STUDIA UBB CHEMIA, LX, 3, 2015 (p. 283-288)
(RECOMMENDED CITATION)

Dedicated to prof. dr. I. C. Popescu
on the occasion of his 70th anniversary

ANTIMICROBIAL ACTIVITY SCREENING OF BENZOTHIAZOLYL-PHENOTHIAZINE DERIVATIVES

ADRIANA GROZAV[a*], VALENTIN ZAHARIA[a], CASTELIA CRISTEA[b], NICODIM IOSIF FIT[c]

ABSTRACT. The antimicrobial activity of a series of benzothiazolyl-10*H*-phenothiazine derivatives against Gram positive Bacteria (*Staphylococcus aureus* and *Bacillus cereus*), Gram negative Bacteria (*Escherichia coli*, *Salmonella enteritidis*, *Pseudomonas aeruginosa*) and fungus *Candida albicans* respectively, was screened using diffusion method and minimal inhibitory concentrations (MIC) were assessed by broth serial dilution procedure. *Candida albicans*, *Bacillus cereus* and *Pseudomonas aeruginosa* were proved to be susceptible to the benzothiazolyl-10*H*-phenothiazine derivatives (MIC 0.02-1.3 μg/mL)

Key words: *phenothiazine, benzothiazole, antibacterial, diffusion, broth dilution, minimal inhibitory concentration*

INTRODUCTION

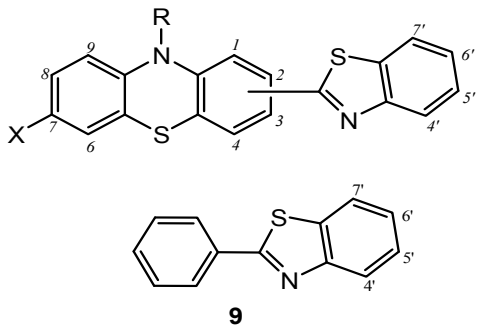
Phenothiazine and benzothiazole are heterocyclic aromatic structures largely employed as pharmacophoric units. A careful selection of the substitution pattern of the phenothiazine unit enabled a large spectrum of biological activities such as neuroleptic, antimicrobial, anthelmintic, etc. [1,2], while benzothiazole derivatives were also screened as antimicrobial [3], anticancer [4], antihelmintic [5], and antidiabetic [6] agents.

[a] *Faculty of Pharmacy, "Iuliu Haţieganu" University of Medicine and Pharmacy, RO-400012, Victor Babes 41, Cluj-Napoca, Romania.*
[*] *Corresponding author: adriana.ignat@umfcluj.ro*
[b] *Babes-Bolyai" University, Faculty of Chemistry and Chemical Engineering, RO-400028, Cluj-Napoca, Romania.*
[c] *Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Veterinary Medicine, Microbiology Department, Cluj Napoca, RO-400337, Romania.*

Continuing our research in the biological activity evaluation of new synthetic compounds containing phenothiazine and thiazole units assembled in the same molecular structure [7-10], we report here the antimicrobial screening of a series of new derivatives containing joint phenothiazine and thiazole units [11].

RESULTS AND DISCUSSIONS

The series of benzothiazolyl-phenothiazine derivatives selected for the evaluation of antimicrobial activity contain different substitution patterns at the phenothiazine unit as shown in Figure 1. C-substituted regioisomer series cover derivatives with benzothiazol-2-yl unit attached in positions 1-4 of the phenothiazine moiety and the heterocyclic nitrogen atom in an NH (**1**), or N-methyl group (**2-5**). Symmetrical 3,7-disubstituted phenothiazine derivatives contain two benzothiazolyl groups (**6**), while unsymmetrical derivatives include a halogene (**7**), or a 2-cyano-vinyl group (**8**) apart the benzothiazole substituent. 2-Phenyl-benzothiazole (**9**) was also included in the tested series for a relative examination of the properties induced by the benzothiazole unit.



9

1 R= -H, 1-thiazolyl, X= -H
2 R= -CH_3, 1-thiazolyl, X= -H
3 R= -CH_3, 2-thiazolyl, X= -H
4 R= -CH_3, 3-thiazolyl, X= -H
5 R= -CH_3, 4-thiazolyl, X= -H
6 R= -CH_3, 3-thiazolyl, X= -thiazolyl
7 R= -CH_3, 3-thiazolyl, X= -Br
8 R= -CH_3, 3-thiazolyl, X= -CH=CH-CN

Figure 1. Structure of synthetic derivatives subjected to the antimicrobial activity screening

The *in vitro* antimicrobial susceptibility of Gram positive bacteria: *Staphylococcus aureus* and *Bacillus cereus*, Gram negative bacteria: *Escherichia coli*, *Salmonella enteritidis*, *Pseudomonas aeruginosa* and *Candida albicans* fungus respectively, was screened using diffusion method; minimuml inhibitory concentration (MIC) of compounds **1-9** were determined using broth serial dilution procedure.

The examination of the inhibition zones resulted by diffusion procedure pointed out an intermediate response of *S. aureus, B. cereus*, *P.*

aeruginosa (Table 1) to the tested compounds **1-9**, while the rest of the tested bacterial strains seemed resistant. Based on this method, 3-(benzothiazol-2-yl)-10-methy-10*H*-phenothiazine **4** was observed to be the most efficient of the series, even more efficient than amoxicillin against *B. cereus* and with an antifungal activity slightly better than miconazole against *C. albicans.*

Table 1. Inhibition zone diameter (mm) obtained by diffusion procedure using compounds **1-9** in solution 63 mM in DMSO[a].

Compound (25 µl/disc)	*S. aureus*	*B. cereus*	*P. aeruginosa*	*C. albicans*
1	6	6	0	0
2	6	0	0	0
3	6	6	0	6
4	12	18	10	25
5	6	6	0	6
6	6	7	6	6
7	6	7	7	8
8	9	8	6	0
9	7	7	12	7
Amoxicillin[b]	40	9	0	-
Miconazole[c]				21

[a]DMSO produced no inhibition zone; [b]25µg/disc; [c]10 µg/disc.

The minimum inhibitory concentration (MIC) determined by broth dilution using ten concentrations derived from serial twofold dilution indicate antifungal and antibacterial activity for each compound in the series with MIC between 0.02-1.3 µg/ml. As it may be seen from figure 2, in the mono-benzothiazolyl-phenothiazine series characterized by enhanced antimicrobial activity, 1-, 2- and 3-substituted phenothiazine derivatives (**1, 2, 3** and **4)** were the most effectives against *C. albicans* (with MIC around 0.02-0.04 µg/ml), while *B. cereus* was effectively inhibited by 1- and 3-benzothiazolyl-phenothiazines (**1, 2** and **4)**. When the benzothiazolyl substituent was situated in position 4 of the phenothiazine unit (**5)** the observed activity against *B. cereus* appeared largely diminished (MIC ≈1.3 µg/mL).

A lower susceptibility of Gram negative bacteria to benzothiazolyl-phenothiazine derivatives **1-8** may be observed in figure 3. The best results were achieved in the inhibition of *P. aeruginosa. E. coli* and *S. enteritidis* were susceptible to the unsymmetrical 3,7-disubstituted benzothiazolyl-phenothiazine derivatives containing bromine **7**, or 2-cyano-vinyl group **8** (MIC 0.8 and 0.4 µg/ml respectively).

The antimicrobial activity of 2-phenyl-benzothiazole **9** was observed to be less effective as compared to benzothiazolyl-phenothiazine derivatives **1-8** in each case.

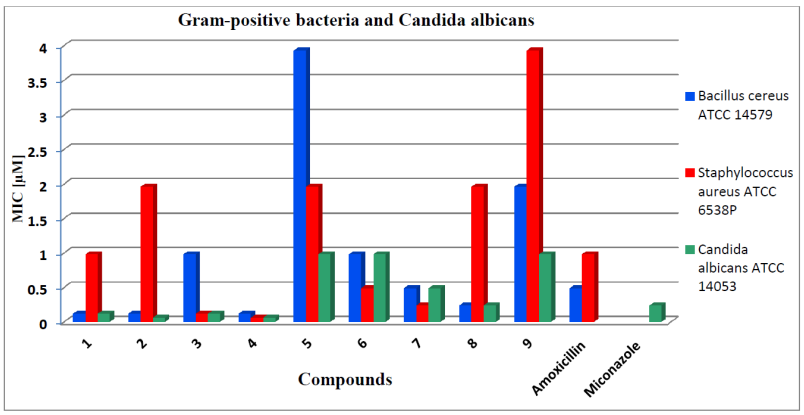


Fig. 2. Antimicrobial activity of benzothiazolyl-phenothiazine derivatives evaluated using broth dilutions method (tested serial concentrations: 0.06, 0.12, 0.24, 0.49, 0.98, 1.96, 3.93, 7.85, 15.75 and 31.5 µM).

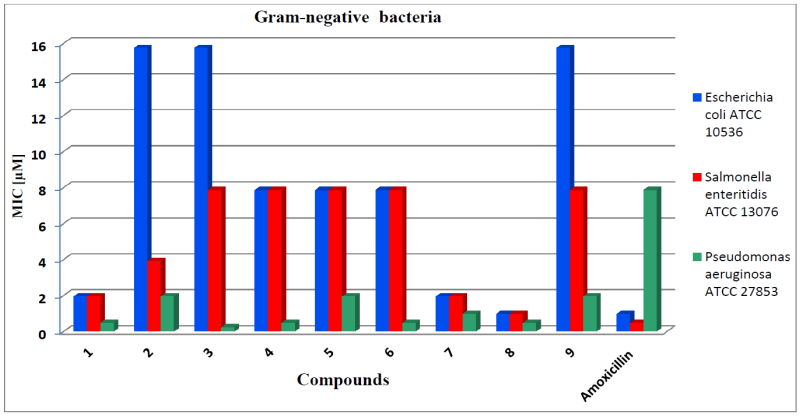


Fig 3. Antibacterial activity of benzothiazolyl-phenothiazine derivatives evaluated using broth dilutions method (tested serial concentrations: 0.06, 0.12, 0.24, 0.49, 0.98, 1.96, 3.93, 7.85, 15.75 and 31.5 µM).

CONCLUSIONS

Based on the described *in vitro* antimicrobial susceptibility test, the synthetic benzothiazolyl-phenothiazine derivatives developed a better antimicrobial activity as compared to 2-phenyl-benzothiazole.

Mono 1-, 2- and 3-benzothiazolyl-phenothiazine derivatives were effective against *C. albicans*, *B. cereus* and *P. aeruginosa*. Connecting the benzothiazolyl substituent in position 4 of the phenothiazine unit diminishes this antimicrobial activity.

Symmetrical 3,7-disubstituted phenothiazine derivatives containing two benzothiazolyl groups were less efficient than mono 3-substituted derivative.

S. aureus, *E. coli*, and *S. enteritidis* were less susceptible to the antibacterial benzothiazolyl-phenothiazine derivatives.

EXPERIMENTAL

Materials:

Gram positive bacterial strains: *Staphylococcus aureus* ATCC 6538P and *Bacillus cereus* ATCC 14579.

Gram negative bacterial strains: *Escherichia coli* ATCC 10536, *Salmonella enteritidis* ATCC 13076 and *Pseudomonas aeruginosa* ATCC 27853

Fungus: *Candida albicans* ATCC 14053

Mueller Hinton Agar (Merck, Germany)

Sabouraud dextrose agar (Merck, Germany)

Antimicrobial tests:

a) The Diffusion method was carried out according to standard methods described by CLSI-M02-A10 [12] and Markey and co. [13]. A bacteria culture which has been adjusted to 0.5 McFarland standard, 10^6UFC/ml in physiological solution was used to inoculate Muller Hinton agar plates, followed by incubation at 37±2^{0}C for 24 hours. For the susceptibility tests, on each test plate were made wells (5 mm diameter) in radial disposition and 25 µl from 63 mM solution of synthetic compounds in DMSO were disposed. Seven compounds were tested on a plate. The standard commercial antibiotic amoxicillin (25µg**)** was used for positive control and DMSO solvent for negative control. After incubation at 37±2^{0}C for 24 hours the plates were examined for inhibition zone, which was measured and recorded in mm.

The fungus was cultivated and tested on Sabouraud dextrose agar with incubation at 37±2^{0}C for 48h in aerobic conditions. The tests were repeated three times to ensure reliability.

b) Broth dilution procedure was carried out according to the protocols previously described [12,13] with minor modifications according to the properties of tested compounds. MIC was determined using microdilution trays with 96 wells. In each well were introduced 50µl Mueller Hinton broth and 50 µl of diluted antimicrobial agents. Serial two fold dilutions were prepared starting with a stock solution 63 mM in DMSO and distilled water. 5 µl suspension of bacteria in physiological solution standardized to 0.5 McFarland was added in each well and the plates were incubated at 37±2^{0}C for 24 h under aerobic conditions. Each tray included a growth control well and a sterility (uninoculated) well.

For the fungus Sabouraud dextrose broth was used and incubation was performed at 37±2^{0}C for 48h in aerobic conditions.

The amount of growth in the wells containing the antimicrobial agent was compared with the amount of growth in the growth-control well.

ACKNOWLEDGMENTS

This paper was published under the frame of European Social Found, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/136893" (A. Grozav).

REFERENCES

1. K. Pluta, B. Morak-Młodawska, M. Jeleń, *Eur. J. Med. Chem.,* **2011**, *8*, 3179.
2. K.V. Tandon, H.K. Maurya, A. Tripathi, G.B. ShivaKeshava, P.K. Shukla, P. Srivastava, D. Panda, *Eur J. Med. Chem.*, **2009**, 1086.
3. S. Gupta, N. Ajmera, N. Gautam, R. Sharma, D. Gauatam, *Ind. J. Chem.,* **2009**, *48B*, 853. b) R.M. Kumbhare, V.N. Ingle, *Ind. J. Chem*., **2009**, *48B*, 996. c) Y. Murthi, D. Pathak, *J. Pharm. Res*., **2008**, *7*(3), 153.
4. H.L.K. Stanton, R. Gambari, H.C. Chung, C.O.T. Johny, C. Filly, S.C.C. Albert, *Bioorg. Med. Chem*., **2008**, *16*, 3626. b) I. Hutchinson, M.S. Chua, H.L. Browne, V. Trapani, T.D. Bradshaw, A.D. Westwell, *J. Med. Chem.,* **2001**, *44*, 1446.
5. M. Sreenivasa, E. Jaychand, B. Shivakumar, K. Jayrajkumar, J. Vijaykumar, *Arch. Pharm. Sci. Res*., **2009**, *1*(2), 150.
6. S. Pattan, C. Suresh, V. Pujar, V. Reddy, V. Rasal, B. Koti, *Ind. J. Chem*., **2005**; *44B*, 2404-2408.
7. A. Ignat, T. Lovasz, M. Vasilescu, E. Fischer-Fodor, C.B. Tatomir, C. Cristea, L. Silaghi-Dumitrescu, V. Zaharia, *Arch. Pharm. Chem. Life Sci.,* **2012**, *345*, 574.
8. V. Zaharia, A. Ignat, N. Palibroda, B.Ngameni, V. Kuete, C.N. Fokunang, M.L. Moungang, B.T. Ngadjui, *Eur. J. Med. Chem.*, **2010**, *45*, 5080.
9. A. Ignat, V. Zaharia, C. Mogosan, N. Palibroda, C. Cristea, L. Silaghi-Dumitrescu, *Farmacia,* **2010**, *58*, 290.
10. A. Grozav, L.I. Găină, V. Pileczki, O. Crisan, L.Silaghi-Dumitrescu, B. Therrien, V. Zaharia, I. Berindan-Neagoe, *Int. J. Mol. Sci.,* **2014**, *15*, 22059.
11. B. Brem, E. Gal, C. Cristea, L. Găină, A. Grozav, V. Zaharia, L. Silaghi-Dumitrescu, *Studia UBB Chemia*, **2015**, *60* (2), 371.
12. CLSI. Methods for Dilution Antimicrobial Susceptibility Tests f or Bacteria That Grow Aerobically; Approved Standard–Ninth Edition. CLSI document M07-A9. Wayne, PA: Clinical and Laboratory Standards Institute, **2012**.
13. B.K. Markey, F.C. Leonard, M. Archambault, A. Cullinane, D. Maguire. Clinical Veterinary Microbiology. Elsevier, Edinburgh, London, New York, Oxford, Philadelphia, St Louis, Sydney, Toronto, **2013**.