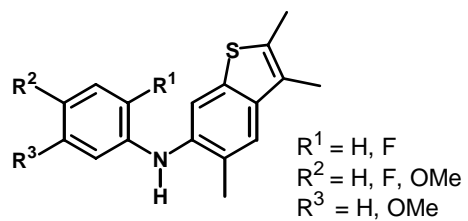


Screening of antimicrobial activity of diarylamines in the 2,3,5-trimethylbenzo[*b*]thiophene series: a structure-activity evaluation study

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In vitro antimicrobial data against Gram positive, Gram negative bacteria and *Candida albicans* are presented.

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

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Abstract- Gram positive (*Bacillus cereus*, *Bacillus 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-trimethyl benzo[*b*]thiophene series bearing different substituents, synthesised 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 (3mol%), Cs₂CO₃ (1.4 equiv) and BINAP (4mol%) in toluene] it was possible to obtain either primary amines⁸ 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 – (*Escherichia coli* and *Pseudomonas aeruginosa*) and two Gram + bacteria (*Bacillus subtilis* and *Bacillus cereus*) and antifungal using *Candida albicans* was performed for the diarylamines **4-8** and for their benzo[*b*]thiophene

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precursors **1-3**, to determine the importance of the diarylamine skeleton in this series. The minimal inhibitory concentration (MIC in $\mu\text{g/mL}$) 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 $\mu\text{g/mL}$) in DMSO was prepared and graded dilutions of the tested compounds were incorporated in a cavity (depth 3mm, diameter 4mm) 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 24h in duplicate. In the same conditions different concentrated solutions of Ampiciline, Chloramphenicol (antibacterial) and Cyclohexamide (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 + 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 – or Gram + bacteria (MIC 200 $\mu\text{g/mL}$). The introduction of a OMe group in the *para* position (compound **5**) lowers the MIC to 100 $\mu\text{g/mL}$ for *B. cereus* and to 50 $\mu\text{g/mL}$ for *B. subtilis* but increases it to 1000 $\mu\text{g/mL}$ for Gram – 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 + 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 $\mu\text{g/mL}$. Although the MICs were higher than the values obtained for ampiciline it is the first time that this type of compounds was submitted to antibacterial studies. Diarylamines **5** and **6** were also submitted to anti-fungi preliminary studies using *Candida albicans* and the MIC obtained for compound **5** is comparable to the obtained for cyclohexamide (anti-fungal).

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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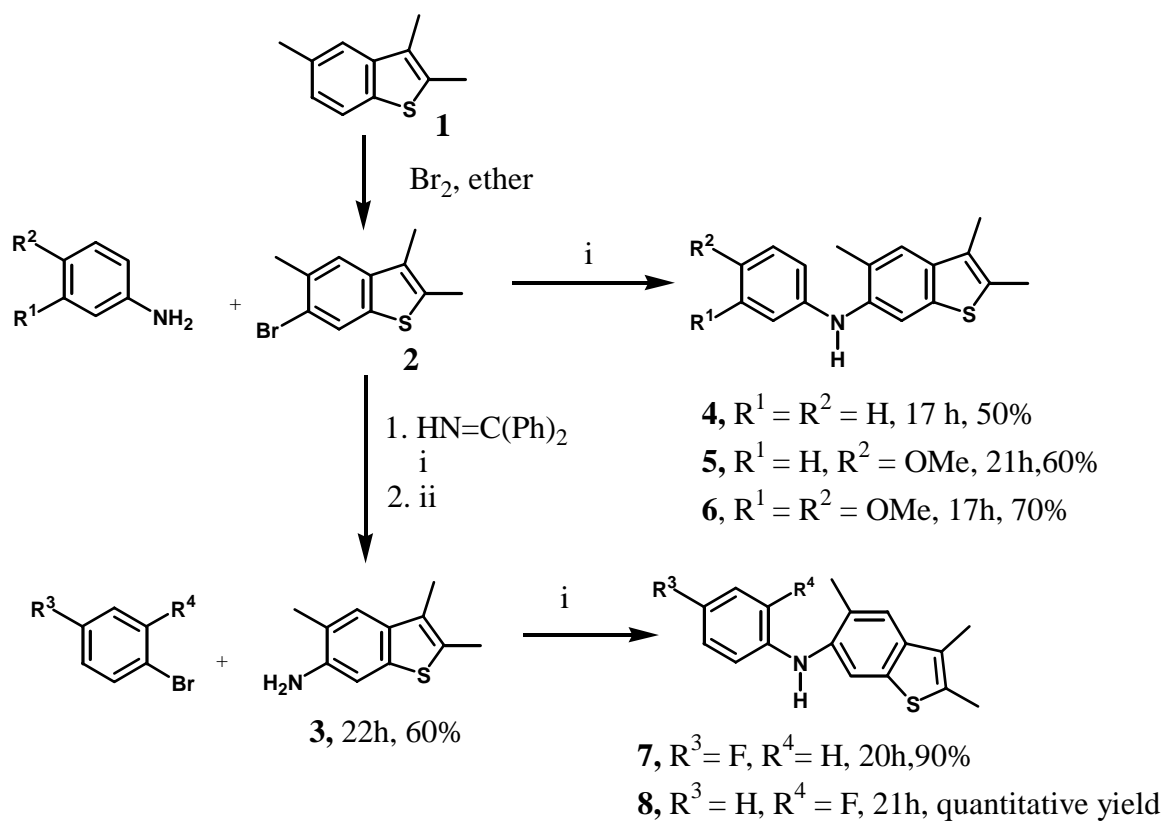
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Table 1. Antimicrobial activity of the synthesized compounds.

Compounds	Microorganism	MIC ($\mu\text{g/mL}$) (Zone of inhibition) (mm)
1	<i>B. cereus</i> (CECT 148)	-
	<i>B. subtilis</i> (CECT 498)	-
	<i>E. coli</i> (CECT 101)	-
	<i>P. aeruginosa</i> (CECT 108)	-
	<i>C. albicans</i> (CECT 1394)	-
2	<i>B. cereus</i>	1000 (12)
	<i>B. subtilis</i>	500 (18)
	<i>E. coli</i>	-
	<i>P. aeruginosa</i>	-
	<i>C. albicans</i>	-
3	<i>B. cereus</i>	500 (10)
	<i>B. subtilis</i>	500 (17)
	<i>E. coli</i>	-
	<i>P. aeruginosa</i>	-
	<i>C. albicans</i>	1000 (13)
4	<i>B. cereus</i>	200 (18)
	<i>B. subtilis</i>	200 (8)
	<i>E. coli</i>	200 (4)
	<i>P. aeruginosa</i>	200 (16)
5	<i>B. cereus</i>	100 (4)
	<i>B. subtilis</i>	50 (19)
	<i>E. coli</i>	1000 (4)
	<i>P. aeruginosa</i>	1000 (8)
	<i>C. albicans</i>	12.5 (6)
6	<i>B. cereus</i>	200 (24)
	<i>B. subtilis</i>	200 (10)
	<i>E. coli</i>	-
	<i>P. aeruginosa</i>	1000 (10)
	<i>C. albicans</i>	25 (8)
7	<i>B. cereus</i>	200 (12)
	<i>B. subtilis</i>	200 (10)
	<i>E. coli</i>	-
	<i>P. aeruginosa</i>	-
8	<i>B. cereus</i>	200 (9)
	<i>B. subtilis</i>	50 (8)
	<i>E. coli</i>	-
	<i>P. aeruginosa</i>	-
Ampicillin	<i>B. cereus</i>	3.13 (13)
	<i>B. subtilis</i>	12.5 (10)
	<i>E. coli</i>	6.25 (15)
	<i>P. aeruginosa</i>	6.25 (12)
Choramphenicol	<i>B. cereus</i>	3.13 (8)
Cyclohexamide	<i>C. albicans</i>	12.5 (5)

CECT-Spanish type culture collection of Valencia University



i) $\text{Pd}(\text{OAc})_2$ (3mol%), Cs_2CO_3 (1.4equiv.), BINAP (4mol%), dry toluene, 100°C , Ar
 ii) HCl 2M/THF

Scheme 1. Synthesis of diarylamines and their precursors in the 2,3,5-trimethylbenzo[*b*]thiophene series.⁸