Scientific research article / http://dx.doi.org/10.15446/rcciquifa.v51n2.98279

Biological activities of 1,4-naphthoquinones derivatives against *T. cruzi* and *L. amazonensis*

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Received: September 15, 2021
Corrected: November 10, 2021
Accepted: November 17, 2021

Summary

Introduction: Chagas disease and Leishmaniasis are neglected diseases caused by the *Trypanosoma cruzi* and kentoplastid parasites *Leishmania spp*. Parasitic diseases cause great impact on social and economic, affecting millions of people in the world and represent a major global health problem. In the search for new alternatives for the treatment of Leishmaniasis and Chagas disease, strategies have been used to discover new active molecules, because there is an urgent need for the development of new drugs. In this scenario, 1,4-naphthoquinones have shown notable activity in the context of neglected diseases. **Aim:** To synthesis of 1,4-naphthoquinones derivatives and evaluated these compounds against *Trypanosoma cruzi* epimastigotes, *Leishmania* promastigotes (*Leishmania amazonensis*) and cytotoxicity to $LLCMK_2$ cells. **Results:** Nine 1,4-naphthoquinones derivatives were synthesized using 2-Bromo-1,4-naphthoquinone (**1**), 1,4-Naphthoquinone (**5**) and 2-Hydroxi-1,4-naphthoquinone (**9**) as starting material. Derivative **6a** exhibited excellent trypanocidal activity, IC_{50} of 0.25 ± 0.02 **μM**, superior potency compared with the reference drug Benznidazol. Besides, these compounds displayed low activity against promastigote from *L. amazonensis.* **Conclusion:** The results indicate that compound **6a** may have potential for agent against Chagas disease.

Keywords: Naphthoquinone, *Trypanosoma cruzi*, *Leishmania*, Chagas diseases.

Resumen

Actividades biológicas de derivados de 1,4-naftoquinonas contra *T. cruzi* y *L. amazonensis*

Introducción: la enfermedad de Chagas y la leishmaniasis son enfermedades desatendidas causadas por los parásitos *Trypanosoma cruzi* y kentoplastid *Leishmania spp*. Las enfermedades parasitarias tienen un gran impacto social y económico, afectan a millones de personas en el mundo y representan un importante problema de salud mundial. En la búsqueda de nuevas alternativas para el tratamiento de la leishmaniasis y la enfermedad de Chagas, se han utilizado estrategias para descubrir nuevas moléculas activas, porque existe una necesidad urgente de desarrollo de nuevos fármacos. En este escenario, las 1,4-naftoquinonas han mostrado una notable actividad en el contexto de enfermedades desatendidas. **Objetivo**: sintetizar derivados de 1,4-naftoquinonas y evaluación de estos compuestos frente a epimastigotes de *Trypanosoma cruzi*, promastigotes de *Leishmania* (*Leishmania amazonensis*) y citotoxicidad a células $LLCMK_2$. **Resultados:** se sintetizaron nueve derivados de 1,4-naftoquinonas usando 2-bromo-1,4-naftoquinona (**1**), 1,4-naftoquinona (**5**) y 2-hidroxi-1,4-naftoquinona (**9**) como material de partida. El derivado **6a** exhibió una excelente actividad tripanocida, CI_{50} de 0,25 ± 0,02 μM, potencia superior en comparación con el fármaco de referencia Benznidazol. Además, estos compuestos mostraron una baja actividad contra el promastigote de *L. amazonensis*. **Conclusión:** los resultados indican que el compuesto **6a** puede tener potencial como agente contra la enfermedad de Chagas.

Palabras clave: Naftoquinona, *Trypanosoma cruzi*, *Leishmania*, enfermedad de Chagas.

Resumo

Atividades biológicas de derivados de 1,4-naftoquinonas contra *T. cruzi* e *L. amazonensis*

Introdução: a doença de Chagas e a leishmaniose são doenças negligenciadas causadas pelos parasitas *Trypanosoma cruzi* e kentoplastídeos *Leishmania spp*. As doenças parasitárias causam grande impacto social e econômico, afetando milhões de pessoas no mundo e representam um dos maiores problemas de saúde global. Na busca por novas alternativas para o tratamento da Leishmaniose e da doença de Chagas, estratégias têm sido utilizadas para descobrir novas moléculas ativas, porque

há urgência no desenvolvimento de novos fármacos. Nesse cenário, as 1,4-naftoquinonas têm mostrado notável atividade no contexto das doenças negligenciadas. **Objetivos:** sintetizar derivados de 1,4-naftoquinonas e avaliar esses compostos contra epimastigotas de *Trypanosoma cruzi*, promastigotas de *Leishmania* (*Leishmania amazonensis*) e citotoxicidade para células $LLCMK_2$. **Resultados:** nove derivados de 1,4-naftoquinonas foram sintetizados usando 2-Bromo-1,4-naftoquinona (**1**), 1,4-Naftoquinona (**5**) e 2-Hidroxi-1,4-naftoquinona (**9**) como material de partida. O derivado **6a** exibiu excelente atividade tripanocida, IC_{50} de 0,25 ± 0,02 μM, potência superior em comparação com o medicamento de referência Benzonidazol. Além disso, esses compostos apresentaram baixa atividade contra a forma promastigota de *L. amazonensis*. **Conclusão:** os resultados indicam que o composto **6a** pode ter potencial para agente contra a doença de Chagas.

Palavras-chave: Naftoquinona, *Trypanosoma cruzi*, *Leishmania*, doença de Chagas.

Introduction

Parasitic diseases are a heterogeneous group of infections which may be caused by protozoa, helminths or ectoparasites [1]. International travel and shifting patterns of immigration have increased the importance of awareness of the major clinical syndromes associated with infections due to parasites [2]. Protozoan parasitic infections transmitted by insect vector pose a significant global health problem [3], such Chagas diseases and Leishmaniasis.

Chagas disease, a vector-borne parasitosis caused by *Trypanosoma cruzi* [4], the great number of people infected with *T. cruzi* and the millions in risk of being infected together with the low efficacy of the actual treatments make of this disease one of the major health problems in Latin America [5]. Chagas disease has two successive phases: acute and chronic. Acute *T. cruzi* infection leads to focal myocarditis with accompanying necrosis of infected myocytes and reparative interstitial fibrosis. During the chronic disease, parasitaemia drastically decreases and parasites are barely detected [6]. Only two drugs, the 2-nitroimidazole benznidazole and the 5-nitrofuran nifurtimox, are licensed for the treatment of Chagas disease, although their safety and efficacy profile are far from ideal [7].

Leishmaniasis is a disease caused by intracellular protozoan parasites of the genus *Leishmania* [8]. The three main clinical manifestations of this illness are cutaneous, mucocutaneous and visceral [9]. The control of Leishmaniasis has been based on che-

motherapy with pentavalent antimonials for more than 70 years. Meglumine antimoniate is a first-line drug, but use of this therapeutic is limited by its high cost and toxicity [10].

The impact of natural products on drug discovery is considerable, not only for cancer but also for parasitic infections [11]. Naphthoquinones constitute a structurally diverse class of phenolic compounds with wide applications in the fields of pharmacy and medicine [12] and are of key importance in organic synthesis and medicinal chemistry. In the last few years, various synthetic routes have been developed to prepare bioactive compounds derived [13]. The biological activity of quinones has been related to their redox properties and their capacity to accept one or two electrons to form the corresponding radical-anion ($Q^{\cdot-}$) and hydroquinone radical dianion (Q^{2-}). These intermediate species interact with crucial cellular molecules such as oxygen, DNA and proteins modifying their biological activity [14]. Beyond these important features, the *T. cruzi* mitochondrion is known to be deficient in reactive oxygen and nitrogen species detoxification, being especially sensitive to oxidative stress conditions [15].

The biological activity of 1,4-naphthoquinones derivatives have been reported to exhibit a variety of pharmacological properties involving antimicrobial [16], anticancer [17], antioxidant [18], trypanocidal [19], leishmanicidal [20], anti-inflammatory [21], herbicidal [22], antiplasmodial [23], fungicidal [24].

Considering a variety of resistance mechanisms to antimonials in *Leishmania* [9], a limited efficacy of agents therapeutics in the treatment of Chagas disease, and investigation by different research groups to discover new molecules that can be used such strategies for Chagas disease and leishmaniasis chemotherapy. Our lab has been interested in the synthesis and pharmacological properties of 1,4-naphthoquinone derivatives. Thus, we synthesized nine compounds to evaluate their leishmanicidal, trypanocidal activity and cytotoxic properties.

Materials and methods

Chemistry

The carboxylic acids **2a-d** and 7 were purchased from Sigma-Aldrich and used without further purification. The reagents **4** and **8** were obtained from cardanol as described in the literature [25, 35]. Purifications of compounds were made by column chromatography (CC) using Merck silica gel 60 (230 e 400 mesh) and a mixture of hexane and ethyl acetate was used for elution. All the reactions were monitored by TLC using Merck silica gel 60. ^{1}H (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded

with a Bruker DPX-300 spectrometer using $CDCl^3$, or DMSO-d^6 as the solvent with TMS as an internal standard. Chemical shifts are reported in ppm and coupling constants (J) in Hertz. Mass spectra (EI, 70 eV) were run on a Shimadzu CGMS QP2010 Plus gas chromatography mass spectrometer. The main fragments were described as a relation between atomic mass units and the charge (m/z) and the relative abundance in percentage of the base-peak intensity. Infrared spectra were recorded on Perkin-Elmer model 783 in a KBr cell for liquid (film) or KBr pellets for solids and the absorption wave numbers expressed in cm^{-1}. HRMS was acquired using a UFLC Shimadzu LC-20AD apparatus, with and IES-Q-QTOF-microTOF III detector (Bruker Daltonics) in chemical ionization positive ion mode (*m/z* 120-1200). Melting points were determined by MQAPF-301 model equipment.

General procedure

The general procedure for the synthesis of 1,4-naphthquinone derivatives (**3a-3d**) was as described in literature [26, 32]. A solution of 1 g $(NH^4)_2S_2O_8$ in H_2O (10 mL) was added dropwise over 90-120 min to a stirred suspension of H_2O (100 mL), CH_3CN (20 mL), $AgNO_3$ (0.25 g), 2-Bromo-[1,4]naphthoquinone (0,2 g) and 1,5 mmol carboxylic acid (Palmitic, linoleic, 3-(4-Methoxy-phenyl)-propionic, oleic) at 65-75 °C. The resulting mixture was stirred for another 30 min. Then the material was brought to room temperature, washed with $NaHCO_3$ and dried with $MgSO_4$, was filtered, concentrated under reduced pressure and purified by flash chromatography on silica gel with hexane/ethyl acetate.

2-Bromo-3-pentadecyl-[1,4]naphthoquinone (3a)

This compound was prepared according to general procedure. Yield: 66 % (210 mg); Yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.85 (t, *J* = 9.0 Hz, 3H, CH_3), 1.00-1.50 (m, 24H, CH_2), 1.54 (m, 2H, CH_2), 2.81 (t, *J* = 9.0 Hz, 2H, CH_2), 7.08-7.73 (m, 2H, CH), 8.06-8.15 (m, 2H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.0 (CH_3), 22.6 (CH_2), 27.7 (CH_2), 31.6 (CH_2), 29.0-30.0 (CH_2), 31.9 (CH_2), 127.0 (CH), 127.4 (CH), 131.2 (C), 131.6 (C), 133.8 (CH), 134.0 (CH), 138.6 (C), 152.2 (C), 177.7 (C=O), 181.7 (C=O). MS (EI^+): m/z (%) 448,30 [$M^{+\bullet}$+2] (56,91); 447,30 [$M^{+\bullet}$+1] (55,97); 446,30 [$M^{+\bullet}$] (16,05); 253,05 (79,96); 252,00 (77,94); 251,05 (82,13); 173,10 (100,00). HRMS (ESI): m/z = not ionizable $[M+H]^+$.

2-Bromo-3-heptadecyl-[1,4]naphthoquinone (3b)

Compound was prepared according to general procedure. Yield: 47 % (187 mg), yellow solid, mp = 58-59 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.85 (t, *J* = 6.0 Hz, 3H, CH_3); 1.00-1.70 (m, 30H, CH_2); 2.81 (t, *J* = 9.0 Hz, 2H, CH_2); 7.68-7.76 (m, 2H, CH); δ 7.68-7.76 (m, 2H, CH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14,0 (CH_3); 22,6 (CH^2); 27,7

(CH_2); 28,0-30,0 (CH_2); 31,6 (CH_2); 31,9 (CH_2); 127,0 (CH); 127,4 (CH); 131,2 (C); 131,6 (C); 133,8 (CH); 134,0 (CH); 138,6 (C); 152,2 (C); 177,7 (C=O); 181,7 (C=O). MS (EI^+): m/z (%): 474 [$M^{+\bullet}$] (25); 476 [$M^{+\bullet}$+2] (26); 475 [$M^{+\bullet}$+1] (7); 448 (42); 447 (12); 446 (41); 253 (80); 252 (75); 251 (82); 173 (100). HRMS (ESI) m/z: not ionizable $[M+H]^+$.

2-Bromo-3-[2-(4-methoxy-phenyl)-ethyl]-[1,4]naphthoquinone (3c)

Compound was prepared according to general procedure. Yield: 10 % (20 mg), yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.78 (t, J = 9.0 Hz, 2H, CH_2); 3.08 (t, J = 9.0 Hz, 2H, CH_2); 3.77 (s, 3H, OCH_3); 6.82 (d, J = 9.0 Hz, 2H, CH); 7.19 (d, J = 9.0 Hz, 2H, CH); 7.71-7.76 (m, 2H, CH); 8.08-8.16 (m, 2H CH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 32.8 (CH_2), 34.1 (CH_2), 56.2 (OCH_3), 113.9 (CH), 127.0 (CH), 127.5 (CH), 129.4 (CH), 131.1 (C), 131.5 (C), 132.7 (C), 133.8 (CH), 134.1 (CH), 139.1 (C), 150.9 (C), 158.9 (C), 177.7 (C=O), 181.5 (C=O). MS (EI^+): m/z (%): 370 [$M^{+\bullet}$] (2); 371 [$M^{+\bullet}$+1] (0,5); 372 [$M^{+\bullet}$+2] (1); 135 (19); 121 (100); 77 (13). HRMS (ESI) m/z: not ionizable $[M+H]^+$.

2-Bromo-3-heptadecyl-8(Z)-enyl-[1,4]naphthoquinone (3d)

Compound was prepared according to general procedure. Yield: 10 % (40 mg), yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.85 (t, J = 9.0 Hz, 3H, CH_3), 1.10-1.50 (m, 20H, CH_2), 1.54 (qt, J = 9.0 Hz, 2H, CH_2), 1.90-2.10 (m, 4H, CH_2), 2.81 (t, J = 9.0 Hz, 2H, CH_2), 5.27-5.40 (m, 2H, CH), 7.70-7.73 (m, 2H, CH), 8.08-8.15 (m, 2H, CH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14,0 (CH_3); 22,6 (CH_2); 27,1 (CH_2); 27,2 (CH_2); 27,7 (CH_2); 28,0-30,0 (CH_2); 31,6 (CH_2); 31,8 (CH_2); 127,0 (CH); 127,4 (CH); 129,7 (CH); 130,0 (CH); 131,2 (C); 131,6 (C); 133,8 (CH); 134,0 (CH); 138,6 (C); 152,2 (C); 177,7 (C=O); 181,7 (C=O). MS (EI^+): m/z (%): 472 [$M^{+\bullet}$] (22); 473 [$M^{+\bullet}$+1] (8); 474 [$M^{+\bullet}$+2] (21); 253 (58); 252 (45); 251 (61); 55 (100). HRMS (ESI) m/z: not ionizable $[M+H]^+$.

N-(1,4-dioxo-1,4-dihydro-naphthalen-2-yl)-3,4,5-trimethoxy-benzamide (6a)

Compound was prepared as described previously in literature [27]. 2-amino-1,-4-naphthoquinone (6) (215 mg, 1.2 mmol) was dissolved in freshly distilled THF (15 mL). NaH (100 mg, 4.2 mmol) was added to the solution and the mixture was stirred at room temperature for 30 mins. 3,4,5-trimethoxy-benzoyl chloride (0.27 mL, 2.14 mmol) was added and the mixture was stirred for 5 mins. THF was removed under vacuum and the mixture was washed with ice-water (10 g ice and 10 mL water). The ice-water mixture was extracted with CH_2Cl_2 (30 mL, 20 mL consecutively). The combined organic phase was washed with water (3 × 20 mL), saturated NaCl solution (3 × 20 mL), and then dried over anhydrous $MgSO_4$. The crude material was puri-

fied via column chromatography. Yield: 20 % (89 mg), yellow solid, mp = 167-168 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.91 (s, 6H, CH_3), 3.88 (s, 3H, CH_3), 7.08 (s, 1H, CH), 7.69-7.76 (m, 2H, CH), 8.06-8.08 (m, 2H, CH), 9.05 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 56.4 (OCH_3), 60.9 (OCH_3), 104.7 (CH), 117.1 (CH), 126.4 (CH), 126.6 (CH), 128.4 (C), 129.9 (C), 132.2 (C), 133.3 (CH), 135.1 (CH), 140.0 (C), 142.2 (C), 153.4 (C), 165.4 (C), 181.2 (C=O), 185.1 (C=O). MS (EI^+): m/z (%): 77,10 (9,24); 152,10 (10,81); 167,15 (9,69); 195,10 (100,00); 367,20 [$M^{+\bullet}$] (27,60). HRMS (ESI) m/z calcd for $C^{20}H^{17}NO^6$ +H: 368,1134, Found: 368,1153 [$C_{20}H_{17}NO_6$ +H] $^+$.

3,4,5-trimethoxy-benzoic acid 1,4-dioxo-1,4-dihydro-naphthalen-2-yl ester (9a)

Compound was prepared as described previously in literature [28]. To a solution of 200 mg (1,1 mmol) of 2-Hydroxy-1,4-naphthoquinone (**9**) in CH_2Cl_2 (50 mL), 40 mL of aqueous NaOH solution (10 %) were added and the solution was stirred for 4 h at room temperature. Then 330 mg (1,4 mmol) of the 3,4,5-trimethoxy-benzoyl chloride (**8**) were added and stirring was maintained for 48 h. The reaction mixture was washed with water (3 x 50 mL) and the organic phase was dried over $MgSO_4$ and evaporated under reduced pressure. The crude material was purified via column chromatography. Yield: 94 % (380 mg), yellow solid, mp = 138-140 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.92 (s, 6H, OCH_3), 3.94 (s, 3H, OCH_3), 6.91 (s,1H, CH), 7.41 (s, 2H, CH), 7.76-7.79 (m, 2H, CH), 8.11-8.14 (m, 2H, CH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 56.3 (OCH_3), 61.0 (OCH_3), 107.9 (2CH), 122.4 (C), 126.1 (CH), 126.5 (CH), 127.0 (CH), 131.1 (C), 132.0 (C), 134.0 (CH), 134.4 (CH), 153.1 (3C), 154.7 (C), 163.3 (C), 178.6 (C), 184.5 (C). MS (EI^+): m/z (%): 368 [$M^{+\bullet}$] (7); 196 (11); 195 (100); 167 (7). HRMS (EI) m/z calcd for $C_{20}H_{16}0_7$: 368,0896 Found: 368,0895 [$C_{20}H_{16}0_7$]$^+$.

2-[7-(3-Methoxy-phenyl)-heptyl]-[1,4]naphthoquinones (5a)

Compound was prepared according to general procedure with 1,4-naphthoquinone 5 and carboxylic acid 8. Yield: 40 % (188 mg), yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.10-1.70 (m, 10H, CH_2), 2.55 (m, 4H, CH_2), 3.77 (s, 3H, CH_3), 6.69-6.73 (m, 3H, CH), 6.76 (s, 1H, CH), 7.16 (t, J = 6.0 Hz, 1H, CH), 7.67-7.72 (m, 2H, CH), 8.02-8.09 (m, 1H, CH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 27.9 (CH_2), 29.0-30.0 (CH_2), 31.2 (CH_2), 35.9 (CH_2), 55.1 (CH_3), 110.8 (CH), 114.1 (CH), 120.8 (CH), 126.0 (CH), 126.5 (CH), 129.1 (CH), 132.1 (C), 132.3 (C), 133.5 (CH), 133.5 (CH), 134.7 (CH), 144.4 (C), 151.9 (C), 159.5 (C), 185.2 (C). MS (EI^+): m/z (%): 362 [$M^{+\bullet}$] (52); 173 (65); 122 (100); 91 (45). HRMS (ESI) m/z calcd for $C_{24}H_{26}0_3$ + H: 363,1916, Found: 363,1906 [$C^{24}H^{26}0^3$ + H]$^+$.

2-(3-Pentadecyl-phenoxy)-[1,4]naphthoquinone (4a) and 2,3-Bis-(3-pentadecyl-phenoxy)-[1,4]naphthoquinone (4b)

The Compounds were prepared as described previously in literature [29, 30]. A stirred mixture of 3-Pentadecyl-phenol **4**, (128 mg, 0.4 mmol) and DMF (10 mL). After 1 min, K_2CO_3, (175 mg, 1.3 mmol) was added and the reaction mixture was stirred for 1 h at room temperature. 2-Bromo-1,4-naphthoquinone 1, (100 mg, 0.42 mmol) was added. After standing for 3 h, the mixture was treated with cold water. The reaction mixture was washed with water (3 x 50 mL) and extracted with hexane. The combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified via preparative silica gel plates GF – 500 microns Uniplat™ using hexane/ethyl acetate 8:1 to give **4a** and **4b**. The fraction with Rf = 2.6 afforded 20 mg (12 % yield) of a yellow solid, which was identified as 2-(3-Pentadecyl-phenoxy)-[1,4]naphthoquinones (**4a**), mp 50 – 51 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.85 (t, J = 6.0 Hz, 3H, CH_3), 1.00-1.70 (m, 26H, CH_2), 2.61 (t, J = 9.0 Hz, 2H, CH_2), 5.95 (s,1H, CH), 6.91 (d, J = 9.0 Hz, 1H, CH), 6.93 (s, 1H, CH), 7.09 (d, J = 9.0 Hz, 1H, CH), 7.33 (t, J = 9.0 Hz, 1H, CH), 7.72-7.75 (m, 2H, CH), 8.03-8.20 (m, 2H, CH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.0 (CH_3), 22.6 (CH_2), 28.0-30.0 (CH_2), 31.1 (CH_2), 31.9 (CH_2), 35.6 (CH_2), 113.2 (C), 118.0 (CH), 120.8 (CH), 126.2 (CH), 126.6 (CH), 130.0 (CH), 131.1 (C), 131.9 (CH), 133.4 (CH), 134.3 (CH), 145.9 (C), 152.6 (C), 160.6 (C), 185.0 (C). MS (EI^+): m/z (%): 460 [$M^{+\bullet}$] (100); 305 (8); 264 (23); 247 (13). HRMS (ESI) m/z: calcd for $C_{31}H_{40}0_3$ +H: 461.3055, Found 461.3038 [$C_{31}H_{40}0_3$ + H]$^+$. The fraction with Rf = 2,9 afforded 30 mg (10 % yield) of a yellow solid, which was identified as 2,3-Bis-(3-pentadecyl-phenoxy)-[1,4]naphthoquinone (4b), mp 58 – 59 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.86 (t, J = 6.0 Hz, 6H, CH_3); 1.00-1.60 (m, 52H, CH_2); 2.47 (t, J = 6.0 Hz, 4H, CH_2); 6.66 (d, J = 6.0 Hz, 2H, CH); 6.67 (s, 2H, CH); 6.82 (d, J = 6.0 Hz, 2H, CH); 7.08 (t, J = 6.0 Hz, 2H, CH), 7.73-7.76 (m, 2H, CH), 8.09-8.12 (m, 2H, CH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.1 (CH_3), 22.6 (CH_2), 29.0-30.0 (CH_2), 31.1 (CH_2), 31.9 (CH_2), 35.7 (CH_2), 113.6 (CH), 116.6 (CH), 123.6 (CH), 126.7 (CH), 128.9 (CH), 130.8 (C), 134.1 (CH), 144.7 (C), 146.0 (C), 156.4 (C), 180.5 (C). MS (EI^+): m/z (%): 763 [$M^{+\bullet}$] (15); 762 (27); 460 (13); 108 (100); 57 (29). HRMS (ESI): m/z = not ionizable [M+H]$^+$.

Biological activities

Parasites and Cell Cultures

The experiments of anti-parasitic activity were performed with the Y strain of *Trypanosoma cruzi* (epimastigotes) and promastigotes of *Leishmania amazonensis* (WHOM/

BR/75/JOSEFA strain). Epimastigote forms were cultivated in Liver Infusion Tryptose (LIT) medium supplemented with 10 % heat-inactivated fetal bovine serum (FBS; Gibco Invitrogen, Grand Island, NY, USA), kept at 28 °C, and maintained by weekly transfers. The promastigote forms of *L. amazonensis* were maintained in culture at 25 °C with weekly transfers to fresh Warren's medium supplemented with 10 % FBS. In order to assess the cytotoxicity of the compounds, $LLCMK_2$ cells (Macaca mulatta epithelial kidney cells) were maintained in Dulbecco's modified Eagle medium (DMEM; Gibco Invitrogen), pH 7.4, supplemented with 2 mM L-glutamine, 10 % FBS, and 50 mg/L gentamicin at 37 °C in a humidified 5 % CO_2 atmosphere.

Anti-proliferative Activity against Epimastigote Forms (Trypanosoma cruzi)

Epimastigotes (1×10^6 parasites/mL) in the exponential phase of growth (96 h) were harvested and incubated in the presence of LIT supplemented with 10 % FBS added or not to increasing concentrations of the drug candidates. Parasites incubated at 28 °C in 96-well flat-bottom plates were afterwards counted in a Neubauer hemocytometer under light microscopy. The IC_{50} (concentration that inhibited 50 % of parasite growth) were determined by regression analysis of the data.

Anti-proliferative Activity against Promastigote Forms (Leishmania amazonensis)

Promastigotes (1×10^6 cells/mL) at exponential phase of growth (48 h cultures) were inoculated in a 96-well plate in the absence or presence of different concentrations of the drug candidates. Activity against promastigote forms was evaluated after 72 h by using the XTT method, which consists on the incubation of the cultures on the presence of a combination of the tetrazolium compound 2,3-Bis-(2-Methoxy-4-Nitro-5- Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide (XTT, Sigma) and the electron coupling reagent phenazine methosulphate (PMS, Sigma). After the treatment of the parasites, 100 μL of the mixture of XTT (0,5 mg/mL) and PMS (0,06 mg/mL) was added to each well, the plate was incubated for 4 h protected from light at 28 °C and the absorbance measured at 450 nm in a microplate reader (Bio Tek – Power Wave XS). By comparing the absorbance in control untreated parasites with the treated ones, the inhibitory activity was determined. The IC_{50} (concentration that inhibited 50 % of parasite growth) were determined by regression analysis of the data.

Cytotoxicity Assay

To evaluate the cytotoxicity of the compounds, the MTT assay was applied as previously described [31]. This colorimetric assay is based on the ability of viable mitochondria to convert MTT, a water-soluble tetrazolium salt (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), into an insoluble purple- colored formazan precipitate. $LLCMK_2$ cells were collected from confluent cultures, plated in 96-well plates, and

incubated at 37°C in a humid 5 % CO_2 atmosphere. After 24 h, the medium was replaced with new DMEM that contained concentrations of the compounds that ranged from 3.7 to 73.6 μM. Following 96 h incubation, the cells were washed in PBS, and 50 μL of MTT (2 mg/mL) was added to each well. The formazan crystals were solubilized in DMSO, and absorbance was read at 570 nm in a microplate reader (Bio Tek – Power Wave XS). The concentration that diminished 50 % of the absorbance value observed in the control represented the CC^{50} (cytotoxic concentration for 50 % of the cells).

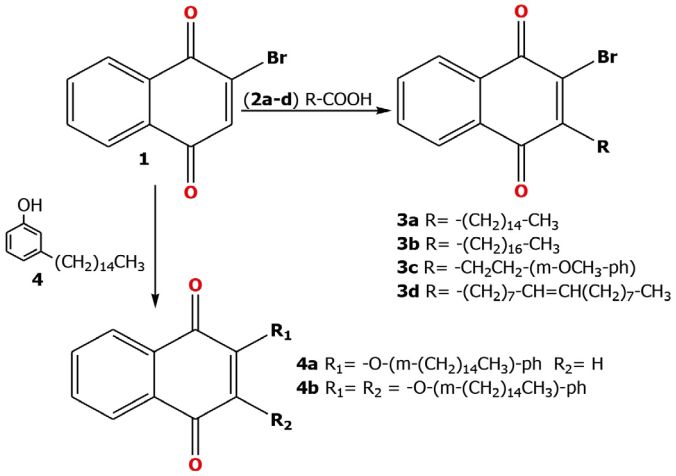
Statistical Analysis

All of the quantitative experiments were conducted in at least three independent experiments in duplicate. The statistical analyses were performed using GraphPad Prism 5.0 software. The data were analyzed using one-way analysis of variance (Anova), and the Tukey post hoc test was used to compare means when appropriate. Values of p, 0.05 were considered statistically significant.

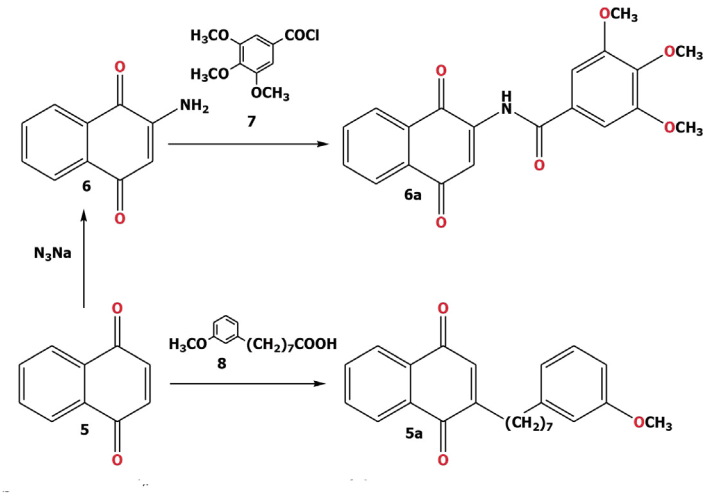
Results and discussion

Chemistry

The 1,4-naphthoquinones derivatives **3a-d** (Scheme 1) were obtained from compound **1** by coupling reaction with carboxylic acid **2a-d** (Palmitic, linoleic, 3-(4-Methoxy--phenyl)-propionic, oleic) using ammonium persulfate ($(NH_4)_2S_2O_8$) and silver nitrate ($AgNO_3$), following conditions previously reported [26; 32].



Scheme 1. Synthetic routes to compounds **3a-d** and **4a-b.**

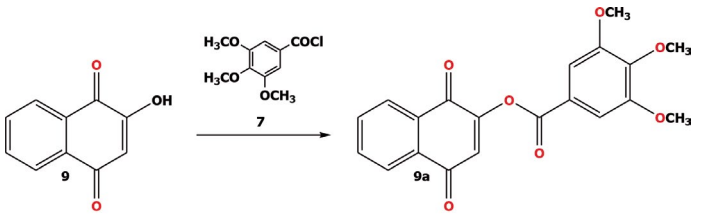


Scheme 2. Synthetic routes to compounds **5a** and **6a**.

On the other hand, treatment of compound **1** with phenol **4** (saturated cardanol) obtained two compounds (**4a-b**; Scheme 1) by nucleophilic substitution of the bromo atom attached to the C-2 and for addition of phenol in C-3 using K_2CO_3 and DMF [29]. The compound **6a** (Scheme 2) were obtained by reaction of compound **6** and **7** in NaH/THF as described in literature [27].

The 1,4-naphthoquinone (**5**) was used to prepare **5a** as described previously [26; 32] with 8-(3-methoxyphenyl) octanoic acid **8**. The condensation reaction of naphthoquinone 9 with 3,4,5-trimethoxybenzoyl chloride (**7**) in the presence of K_2CO_3 (Scheme 3), gave the compound **9a** [28].

The 1,4-naphthoquinone derivatives (**3a-d**, **4a-b**, **5a**, **6a** and **9a**) were obtained in 10-94 % yields and were further fully characterized by ^1H, ^{13}C, mass spectrometry and HRMS, and the data are compatible with their structures, the analytical data for all compounds are shown in appendix.



Scheme 3. Synthetic route to compound **9a**.

In vitro biological activity

In the present study, nine 1,4-naphthoquinone derivatives (Figure. 1) were synthesized and investigated for their potential as trypanocidal and leishmanicidal agents.

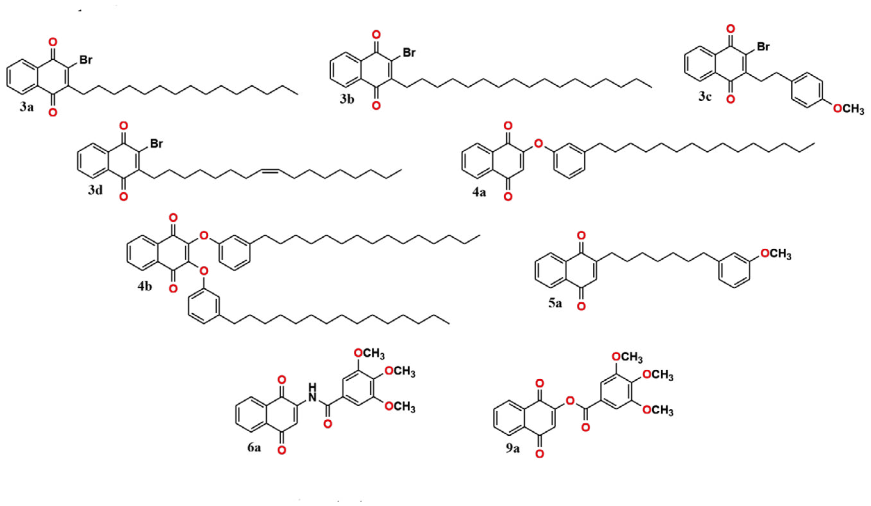


Figure. 1. 1,4-naphthoquinone derivatives tested against *T. cruzi* and *L. amazonensis.*

In order to obtain preliminar information, the *in vitro* activity of compounds **3a-d**, **4a-b**, **5a**, **6a** and **9a** was evaluted against epimastigote forms of *T. cruzi* and promastigote forms Y of *L. amazonensis*.

Trypanocidal activity of 1,4-naphthoquinones derivatives on epimastigotes proliferation

The synthesized 1,4-naphthoquinone derivatives **3a-d, 4a-b, 5a, 6a** and **9a** were evaluated against epimastigotes forms of *T. cruzi*. Table 1 shows IC_{50} values for the *in vitro* trypanocidal assay, the CC50 for the cytotoxicity to $LLCMK_2$ cells (MTT method) and the SI for each compound.

Some of these compounds showed good biological activity against epimastigotes form of *T. cruzi*. Among them, compound **6a** was the most potent of 1,4-naphthoquinone derivatives, showing an IC_{50} value of 0.25 ± 0.02 μM, being 35.0 times more active than Benznidazol (IC_{50} = 8.80 ± 0.40 μM) when assayed. However, the cytotoxicity of CC_{50} 6.53 ± 2.69 (SI = 26.12) was observed.

In 2013, Salomão et al. evaluated the efficacy of sixteen 1,4-naphthoquinones against the infective bloodstream trypomastigote forms of *T. cruzi* and the prototype 1,4-naphthoquinone **5**, demonstrated higher activity than Benznidazol (IC_{50} = 26.0 ± 4.0 μM) [33]. The compound **5** obtained an efficacy with IC_{50} = 0.79 ± 0.02 μM and was compared with other derivatives such 2-Bromo-1,4-naphthoquinone 1 (IC_{50} = 1.37 ± 0.03 μM) and 2-Hydroxi-1,4-naphthoquinone 9 (IC_{50} = 563.18 ± 83.28 μM). Among the most active compounds on trypomastigotes, the compound 5 was selected for further studies and was the most active against epimastigotes forms of *T. cruzi* with IC_{50} = 0.26 ± 0.05 μM. Comparing the compound 5 (IC_{50} = 0.26 ± 0.05 μM) and derivative 6a (IC_{50} = 0.25 ± 0.02 μM), we noted that the activity against proliferation of *T. cruzi* epimastigotes was equivalent, evidencing that amination reaction at the 2' position of the 1,4-napthoquinone followed of the *N*-acylation reaction with 3,4,5-trimethoxybenzoyl chlorid had no pronounced trypanocidal action.

In addition, compound **9** (lawsone) as prototype was active on culture growth of the Tulahuén strain epimastigotes form of *T. cruzi* with IC_{50} = 20.0 ± 6.0 μM [34]. When we acylation reaction of lawsone with 3,4,5-trimethoxybenzoyl chlorid, obtaining the compound **9a**, was observed the increased of the trypanocidal activity (IC_{50} = 5.06 ± 0.44 μM) in 4.0 times. Comparison between compounds **6a** (IC_{50} = 0.25 ± 0.02 μM) and **9a** (IC_{50} = 5.06 ± 0.44 μM) the activity is significantly increased, the presence of NH group had a positive effect on the trypanocidal activity.

Considering the structural of compounds **3a-d** and when compared, the substitution of the alkyl or allyl chain (e.g. **3a, 3b, 3d**) for phenyl ring with methoxy groups (e.g. **3c**) result in increased trypanocidal activity. It is interesting to note that increase in the length of the carbon chain decrease activity, the compound **3b** (IC_{50} = 9.46 ± 4.75 μM) was 2.0 times less active than the compound **3a** (IC_{50} = 4.58 ± 0.47 μM). In contrast, the comparison of the compounds **3b** (IC_{50} = 9.46 ± 4.75 μM) and **3d** (IC_{50} = 4.01 ± 1.19 μM), the presence of a double bond in the side chain increase the activity. Comparing the activity of compound precursor **1** (IC_{50} = 1.37 ± 0.03 μM) with **3c** (IC_{50} = 0.71 ± 0.32 μM), the effect of methoxy group resulted in an increase in activity.

The activities of the compounds **4a** (IC_{50} = 87.59 ± 20.72 μM) and **4b** (IC_{50} > 100 μM) gave low activity.

Table 1. Values of activities for 1,4-naphthoquinone derivatives **3a-d**, **4a-b**, **5a**, **6a** and **9a**, *in vitro* Trypanocidal activity (IC_{50} μM) against epimastigote form *T. cruzi*, cytotoxicity (CC_{50} μM, LLCMK^2 cells) and selectivity index (SI).

Compound	CC_{50}[a] (μM)	IC_{50}[b] (μM)	SI[c]
3a	59.67 ± 18.96	4.58 ± 0.47-	13.03
3b	10.19 ± 1.04	9.46 ± 4.75	1.08
3c	8.41 ± 0.09	0.71 ± 0.32	11.84
3d	6.96 ± 0.59	4.01 ± 1.19	1.73
4a	64.14 ± 6.60	87.59 ± 20.72	0.73
4b	44.68 ± 11.11	> 100	-
5a	8.55 ± 0.39	4.75 ± 4.19	1.80
6a	6.53 ± 2.69	0.25 ± 0.02	26.12
9a	6.1 ± 3.64	5.06 ± 0.44	1.20
Benznidazol	ND	8.80 ± 0.40	-

[a]CC_{50}: concentration that kills 50 % of $LLCM_{K2}$ cells, 96 h after incubation with the compounds determined by the MTT method. [b]IC_{50}: concentration that inhibits 50 % of the parasite growth in relation to control cultures with no drugs. [c]SI: Selectivity Index ¼ CC_{50}/IC_{50}. ND: not determined.

Leishmanicidal activity

All the synthesized compounds were tested against promastigote form *L. amazonensis* (Table 2) and only compounds **3c, 3d, 5a, 6a** and **9a** exhibited activity leishmanicidal with values between 0.16 and 2.49 μM. In terms of the most active of the naphthoquinones derivatives, compound **3c** showed the best IC_{50} 0.16 ± 0.05 μM, but is less active in comparison with the reference compound (Amphotericin B, IC_{50} = 0.06 μM).

Table 2. Values of activities for 1,4-naphthoquinone derivatives **3a-d**, **4a-b**, **5a**, **6a** and **9a**, in vitro Leishmanicidal activity (IC_{50} μM) against promastigote form *L. amazonensis*, cytotoxicity (CC_{50} μM, $LLCMK_2$ cells) and selectivity index (SI).

Compound	CC_{50}[a] (μM)	IC_{50}[b] (μM)	SI[c]
3a	59.67 ± 18.96	> 100	-

(*Continued*)

Compound	CC_{50}[a] (μM)	IC_{50}[b] (μM)	SI[c]
3b	10.19 ± 1.04	> 100	-
3c	8.41 ± 0.09	0.16 ± 0.05	52.56
3d	6.96 ± 0.59	0.31 ± 0.17	22.45
4a	64.14 ± 6.60	> 100	-
4b	44.68 ± 11.11	> 100	-
5a	8.55 ± 0.39	2.49 ± 3.29	3.43
6a	6.53 ± 2.69	0.17 ± 0.06	38.41
9a	6.1 ± 3.64	1.5 ± 0.74	4.07
Amphotericin B	ND	0.06	-

[a]CC_{50}: concentration that kills 50 % of $LLCMK_2$ cells, 96 h after incubation with the compounds determined by the MTT method. [b]IC_{50}: concentration that inhibits 50 % of the parasite growth in relation to control cultures with no drugs. [c]SI: Selectivity Index ¼ CC_{50}/IC_{50}. ND: not determined.

Conclusions

In conclusion, the synthesis of 1,4-napthoquinone derivatives was prepared and biologically assayed against *T. cruzi* and *L. amazonensis* in order to obtain an action profile for these compounds. The observed results were considered as preliminary and the active compounds **3a, 3c, 3d, 5a, 6a** and **9a** showed moderate to excellent trypanocidal activity. In particular, the compound **6a** exhibited better activity against epimastigotes forms of *T. cruzi* than benznidazole. On the other hand, the activity of 1,4-napthoquinone derivatives against promastigote forms *L. amazonensis* was not more effective than the Amphotericin B. Based on the result of this work, the compound 6a merits further studies in amastigotes and trypomastigotes to investigate the mechanism of trypanocidal action in both parasite forms and its potential for agents against Chagas disease.

Disclosure Statement

No potential conflict of interest was reported by the authors.

Acknowledgements

The authors would like to thank the FUNDECT-MS, CAPES-Brazil, the PROPP--UFMS and the Institute of Chemistry of UFMS for the financial support and infrastructure.

References

1. F.F. Norman, B. Monge-Maillo, A. Martínez-Pérez, J.A. Perez-Molina, R. López-Vélez, Parasitic infections in travelers and immigrants: part I protozoa, *Future Microbiol.*, **10**(1), 69-86 (2015).

2. B. Munksgaard, Parasitic infections, *Am. J. Transplant.*, **4**(s10), 142-155 (2004).

3. B. Singh, S. Varikuti, G. Halsey, G. Volpedo, O.M. Hamza, A.R. Satoskar, Host-directed therapies for parasitic diseases, *Future Med. Chem.*, **11**(15), 1999-2018 (2019).

4. C. Vergara, G. Muñoz, G. Martínez, W. Apt, I. Zulantay, Detection of *Trypanosoma cruzi* by PCR in adults with chronic Chagas disease treated with nifurtimox, *PLoS One*, **14**(8), e0221100 (2019).

5. J.A. Castillo-Garit, O. del Toro-Cortés, M.C. Vega, M. Rolón, A. Rojas de Arias, G.M. Casañola-Martin, J.A. Escario, A. Gómez-Barrio, Y. Marrero-Ponce, F. Torrens, C. Abad, Bond-based bilinear indices for computational discovery of novel trypanosomicidal drug-like compounds through virtual screening, *Eur. J. Med. Chem.*, **96**, 238-244 (2015).

6. T.G. Melo, D. Adesse, M.N. Meirelles, M.C.S. Pereira, *Trypanosoma cruzi* down--regulates mechanosensitive proteins in cardiomyocytes, *Mem. Inst. Oswaldo Cruz*, **114**, e180593 (2019).

7. A.C.M.V. Trompowsky, T.R. Conde, R.C. Lemos, B.M.C. Quaresma, M.C.S. Pitombeira, A.S. Carvalho, N. Boechat, K. Salomão, S.L. Castro, H.P.S. Zamith, *In vitro* genotoxicity of nitroimidazoles as a tool in the search of new trypanocidal agents, *Mem. Inst. Oswaldo Cruz*, **114**, e190017 (2019).

8. F.J.A. Santos, L.S. Silva, J.E. Santo Júnior, T.G.R. Mesquita, M.L.G. Souza, M.C. Andrade Júnior, S. Talhari, R. Ramasawmy, Single nucleotide polymorphisms of the genes IL-2, IL-2RB, and JAK3 in patients with cutaneous leishmaniasis

caused by *Leishmania* (V) *guyanensis* in Manaus, Amazonas, Brazil, *PLoS One*, **14**(8), e0220572 (2019).

9. D.S. Moreira, M.V. Xavier, S.M.F. Murta, Ascorbate peroxidase over expression protect *Leishmania braziliensis* against trivalente antimony effects, *Mem. Inst. Oswaldo Cruz*, **113**(12), e180377 (2018).

10. B.B. Cota, L.G. Tunes, D.N.B. Maia, J.P. Ramos, D.M. Oliveira, M. Hohlhoff, T.M. Alves, E.M. Souza-Fagundes, F.F. Campos, C.L. Zani, Leishmanicidal compounds of *Nectria pseudotrichia*, an endophytic fungus isolated from the plant *Caesalpinia echinata* (Brazilwood), *Mem. Inst. Oswaldo Cruz*, **113**(2), 102-110 (2018).

11. C.M. Cascabulho, M. Meuser-Batista, K.C.G. Moura, M.C. Pinto, T.L.A. Duque, K.C. Demarque, A.C.R. Guimarães, P.P.A. Manso, M. Pelajo-Machado, G.M. Oliveira, S.L. Castro, R.F.S. Menna-Barreto, Antiparasitic and anti-inflammatory activities of β-Lapachone-derived naphthoimidazoles in experimental acute *Trypanosoma cruzi* infection, *Mem. Inst. Oswaldo Cruz*, **115**, e190389 (2020).

12. A. Arora, D. Gupta, D. Rastogi, M. Gulrajani, Naphthoquinone colorants from *Arnebia nobilis* **Rech.f**, *Coloration Technology*, **128**(5), 350-355 (2012).

13. E.N. Silva Júnior, G.A.M. Jardim, C. Jacob, U. Dhawa, L. Ackermann, S.L. Castro, Synthesis of quinones with highlighted biological applications: A critical update on the strategies towards bioactive compounds with emphasis on lapachones, *Eur. J. Med. Chem.*, **179**, 863-915 (2019).

14. E. Leyva, L.I. Lopez, S.E. Loredo-Carrillo, M. Rodriguez-Kessler, A. Montes-Rojas, Synthesis, spectral and electrochemical characterization of novel, *J. Fluor. Chem*., **132**, 94-101 (2011).

15. R.F.S. Menna-Barreto, R.L.S. Gonçalves, E.M. Costa, R.S.F. Silva, A.V. Pinto, M.F. Oliveira S.L. de Castro, The effects on *Trypanosoma cruzi* of novel synthetic naphthoquinones are mediated by mitochondrial dysfunction, *Free Radic. Biol. Med.*, **47**(5), 644-653 (2009).

16. M. Janeczko, O. M. Demchuk, D. Strzelecka, K. Kubinski, M. Maslyk, New family of antimicrobial agents derived from 1,4-naphthoquinone, *Eur. J. Med. Chem.*, **124**, 1019-1025 (2016).

17. I.A. Schepetkin, A.S. Karpenko, A.I. Khlebnikov, M.O. Shibinska, I.A. Levandovsky, L.N. Kirpotina, N.V. Danilenko, M.T. Quinn, Synthesis, anticancer activity, and molecular modeling of 1,4-naphthoquinones that inhibit MKK7 and Cdc25, *Eur. J. Med. Chem.*, **183**, 111719 (2019).

18. M.A. Berghot, E.M. Kandeel, A.H. Abdel-Rahman, M. Abdel-Motaal, Synthesis, antioxidant and cytotoxic activities of novel naphthoquinone derivatives from 2,3-dihydro-2,3-epoxy-1,4-naphthoquinone, *Med. Chem.*, **4**(3), 381-388 (2014).

19. A.O. Silva, R.S. Lopes, R.V. Lima, C.S.S. Tozatti, M.R. Marques, S. Albuquerque, A. Beatriz, D.P. Lima, Synthesis and biological activity against *Trypanosoma cruzi* of substituted 1,4-naphthoquinones, *Eur. J. Med. Chem.*, **60**, 51-56 (2013).

20. A.A.S. Naujorks, A.O. Silva, R.S. Lopes, S. Albuquerque, A. Beatriz, M.R. Marques, D.P. Lima, Novel naphthoquinones derivatives and evaluation of their trypanocidal and leishmanicidal activities, *Org. Biomol. Chem.*, **13**(2), 428-437 (2015).

21. K. Kobayashi, S. Nishiumi, M. Nishida, M. Hirai, T. Azuma, H. Yoshida, Y. Mizushina, M. Yoshida, Effects of quinone derivatives, such as 1,4-naphthoquinone, on DNA polymerase inhibition and anti-inflammatory action, *Med. Chem.*, **7**(1), 37-44 (2011).

22. P.J. Jewess, J. Higgins, K.J. Berry, S.R. Moss, A.B. Boogaard, B.P.S. Khambay, Herbicidal action of 2-hydroxy-3-alkyl-1,4-naphthoquinones, *Pest Manag. Sci.*, **58**(3), 234-243 (2002).

23. E. Pérez-Sacau, A. Estéves-Braun, A.G. Ravelo, D.G. Yapu, A.G. Turba, Antiplasmodial activity of naphthoquinones related to lapachol and β-lapachone, *Chem. Biodivers.*, **2**(2), 264-274 (2005).

24. N. Jacobsen, A. Wengel, Fungicidal activity of 2-(1-alkenyl)-3-hydroxy-1,4-naphthoquinones and related compounds, *Pestic. Sci.*, **17**, 686-690 (1986).

25. M.B. Graham, J.H.P. Tyman, Ozonization of phenols from *Anacardium occidentale* (cashew), *J. Am. Oil Chem. Soc.*, **79**, 725-732 (2002).

26. B. Liu, L. Gu, J. Zhang, Synthesis of vitamin-K derivatives with different lengths of the alkyl side chain, *Recueil des Travaux Chimiques des Pays-Bas*, **110**(4), 99-103 (1991).

27. Y. Brandy, E. Akinbove, M. Lewis, C. Mouamba, S. Mack, R.J. Butcher, A.J. Anderson, O. Bakare, Synthesis and characterization of novel unsymmetrical and symmetrical 3-halo- or 3-methoxy-substituted 2-dibenzoylamino-1,4-naphthoquinone derivatives, *Molecules*, **18**, 1973-1984 (2013).

28. S.E. Hage, M. Ane, J.L. Stigliani, M. Marjorie, H. Vial, G. Baziard-Mouysset, M. Payard, Synthesis and antimalarial activity of new atovaquone derivatives, *Eur. J. Med. Chem.*, **44**, 4778-4782 (2009).

29. R.A. Tapia, C.O. Salas, K. Vázquez, C. Espinosa-Bustos, J. Soto-Delgado, J. Varela, E. Birriel, H. Cerecetto, M. González, M. Paulino, Synthesis and biological characterization of new aryloxyindole-4,9-diones as potent trypanosomicidal agents, *Bioorg. Med. Chem. Lett.*, **24**, 3919-3922 (2014).

30. M.L. Bolognesi, F. Lizzi, R. Perozzo, R. Brun, A. Cavalli, Synthesis of a small library of 2-phenoxy-1,4-naphthoquinone and 2-phenoxy-1,4-naphthoquinone derivatives bearing anti-trypanosomal and anti-leishmanial activity, *Bioorg. Med. Chem. Lett.*, **18**, 2272-2276 (2008).

31. J.H.S. Rodrigues, T. Ueda-Nakamura, A.G. Corrêa, D.P. Sangi, C.V.A. Nakamura, Quinoxaline derivative as potent chemtherapeutic agent, alone or in combination with benznidazole, against Trypanosoma cruzi, *PLoS One*, **9**(1), e85706 (2014).

32. G. Naturale, M. Lamblin, C. Commandeur, F.X. Felpin, J. Dessolin, Direct C-H alkylation of naphthoquinones with amino acids through a revisited Kochi-Anderson radical decarboxylation: Trends in reactivity and applications, *Eur. J. Org. Chem.*, **29**, 5774-5788 (2012).

33. K. Salomão, N.A. Santana, M.T. Molina, S.L. Castro, R.F.S. Menna-Barreto, *Trypanosoma cruzi* mitochondrial swelling and membrane potential colapse as primary evidence of the mode of action of naphthoquinones analogues, *BMC Microbiol.*, **13**, 196 (2013).

34. A. Morello, M. Pavani, J.A. Garbarino, M.C. Chamy, C. Frey, J. Mancilla, A. Guerrero, Y. Repetto, J. Ferreira, Effects and mode of action of 1,4-naphthoquinones isolated from *Calceolaria sessilis* on tumoral cells and *Trypanosoma* parasites, *Comp. Biochem. Physiol.*, **112C**, 119-128 (1995).

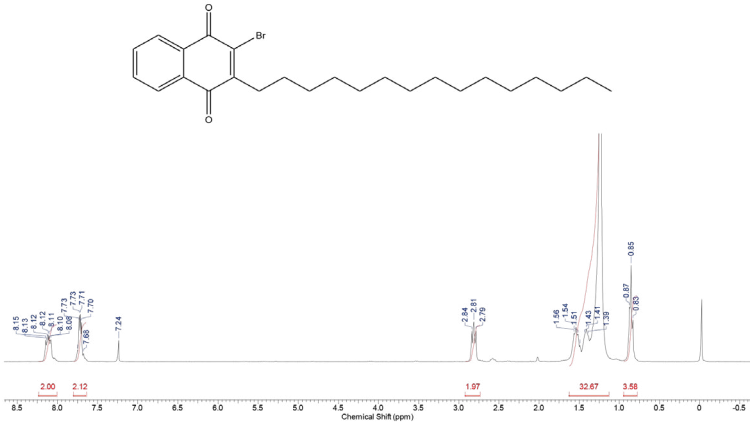
35. J.H.P. Tyman, I.E. Bruce, Synthesis and characterization of polyethoxylated surfactants derived from phenolic lipids, *J. Surfactants Deterg.*, **6**, 291-297 (2003).

How to cite this article

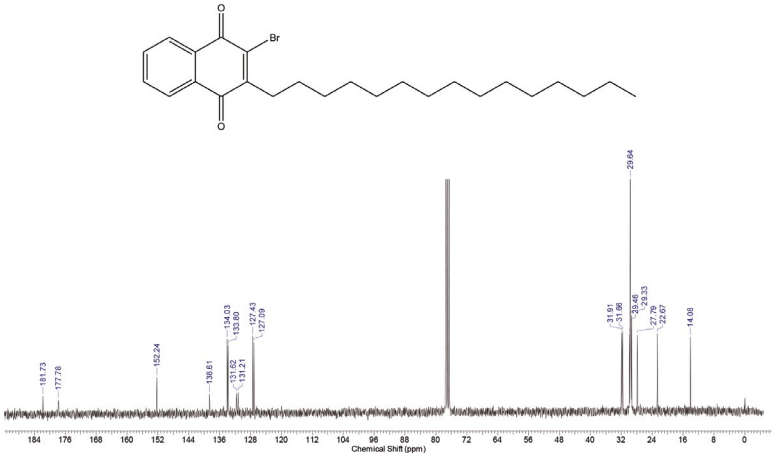
A.O. da Silva, R. da Silva-Lopes, A.A. dos Santos-Naujorks, C. Vataru-Nakamura, D. Pires de Lima, Synthesis, biological activities of 1,4-naphthoquinones derivatives against *T. cruzi* and *L. amazonensis*, *Rev. Colomb. Cienc. Quim. Farm.*, **51**(2), 752-786 (2022). http://dx.doi.org/10.15446/rcciquifa.v51n2.98279

Appendix

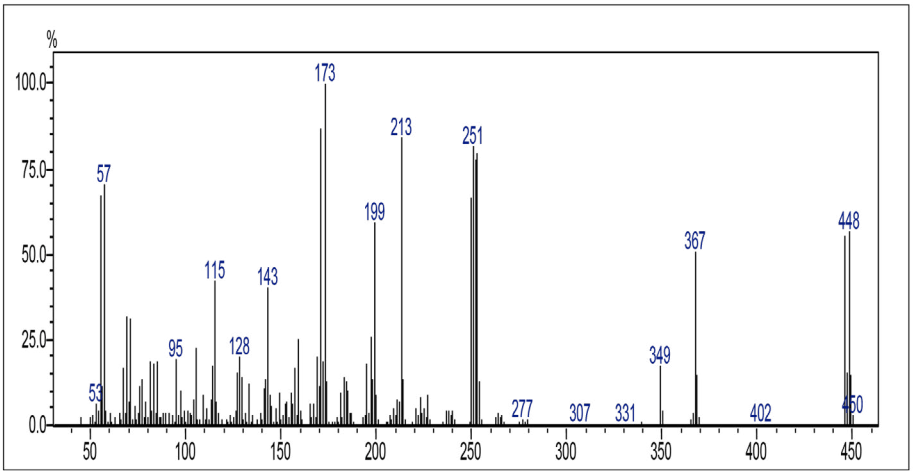
^{1}H-NMR spectrum of Compound 3a.



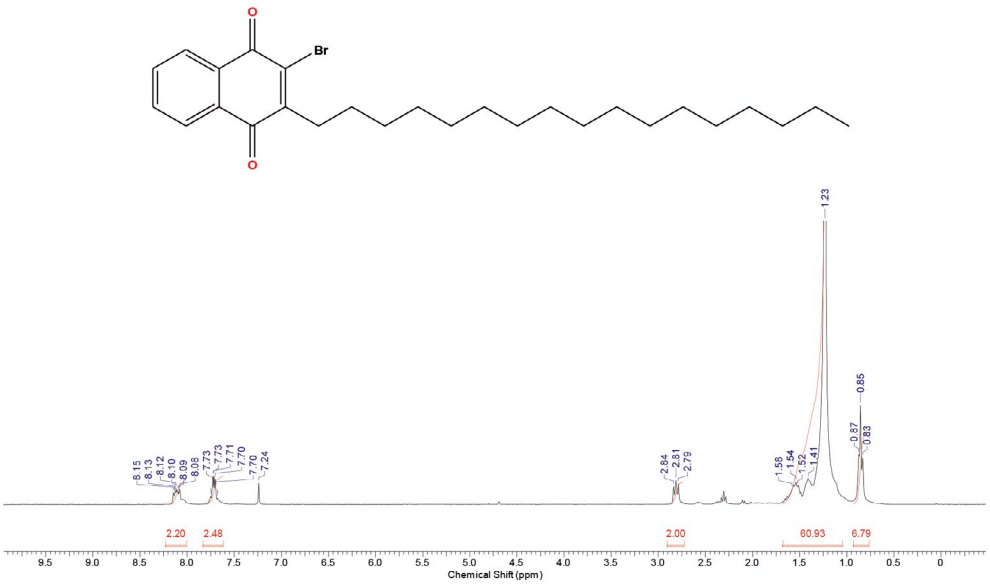
^{13}C-NMR spectrum of compound 3a.



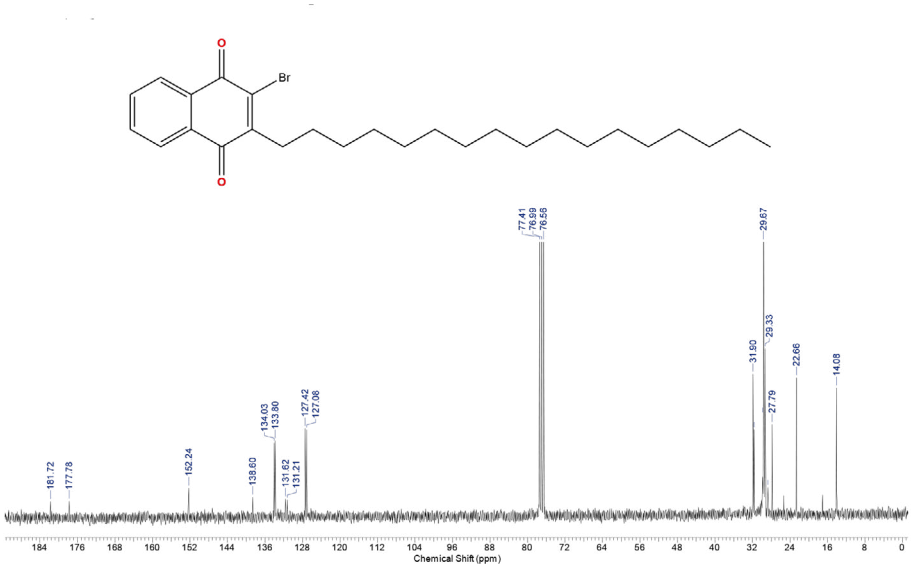
Mass spectrum of compound 3a.



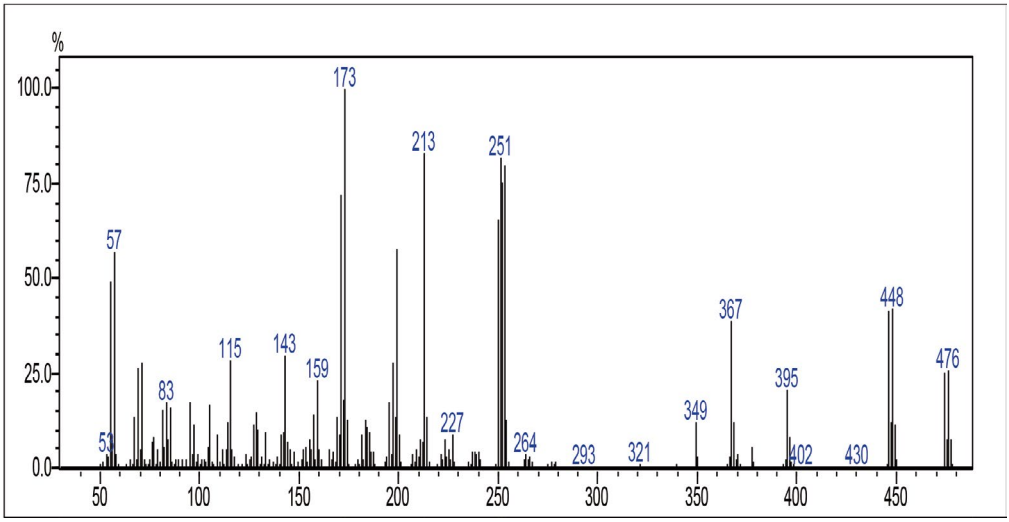
^{1}H-NMR spectrum of Compound 3b.



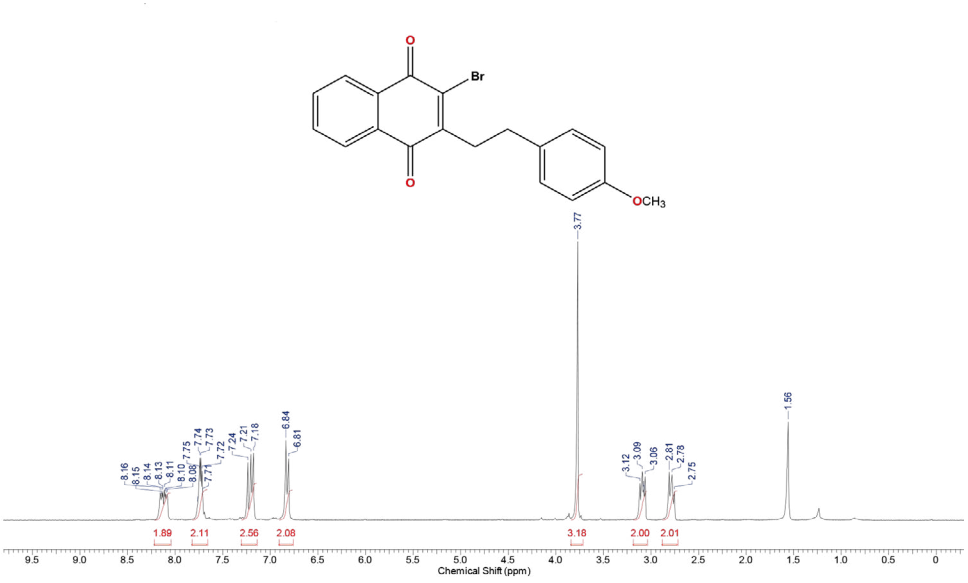
^{13}C-NMR spectrum of compound 3b.



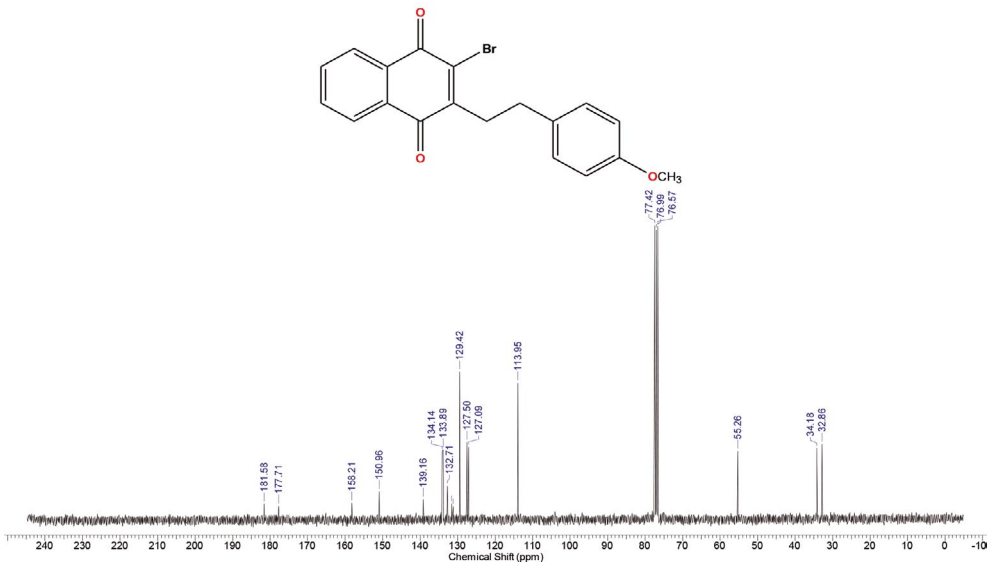
Mass spectrum of compound 3b.



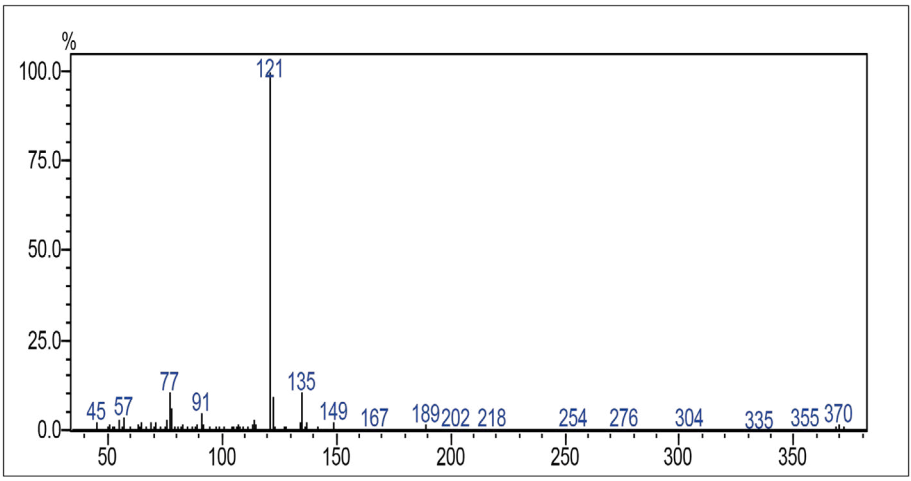
^{1}H-NMR spectrum of Compound 3c.



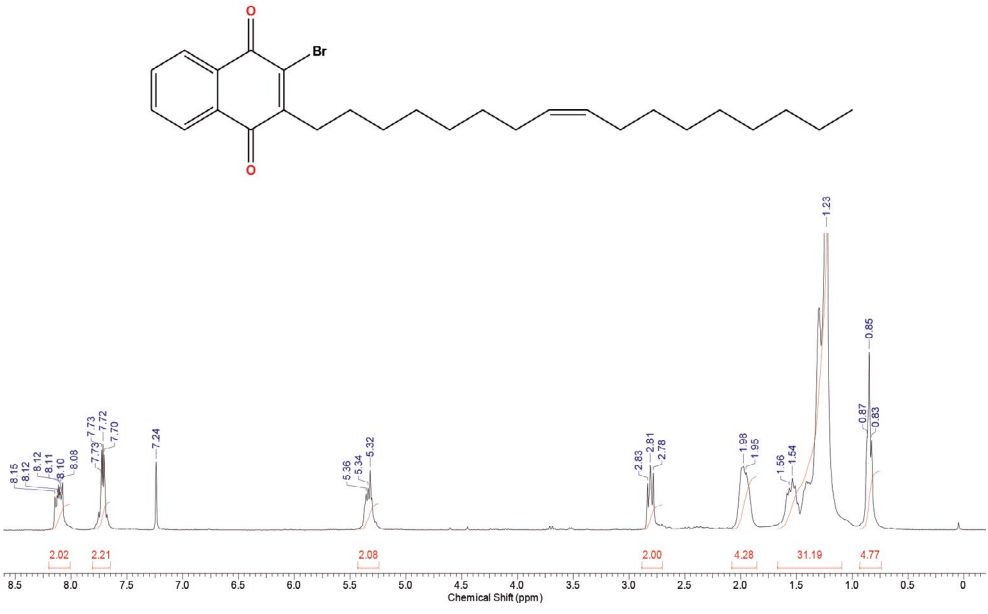
^{13}C-NMR spectrum of compound 3c.



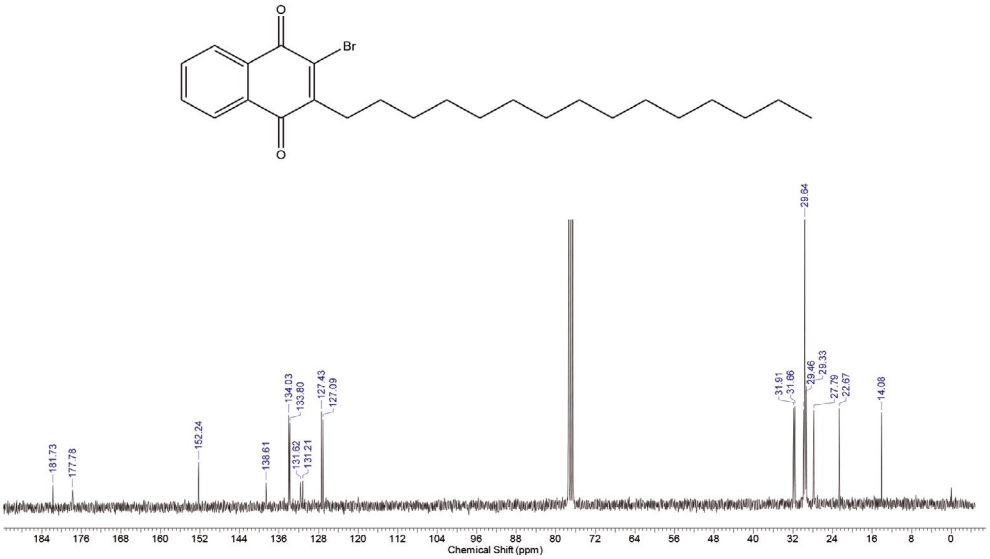
Mass spectrum of compound 3c.



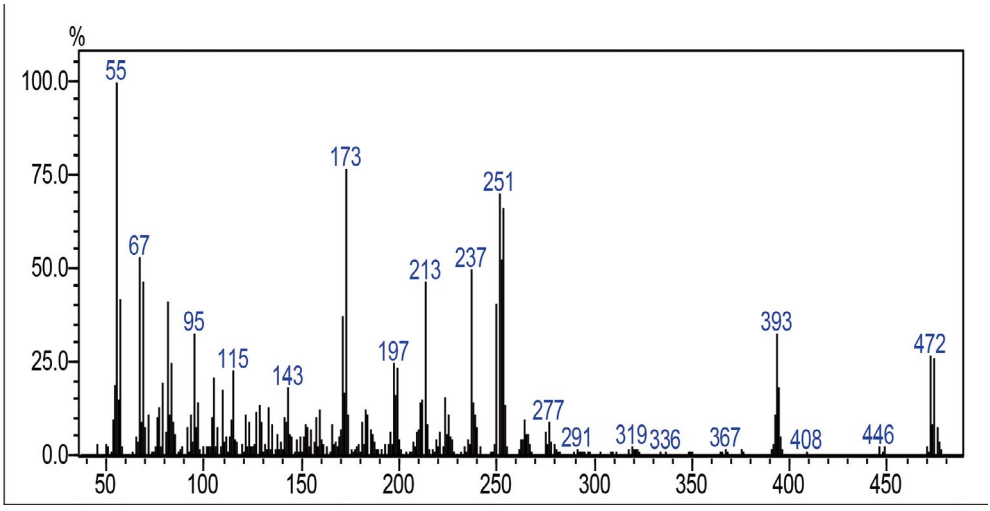
^{1}H-NMR spectrum of Compound 3d.



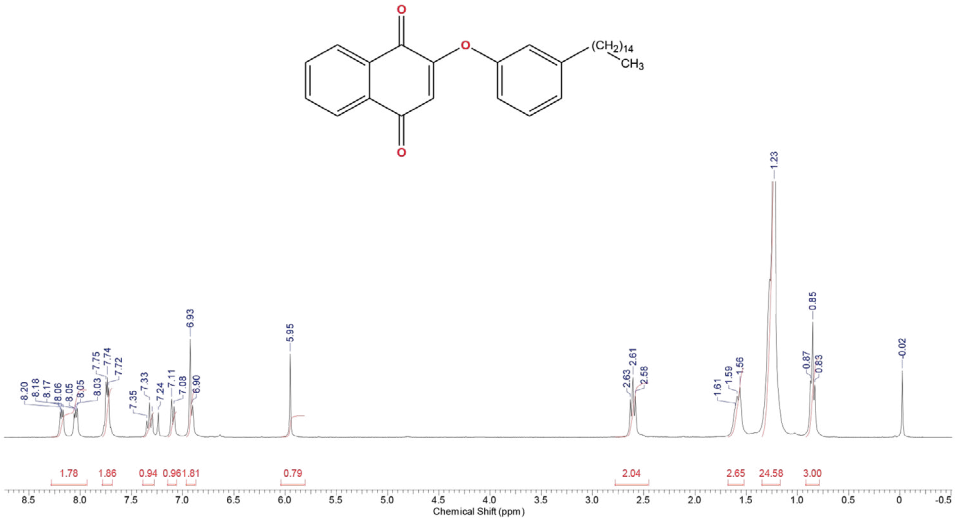
^{13}C-NMR spectrum of compound 3d.



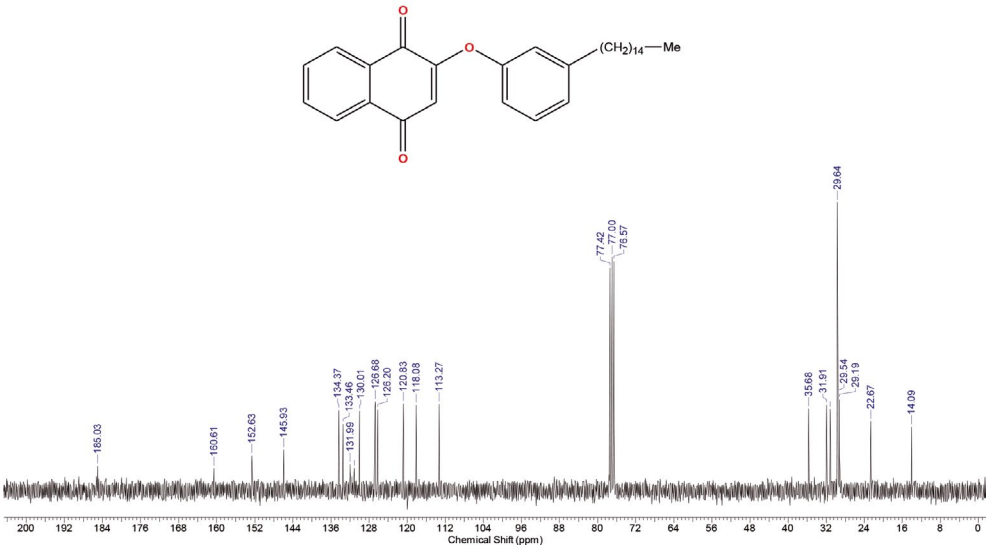
Mass spectrum of compound 3d.



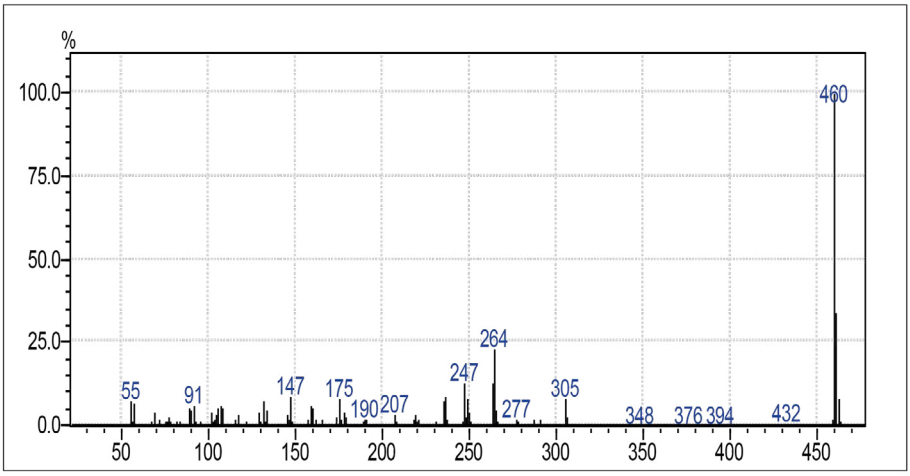
^{1}H-NMR spectrum of Compound 4a.



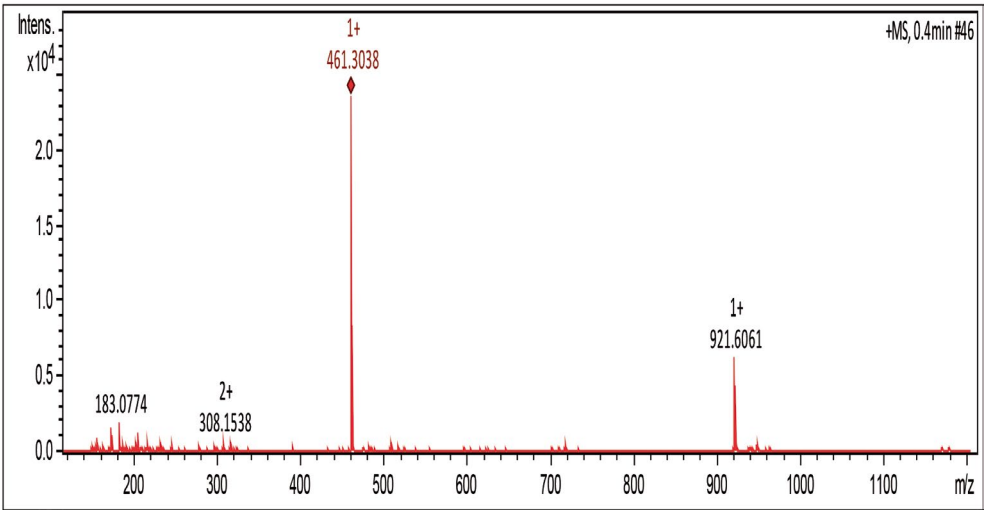
^{13}C-NMR spectrum of compound 4a.



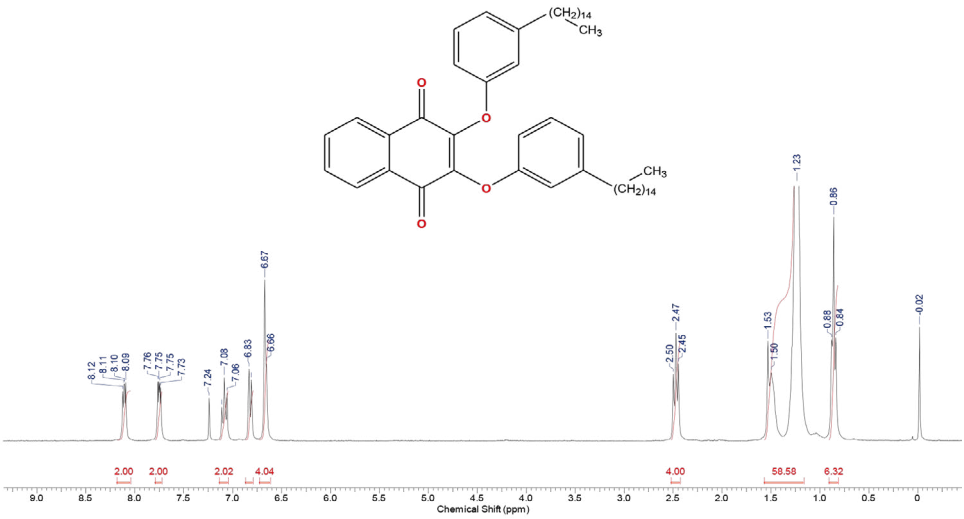
Mass spectrum of compound 4a.



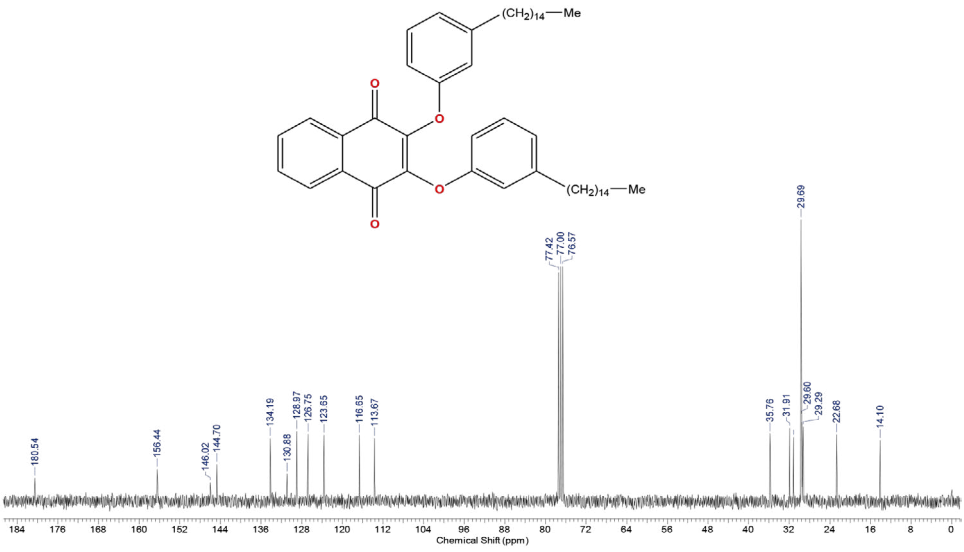
Mass spectrum (ESI-HRMS) of compound 4a.



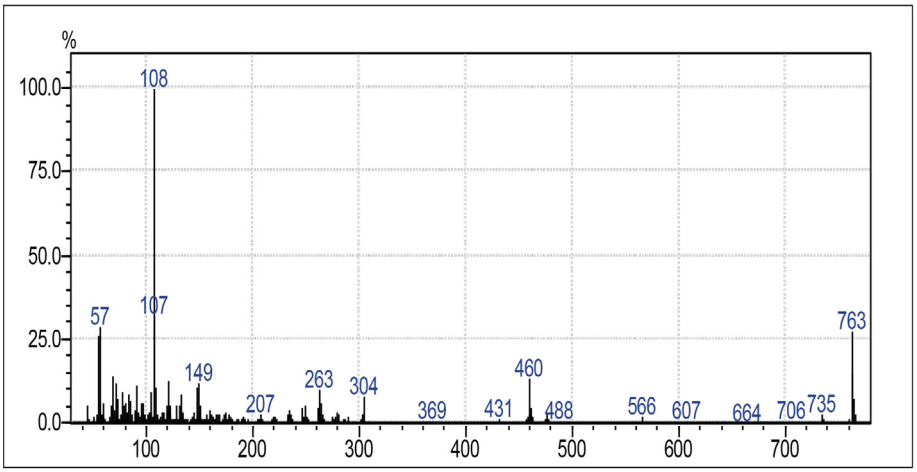
^{1}H-NMR spectrum of Compound 4b.



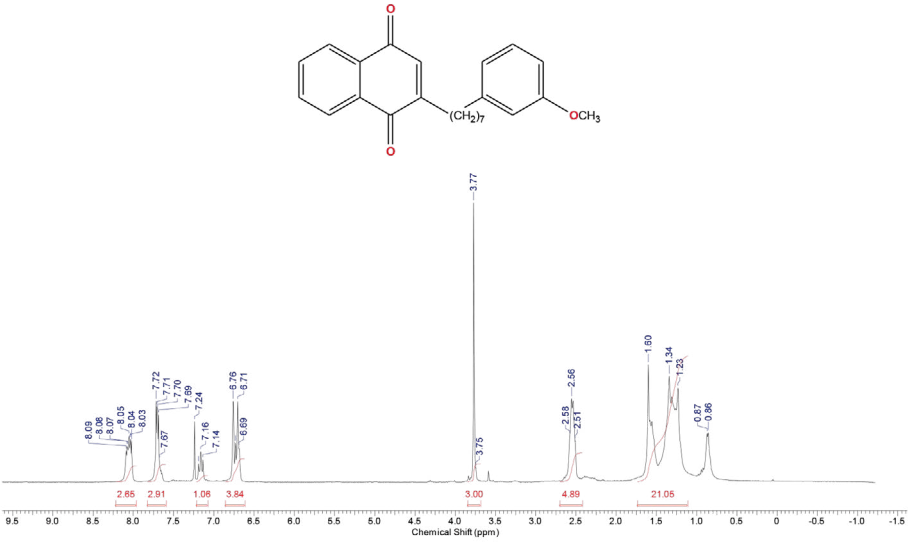
^{13}C-NMR spectrum of compound 4b.



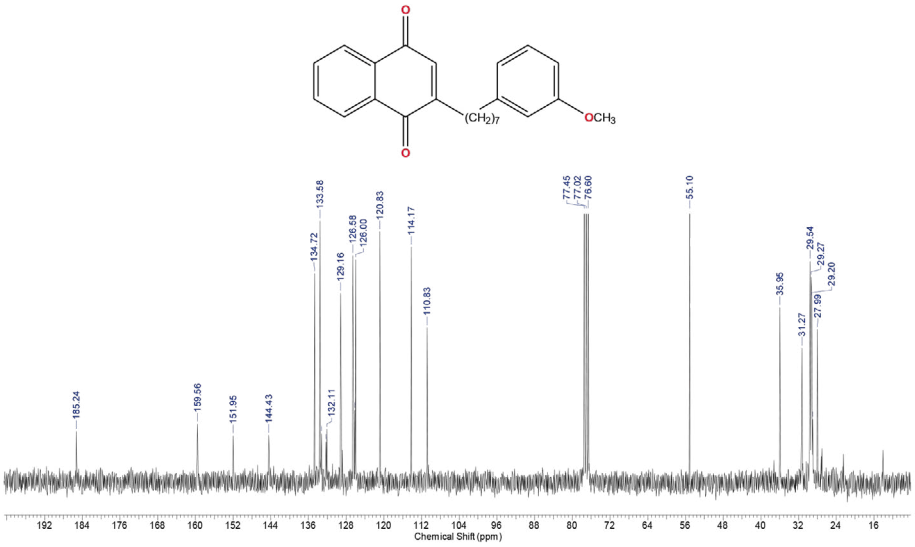
Mass spectrum of compound 4b.



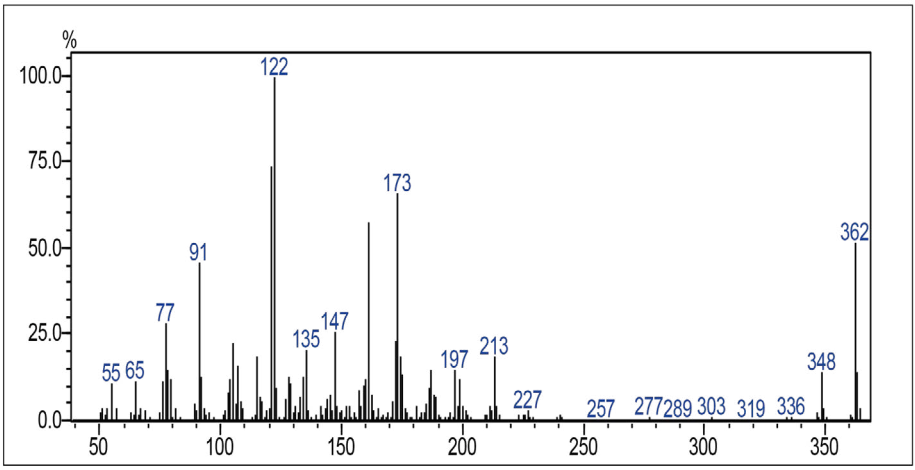
^{1}H-NMR spectrum of Compound 5a.



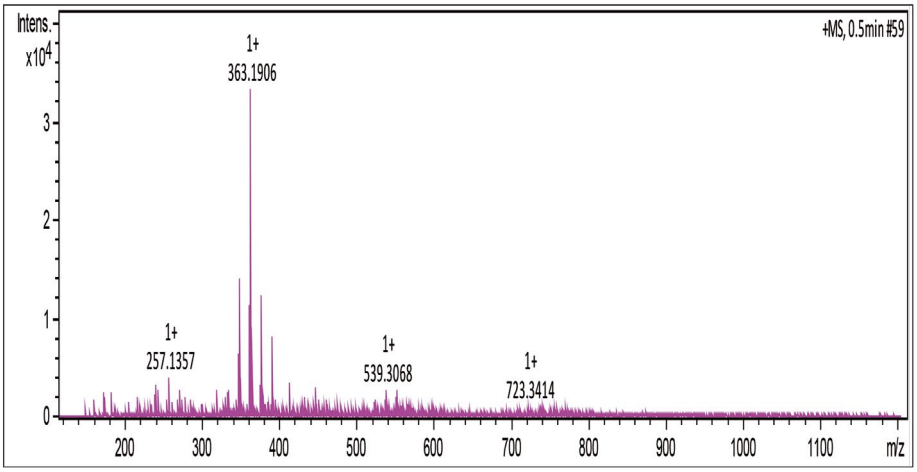
^{13}C-NMR spectrum of compound 5a.



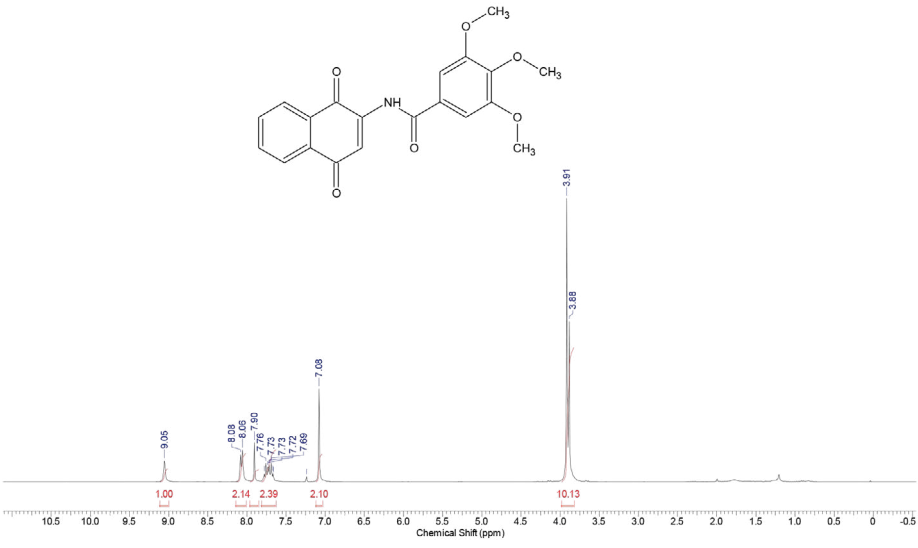
Mass spectrum of compound 5a.



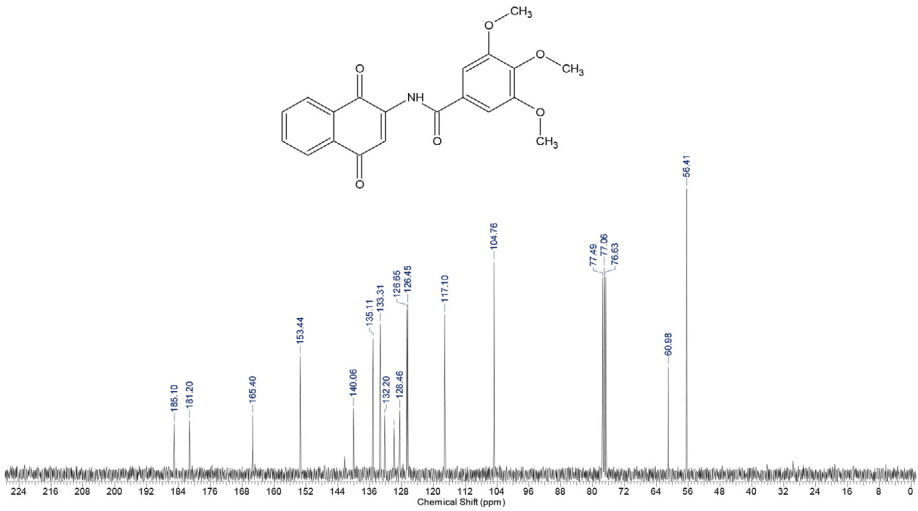
Mass spectrum (ESI-HRMS) of compound 5a.



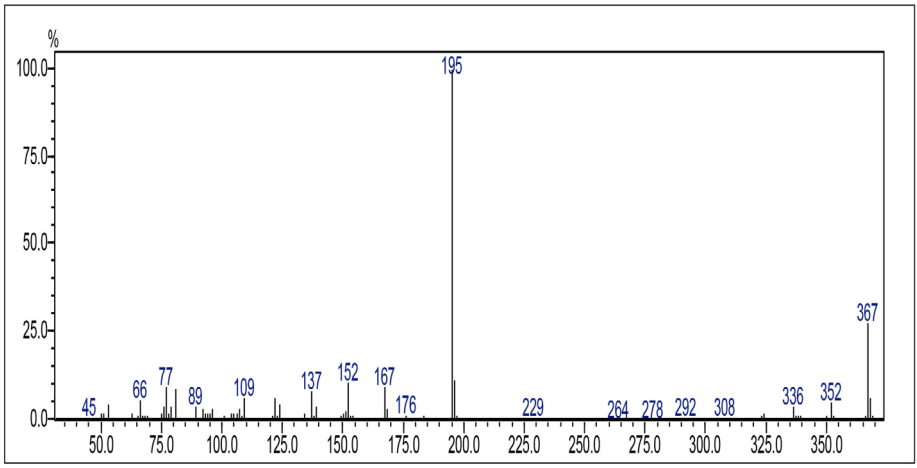
^{1}H-NMR spectrum of Compound 6a.



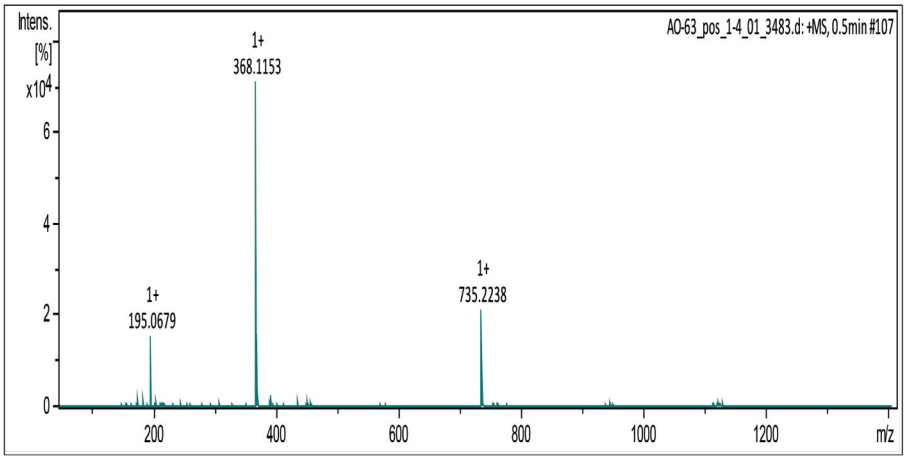
^{13}C-NMR spectrum of compound 6a.



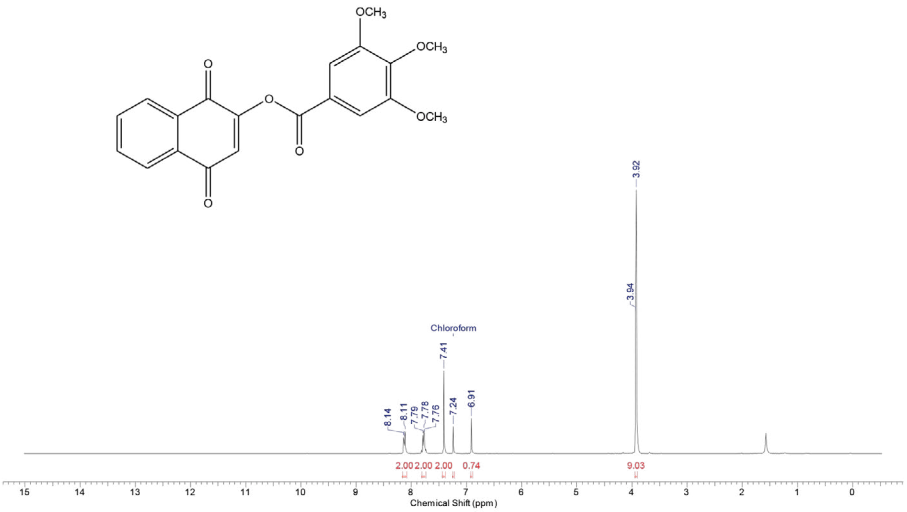
Mass spectrum of compound 6a.



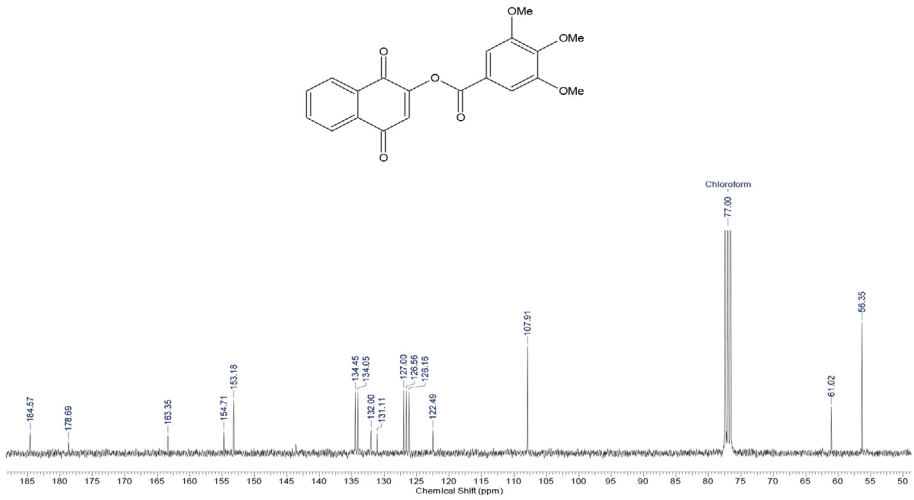
Mass spectrum (ESI-HRMS) of compound 6a.



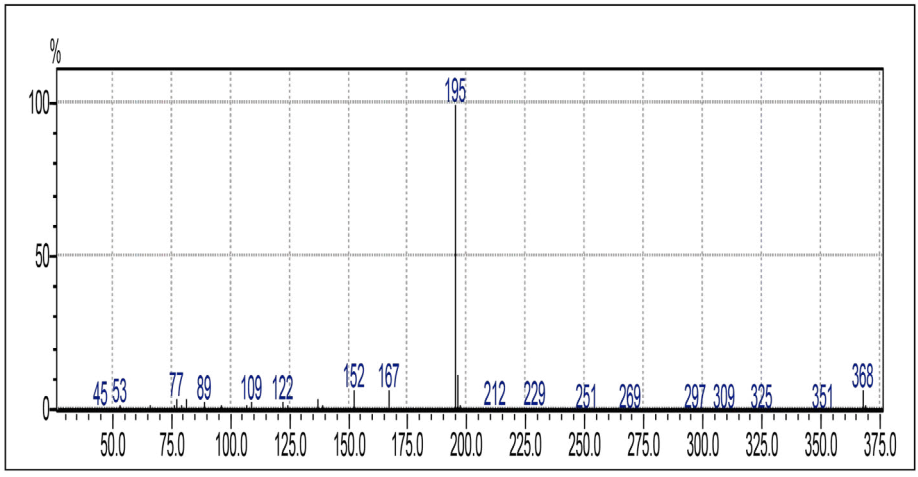
^{1}H-NMR spectrum of compound 9a.



^{13}C-NMR spectrum of compound 9a.



Mass spectrum of compound 9a.



Mass spectrum (ESI-HRMS) of compound 9a.