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Artigo

SYNTHESIS AND BIOLOGICAL ACTIVITY OF SULFUR COMPOUNDS SHOWING STRUCTURAL ANALOGY WITH COMBRETASTATIN A-4

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We extended our previous exploration of sulfur bridges as bioisosteric replacements for atoms forming the bridge between the aromatic rings of combretastatin A-4. Employing coupling reactions between 5-iodo-1,2,3-trimethoxybenzene and substituted thiols, followed by oxidation to sulfones with *m*-CPBA, different locations for attaching the sulfur atom to ring A through the synthesis of nine compounds were examined. Antitubulin activity was performed with electrophoretically homogenous bovine brain tubulin, and activity occurred with the 1,2,3-trimethoxy-4-[(4-methoxyphenyl)thio]benzene (**12**), while the other compounds were inactive. The compounds were also tested for leishmanicidal activity using promastigote forms of Leishmania braziliensis (MHOM/BR175/M2904), and the greatest activity was observed with 1,2,3-trimethoxy-4-(phenylthio)benzene (**10**) and 1,2,3-trimethoxy-4-[(4-methoxyphenyl) sulfinyl]benzene (**15**).

Keywords: combretastatin A-4; antitubulin activity; leishmanicidal activity.

INTRODUCTION

Microtubules are intracellular polymers assembled from α,β-tubulin heterodimers. These organelles have a number of essential cellular functions, including chromosome segregation, the maintenance of cell shape, transport, motility and, organelle distribution.[1,2] Inhibition of the formation of microtubules leads to cell cycle arrest and promotes vascular disruption. Inhibition of the formation of new tumor blood vessels (antiangiogenic agents) or destruction of vessels already present in tumors (vascular disrupting agents, VDA) are areas of extensive research in the development of new anticancer drugs.[3]

Research in the field of VDAs is focused on compounds capable of acting upon the processes of polymerization and depolymerization of tubulin, since microtubule integrity plays a major role in maintaining the structural integrity of blood vessels.[4] The natural product combretastatin A-4 (CA-4, Figure 1) was isolated from the bark of the African tree *Combretum caffrum*. Despite its low molecular weight and structural simplicity, CA-4 is one of the most powerful inhibitors of tubulin polymerization.[5,6] Studies examining various spacer groups that link the two aromatic rings of CA-4 have shown that biological activity does not require the *cis*-stilbene configuration of CA-4.[7] The synthesis of 1,2,3-trimethoxy-5-[(4-methoxyphenyl)thio]benzene (**1**) and derivatives **2** and **3** (Figure 1) showed that the sulfur atom is an interesting alternative as a spacer group.[7]

Protozoans of the genus *Leishmania* cause a wide spectrum of clinical disease in humans (especially leishmaniasis) and are a major public health risk in several countries.[8] Studies of potential leishmanicidal activity of combretastatins are scarce. Combretastatins and heterocombretastatins have previously been tested for *in vitro* activity in three different species of *Leishmania* (*L. amazonensis*, *L. braziliensis* and *L. donovani*).[9] Two of the most active, SAAS-41 and SAAS-59 (Figure 2) were also tested for *in vivo* activity in mice infected with amastigotes of the species *L. amazonensis*, resulting in very significant reductions in lesion size and parasite load.[10]

In the present study, we synthesized analogues of compounds **1**, **2** and **3** with the sulfur atom binding at position 4 of the A ring,

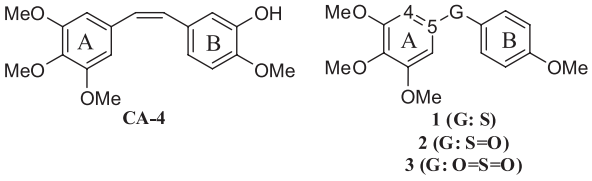

Figure 1. *Structures of compounds* **1***,* **2** *and* **3**

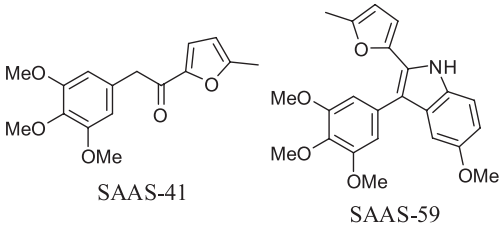

Figure 2. *Structures of SAAS-41 e SAAS-59*

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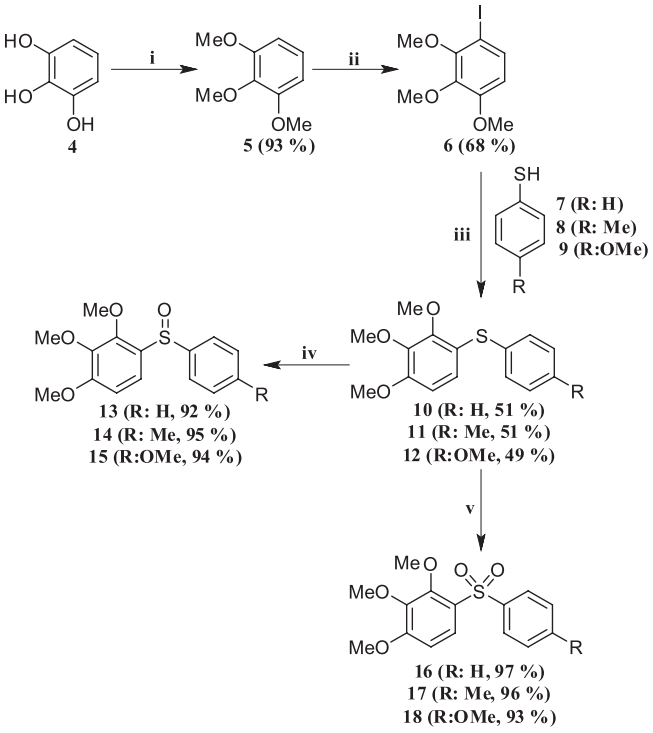
and effects of these compounds on tubulin polymerization and their activity against promastigote forms of *L. braziliensis* were evaluated.

RESULTS AND DISCUSSION

The CA-4 analogues were synthesized via the synthetic route shown in Scheme 1. In the first step, compound **5** was obtained by permethylation of pyrogallol (**4**) using CH_3I and base. Compound **5** was then subjected to iodination with NIS and TFA to furnish compound **6**. The coupling reaction of **6** with the thiols was catalyzed by neocuproine–Cu^+ and *t*-NaOBu under a nitrogen atmosphere.[11] The reaction mechanism can be related to that reported by Verma and co-workers.[12] This process was slow, and can be attributed to the steric hindrance caused by methoxyl groups. The sulfoxides and sulfones were obtained at a high yield by oxidation of sulfides with *m*-CPBA.[13] The preparation of compounds **10** and **16** was reported in the literature,[14-16] but neither of the compounds were synthesized by the procedures described here, nor were they examined for potential cytotoxic and antitubulin activities. The structure of all compounds was confirmed by spectroscopic methods.



Scheme 1. *Synthesis of CA-4 analogues. Reagents and conditions: (i) CH_3I, K_2CO_3, acetone, Δ; (ii) NIS, TFA, acetonitrile, r.t.; (iii) neocuproine, CuI, t-NaOBu, toluene, 110 °C; (iv) 1 equiv. m-CPBA, CH_2Cl_2; (v) 2 equiv. m-CPBA, CH_2Cl_2*

Although the previously prepared sulfone and sulfoxide[7] were not significantly active as antitubulin compounds, we decided to prepare similar derivatives with the sulfur atom attached to different positions of ring A, in the hope that this maneuver would lead to better activity.

However, only compound **12** showed any activity as an inhibitor of tubulin assembly (IC_{50}= 16 ± 2 (SD) μM), but **12** was much less active than either CA-4 or **1** (Table 1). All other compounds tested showed no effect on tubulin assembly. By comparing compounds **10**, **11** and **12** with **1**, **2** and **3**, it is clear that moving the bridge from position 5 to 4 in the A ring is highly deleterious for antitubulin activity. This probably affects the relative positions of the methoxyl groups in ring A, which are essential for activity, preventing formation of optimally active conformations for compounds **10**, **11** and **12**.[17,18] With respect to the substituent on ring B, the methoxyl group contributes to the weak antitubulin activity of compound **12** compared with other newly synthesized sulfides.[17]

Table 1. Evaluation of compounds **10**-**18** for antitubulin and leishmanicidal (*L. braziliensis*) activities, with antitubulin activities of CA-4 and **1**-**3**[a]

Compounds	Tubulin polymerization IC_{50} (μM) ± SD	Antileishmanial[b] IC_{50} (μM)
10	>40	14.3
11	>40	40.3
12	16 ± 2	27.9
13	>40	62.6
14	>40	67.2
15	>40	15.2
16	>40	NC[c]
17	>40	91.7
18	>40	31.5
CA-4	1.0 ± 0.1	-
1	1.2 ± 0.1	-
2	31 ± 3	-
3	>40	-

[a] Ref. 7. [b] Positive control: amphotericin B (IC_{50}, 22 μM). [c] Not calculated.

The new compounds were examined for leishmanicidal activity on promastigotes of *L. braziliensis* (Table 1), in comparison with a known active agent, amphotericin B, which had an IC_{50} of 22 μM. Compounds **11**, **13**, **14** and **17** were 2-4.5 fold less active than amphotericin B, while **12** and **18** were nearly as active (IC_{50}'s, 28 and 32 μM, respectively). However, compounds **10** and **15** (IC_{50}'s, 14 and 15 mM, respectively) were more active than amphotericin B.

CONCLUSION

Nine analogs of CA-4 with a sulfur atom bridge at position 4, as opposed to position 5, of the A ring were synthesized, seven of which are new compounds. Antitubulin tests for all compounds demonstrated that modifying the bridge position greatly reduced antitubulin activity. We are continuing to synthesize additional sulfur analogues to better understand how different ring attachment patterns and different groups affect the activity of this class of compounds. Compounds **10** and **15** are promising leads for designing new drugs to combat leishmanial diseases, as shown by their greater activity as compared with amphotericin B.

SUPPLEMENTARY MATERIAL

Available at http://quimicanova.sbq.org.br, in PDF format, with free access.

EXPERIMENTAL

Chemistry

General

All melting points were determined using a Uniscience of Brazil model 498 instrument. FT-IR spectra were obtained using the KBr pellet method or in a film of the compound performed with a FTIR

MB100 Boomen spectrophotometer. NMR spectra were recorded in $CDCl_3$ solutions on a Bruker DPX-300 instrument. All chemical shifts (d) are referenced to $CDCl_3$. High resolution mass spectrometry (HRMS) analyses were performed using an Agilent 6520 Accurate-Mass Q-TOF LC/MS System, equipped with a dual electro-spray source, operated in the positive-ion mode. The mass spectra obtained by electron ionization (EI-MS) were measured using a Shimadzu GCMS-QP2010 Plus gas chromatograph mass spectrometer. The reactions were monitored by TLC on silica gel-precoated aluminum sheets (UV_{254}). The solvents employed in the reactions and silica gel column chromatography were previously purified and dried according to procedures found in the literature.[19] Purification of compounds was performed by column chromatography, using stationary phase silica gel 60 (0.035-0.075 mm). All reagents were analytical grade.

*Synthesis of 1,2,3-trimethoxybenzene (**5**)*

K_2CO_3 (15.0 g, 108.5 mmol) and CH_3I (5 mL, 79.4 mmol) was added to a solution of pyrogallol (**4**) (commercial reagent, 2.0 g, 15.9 mmol) in acetone (60 mL) . The reaction mixture was refluxed for 24 h and cooled to r.t. The reaction mixture was filtered and concentrated under reduced pressure. The residue was placed into a dropping funnel and extracted with AcOEt (70 mL). The organic layer was washed with H_2O (2 x 50 mL) and brine (50 mL) and dried over anhydrous $MgSO_4$, and the solvent was evaporated under reduced pressure. Yield 93%; white solid; m.p. 43-44 °C (Lit. 42-43 °C).[20] IR (KBr) ν_{max}/cm^{-1}: 3016 (aromatic CH), 2835 (methyl CH), 1596 (C=C), 1253, 1110 (C-O). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.82 (s, 3H, OCH_3), 3.83 (s, 6H, OCH_3), 6.55 (d, *J* = 8.3 Hz, 2H, Ar-H), 6.96 (t, *J* = 8.3 Hz, 1H, Ar-H). ^{13}C NMR ($CDCl_3$) δ (ppm): 55.8 (OCH_3), 60.6 (OCH_3), 105.0 (CH), 123.4 (CH), 137.9 (C), 153.3 (C). EI-MS *m/z* (%): 168 [M+] (100), 153 (87), 125 (46), 110 (61), 93 (46).

*Synthesis of 5-iodo-1,2,3-trimethoxybenzene (**6**)*

A mixture of 1,2,3-trimethoxybenzene (**5**) (5.0 g, 29.7 mmol), NIS (7.3 g, 32.7 mmol) and TFA (0.7 mL, 8.9 mmol) in 120 mL of CH_3CN was stirred at r.t. for 5 h. The reaction mixture was concentrated under reduced pressure. An aqueous solution of 5% Na_2SO_3 (50 mL) was added to the residue, and the mixture extracted with EtOAc (2 x 75 mL). The organic layer was washed with H_2O (2 x 50 mL) and brine (50 mL) then dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure. Purification was performed by flash chromatography. Yield 68%; colorless solid; m.p. 40 °C (Lit. 42 °C).[21] IR (KBr) ν_{max}/cm^{-1}: 3016 (aromatic CH), 2835 (methyl CH), 1569 (C=C), 1288, 1083 (C-O). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.82 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 6.48 (d, *J* = 8.9 Hz, 1H, Ar-H), 7.37 (d, *J* = 8.9 Hz, 1H, Ar-H). ^{13}C NMR ($CDCl_3$) δ (ppm): 55.7 (OCH_3), 60.3 (OCH_3), 60.4 (OCH_3), 80.8 (C-I), 109.4 (CH), 132.0 (CH), 142.1 (C), 152.8 (C), 153.9 (C). EI-MS *m/z* (%): 294 [M+] (100), 279 (29), 236 (14), 124 (23), 109 (19).

General synthesis of diaryl sulfides

Sodium NaOBu-*t* (6 mmol), CuI (0.4 mmol), neocuproine (0.4 mmol), the thiol (4.5 mmol) and 5-iodo-1,2,3-trimethoxybenzene (**6**) (4 mmol) dissolved in anhydrous toluene (70 mL) were added to a round bottom flask under an N_2 atmosphere. The reaction mixture was heated for 70 h at 110 ºC. The mixture was cooled and filtered, and the solid washed with toluene. The solvent was evaporated under reduced pressure, and the resulting material purified by flash chromatography.

*1,2,3-trimethoxy-4-(phenylthio)benzene (**10**)*

Yield 51%; colorless oil. IR (film) ν_{max}/cm^{-1}: 3055 (aromatic CH), 2835 (methyl CH), 1577 (C=C), 1091 (C-O). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.82 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 6.64 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.02 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.17 (m, 1H, Ar-H), 7.24 (m, 4H, Ar-H). ^{13}C NMR ($CDCl_3$) δ (ppm): 56.0 (OCH_3), 60.9 (OCH_3), 61.1 (OCH_3), 107.9 (CH), 119.7 (C-S), 126.1 (CH), 128.8 (CH), 128.9 (CH), 129.2 (CH), 137.0 (C-S), 142.9 (C), 153.6 (C), 154.2 (C). EI-MS *m/z* (%): 278 [M+2] (6), 277 [M+1] (17), 276 [M+] (100), 261 (20), 246 (10), 147 (13), 109 (6), 91 (17). HRMS [ESI–MS]: $C_{15}H_{16}O_3S$ $[M+H]^+$ *m/z*, calc. 277.08929, found 277.08939.

*1,2,3-trimethoxy-4-[(4-methylphenyl)thio]benzene (**11**)*

Yield 51%; colorless oil. IR (film) ν_{max}/cm^{-1}: 3035 (aromatic CH), 2835 (methyl CH), 1577 (C=C), 1091 (C-O). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.29 (s, 3H, CH_3), 3.82 (s, 6H, OCH_3), 3.86 (s, 3H, OCH_3), 6.59 (d, *J* = 8.7 Hz, 1H, Ar-H), 6.88 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.08 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.18 (d, *J* = 8.2 Hz, 2H, Ar). ^{13}C NMR ($CDCl_3$) δ (ppm): 21.0 (CH_3), 56.0 (OCH_3), 60.9 (OCH_3), 61.0 (OCH_3), 107.9 (CH), 121.2 (C-S), 127.3 (CH), 129.8 (CH), 130.6 (CH), 132.5 (C-S), 136.6 (C), 142.8 (C), 152.9 (C), 153.6 (C). EI-MS *m/z* (%): 292 [M+2] (7), 291 [M+1] (19), 290 [M+] (100), 275 (20), 260 (13), 161 (14), 123 (7), 105 (35). HRMS [ESI–MS]: $C_{16}H_{19}O_3S$ $[M+H]^+$ *m/z*, calc. 291.10494, found 291.10505.

*1,2,3-trimethoxy-4-[(4-methoxyphenyl)thio]benzene (**12**)*

Yield 49%; colorless solid; m.p. 45 °C. IR (KBr) ν_{max}/cm^{-1}: 3062 (aromatic CH), 2831 (methyl CH), 1589 (C=C), 1245, 1091 (C-O). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.81 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 6.57 (d, *J* = 8.5 Hz, 1H, Ar-H), 6.71 (d, *J* = 8.5 Hz, 1H, Ar-H), 6.88 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.36 (d, *J* = 8.2 Hz, 2H, Ar-H). ^{13}C NMR ($CDCl_3$) δ (ppm): 55.4 (OCH_3), 56.1 (OCH_3), 60.9 (OCH_3), 107.9 (CH), 114.9 (CH), 123.3 (C-S), 125.2 (CH), 134.1 (CH), 135.4 (C-S), 142.8 (C), 151.8 (C), 153.0 (C), 159.4 (C). EI-MS *m/z* (%): 308 [M+2] (7), 307 [M+1] (18), 306 [M+] (100), 291 (15), 275 (8), 260 (13), 139 (15), 121 (33). HRMS [ESI–MS]: $C_{16}H_{19}O_4S$ $[M+H]^+$ *m/z*, calc. 307.09986, found 307.10007.

General synthesis of sulfoxides

m-CPBA 70-75%, balance 3-chlorobenzoic acid and water (0.3 mmol) was slowly added to a solution of diaryl sulfide (0.3 mmol) and 17 mL of anhydrous CH_2Cl_2 . The reaction mixture was stirred for 24 h at r.t. Sulfoxide formation was observed by TLC. The mixture was transferred to a dropping funnel, and CH_2Cl_2 (30 mL) added. The organic layer was washed with a saturated aqueous solution of Na_2CO_3 (2 x 20 mL) and brine (20 mL) then dried over anhydrous $MgSO_4$. The solvent was evaporated at reduced pressure, and the resulting material subjected to flash chromatography for purification.

*1,2,3-trimethoxy-4-(phenylsulfinyl)benzene (**13**)*

Yield 92%; white solid; m.p. 47 °C. IR (KBr) ν_{max}/cm^{-1}: 3008 (aromatic CH), 2839 (methyl CH), 1577 (C=C), 1087 (C-O), 1041 (S=O). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.75 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 6.82 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.44 (m, 3H, Ar-H), 7.52 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.69 (m, 2H, Ar-H). ^{13}C NMR ($CDCl_3$) δ (ppm): 56.1 (OCH_3), 60.8 (OCH_3), 107.5 (CH), 120.0 (CH), 125.3 (CH), 129.0 (CH), 130.0 (C-S), 130.8 (CH), 141.6 (C), 146.0 (C-S), 150.1 (C), 156.3 (C). EI-MS *m/z* (%): 294 [M+2] (0,10), 293 [M+1] (0.13), 292 [M+] (0.75), 276 (100), 261 (20), 246 (10), 147 (14), 109 (8), 91 (20). HRMS [ESI-MS]: $C_{15}H_{17}O_4S$ $[M+H]^+$ *m/z*, calc. 293.08421, found 293.08415.

*1,2,3-trimethoxy-4-[(4-methylphenyl)sulfinyl]benzene (**14**)*

Yield 95%; colorless solid; m.p. 103 °C. IR (KBr) ν_{max}/cm^{-1}:

3004 (aromatic CH), 2835 (methyl CH), 1577 (C=C), 1087 (C-O), 1037 (S=O). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.36 (s, 3H, CH_3), 3.74 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 6.82 (d, *J*= 8.8 Hz, 1H, Ar-H), 7.24 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.53 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.57 (d, *J* = 8.2 Hz, 2H, Ar-H). ^{13}C NMR ($CDCl_3$) δ (ppm): 21.4 (CH_3), 56.1 (OCH_3), 60.8 (OCH_3), 107.5 (CH), 119.8 (CH), 125.5 (CH), 129.7 (CH), 130.1 (C-S), 141.3 (C), 141.7 (C), 142.8 (C-S), 150.1 (C), 156.2 (C). EI-MS *m/z* (%): 306 [M+] (0.43), 290 (100), 275 (19), 260 (13), 161 (15), 123 (9), 105 (39). HRMS [ESI-MS]: $C_{16}H_{19}O_4S$ $[M+H]^+$ *m/z*, calc. 307.09986, found 307.10029.

*1,2,3-trimethoxy-4-[(4-methoxyphenyl)sulfinyl]benzene (**15**)*

Yield 94%; white solid; m.p. 73 °C. IR (KBr) ν_{max}/cm^{-1}: 3051 (aromatic CH), 2839 (methyl CH), 1577 (C=C), 1245, 1087 (C-O), 1041 (S=O). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.70 (s, 3H, OCH_3), 3.81 (s, 6H, OCH_3), 3.89 (s, 3H, OCH_3), 6.84 (d, *J* = 8.9 Hz, 1H, Ar-H), 6.95 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.56 (d, *J* = 8.9 Hz, 1H, Ar-H), 7.60 (d, *J* = 8.7 Hz, 2H, Ar-H). ^{13}C NMR ($CDCl_3$) δ (ppm): 55.4 (OCH_3), 56.1 (OCH_3), 60.8 (OCH_3), 60.9 (OCH_3), 107.4 (CH), 114.5 (CH), 119.7 (CH), 127.6 (CH), 130.1 (C-S), 137.2 (C-S), 141.7 (C), 149.9 (C), 156.2 (C), 161.8 (C). EI-MS *m/z* (%): [M+] (not observed), 306 (100), 291 (16), 276 (9), 260 (14), 139 (17), 121 (42). HRMS [ESI-MS]: $C_{16}H_{19}O_5S$ $[M+H]^+$ *m/z*, calc. 323.09477, found 323.09538.

General synthesis of sulfones

Diaryl sulfide (0.3 mmol) was dissolved in anhydrous CH_2Cl_2 (17 mL), and *m*-CPBA (0.6 mmol) was slowly added to the solution. The reaction mixture was stirred for 24 h at r.t. Sulfoxide formation to completion was observed by TLC. The mixture was transferred to a dropping funnel, and CH_2Cl_2 (30 mL) was added. The organic layer was washed with a saturated aqueous solution of Na_2CO_3 (2 x 20 mL) and brine (20 mL) and dried over anhydrous $MgSO_4$. The solvent was evaporated at reduced pressure, and the resulting material was subjected to flash chromatography for purification.

*1,2,3-trimethoxy-4-(phenylsulfonyl)benzene (**16**)*

Yield 97%; yellow solid; m.p. 63 °C (Lit. 65.5-66.5 °C).[14] IR (KBr) ν_{max}/cm^{-1}: 3093 (aromatic CH), 2842 (methyl CH), 1581 (C=C), 1303, 1145 (S=O), 1095 (C-O). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.75 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 6.77 (d, *J* = 8.9 Hz, 1H, Ar-H), 7.53 (m, 3H, Ar-H), 7.87 (d, *J* = 8.9 Hz, 1H, Ar-H), 7.95 (d, *J* = 7.2 Hz, 2H, Ar-H). ^{13}C NMR ($CDCl_3$) δ (ppm): 56.2 (OCH_3), 60.8 (OCH_3), 61.2 (OCH_3), 106.3 (CH), 124.8 (CH), 126.9 (C-S), 127.9 (CH), 128.6 (CH), 132.8 (CH), 142.2 (C-S), 142.6 (C), 151.9 (C), 158.7 (C). EI-MS *m/z* (%): 310 [M+2] (7), 309 [M+1] (17), 308 [M+] (100), 293 (6), 152 (24), 137 (19), 125 (25), 109 (15), 91 (44), 77 (25). HRMS [ESI-MS]: $C_{15}H_{17}O_5S$ $[M+H]^+$ *m/z*, calc. 309.07912, found 309.07931.

*1,2,3-trimethoxy-4-[(4-methylphenyl)sulfonyl]benzene (**17**)*

Yield 96%; white solid; m.p. 125 °C. IR (KBr) ν_{max}/cm^{-1}: 3093 (aromatic CH), 2842 (methyl CH), 1581 (C=C), 1307, 1141 (S=O), 1095 (C-O). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.41 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 6.76 (d, *J* = 8.9 Hz, 1H, Ar-H), 7.28 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.83 (d, *J* = 8.9 Hz, 1H, Ar-H), 7.85 (d, *J* = 8.2 Hz, 2H, Ar-H). ^{13}C NMR ($CDCl_3$) δ (ppm): 21.5 (CH_3), 56.2 (OCH_3), 60.8 (OCH_3), 61.2 (OCH_3), 106.2 (CH), 124.7 (CH), 127.3 (C-S), 127.9 (CH), 129.2 (CH), 139.3 (C-S), 142.6 (C), 143.6 (C), 151.9 (C), 158.5 (C). EI-MS *m/z* (%): 324 [M+2] (7), 323 [M+1] (18), 322 [M+] (96), 215 (15), 139 (32), 109 (23), 105 (100), 91 (38), 77 (29), 65 (24). HRMS [ESI-MS]: $C_{16}H_{19}O_5S$ $[M+H]^+$ *m/z*, calc. 323.09477, found 323.09524.

*1,2,3-trimethoxy-4-[(4-methoxyphenyl)sulfonyl]benzene (**18**)*

Yield 93%; white solid; m.p. 131-132 °C. IR (KBr) ν_{max}/cm^{-1}: 3020 (aromatic CH), 2838 (methyl CH), 1577 (C=C), 1303, 1137 (S=O), 1265, 1091 (C-O). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.79 (s, 6H, OCH_3), 3.85 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 6.75 (d, *J* = 9.0 Hz, 1H, Ar-H), 6.95 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.83 (d, *J* = 9.0 Hz, 1H, Ar-H), 7.89 (d, *J* = 8.9 Hz, 2H, Ar-H). ^{13}C NMR ($CDCl_3$) δ (ppm): 55.5 (OCH_3), 56.2 (OCH_3), 60.8 (OCH_3), 61.3 (OCH_3), 106.2 (CH), 113.8 (CH), 124.6 (CH), 127.6 (C-S), 130.2 (CH), 133.9 (C-S), 142.6 (C), 151.8 (C), 158.4 (C), 163.1 (C). EI-MS *m/z* (%): 340 [M+2] (7), 339 [M+1] (18), 338 [M+] (100), 215 (18), 155 (29), 137 (29), 121 (96), 109 (19), 77 (27). HRMS [ESI-MS]: $C_{16}H_{19}O_6S$ $[M+H]^+$ *m/z*, calc. 339.08969, found 339.09027.

Biological activity

Antitubulin activity

The tubulin assembly assay was performed with electrophoretically homogenous bovine brain tubulin.[22] The assembly assay was performed with 10 mM (1.0 mg/mL) tubulin in 0.8 M monosodium glutamate (pH 6.6 with HCl in a 2.0 M stock solution), 0.4 mM GTP, and 4% (v/v) dimethyl sulfoxide (as the compound solvent).[23] Tubulin and varying compound concentrations were preincubated without GTP for 15 min at 30 °C, samples were placed on ice, and GTP was added. The samples were transferred to 0 °C cuvettes in Beckman DU7400 and DU7500 recording spectrophotometers equipped with electronic temperature controllers. After baselines were established at 350 nm, the temperature was jumped to 30 °C (less than 1 min), and sample turbidity was followed for 20 min. The IC_{50} was the compound concentration that reduced the turbidity reading at 20 min by 50% relative to a control reaction mixture without compound.

Leishmanicidal activity

Promastigote forms of *Leishmania braziliensis* (MHOM/BR175/M2904) were used to evaluate leishmanicidal activity. The axenic culture medium 199 supplemented with 5% newborn calf serum was used. Six days after the initial inoculation, promastigote forms were obtained and incubated in 96-well microtiter plates (10^6 parasites/well). The compounds were added at different concentrations (0.5, 2.0, 8.0 and 32 μM), and incubation continued for 24 h at 23 °C. The positive control used was amphotericin B (at the same concentrations as the tested compounds), while dimethyl sulfoxide at 1% in physiologic solution was used as a negative control. The determination of biological activity was performed using a colorimetric technique following the addition of a tetrazolium salt (MTT).[24]

ACKNOWLEDGMENTS

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REFERENCES

1. Carvalho, P.; Tirnauer, J. S.; Pellman, D.; *Trends Cell Biol.* **2003**, *13*, 229.

2. Hadfield, J. A.; Ducki, S.; Hirst, N.; McGown, A. T.; *Prog. Cell Cycle Res.* **2003**, *5*, 309.
3. Pilat, M. J.; LoRusso, P. M.; *J. Cell. Biochem.* **2006**, *99*, 1021.
4. Lippert, J. W.; *Bioorg. Med. Chem.* **2007**, *15*, 605.
5. Lin, C. M.; Singh, S. B.; Chu, P. S.; Dempcy, R. O.; Schmidt, J. M.; Pettit, G. R.; Hamel, E.; *Mol. Pharmacol.* **1988**, *34*, 200.
6. Pettit, G. R.; Singh, S. B.; Hamel, E.; Lin, C. M.; Alberts, D. S.; Garcia-Kendall, D.; *Experientia* **1989**, *45*, 209.
7. Barbosa, E. G.; Bega, L. A.; Beatriz, A.; Sarkar, T.; Hamel, E.; Do Amaral, M. S.; De Lima, D. P.; *Eur. J. Med. Chem.* **2009**, *44*, 2685.
8. Leifso, K.; Cohen-Freue, G.; Dogra, N.; Murray, A.; Mcmastera, W. R.; *Mol. Biochem. Parasitol.* **2007**, *152*, 35.
9. Del Rey, B.; Ramos, A. C.; Caballero, E.; Inchaustti, A.; Yaluff, G.; Medarde, M.; De Arias, A. R.; Feliciano, A. S.; *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2711.
10. Nakanishi, W.; Hayashi, S.; Uehara, T.; *Eur. J. Org. Chem.* **2001**, 3933.
11. Bates, C. G.; Gujadhur, R. K.; Venkataraman, D. A.; *Org. Lett.* **2002**, *4*, 2803.
12. Verma, A. K.; Singh, J.; Chaudhary, R.; *Tetrahedron Lett.* **2007**, *48*, 7199.
13. Clennan, E. L.; Chen, X.; *J. Am. Chem. Soc.* **1989**, *111*, 5787.
14. Horner, L.; Gowecke, S.; *Chem. Ber.* **1961**, *94*, 1267.
15. Kita, Y.; Takada, T.; Mihara, S.; Tohma, H.; *Synlett* **1995**, *2*, 211.
16. Kita, Y.; Takada, T.; Mihara, S.; Whelan, B. A.; Tohma, H.; *J. Org. Chem.* **1995**, *60*, 7144.
17. Cushman, M.; Nagarathnam, D.; Gopal, D.; Chakraborti, A. K.; Lin, C. M.; Hamel, E.; *J. Med. Chem.* **1991**, *34*, 2579.
18. Cushman, M.; Nagarathnam, D.; Gopal, D.; He, H. M.; Lin, C. M.; Hamel, E.; *J. Med. Chem.* **1992**, *35*, 2293.
19. Perrin, D. D. In *Purification of Laboratory Chemicals*; Armarego, W. L. F., ed.; Pergamon Press: Oxford, 1988.
20. Yang, J.; Weng, L.; Zheng, H.; *Synth. Commun.* **2006**, *36*, 2401.
21. Wirth, H. O.; Königstein, O.; Kern, W.; *Justus Liebigs Ann. Chem.* **1960**, *634*, 84.
22. Hamel, E.; Lin, C. M.; *Biochemistry* **1984**, *23*, 4l73.
23. Hamel, E.; *Cell Biochem. Biophys.* **2003**, *38*, 1.
24. Muelas-Serrano, S.; Nogal-Ruiz, J. J.; Gómez-Barrio, A.; *Parasitol. Res.* **2000**, *86*, 999.

SYNTHESIS AND BIOLOGICAL ACTIVITY OF SULFUR COMPOUNDS SHOWING STRUCTURAL ANALOGY WITH COMBRETASTATIN A-4

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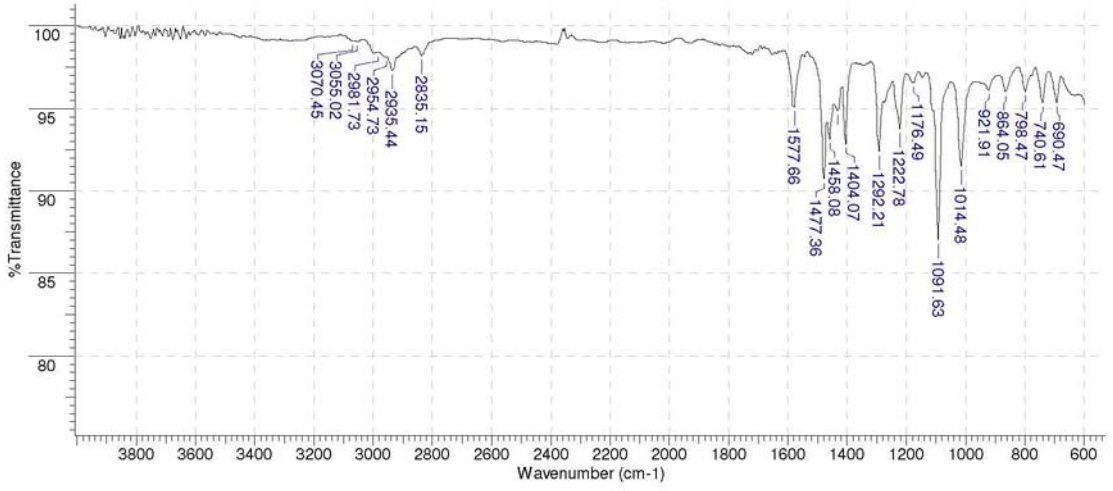

Figure 1S. *IR (film) spectrum of* ***10***

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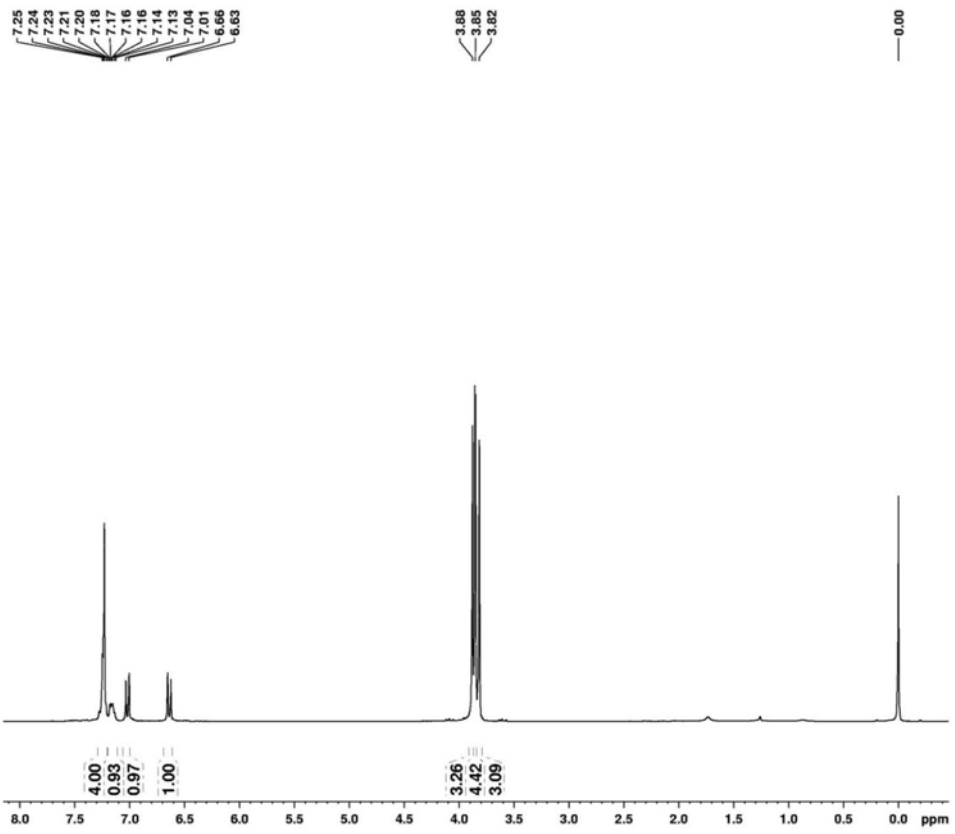

Figure 2S. *^{1}H NMR spectrum of **10**, 300 MHz, $CDCl_3$*

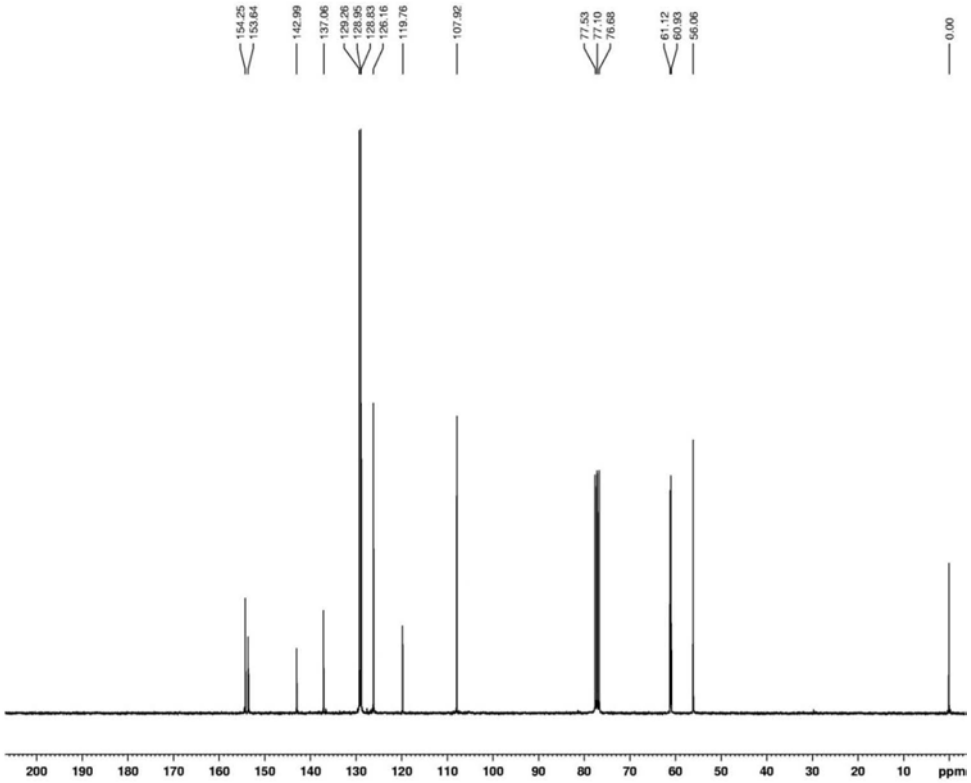

Figure 3S. *^{13}C NMR spectrum of **10**, 75 MHz, $CDCl_3$*

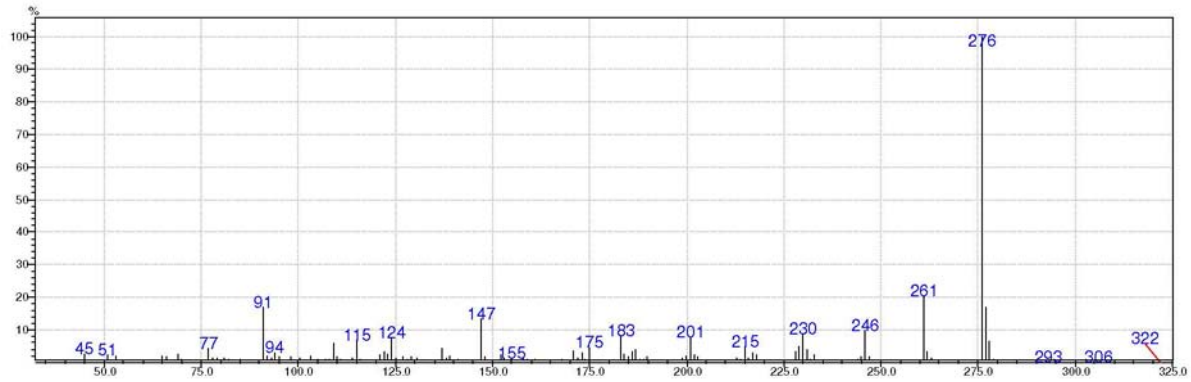

Figure 4S. *EI-MS spectrum of* ***10***

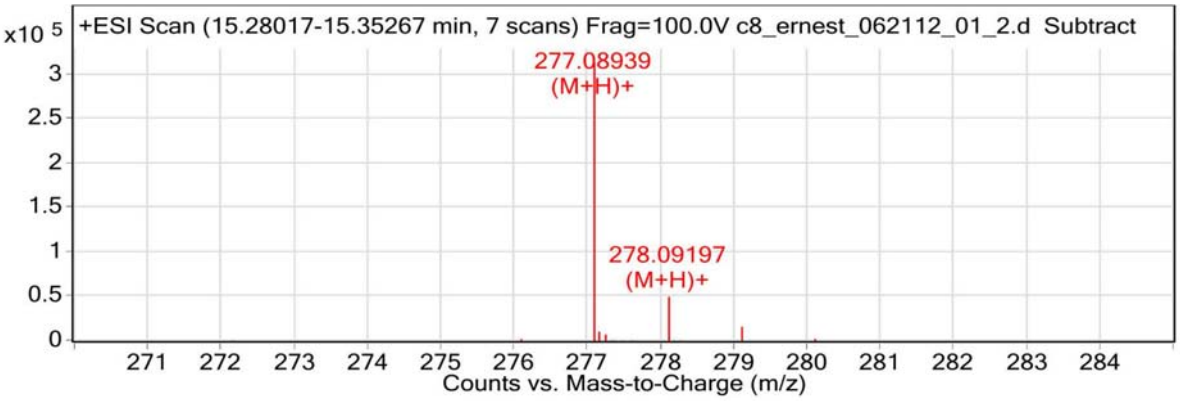

Figure 5S. *HRMS (ESI-MS) spectrum of* ***10***

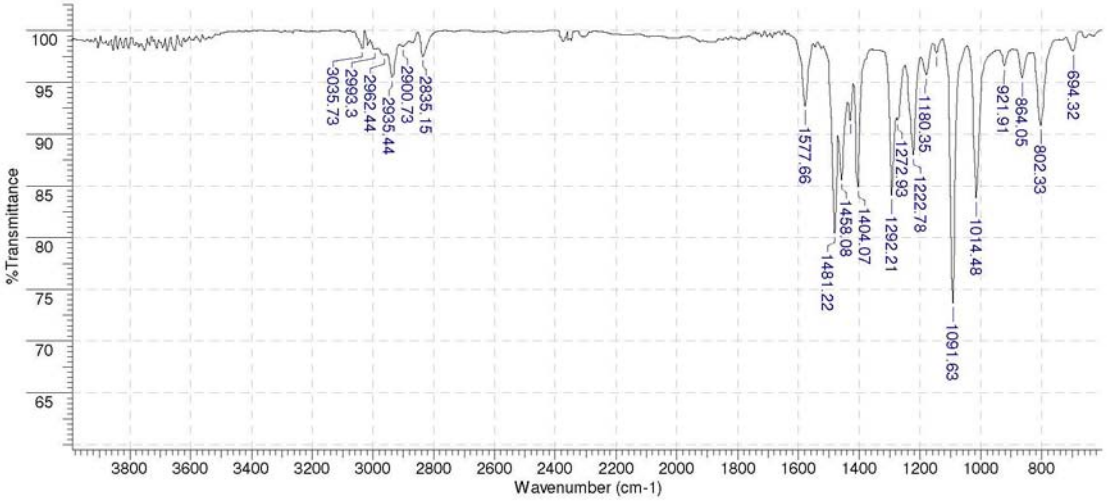

Figure 6S. *IR (film) spectrum of* ***11***

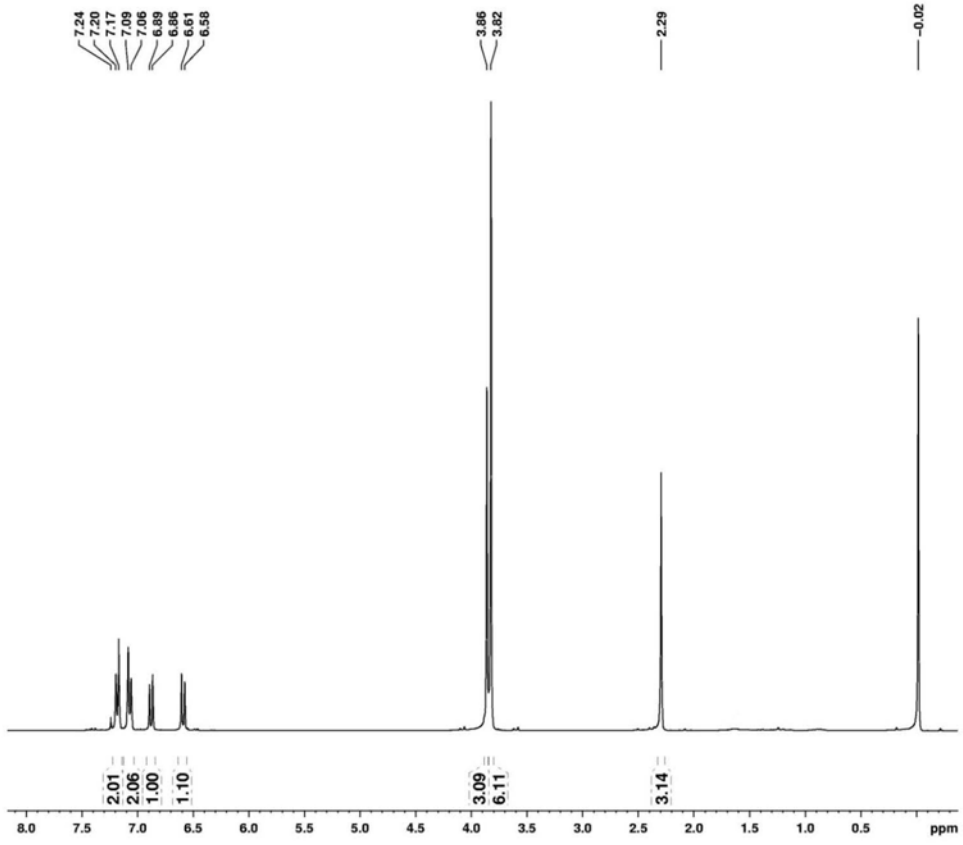

Figure 7S. *[1]H NMR spectrum of* ***11****, 300 MHz, $CDCl_3$*

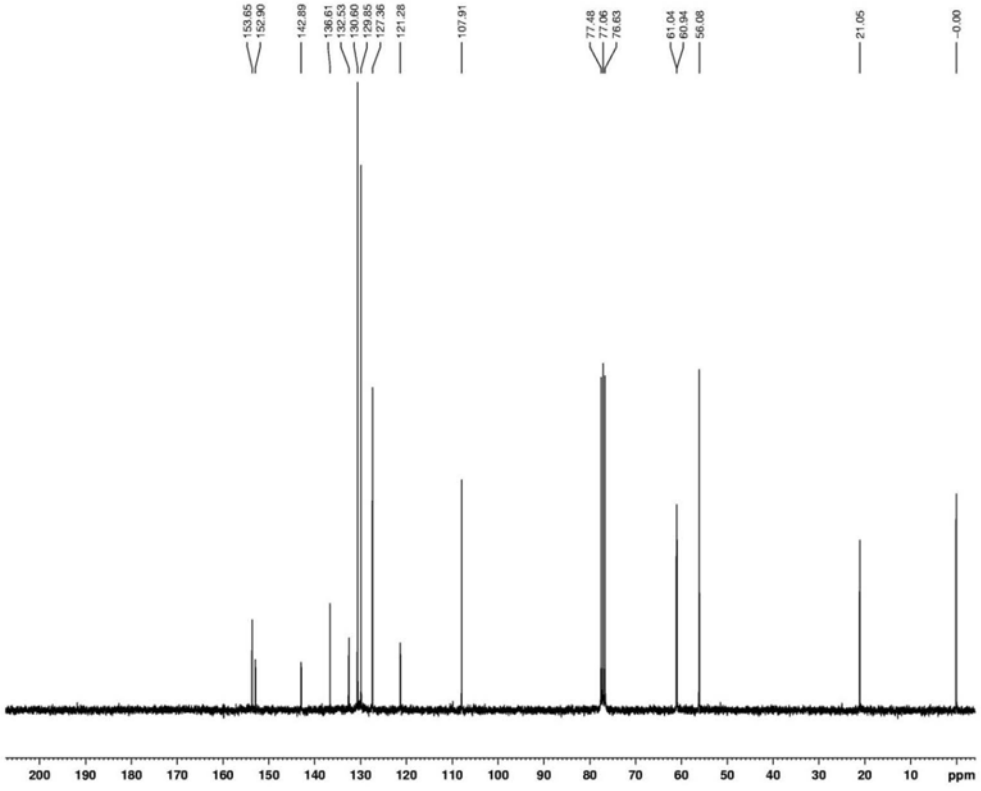

Figure 8S. *[13]C NMR spectrum of* ***11****, 75 MHz, $CDCl_3$*

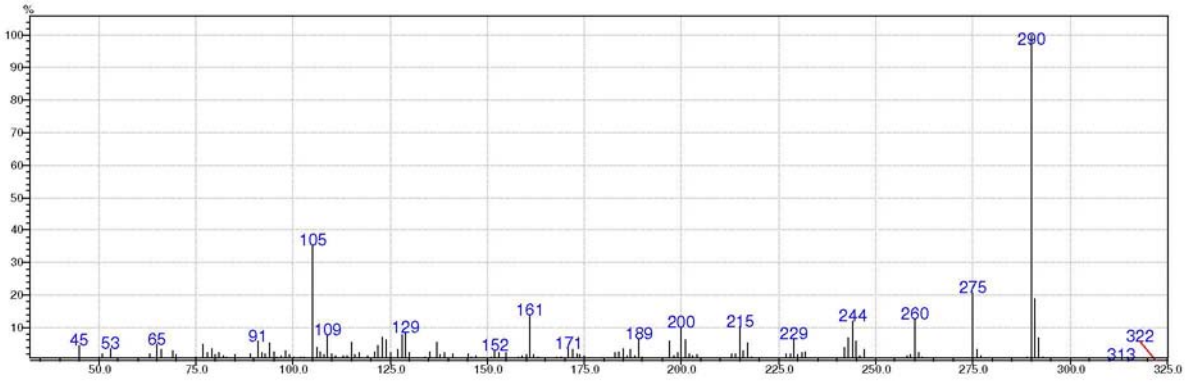

Figure 9S. *EI-MS spectrum of* ***11***

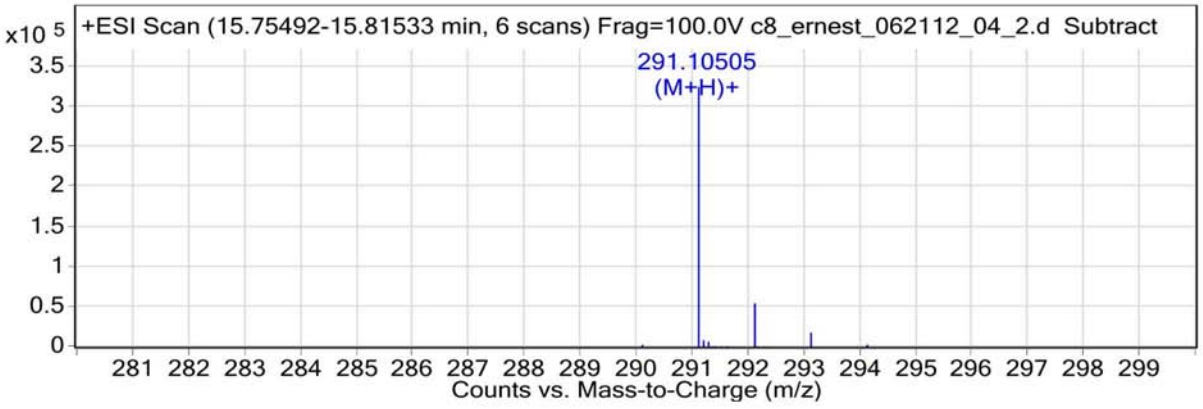

Figure 10S. *HRMS (ESI-MS) spectrum of* ***11***

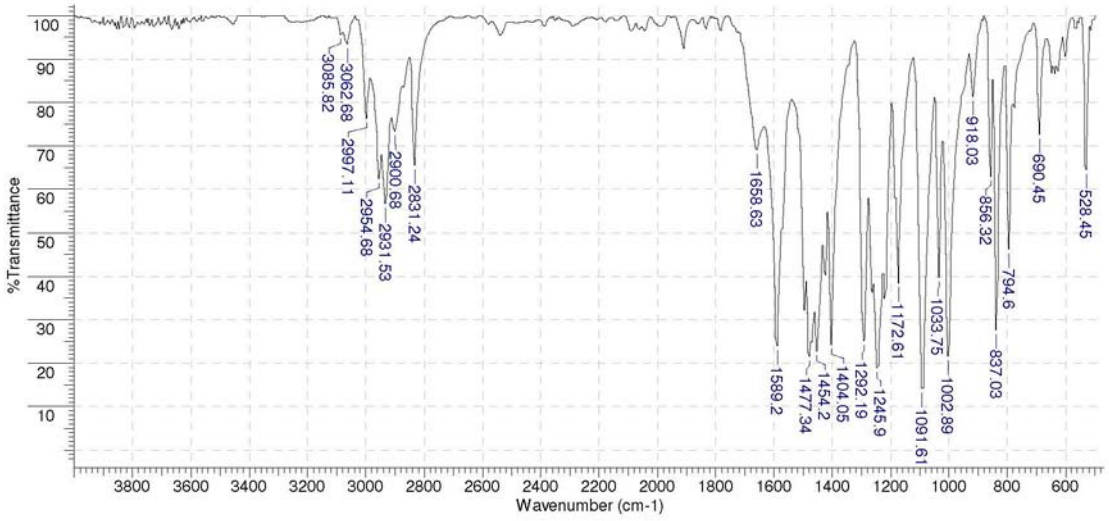

Figure 11S. *IR (KBr) spectrum of* ***12***

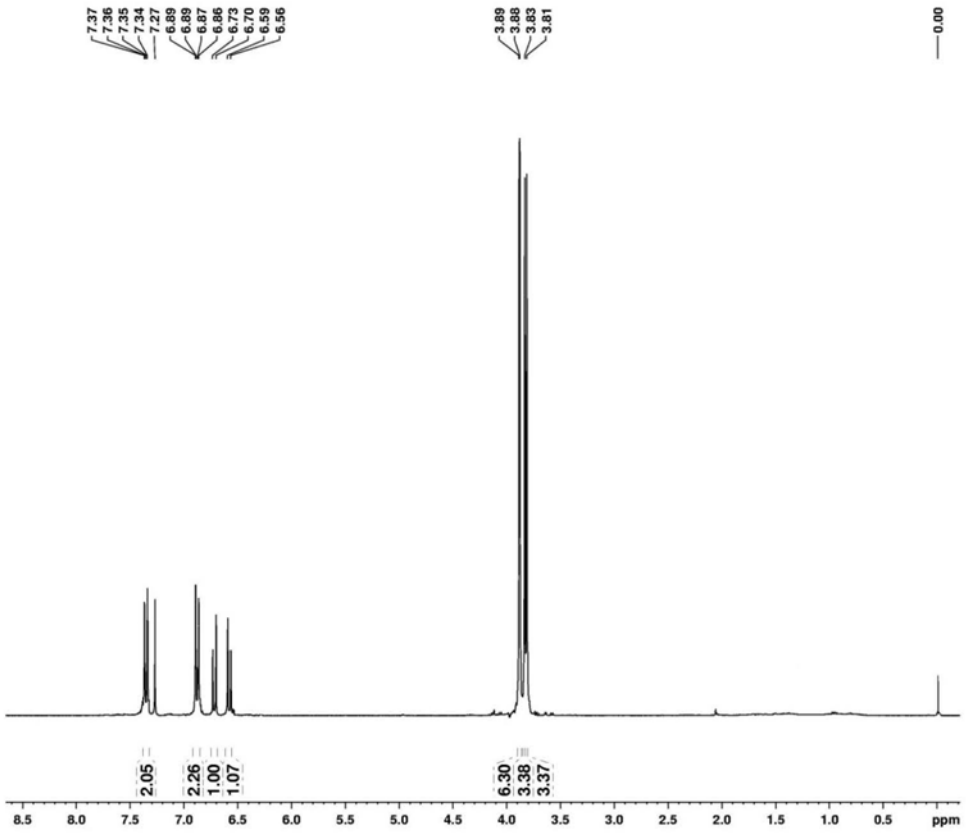

Figure 12S. *[1]H NMR spectrum of* ***12****, 300 MHz,* $CDCl_3$

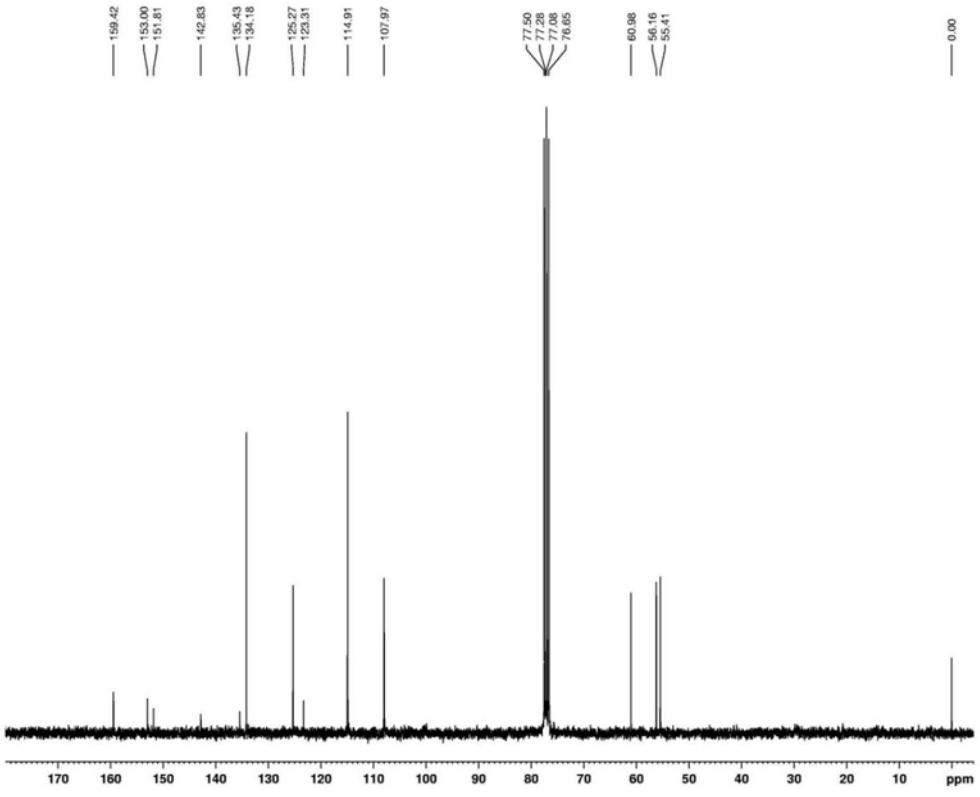

Figure 13S. *[13]C NMR spectrum of* ***12****, 75 MHz,* $CDCl_3$

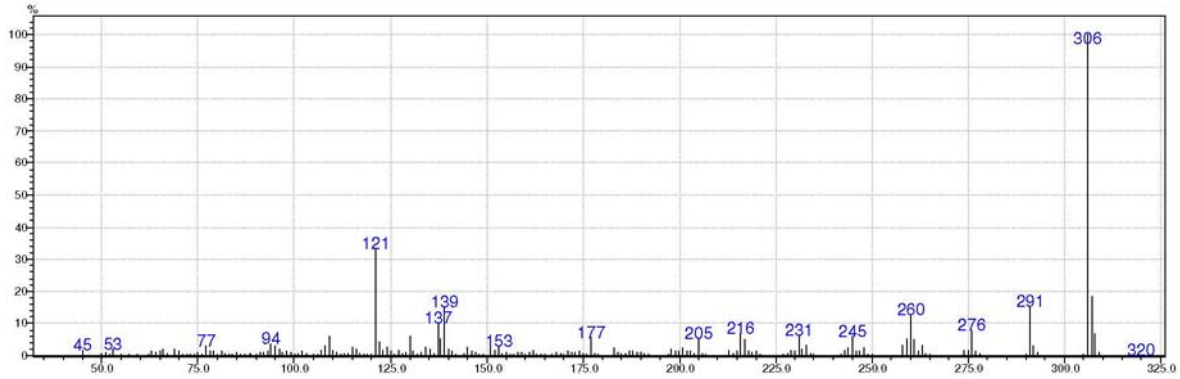

Figure 14S. *EI-MS spectrum of* ***12***

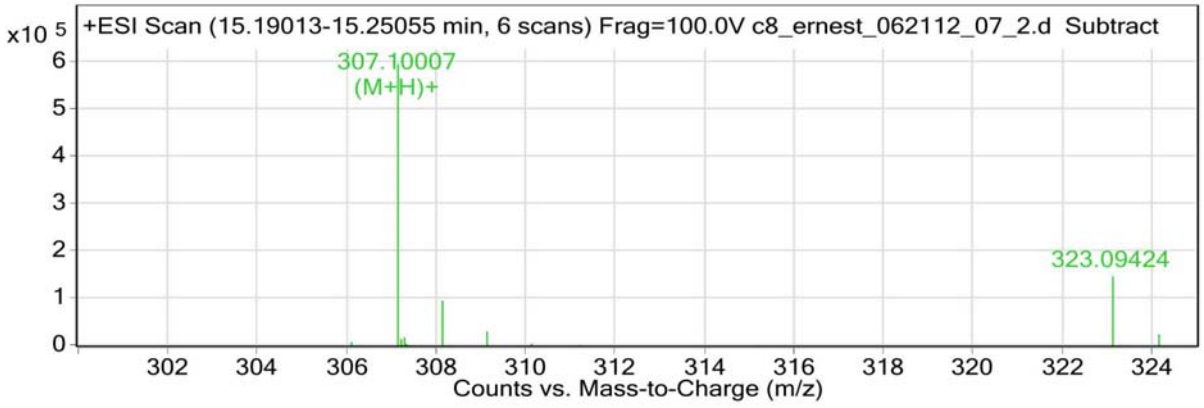

Figure 15S. *HRMS (ESI-MS) spectrum of* ***12***

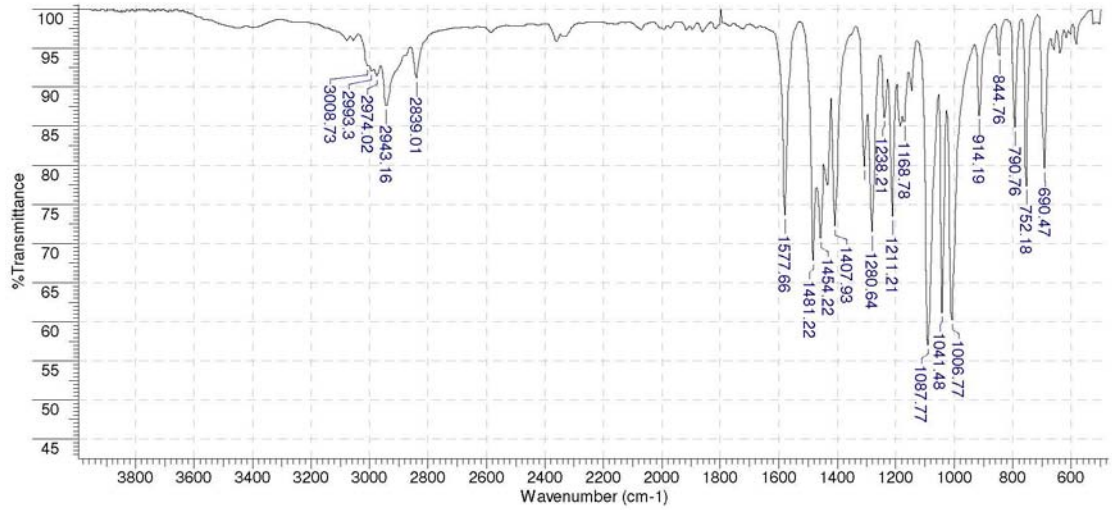

Figure 16S. *IR (film) spectrum of* ***13***

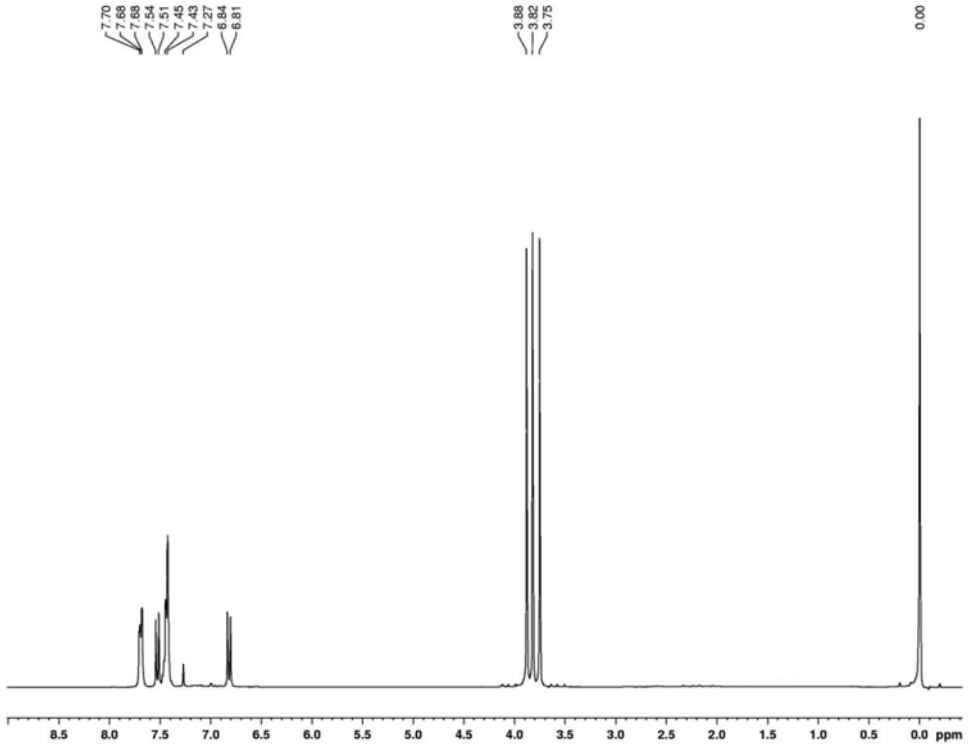

Figure 17S. *[1]H NMR spectrum of* ***13****, 300 MHz, $CDCl_3$*

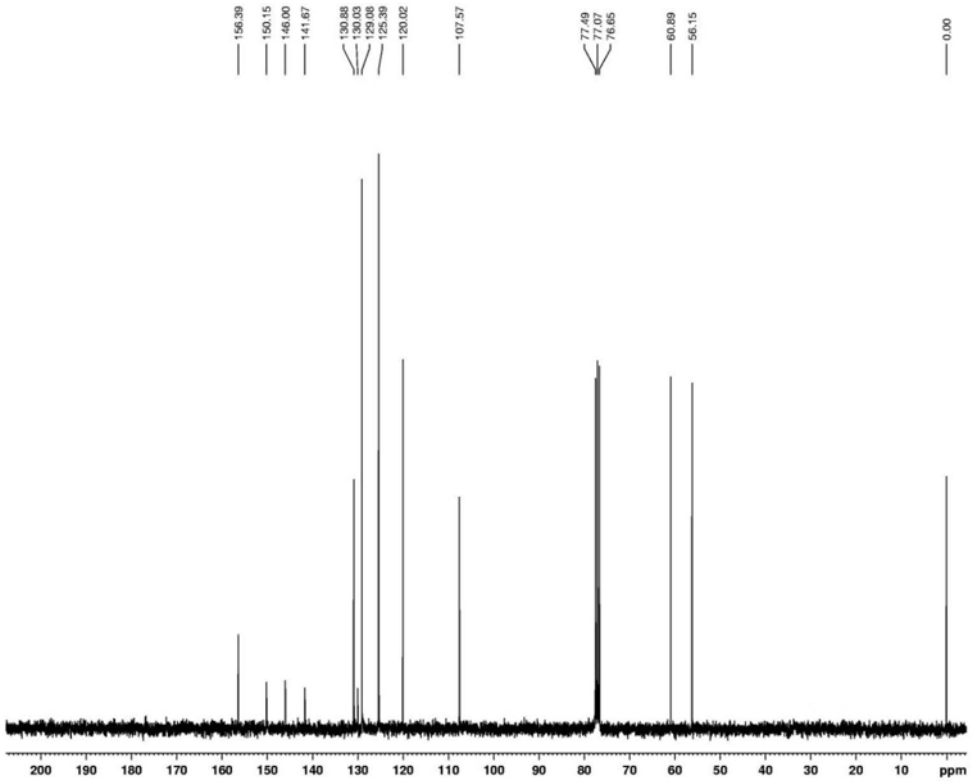

Figure 18S. *[13]C NMR spectrum of* ***13****, 75 MHz, $CDCl_3$*

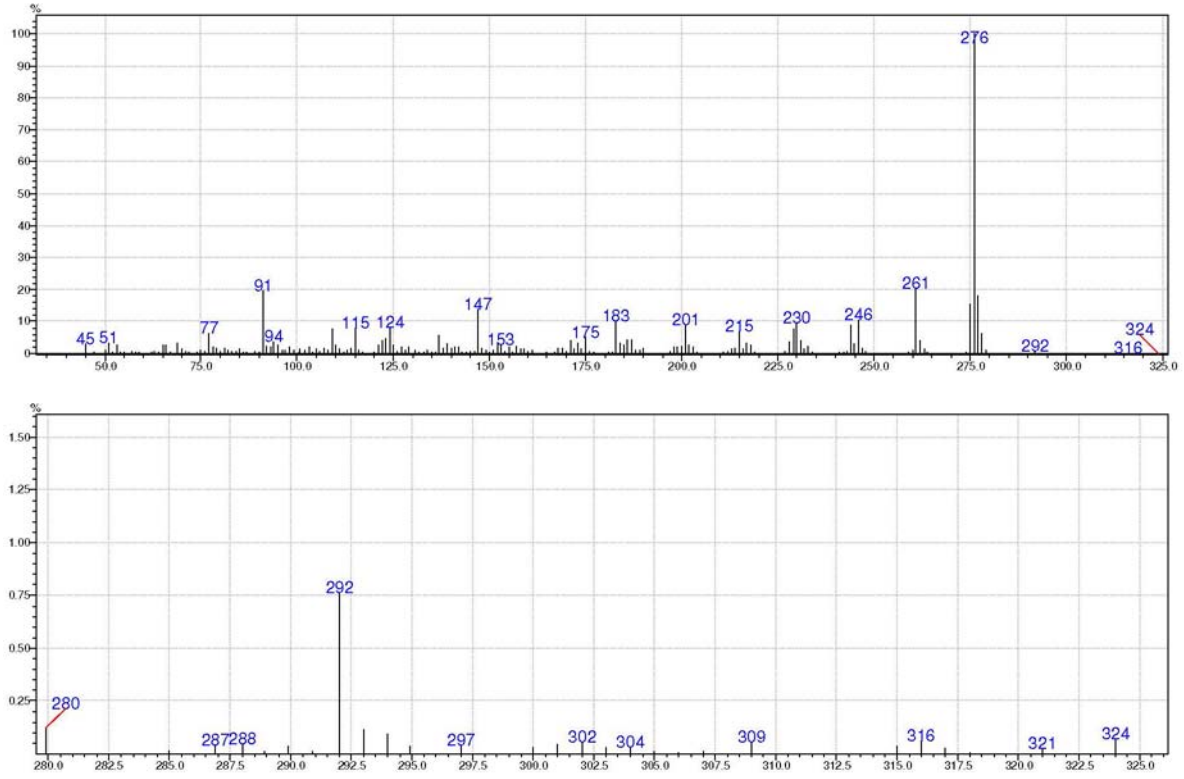

Figure 19S. *EI-MS spectrum of* ***13***

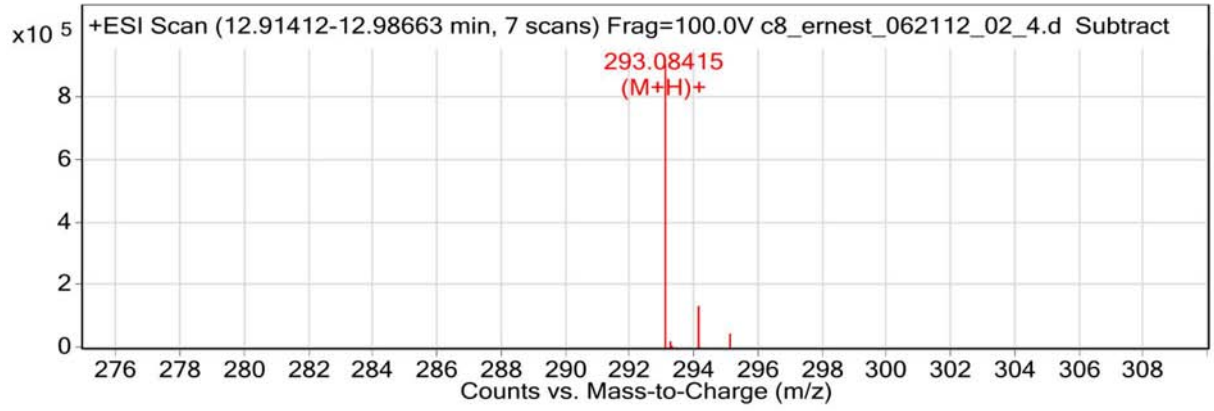

Figure 20S. *HRMS (ESI-MS) spectrum of* ***13***

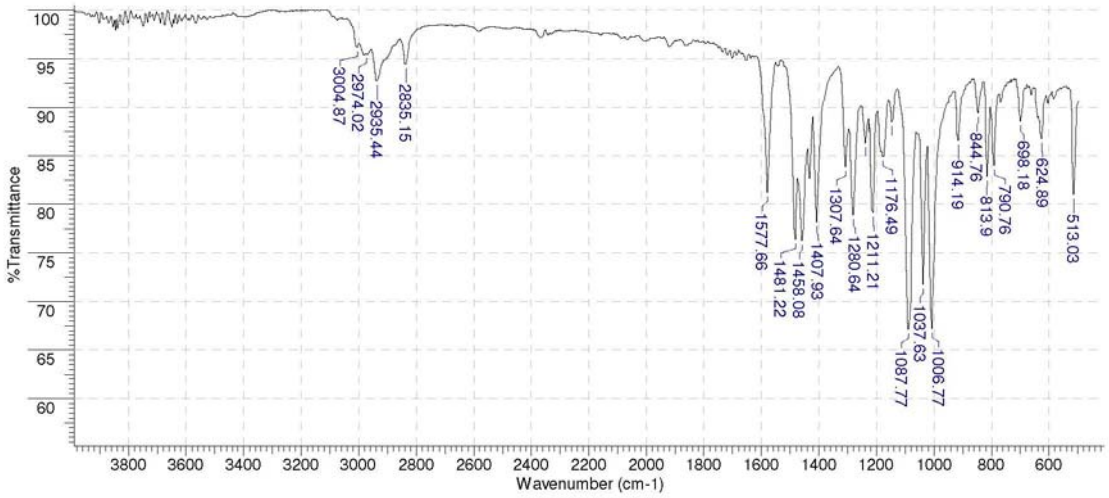

Figure 21S. *IR (KBr) spectrum of* ***14***

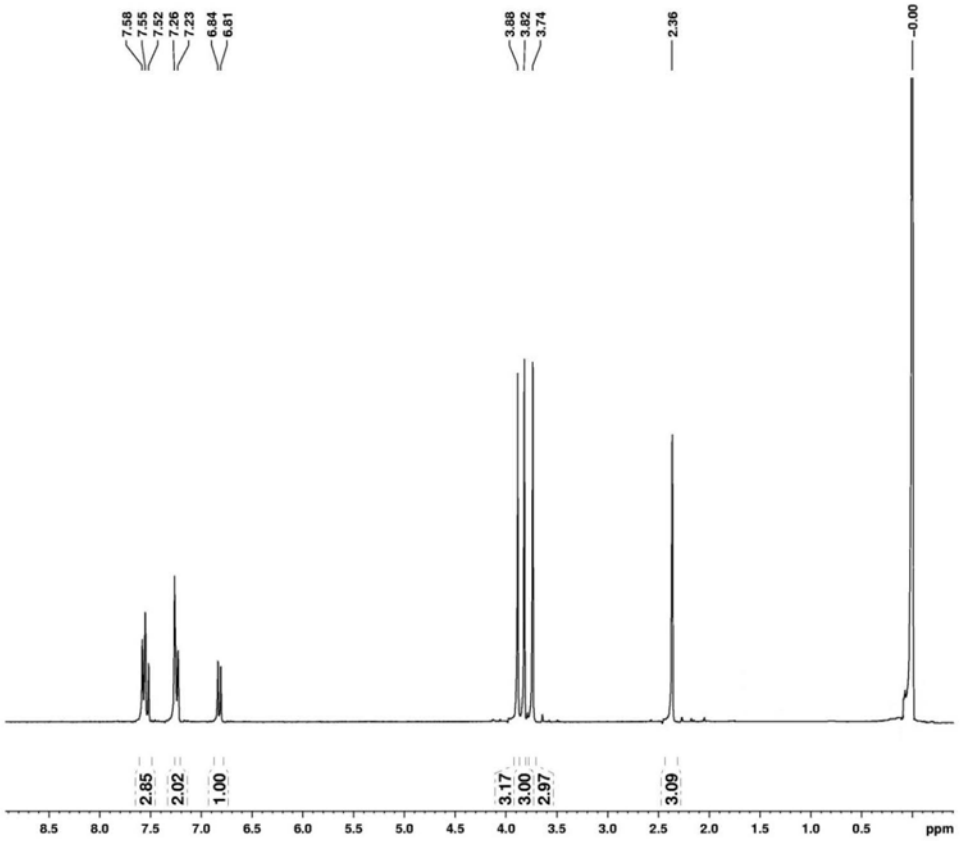

Figure 22S. *^{1}H NMR spectrum of* ***14****, 300 MHz, $CDCl_3$*

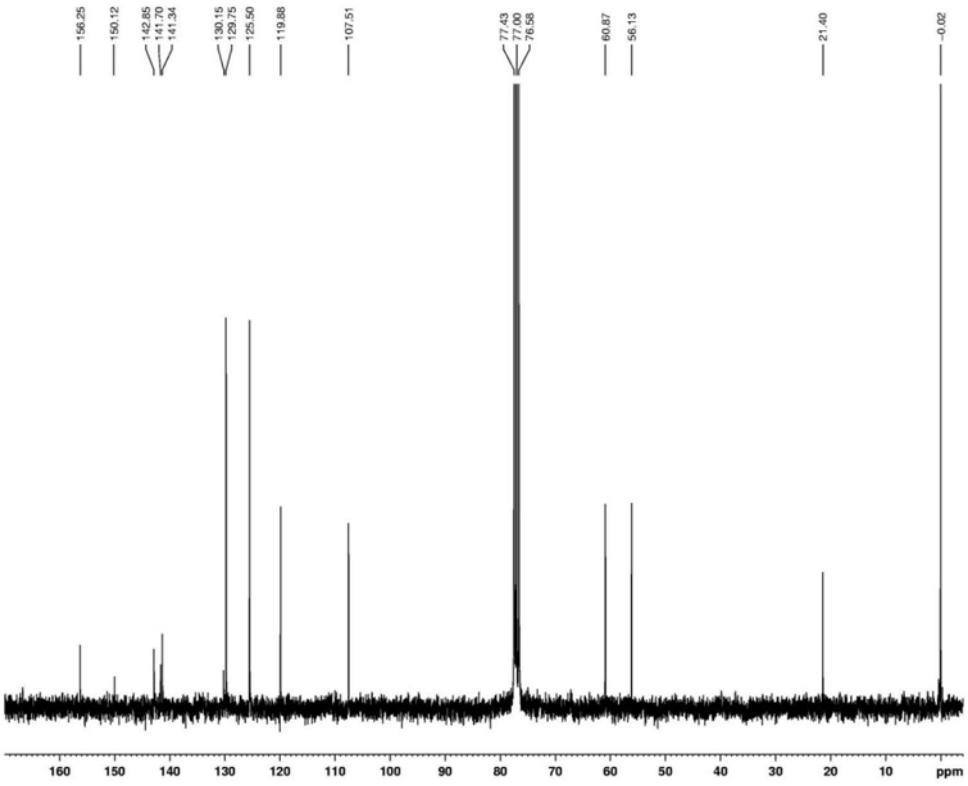

Figure 23S. *^{13}C NMR spectrum of **14**, 75 MHz, $CDCl_3$*

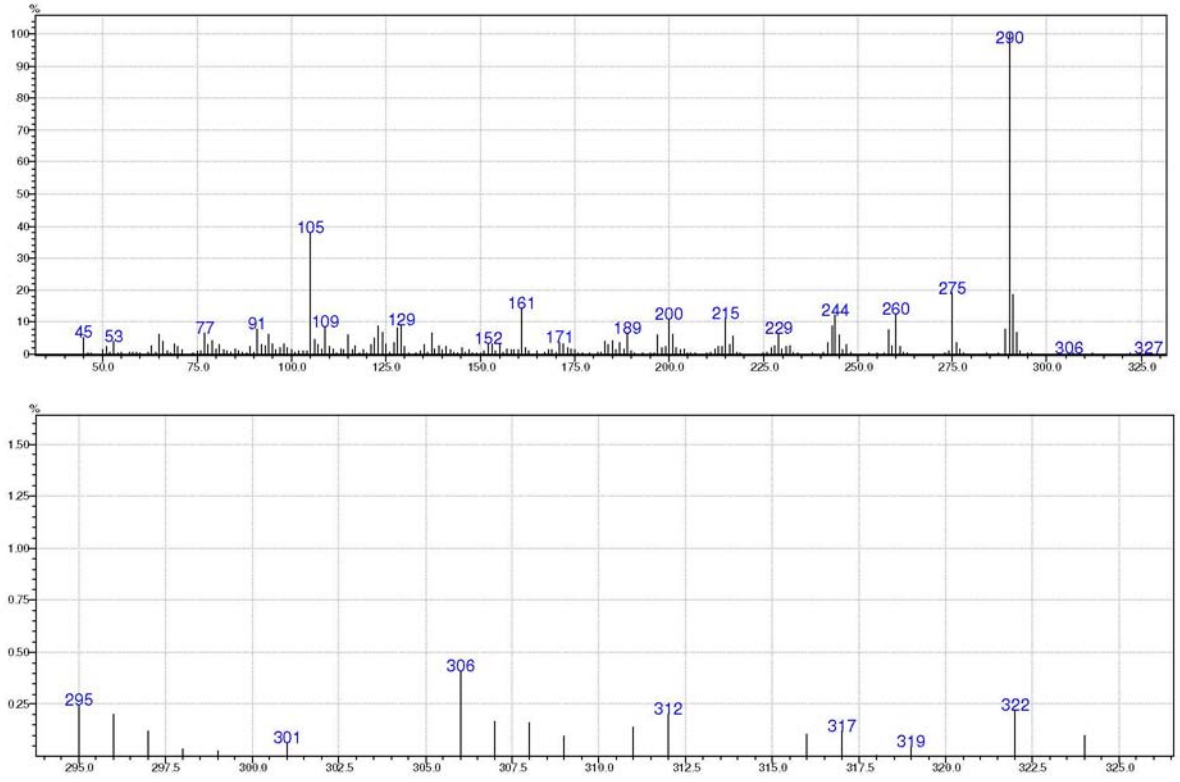

Figure 24S. *EI-MS spectrum of **14***

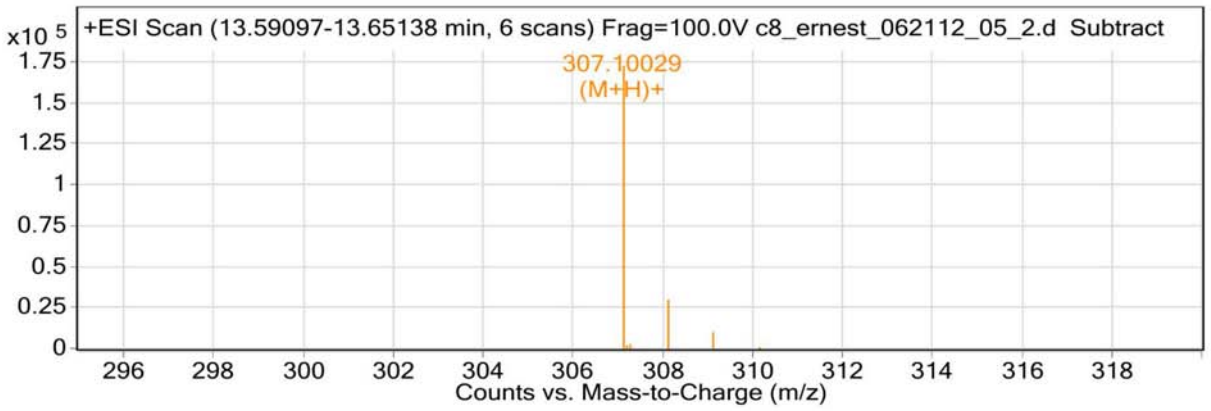

Figure 25S. *HRMS (ESI-MS) spectrum of* ***14***

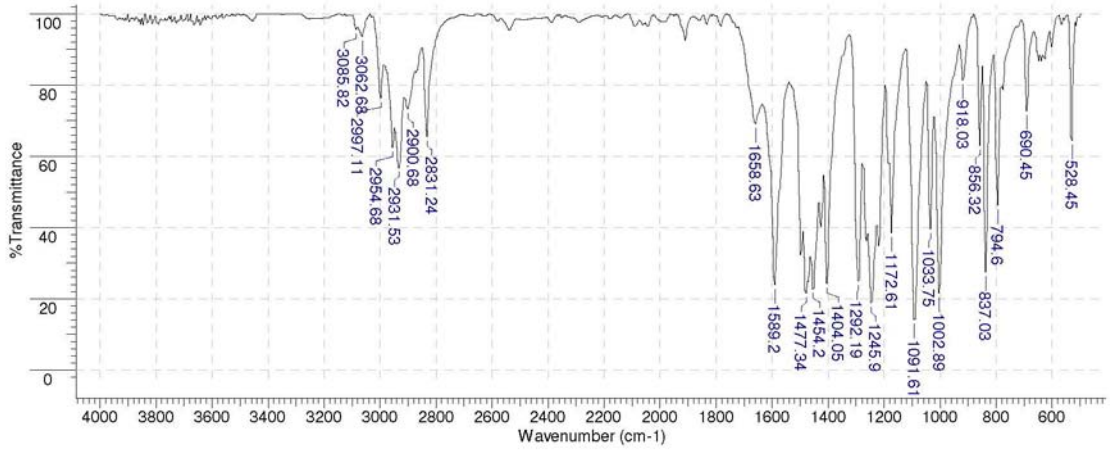

Figure 26S. *IR (KBr) spectrum of* ***15***

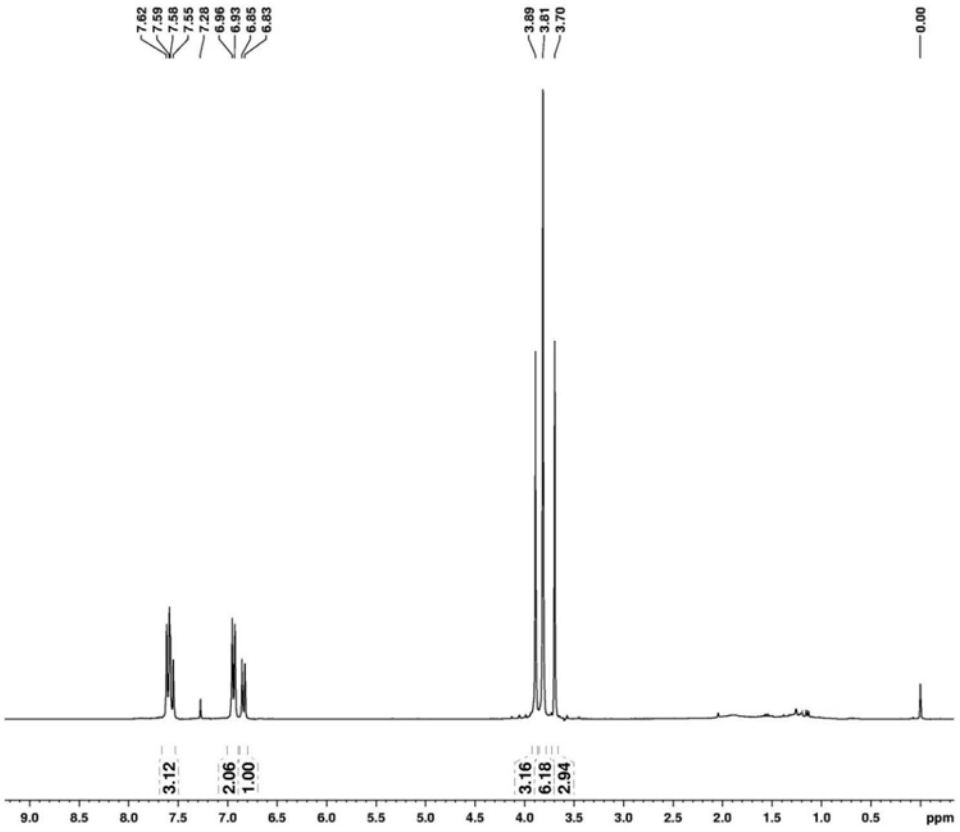

Figure 27S. *^{1}H NMR spectrum of **15**, 300 MHz, $CDCl_3$*

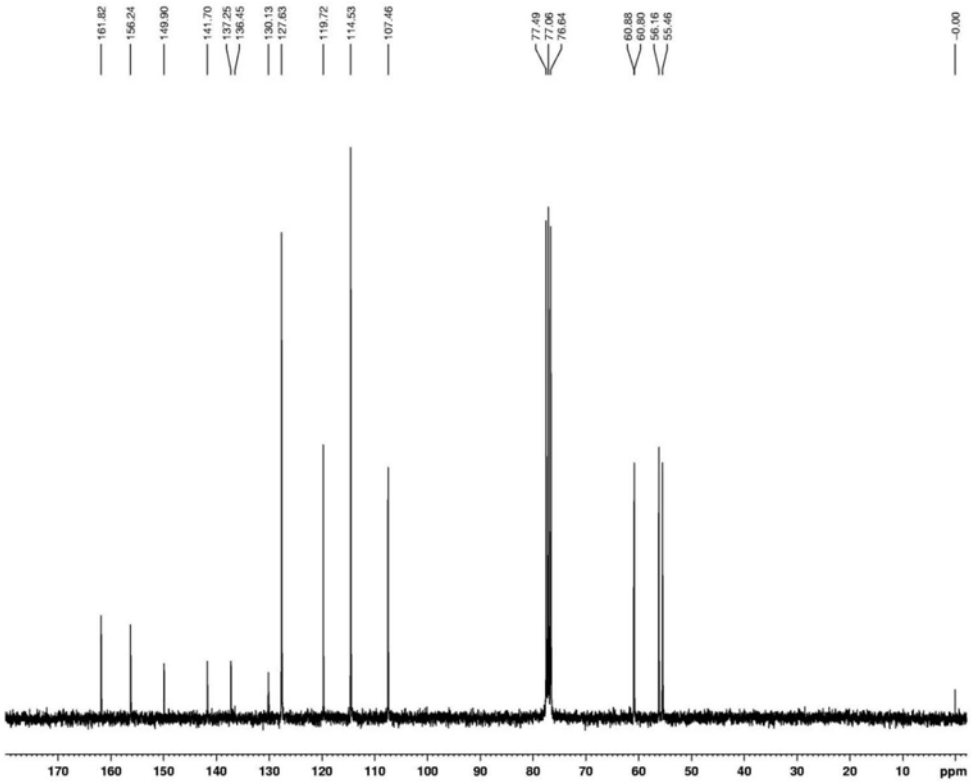

Figure 28S. *^{13}C NMR spectrum of **15**, 75 MHz, $CDCl_3$*

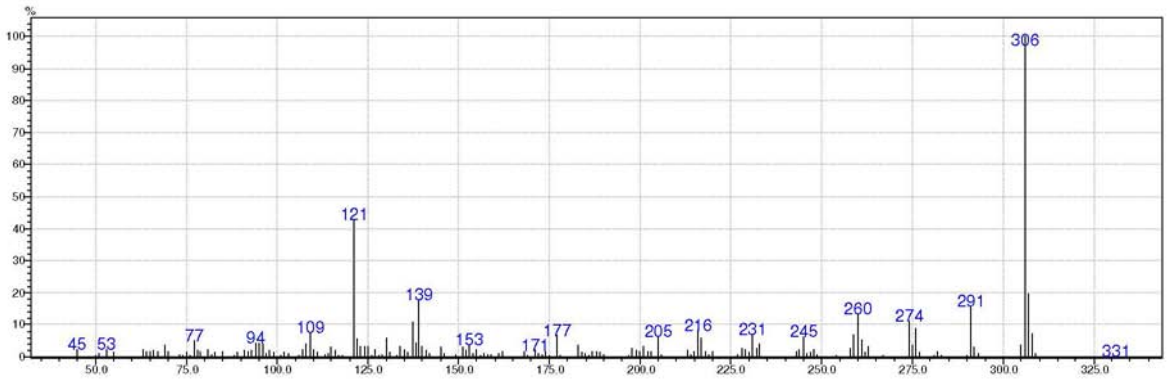

Figure 29S. *EI-MS spectrum of* ***15***

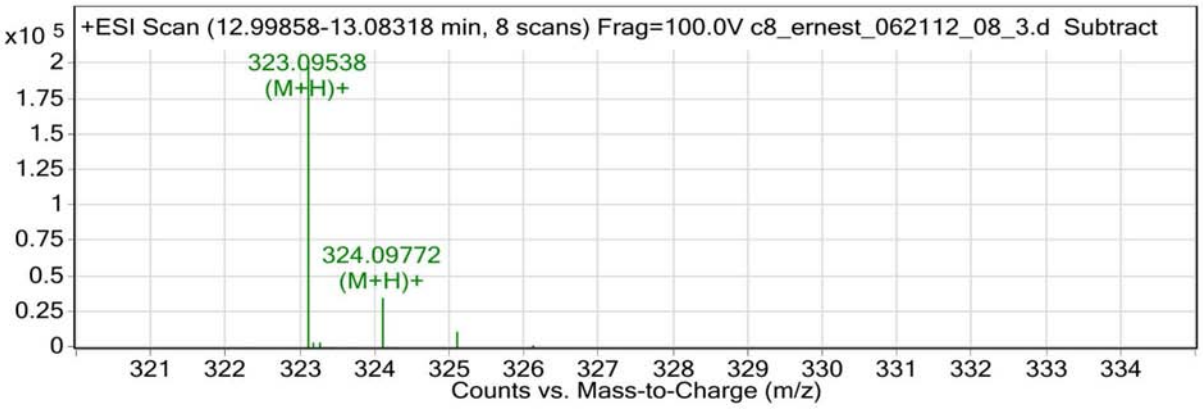

Figure 30S. *HRMS (ESI-MS) spectrum of* ***15***

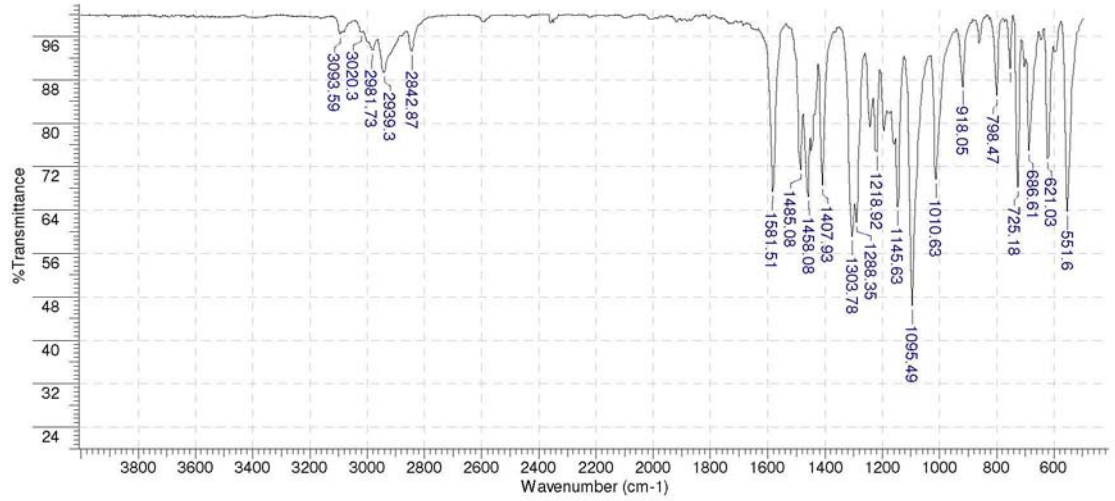

Figure 31S. *IR (KBr) spectrum of* ***16***

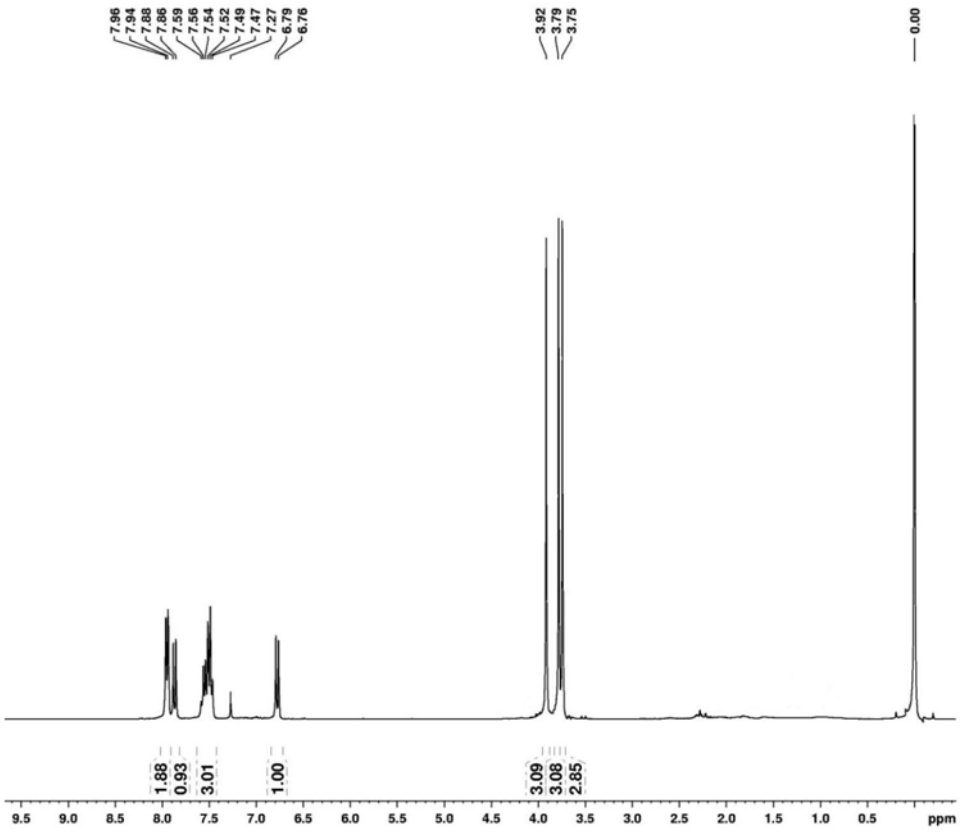

Figure 32S. *^{1}H NMR spectrum of **16**, 300 MHz, $CDCl_3$*

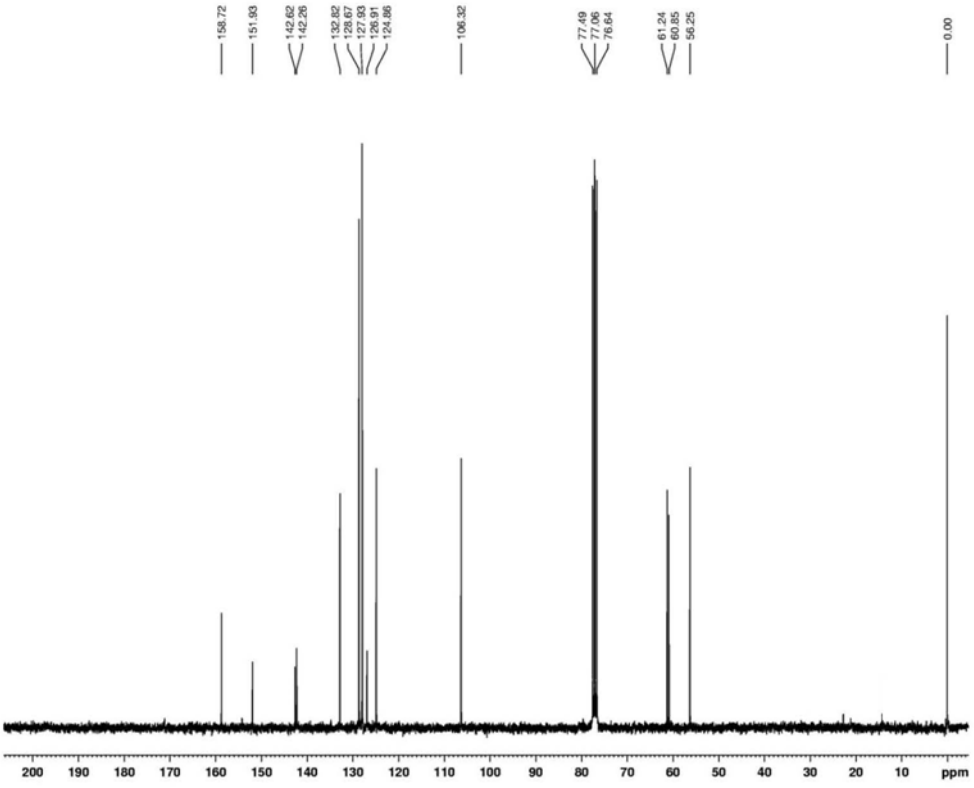

Figure 33S. *^{13}C NMR spectrum of **16**, 75 MHz, $CDCl_3$*

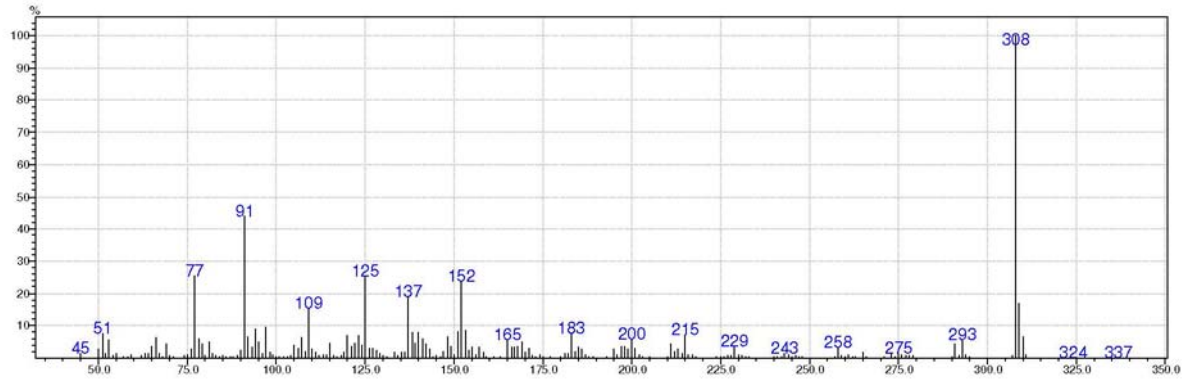

Figure 34S. *EI-MS spectrum of* ***16***

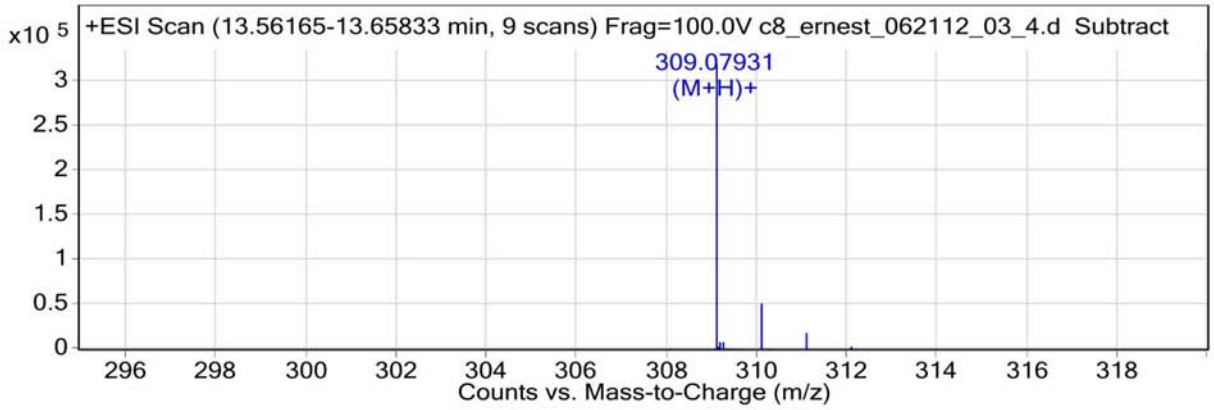

Figure 35S. *HRMS (ESI-MS) spectrum of* ***16***

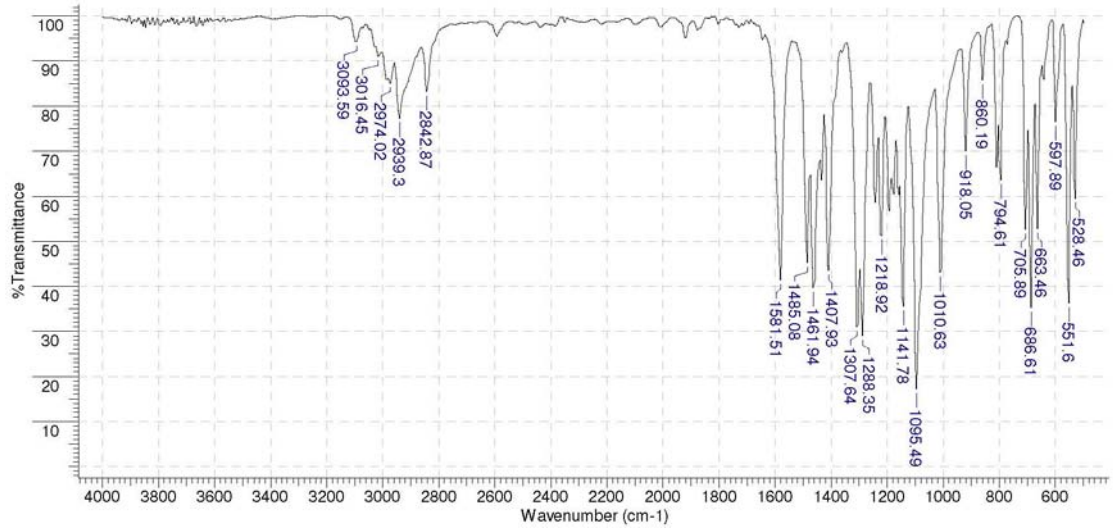

Figure 36S. *IR (KBr) spectrum of* ***17***

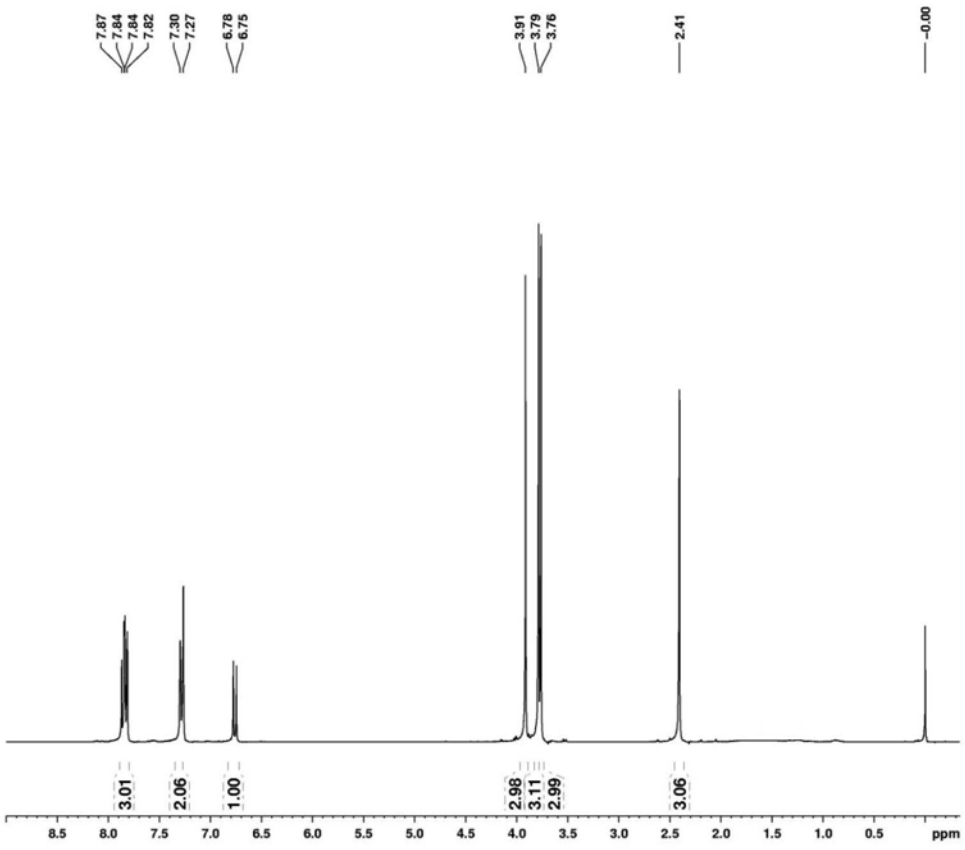

Figure 37S. *^{1}H NMR spectrum of **17**, 300 MHz, $CDCl_3$*

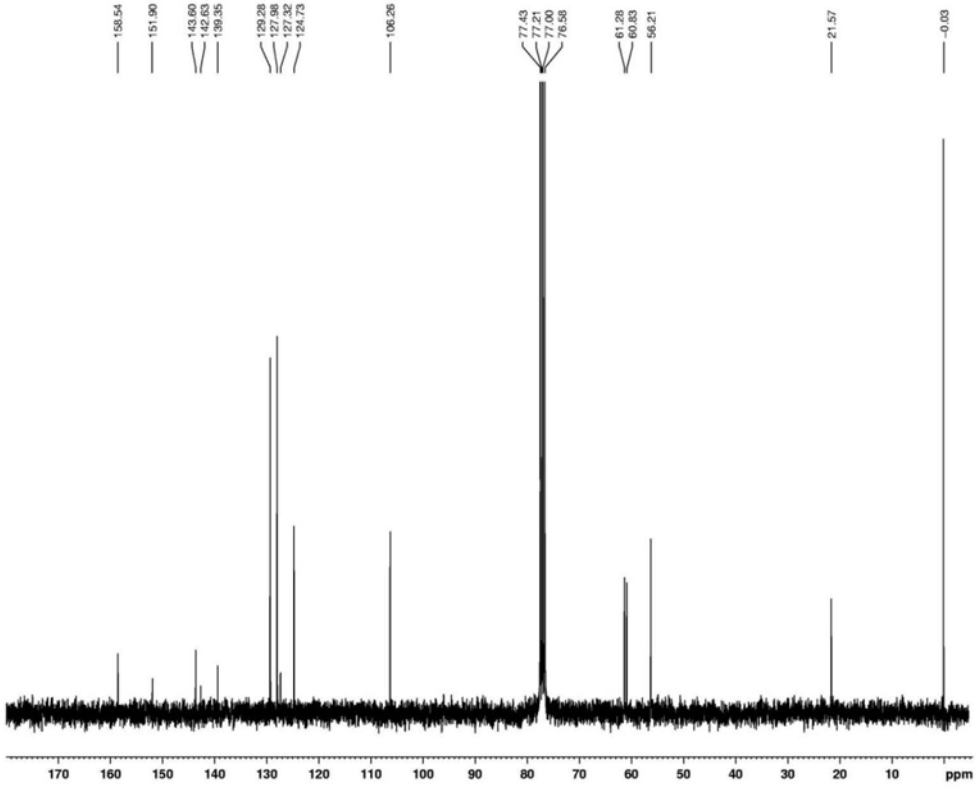

Figure 38S. *^{13}C NMR spectrum of **17**, 75 MHz, $CDCl_3$*

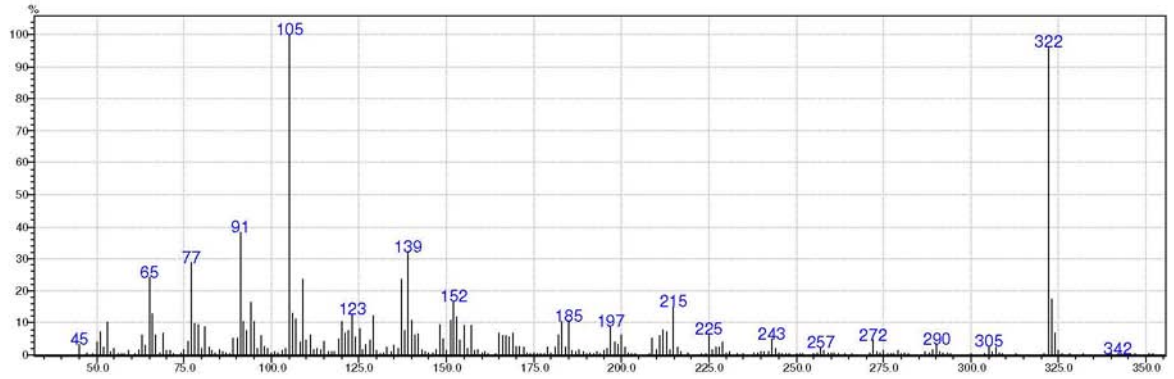

Figure 39S. *EI-MS spectrum of* ***17***

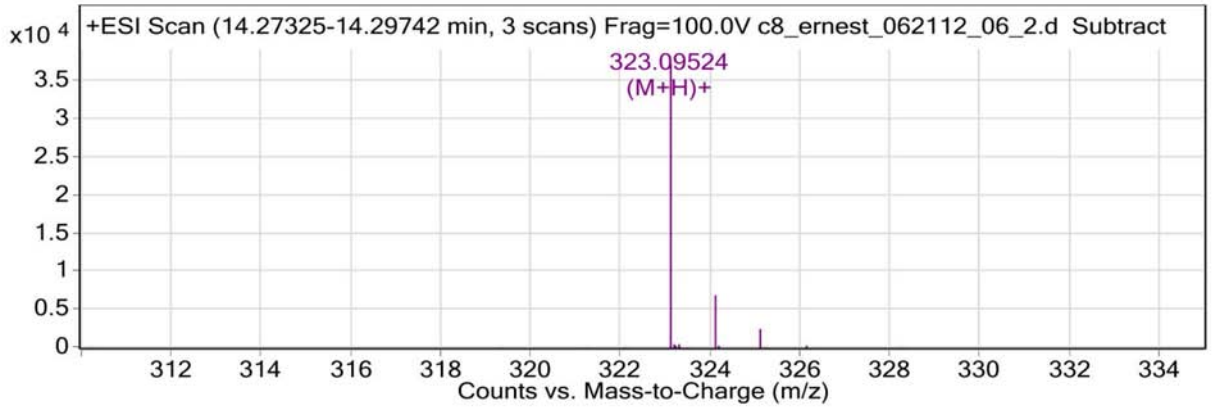

Figure 40S. *HRMS (ESI-MS) spectrum of* ***17***

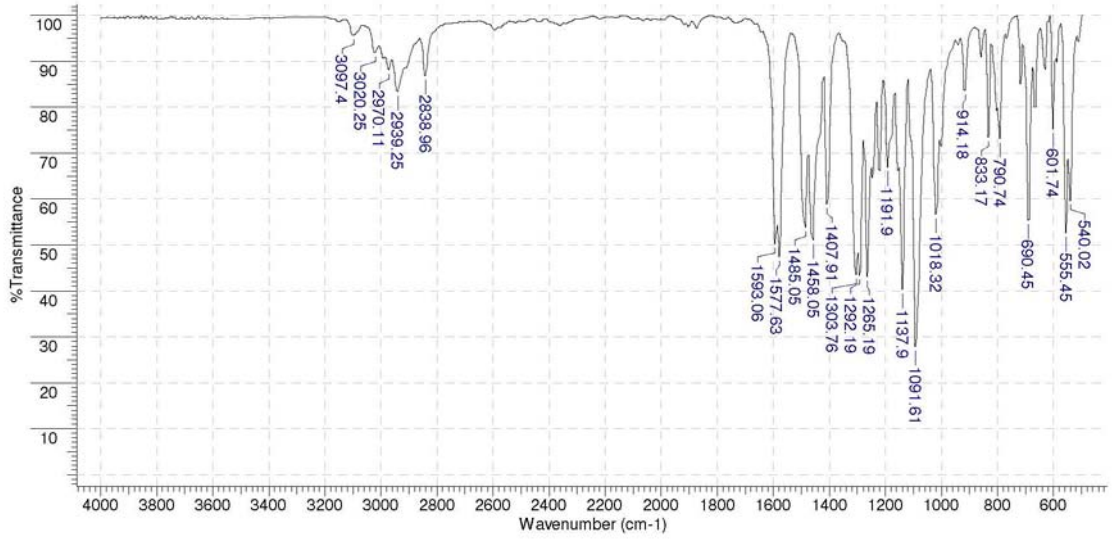

Figure 41S. *IR (KBr) spectrum of* ***18***

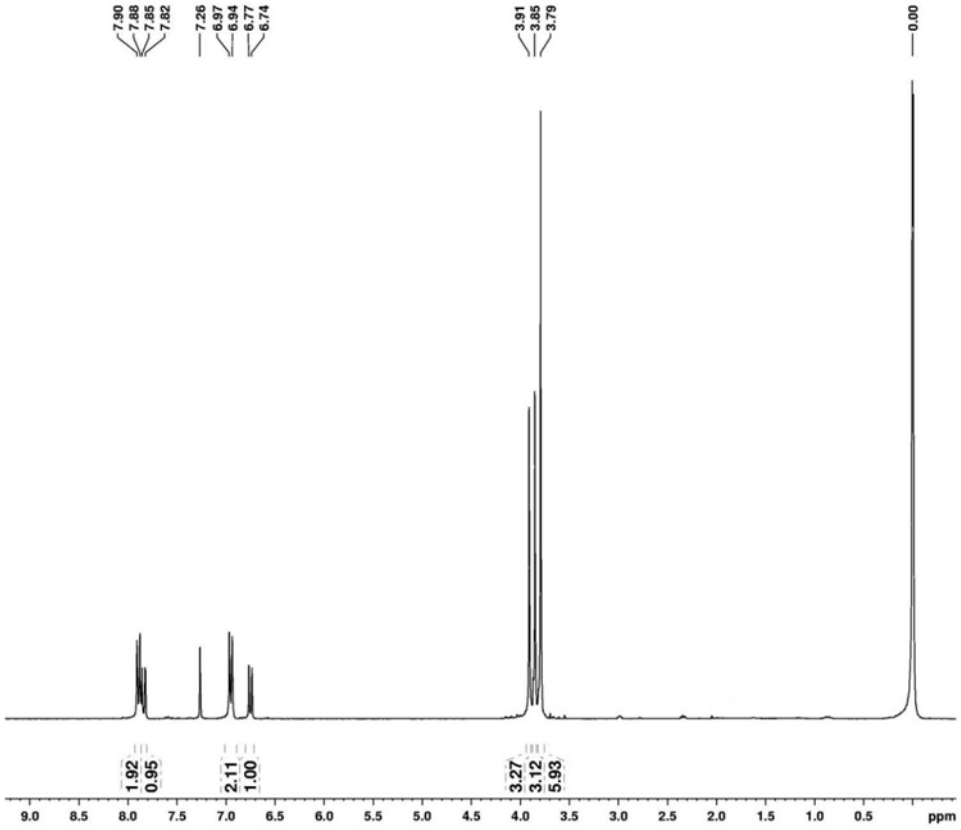

Figure 42S. *^{1}H NMR spectrum of **18**, 300 MHz, $CDCl_3$*

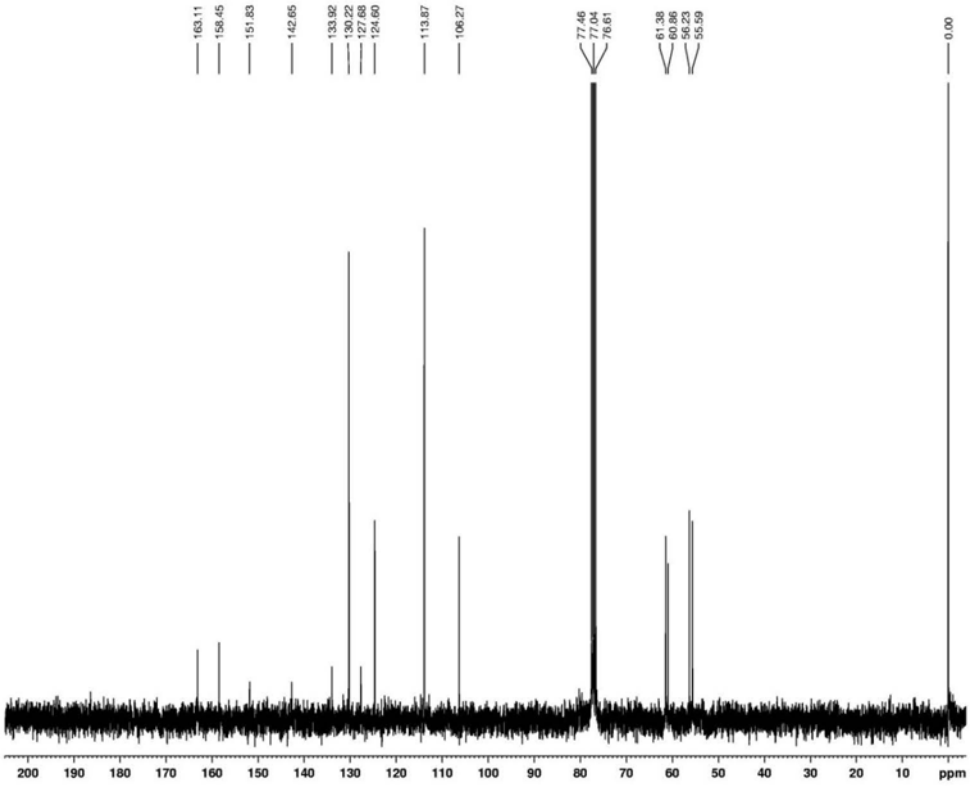

Figure 43S. *^{13}C NMR spectrum of **18**, 75 MHz, $CDCl_3$*

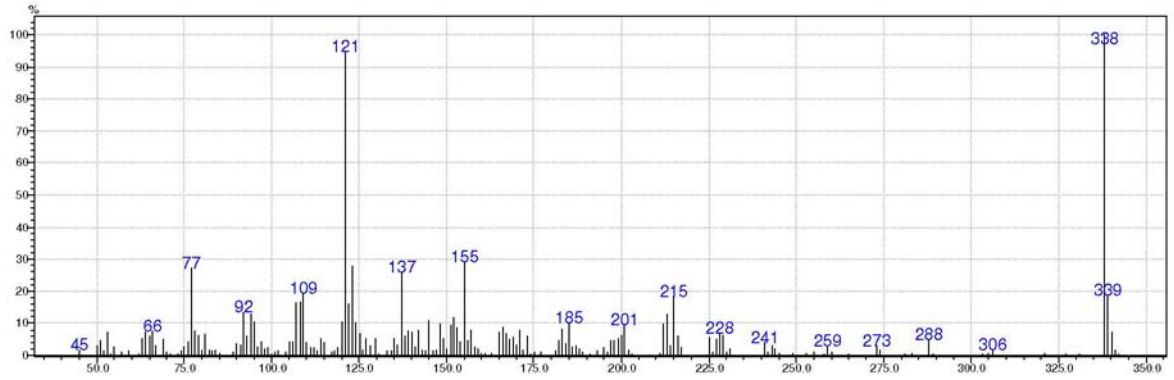

Figure 44S. *EI-MS spectrum of* ***18***

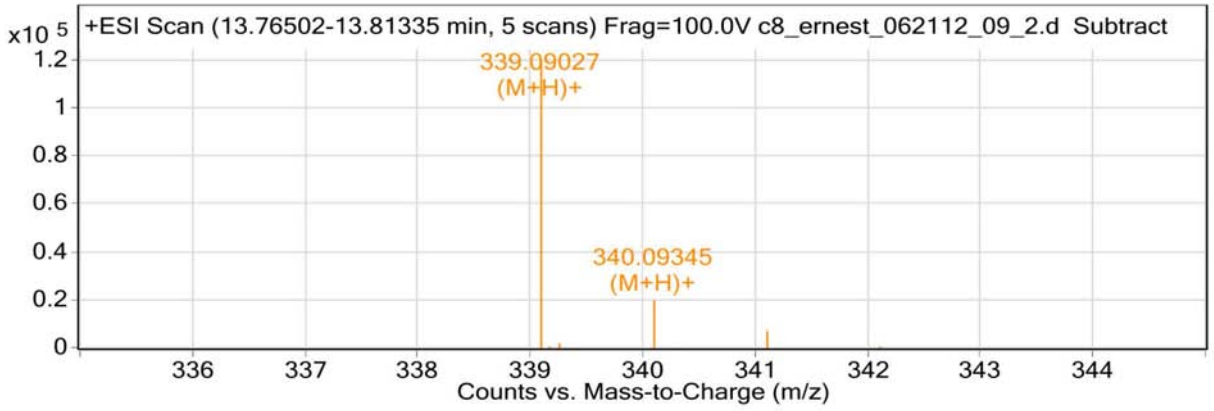

Figure 45S. *HRMS (ESI-MS) spectrum of* ***18***