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Original article

2-Aminobenzothiazole derivatives: Search for new antifungal agents

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ABSTRACT

A new series of 6-substituted 2-aminobenzothiazole derivatives were synthesized and screened *in vitro* as potential antimicrobials. Almost all the compounds showed antifungal activity. In particular, compounds **1n**,**o**, designed on the basis of molecular modeling studies, were the best of the series, showing MIC values of 4–8 μg/mL against *Candida albicans*, *Candida parapsilosis* and *Candida tropicalis*. None of the two compounds did show any cytotoxicity effect on human THP-1 cells.

1. Introduction

One of the major problems we are facing today in the context of infectious diseases is the relentless increase and spread of antimicrobial resistance. Thus, studies for the identification of novel targets and drugs for the treatment of infectious diseases are at the forefront. In this perspective, for example, we recently determined the X-ray crystallographic structure of *Enterococcus faecalis* thymidylate synthase, which should be a potential target for antibacterial therapy [1]. Many heterocyclic nuclei, such as 1,3,4-thiadiazole, benzimidazole, 1,3,5-triazine, and benzothiazole have been recently reviewed as antimicrobial agents [2,3]. Our attention was focused to the benzothiazole nucleus [4]. In fact, benzothiazole derivatives possess a wide spectrum of biological applications such as antitumor, schictosomicidal, anti-inflammatory, anticonvulsant, antidiabetic, antipsychotic, diuretic, and antimicrobial activities [5]. In the past, our research group was interested in the synthesis and microbiological screening of a series of 2-mercapto-1,3-benzothiazole derivatives, some of which showed antibacterial activity against Gram-positive and negative bacterial strains [6]. Results showed that the SH moiety at the 2 position of the heterocyclic nucleus led to a remarkable antibacterial activity. In a search for new leads toward potent antimicrobial agents, given the isosteric relationship existing between SH and NH_2 groups, following a previous work [7], we synthesized a series of 2-amino-1,3-benzothiazoles (Fig. 1) and tested their *in vitro* antimicrobial activity. It was reported, indeed, that several 2-aminobenzothiazole derivatives, variously substituted, showed antifungal activity, even though much lower than those of the reference antifungal agent used [8]. In particular, as it was already done for the series of 2-mercapto-1,3-benzothiazole derivatives [6], substitutions at position 6 of the aryl moiety was investigated. QSAR and docking studies gave valuable hints assessing lipophilicity and steric hindrance as main molecular determinants most likely affecting the newly synthesized benzothiazole derivatives in their antifungal activity.

2. Results and discussion

2.1. Chemistry

Compounds **1a** and **1j**–**m** were commercially available. Compounds **1b**–**i**,**n**,**o** (Table 1) were synthesized as depicted in Scheme 1. Alkyl and aryl alcohols **2e**–**h**,**n** were reacted with 4-nitrophenol under Mitsunobu conditions [9–12] to give their nitro derivatives

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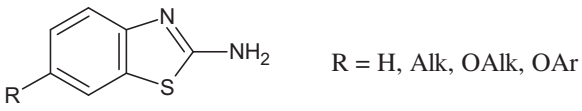


Fig. 1. Structures of 2-amino-1,3-benzothiazole derivatives (**1a**–**o**).

3e–**h**,**n**, which were reduced by catalytic hydrogenation to give anilines **6e**–**h**,**n**. Anilines **6b**–**d**,**o** were commercially available. Aniline **6i** was obtained by reducing nitro derivative **4** to aniline **5** and then by submitting the latter to a Mitsunobu reaction with phenol. 2-Aminobenzothiazole derivatives **1b**–**i**,**n**,**o** were prepared via thiocyanation of **6e**–**i**,**n**,**o** [13]. In this reaction ammonium thiocyanate and bromine were used to generate thiocyanogen in situ [14].

2.2. Antimicrobial studies

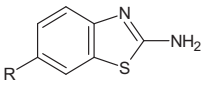
2.2.1. Antibacterial studies

According to the Clinical Laboratory Standards Institute (CLSI) guidelines [15] compounds **1a**–**o** (Table 1) were tested against Gram-positive and Gram-negative bacteria belonging to the ATCC collection (*Staphylococcus aureus* 29213, *E. faecalis* 29212, *Escherichia coli* 25922) using Norfloxacin (NRF) as reference drug. The results, expressed as MIC (μg/mL), are listed in Table 2. The antibacterial screening revealed that the compounds showed very low to no activity against all the bacterial strains tested, thus underlying that isosteric substitution SH/NH_2 brought to a loss of the antibacterial activity, as evidenced by comparing **1b**,**c**,**j**–**l** with their corresponding isosters previously reported [6].

2.2.2. Antifungal studies

Compounds **1a**–**o** (Table 1) were screened, according to CLSI guidelines [16], against a panel of fungi strains (*Candida albicans* 10231, *Candida parapsilosis* 22019, *Candida tropicalis* 750, *Candida krusei* 6258) belonging to the ATCC collection. Fluconazole was used as reference drug. The results, expressed as MIC (μg/mL), are listed in Table 3. All the 6-substituted 2-amino-1,3-mercapto benzothiazole derivatives show slight to high antifungal activity against all the *Candida* spp. tested, while unsubstituted **1a** was inactive. The electron-withdrawing effect at the 6-position of the benzothiazole moiety of halogens, such as a chlorine and fluorine atom (**1j**,**k**, Fig. 2) and a trifluoromethyl group (**1l**, Fig. 2) was first investigated. These three compounds were slightly active against all *Candida* spp. tested. In particular, **1l** was the most active of them, especially against *C. albicans*. Then, we introduced an alkoxy or aryloxy moiety (**1b**–**h**) in the same position. Results showed that antifungal activity enhanced with the increase of steric hindrance at position 6 of the heterocycle. In fact, compounds **1b** and **1c** were slightly active while phenoxy and benzyloxy derivatives (**1d** and **1e**, respectively) were much more potent. Compound **1d** was equipotent to **1c** against *C. albicans* but was much more active against *C. parapsilosis*, *C. tropicalis* and *C. krusei* (MIC values: 16, 32, 32 vs 128, 128, 256 μg/mL, respectively). The benzyloxy derivative **1e** was more active than the others against *C. albicans* and showed high activity also against *C. parapsilosis* and *C. tropicalis.* Thus, we investigated a possible homologation of the alkyl chain with one to three methylene moieties obtaining compounds **1f**, **1g** and **1h**, respectively. They were generally less active than compound **1e** evidencing that elongation of the alkyl chain was detrimental for activity. In particular, compound **1h** was nearly inactive against fungi. Compound **1i** was less active than its position isomer **1e**. This finding suggests that conceivably moving the oxygen atom is detrimental for activity. Among the series of alkoxy derivatives, we also tested the well-known drug riluzole (**1m**), the only FDA-approved drug to treat amyotrophic lateral sclerosis [17]. It was just slightly active against all the species of *Candida*. Finally, compounds **1n**,**o**, suggested by modeling studies (see below), were indeed the most active of the series against *C. albicans*, *C. parapsilosis* and *C. tropicalis.* It is noteworthy that compound **1n** was even as potent as the reference on *C. tropicalis*.

Table 1
Structures of compounds **1a**–**o**.



Compd	R
1a	H
1b	Me–O
1c	Et–O
1d	Ph–O
1e	Bn–O
1f	$Ph(CH_2)_2$–O
1g	$Ph(CH_2)_3$–O
1h	$Ph(CH_2)_4$–O
1i	$PhOCH_2$
1j	F
1k	Cl
1l	CF_3
1m	CF_3O
1n	4-Cl–Bn–O
1o	4-Cl–Ph–O

2.3. Molecular modeling studies

A molecular modeling study was carried out to better perceive and evaluate the biological profile within the series under study. In the first step we make use of classical 2D-QSAR to gain a sound regression model, showing the dependence of the antifungal activity on lipophilicity. In particular, as proposed by Hansch [18], pMIC data showed parabolic dependence by hydrophobic chemotype of benzothiazoles, as scored by the CLog*P* values (Fig. 2, Table 4). It has to be pointed out that model comprising compounds **1b**–**m** was indeed statistically significant ($r^2 = 0.624$), but a valuable increase of the explained variance ($r^2 = 0.750$) was achieved excluding from the regression model **1d**, the lone derivative bearing the phenyl ring directly branched to the oxygen at position 6 of the aryl moiety. Afterward, the antifungal activity was interpreted through docking experiments. Lanosterol 14α-demethylase (CYP51) is a member of the cytochrome P450 superfamily, which catalyzes the oxidative removal of the 14α-methyl group of lanosterol to give $\Delta^{14,15}$-desaturated intermediates in ergosterol biosynthesis [19]. The development of inhibitors of CYP51 in fungi has provided a rich source of drugs, such as clotrimazole, ketoconazole, fluconazole, and itraconazole [20], responsible for the cell growth inhibition due to ergosterol depletion. The same cytochrome is also targeted by drugs (i.e. benzothiazines and benzoxazines) [20–22] characterized by a molecular scaffold similar to the benzothiazoles here presented. Moreover, the importance of CYP51 as a plausible target in the treatment of *C. albicans* infections has been already and successfully reported [23–25]. On the basis of this evidence, compounds **1b**–**i** were therefore docked into the catalytic site of the homology based model of *C. albicans* CYP51 (CA-CYP51) [26,27] which has already been successfully used in a previous study on antifungal agents [7]. Docking binding poses highlighted some interesting features that might be in charge of the antifungal activity of the