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Screening of antimicrobial activity of diarylamines in the 2,3,5-trimethylbenzo[*b*]thiophene series: a structure–activity evaluation study

Isabel C. F. R. Ferreira,[a,*] Ricardo C. Calhelha,[a] Letícia M. Estevinho[a] and Maria-João R. P. Queiroz[b]

[a]*Escola Superior Agrária, Instituto Politécnico de Bragança, Campus de Sta. Apolónia, Apartado 1172, 5301-854 Bragança, Portugal*
[b]*Departamento de Química, Campus de Gualtar, Universidade do Minho, 4710-057 Braga, Portugal*

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Abstract—Gram positive (*Bacillus cereus*, *B. subtilis*), Gram negative (*Pseudomonas aeruginosa*, *Escherichia coli*) bacteria, and *Candida albicans* as a representative of fungi were used for screening the in vitro antimicrobial activity of diarylamines in the 2,3,5-trimethylbenzo[*b*]thiophene series bearing different substituents, synthesized by us using the palladium-catalyzed C–N coupling methodology. The minimal inhibitory concentration (MIC) and structure–activity relationships (SARs) were evaluated.

The enhance prevalence of infectious diseases is becoming a world wide problem. Additionally, the resistance problem demands that a renewed effort should be made to seek antimicrobial agents effective against pathogenic microorganisms resistant to current treatment. Benzo[*b*]thiophenes are important heterocycles as biological active molecules.[1–5] Recently we have been interested in the palladium-catalyzed aryl amination of benzo[*b*]thiophenes either in the benzene or in the thiophene ring to obtain the corresponding diarylamines and in some cases the tetracyclic aromatic compounds resulting from intramolecular cyclizations.[6,7] We have been able to establish that under the same C–N coupling conditions [$Pd(OAc)_2$ (3 mol%), Cs_2CO_3 (1.4 equiv), and BINAP (4 mol%) in toluene] it was possible to obtain either primary amines[8] or diarylamines[7,8] in the benzene ring of the benzo[*b*]thiophene moiety.

Herein, we describe the structure–activity relationship of a novel series of diarylamines as antimicrobial agents. Differently substituted diarylamines derivatives of 2,3,5-trimethylbenzo[*b*]thiophene **1** were obtained by C–N palladium-catalyzed cross-couplings in good to high yields (50%-quantitative yield) using either the bromo compound **2** or the amino derivative **3** as coupling components.[8] The latter was prepared from compound **2** using also a C–N palladium-catalyzed cross-coupling with benzophenone imine, followed by acidic hydrolysis in a 60% overall yield (Scheme 1).[8]

A screening of antibacterial activities using two Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and two Gram positive bacteria (*Bacillus subtilis* and *B. cereus*) and antifungal using *Candida albicans* was performed for the diarylamines **4**–**8** and for their benzo[*b*]thiophene precursors **1**–**3**, to determine the importance of the diarylamine skeleton in this series. The minimal inhibitory concentration (MIC in μg/mL) was determined (Table 1) using an adaptation of agar streak dilution method based on radial diffusion.[9,10] Suspensions of the microorganism were prepared to contain approximately 10^8 cfu/mL and the plates were inoculated. A stock solution of the synthesized compound (1000 μg/mL) in DMSO was prepared and graded dilutions of the tested compounds were incorporated in a cavity (depth 3 mm, diameter 4 mm) made in the center of the petridish (nutrient agar for antibacterial activity and sabouraud dextrose agar medium for antifungal activity). The plates were incubated at 37 °C (bacteria) or 30 °C (fungi) for 24 h in duplicate. In the same conditions different concentrated solutions of Ampicillin, Chloramphenicol (antibacterial), and Cyclohexamide

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* Corresponding author. Tel.: +351 273 303219; fax: +351 273 325405; e-mail: iferreira@ipb.pt

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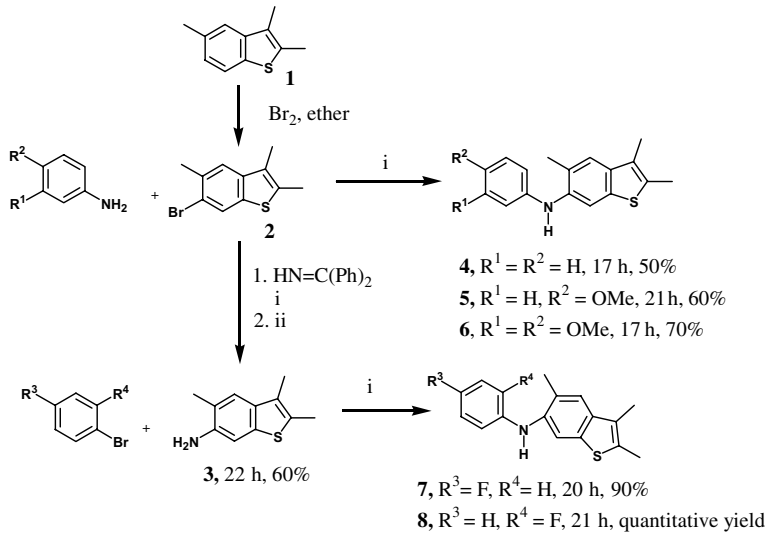

Scheme 1. Synthesis of diarylamines and their precursors in the 2,3,5-trimethylbenzo[*b*]thiophene series.[8] Reagents and conditions: (i) $Pd(OAc)_2$ (3 mol%), Cs_2CO_3 (1.4 equiv), BINAP (4 mol%), dry toluene, 100 °C, Ar; (ii) HCl 2 M/THF.

Table 1. Antimicrobial activity of the synthesized compounds

Compounds	Microorganism	MIC (μg/mL) (zone of inhibition) (mm)
1	*B. cereus* (CECT 148)	Not active
	B. subtilis (CECT 498)	Not active
	E. coli (CECT 101)	Not active
	P. aeruginosa (CECT 108)	Not active
	C. albicans (CECT 1394)	Not active
2	*B. cereus*	1000 (12)
	B. subtilis	500 (18)
	E. coli	Not active
	P. aeruginosa	Not active
	C. albicans	Not active
3	*B. cereus*	500 (10)
	B. subtilis	500 (17)
	E. coli	Not active
	P. aeruginosa	Not active
	C. albicans	1000 (13)
4	*B. cereus*	200 (18)
	B. subtilis	200 (8)
	E. coli	200 (4)
	P. aeruginosa	200 (16)
5	*B. cereus*	100 (4)
	B. subtilis	50 (19)
	E. coli	1000 (4)
	P. aeruginosa	1000 (8)
	C. albicans	12.5 (6)
6	*B. cereus*	200 (24)
	B. subtilis	200 (10)
	E. coli	Not active
	P. aeruginosa	1000 (10)
	C. albicans	25 (8)
7	*B. cereus*	200 (12)
	B. subtilis	200 (10)
	E. coli	Not active
	P. aeruginosa	Not active
8	*B. cereus*	200 (9)
	B. subtilis	50 (8)
	E. coli	Not active
	P. aeruginosa	Not active
Ampicillin	*B. cereus*	3.13 (13)
	B. subtilis	12.5 (10)
	E. coli	6.25 (15)
	P. aeruginosa	6.25 (12)
Chloramphenicol	*B. cereus*	3.13 (8)
Cyclohexamide	*C. albicans*	12.5 (5)

CECT—Spanish type culture collection of Valencia University.

(antifungal) in DMSO were used as standards. The MIC was considered to be the lowest concentration of the tested compound, which inhibits growth of bacteria or fungi on the plate. The diameters of the inhibition zones corresponding to the MICs are presented in Table 1. Positive control using only inoculation and negative control using only DMSO in the cavity were carried out. From analysis of Table 1 we can conclude that 2,3,5-trimethylbenzo[*b*]thiophene **1** is not effective against none of the microorganisms tested. Insertion of a bromine atom in the 6-position (compound **2**) shows activity only for Gram positive bacteria but with high MICs. The substitution of the halogen atom by an amino group (compound **3**) lowers the MIC for *B. cereus* and turns it active against *C. albicans* but still with high MICs. Diarylamine **4**, without substituents in the phenyl ring, shows activity either against Gram negative or Gram positive bacteria (MIC 200 μg/mL). The introduction of a OMe group in the *para* position (compound **5**) lowers the MIC to 100 μg/mL for *B. cereus* and to 50 μg/mL for *B. subtilis* but increases it to 1000 μg/mL for Gram negative bacteria. The introduction of an additional OMe group (compound **6**) makes the compound inactive against *E. coli*.

Compounds with a F atom in the phenyl ring (**7** and **8**) show selectivity against Gram positive bacteria. The presence of the F atom in the *ortho* position (**8**) instead of in the *para* position (**7**) lowers the MIC for *B. subtilis* from 200 to 50 μg/mL. Although the MICs were higher than the values obtained for ampicillin it is the first time that this type of compounds was submitted to antibacterial studies. Diarylamines **5** and **6** were also submitted to antifungi preliminary studies using *Candida albicans* and the MIC obtained for compound **5** is comparable to the obtained for cyclohexamide (antifungal).

In conclusion, a new diarylamine series as antimicrobial agents based on the 2,3,5-trimethylbenzo[*b*]thiophene moiety has been developed. The diarylamine skeleton and the different substituents (H, 1 OMe, 2 OMe, or F) proved to be important for activity, changing selectivities and MICs, when compared with the functionalized (Br and NH_2) benzo[*b*]thiophene precursors.

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