Synthesis and antimicrobial activity studies of ortho-chlorodiarylamines and heteroaromatic tetracyclic systems in the benzo[\(b\)]thiophene series

Maria-João R. P. Queiroz,\(^{a,*}\) Isabel C. F. R. Ferreira,\(^{b}\) Yannick De Gaetano,\(^{a,c}\) Gilbert Kirsch,\(^{c}\) Ricardo C. Calhelha\(^{b}\) and Letícia M. Estevinho\(^{b}\)

\(^{a}\)Departamento de Química, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

\(^{b}\)Escola Superior Agrária—Instituto Politécnico de Bragança, Campus de Sta Apolónia, 5301-855 Bragança, Portugal

\(^{c}\)Laboratoire d’Ingénierie Moléculaire et Biochimie Pharmacologique, Faculté des Sciences, Université de Metz, 1, bd Arago Metz Technopole, 57078 Metz Cedex 3, France

Received 10 March 2006; revised 19 June 2006; accepted 20 June 2006

Available online 14 July 2006

Abstract—Ortho-Chlorodiarylamines in the 2,3,7-trimethylbenzo[\(b\)]thiophene series were prepared in high yields (70–85%) by C–N palladium-catalyzed cross-coupling using P(\(t\)-Bu)\(_3\) as ligand and NaOt-Bu as base. A palladium-assisted C–C intramolecular cyclization of the coupling products gave thienocarbazoles and the dechlorinated diarylamines. Studies of antimicrobial activity of the compounds obtained, against representative species of bacteria (\textit{Escherichia coli}, \textit{Pseudomonas aeruginosa}, \textit{Bacillus cereus} and \textit{Bacillus subtilis}) and fungi (\textit{Candida albicans}), were performed. We have also included in the biological assays some pyridine derivatives previously prepared by us, and it was possible to establish some structure–activity relationships (SARs).

\(\copyright\) 2006 Elsevier Ltd. All rights reserved.

1. Introduction

In recent years we have been interested in palladium-catalyzed C–N cross-couplings in the benzo[\(b\)]thiophene series.\(^{1,2}\) We reported some general conditions to obtain diarylamines functionalizing the benzene ring of the benzo[\(b\)]thiophene system with arenes bearing electron-donating or -withdrawing groups.\(^{1b}\) The antimicrobial activity of diarylamines derivatives of 2,3,5-trimethylbenzo[\(b\)]thiophene was already studied by us.\(^{3}\)

For the synthesis of ortho-bromodiarylamines in the benzo[\(b\)]thiophene series we have established different conditions for the functionalization of the benzene or the thiophene ring. The latter diarylamines were obtained in moderate to good yields and gave the corresponding tetracyclic thienocarbazoles and indolobenzo[\(b\)]thiophenes using a palladium-assisted intramolecular cyclization.\(^{1c}\)

Bedford and Cazin described the synthesis of ortho-chlorodiphenylamines in high yields by C–N palladium-catalyzed cross-coupling of halobenzenes with ortho-chloroanilines using P(\(t\)-Bu)\(_3\) as ligand and NaOt-Bu as base.\(^{4}\)

In this work, we describe the synthesis of ortho-chlorodiatrylamine derivatives of 2,3,7-trimethylbenzo[\(b\)]thiophene in high yields, using Bedford’s conditions and the palladium-assisted C–C intramolecular cyclization to thienocarbazoles. Using the same type of reactions we have already reported the synthesis of an ortho-chloroheteroarylamine from 3-bromo-2-chloropyridine and 6-amino-2,3,7-trimethylbenzo[\(b\)]thiophene and its cyclization to the first thieno-\(\delta\)-carboline.\(^{5}\) The latter compound has already shown antiproliferative activity upon photoactivation, in leukaemia and in solid tumour cell lines.\(^{6}\)

Herein we report studies of the antimicrobial activity of the compounds prepared including also the pyridine.

Keywords: Benzothiophenes; ortho-Chlorodiarylamines; Palladium; Thienocarbazoles; Thienocarboline; Antimicrobial activity.

\(^{*}\) Corresponding author. Tel.: +351 253604378; fax: +351 253678983; e-mail: mjrpq@quimica.uminho.pt

0968-0896/$ - see front matter \(\copyright\) 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.06.035
derivatives previously prepared by us, in order to establish some structure–activity relationships (SARs).

2. Results and discussion

2.1. Synthesis

The ortho-chlorodiarylamines 1a and 1b were prepared in high yields, by palladium-catalyzed C–N cross-coupling of the 6-bromo-2,3,7-trimethylbenzo[b]thiophene with ortho-chloroanilines, using Bedford’s conditions (Scheme 1).

The diarylamines 1a and 1b were cyclized to the corresponding thienocarbazoles using a palladium-assisted C–C intramolecular cyclization (Scheme 2) applying the Maes conditions that had been used in the cyclization of 3-chloro-2-(4-piridinylamino)pyridine, needing in our case a bigger amount of Pd(OAc)$_2$. This reaction can be seen as an intramolecular C–H activation by a Pd(II) complex, resulting of oxidative addition of compounds 1 to Pd(0), presumably by an electrophilic displacement mechanism, to give a six-membered pallacycle which subsequently yields the thienocarbazoles 2 by reductive elimination, as suggested for the synthesis of carbazoles from ortho-chlorodiphenylamines.

Scheme 1. Synthesis of ortho-chlorodiarylamines in the 2,3,7-trimethylbenzo[b]thiophene series. Reagents and condition: (i) Pd(OAc)$_2$ (5 mol%), P($t$-Bu)$_3$ (7 mol%), NaO-$t$-Bu (5 equiv), dry toluene, 105 °C, Ar.

Scheme 2. Intramolecular cyclization to thienocarbazoles 2 and thienocarboline 4. Reagents and condition: (i) Pd(OAc)$_2$ (40 mol%), P($t$-Bu)$_3$ (30 mol%), K$_3$PO$_4$ (10 equiv), dry dioxane, 120 °C, 20 h, Ar.
In the synthesis of thienocarbazoles 2, the dechlorinated diarylamines 3a and 3b were obtained as major products and the starting ortho-chlorodiarylamines were also isolated. In the cyclization of 1b, two thienocarbazoles 2b and 2c were formed (Scheme 2). The latter can be the result of the electrophilic attack of Pd(OAc)$_2$ on the aromatic rings of 3b with extrusion of Pd(0).$^{1a}$

As already reported by us the cyclization of the ortho-chlorodiarylamine 1c gave the thienocarbone 4 in high yield by the same method (Scheme 2).$^5$

### 2.2. In vitro antimicrobial activity evaluation

An evaluation of the antibacterial activity using two Gram-negative (Escherichia coli and Pseudomonas aeruginosa) and two Gram-positive bacteria (Bacillus subtilis and Bacillus cereus) and the antifungal activity using Candida albicans as a representative species of fungi was assessed for compounds 1a–c, 2a,b, 3b and 4. The minimal inhibitory concentration (MIC in µg/mL) was determined using an adaptation of agar streak dilution method based on radial diffusion.$^{10,11}$ In the same conditions different concentrated solutions of ampicillin (antibacterial) and cycloheximide (antifungal) 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. The compounds tested are not active against Bacillus cereus starting from 1000 µg/mL of each compound.

From the analysis of Table 1 it is possible to establish some SARs. The only active compound against E. coli in the concentrations tested is the ortho-chlorodiarylamine 1b (MIC 12.5 µg/mL), the methoxy group being the responsible for the activity. Against Gram + bacteria the MICs for 1b are much lower than those for 1a. Comparing 1b with 1c (the pyridine derivative) the latter shows to be more active against B. cereus (MIC 3.13 µg/mL) but less active against B. subtilis. Against C. albicans 1b and 1c present the same MIC (25 µg/mL) which is lower than the MIC obtained for 1a (50 µg/mL).

Comparing 1b with the corresponding dechlorinated diarylamine 3b, the MICs for the latter are much more lower for B. cereus and for C. albicans (0.05 µg/mL) and even lower than those for ampicillin and cycloheximide.

Among the cyclized products 2a and 2b, the methoxylated thienocarbazole 2b presents the lower MICs. The thieno-δ-carboline 4 presents better results than the corresponding thienocarbazole 2a for B. cereus and C. albicans and the same MIC for B. subtilis which is lower than the MIC for ampicillin.

### 3. Conclusion

**4.1. Materials and methods**

Melting points were determined on a Gallenkamp apparatus and are uncorrected. The $^1$H NMR spectra were measured on a Varian Unity Plus at 300 MHz. Spin–spin decoupling was used to assign the signals. The $^{13}$C NMR spectra were measured in the same instrument at 75.4 MHz (using DEPT $\theta$ 45°).

Elemental analyses were determined on a LECO CHNS 932 elemental analyser. Mass spectra (EI) and HRMS were made by the mass spectrometry service of University of Vigo-Spain.

### Table 1. Antimicrobial activity of compounds 1a–c, 2a,b, 3b and 4

<table>
<thead>
<tr>
<th>Compounds</th>
<th>MIC in µg/mL (zone of inhibition in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. coli CECT 101</td>
</tr>
<tr>
<td>1a</td>
<td>Not active$^a$</td>
</tr>
<tr>
<td>1b</td>
<td>12.5 (6)</td>
</tr>
<tr>
<td>1c</td>
<td>Not active$^a$</td>
</tr>
<tr>
<td>3b</td>
<td>Not active$^a$</td>
</tr>
<tr>
<td>2a</td>
<td>Not active$^a$</td>
</tr>
<tr>
<td>2b</td>
<td>Not active$^a$</td>
</tr>
<tr>
<td>4</td>
<td>Not active$^a$</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>6.25 (15)</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>—</td>
</tr>
</tbody>
</table>

$^a$ Not active starting from 1000 µg/mL.

CECT-Spanish type culture collection of Valencia University.
Column chromatography was performed on Macherey–Nagel silica gel 230–400 mesh. Petroleum ether refers to the boiling range 40–60 °C. Ether refers to diethyl ether. When solvent gradient was used, the increase of polarity was done gradually from neat petroleum ether to mixtures of ether/petroleum ether increasing 10% of ether until the isolation of the product. Preparative layer chromatography (PLC) was performed in 20 × 20 cm² plates Macherey–Nagel silica plates layer 2 mm SIL G-200 UV254. P(t-Bu)₃ was purchased from Strem as an hexane solution.

For the in vitro antimicrobial activity, suspensions of the microorganisms were prepared to contain approximately 10⁸ cfu/mL and the plates were inoculated. A stock solution of the synthesized compound (100 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 vs dextrose agar medium for antifungal activity). The plates were incubated at 37 °C (for bacteria) and at 30 °C (for fungi) for 24 h in duplicate. Positive control using only inoculation and negative control using only DMSO in the cavity were carried out.

4.2. General procedure for the synthesis of ortho-chlorodiarylamines 1a and 1b

In a dry Schlenk tube it was poured under Ar with stirring, dry dioxane (6–8 mL), Pd(OAc)₂ (40 mol %), P(t-Bu)₃ (30 mol %), NaOr-Bu (5 equiv) and the amine (2-chloroaniline or 2-chloro-m-anisidine hydrochloride). The mixture was heated under Ar for 20 h at 120 °C. After cooling ethyl acetate was added and the mixture was filtered. Removal of solvents gave an oil which was submitted to PLC (several elutions) to give thienocarbazoles 2 and the diarylamines 3. The starting materials were also isolated as the less polar products.

4.2.1. 6-(2-Chloro-5-methoxyphenyl)amino-2,3,7-trimethylbenzo[b]thiophene (1b). From 6-bromo-2,3,7-benzo[b]thiophene (150 mg, 0.590 mmol) and 2-chloro-m-anisidine hydrochloride (115 mg, 0.590 mmol) and heating for 2 h, compound 1b was isolated as a colourless solid (135 mg, 70%). Crystallization from ether/petroleum ether gave colourless crystals, mp 152–154 °C. ¹H NMR (CDCl₃): 2.32 (3H, s, ArCH₃), 2.42 (3H, s, ArCH₃), 2.52 (3H, s, ArCH₃), 3.65 (3H, s, OCH₃), 5.95 (1H, br s, N–H), 6.20 (1H, d, J = 3 Hz, H-6’), 6.29 (1H, dd, J = 8.7 and 3 Hz, H-4’), 7.24 (1H, d, J = 8.7 Hz, H-3’), 7.28 (1H, d, J = 8.4 Hz, ArH), 7.45 (1H, d, J = 8.4 Hz, ArH) ppm. ¹³C NMR (CDCl₃): 11.45 (CH₃), 13.84 (CH₃), 15.95 (CH₃), 55.32 (OCH₃), 99.77 (CH), 103.97 (CH), 111.58 (C), 119.42 (CH), 122.75 (CH), 126.95 (C), 127.73 (C), 133.35 (C), 134.03 (C), 138.55 (C), 139.78 (C), 143.45 (C), 159.35 (C) ppm. MS m/z (%): 334 (8), 333 (M⁺ 35Cl, 38), 332 (22) 331 (M⁺ 36Cl, 100). Anal. Calcld for C₁₇H₁₇NS: C, 76.36; H, 6.41; N, 5.24; S, 11.99. Found: C, 76.56; H, 6.23; N, 4.89; S, 11.72. Thienocarbazole 2a was obtained as a white solid (18.5 mg, 30%), mp 210–211 °C. ¹H NMR (CDCl₃): 2.29 (3H, s, ClH), 2.41 (3H, s, ClH), 2.49 (3H, s, ClH), 5.48 (1H, s, NH), 6.79–6.86 (3H, m, ArH), 7.18–7.29 (3H, m, ArH), 7.41 (1H, d, J = 8.4 Hz, ArH). ¹³C NMR (CDCl₃): 11.45 (CH₃), 13.80 (CH₃), 15.97 (CH₃), 115.42 (2 × CH), 119.21 (CH), 119.24 (CH), 120.51 (CH), 124.50 (C), 127.69 (C), 129.23 (2 × CH), 132.34 (C), 135.80 (C), 137.34 (C), 139.90 (C), 145.78 (C) ppm. Anal. Calcld for C₁₇H₁₅NS: C, 76.36; H, 6.41; N, 5.24; S, 11.99. Found: C, 76.56; H, 6.23; N, 4.89; S, 11.72. Thienocarbazole 2a was obtained as a white solid (18.5 mg, 30%), mp 210–211 °C. ¹H NMR (CDCl₃): 2.42 (3H, s, ClH), 2.54 (3H, s, ClH), 2.70 (3H, s, ClH), 7.23–7.29 (1H, m, Ar–H), 7.40–7.46 (2H, m, Ar–H), 7.86 (1H, s, NH), 8.11–8.16 (2H, m, Ar–H) ppm. ¹³C NMR (CDCl₃): 11.80 (CH₃), 14.01 (CH₃), 15.17 (CH₃), 109.69 (CH), 110.51 (CH), 111.59 (C), 119.23 (CH), 120.24 (CH), 122.24 (C), 124.20 (C), 125.70 (CH), 127.46 (C), 130.19 (C), 135.16 (C), 136.61 (C), 136.87 (C), 140.33 (C) ppm. MS m/z (%): 267 (M⁺ 2+, 6), 266 (M⁺ 1+, 20), 265 (M⁺, 100), 250 (19); calcld for C₁₇H₁₅NS: 265,9025. Found M⁺: 265.9017.
4.3.2. 7-Methoxy-2,3,10-trimethyl-9H-thieno[2,3-b]carbazole (2c), 5-methoxy-2,3,10-trimethyl-9H-thieno[2,3-b] carbazole (2c) and 6-(3-methoxyphenyl)amino-2,3,7-trimethylbenzo[b]thiophene (3b). From compound 1b (50.0 mg, 0.151 mmol) and PLC (35% ether/petroleum ether), diarylamine 3b was isolated as a white solid (18.0 mg, 40%), mp 103–105°C. 1H NMR (CDCl3): 2.30 (3H, s, Ar-CH3), 2.41 (3H, s, Ar-CH3), 2.50 (3H, s, Ar-CH3), 3.75 (3H, s, OCH3), 5.48 (1H, br s, NH), 6.34–6.42 (3H, m, ArH), 7.12 (1H, t, J = 8 Hz, H-5'), 7.28 (1H, d, J = 8.4 Hz, ArH), 7.41 (1H, d, J = 8.4 Hz, ArH). 13C NMR (CDCl3): 11.44 (CH3), 13.80 (CH3), 15.97 (CH3), 55.12 (OCH3), 101.06 (CH), 104.44 (CH), 108.10 (CH), 112.07 (CH), 124.96 (C), 137.55 (C), 139.80 (C), 147.35 (C), 160.77 (C). MS m/z (%): 299 (M+2, 7), 298 (M+1, 21), 297 (M+, 100). Anal. Calcd for C18H17N6: C, 72.69; H, 6.44; N, 4.71; S, 10.54. HRMS C18H17N6S: calcd M+ 295.1031; found 295.1035.

Thienocarbazole 2c was isolated as a white solid (4.50 mg, 10%), mp 214–216°C. 1H NMR (CDCl3): 2.44 (3H, s, ArCH3), 2.54 (3H, s, ArCH3), 2.69 (3H, s, ArCH3), 4.15 (3H, s, OCH3), 6.72 (1H, d, J = 7.9 Hz, ArH), 7.07 (1H, d, J = 7.9 Hz, ArH), 7.36 (1H, t, J = 7.9 Hz, H-7), 7.89 (1H, br s, NH), 8.35 (1H, s, H-4). MS m/z (%): 297 (M+2, 10), 296 (M+1, 16), 295 (M+, 70). HRMS C18H17N6S: calcd M+ 295.1031; found 295.1034.

Acknowledgments

The authors thank the Foundation for the Science and Technology (Portugal) for financial support to Centro de Química—Univ. Minho, CIMO-ESEDIPAR (Univ. Metz→Univ. Minho) for financial support of Yannick De Gaetano in Portugal. References and notes


