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Article

Application of the Negishi Reaction in the Synthesis of Thiophene-Based Lignans Analogues with Leishmanicidal Effects

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As lignanas tetraidrofurânicas são metabólitos secundários com reconhecida atividade antiprotozoária. Na literatura, há vários relatos sobre os efeitos anti-parasitários de análogos sintéticos de lignanas contendo pontes de enxofre. Neste trabalho, foi realizada a síntese de análogos tiofênicos de lignanas com o uso de uma estratégia sintética seletiva e de alto desempenho baseada na reação de acoplamento cruzado de Negishi. Os derivados sintéticos foram obtidos de maneira rápida e apresentaram alto grau de pureza e baixa toxicidade para uma linhagem celular de mamífero e atividade leishmanicida com diferentes potências.

Lignans represent a well-known group of natural products with anti-protozoal activity. In the literature there are many examples of the anti-parasitic activity of synthetic analogues of lignans containing sulphur bridges. In this work, we have obtained thiophene-based analogues by using a selective and high performance synthetic strategy based on the Negishi cross-coupling reaction. The derivatives were quickly obtained and showed great purity, low toxicity toward a mammalian cell line, and leishmanicidal activity with different potencies.

Keywords: thiophene, leishmanicidal, Negishi reaction, *cross*-coupling, green chemistry

Introduction

Leishmaniasis is a serious disease caused by different protozoan parasites of Leishmania genus and this disease can be represented by a large spectrum of symptoms such as cutaneous lesion and fatal visceral infection.[1] Data from World Health Organization (WHO) estimated that leishmaniasis threatens 350 millions of people and it is responsible for about 2 million clinical cases each year in 88 countries.[2] The countries with high prevalence of leishmaniasis are subtropical and development countries, for example, India, Sudan, Bangadesh, Nepal and Brazil.[2]

The treatment of leishmaniasis is based on pentavalent antimony compounds, which may be represented by sodium stibogluconate (Pentostam®) or meglumine antimoniate (Glucantime®). These antimony compounds are widely prescribed despite their severe side effects in the heart, kidney, pancreas and liver, high cost, difficult administration and increase of parasite resistance.[3-5] Other drugs such as amphotericin B, pentamidine and metilfosine are used in leishmaniasis treatment. However, the clinical use of these drugs are limited because of their toxicity, adverse side effects and high cost.[4,6]

Currently, there is an important demand for the development of new treatments against leishmaniasis, which have primarily features as inexpensive, potent, safe and easy administration. The drugs of choice for the treatment of leishmaniasis, such as pentavalent antimonials (sodium stibogluconate and meglumine antimonates), miltefosine, and pentamidine are toxic to the host. Some forms of administration (injections) or different formulations developed to reduce toxicity, increase the

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expense of the treatment and makes the use of the drug less practical in developing nations, where leishmaniasis are endemic and cost is a major factor.[7,8]

Therefore, in order to solve these problems, studies aiming the discovery of new drugs against leishmaniasis are necessary and may provide treatments that could be more efficient for population and reduce the economic impact of this disease in endemic countries. Lignans and neolignanas are included in the potential natural products for the treatment of neglected diseases.[9,10] From the Brazilian biodiversity, there are some papers that report the anti-protozoal activity of lignans from *Virola pavonis* and *Virola surinamensis*.[11,12] For example, Barata *et al.* showed the highest selective activity for lignans analogues with sulphur bridges than those structures isolated from *V. pavonis*.[11]

Recently, some new compounds containing the thiophene ring in their structure have demonstrated high leishmanicidal activity. For instance, Takahashi *et al.* have isolated thiophene derivatives with leishmanicidal activity from *Porophyllum ruderale* (Jacq.) Cass. These molecules have exhibited strong activity against promastigote and axenic amatigote forms of *L. amazonensis.*[13] On the other hand, Ram *et al.* have synthesized some potent thiophenes and thieno[3,2-c]pyran-4-ones displaying 95-100% growth inhibition of promastigotes of *L. donovani*.[14] Similarly, Mohareb *et al.* have also investigated the anti-leishmanial activity of new aromatic thiophenes.[15]

Due to the growing interest on thiophene-based compounds presenting biological activity, the preparation of functionalized derivatives is of great importance. In this context, the construction of carbon-carbon bonds using selective strategies has been a major target on synthetic organic chemistry. Palladium catalysed coupling reactions are among the most important reactions for the functionalization of aromatics and heterocyclic substrates.[16] The Nobel Prize in Chemistry 2010 was awarded jointly to Richard F. Heck, Ei-ichi Negishi and Akira Suzuki for their contributions on the development of efficient and versatile palladium-catalyzed coupling reactions.[17]

Among the strategies commonly used for doing new C-C bonds, the Negishi cross-coupling reaction has been highlighted, mainly in the preparation of biaryl compounds.[18] In addition, this strategy has already been successfully used for the arylation of tiophenes, and different methodologies have been applied to improve this reaction.[19] For example, Genov *et al.* have reported a greener protocol by using a microwave assisted Negishi cross-coupling reaction that led to the rapid enantioselective synthesis of binaphthalene derivatives in reasonable to excellent yields in few minutes.[20]

In this work, we have applied the Negishi reaction in the synthesis of thiophene-based analogues of lignans 2-(4-chlorophenyl)-5-phenylthiophene (**5a**) and 2,5 bis(2- methoxyphenyl)thiophene (**5b**) and evaluated their leishmanicidal effect against *Leishmania amazonensis* promastigotes. In addition, we have also evaluated the impact of these compounds on mammalian cell viability.

Experimental

General procedure for the preparation of (**3a-3b**)

To a 30 mL sealed flask equipped with a magnetic stirring bar and under a nitrogen atmosphere was added a solution of thiophene (5 mmol) in THF (10 mL). This solution was cooled to −78 °C and *n*-BuLi (5.5 mmol, 1.1 equiv; C = 2.47 mol L^{-1}) was added dropwise. After the addition was complete, the reaction mixture was warmed to 0 °C and stirred at this temperature for 1 h. Then this solution was cooled to −40 °C and a solution of $ZnCl_2$ in THF was added (1 mol L^{-1}, 5.5 mmol, 5.5 mL). After 15 min, a solution of $Pd_2(dba)_3$ (2 mol%) e P(*o*furil)$_3$ (4 mol%) in THF (2.5 mL) and a solution of corresponding aryl halide (7.5 mmol, 1.5 equiv.) were added and irradiated at 100 °C, in a microwave Antoon Paar Monowave 3000. After 45 min, the reaction mixture was quenched with saturated aqueous NH_4Cl (40 mL) and extracted with AcOEt (3 × 50 mL). The solvent was evaporated under vacuum and the crude product was purified by flash chromatography (Hex.).

2-(2-methoxyphenyl)thiophene (**3a**):[21] From 2-iodoanisole; yield 89%; brown solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54-7.52 (dd, 1H, J^1 7.6, J^2 1.6 Hz, C_6H_4), 7.39-7.38 (dd, 1H, J^1 3.6, J^2 1.2 Hz, H3), 7.21-7.19 (dd, 1H, J^1 5.2, J^2 1.2 Hz, H5), 7.14-7.12 (m, 1H, C_6H_4), 6.98-6.96 (dd, 1H, J^1 5.2, J^2 3.6 Hz, H4), 6.90-6.84 (m, 2H, C_6H_4), 3.79 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.4, 139.3, 128.5, 128.2, 126.7, 125.3, 125.2, 123.2, 120.8, 111.5, 55.4; MS (ESI, 70 eV) *m/z* 190 (M^+, 100%), 175 (42%), 157 (18%), 147 (65%), 131 (30%), 121 (10%), 115 (17%), 103 (15%), 77 (15%), 69 (12%), 63 (10%); HRMS (ESI) *m/z* 191.0522 ([M + H]$^+$ calcd. for $C_{11}H_{10}SO$: 191.0531).

2-phenylthiophene (**3b**):[22] From iodobenzene; yield 85%; brown solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51-7.49 (m, 2H), 7.27-7.23 (m, 2H), 7.19-7.13 (m, 3H), 6.96-6.94 (dd, 1H, J^1 4.8, J^2 3.6 Hz, H4); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.3, 134.3, 128.8 (2C), 127.9, 127.3, 125.8 (2C), 124.7, 123.0; MS (ESI, 70 eV) *m/z* 160 (M^+, 100%), 128 (13%),

121 (5%), 115 (40%), 89 (8%), 77 (6%), 63 (7%); HRMS (ESI) *m/z* 161.0433 ([M + H]$^+$ calcd. for $C_{10}H_8O$: 161.0425).

General procedure for the preparation of (**5a-5b**)

To a 30 mL sealed flask equipped with a magnetic stirring bar and under a nitrogen atmosphere was added a solution of 2-aryl-thiophene (**3a** or **3b**) (2.5 mmol) in THF (5 mL). This solution was cooled to −78 °C and *n*-BuLi (2.75 mmol, 1.1 equiv; C = 2.47 mol L^{-1}) was added dropwise. After the addition was complete, the reaction mixture was warmed to 0 °C and stirred at this temperature for 1 h. Then this solution was cooled to −40 °C and a solution of $ZnCl_2$ in THF was added (1 mol L^{-1}, 2.75 mmol, 2.75 mL). After 15 min, a solution of $Pd_2(dba)_3$ (2 mol%) e P(*o*furil)$_3$ (4 mol%) in THF (2.5 mL) and a solution of corresponding aryl halide (3.75 mmol, 1.5 equiv.) were added and irradiated at 100 °C. After 45 min, the reaction mixture was quenched with saturated aqueous NH_4Cl (30 mL) and extracted with AcOEt (3 × 30 mL). The solvent was evaporated under vacuum and the crude product was purified by flash chromatography (Hex/AcOEt).

2-(4-chlorophenyl)-5-phenylthiophene (**5a**):[23] From 1-chloro-4-iodobenzene; yield 81%; yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, 2H, *J* 7.2 Hz), 7.45 (d, 2H, *J* 8 Hz), 7.32-7.17 (m, 7H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.0, 142.1, 134.0, 133.1, 132.7, 129.0 (2C), 128.9 (2C), 127.6, 126.7 (2C), 125.6 (2C), 124.3, 124.0; MS (ESI, 70 eV) *m/z* 272 (37%), 270 (M$^+$, 100%), 234 (15%), 202 (11%), 189 (5%), 154 (9%), 135 (8%), 121 (12%), 77 (6%), 63 (5%); HRMS (ESI) *m/z* 271.0351 ([M + H]$^+$ calcd. for $C_{16}H_{11}SCl$: 271.0348).

2,5-bis(2-methoxyphenyl)thiophene (**5b**):[24] From 2-iodoanisole; yield 88%; green solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60-7.57 (dd, 2H, J^1 7.6, J^2 1.6 Hz, C_6H_4), 7.40 (s, 2H, H3 and H4), 7.17-7.13 (m, 2H, C_6H_4), 7.17-7.13 (m, 2H, C_6H_4), 6.92-6.87 (m, 4H, C_6H_4), 3.83 (s, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.6 (2C), 139.1 (2C), 128.3 (2C), 128.1 (2C), 125.6 (2C), 123.5 (2C), 120.8 (2C), 111.5 (2C), 55.4 (2C); MS (ESI, 70 eV) *m/z* 296 (M$^+$, 100%), 281 (28%), 266 (13%), 253 (10%), 237 (12%), 205 (15%), 131 (11%), 121 (12%), 77 (4%); HRMS (ESI) *m/z* 297.0956 ([M + H]$^+$ calcd. for $C_{18}H_{16}O_2S$: 297.0949).

Leishmanicidal assay

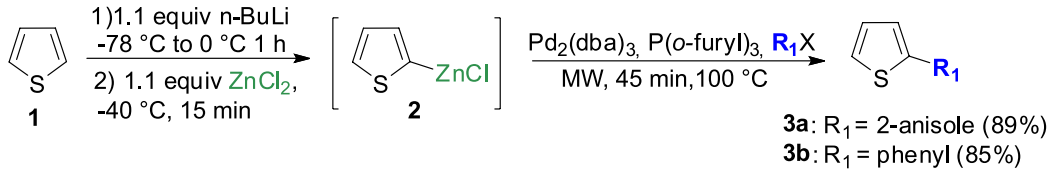
Promastigote forms of *Leishmania amazonensis* were cultured in Scheneider's medium (Sigma-Aldrich, USA) supplemented with 20% fetal bovine serum, 1% L-glutamine, 10 UI penicillin and 10 μg mL^{-1} streptomicin at 24 °C. The leishmanicidal activity of compounds was evaluated by colorimetric MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) was used as previously described by Mossman.[25] Briefly, log phase promastigotes of *Leishmania amazonensis* were seeded in 96-well tissue culture plates (1.0 × 10^6 well^{-1}). Then, the parasites were treated with 37, 111, 222, 369 μmol L^{-1} of compound **5a** or 34, 101, 202, 337 μmol L^{-1} of compound **5b** previously diluted in Schneider's medium with dimethyl-sulfoxide (DMSO) for 48 h. Each concentration was performed in triplicate and two independent experiments were executed. The used concentration of DMSO was not higher than 0.1%. DMSO solution and amphotericin B (Amp, 2.2 μmol L^{-1}) were used as negative and positive controls of cell death, respectively. After all treatments, cells were incubated with MTT solution (5 mg mL^{-1}; 10 μL well^{-1}) by 4 h at 24 °C. The plate was centrifuged at 400 × g for 10 min and the supernatants were then discarded. The formazan crystals produced by viable cells were solubilized with 200 μL of DMSO. The absorbance readout of each well was determined using a spectrophotometer (Spectramax Plus, Molecular Devices) at 570 nm. The leishmanicidal effects of compounds (**5a** and **5b**) was determined by calculation of the 50% inhibitory (IC_{50}) using a logarithm regression analysis.

Mammalian cell viability assay

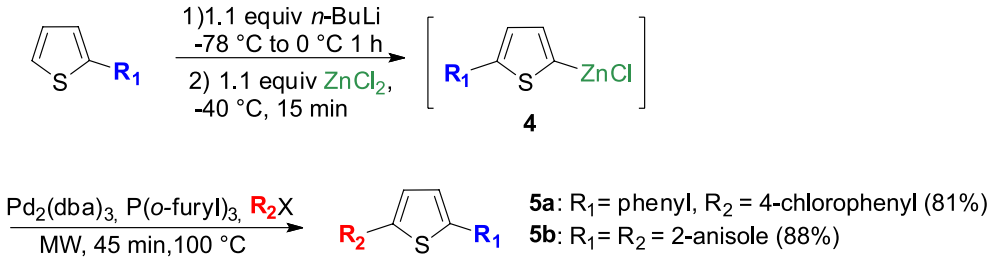
In order to evaluate the *in vitro* toxic effect of compounds **5a** and **5b** on mammalian cells, a cell lineage of mouse macrophage (J744A.1 cells) were used. J744A.1 cells (10^5 cells well^{-1}) were incubated in 96-well plates at 37 °C and 5% CO_2 in complete RPMI 1640 media containing 10% of fetal bovine serum, 1% L-glutamine, 10 UI penicillin, and 10 μg mL^{-1} streptomycin. After 24 h, the cells were washed with PBS and treated with 37, 111, 222, 369 μmol L^{-1} of compound **5a** or 34, 101, 202, 337 μmol L^{-1} of compound **5b** previously diluted in RPMI-1640 medium with DMSO. The 96-well plates were incubated at CO_2 5%, and 37 °C, for 48 h. The used DMSO concentration was not higher than 0.1%. Macrophages cells viability was determined by the MTT assay, as described above.

Statistical analysis

Results are presented as mean ± S.D. of experiments. Differences between groups were evaluated by analyses of variance (one-way ANOVA) followed by Bonferroni's test. Statistical differences were considered to be significant at $p < 0.05$.



Scheme 1. Synthesis of 2-arylthiophenes **3a** and **3b**.



Scheme 2. Synthesis of 2,5-diarylthiophenes **5a** and **5b.**

Results and Discussion

In our synthetic approach to thiophene-based analogues of lignans, we have planned to apply the Negishi cross-coupling reaction for the double arylation of the thiophene ring. Thus, we initiated our study by investigating the preparation of the organozinc reagent (**2**) and its palladium catalyzed cross-coupling reaction with aryl-iodines.

Reaction of thiophene (**1**) with 1.1 equivalents of *n*-BuLi in THF (–78 to 0 °C) led to the full lithiation of the starting material within 1 h. The organolithium derivative was then transmetalated with $ZnCl_2$ (1.1 equiv) at –40 °C to afford the organozinc reagent **2**. After that, a palladium-catalyzed reaction ($Pd_2(dba)_3$) of **2** with 2-iodoanisole was studied. Best results were obtained when the cross-coupling reaction was performed in a microwave reactor (45 min at 100 °C) and afforded the desired 2-(4-methoxyphenyl)thiophene (**3a**) in 89% yield. Similarly, when 2-iodobenzene was used as substrate, the 2-phenylthiophene (**3b**) was isolated in 85% yield (Scheme 1).

Aiming to synthesize the 2,5-diarylthiophenes, we have applied the same protocol for the arylation of **3a** and **3b**. Thus, the 2-arylthiophenes **3a** and **3b** were smoothly lithiated with *n*-BuLi in THF. After the transmetallation step with $ZnCl_2$ (1.1 equiv), the corresponding organozinc reagents of type **4** were reacted in the microwave reactor for 45 min at 100 °C with 1-chloro-4-iodobenzene and 2-iodoanisole in the presence of $Pd_2(dba)_3$ and the ligand P(*o*-furyl)$_3$. After the reaction work up and chromatographic purification, the corresponding 2-(4-chlorophenyl)-5-phenylthiophene (**5a**) and 2,5 bis(2- methoxyphenyl) thiophene (**5b**) were isolated in 81% and 88% yields, respectively (Scheme 2).

After the synthesis, we evaluated the leishmanicidal activity of two compounds (**5a** and **5b**) on the promastigote forms of *Leishmania amazonensis* (Figure 1). The results showed that the parasites were distinct susceptible to treatment of both compounds. The treatment of promatigotes with compound **5a** promoted a slight decrease in their cell viability compared to the positive control

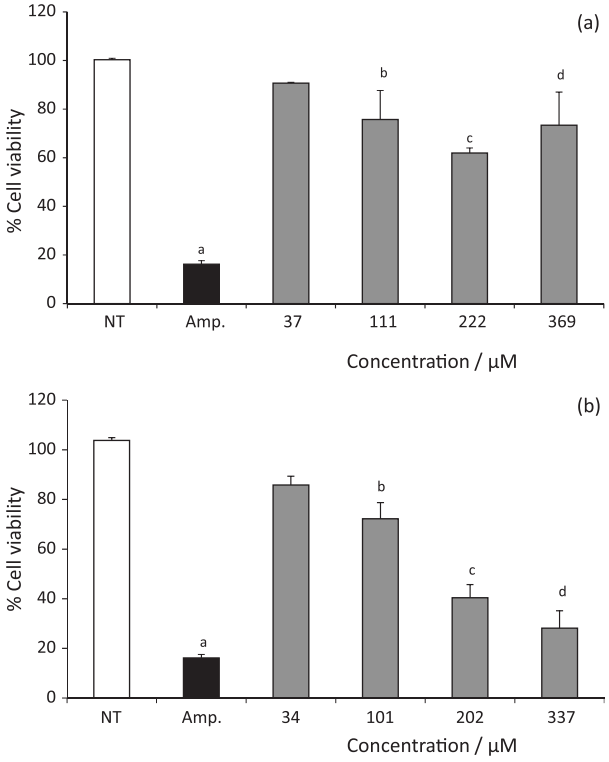


Figure 1. Compounds **5a** and **5b** induce different levels of cell death on promastigote form of *L. amazonensis*. NT: Not treated. Amp: amphotericin B (2.2 µM). Different letters on the graphic indicate significant ($P < 0.05$).

(Amp). Moreover, all concentrations of compound **5a** showed similar leishmanicidal effects and its IC_{50} was 526 μM.

The efficiency of the leishmanicidal activity of compound **5b** was greater than the activity of compound **5a**, however its efficiency was moderate compared to Amp. The leishmanicidal effect of compound **5b** on parasites was concentration-dependent, and the optimum effect was observed at ca. 202 μM, which showed similar activity to Amp (2.2 μM). Moreover, the IC_{50} of compound **5b** was around three times smaller (166 μM) than IC_{50} of compound **5a** (526 μM). Therefore, these results indicate that the compound **5b** presents better leishmanicidal effects than compound **5a** and this could be associated with the structural differences between these two compounds, since their substituent (methoxy) and/or the position of aromatic rings of these groups are different.

Several drugs used to treat leishmaniasis present significant toxic effects to the patients, such as amphotericin B.[19,20] Hence, the next step was to verify the impact of compounds **5a** and **5b** on the viability of J744A.1 macrophages. Interestingly, both compounds and negative control (non-treated macrophages) showed similar levels of cell death on macrophage (Figure 2). This set of results shows that the compounds **5a** and **5b** were less effective in causing death of leishmania compared to amphotericin B, but they were not toxic to J744A.1 cell line. The macrophage is a host cell target for leishmania and participates of immune defense against this parasite.[26] Thus, it is important for a good leishmanicidal activity to be selectively toxic against the parasite cell, causing no or little damage to host cells, as described for compound **5b** (Figure 2).[27]

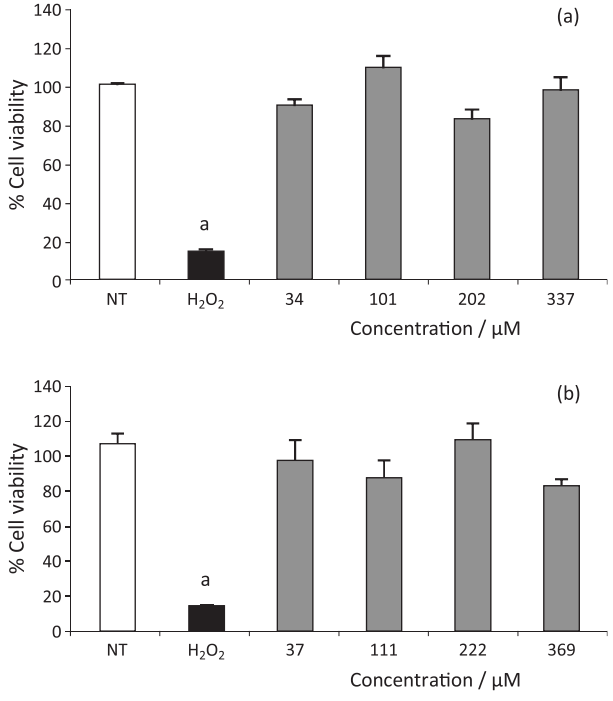


Figure 2. Treatment of J774A.1 cell line with compounds **5a** (a) and **5b** (b) does not cause cell death. H_2O_2: hydrogen peroxide (an inducer of cell death); NT: not treated. Letter "a" on the graphic indicate significant ($P < 0.05$).

In the literature, there are other studies showing that thiophene compounds present important activity against *Leishmania* spp. and other protozoans. For example, Gonzalez *et al.* have described the synthesis of bis-2,5-[4-guanidinophenyl]thiophenes, dicationic diguanidines related to the anti-protozoans furamidine and pentamidine, and their activity against some parasites.[28] In addition, a recent work from Bonano *et al.*[29] showed a screening of a compound library of estrogen receptor modulator analogs based on 2-arylbenzothiophene structure. It was described that optimal anti-leishmanial potency is dependent on the presence of two basic side chains in this skeleton, but the presence of phenols are not required for this activity. Takahashi *et al.* have demonstrated that two thiophene derivatives isolated from *Porophyllum ruderale* inhibited the growth of the promastigote and axenic amastigote forms of *L. amazonensis.*[11] Moreover, the same research group have also demonstrated the potential leishmanicidal effect of these compounds against intracellular amastigote forms.[30] However, in comparison to our data, the structures of these two thiophene, 5-methyl-2,2':5',2"-terthiophene and 5'-methyl-[5-4(4-acetoxy-1-butynyl)]-2,2' bi-thiophene, are very different from our compounds, mainly due to the presence of two or more rings in the thiophene structure. This fact can be related to the higher leishmanicidal effect from these two thiophenes when compared with our results.

Other study demonstrated the effect of many classes of diphenyl furans and thiophenes against *Leishmania donovani in vitro*. Among these synthetic analogues, the 2,5-diphenyl thiophenes with diamidines substituent were those that possess the most leishmanicidal activity from the compounds against *L. donovani* amastigote forms.[20]

In these context, the results of our study may provide some insights as to structure activity relationship of 2,5-diphenyl thiophenes compounds. Our studies and those from Brendle *et al.*[20] suggest that the structure of a 2,5-diphenyl thiophene unit bearing aromatic rings appears to be essential for the leishmanicidal effect of these compounds. However, the presence of substituents on the phenyl rings interfere in the anti-parasite effects. The presence of methoxy group (**5b**) provide more leishmanicidal effects than the chloro substituent (**5b**). These data suggest that the presence of a methoxy group in the aromatic ring is important for the increase of the leishmanicidal activity. Also, these modifications did not

induce cytotoxicity in the mammal cells. However, the presence of diamidines substituents in the thiophenes increased their leishmanicidal activity, but in some cases increased cytotoxicity in macrophage J774A.1 cell line were observed.[20]

It is interesting to note that the thiophenes can be a source of compounds to the development of new drugs against leishmaniasis and other protozoan diseases. This fact has been reported in the patent WO 2009051796,[31] where diazaaryl thiophene and selenophene compounds showed significant leishmanicidal effect on amastigote forms of *L. donovani* and other protozoan such as *Trypanossoma brucei* and *Plasmodium falciparum*. Thus, the synthetic routes to obtain 2,5-diaryl thiophenes are important to help the development of new compounds to protozoan diseases. In addition, many authors showed the potential leishmanicidal or antiparasitic effects of several thiophene compounds isolated from plants, which confirm the importance of additional studies with this class of structures against protozoan agents.[11,20,28,30]

Other important aspect of our study is that we demonstrated the leishmanicidal effect in *Leishmania amazonensis*. These parasites are responsible to induce localized cutaneous leishmaniasis (LCL) and diffuse cutaneous leishmaniasis (DCL), while other reported studies demonstrated leishmanicidal effect in *Leishmania donovani,* that is known to cause visceral leishmaniasis or mucosal leishmaniasis, mainly in immunocompromised patients (HIV-positive patients and other subjects immunosuppressive).[20,32] Biological differences between these parasites and the different mechanism of pathogenesis should be considered when comparing our studies and others in the literature.

Further studies are necessary to investigate the effect of 2,5-diphenyl thiophenes in leishmaniasis, mainly because it is a complex disease and involves modulation of the immune system. In addition, some aspects related to *in vivo* administration and efficacy of these compounds by different doses, routes and/or formulations should be evaluated, as absorption, leishmanicidal effect, half-life, excretion and possible toxicological effects. Therefore, the investigation of these 2,5-diphenyl thiophenes in an *in vivo* model of leishmaniasis and evaluation of impact of these drugs in a biological system, mainly in immune system is important to help in the development of new treatments to be used against leishmaniasis.

Conclusion

In summary, the application of the Negishi cross-coupling reaction in the microwave assisted synthesis of thiophene-based analogues of lignans has allowed the isolation of the desired 2,5-diarylthiophenes in good yields. In addition, the synthesized compounds appeared to induce selective leishmanicidal effect with low (compound **5a**) or moderate (compound **5b**) potencies compared to Amp. Therefore, this synthetic strategy will be helpful for the development of new drugs against cutaneous leishmaniasis. The scope of this methodology and its applicability toward the fast synthesis of other bioactive thiophenes with improved leishmanicidal activity are currently being investigated in our laboratories.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

References

1. David, C. V.; Craft, N.; *Dermatol. Ther.* **2009**, *22*, 491.
2. World Health Organization (WHO); *Control of the Leishmaniasis*, Technical Report Series; No. 949, Geneva, 2010.
3. Clem, A.; *J. Glob. Infect. Dis*. **2010**, *2*, 124.
4. Rath, S.; Trivelin, L. A.; Imbrunito, T. R.; Tomazela, D. M.; Jesús, M. N.; Marzal, P. C.; Andrade Junior, H. F.; Tempone, A. G.; *Quim. Nova* **2003**, *26*, 550.
5. Rojas, R.; Valderrama, L.; Valderrama, M.; Varona, M. X.; Ouellette, M.; Saravia, N. G.; *J. Infect. Dis.* **2006**, *193*, 1375.
6. Croft, S. L.; Coombs, G. H.; *Trends Parasitol.* **2003***, 19,* 502.
7. Vendrametto, M. C.; Santos, A. O.; Nakamura, C. V.; Dias Filho, B. P.; Cortez, D. A.; Ueda-Nakamura, T.; *Parasitol. Int.* **2010**, *59*, 154.
8. Brendle, J. J.; Outlaw, A.; Kumar, A.; Boykin, D. W.; Patrick, D. A.; Tidwell, R. R.; Werbovetz, K. A.; *Antimicrob. Agents Chemother*. **2002**, *46*, 797.
9. Schmidt, T. J.; Khalid, S. A.; Romanha, A. J.; Alves, T. M.; Biavatti, M. W.; Brun, R.; da Costa, F. B.; de Castro, S. L.; Ferreira, V. F.; de Lacerda, M. V.; Lago, J. H.; Leon, L. L.; Lopes, N. P.; Amorim, R. C. N.; Niehues, M.; Ogungbe, I. V.; Pohlit, A. M.; Scotti, M. T.; Setzer, W. N.; Soeiro, M. N. C.; Steindel, M.; Tempone, A. G.; *Curr. Med. Chem.* **2012**, *19,* 2128**.**
10. Schmidt, T. J.; Khalid, S. A.; Romanha, A. J.; Alves, T. M.; Biavatti, M. W.; Brun, R.; da Costa, F. B.; de Castro, S. L.; Ferreira, V. F.; de Lacerda, M. V.; Lago, J. H.; Leon, L. L.; Lopes, N. P.; Amorim, R. C. N.; Niehues, M.; Ogungbe, I. V.; Pohlit, A. M.; Scotti, M. T.; Setzer, W. N.; Soeiro, M. N. C.; Steindel, M.; Tempone, A. G.; *Curr. Med. Chem.* **2012**, *19,* 2176.
11. Barata, L. E. S.; Santos, L. S.; Ferri, P. H.; Paine, A.; Crofts, S. L.; Philipson, J. D.; *Phytochemistry* **2000**, *55*, 589.
12. Lopes, N. P.; Chicaro, P.; Albuquerque, S.; Yoshida, M.; Kato, M. J.; *Planta Med*. **1998**, *64*, 667.

13. Takahashi, H. T.; Novello, C. R.; Nakamura, T. U.; Filho, B. P. D.; Mello, J. C. P.; Nakamura, C. V.; *Molecules* **2011**, *16*, 3469.
14. Ram, V. J.; Goel, A.; Shukla, P. K.; Kapil, A.; *Bioorg. Med. Chem. Lett.* **1997**, *7*, 3101.
15. Mohareb, R. M.; Fahmy, A. A.; *Eur. Chem. Bull*. **2013**, *2*, 545.
16. Magano, J.; Dunetz, J. R.; *Chem. Rev*. **2011**, *111*, 2177; Wu, X.-F.; Neumann, H.; Beller, M.; *Chem. Rev*. **2013**, *113*, 1; Li, H.; Seechurn, C. C. C. J.; Colacot, T. J.; *ACS Catal*. **2012**, *2*, 1147; Amaral, M. F. Z. J.; Deliberto, L. A.; Souza, C. R.; Naal, R. M. Z. G.; Naal, Z.; Clososki, G. C.; *Tetrahedron* **2014**, *70*, 3249.
17. http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2010/, accessed in February 2014.
18. King, A. O.; Okukado, N.; Negishi, E.; *J. Chem. Soc., Chem. Commun.* **1977**, 683; Kanomata, N.; Suzuki, J.; Kubota, H.; Nishimura, K.; Enomoto, T.; *Tetrahedron Lett*. **2009**, *50*, 2740; Kürti, L.; Czakó, B.; *Strategic Applications of Named Reactions in Organic Synthesis*, Elsevier Academic Press: California, **2005**.
19. Piller, F. M.; Knochel, P.; *Org. Lett*. **2009**, *11*, 445; Kunz, T.; Knochel, P.; *Chem. Eur. J.* **2011**, *17*, 866; Bayh, O.; Awad, H.; Mongin, F.; Hoarau, C.; Trécourt, F.; Quéguiner, G.; Marsais, F.; Blanco, F.; Abarca, B.; Ballesteros, R.; *Tetrahedron* **2005**, *61*, 4779; Tanaka, S.; Tanaka, D.; Sugie, A.; Mori, A.; *Tetrahedron Lett*. **2012**, *53*, 1173; Hornillos, V.; Giannerini, M.; Vila, C.; Fañanás-Mastral, M.; Feringa, B. L.; *Org. Lett*. **2013**, *15*, 5114; Sanchis, E. F.; Guirao, F. J. A.; Santos, A. S.; Lázaro, F. F.; *J. Org. Chem*. **2007**, *72*, 3589; Zeni, G.; Alves, D.; Braga, A. L.; Stefani, H. A.; Nogueira, C. W.; *Tetrahedron Lett*. **2004**, *45*, 4823.
20. Genov, M.; Almorin, A.; Espinet, P.; *Tetrahedron: Asymmetry* **2007**, *18*, 625.
21. Demir, S. A.; Reis, O.; Emrullahoglu, M.; *J. Org. Chem*. **2003**, *68*, 578.
22. Guchhait, S. K.; Kashyap, M.; Saraf, S.; *Synthesis* **2010**, *7*, 1166; Liu, W-D.; Chi, C-C.; Pai, I-F.; Wu, A-T.; Chung, W-S.; *J. Org. Chem*. **2002**, *67*, 9267.
23. Leising, F.; Mignani, G.; *J. Heterocyclic Chem*. **1994**, *31*, 1005.
24. Chandra, R.; Kung, M-P.; Kung, H. F.; *Bioorg. Med. Chem. Lett*. **2006**, *16*, 1350.
25. Mosmann, T.; *J. Immunol. Methods* **1983**, *65*, 55.
26. Murray, H. W.; *J. Exp. Med.* **1999**, *189*, 741.
27. Iniguez, E.; Sánchez, A.; Vasquez, M. A.; Martínez, A.; Olivas, J.; Sattler, A.; Sánchez-Delgado, R. A.; Maldonado, R. A.; *J. Biol. Inorg. Chem.* **2013**, *18*, 779.
28. Gonzalez, J. L.; Stephens, C. E.; Wenzler, T.; Brun, R.; Tanious, F. A.; Wilson, W. D.; Barszcz, T.; Werbovetz, K. A.; Boykin, D. W.; *Eur. J. Med. Chem.* **2007,** *42*, 552.
29. Bonano, V. I.; Yokoyama-Yasunaka, J. K. U.; Miguel, D. C.; Jones, S. A.; Dodge, J. A.; Uliana, S. R. B.; *Chem. Biol. Drug Des.* **2014**, *83*, 289.
30. Takahashi, H. T.; Britta, E. A.; Longhini, R.; Ueda-Nakamura, T.; Palazzo de Mello, J. C.; Nakamura, C. V.; *Planta Med*. **2013**, *79*, 330.
31. Boykin, D. W.; Brun, R.; Ismail, M. A.; Kumar, A.; Stephens, C. E.; Tidwell, R. R.; Werbovetz, K.; Wilson, W. D.; *PCT/US2008/011870* 2008 *(WO2009051796)*.
32. Jarvis, J. N.; Lockwood, D. N.; *Curr. Opin. Infect. Dis.* **2013**, *26*, 1.

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Supplementary Information

Application of the Negishi Reaction in the Synthesis of Thiophene-Based Lignans Analogues with Leishmanicidal Effects

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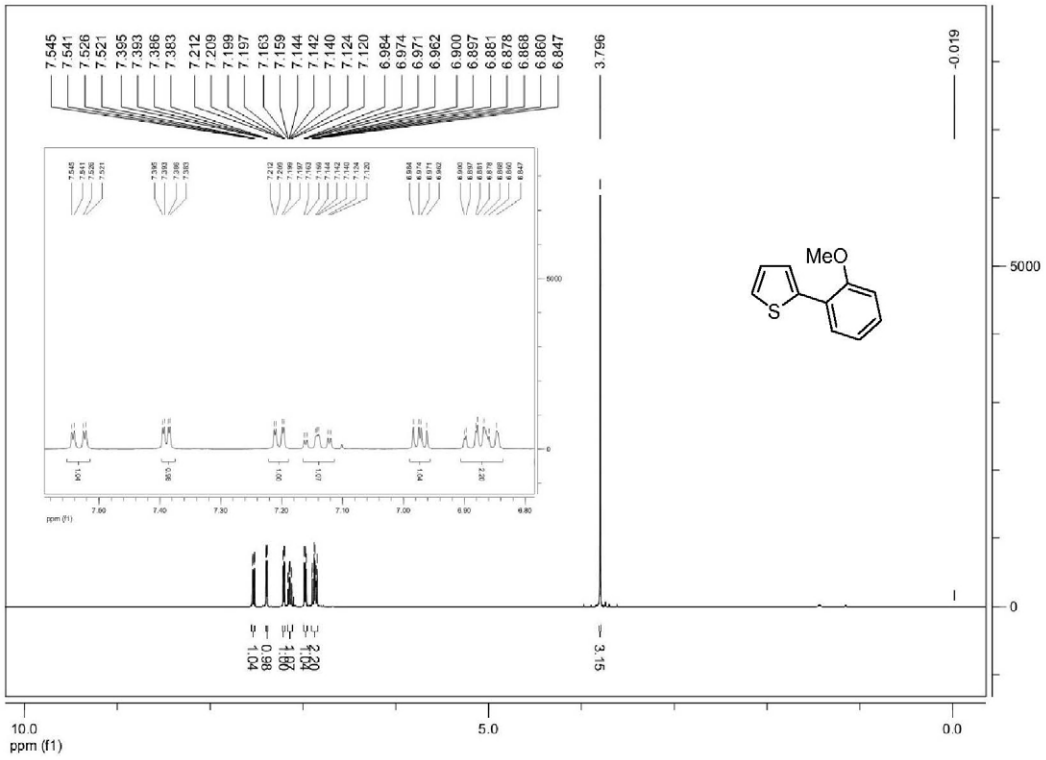

Figure S1. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of 2-(2-methoxyphenyl)thiophene (**3a**).

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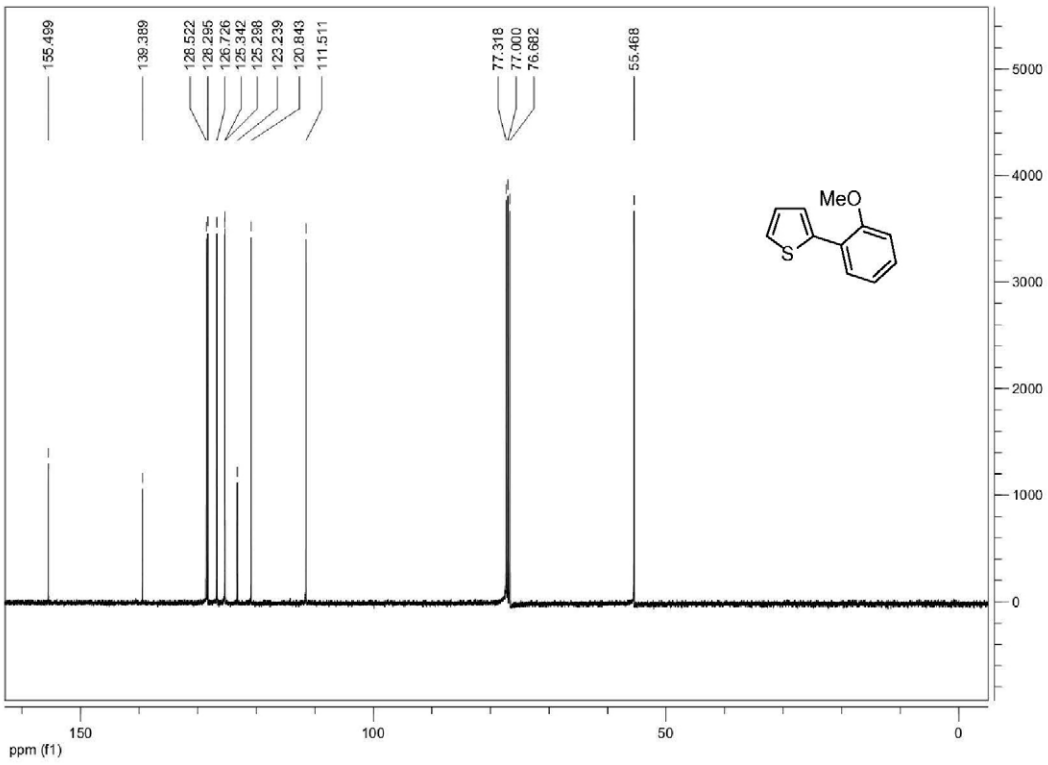

Figure S2. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of 2-(2-methoxyphenyl)thiophene (**3a**).

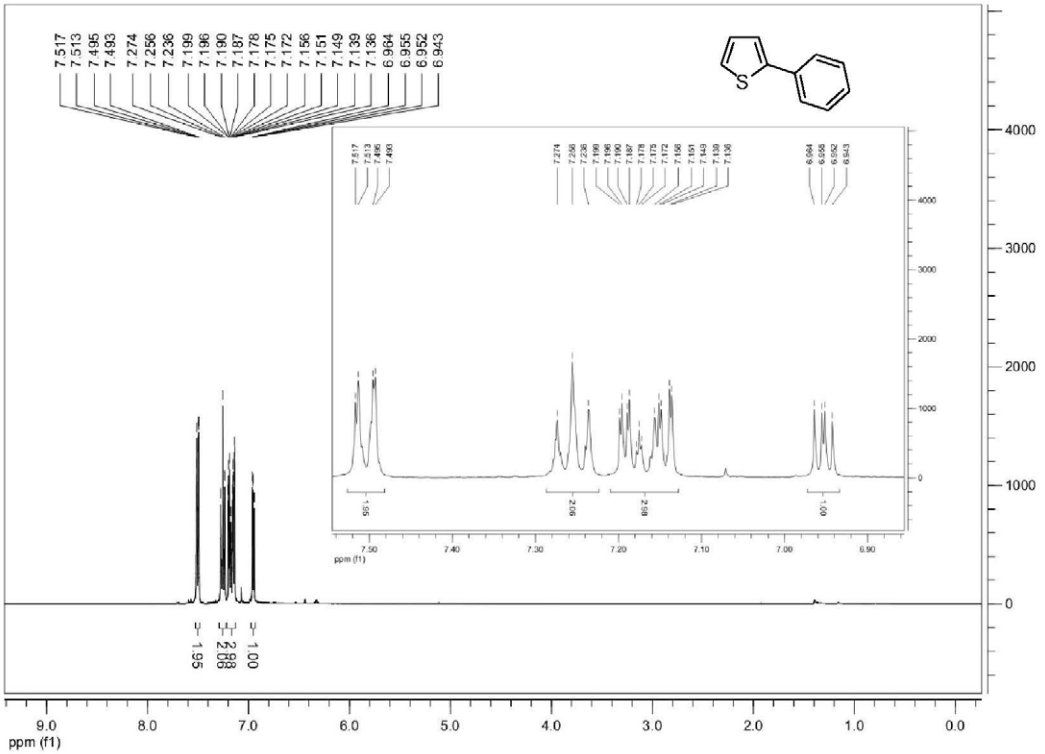

Figure S3. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of 2-phenylthiophene (**3b**).

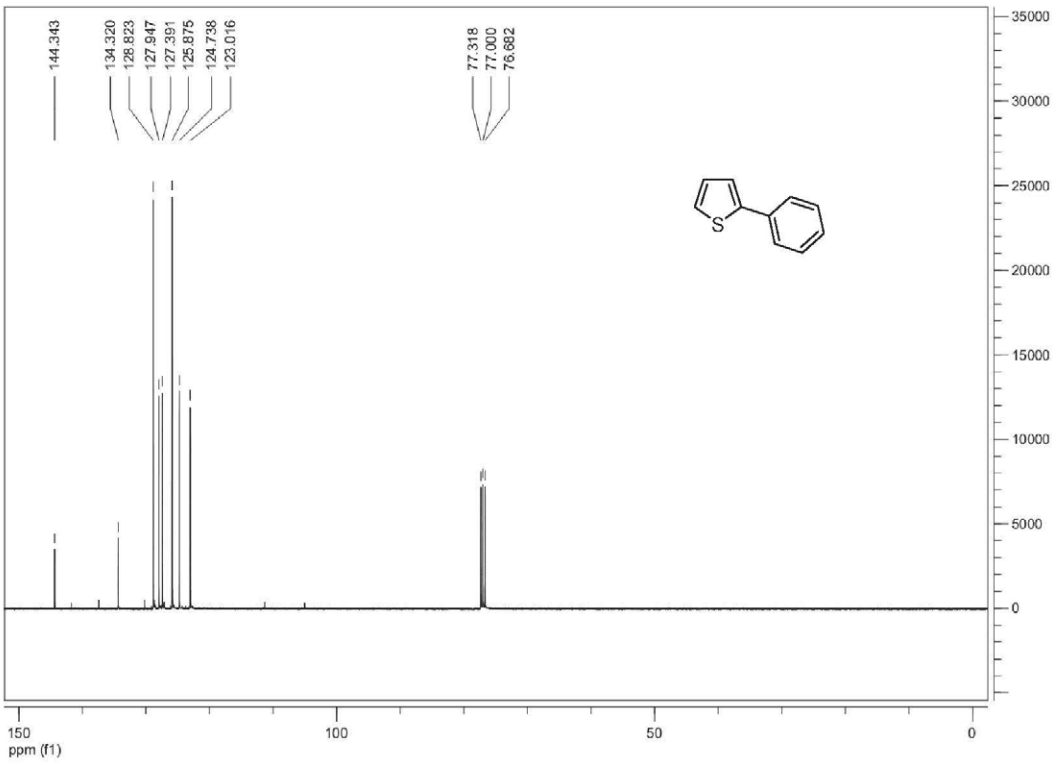

Figure S4. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of 2-phenylthiophene (**3b**).

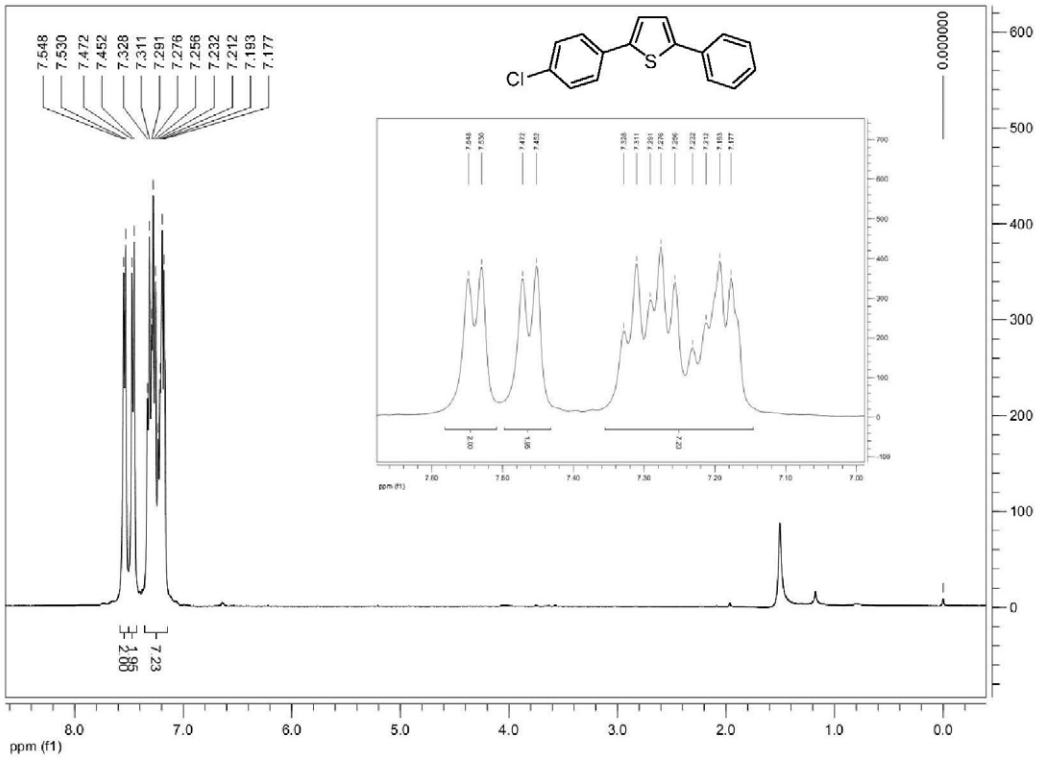

Figure S5. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of 2-(4-chlorophenyl)-5-phenylthiophene (**5a**).

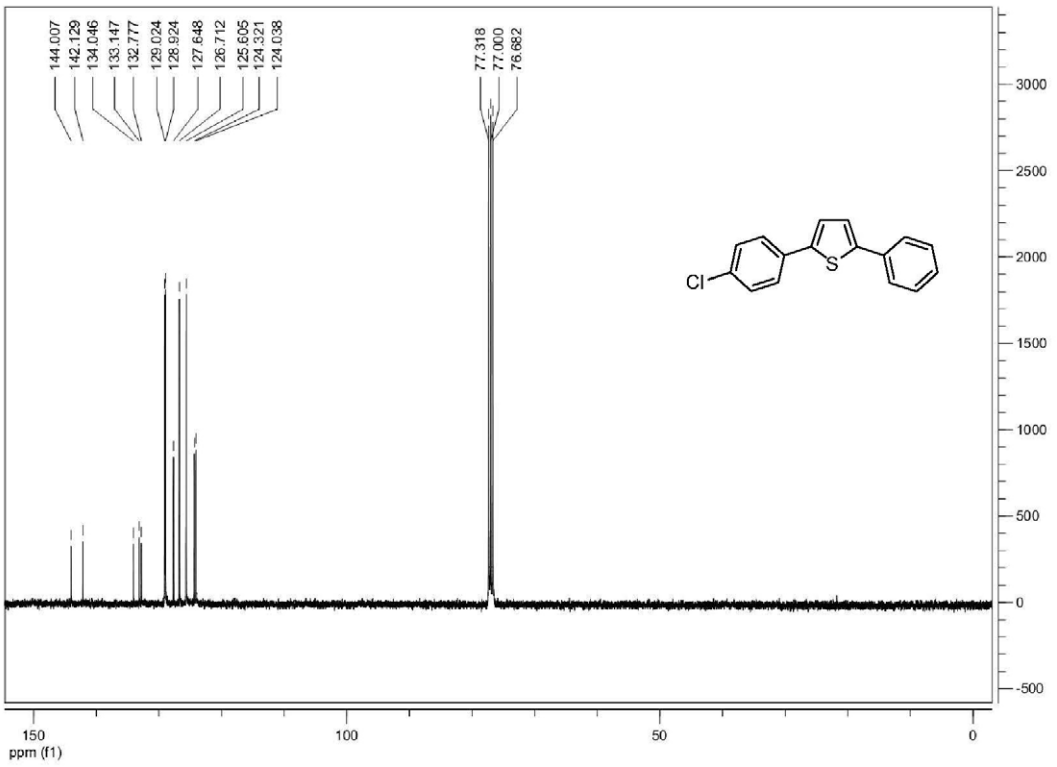

Figure S6. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of 2-(4-chlorophenyl)-5-phenylthiophene (**5a**).

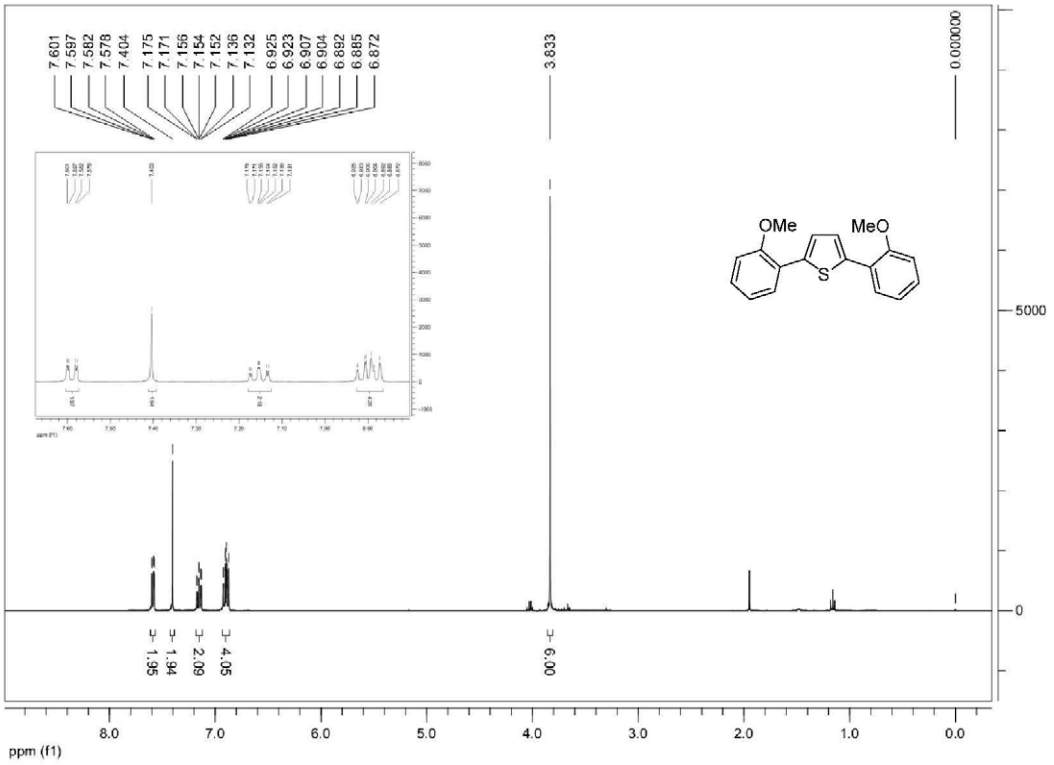

Figure S7. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of 2,5-bis(2-methoxyphenyl)thiophene (**5b**).

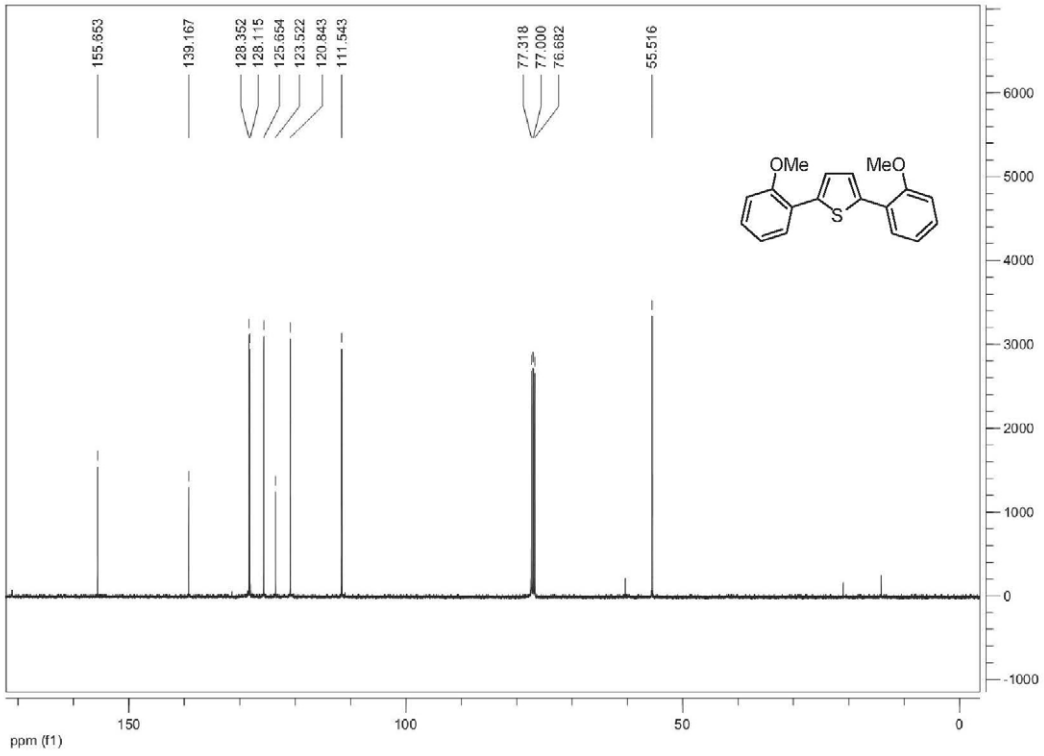

Figure S8. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of 2,5-bis(2-methoxyphenyl)thiophene (**5b**).

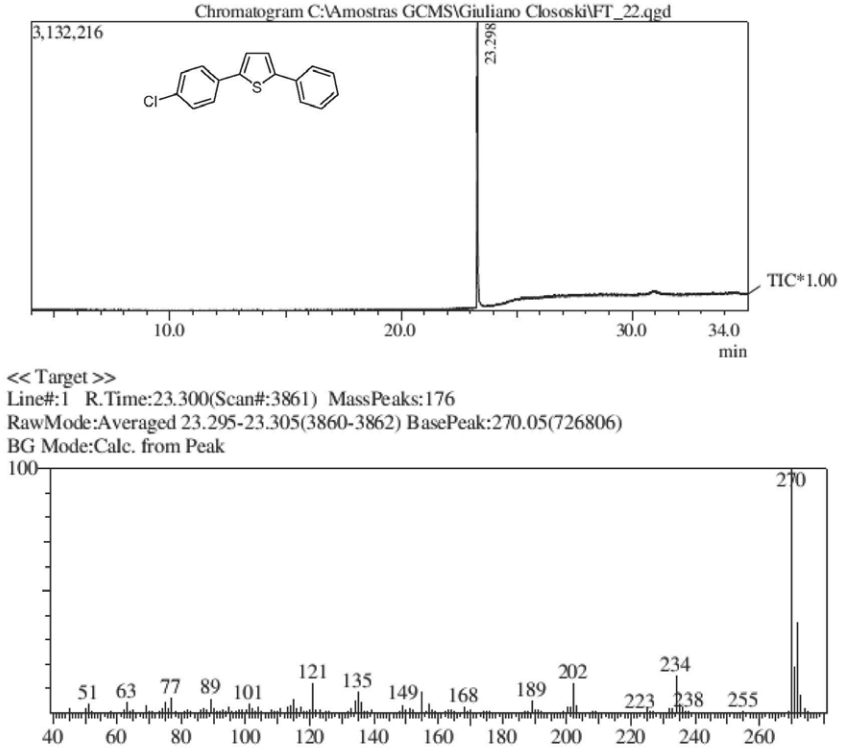

Figure S9. GC-MS analysis (ESI, 70 eV) of 2-(4-chlorophenyl)-5-phenylthiophene (**5a**).

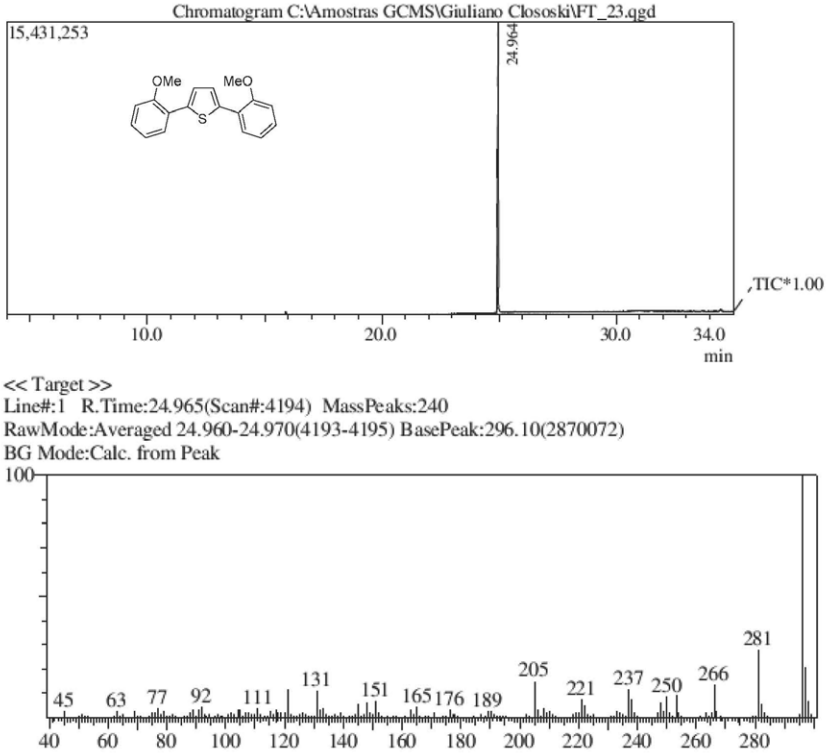

Figure S10. GC-MS analysis (ESI, 70 eV) of 2,5-bis(2-methoxyphenyl)thiophene (**5b**).