 8-position of the quinobenzothiazine ring **1l**. The presence of additional substituents in various positions of the aromatic or heteroaromatic rings of biologically or pharmacologically active compounds affects electron density distribution and spatial structure of the molecules. Changes in these parameters may alter drug transport in the body and a match between the drug and receptor, thereby altering the strength of drug action (22). In order to increase lipophilic properties of the examined compounds, butyl or decyl groups were introduced into the 5-position of the quinobenzothiazine ring (compounds **1**(**m**–**n**)). The values of lipophilicity parameter $LogP_{TLC}$ for compounds **1**(**a**–**n**) are within the 2.12–3.15 range. $LogP_{TLC}$ parameters for compounds **1m** and **1n** are 2.65 and 3.15, respectively (23).

The investigated compounds demonstrated the highest activity against *S. aureus*, *E. faecalis* and *E. coli*. Concerning *P. aeruginosa*, only derivatives **1d** and **1e** with chlorine or fluorine atom at the C9 atom (MIC values: 180 and 240 μg/mL, respectively) were active. In the case of *E. faecalis* it was derivative **1d**, with chlorine atom at C9, which was the most active (MIC 4 μg/mL), followed by compound **1a** having no additional substituents (MIC 6 μg/mL) and compound **1e** with a fluorine atom at C9 of the chinobenzothiazine ring (MIC 9 μg/mL). Compound **1d** also demonstrates the highest activity against *E. coli* (MIC 6 μg/mL). Compounds **1c, 1h** and **1k** with various substituents (CH_3, OH, NH_2) at C11 position did not exhibit activity against any tested bacterial strain within the examined concentration range. Such a result may suggest that the thiazine nitrogen atom exerts major impact on the antibacterial activity of the examined compounds and that the spatial hindrance of substituents at the 11-position with respect to NH group may significantly limit this activity. Concerning antifungal activity, compounds **1m** and **1n** (MIC 108 and 42 μg/mL, respectively) demonstrated such activity in the examined concentration range. Only compounds with long aliphatic chains at the N5 quinoline nitrogen atom and with highest lipophilicity parameters ($LogP_{TLC}$) showed antifungal activity in the examined concentration range.

The results obtained herein show that searching for improved antimicrobial agents with quinobenzothiazine structural fragment is promising.

Acknowledgment

The work was supported by the Medical University of Silesia (grant KNW-2-041/10).

REFERENCES

1. Gupta R.R., Kumar M., Synthesis, properties and reactions of phenothiazines, in, Phenothiazines and 1,4-Benzothiazines: Chemical and Biomedical Aspects, Gupta R.R. Ed., pp. 1–146, Elsevier, Amsterdam 1988.
2. Dominguez J.N., López S., Charris S.J., Iarruso L., Lobo G., Semenov A., Olson J.E., Rosenthal P.J.: J. Med. Chem. 40, 2726 (1997).
3. Lin G., Midha K.K., Hawes E.M.: J. Heterocycl. Chem. 28, 215 (1991).
4. Kaatz G.W., Moudgal V.V., Seo S.M., Kristiansen J.E.: Antimicrob. Agents Chemother. 47, 719 (2003).
5. Viveiros M., Amaral L.: Int. J. Antimicrob. Agents 17, 225 (2001).
6. Amaral L., Kristiansen J.E.: Int. J. Antimicrob. Agents 14, 173 (2000).
7. Motohashi N., Kawase M., Saito S., Sakagami H.: Curr. Drug Targets 1, 237 (2000).
8. Sharma S., Srivastava V.K., Kumar A.: Pharmazie 60, 18 (2005).
9. Isaacson E.I.: Central nervous system depressants, in Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical

Chemistry, 10 edn., Delgado J.N., Remers W.A. Eds., pp. 435–461, Lippincott-Raven Publishers, Philadelphia 1998.
10. Zięba A., Sochanik A., Szurko A., Rams M., Mrozek A., Cmoch P.: Eur. J. Med. Chem. 45, 4733 (2010).
11. Chambers H.F.: Clin. Microbiol. Rev. 10, 781 (1997).
12. Livermore D.M.: Int. J. Antimcrob. Agents. 16, 3 (2000).
13. CDC.: Staphylococcus aureus resistant to vancomycin – United States. MMWR 51, 565 (2002).
14. Fung H.B., Kirschenbaum H.L., Ojofeitimi B.O.: Clin. Ther. 23, 356 (2001).
15. Wright G.D., Sutherland A.D.: Trends Mol. Med. 13, 260 (2007).
16. Girdhar A., Jain S., Jain N., Girdhar S.: Acta Pol. Pharm. Drug Res. 67, 211 (2010).
17. Zięba A., Wojtyczka R.D., Kępa M., Idzik D.: Folia Microbiol. 55, 3 (2010).
18. Zięba A., Maślankiewicz A., Suwińska K.: Eur. J. Org. Chem. 2947 (2000).
19. Zięba A., Suwińska K.: Heterocycles 68, 495 (2006).
20. CLSI (NCCLS): Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 8 edn., Approved standard. NCCLS, Wayne, Pa 2009.
21. CLSI (NCCLS): Reference method for broth dilution antifungal susceptibility testing of yeasts, 3 edn., Approved standard. NCCLS, Wayne, Pa 2008.
22. Patrick G.L., An Introduction to Medicinal Chemistry, 3 edn., pp. 271–298, Oxford University Press, Oxford 2005.
23. Zięba A., Prus W.: Acta Chromatrographica 3, 369 (2009).

Received: 23. 03. 2011