



Structure-antimicrobial activity relationship of a series of functionalized arylbenzothiazoles

Fatma Abdmouleh*^{1,2}, Mehdi El Arbi², Maité Sylla-Iyarreta Veitia*¹, Mamdouh Ben Ali² and Clotilde Ferroud¹

- ¹ Equipe de Chimie Moléculaire, Laboratoire Génomique, bioinformatique et chimie moléculaire GBCM, EA 7528, Conservatoire national des arts et métiers, 2 rue Conté, 75003, Paris France ; HESAM Université. maite.sylla@lecnam.net (M.S-IV), clotilde.ferroud@lecnam.net (C.F.)
- ² Laboratoire de Biotechnologie Microbienne et d'Ingénierie des Enzymes (LBMIE). Centre de Biotechnologie de Sfax, Université de Sfax. Route de Sidi Mansour Km 6, BP 1177, 3018 Sfax, Tunisie. mehdi_arbi@yahoo.fr (M.E.); mamdouh.benali@cbs.rnrt.tn (M.B.)
- * Author to whom correspondence should be addressed; E-Mail: maite.sylla@lecnam.net (M.S-IV), Tel.: +33-1-58 80 84 82, abdmouleh.fatma91@gmail.com (F.A)

Received: / Accepted: / Published:

Abstract: The antibacterial activity of a series of functionalized arylbenzothiazoles was investigated against Gram-positive pathogens including *Staphylococcus aureus* (ATCC 9144), *Micrococcus luteus* (LB14110), *Salmonella enterica* (NCTC 6017) and Gram-negative foodborne *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 8739). The antifungal activity was also evaluated against the opportunistic pathogenic yeast *Candida albicans* (ATCC 2091). The results displayed that these compounds exhibit a good antimicrobial activity compared with fusidic acid. The structure-antimicrobial activity relationships are also discussed.

Keywords: arylbenzothiazoles, antibacterial activity, antifungal

1. Introduction

Benzothiazole (BTZ) belongs to the family of bicyclic heterocyclic compounds bearing a benzene ring fused with a five-membered ring containing nitrogen and sulfur atoms. Benzothiazole is a major scaffold with many biological applications such as antimicrobial, antimalarial, anticonvulsant, anti-inflammatory and analgesic activities. Furthermore, benzothiazoles are present in natural compounds and have been used in the treatment of neurodegenerative disorders, local brain ischemia

and cancer. Some marketed drugs containing a benzothiazole moiety are illustrated in figure 1. [1-4]

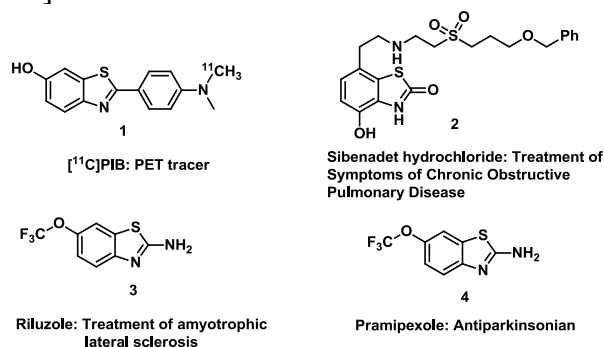
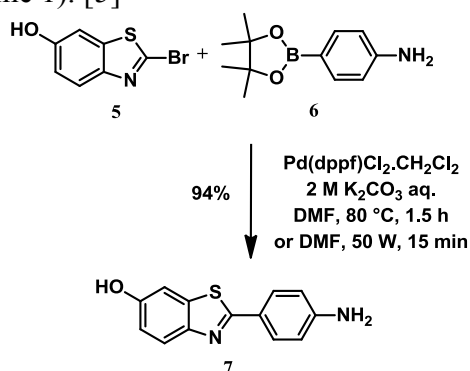


Figure 1: Marketed benzothiazoles

In 2013, encouraged by the high interest in [^{11}C]PIB for research into the diagnosis of Alzheimer's disease, we decided to develop a direct synthesis of the precursor **7** of [^{11}C]PIB from commercially available reagents, by a Suzuki-Miyaura coupling reaction. We described a one-step cross-coupling reaction between unprotected 2-bromo-6-hydroxybenzothiazole **5** and 4-aminophenyl boronic acid pinacol ester **6** using $\text{Pd}(\text{dppf})\text{Cl}_2 \cdot \text{CH}_2\text{Cl}_2$ as catalyst leading to rapid and total conversion of the starting material without affecting the hydroxyl and amino groups (Scheme 1). [5]



Scheme 1: Suzuki-Miyaura coupling reaction leading to the precursor **7** of the radiochemical tracer [^{11}C]PIB **1**.

Inspired by the therapeutic potentialities of the benzothiazole scaffold, we are interested in studying the antibacterial and antifungal activity of a series of arylbenzothiazoles previously synthesized in our laboratory. We report herein the evaluation of the antibacterial activity of these arylbenzothiazoles using pathogens bacteria Gram positive and Gram negative. The antifungal activity against the opportunist yeast *Candida Albicans* is also described. The structure-antimicrobial activity relationships are also discussed.

2. Results and Discussion

Biological studies

Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC)

A series of functionalized arylbenzothiazoles was screened for antibacterial activity against Gram-positive and Gram-negative pathogens strains *Micrococcus luteus*, *Staphylococcus aureus* and Gram-negative strains *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella*

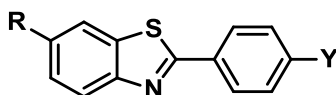
enterica were used for inhibitory tests, using levofloxacin and fusidic acid, a broad-spectrum antibiotic, as a control. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values are presented in Table 1. The MIC and MBC values for levofloxacin and fusidic acid were found to be $< 1.52 \mu\text{g/mL}$ and ranging from $12.5 \mu\text{g}$ to $25 \mu\text{g/mL}$.

The functionalized aryl benzothiazoles showed a good antimicrobial activity. They seem to be more bacteriostatic than bactericidal, since the MBC/MIC ratio is greater than or equal to four (≥ 4). The obtained results are comparable with fusidic acid.

Even if no significant difference in activity against Gram-positive or Gram-negative bacteria was observed, Gram-positive strains seem to be more sensitive than Gram-negative strains. Consequently, these results suggest a potential use against Gram-positive bacterial infections for these compounds particularly against *Micrococcus luteus*.

As shown in Table 1 compound **7** displayed the best antimicrobial activity with MIC values between 6.25 to $12.5 \mu\text{g/mL}$ against Gram-positive pathogens *Micrococcus luteus* and *Staphylococcus aureus*. Gram-negative foodborne *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella enterica* were also sensitive to compound **7** with MIC values between 12.25 and $25 \mu\text{g/mL}$. In the evaluation of the antifungal activity against the opportunistic pathogenic yeast *Candida albicans*, compound **7** also showed the best activity with a MIC ranging between 12.5 to $25 \mu\text{g/mL}$. These results suggest that combined functionalization of a hydroxyl group with an amino group has a positive influence on antibacterial and antifungal activity.

When the hydroxy group of compound **7** is replaced by a methoxy group (compound **9**) or a nitro group (compound **12**), no difference in activity was observed against *Micrococcus luteus* (MIC values between 6.25 - 12.5). However, the antimicrobial activity decreases for most pathogens. In addition, the introduction of amino group (compound **14**) doesn't have a positive influence on the activity. These results could suggest that a hydroxy group may be required to obtain excellent antimicrobial activity.

Table 1. Antimicrobial activities of a series of functionalized arylbenzothiazoles. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) in µg/mL.

Cpd. Num	R, Y		Gram (+)		Gram (-)			Yeast
			<i>Micrococcus</i>	<i>Staphylococcus</i>	<i>E. Coli</i>	<i>Pseudomonas</i>	<i>Salmonella</i>	<i>Candida</i>
7	OH, NH ₂	MIC	[6,25-12,5]	[6,25-12,5]	[12,5-25]	[12,5-25]	[12,5-25]	[12,5-25]
		MBC	100	100	>100	>100	100	>100
8	OH, NO ₂	MIC	[12,5-25]	[12,5-25]	[12,5-25]	[25-50]	[25-50]	[25-50]
		MBC	100	>100	>100	>100	>100	>100
9	OMe, NH ₂	MIC	[6,25-12,5]	[25-50]	[25-50]	[25-50]	[25-50]	[25-50]
		MBC	>100	>100	>100	>100	>100	>100
10	Me, NO ₂	MIC	[6,25-12,5]	[25-50]	[12,5-25]	[12,5-25]	[25-50]	[25-50]
		MBC	>100	>100	>100	>100	>100	>100
11	OMe, NO ₂	MIC	[6,25-12,5]	[25-50]	[12,5-25]	[25-50]	[50-100]	[50-100]
		MBC	>100	>100	100	>100	>100	>100
12	NO ₂ , NH ₂	MIC	[6,25-12,5]	[12,5-25]	[25-50]	[12,5-25]	[25-50]	[25-50]
		MBC	>100	>100	100	>100	50	100
13	NO ₂ , NO ₂	MIC	[6,25-12,5]	[12,5-25]	[25-50]	[25-50]	[25-50]	[25-50]
		MBC	>100	>100	>100	>100	>100	>100
14	NH ₂ , NH ₂	MIC	[12,5-25]	[12,5-25]	[12,5-25]	[25-50]	[25-50]	[25-50]
		MBC	100	>100	>100	100	100	100
Levofloxacin		MIC	<1,52	<1,52	<1,52	<1,52	<1,52	<1,52
		MBC	<1,52	<1,52	<1,52	<1,52	<1,52	<1,52
Fusidic acid		MIC	[12,5-25]	[12,5-25]	[12,5-25]	[12,5-25]	[12,5-25]	[12,5-25]
		MBC	100	100	>100	>100	>100	>100

3. Materials and Methods

Experimental

In vitro antibacterial activity

Bacteria and growth conditions

Microorganism growth inhibition assays were performed using LB (1% Bactotryptone, 0.5% Yeast extract, 0.5% NaCl) cultures of *Gram-positive pathogens*: *Staphylococcus aureus* (ATCC 9144), *Micrococcus luteus* (LB14110) and *Gram-negative pathogens*: *Salmonella enterica* (NCTC 6017), *Pseudomonas aeruginosa*

(ATCC 9027), *Escherichia coli* (ATCC 8739). The antifungal activity was investigated against the opportunistic pathogenic yeast *Candida albicans* (ATCC 2091).

Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC)

The synthesized compounds, dissolved in dimethylsulfoxide (DMSO), were tested in triplicate, using microplate dilution method. Minimal inhibitory concentrations (MICs) of compounds were determined according the National Committee for Clinical Laboratory

Standard (NCCLS, 2002). The test was performed in sterile 96-well microplates.

Serial two fold dilutions of each sample to be evaluated were made to yield volumes of 100 μ L per well with final concentrations ranging from 200 to 1,152 μ g/mL. 100 μ L of bacteria suspension with a concentration of 10⁷ CFU/mL were added to each well. Negative control wells contained bacteria only in LB broth medium. After incubation at 37 °C for 20 h, the minimal inhibitory concentrations (MICs) were recorded as the lowest concentration of compound in the medium that showed no microbial growth. Then, 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was added to the wells to facilitate reading of the plates. In case of microbial growth, MTT turns to blue, otherwise the medium remains yellow. Solvent medium and positive growth controls were also run simultaneously. Then from each tube, one loopful was cultured on plate count agar and incubated for 24 h at 30°C. The lowest concentration of the compound supporting no colony formation was defined as the MBC. Levofloxacin, a broad-spectrum antibiotic and fusidic acid, a narrow spectrum antibiotic were used as references.

The inhibitory activity of the tested compounds was calculated according to the formula:

$$IA (\%) = 100 - 100 (OD 600 (x) / OD 600 (i))$$

where (x) is the microbial culture containing the inhibitor and (i) is the microbial culture without inhibitor.

4. Conclusions

We discussed in this article the antibacterial and antifungal activity of a series of arylbenzothiazoles. The synthesized compounds displayed a good antimicrobial activity and these results validate the potentialities of the benzothiazole scaffold in drug discovery process.

5. Acknowledgments

We gratefully acknowledged Campus France (PHC UTIQUE 2017 37094WH) and the Tunisian Ministry of Higher Education and Scientific Research and Technology for financial support.

Author Contributions

All authors contributed to the drafting and revision of the article and approved the final version.

Conflicts of Interest

The authors declare no conflict of interest.

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