Synthesis of β-benzo[*b*]thienyldehydrophenylalanine derivatives by one pot palladium-catalyzed borylation and Suzuki coupling (BSC) and metal-assisted intramolecular cyclization. Studies of fluorescence and antimicrobial activity.

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Palladium-catalyzed borylation and Suzuki coupling (BSC) in a one pot procedure was successfully applied to the synthesis of several β-substituted dehydrophenylalanines in the benzo[*b*]thiophene series maintaining the stereochemistry of the starting materials. Bromobenzo[*b*]thiophenes bearing an *ortho* EDG (OMe or Me) were used as the components to be borylated with pinacolborane. Pure stereoisomers of β-bromodehydrophenylalanines were used as the other Suzuki coupling component. Treatment of the methyl ester of *N*-(*tert*-butoxycarbonyl)-(*Z*)-β-(2,3,5-trimethylbenzo[*b*]thien-6-yl)dehydrophenylalanine thus obtained, with $Pd(OAc)_2$ and $Cu(OAc)_2$ in DMF at 160 °C gave two indole derivatives (1:3). The major product resulting from isomerization and cyclization and the minor product resulting from direct

cyclization (thienoindole). Reaction at 100 °C gave the same products in similar amounts. Using as starting material the methyl ester of *N*-(*tert*-butoxycarbonyl)-(*Z*)-β-(2,3,7-trimethylbenzo[*b*]thien-6-yl)dehydrophenylalanine gave only one product, resulting from isomerization and cyclization at 100 °C. Two of the cyclized compounds were submitted to fluorescence studies; the thienoindole could be used as a fluorescent probe. Preliminary studies of antimicrobial activity were performed on the precursors and on the cyclized products.

Introduction

In recent years we have been interested in the linkage of dehydroamino acid derivatives to benzo[*b*]thiophenes and in the study of the fluorescence and antimicrobial activity of the resulting products. Using Suzuki coupling we were able to synthesize either β-benzo[*b*]thienyl or β, β-bis(benzo[*b*]thienyl)dehydroamino acid derivatives.[1,2] The latter were cyclized to the corresponding dehydroprolines using a metal-assisted intramolecular cyclization and the fluorescence properties of one of them were studied.[2b]

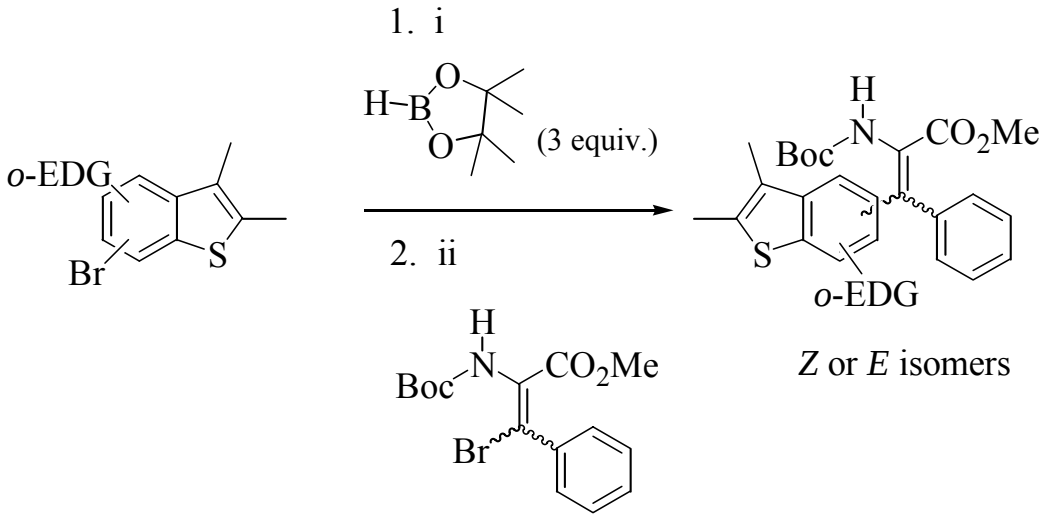
Here we describe the application of the one pot borylation Suzuki coupling (BSC) to the synthesis of new benzo[*b*]thienyldehydrophenylalanine derivatives from methylated or methoxylated benzo[*b*]thiophenes and brominated dehydrophenylalanines. This method was first applied by Baudoin *et al.* [3] to the synthesis of 2,2'-disubstituted biaryl compounds. Substituted halobenzenes were submitted to borylation using pinacolborane, followed by *in situ* Suzuki cross-coupling with a second 2-substituted halobenzene. The authors postulated that the borylation should be performed on the component bearing an *ortho*-EDG and the other coupling component having an *ortho*-EWG. The method is also based on the use of the electron-rich sterically hindered 2-(dicyclohexylphosphino)biphenyl as ligand.[4] Recently we applied this reaction to the synthesis of 2-methyl-2'-nitrobiaryl compounds in the benzo[*b*]thiophene series to obtain thienocarbazoles.[5] More recently another application of the BSC to the synthesis of substituted biaryl compounds appeared, using DPEphos as ligand and CsF as base in the Suzuki coupling.[6]

In this work the brominated Suzuki coupling component is not aromatic and this constitutes a novel application of the BSC reaction as reported by us previously.[7] The C-N metal-assisted cyclization[2] of differently *ortho*-methylated *Z*-β-benzo[*b*]thienyldehydrophenylalanine derivatives was studied. A thienoindole thus obtained showed to be fluorescent. Preliminary antimicrobial studies were performed either on the precursors or on the cyclized products.

Results and Discussion

Synthesis

Several benzo[*b*]thienyldehydrophenylalanines were prepared by palladium-catalyzed BSC reaction of bromobenzo[*b*]thiophenes *ortho*-substituted with an EDG group (OMe or Me) and pure stereoisomers of the methyl ester of *N*-(*tert*-butoxycarbonyl)-β-bromodehydrophenylalanine, Boc-*(Z)*-ΔPhe-(β-Br)-OMe ***(Z)*-1** and Boc-*(E)*-ΔPhe-(β-Br)-OMe ***(E)*-1** already prepared by us.[1] The bromobenzo[*b*]thiophenes were borylated with pinacolborane followed by Suzuki coupling with ***(Z)*-1** or ***(E)*-1** in a one pot procedure (Scheme 1, Table 1).



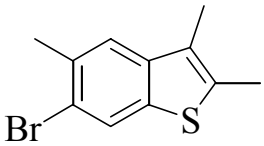
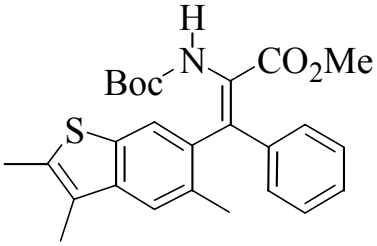
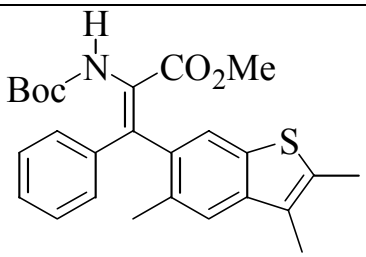
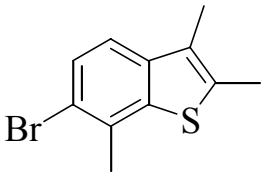
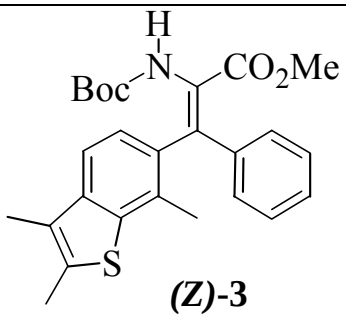
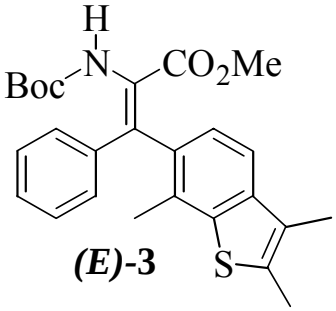
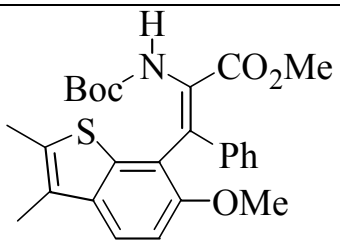
***(Z)*-1** or ***(E)*-1**

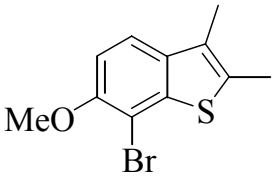
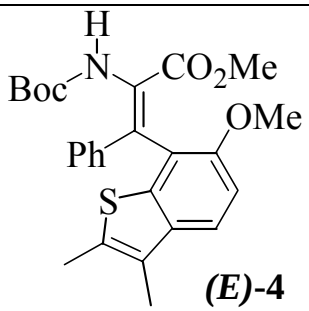
i) $Pd(OAc)_2$ (5 mol%), 2-(dicyclohexylphosphino)biphenyl (20 mol%), NEt_3 (4 equiv.), dry dioxane, 80 °C, 1 h, Ar.

ii) $Ba(OH)_2.8H_2O$ (3 equiv.), H_2O, 100 °C, 1 h 30 min.

Scheme 1

Table 1: Starting materials and product yields of BSC reaction.

Benzo[*b*]thiophene	**dehydrophenylalanine**	**Products**	**Yield (%)**
	***(Z)*-1**	 ***(Z)*-2**	61
	***(E)*-1**	 ***(E)*-2**	30
	***(Z)*-1**	 ***(Z)*-3**	52
	***(E)*-1**	 ***(E)*-3**	43
	***(Z)*-1**	 ***(Z)*-4**	54

	***(E)*-1**	 ***(E)*-4**	40

In all cases debrominated benzo[*b*]thiophenes were isolated as by-products (25-50%). When the two components were aromatic the component to be borylated needs an EDG and the other Suzuki coupling component requires an EWG.[3, 5] In our case however the carbamate group of the dehydroamino acid derivative has a slight electron donating effect and this can affect the product yields which were moderate to good (Table 1). The products maintained the stereochemistry of the starting materials as observed by NOE difference experiments. Enhancements on the phenyl protons for the *E* isomers and on the *ortho*-methyl or methoxy and on the benzo[*b*]thienyl protons for the *Z* isomers were observed irradiating the αNH as shown as examples in Fig. 1 and 2 for compounds ***(Z)*-3** and ***(E)*-3**. In Fig.1 we observe an enhancement of the 7-Me (δ 2.39 ppm) and of the doublet of 5-H (δ 7.06 ppm). In Fig 2 the NOE enhancement is only observed for the phenyl protons.

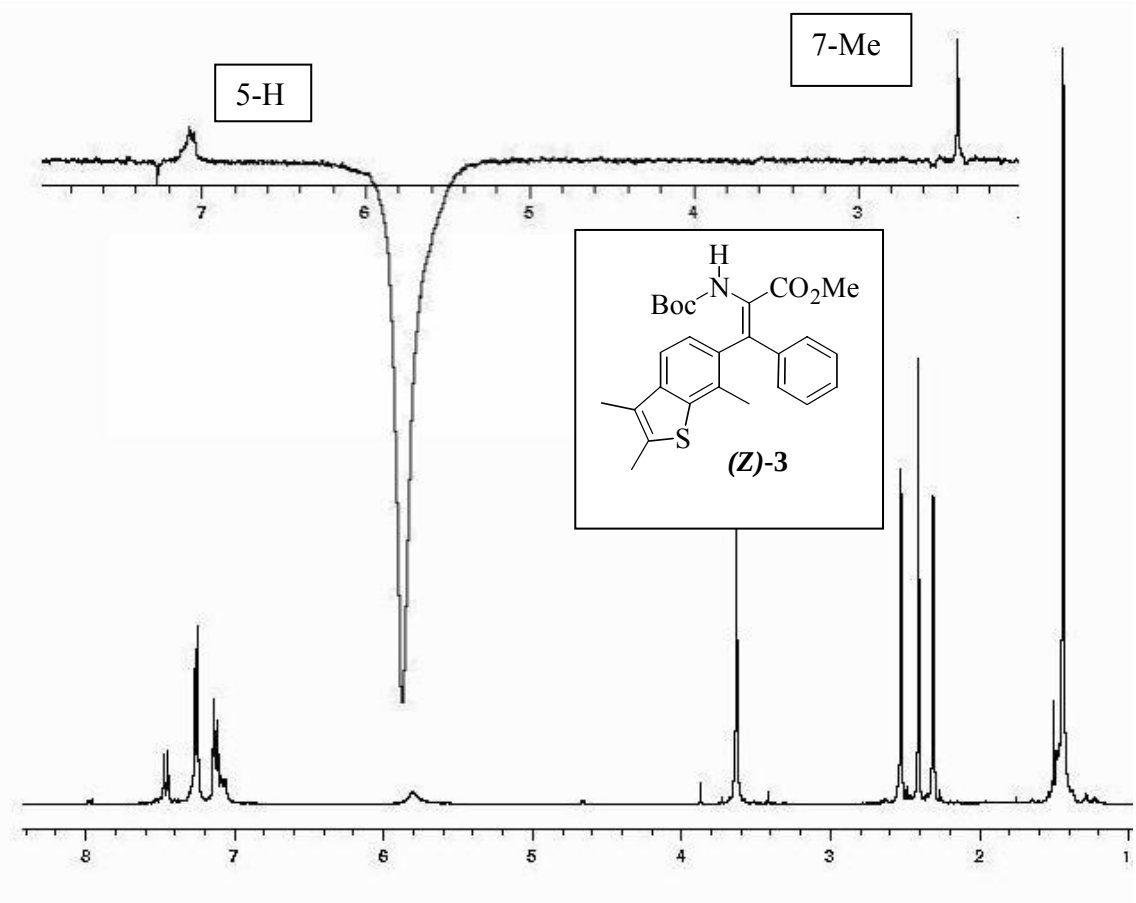


Fig. 1- ^{1}H-NMR ($CDCl_3$) spetrum and NOE difference experiment irradiating the αNH of compound **(*Z*)-3**.

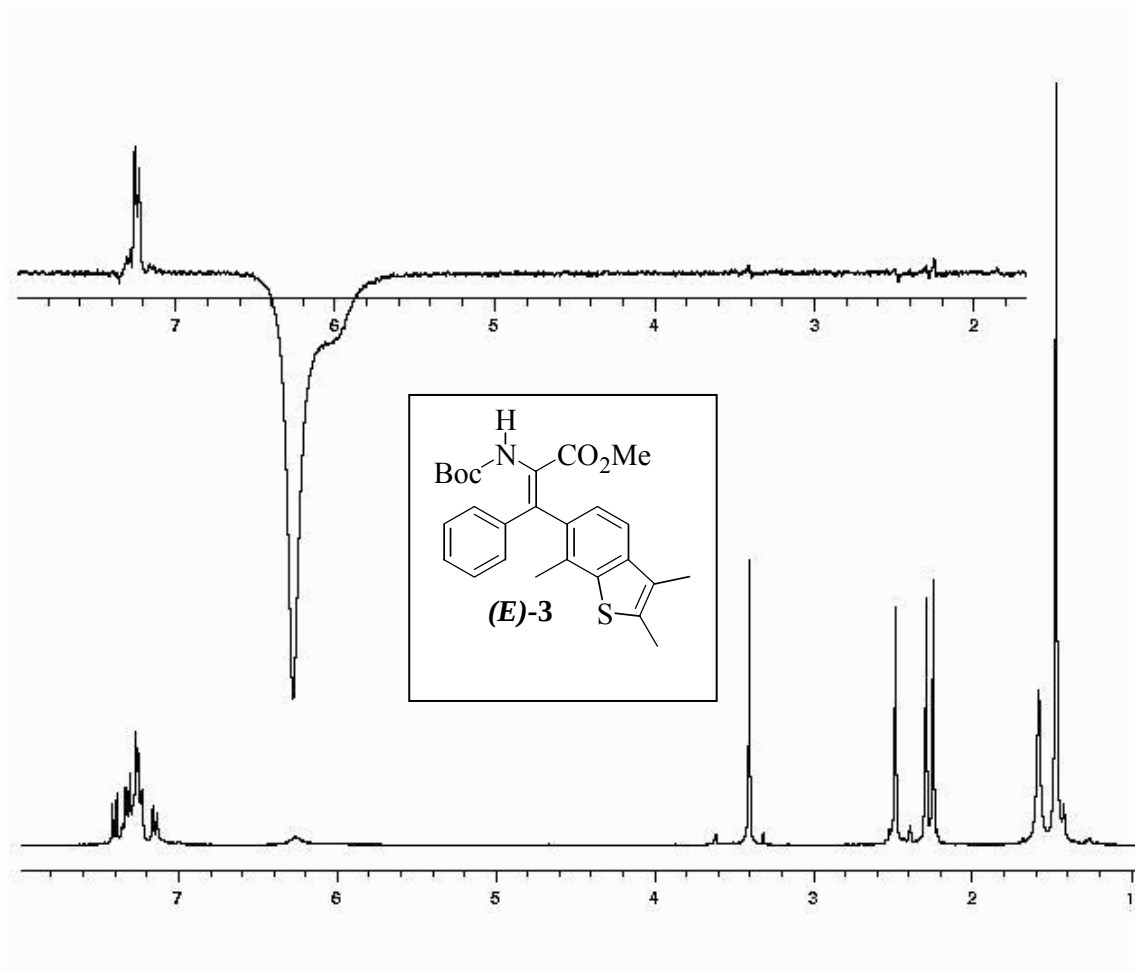
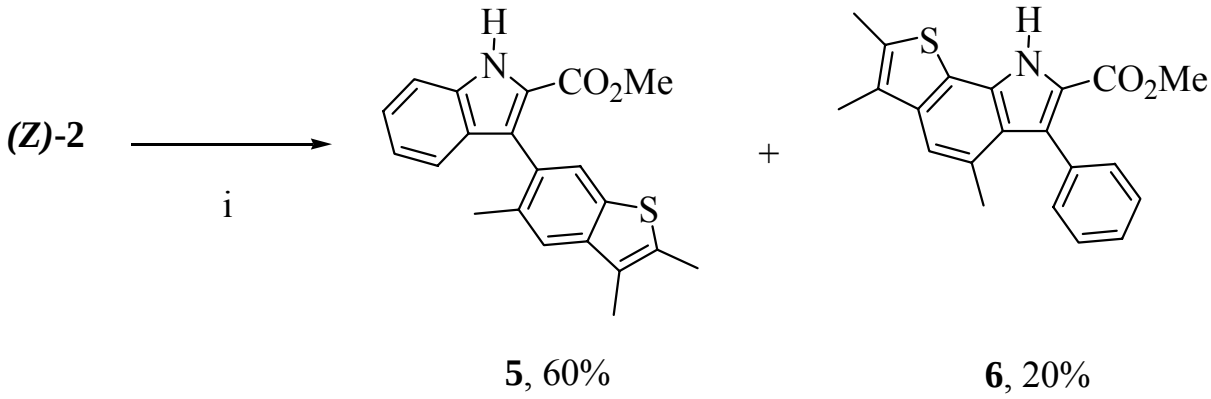


Fig. 2- ^{1}H-NMR ($CDCl_3$) spetrum and NOE difference experiment irradiating the αNH of compound **(*E*)-3**.

In order to obtain cyclic amino acids, compound ***(Z)*-2** and ***(Z)*-3** were submitted to our cyclization conditions[2] (Scheme 2 and 3). Compound ***(Z)*-2** gave two cyclized products resulting either from isomerization followed by cyclization giving indole **5** (major product), or from direct cyclization giving thienoindole **6**. The reaction was also performed at 100 ^{o}C for 6h but the same compounds were obtained in similar amounts.



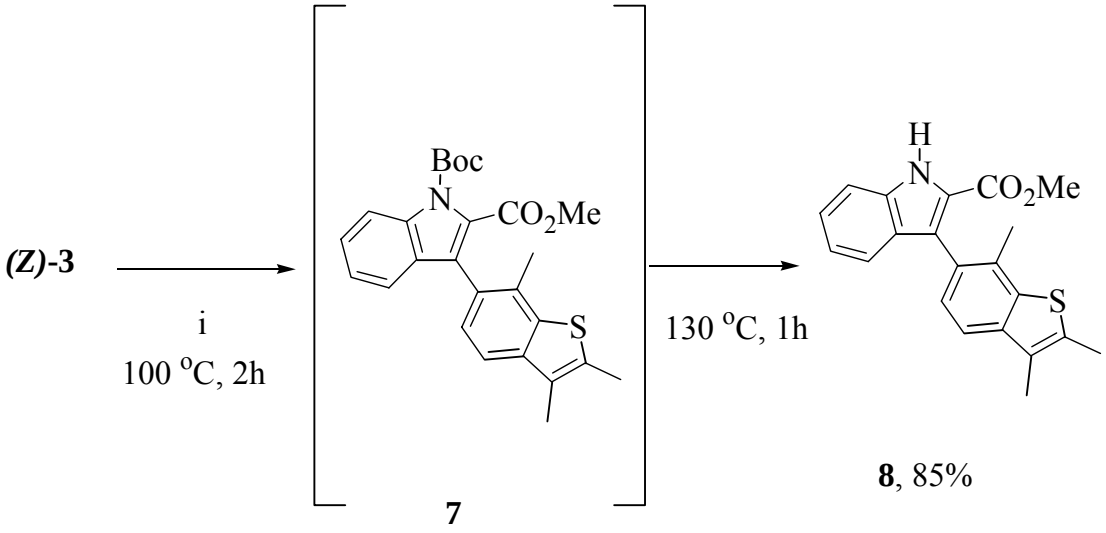
5, 60% **6**, 20%

i) $Pd(OAc)_2$ (50 mol%), $Cu(OAc)_2.H_2O$ (3 equiv.), DMF, 160 °C, 2h 30min.

Scheme 2

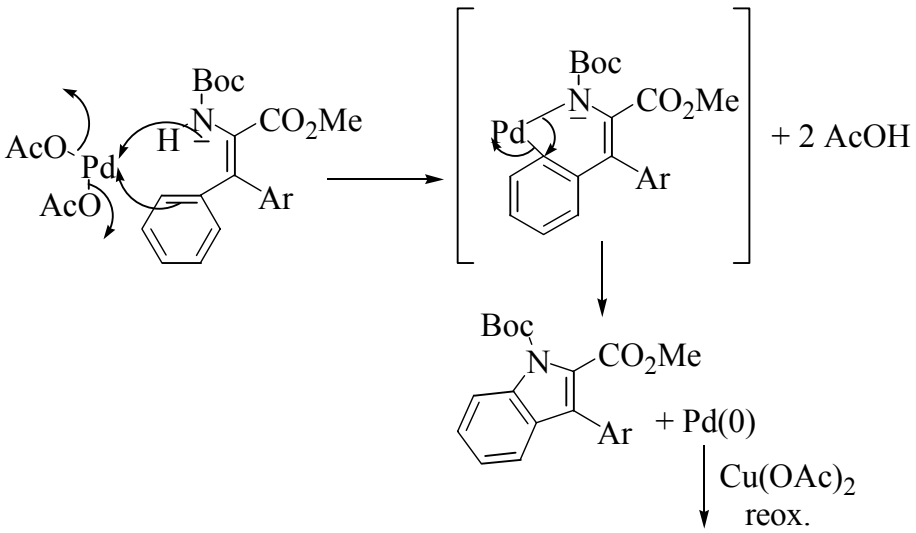
Compound ***(Z)*-3** was also submitted to the same conditions heating at 100 °C for 2h but a cyclized intermediate **7**, resulting from isomerization and cyclization without *N*-deprotection was formed (this compound was isolated from an independent experiment stopping the reaction at that time). Heating at 130 °C for an additional hour gave the corresponding *N*-deprotected dehydroproline derivative in high yield. In this case no product resulting from direct cyclization of the starting material was isolated. This could be due to the much more favorable cyclization on the phenyl ring than on position 5 of the benzo[*b*]thiophene moiety.

We believe that the mechanism of cyclization involves an electrophylic attack of Pd(II) on the aromatic ring and a nucleophylic attack of nitrogen forming a palladacycle. The indole being formed after Pd(0) extrusion. The role of the $Cu(OAc)_2$ can be the reoxidation of Pd(0) avoiding the use of stoichiometric amounts of $Pd(OAc)_2$ (Scheme 4). In our case *N*-deprotection also occurs depending on the reaction conditions (temperature and/or time).



i) $Pd(OAc)_2$ (50 mol%), $Cu(OAc)_2.H_2O$ (3 equiv.), DMF.

Scheme 3



Scheme 4

***In vitro* antimicrobial activity**

A screening of antibacterial activities using two Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and two Gram positive bacteria (*Bacillus subtilis* and *Bacillus cereus*) and antifungal using *Candida albicans* as a representative of fungi was assessed for compounds **(*Z*)-2**, **(*Z*)-3**, **5**, **6**, **7** and **8**. The minimal inhibitory concentration (MIC in μg/mL) was determined (Table 2) using an adaptation of agar streak dilution method based on radial

diffusion.[8] In the same conditions different concentrated solutions of Ampicillin (antibacterial) and Cyclohexamide (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 2. The compounds tested are not active against *Pseudomonas aeruginosa* starting from DMSO solutions of 6000 μg/mL of each compound. Compounds **(*Z*)-2**, **(*Z*)-3** are also not active against *Escherichia coli* but are the only ones active against *Candida albicans*, being **(*Z*)-2** (MIC 6 μg/mL) more active than **(*Z*)-3** (MIC 600 μg/mL) and than Cyclohexamide (MIC 12.5 μg/mL). Against Gram positive bacteria **(*Z*)-2** is more active against *B. cereus* but **(*Z*)-3** shows a lower MIC against *B. subtilis*, even lower than Ampicillin.

Against *Escherichia coli* indole **7** and **8** are more active (MIC 0.06 μg/mL) than thienoindole **6** (MIC 0.6 μg/mL). All the cyclized products are active against Gram positive bacteria, presenting lower MICs than their precursors **(*Z*)-2**, **(*Z*)-3** and Ampicillin, indole **7** being the most active (MIC 0.006 μg/mL).

Table 2. Antimicrobial activity of compounds **(*Z*)-2, (*Z*)-3, 5, 6, 7** and **8**

Compounds	MIC in μg/mL (Zone of inhibition in mm)			
	E. coli CECT 101	*B. cereus* CECT148	*B. subtilis* CECT498	*C. albicans* CECT 1394
(*Z*)-2	not active[a]	6 (10)	60 (11)	6 (8)
(*Z*)-3	not active[a]	60 (12)	6 (8)	600 (9)
Indole **5**	600 (11)	0.6 (13)	0.6 (9)	not active[a]
Thienoindole **6**	0.6 (7)	0.06 (10)	0.06 (9)	not active[a]
Indole **7**	0.06 (8)	0.006 (10)	0.006 (13)	not active[a]
Indole **8**	0.06 (11)	0.06 (9)	0.06 (8)	not active[a]
Ampicillin	6.25 (15)	3.13 (13)	12.5 (10)	_____
Cyclohexamide	_____	_____	_____	12.5 (5)

CECT-Spanish type culture collection of Valencia University
[a] Not active starting from 6000 μg/mL

Fluorescence studies

The fluorescence of compounds **5** and **6** was studied (Fig 3). Thienoindole **6** showed a λ_{em}= 393 nm and a relative quantum yield of fluorescence in dichloromethane Φ_{dcm} = 0.33, using the

standard method[9] with 9,10-diphenylanthracene in EtOH ($2x10^{-5}$ M) as reference (Φ_{EtOH} = 0.95)[10] using λ_{exc} = 333 nm. Indole **5** showed a λ_{em} = 408 nm and a relative quantum yield of fluorescence in dichloromethane Φ_{dcm} = 0.0067 using the same method and λ_{exc} = 296 nm. The excitation wavelengths (λ_{exc}) were chosen from the UV spectra of the compounds (see experimental). From the results obtained it can be concluded that thienoindole **6** can be used as a fluorescent probe.

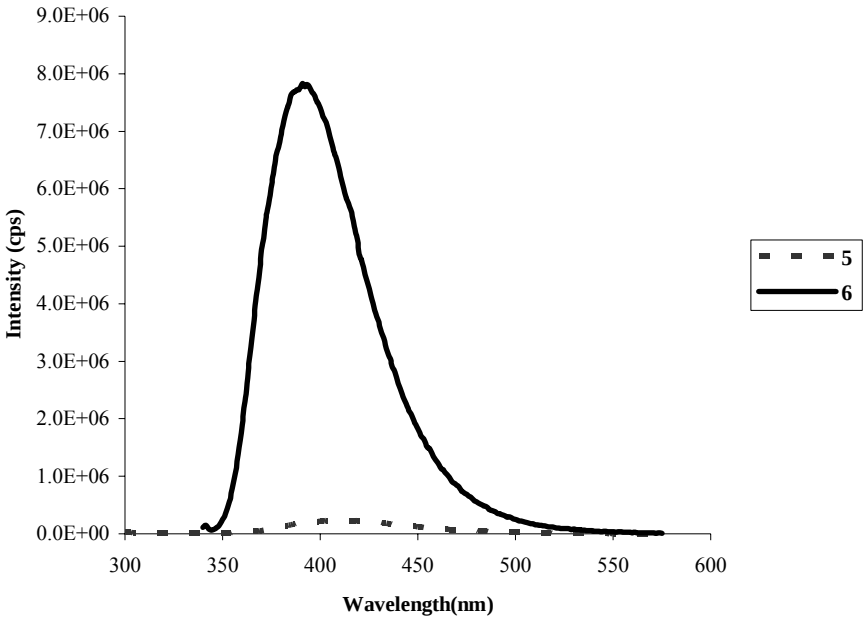


Fig. 3. Fluorescence spectra of compounds **5** and **6** in dichloromethane ($1.6x10^{-6}$ M).

Conclusions

Several new β-benzo[*b*]thienyldehydrophenylalanines were synthesized in moderate to good yields from *ortho*-substituted bromobenzo[*b*]thiophenes and pure stereoisomers of β-brominated dehydrophenylalanines by one pot borylation and Suzuki coupling (BSC), maintaining the stereochemistry of the starting materials. Two of the *Z* isomers obtained, **(*Z*)-2** and **(*Z*)-3,** were submitted to C-N metal-assisted cyclization giving dehydroprolines (indoles and a thienoindole). From preliminary antimicrobial studies on the precursors and on the cyclized products it is possible to conclude that all the compounds were active against the Gram positive bacteria (*B. cereus* and *B. subtilis*), the cyclized products being more active (lower MICs). The latter were also active against *E. coli* despite presenting very different MICs and in turn the precursors were

the only compounds active against *Candida albicans*. Fluorescence studies on compounds **5** and **6** show that the thienoindole **6** can be used as a fluorescent probe.

Experimental Section

General Remarks: 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 techniques were used to assign the signals. NOE difference experiments were performed to determine the stereochemistry of the products. The ^{13}C NMR spectra were measured in the same instrument at 75.4 MHz (using DEPT θ 45º). The UV spectra were recorded on a Schimadzu UV-250 1PC, UV-vis recording spectrophotometer.

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.

Column chromatography was performed on Macherey-Nagel silica gel 230-400 mesh. Petroleum ether refers to the boiling range 40-60 ^{o}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.

For the *in vitro* antimicrobial activity, 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 (6000 μg/mL) in DMSO was prepared and graded dilutions (10x) 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 ^{o}C (for bacteria) and at 30 ^{o}C (for fungi) for 24h in duplicate. Positive control using only inoculation and negative control using only DMSO in the cavity were carried out.

The fluorescence studies were performed on a spectrofluorimeter Spex Fluorolog 1680 Double Spectrometer. Samples quantum yields are given by $\Phi_S = \left[\left(A_r F_S n_S^2\right)/\left(A_S F_r n_r^2\right)\right]\Phi_r$ where A is the absorbance at the excitation wavelength, F the integrated emission area and n the refraction index of the solvents used. Subscripts refer to the reference (r) or sample (s) compound.

Compounds **(*Z*)-1** and **(*E*)-1** were already described by us.[1] The 6-bromotrimethylbenzo[*b*]thiophenes were prepared according to a procedure described by others[11a] and previously used by us.[11b]

7-Bromo-2,3-dimethyl-6-methoxybenzo[*b*]thiophene and **5-bromo-2,3-dimethyl-6-methoxy-benzo[*b*]thiophene**: To a cooled solution 0 ºC of 6-methoxy-2,3-dimethylbenzo[*b*]thiophene[12] (3.00 mmol, 0.500 g) in dry ether (30 mL) protected against light it was added 1 equiv. of Br_2 (3.00 mmol, 0.154 mL) and a small amount of Fe. The reaction was followed by ^{1}H-NMR and it was left stirring for 1h. Iced water (10mL) was added and the mixture was stirring for 10 min. The phases were separated and the aqueous phase was extracted with more ether (2x30 mL). The organic phases were collected, washed with a 5% solution of sodium sulfite and a 10% solution of sodium carbonate and dried ($MgSO_4$). Removal of the solvent gave a solid that was submitted to column chromatography using a petroleum ether. **5-Bromo-2,3-dimethyl-6-methoxybenzo[*b*]thiophene** (62.0 mg, 8 %) was isolated as the less polar product as a white solid, m.p. 119 – 120 ºC. ^{1}H NMR ($CDCl_3$): δ 2.24 (s, 3 H, $ArCH_3$),

2.45 (s, 3 H, $ArCH_3$), 3.95 (s, 3 H, OCH_3), 7.25 (s, 1 H, 7-H), 7.74 (s, 1 H, 4-H) ppm. ^{13}C NMR ($CDCl_3$): δ11.29 (CH_3), 13.65 (CH_3), 56.44 (OCH_3), 104.53 (CH), 109.34 (C), 125.27 (CH), 125.94 (C), 132.40 (C), 136.01 (C), 138.08 (C), 152.54 (C) ppm. $C_{11}H_{11}BrOS$ (271.17): calc. C 48.72, H 4.09, S 11.82; found C 48.87, H 4.23, S 11.60; followed by **7-bromo-2,3-dimethyl-6-methoxybenzo[*b*]thiophene** (484 mg, 60%) isolated as a white solid, m.p. 120–122 ºC. ^1H NMR ($CDCl_3$): δ 2.26 (s, 3 H, $ArCH_3$), 2.46 (s, 3 H, $ArCH_3$), 3.97 (s, 3 H, OCH_3), 7.00 (d, J = 8.4 Hz, 1 H, 5-H), 7.48 (d, J = 8.4 Hz, 1 H, 4-H) ppm. ^{13}C NMR ($CDCl_3$): δ11.60 (CH_3), 13.67 (CH_3), 57.05 (OCH_3), 103.97 (C), 109.73 (CH), 120.39 (CH), 127.39 (C), 132.55 (C), 135.85 (C), 141.55 (C), 152.74 (C) ppm. $C_{11}H_{11}BrOS$ (271.17): calc. C 48.72, H 4.09, S 11.82; found C 49.00, H 4.27, S 11.74.

General procedure for *one-pot* BSC Reaction: A dried Schlenk tube was charged under Ar with dry dioxane (2 mL), the *ortho*-methylated or methoxylated bromobenzo[*b*]thiophene (0.5 mmol) was added and the mixture heated for 5 min. at 80 ºC. Triethylamine (4 equiv.), $Pd(OAc)_2$ (5 mol%), 2-(dicyclohexylphosphino)biphenyl (20 mol%) and pinacolborane (3 equiv.) were added, and the mixture was left with rapid stirring at 80 ºC for 1h. After cooling, a pure stereoisomer of β-bromodehydrophenylalanine derivative (1 equiv.) and $Ba(OH)_2.8H_2O$ (3 equiv.) were added, and the mixture was heated at 100 ºC for 1h 30 min. Water and ethyl acetate were added, the phases were separated, and then the aqueous phase was extracted with more ethyl acetate. The organic phases were collected, dried ($MgSO_4$), filtered and then the solvent was evaporated at reduced pressure to give a brown solid, that was subjected to column chromatography.

Boc-(*Z*)-ΔPhe[β-(2,3,5-trimethylbenzo[*b*]thien-6-yl)]-OMe (*Z*)-2: The procedure described above was followed using compound 6-bromo-2,3,5-trimethylbenzo[*b*]thiophene (0.500 mmol, 128 mg) and Boc-(*Z*)-ΔPhe(β-Br)-OMe (0.410 mmol, 147 mg), but adding water (200 μL) in the second step. Column chromatography using solvent gradient from neat petroleum ether to 30% diethyl ether/petroleum ether, gave product **(*Z*)-2** (112 mg, 61%) as an oil. Crystallization from diethyl ether/*n*-hexane gave light-yellow solid, mp 154-156 ºC. ^1H NMR ($CDCl_3$): δ = 1.44 (s, 9H, CH_3 Boc), 2.31 (s, 3H, 5-CH_3), 2.35 (s, 3H, $ArCH_3$), 2.49 (s, 3H, $ArCH_3$), 3.64 (s, 3H, OCH_3), 5.80 (s, 1H, NH), 7.13-7.16 (m, 2H, ArH), 7.24-7.29 (m, 3H, ArH), 7.48 (broad s, 1H, ArH), 7.51 (s, 1H, ArH) ppm. ^{13}C NMR ($CDCl_3$): δ = 11.25 (CH_3), 13.76 (CH_3), 19.57 (CH_3), 27.99 ($C(CH_3)_3$), 52.03 (OCH_3), 81.22 ($OC(CH_3)_3$), 122.87 (CH), 123.20 (CH), 126.50 (C), 126.73 (C), 127.56 (CH), 128.00 (CH), 128.41 (CH), 132.28 (C), 133.32 (C), 135.21 (C), 135.91 (C), 139.17 (C), 141.22 (C), 152.48 (C=O), 166.30 (C=O) ppm. MS: *m / z* (%) = 451 (22) [M^+], 351 (100) [M^+ - Boc], 291 (34) [(M^+-Boc)-CO_2CH_3]. HRMS: calcd. for $C_{26}H_{29}NO_4S$ [M^+] 451.1817; found 451.1801.

Boc-(*E*)-ΔPhe[β-(2,3,5-trimethylbenzo[*b*]thien-6-yl)]-OMe (*E*)-2: The procedure described above was followed using compound 6-bromo-2,3,5-trimethylbenzo[*b*]thiophene (0.460 mmol, 117 mg) and Boc-(*E*)-ΔPhe(β-Br)-OMe (0.380 mmol, 136 mg), but adding water (200 μL) in the second step. Column chromatography using solvent gradient from neat petroleum ether to 30% diethyl ether/petroleum ether, gave product **(*E*)-2** (51.0 mg, 30%) as an oil. Crystallization from petroleum ether gave white crystals, mp 156-157 ºC. ^1H NMR ($CDCl_3$): δ = 1.48 (s, 9H, CH_3 Boc), 2.13 (s, 3H, $ArCH_3$), 2.26 (s, 3H, $ArCH_3$), 2.48 (s, 3H, $ArCH_3$), 3.39 (s, 3H, OCH_3), 6.26 (s, 1H, NH), 7.24-7.34 (m, 6H, ArH), 7.51 (s, 1H, ArH) ppm. ^{13}C NMR ($CDCl_3$): δ = 11.35 (CH_3), 13.82 (CH_3), 20.25 (CH_3), 28.18 ($C(CH_3)_3$), 52.00 (OCH_3), 81.21 ($OC(CH_3)_3$), 122.45 (CH), 122.54 (CH), 126.40 (C), 126.67 (C), 128.09

(CH), 128.63 (CH), 129.38 (CH), 132.98 (C), 134.29 (C), 135.06 (C), 137.52 (C), 140.98 (C), 152.99 (C=O), 166.24 (C=O) ppm. $C_{26}H_{29}NO_4S$ (451.58): calcd. C 69.15, H 6.47, N 3.10, S 7.10; found C 68.90, H 6.54, N 3.14, S 7.03.

Boc-(*Z*)-ΔPhe[β-(2,3,7-trimethylbenzo[*b*]thien-6-yl)]-OMe (*Z*)-3: The procedure described above was followed using compound 6-bromo-2,3,7-trimethylbenzo[*b*]thiophene (0.500 mmol, 128 mg) and Boc-(*Z*)-ΔPhe(β-Br)-OMe (0.500 mmol, 178 mg), but adding water (200 μL) in the second step. Column chromatography using solvent gradient from neat petroleum ether to 30% diethyl ether/petroleum ether, gave product **(*Z*)-3** (122 mg, 52%) as an oil. Crystallization from petroleum ether gave white crystals, mp 149-150 ºC. ^{1}H NMR ($CDCl_3$): δ = 1.42 (s, 9H, CH_3 Boc), 2.31 (s, 3H, $ArCH_3$), 2.39 (s, 3H, 7-CH_3), 2.53 (s, 3H, $ArCH_3$), 3.62 (s, 3H, OCH_3), 5.80 (s, 1H, NH), 7.06 (d, *J* = 8.4 Hz, 1H, 5-H), 7.09-7.13 (m, 2H, ArH), 7.22-7.28 (m, 3H, ArH), 7.45 (d, *J* = 8.4 Hz, 1H, 4-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 11.42 (CH_3), 13.84 (CH_3), 17.59 (CH_3), 28.08 (C(*C*H_3)$_3$), 52.07 (OCH_3), 81.33 (O*C*(CH$_3$)$_3$), 119.44 (CH), 126.53 (C), 126.93 (CH), 127.60 (CH), 127.90 (C), 128.06 (CH), 128.52 (CH), 130.04 (C), 133.02 (C), 134.78 (C), 139.45 (C), 139.55 (C), 140.63 (C), 152.57 (C=O), 166.38 (C=O) ppm. $C_{26}H_{29}NO_5S$ (467.58): calcd. C 69.12, H 6.47, N 3.10, S 7.10; found C 69.15, H 6.63, N 3.11, S 6.91.

Boc-(*E*)-ΔPhe[β-(2,3,7-trimethylbenzo[*b*]thien-6-yl)]-OMe (*E*)-3: The procedure described above was followed using compound 6-bromo-2,3,7-trimethylbenzo[*b*]thiophene (0.310 mmol, 79.0 mg) and Boc-(*E*)-ΔPhe(β-Br)-OMe (0.310 mmol, 111 mg), but adding water (125 μL) in the second step. Column chromatography using solvent gradient from neat petroleum ether to 30% diethyl ether/petroleum ether, gave product **(*E*)-3** (41 mg, 43%) as an oil. Crystallization from petroleum ether gave light-yellow crystals, mp 172-173 ºC. ^{1}H NMR ($CDCl_3$): δ = 1.47 (s, 9H, CH_3 Boc), 2.24 (s, 3H, $ArCH_3$), 2.29 (s, 3H, $ArCH_3$), 2.48 (s, 3H, $ArCH_3$), 3.40 (s, 3H, OCH_3), 6.24 (s, 1H, NH), 7.15 (d, *J* = 8.1 Hz, 1H, ArH), 7.23-7.33 (m, 5H, ArH), 7.40 (d, *J* = 8.1 Hz, 1H, ArH) ppm. ^{13}C NMR ($CDCl_3$): δ = 11.48 (CH_3), 13.84 (CH_3), 18.16 (CH_3), 28.17 (C(*C*H_3)$_3$), 52.01 (OCH_3), 81.21 (O*C*(CH$_3$)$_3$), 118.31 (CH), 126.43 (CH), 126.59 (C), 127.81 (C), 128.04 (CH), 128.52 (C), 128.65 (CH), 129.28 (CH), 130.18 (C), 133.76 (C), 133.94 (C), 138.17 (C), 139.17 (C), 140.52 (C), 152.97 (C=O), 166.27 (C=O) ppm. $C_{26}H_{29}NO_4S$ (451.58): calcd. C 69.15, H 6.47, N 3.10, S 7.10; found C 69.19, H 6.60, N 3.14, S 7.11.

Boc-(*Z*)-ΔPhe[β-(2,3-dimethyl-6-methoxybenzo[*b*]thien-7-yl)]-OMe (*Z*)-4: The procedure described above was followed using compound 7-bromo-2,3-dimethyl-6-methoxybenzo[*b*]thiophene (0.400 mmol, 108 mg) and Boc-(*Z*)-ΔPhe(β-Br)-OMe (0.400 mmol, 142 mg), but adding water (160 μL) in the second step. Column chromatography using solvent gradient from neat petroleum ether to 40% diethyl ether/petroleum ether, gave product **(*Z*)-4** (100 mg, 54%) as an yellow solid. Recrystallization from petroleum ether gave yellow crystals, mp 104-106 ºC. ^{1}H NMR ($CDCl_3$): δ = 1.41 (s, 9H, CH_3 Boc), 2.26 (s, 3H, $ArCH_3$), 2.37 (s, 3H, $ArCH_3$), 3.59 (s, 3H, OCH_3), 3.74 (s, 3H, 6-OCH_3), 5.86 (s, 1H, NH), 7.04 (d, *J* = 8.4 Hz, 1H, ArH), 7.20-7.24 (m, 5H, ArH), 7.53 (d, *J* = 8.4 Hz, 1H, ArH) ppm. ^{13}C NMR ($CDCl_3$): δ = 11.33 (CH_3), 13.57 (CH_3), 28.03 (C(*C*H_3)$_3$), 52.08 (OCH_3), 56.48 (OCH_3), 80.86 (O*C*(CH$_3$)$_3$), 109.79 (CH), 119.50 (C), 121.93 (CH), 126.38 (C), 127.48 (CH), 127.77 (CH), 127.82 (C), 128.39 (CH), 132.46 (C), 135.62 (C), 138.18 (C), 139.75 (C), 152.70 (C), 153.77 (C=O), 166.25 (C=O) ppm. $C_{26}H_{29}NO_5S$ (467.58): calcd. C 66.79, H 6.25, N 3.00, S 6.86; found C 66.93, H 6.62, N 2.96, S 6.63.

Boc-(*E*)-ΔPhe[β-(2,3-dimethyl-6-methoxybenzo[*b*]thien-7-yl)]-OMe (*E*)-4: The procedure described above was followed using compound 7-bromo-2,3-dimethyl-6-methoxybenzo[*b*]thiophene (0.340 mmol, 92.0 mg) and Boc-

(*E*)-ΔPhe(β-Br)-OMe (0.340 mmol, 119 mg), but adding water (134 μL) in the second step. Column chromatography using solvent gradient from neat petroleum ether to 40% diethyl ether/petroleum ether, gave product **(*E*)-4** (63.0 mg, 40 %) as an yellow solid. Recrystallization from petroleum ether gave yellow crystals, mp 135-136 ºC. ^{1}H NMR ($CDCl_3$): δ = 1.46 (s, 9H, CH_3 Boc), 2.22 (s, 3H, $ArCH_3$), 2.36 (s, 3H, $ArCH_3$), 3.44 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 6.22 (s, 1H, NH), 6.99 (d, *J* = 8.7 Hz, 1H, ArH), 7.26-7.37 (m, 5H, ArH), 7.45 (d, *J* = 8.7 Hz, 1H, ArH) ppm. ^{13}C NMR ($CDCl_3$): δ = 11.34 (CH_3), 13.66 (CH_3), 28.15 ($C(CH_3)_3$), 51.79 (OCH_3), 56.64 (OCH_3), 80.92 ($OC(CH_3)_3$), 109.56 (CH), 121.12 (CH), 122.29 (C), 126.26 (C), 127.68 (C), 127.80 (C) 128.07 (C), 128.48 (CH), 129.22 (CH), 132.04 (CH), 135.37 (C), 137.26 (C), 140.02 (C), 152.96 (C), 153.57 (C=O), 165.33 (C=O) ppm. $C_{26}H_{29}NO_5S$ (467.58): calcd. C 66.79, H 6.25, N 3.00, S 6.86; found C 66.54, H 6.33, N 3.07, S 6.91.

General Procedure for the synthesis of dehydroprolines: To a solution of the (Z)-benzo[*b*]thienyldehydrophenylalanines (0.1 M) in DMF were added $Pd(OAc)_2$ (50mol%) and $Cu(OAc)_2.H_2O$ (3 equiv.), and the mixture was heated at 100ºC, 130ºC or 160ºC, monitoring the reaction by TLC. Ethyl acetate (50 mL) was then added and the organic layer washed with water and brine (2 x 25 mL), dried with $MgSO_4$ ant the solvents were evaporated at reduce pressure to give an oil that was submitted to column chromatography.

Methyl 3-(phenyl)-1*H*-4,6,7-trimethylbenzo[*b*]thien[7,6-*b*]pyrrole-2-carboxylate (6) and Methyl 3-(2,3,5-trimethylbenzo[*b*]thien-6-yl)indole-2-carboxylate (5): The procedure described above was applied using compound **(*Z*)-2** (0.177 mmol, 80.0 mg) and heating for 2h 30 min. at 160ºC. Column chromatography using solvent gradient from neat petroleum ether to 30% diethyl ether / petroleum ether, gave product **6** (12.0 mg, 20%) as a white solid, m.p. 215-217 ºC, as the less-polar product. UV(CH_2Cl_2): λ_{max} (ε $mol^{-1}dm^3cm^{-1}$) 333 (14750) 266 (30938) nm. ^{1}H NMR ($CDCl_3$): δ = 2.10 (s, 3H, Ar-CH_3), 2.32 (s, 3H, Ar-CH_3), 2.54 (s, 3H, Ar-CH_3), 3.74 (s, 3H, OCH_3), 7.07 (s, 1 H, ArH), 7.41 (s, 5 H, ArH), 9.07 (s largo, 1H, NH) ppm. ^{13}C NMR ($CDCl_3$): δ = 11.80 (CH_3), 13.84 (CH_3), 20.62 (CH_3), 51.64 (OCH_3), 116.40 (CH), 118.53 (C), 121.87 (C), 123.47 (C), 126.82 (C), 127.14 (CH), 127.28 (CH), 128.41 (C), 129.83 (C), 130.50 (CH), 132.09 (C), 135.88 (C), 139.94 (C), 162.23 (C=O). MS: *m / z* (%) = 349 (85) [M^+], 318 (26) [M^+ - OCH_3], 317 (100), 288 (28). HRMS: calc. for $C_{21}H_{19}NO_2S$ [M^+] 349.1137; found 349.1130. Product **5** eluted next with 40% diethyl ether / petroleum ether and was isolated as a white solid (37.0 mg, 60%), m.p. 267-269 ºC. UV(CH_2Cl_2): λ_{max} (ε mol^{-1} dm^3cm^{-1}) 296 (20875) 240 (48125) ^{1}H NMR ($CDCl_3$): δ = 2.23 (s, 3 H, Ar-CH_3), 2.35 (s, 3 H, Ar-CH_3), 2.52 (s, 3 H, Ar-CH_3), 3.75 (s, 3 H, OCH_3), 7.12 (ap.t, 1H, ArH), 7.33-7.49 (m, 3H, ArH), 7.54 (s, 1H, ArH), 7.63 (s, 1H, ArH), 9.04 (s largo, 1H, NH) ppm. ^{13}C NMR ($CDCl_3$): δ = 11.43 (CH_3), 13.84 (CH_3), 20.40 (CH_3), 51.84 (OCH_3), 111.64 (CH), 120.72 (CH), 121.78 (CH), 121.93 (CH), 123.43 (C), 123.71 (CH), 123.78 (C), 125.78 (CH), 126.69 (C), 128.54 (C), 129.33 (C), 133.38 (C), 133.84 (C), 135.07 (C), 135.70 (C), 140.80 (C), 162.46 (C=O). MS: m/z (%) = 349 (100) [M^+], 318 (15) [M^+ - OCH_3], 317 (41). HRMS: calc. for $C_{21}H_{19}NO_2S$ [M^+] 349.1137; found 349.1126.

Methyl *N*-(*tert*-butoxycarbonyl)-3-(2,3,7-trimethylbenzo[*b*]thien-6-yl)indole-2-carboxylate (7): The procedure described above was applied using compound **(*Z*)-3** (0.310 mmol, 140 mg) and heating for 2h at 100ºC. Column chromatography using solvent gradient from neat petroleum ether to 10% diethyl ether / petroleum ether, gave compound (70.0 mg, 50%) as an oil. ^{1}H NMR ($CDCl_3$): δ = 1.70 (s, 9 H, CH_3 Boc), 2.37 (s, 3 H, Ar-CH_3), 2.38 (s, 3 H, Ar-CH_3), 2.56 (s, 3 H, Ar-CH_3), 3.72 (s, 3 H, OCH_3), 7.25-7.27 (m, 2 H, ArH), 7.32 (d, *J* = 8.1 Hz, 1 H, ArH), 7.42-7.49 (m, 1 H, ArH), 7.53 (d, *J* = 8.1 Hz, 1 H, ArH), 8.23 (d, *J* = 8.4 Hz, 1 H, ArH) ppm. ^{13}C NMR ($CDCl_3$): δ =

11.47 (CH_3), 13.82 (CH_3), 18.38 (CH_3), 27.91 ($C(CH_3)_3$), 52.19 (OCH_3), 84.67 ($OC(CH_3)_3$), 115.10 (CH), 118.40 (CH), 121.31 (CH), 123.33 (CH), 125.72 (C), 125.84 (C), 126.53 (CH), 127.03 (CH), 127.54 (C), 127.89 (C), 129.25 (C), 130.78 (C), 134.00 (C), 135.93 (C), 138.91 (C), 140.72 (C), 149.30 (C=O), 163.03 (C=O). MS: m/z (%) = 449 (22) [M^+], 349 (100) [M^+ - Boc], 317 (43). HRMS: calc. for $C_{26}H_{27}NO_4S$ [M^+] 449.1650; found 449.1661.

Methyl 3-(2,3,7-trimethylbenzo[*b*]thien-6-yl)indole-2-carboxylate (8): The procedure described above was applied using compound **(*Z*)-3** (0.137 mmol, 62.0 mg) and heating for 1h at 130ºC. Column chromatography using solvent gradient from neat petroleum ether to 20% diethyl ether / petroleum ether, gave compound **8** (41.0 mg, 85%) as a yellow solid, m.p. 230 -232 ºC. ^{1}H NMR ($CDCl_3$): δ = 2.34 (s, 3 H, Ar-CH_3), 2.38 (s, 3 H, Ar-CH_3), 2.56 (s, 3 H, Ar-CH_3), 3.77 (s, 3 H, OCH_3), 7.14 (broad t, *J* = 6.9 Hz, 1H, ArH), 7.33-7.41 (m, 3 H, ArH), 7.48 (d, *J* = 9.0 Hz, 1H, ArH), 7.54 (d, *J* = 8.1 Hz, 1 H, ArH), 9.21 (s largo, 1 H, NH) ppm. ^{13}C NMR ($CDCl_3$): δ = 11.52 (CH_3), 13.84 (CH_3), 18.33 (CH_3), 51.84 (OCH_3), 111.70 (CH), 118.09 (CH), 120.70 (CH), 122.04 (CH), 123.50 (C), 125.73 (CH), 127.55 (CH), 127.90 (C), 128.62 (C), 130.54 (C), 133.49 (C), 135.70 (C), 138.75 (C), 140.20 (C), 162.56 (C=O). MS: *m / z* (%) = 349 (100) [M^+], 318 (17) [M^+ - OCH_3], 317 (46), 288 (22). HRMS: calc. for $C_{21}H_{19}NO_2S$ [M^+] 349.1137; found 349.1142.

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