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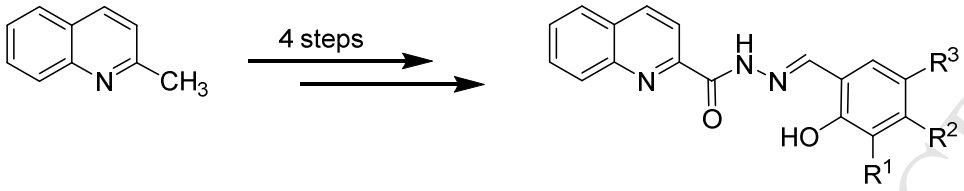
Graphical Abstract



	Leishmanicidal activity, EC_{50} (uM)	Trypanosomal activity, EC_{50} (uM)
6a R^1=H, R^2=H, R^3=H	-	4.8
6b R^1=OH, R^2=H, R^3=H	21.2	-
6c R^1=H, R^2=OH, R^3=H	2.6	4.6
6e R^1=OH, R^2=OH, R^3=OH	47.6	-

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Synthesis, Leishmanicidal, Trypanocidal and Cytotoxic Activity of Quinoline-Hydrazone Hybrids

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Abstract

Cutaneous leishmaniasis and Chagas disease are vector-borne parasitic disease causing serious risks to million people living in poverty-stricken areas. Both diseases are a major health problem in Latin America, and currently drugs for the effective treatment of these diseases have important concerns related with efficacy or toxicity than need to be addressed.

We report herein the synthesis and biological activities (cytotoxicity, leishmanicidal and trypanocidal activities) of ten quinolone-hydrazone hybrids. The structure of the products was elucidated by spectrometric analyses. The synthesized compounds were evaluated against amastigotes forms of *L. (V) panamensis* which is the most prevalent *Leishmania* species in Colombia and *Trypanosoma cruzi* that is the major pathogenic species to humans; in turn, cytotoxicity was evaluated against human U-937 macrophages.

Compounds **6b, 6c** and **8** showed activity against *L. (V) panamensis* with EC_{50} of 6.5 ± 0.8 μg/mL (21.2μM), 0.8 ± 0.0 μg/mL (2.6 μM) and 3.4 ± 0.6 μg/mL (11.1 μM), respectively, while compounds **6a** and **6c** had activity against *T. cruzi*. with EC_{50} values of 1.4 ± 0.3 μg/mL (4.8 μM) and 6.6 ± 0.3 μg/mL (4.6 μM), respectively. Even compound **6a** showed better activity against *T. cruzi* than the standard drug benznidazole with EC_{50} = 10.5 ± 1.8 μg/mL (40.3 μM).

Analysis of the results obtained against leishmaniasis indicates that antiparasite activity is related to the presence of 2-substituted quinoline (isoquinolinic core) and the hydroxyl group in positions 3 and 4 of the aromatic ring. Although the majority of these compounds were highly cytotoxic, the antiparasite activity was higher than cytotoxicity and therefore, they still have potential to be considered as hit molecules for leishmanicidal and trypanocidal drug development.

Keywords: leishmaniasis; Chagas disease; antiprotozoal activity; cytotoxicity; quinoline; hydrazone; hybrids

1. Introduction

Cutaneous leishmaniasis and Chagas disease are causes of high morbidity in several countries of tropical and subtropical regions where these diseases remain a significant health problem affecting mainly people living in poverty-stricken areas. Both are considered by WHO as two of the 17 neglected diseases due to lack of interest by the pharmaceutical industry to develop new or better drugs [1]. Cutaneous leishmaniasis involves a wide spectrum of clinical manifestations, ranging from small nodules, plaques or ulcers in the skin to severe mucosal tissue destruction affecting 1.2 – 1.5 million people around world every year. In the American region the disease can be caused by various species of *Leishmania* that include: *L. panamensis, L. braziliensis*, and *L. guayanensis* (members of the *Viannia* subgenus) and *L. mexicana* and *L. amazonensis* (members of the *Leishmania* subgenus). *L. (V) panamensis* is one of the most prevalent *Leishmania* species involved in human cases of cutaneous leishmaniasis in Colombia. This protozoan parasite is transmitted to humans through the bite of phlebotominae sandflies of the *Lutzomyia* genus [2]. On the other hand, Chagas disease (also named American trypanosomiasis) affects around 10 million people in Latin America. The disease is produced by the protozoan parasite *Trypanosoma cruzi* that is transmitted to the mammalian host through the bite of triatomine bugs belonging to *Triatoma, Rhodnius* and *Panstrongylus* genus [3].

The current chemotherapies are based on old drugs, pentavalent antimonials (meglumine antimoniate and sodium stibogluconate) for cutaneous leishmaniasis or nitroaromatic compounds (benznidazole and nifurtimox) for Chagas disease. However, all of them have various toxic effects on the patients that are associated with high doses and long therapeutic schemes. Moreover, they are no longer as effective as before due to the emergence of drug resistance in the parasite making the problem more complex [4-6].

A quinolinic core is a structural feature of several bioactive compounds. Thus, this core is an interesting constituent for new drugs design. Anti-mycobacterial, anti-microbial, anti-convulsant, anti-inflamatory, anti-tumoral, cardiovascular but also leishmanicidal and trypanocidal, are some biological activities exhibited by compounds having this heteroaromatic ring [7-15]. On the other hand, hydrazones constitute an important type of biologically active compounds which have high ability to elicit an leishmanicidal and trypanocidal activity [16-18]; thus for example, the *4-benzyloxy N´,N´-diethylbenzohydrazone* (figure 1a) is one of the synthetic benzyloxy protected hydrazone series with an Inhibitory Concentration (IC_{50}) of 6.25 µg/mL (21µM) against *(L) major* promastigotes [19]. In turn, the nitro derivative 1b (figure 1) exhibited an IC_{50} value lower than standard drugs pentamidine and amphotericin B when tested on *(L) donovani* promastigotes [20]. Moreover, evaluation of *1H-pyrazole-4-carbohydrazides* derivatives against *(L) amazonensis* promastigotes showed that the most active compounds were those with X = Br, Y = NO_2 and X = NO_2, Y = Cl (figures 1c and 1d) with CI_{50} 20.71

µg/mL (50 µM) and 29.58 µg/mL (80 µM) respectively[21]. Finally, hybrid compounds containing hydrazones and benzofuroxan pharmacophores (figure 1e) showed selective trypanocidal activity with IC_{50} 6.80 µg/mL (19.8 µM) [22] while brazilizone A (figure 1f), a new potent trypanocydal prototype, showed an IC_{50} of 1.92 µg/mL (5.3 µM) [23].

The combination of two pharmacological agents into a single molecule, called hybrid molecule is an emerging strategy in medicinal chemistry and drug discovery research [24,25]. These hybrid molecules may display dual activity but do not necessarily act on the same biological target [26-28]. In the search for new therapeutic alternatives to treat cutaneous leishmaniasis and Chagas disease we designed and synthesized a series of novel hydrazones having quinoline cores and their cytotoxicity and leishmanicidal and trypanocidal activities were evaluated *in vitro* (figure 2).

2. Results and Discussion

2.1. Chemistry

Quinaldine (**1**) was oxidized with selenium oxide to produce the aldehyde **2** in 80% yield [29], which was treated with iodine and methanol in basic solution affording ester **3** in 70% yield [30]. Then, compound **3** was submitted to nucleophilic substitution with hydrazine hydrate obtaining acylhydrazide **4** in 75% yield [31], that was coupled with different hydroxyaldehydes in aqueous medium obtaining hydrazones **6a-6e** in 40-85% yields [32]. The synthetic strategy is summarized in Scheme 1. With this strategy we only perform modifications of aromatic ring of the side chain in order to keep the two pharmacological agents. Hydrazones **7a-7d** and **8** were obtained following the same synthetic strategy starting from compounds 4-quinolinecarboxaldehyde and isoquinoline-1-carboxylic acid, respectively (figure 3).

2.2. Biological activities

The effect of hydrazones on cell growth and viability was assessed in human macrophages (U937 cells) which are the host cells for *L. (V) panamensis* and *T. cruzi* parasites. On the other hand, the antiparasite activity of these compounds was tested on intracellular amastigotes of *L. (V.) panamensis* and *T. cruzi* according to the ability of these compounds to reduce the amount of parasite inside infected macrophages. Results are summarized in Tables 1 and 2.

All novel hydrazones with exception of **7c** and **7d**, were highly cytotoxic to U937 cells showing LC_{50} < 100.0 µg/mL (Table 1). Compound **7d** showed moderate cytotoxicity (LC_{50} > 100.0 µg/mL, 347.3 µM) while compound **7c** had no cytotoxicity (LC_{50} > 200 µg/mL, >650.8 µM). In turn, amphotericin B, benznidazole and meglumine antimoniate showed high, moderate and no cytotoxicity, respectively.

Compounds **6b-6e**, **7a** and **8** showed activity against intracellular amastigotes of *L. (V) panamensis* with percentages of inhibition higher than 50% at concentrations $\leq$ 20 μg/mL (Table 1). The most active compounds was **6c** inhibiting in 64% the amount of intracellular amastigotes at 1.0 μg/mL followed by compound **8** with 62% of inhibition at 5.0 μg/mL. Others compounds showed low activity against *L. (V) panamensis* intracellular amastigotes. As expected, amphotericin B and meglumine antimoniate, standar drugs to treat cutaneous leishmaniasis, demonstrated activity against intracellular amastigotes with percentages of inhibition > 70.0% at 0.05 and 10.0 μg/mL, respectively.

On the other hand, only compound **6a** showed activity against intracellular amastigotes of *T. cruzi*, inhibiting the amount of parasite in 50.4% at concentrations lower than 10 μg/mL (Table 1). Benznidazole, one of the standard drugs to treat Chagas disease was active against intracellular amastigotes of *T. cruzi* inhibiting 87.6% of the parasite growth at 20 μg/mL.

The leishmanicidal and trypanocidal activities were confirmed by determining the effective concentration 50 (EC_{50}) that corresponds to the concentration of drug that gives the half-maximal reduction of the parasite growth (Table 2). Dose-response relationship showed that compounds **6b-6e** and **8** were highly active against intracellular amastigotes of *L. (V) panamensis* with EC_{50} < 20 μg/mL. The most active compound was **6c** with an EC_{50} of 0.8 ± 0.0 μg/mL (2.6 μM), followed by **6b** with an EC_{50} of 6.5 ± 0.8 μg/mL (21.2 μM). As expected again, the leishmanicidal drugs amphotericin B and meglumine antimoniate showed activity with low EC_{50} values. In turn, compounds **6a**, **6c** and **7b** were active against intracellular amastigotes of *T. cruzi* with EC_{50} of 1.4 ± 0.3 (4.8 μM), 6.6 ± 0.3 (4.6 μM) and 20.8 ± 0.5 μg/mL (67.7 μM), respectively (Table 2). In this case, benznidazole showed activity with an EC_{50} of 10.5 $\pm$ 1.8 μg/mL (40.3 μM).

The leishmanicidal activity of hydrazones **6b-6e** and **8** and trypanocidal activity of hydrazones **6a**, **6c** and **7b** were higher than their cytotoxicity. Thus, the IS values calculated for these compounds ranged from 2.6 to 4.7 in leishmanicidal compounds and from 0.5 to 11.6 in trypanocidal compounds (Table 2). As demonstrated elsewhere, amphotericin B and meglumine antimoniate have very high IS values. Although compounds **6c** and **8** showed better activity than meglumine antimoniate, the IS of these compounds is affected by their high cytotoxicity. These results suggest that biological activity of the hydrazones reported here is selective, being more active against *L. (V) panamensis* than U937 cells. On the other hand, only the trypanocidal activity of hydrazones **6a** was also higher than their cytotoxicity with an IS value of 11.6.

According to the results obtained against leishmaniasis, it is interesting to note that compounds with 2-substituted quinoline or isoquinolinic core (**6a-6e** vs **8**) are more active than those with 4-substituted quinoline (**7a-7d**). This higher activity could be due to a possible mechanism of action for these

compounds as iron chelators. In this mechanism, iron complexes with the nitrogen atom in hydrazone and with the oxygen atom in the carbonyl group [33]. In our case, we consider that iron complexes with the 2-substituted quinolone or isoquinolinic nitrogen instead of complexing with the oxygen in the carbonyl group because nitrogen is more basic. The mode of action of iron chelators have been reported for hydrazones in other protozoan diseases [34]. Studies *in vitro* have shown that chelating agents are able to inhibit parasite growth and proliferation by deprivation of iron, which is an essential nutrient for cell growth and division [35].

Other possible mechanisms of action for these compounds may be formulated in terms of conjugated addition of nucleophilic amino acid residues present in target enzymes of *Leishmania* e.g. such cysteine proteases [36], in a Michael addition [37]. An electrophilic conjugated system could be generated from o-hydroxybenzylidene-*N*-acylhydrazone framework due to the ability of this system to be converted into an electrophilic quinone methide intermediate through a pericyclic rearrangement [38]. This mechanism has been reported for other α,β-unsaturated compounds such as lactones, chromones and cinnamic acid esters [39-41].

Results of the present work suggest that the hydroxyl group in the positions 3 and 4 in the aromatic ring is determinant for the activity. The importance of this position may be due to the best molecular recognition ability towards target bioreceptors by forming hydrogen bonds [42].

3. Conclusions

The synthesis, leishmanicidal and trypanocidal screening of 10 quinoline-hydrazone hybrids are reported. Three of them were active against *L. (V) panamensis* (**6b, 6c** and **8)** and two of them against *T. cruzi* (**6a** and **6c**) with EC_{50} values lower than 10 μg/mL, being **6b** and **6a** the most active compounds for *L. (V) panamensis* and *T. cruzi*, respectively. The antiparasite activity and the selectivity showed by these compounds suggest that they have potential as templates for drugs development against these parasites. The presence of 2-substituted quinoline or isoquinolinic core and hydroxyl group in positions 3 and 4 of the aromatic ring increase the leishmanicidal activity.

4. Experimental Section

4.1. Chemical Synthesis

4.1.1. General Remarks

Synthesis of acylhydracides were carried out in a MW domestic oven adapted for the use of a reflux condenser and magnetic stirrer at a constant power (400W). Hydrazones were synthesized in an ultrasonic cleaner (BRANSON). NMR spectra were recorded as DMSO-d_6 solutions on an AMX 300 instrument (Bruker, Billerica, MA, USA) operating at 300 MHz for 1H and 75 MHz for ^{1}C. Chemical

shifts (δ) are expressed in ppm with the solvent peak as reference and TMS as an internal standard; coupling constants (J) are given in Hertz (Hz). High resolution mass spectra were recorded using electrospray ionization mass spectrometry (ESI-MS). A QTOF Premier instrument with an orthogonal Z-spray-electrospray interface (Waters, Manchester, UK) was used operating in the W-mode. The drying and cone gas was nitrogen set to flow rates of 300 and 30 L/h, respectively. Methanol sample solutions (ca. 1×10^{-5} M) were directly introduced into the ESI spectrometer at a flow rate of 10 μL/min. A capillary voltage of 3.5 kV was used in the positive scan mode, and the cone voltage set to *U*c = 10 V. For accurate mass measurements, a 2 mg/L standard solution of leucine enkephalin was introduced via the lock spray needle at a cone voltage set to 85 V and a flow rate of 30 μL/min. IR spectra were recorded on a Spectrum RX I FT-IR system (Perkin-Elmer, Waltham, MA, USA) in KBr disks. Silica gel 60 (0.063–0.200 mesh, Merck, Whitehouse Station, NJ, USA) was used for column chromatography, and precoated silica gel plates (Merck 60 F254 0.2 mm) were used for thin layer chromatography (TLC).

4.1.1.1. Synthetic procedure for methyl quinoline-2-carboxylate preparation (**3**):

Quinoline-2-carbaldehyde **2** (2g, 12.7 mmol) was dissolved in methanol (20 mL) and solutions of KOH (32 mmol, 1.8 g) and iodine (16.5 mmol, 4.2 g) in MeOH (each 6 mL) were successively added at 0°C. The mixture was stirred for a period of 15 minutes and then was concentrated on a rotatory evaporator, and the residue was purified by column chromatography over silica gel eluting with hexane and a mixture of hexane-ethyl acetate (9:1 ratio) to obtain the ester in 70% yield (8.9 mmol, 1.7 g). Monitoring of the reaction progress and product purification was carried out by TLC.

4.1.1.2. Synthetic procedure for quinoline-2-carbohydrazide preparation (**4**):

To a solution of **3** (1.5 g, 8.0 mmol) in ethanol (10 mL) was added hydrazine monohydrate (16 mL of a 80% solution). The reaction mixture submitted to microwave irradiation and maintained under reflux for 30 minutes. Then, the reaction mixture was poured on ice and the resulting precipitate was filtered out affording the title compound in 75% yield (6 mmol, 1.1 g).

4.1.1.3. Synthetic procedure for hydrazones preparation:

A quinoline-2-carbohydrazide **4** (100 mg, 0.53 mmol) solution in water (2 mL) was sonicated for 2 minutes and then aldehyde **5** (0.53 mmol) and acetic acid (0.1 mL) were added dropwise to the reaction mixture. Upon completion of the reaction (20–30 min), the product was filtered, sequentially washed with water (20 mL) and ethyl ether (5 mL), dried in vacuo and recrystallized from ethanol to obtain the corresponding hydrazones in yields ranging from 40% to 85%.

4.1.1.3.1. *N'-[(1E)-(2-hydroxyphenyl)methylidene]quinoline-2-carbohydrazide* (**6a**):

Yield 50% (0.265 mmol, 77.2 mg); light yellow solid, M.p. 185–187°C; IR (cm^{-1}): ν_{max} 3457 (Ar-OH), 3323 (N-H), 1700 (C=O), 1502 (C=N), 1482 (C=C_{Ar}), 1268 ((C=O)-N), 775 (C-H_{Ar}). ^{1}H-NMR (DMSO-d_6): δ 6.90-6.99 (H_{14}, H_{16}, m), 7.33 (H_{15}, $t_{apparent}$, *J* = 8.32 Hz), 7.55 (H_{17}, dd, *J* = 7.74, 1.5 Hz), 7.75 (H_7, $t_{apparent}$, *J* = 7.60 Hz), 7.91 (H_8, $t_{apparent}$, *J* = 7.60 Hz), 8.10 (H_6, d, *J* = 8.14 Hz), 8.20 (H_3, d, *J* = 8.52 Hz), 8.23 (H_9, d, *J* = 8.17 Hz), 8.61 (H_4, d, *J* = 8.52 Hz), 8.89 (H_{11}, s), 11.35 (OH), 12.53 (NH). ^{13}C-NMR ($CDCl_3$): δ 116.94 (C_{12}), 119.07 (C_{14}), 119.54 (C_{16}), 119.96 (C_3), 128.63 (C_7), 128.98 (C_6), 129.46 (C_{17}), 129.75 (C_8), 130.28 (C_5), 131.23 (C_9), 132.14 (C_{15}), 138.60 (C_4), 146.47 (C_2), 149.70 (C_{11}), 150.57 (C_{10}), 158.01 (C_{13}), 161.22 (C=O). EIMS: *m/z* 292.1087 $[M + H]^+$, Calcd for $C_{17}H_{13}N_3O_2$: 292.1086.

4.1.1.3.2. N'-[(1E)-(2,3-dihydroxyphenyl)methylidene]quinoline-2-carbohydrazide (**6b**):

Yield 75% (0.40 mmol, 122.1 mg); light brown solid, M.p. 205-207°C; IR (cm^{-1}): ν_{max} 3382 (Ar-OH), 3289 (N-H), 1683 (C=O), 1503 (C=N), 1471 (C=C_{Ar}), 1274 ((C=O)-N), 772 (C-H_{Ar}). ^{1}H-NMR (DMSO-d_6): δ 6.77 (H_{16}, $t_{apparent}$, *J* = 7.84 Hz), 6.89 (H_{15}, dd, *J* = 7.77, 1.0 Hz), 6.97 (H_{17}, dd, *J* = 7.77, 1.0 Hz), 7.77 (H_7, $t_{apparent}$, *J* = 7.76 Hz), 7.93 (H_8, $t_{apparent}$, *J* = 7.76 Hz), 8.13 (H_6, d, *J* = 8.10 Hz), 8.23 (H_3, H_9, d, *J* = 8.57 Hz), 8.63 (H_4, d, *J* = 8.51 Hz), 8.90 (H_{11}, s), 9.22 (OH), 11.25 (OH), 12.54 (NH). ^{13}C-NMR ($CDCl_3$): δ 118.0 (C_{12}), 119.24 (C_{15}), 119.62 (C_3), 119.67 (C_{16}), 120.78 (C_{17}), 128.70 (C_7), 128.91 (C_5), 129.50 (C_6), 129.69 (C_8), 131.22 (C_9), 138.58 (C_4), 146.12 (C_2), 146.51 (C_{10}), 146.73 (C_{14}), 149.86 (C_{11}), 151.24 (C_{13}), 161.05 (C=O). EIMS: *m/z* 308.1036 $[M + H]^+$, Calcd for $C_{17}H_{13}N_3O_3$: 308.1035.

4.1.1.3.3. N'-[(1E)-(2,4-dihydroxyphenyl)methylidene]quinoline-2-carbohydrazide (**6c**):

Yield 81% (0.43 mmol, 132.0 mg); yellow solid, M.p. 259-262°C; IR (cm^{-1}): ν_{max} 3459 (Ar-OH), 3309 (N-H), 1675 (C=O), 1501 (C=N), 1431 (C=C_{Ar}), 1225 ((C=O)-N), 770 (C-H_{Ar}). ^{1}H-NMR (DMSO-d_6): δ 6.34 (H_{14}, d, *J* = 2.23 Hz), 6.39 (H_{16}, dd, *J* = 8.40, 2.23 Hz), 7.32 (H_{17}, d, *J* = 8.40 Hz), 7.74 (H_7, $t_{apparent}$, *J* = 8.0 Hz), 7.90 (H_8, $t_{apparent}$, *J* = 8.0 Hz), 8.09 (H_6, d, *J* = 8.03 Hz), 8.18 (H_3, d, *J* = 8.56 Hz), 8.22 (H_9, d, *J* = 8.83 Hz), 8.60 (H_4, d, *J* = 8.60 Hz), 8.75 (H_{11}, s), 10.16 (OH), 11.55 (OH), 12.34 (NH). ^{13}C-NMR ($CDCl_3$): δ 103.15 (C_{14}), 108.30 (C_{16}), 111.0 (C_{12}), 119.50 (C_3), 128.60 (C_7), 128.90 (C_6), 129.40 (C_5), 129.73 (C_8), 131.20 (C_9), 132.19 (C_{17}), 138.56 (C_4), 146.50 (C_2), 149.90 (C_{10}), 151.40 (C_{11}), 160.10 (C_{15}), 160.83 (C_{13}), 161.33 (C=O). EIMS: *m/z* 308.1039 $[M + H]^+$, Calcd for $C_{17}H_{13}N_3O_3$: 308.1035.

4.1.1.3.4. *N'-[(1E)-(2,5-dihydroxyphenyl)methylidene]quinoline-2-carbohydrazide* (**6d**):

Yield 47% (0.25 mmol, 76.5 mg); yellow solid, M.p. 229-232°C; IR (cm^{-1}): ν_{max} 3418 (Ar-OH), 3307 (N-H), 1675 (C=O), 1505 (C=N), 1382 (C=C_{Ar}), 1257 ((C=O)-N), 774 (C-H_{Ar}). ^{1}H-NMR (DMSO-d_6): δ 6.98 (H_{17}, d, *J* = 1.72 Hz), 6.75-6.79 (H_{14}, H_{15}, m), 7.76 (H_7, $t_{apparent}$, *J* = 7.52 Hz), 7.92 (H_8, $t_{apparent}$, *J*

= 7.52 Hz), 8.12 (H_6, d, *J* = 8.02 Hz), 8.18-8.26 (H_3, H_9, m), 8.63 (H_4, d, *J* = 8.54 Hz), 8.85 (H_{11}, s), 8.87 (OH), 9.02 (OH), 12.42 (NH). ^{13}C-NMR ($CDCl_3$): δ 114.56 (C_{12}), 117.60 (C_{15}), 119.42 (C_3), 119.58 (C_{14}, C_{17}), 128.69 (C_6), 128.87 (C_5), 129.46 (C_8), 129.69 (C_9), 131.19 (C_4), 138.54 (C_2), 146.50 (C_{10}), 149.98 (C_{11}), 150.37 (C_{16}), 150.88 (C_{13}), 161.0 (C=O). EIMS: *m/z* 308.1037 $[M + H]^+$, Calcd for $C_{17}H_{13}N_3O_3$: 308.1035.

4.1.1.3.5. *N'-[(1E)-(2,3,4-trihydroxyphenyl)methylidene]quinoline-2-carbohydrazide* (**6e**):

Yield 60% (0.32 mmol, 102.8 mg); dark brown solid, M.p. 219-221°C; IR (cm^{-1}): ν_{max} 3461 (Ar-OH, N-H), 1636 (C=O), 1501 (C=N), 1480 (C=C_{Ar}), 1265 ((C=O)-N), 770 (C-H_{Ar}). ^{1}H-NMR (DMSO-d_6): δ 6.42 (H_{16}, d, *J* = 8.32 Hz), 6.79 (H_{17}, d, *J* = 8.32 Hz), 7.76 (H_7, $t_{apparent}$, *J* = 7.40 Hz), 7.92 (H_8, $t_{apparent}$, *J* = 7.40 Hz), 8.12 (H_6, d, *J* = 8.02 Hz), 8.22 (H_9, H_3, d, *J* = 8.43 Hz), 8.50 (OH), 8.62 (H_4, d, *J* = 8.60 Hz), 8.76 (H_{11}, s), 9.49 (OH), 11.59 (OH), 12.38 (NH). ^{13}C-NMR ($CDCl_3$): δ 108.20 (C_{16}), 111.37 (C_{12}), 119.60 (C_3), 121.88 (C_{17}), 128.69 (C_7), 128.82 (C_6), 129.44 (C_5), 129.67 (C_8), 131.20 (C_9), 133.22 (C_{14}), 138.55 (C_4), 146.53 (C_2), 148.13 (C_{11}), 149.40 (C_{10}), 150.03 (C_{15}), 152.30 (C_{13}), 160.72 (C=O). EIMS: *m/z* 324.0988 $[M + H]^+$, Calcd for $C_{17}H_{13}N_3O_4$: 324.0984.

4.1.1.3.6. *N'-[(1E)-(2,3-dihydroxyphenyl)methylidene]quinoline-4-carbohydrazide* (**7a**):

Yield 70% (0.371 mmol, 114.0 mg); brown solid, M.p. 263-267°C; IR (cm^{-1}): ν_{max} 3443 (Ar-OH), 3191 (N-H), 1654 (C=O), 1578 (C=N), 1471 (C=C_{Ar}), 1283 ((C=O)-N), 724 (C-H_{Ar}). ^{1}H-NMR (DMSO-d_6): δ 6.76 (H_{16}, $t_{apparent}$, *J* = 7.84 Hz), 6.89 (H_{15}, dd, *J* = 7.8, 1.3 Hz), 7.03 (H_{17}, dd, *J* = 7.8, 1.3 Hz), 7.74 (H_3, d, *J* = 4.2 Hz), 7.78-7.96 (H_7, H_8, m), 8.13 (H_6, d, *J* = 8.4 Hz), 8.20 (H_9, d, *J* = 8.2 Hz), 8.30 (OH), 8.52 (H_{11}, s), 9.04 (H_2, d, *J* = 4.4 Hz), 10.79 (OH), 12.34 (NH). ^{13}C-NMR ($CDCl_3$): δ 119.20 (C_{12}), 119.82 (C_{15}), 120.16 (C_3), 120.32 (C_{16}), 124.61 (C_{17}), 125.67 (C_5), 128.30 (C_5), 129.87 (C_6, C_9), 130.63 (C_8), 140.18 (C_4), 146.03 (C_{14}), 148.30 (C_{10}), 150.13 (C_{11}), 150.74 (C_2), 163.10 (C_{13}), 168.76 (C=O). EIMS: *m/z* 308.1037 $[M + H]^+$, Calcd for $C_{17}H_{13}N_3O_3$: 308.1035.

4.1.1.3.7. N'-[(1E)-(2,4-dihydroxyphenyl)methylidene]quinoline-4-carbohydrazide (**7b**):

Yield 60% (0.318 mmol, 97.7 mg); yellow solid, M.p. 250-254°C; IR (cm^{-1}): ν_{max} 3381 (Ar-OH), 2856 (N-H), 1632 (C=O), 1509 (C=N), 1466 (C=C_{Ar}), 1235 ((C=O)-N), 762 (C-H_{Ar}). ^{1}H-NMR (DMSO-d_6): δ 6.35 (H_{14}, d, *J* = 1.6 Hz), 6.38 (H_{16}, dd, *J* = 8.5, 1.6 Hz), 7.36 (H_{17}, d, *J* = 8.5 Hz), 7.71 (H_3, d, *J* = 4.2 Hz), 7.77-7.89 (H_7, H_8, m), 8.12 (H_6, d, *J* = 8.4 Hz), 8.19 (H_9, d, *J* = 7.6 Hz), 8.42 (H_{11}, s), 9.02 (H_2, d, *J* = 4.2 Hz), 11.26 (OH), 12.22 (NH). ^{13}C-NMR ($CDCl_3$): δ 103.1 (C_{14}), 108.40 (C_{16}), 110.82 (C_{12}), 120.10 (C_3), 124.70 (C_5), 125.61 (C_7), 128.22 (C_8), 129.83 (C_9), 130.57 (C_6), 131.76 (C_{17}), 140.36 (C_4), 148.31 (C_{10}), 150.36 (C_{11}), 150.71 (C_2), 159.95 (C_{15}), 161.49 (C_{13}), 162.76 (C=O). EIMS: *m/z* 308.1035 $[M + H]^+$, Calcd for $C_{17}H_{13}N_3O_3$: 308.1035.

4.1.1.3.8. N'-[(1E)-(2,5-dihydroxyphenyl)methylidene]quinoline-4-carbohydrazide (**7c**):

Yield 62% (0.329 mmol, 101.0 mg); light brown solid, M.p. 270-276°C; IR (cm^{-1}): ν_{max} 3442 (Ar-OH), 3339 (N-H), 1661 (C=O), 1585 (C=N), 1466 (C=C_{Ar}), 1226 ((C=O)-N), 765 (C-H_{Ar}). ^{1}H-NMR (DMSO-d_6): δ 6.77 (H_{14}, H_{15}, $s_{apparent}$), 7.05 (H_{17}, s), 7.72 (H_3, d, *J* = 4.4 Hz), 7.78-7.89 (H_7, H_8, m), 8.12 (H_6, d, *J* = 8.5 Hz), 8.19 (H_9, d, *J* = 8.3 Hz), 8.27 (OH), 8.49 (H_{11}, s), 9.03 (H_2, d, *J* = 4.4 Hz), 10.22 (OH), 12.30 (NH). ^{13}C-NMR ($CDCl_3$): δ 117.64 (C_{12}), 119.43 (C_{14}, C_{17}), 119.90 (C_{15}), 120.14 (C_3), 124.60 (C_5), 125.71 (C_7), 128.22 (C_9), 129.93 (C_6), 130.60 (C_8), 140.36 (C_4), 148.33 (C_{11}), 148.65 (C_{10}), 150.36 (C_{16}), 150.73 (C_2), 163.10 (C_{13}), 168.83 (C=O). EIMS: *m/z* 308.1034 $[M + H]^+$, Calcd for $C_{17}H_{13}N_3O_3$: 308.1035.

4.1.1.3.9. N'-[(1E)-(2,3,4-trihydroxyphenyl)methylidene]quinoline-4-carbohydrazide (**7d**):

Yield 85% (0.450 mmol, 145.6 mg); light yellow solid, M.p. 285-286°C; IR (cm^{-1}): ν_{max} 3496 (Ar-OH), 3190 (N-H), 1652 (C=O), 1517 (C=N), 1465 (C=C_{Ar}), 1250 ((C=O)-N), 797 (C-H_{Ar}). ^{1}H-NMR (DMSO-d_6): δ 6.42 (H_{16}, d, *J* = 8.6 Hz), 6.84 (H_{17}, d, *J* = 8.6 Hz), 7.73 (H_3, d, *J* = 4.3 Hz), 7.79-7.89 (H_7, H_8, m), 8.12 (H_6, d, *J* = 8.4 Hz), 8.16 (OH), 8.20 (H_9, d, *J* = 8.4 Hz), 8.38 (H_{11}, s), 8.74 (OH), 9.04 (H_2, d, *J* = 4.3 Hz), 11.21 (OH), 12.26 (NH). ^{13}C-NMR ($CDCl_3$): δ 108.3 (C_{17}), 111.16 (C_3), 120.15 (C_{12}), 121.72 (C_{16}), 124.70 (C_5), 125.71 (C_7), 128.20 (C_9), 129.91 (C_6), 130.60 (C_8), 133.2 (C_{14}), 140.31 (C_4), 148.0 (C_{10}), 149.52 (C_{11}), 150.74 (C_{15}), 151.45 (C_2), 162.73 (C_{13}), 168.25 (C=O). EIMS: *m/z* 324.0983 $[M + H]^+$, Calcd for $C_{17}H_{13}N_3O_4$: 324.0984.

4.1.1.3.10. N'-[(1E)-(2,4-dihydroxyphenyl)methylidene]isoquinoline-1-carbohydrazide (**8**):

Yield 65% (0.345 mmol, 105.9 mg); yellow solid, M.p. 260-263°C; IR (cm^{-1}): ν_{max} 3461 (Ar-OH), 3268 (N-H), 1665 (C=O), 1511 (C=N), 1460 (C=C_{Ar}), 1200 ((C=O)-N), 750 (C-H_{Ar}). ^{1}H-NMR (DMSO-d_6): δ 6.38 (H_{14}, H_{16}, m), 7.29 (H_{17}, d, *J* = 8.90 Hz), 7.77 (H_7, ddd, *J* = 8.30, 7.07, 1.13 Hz), 7.86 (H_8, ddd, *J* = 8.30, 707, 1.13 Hz), 8.09 (H_6, H_4 $d_{apparent}$, *J* = 8.03 Hz), 8.61 (H_3, H_{11}, m), 8.91 (H_9, d, *J* = 8.51 Hz), 10.16 (OH), 11.55 (OH), 12.34 (NH). ^{13}C-NMR ($CDCl_3$): δ 103.19 (C_{14}), 108.27 (C_{16}), 110.94 (C_{12}), 124.31 (C_4), 126.28 (C_{10}), 126.72 (C_6), 127.75 (C_7), 129.21 (C_8), 131.35 (C_9), 131.86 (C_{17}), 137.03 (C_5), 141.39 (C_3), 150.41 (C_1), 150.60 (C_{11}), 160.06 (C_{15}), 161.42 (C_{13}), 165.50 (C=O). EIMS: *m/z* 308.1035 $[M + H]^+$, Calcd for $C_{17}H_{13}N_3O_3$: 308.1035.

4.2. Biological Activity Assays

The compounds were subjected to evaluation of *in vitro* cytotoxicity on U937 human cells and leishmanicidal and trypanocidal activities on intracellular amastigotes of *L. (V) panamensis* and *T. cruzi*.

4.2.1. *In vitro* cytotoxicity

The cytotoxic activity of the compounds was assessed based on the viability of the human promonocytic cell line U937 (ATCC CRL-1593.2^{TM}) evaluated by the MTT (*3-(4,5-dimethylthiazol-2-*

yl)-2,5-diphenyltetrazolium bromide) assay following the metodology described previously [43]. In brief, cells were grown in 96-well cell-culture dishes at a concentration of 100,000 cells/mL in RPMI-1640 supplemented with 10% FBS and the corresponding concentrations of the compounds, starting at 200 µg/mL in duplicate. The cells were incubated at 37 °C with 5% CO_2 for 72 h in the presence of the compounds and then the effect of the compounds was determined by measuring the activity of the mitochondrial dehydrogenase by adding 10 µL/well of MTT solution (0.5 mg/mL) and incubating at 37 °C for 3h. The reaction was stopped by adding 100 µL/well of 50% isopropanol solution with 10% sodium dodecyl sulfate and incubating for 30 min. Cell viability was determined based on the quantity of formazan produced according to the intensity of color (absorbance) that registered as optical densities (O.D) obtained at 570 nm in a ELISA reader plate (Bio-Rad, Hercules, CA, USA). Cultured cells in the absence of compounds were used as control of viability (negative control), while meglumine antimoniate and amphotericin B were used as control for cytotoxicity (negative and positive controls, respectively). Assays were performed in two independent assays with three replicates per each concentration tested.

4.2.2. *In vitro* leishmanicidal activity

The activity of compounds was evaluated on intracellular amastigotes of *L. (V) panamensis* transfected with the green fluorescent protein gene (MHOM/CO/87/UA140pIR-GFP) [44]. The effect of each compound was determined according to the inhibition of the infection evidenced by both decrease of the infected cells and decrease of intracellular parasite load. Briefly, U-937 human cells at a concentration of 3×10^5 cells/mL in RPMI 1640 and 0.1 µg/mL of PMA (phorbol-12-myristate-13-acetate) were infected with promastigotes in stationary phase growth in a 15:1 parasites per cell ratio and incubated at 34 °C and 5% CO_2 for 3 h. Cells were washed two times with phosphate buffer solution (PBS) to eliminate not internalized parasites. Fresh RPMI 1640 1 mL was added and cells were incubated during 24 h to guarantee multiplication of intracellular parasites.

After 24 h of infection, the culture medium was replaced by fresh culture medium containing each compound at concentrations 20 µg/mL or lower, (based on the cytotoxicity showed previously by each compound). After 72 h, the inhibition of the infection progress was determined. Cells were removed from the bottom plate with a trypsin/EDTA (250 mg) solution. Recovered cells were centrifuged at 1100 rpm during 10 min at 4 °C, the supernatant was discarded and cells were washed with 1 mL of cold PBS and centrifuged at 1100 rpm during 10 min at 4 °C. The supernatant was discarded and cells were suspended in 500 µL of PBS and analyzed by flow cytometry (FC 500MPL, Cytomics, Brea, CA, US) counting 20.000 events. All determinations for each compound and standard drugs were carried out in triplicate, in two independent experiments [43, 45]. Activity of tested compounds was carried out

in parallel with infection progress in culture medium alone and in culture medium with amphotericin B and meglumine antimoniate as leishmanicidal drugs (positive controls). Compounds that showed growing percentages of inhibition higher than 50% al 20 or less μg/mL were then evaluated at four additional concentrations to determine the effective concentration 50 (EC_{50}). Here, infected cells were exposed against each concentration of synthesized compounds during 72 h, then, cells were removed and tested by flow cytometry as described before.

4.2.3. *In vitro* trypanocidal activity

Compounds were tested on intracellular amastigotes of *T. cruzi*, Tulahuen strain transfected with β-galactosidase gene (donated by Dr. F. S. Buckner, University of Washington) [45]. The activity was determined according to the ability of the compound to reduce the infection of U-937 cells by *T. cruzi*. Similar to the procedure described previously, the trypanocidal activity was initially screened at unique concentration $\leq$ 20 mg/ml. Briefly, 100 μL of U-937 human cells at a concentration of 2.5 x 10^5 cells/mL in RPMI-1640, 10% SFB and 0.1 μg/mL of PMA were placed in each well of 96-wells microplate and then infected with log phase growth epimastigotes in 5:1 (parasites per cell) ratio and incubated at 34°C, 5% CO_2. After 24 hours of incubation, 20 μg/mL of each compound were added to infected cells. After 72h of incubation, the effect of all compounds on viability of intracellular amastigotes was determined measuring the β-galactosidase activity by spectrophotometry adding to each well 100 μM CPRG and 0.1% nonidet P-40. After 3h of incubation, plates were read at 570 nm in a spectrophotometer (Varioskan™ Flash Multimode Reader - Thermo Scientific, USA) and intensity of color (absorbance) was registered as optical densities (O.D). Compounds that showed inhibition percentages higher than 50% were evaluated again at four concentrations selected according to the LC_{50} previously obtained for each compound. Infected cells exposed to benznidazol were used as control for trypanocidal activity (positive control) while infected cells incubated in culture medium alone were used as control for infection (negative control). Non-specific absorbance was corrected by subtracting absorbance (O.D) of the blank. Determinations were done by triplicate in at least two independent experiments [46].

4.2.4. Statistical Analysis

Cytotoxicity was determined according to viability and mortality percentages obtained for each isolated experiment (compounds, amphotericin B, meglumine antimoniate and culture medium alone). The results were expressed as 50 lethal concentrations (LC_{50}) that corresponds to the concentration necessary to eliminate 50% of cells and calculated by Probit analysis [47]. Percentage of viability was calculated by Equation 1, where the O.D of control, corresponds to 100% of viability. In turn, mortality percentage corresponds to 100%–% viability:

$$\% \text{ Viability} = (\text{O.D Exposed cells}) / (\text{O.D Control cells}) \times 100 \quad (1)$$

The degree of toxicity was graded according to the LC_{50} value using the following scale: high cytotoxicity: LC_{50} < 100 μg/mL; moderate cytotoxicity: LC_{50} > 100 to < 200 μg/mL and potentially non-cytotoxicity: LC_{50} > 200 μg/mL.

Leishmanicidal activity was determined according to the percentage of infected cells and parasite load obtained for each experimental condition by flow cytometry. The percentage of infected cells was determined as the number of positive events by double fluorescence (green for parasites and red for cells) using dotplot analysis. On the other hand, the parasitic load was determined by analysis of mean fluorescence intensity (MFI) of fluorescent parasites [43]. The parasite inhibition was calculated by equation 2, where the MFI of control, corresponds to 100% of parasites. In turn, inhibition percentage corresponds to 100% – % Parasites. Results of leishmanicidal activity were expressed as EC_{50} determined by the Probit method [47]:

$$\% \text{ Parasite} = (\text{MFI Exposed parasites}) / (\text{MFI Control parasites}) \times 100 \quad (2)$$

Similarly, trypanocidal activity was determined according to the percentage of infected cells and parasite load obtained for each experimental condition by colorimetry. The parasite inhibition was calculated by equation 3, where the O.D of control corresponds to 100% of parasites. In turn, inhibition percentage corresponds to 100% – % Parasites. Results of trypanocidal activity were also expressed as EC_{50} determined by the Probit method [47]:

$$\% \text{ Parasite} = (\text{O.D Exposed parasites}) / (\text{O.D Control parasites}) \times 100 \quad (3)$$

The leishmanicidal or trypanocidal activities were graded according to the EC_{50} value using the following scale: High activity: EC_{50} < 20 μg/mL, moderate activity: EC_{50} > 20 to < 50 μg/mL; potentially non activity: EC_{50} > 100 μg/mL.

The selectivity index (SI), was calculated by dividing the cytotoxic activity and the leishmanicidal or trypanocidal activity using the following formula: $SI = CL_{50}/CE_{50}$. Cytotoxic compound: LC_{50}<100 μg/mL.

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Conflict of interest

The authors declare no conflict of interest.

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Figure and Scheme legends

Figure 1. Structures of some hydrazones with antiprotozoal activity.

Figure 2. Design of quinoline-hydrazone hybrids as antiprotozoal agents.

Figure 3. 4-quinoline and isoquinoline hydrazone derivatives.

Scheme 1. Synthetic pathway to quinoline-hydrazone hybrids.

Table 1. *In vitro* cytotoxicity and antiprotozoal activity of hydrazones

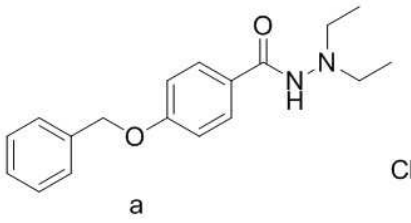
Compound	Cytotoxicity (LC_{50}) [a]	Inhibition intracellular amastigotes growth (%)	
		L. (V) panamensis	***T. cruzi***
6a	16.3 ± 2.2, 56.0	16.8 ± 5.0 [b]	50.4 ± 1.4 [d]
6b	30.6 ± 5.3, 99.6	75.7 ± 14.1 [b]	31.0 ± 2.0 [b]
6c	3.6 ± 0.3, 11.7	64.7 ± 5.6 [e]	41.5 ± 6.0 [d]
6d	48.9 ± 3.8, 159.1	64.0 ± 8.2 [b]	24.0 ± 3.0 [b]
6e	71.5 ± 4.5, 221.2	68.2 ± 2.1 [b]	36.1 ± 1.6 [b]
7a	14.6 ± 1.0, 47.5	47.4 ± 1.2 [c]	27.4 ± 3.2 [b]
7b	10.6 ± 2.1, 34.5	6.4 ± 1.2 [d]	27.7 ± 1.7 [c]
7c	>200.0, >650.8	26.1 ± 3.7 [b]	20.7 ± 3.6 [b]
7d	112.2 ± 4.0, 347.3	21.3 ± 3.5 [b]	15.7 ± 0.8 [b]
8	8.8 ± 1.5, 28.6	62.6 ± 7.8 [d]	24.1 ± 3.7 [c]
Meglumine antimoniate	495.9 ± 55,6	79.4 ± 2.1 [c]	NA[g]
Amphotericin B	42.1 ± 2.0, 45.6	76.0 ± 3.0 [f]	NA[g]
Benznidazole	179.0 ± 4.2, 687.7	NA[g]	87.6 ± 8.4 [b]

Data represent mean value +/- standard deviation; [a] LC_{50}: Lethal Concentration 50 in µg/mL, µM; [b] Dose employed 20 µg/mL; [c] Dose employed: 10 µg/mL; [d] Dose employed: 5.0 µg/mL; [e] Dose employed: 1.0 µg/mL; [f] Dose employed: 0.05 µg/mL; [g] NA: Not applicable.

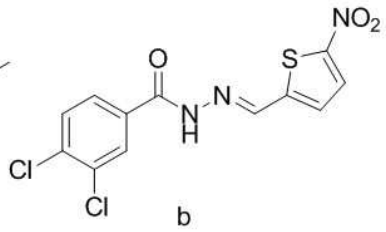
Table 2. Dose-response antileishmanial and antitrypanosomal activities of hydrazones and selectivity

Compound	**EC_{50} [a]**	**IS [b]**
Antileishmanial		
6b	6.5 ± 0.8, 21.2	4.7
6c	0.8 ± 0.0, 2.6	4.5
6d	37.2 ± 3.7, 121.1	2.8
6e	15.4 ± 2.5, 47.6	4.6
8	3.4 ± 0.6, 11.1	2.6
Meglumine antimoniate	6.3 ± 0.9	78.6
Amphotericin B	0.04 ± 0.01, 0.04	1052.5
Antitrypanosomal		
6a	1.4 ± 0.3, 4.8	11.6
6c	6.6 ± 0.3, 4.6	0.5
7b	20.8 ± 0.5, 67.7	0.5
Benznidazole	10.5 ± 1.8, 40.3	17.0

Data represent the mean value +/- standard deviation; [a] EC_{50}: Effective Concentration 50 in μg/mL, μM; [b] IS: Index of Selectivity = LC_{50} / EC_{50}.



a



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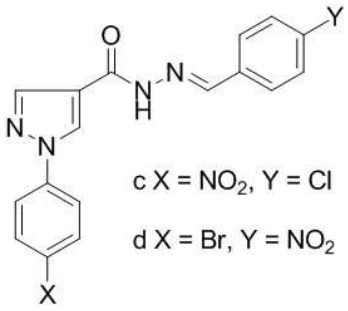
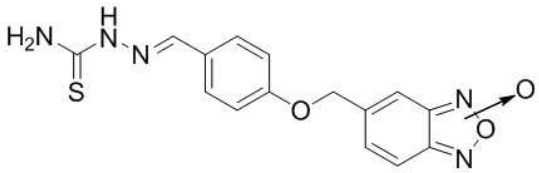


c X = NO_2, Y = Cl

d X = Br, Y = NO_2



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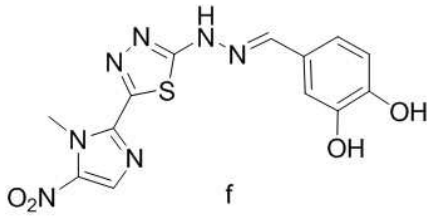
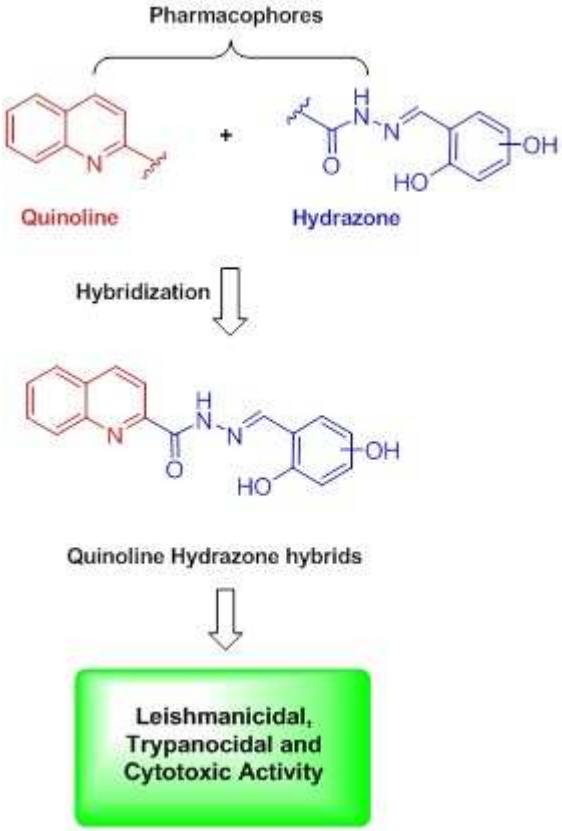
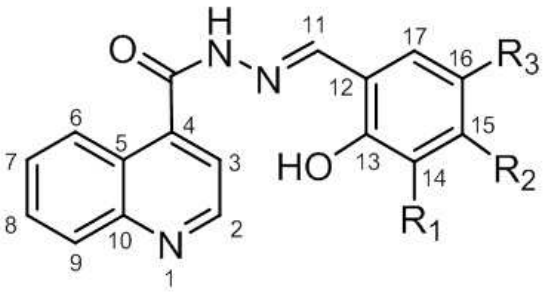
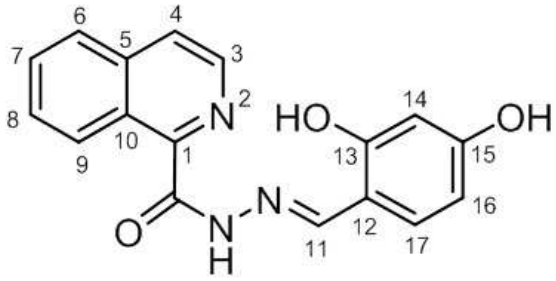


f





7a R^1 = OH, R^2 =H, R^3 = H
7b R^1 =H, R^2 = OH, R^3 = H
7c R^1 =H, R^2 = H, R^3 = OH
7d R^1 = OH, R^2 = OH, R^3 = H



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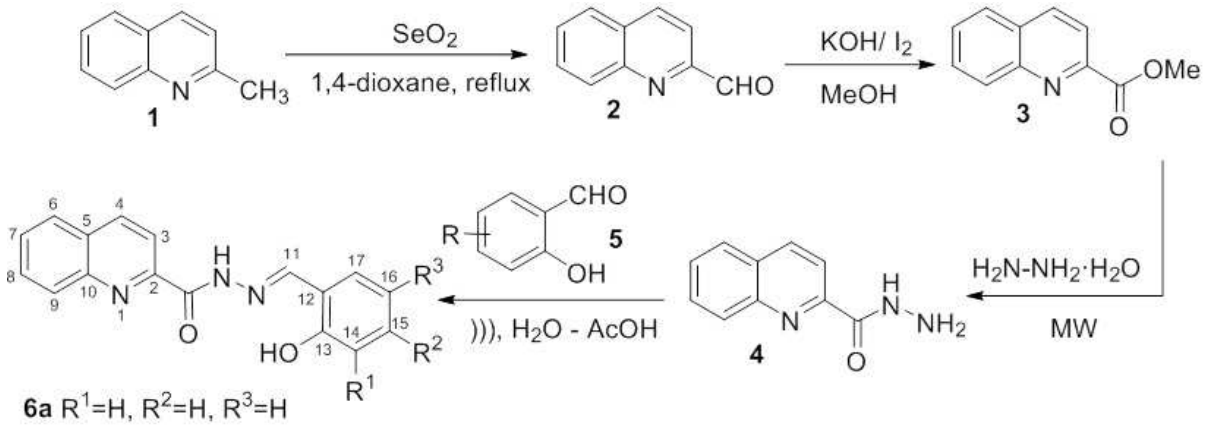

6a R^1=H, R^2=H, R^3=H

6b R^1=OH, R^2=H, R^3=H

6c R^1=H, R^2=OH, R^3=H

6d R^1=H, R^2=H, R^3=OH

6e R^1=OH, R^2=OH, R^3=H

Synthesis, Leishmanicidal, Trypanocidal and Cytotoxic Activity of Quinoline-Hydrazone Hybrids

Highlights

1. We synthesized ten quinoline hydrazones with good yields
2. Five of the compounds obtained were active against *L. (V) panamensis* and three of them against *T. cruzi*
3. The presence of 2-substituted quinoline o isoquinolinic core increase the leishmanicidal activity
4. Hydroxyl group in positions 3 and 4 of the aromatic ring is essential for activity.

Juan Carlos Coa [1], Wilson Castrillón L[1], Wilson Cardona G[1] *, Miguel Carda [2], Victoria Ospina[3], July Andrea Muñoz[3], Iván D. Vélez [3], Sara M. Robledo [3]

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Synthesis, Leishmanicidal, Trypanocidal and Cytotoxic Activity of Quinoline-Hydrazone Hybrids

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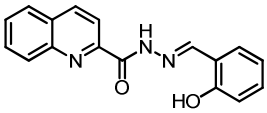
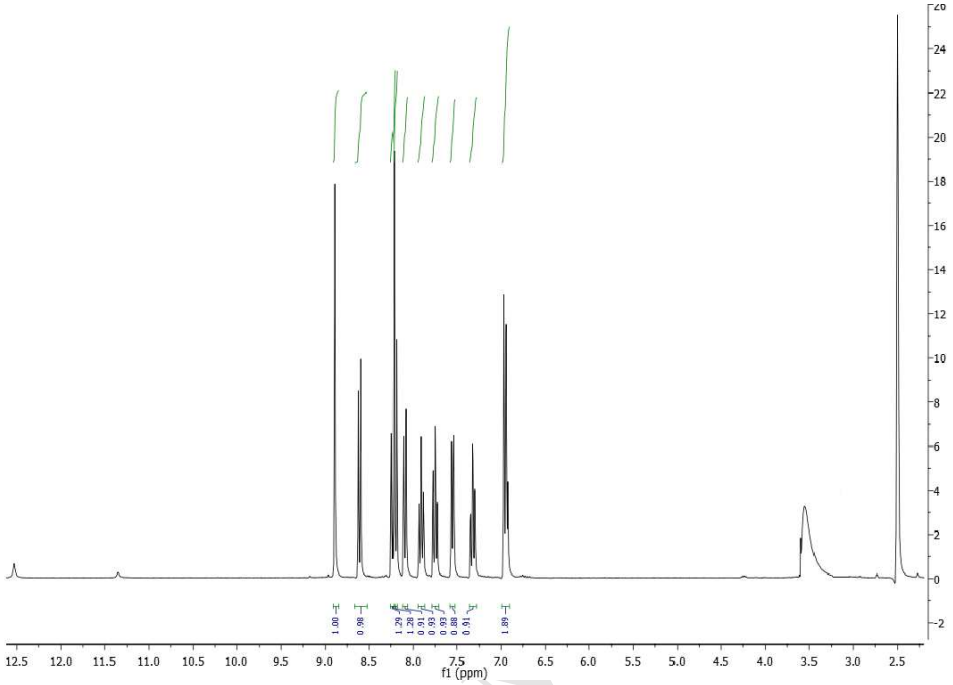
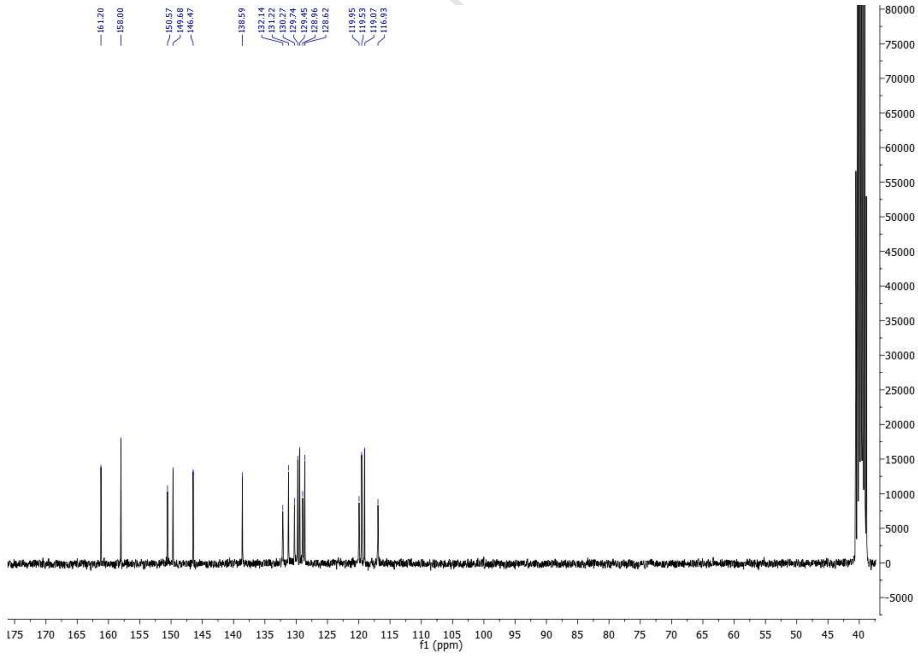
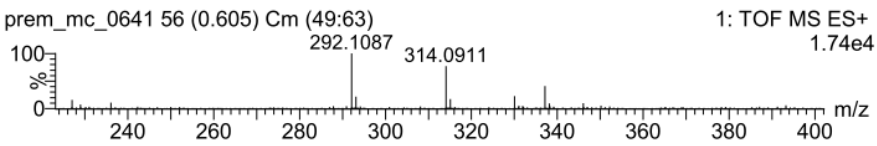
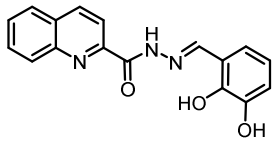
^{1}H, ^{13}C NMR and MS spectra







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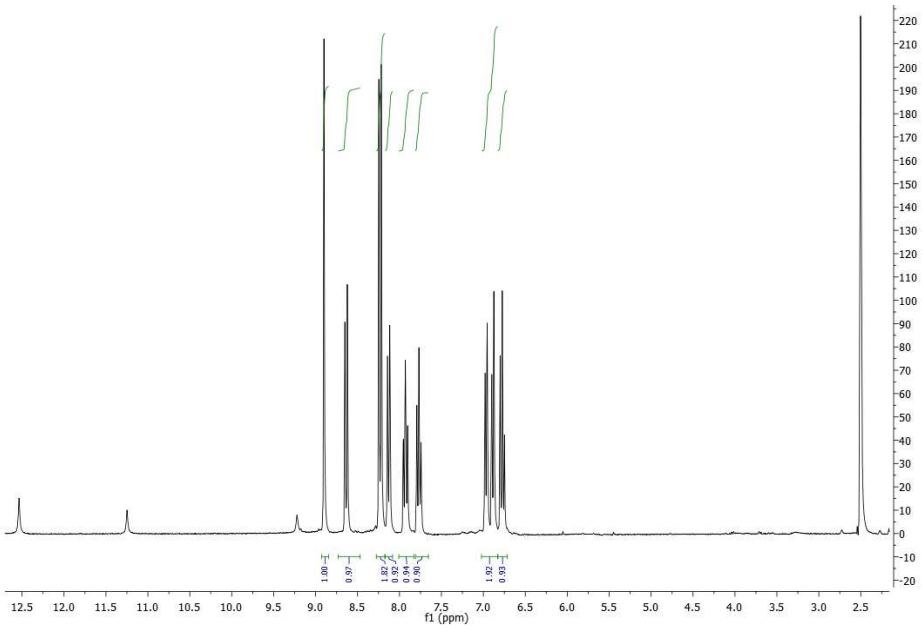
OH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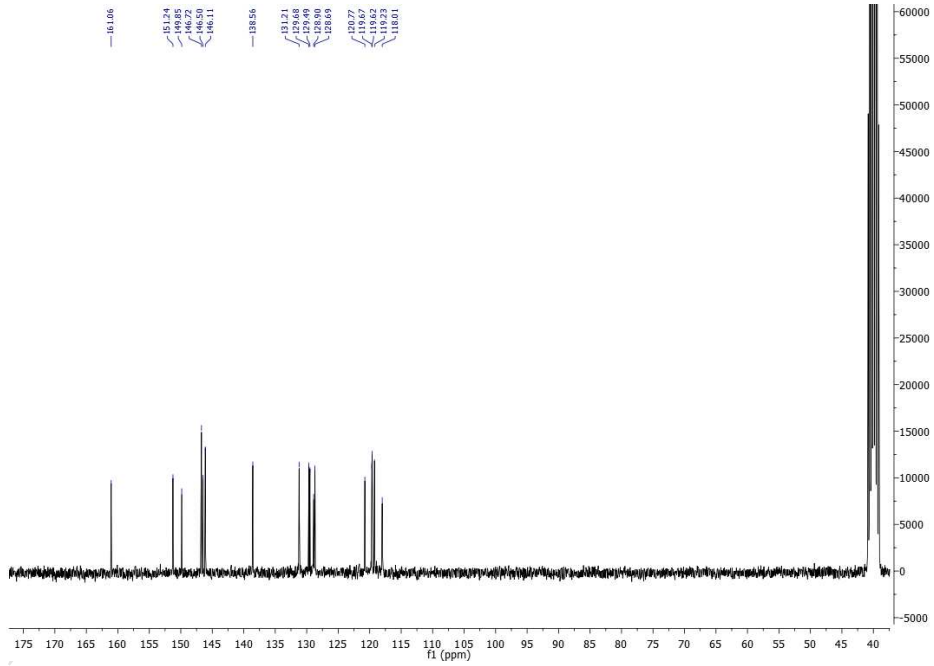
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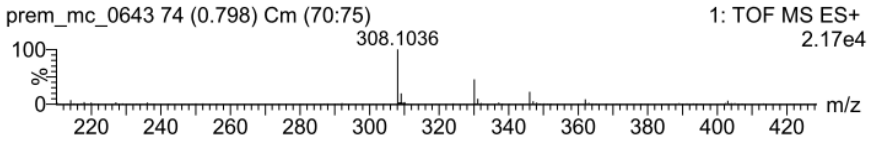
118.01
f1 (ppm)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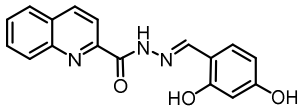
100
%
0
m/z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N
O
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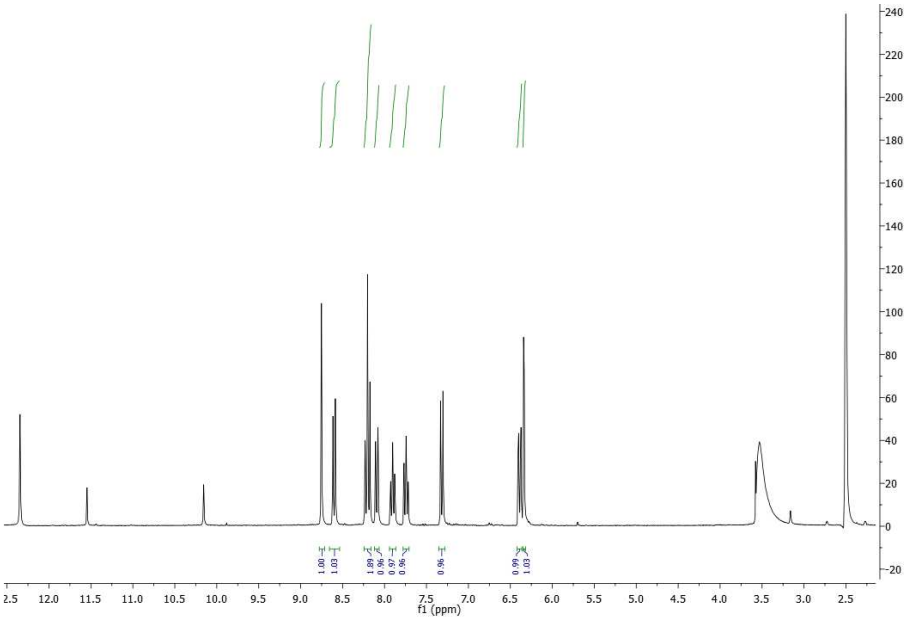
OH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0.96
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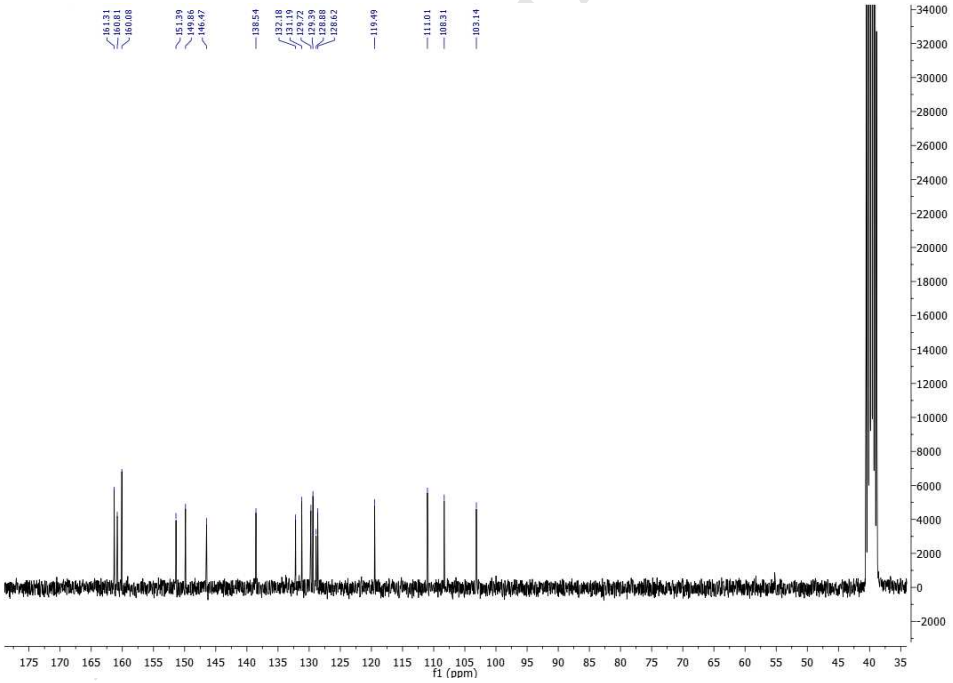
f1 (ppm)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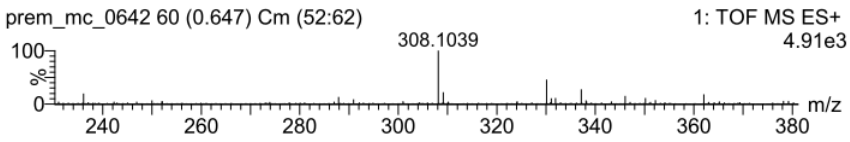
f1 (ppm)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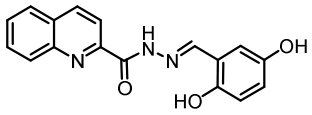
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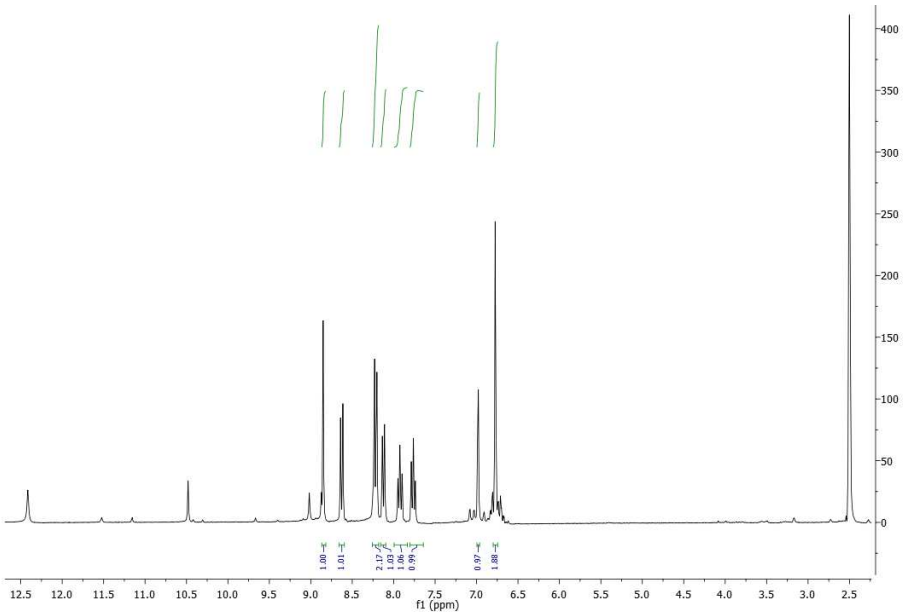
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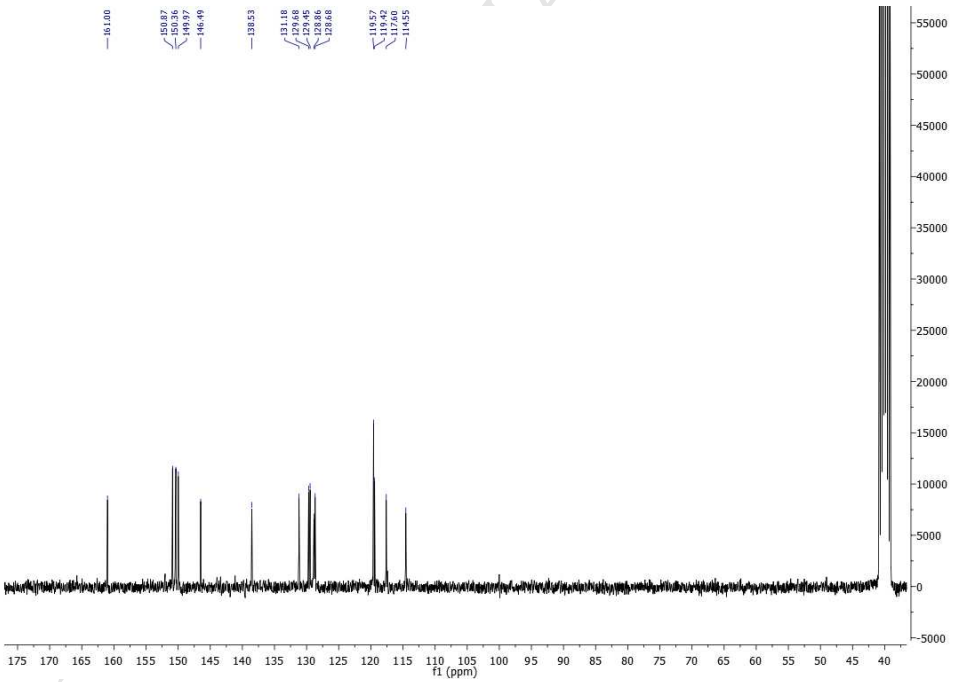
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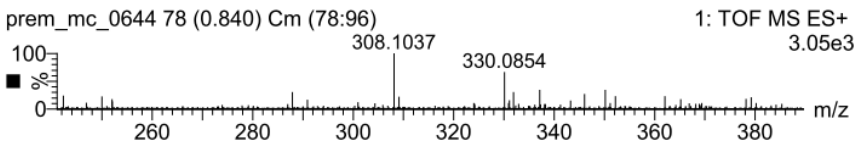
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%
0
m/z
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300
320
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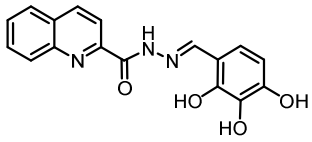
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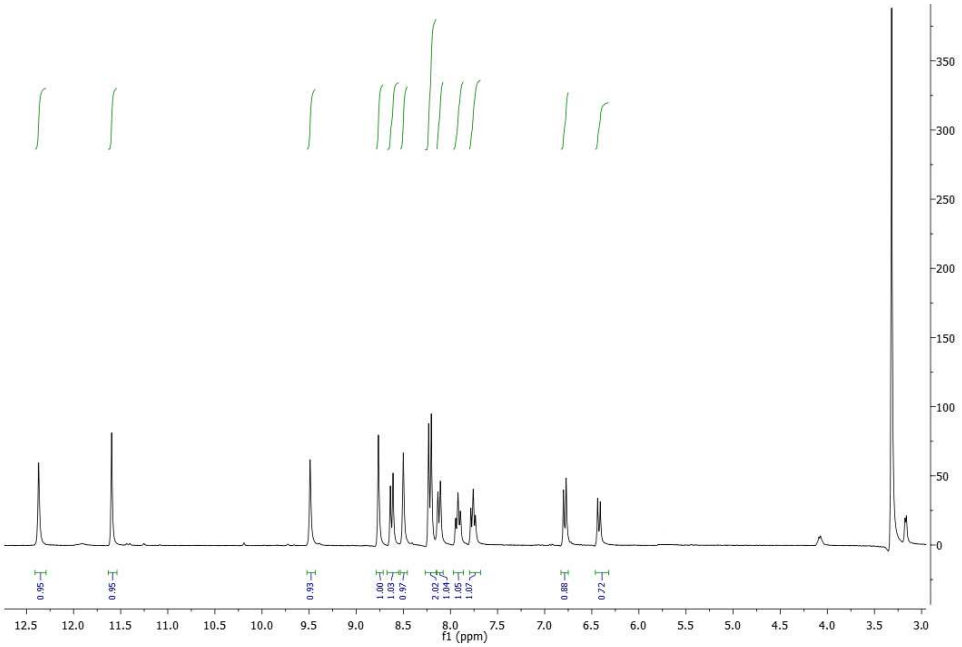
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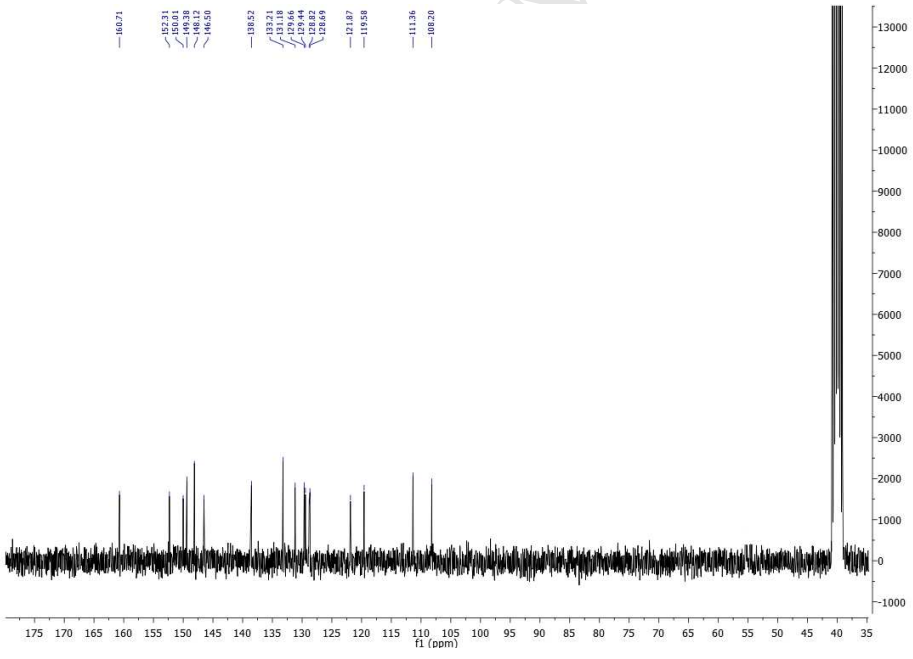
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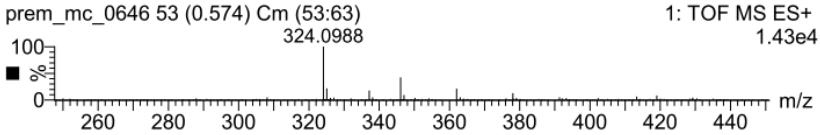
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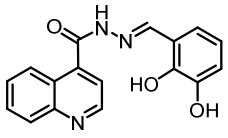
0
m/z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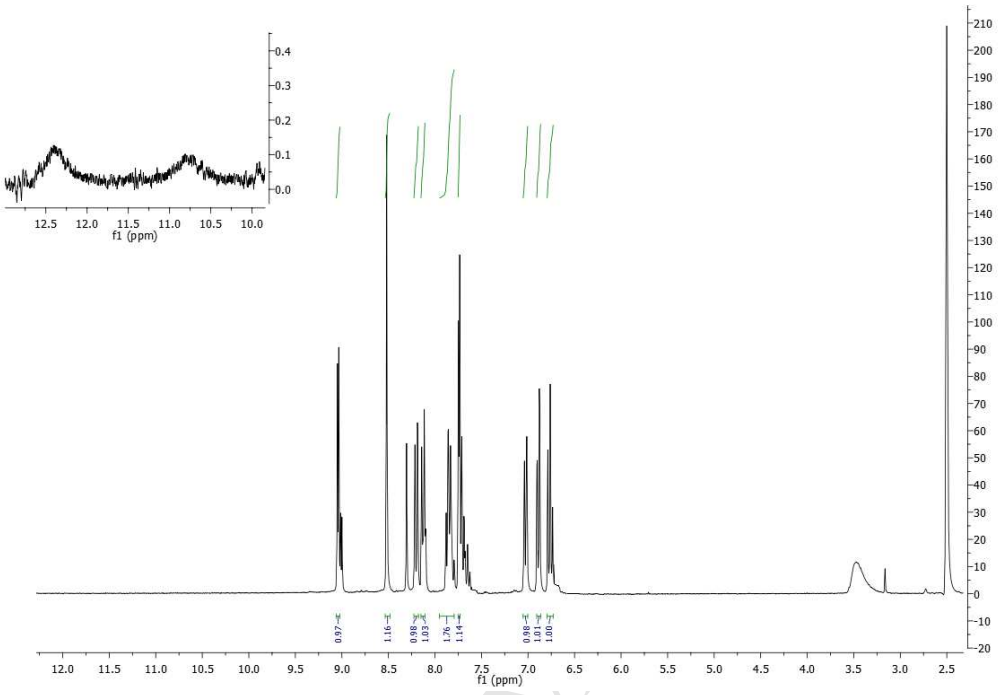
N
N
HO
OH
N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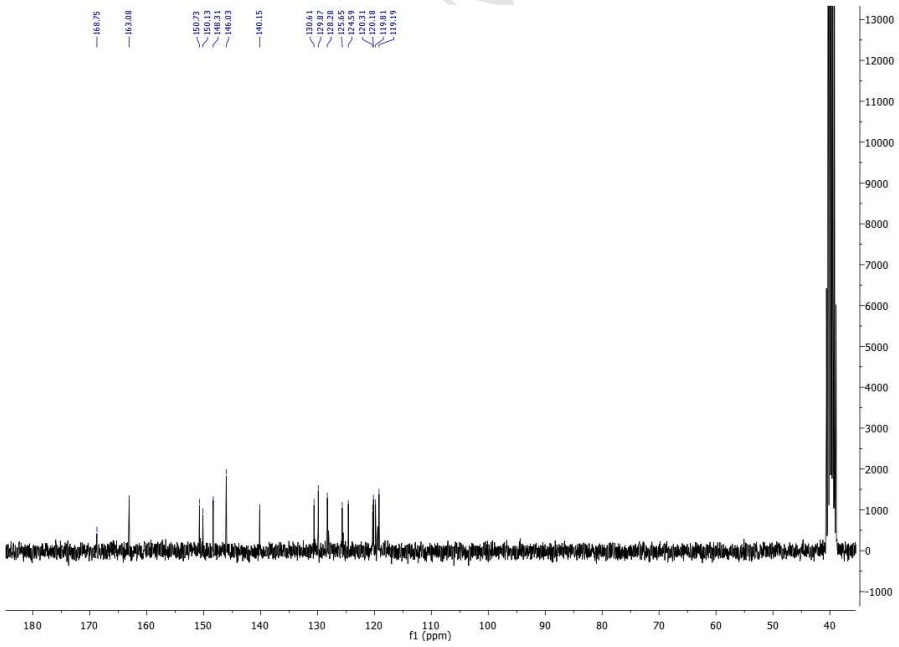
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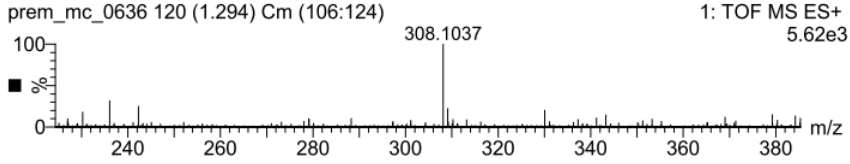
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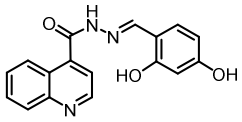
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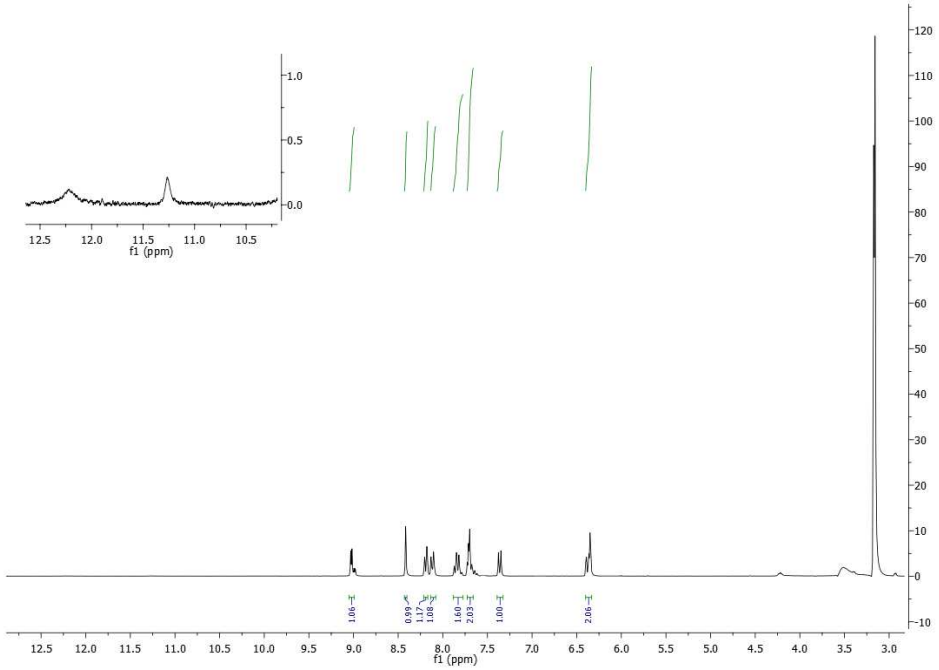
N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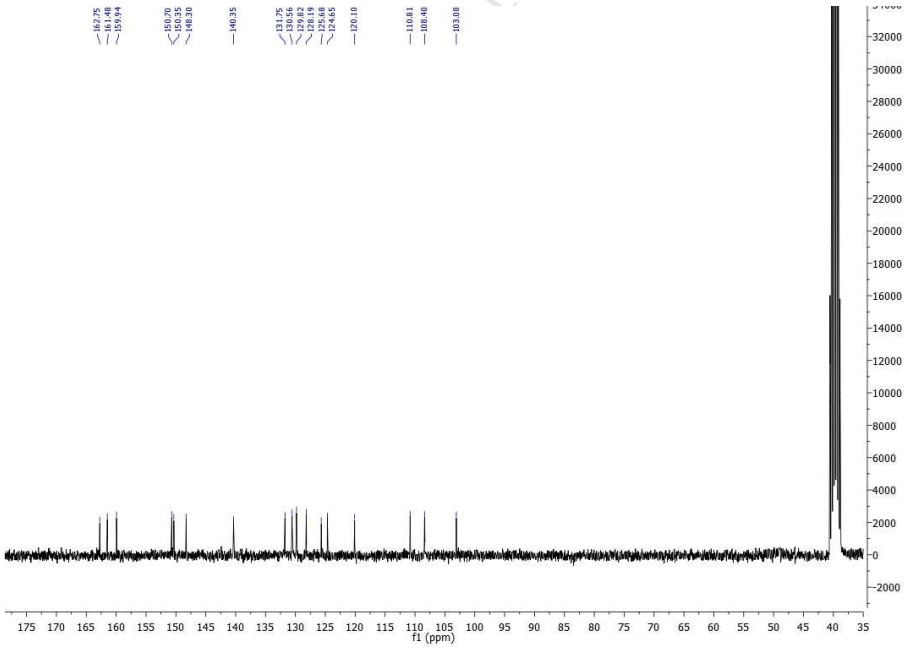
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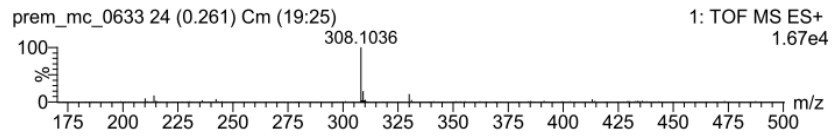
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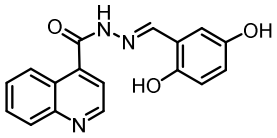
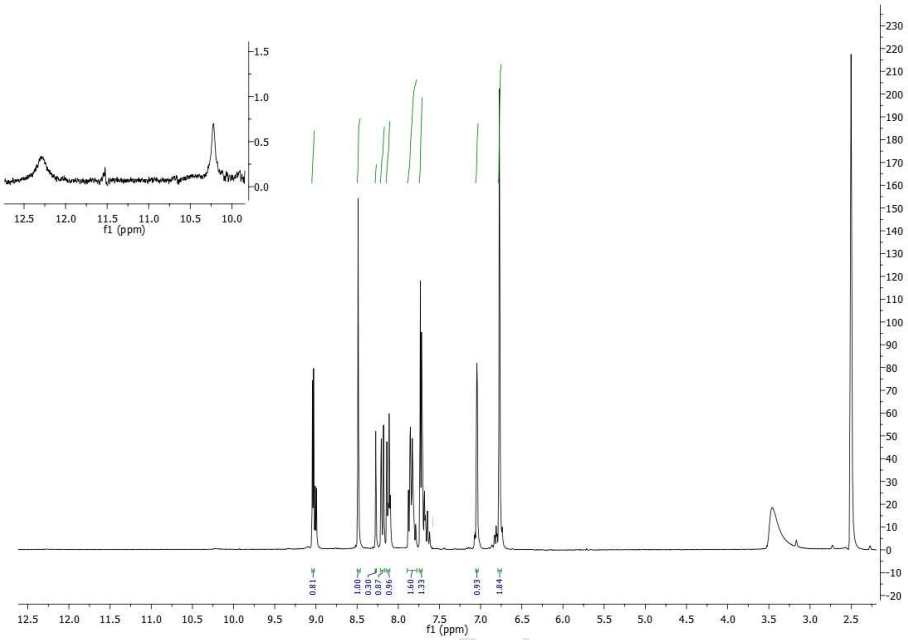
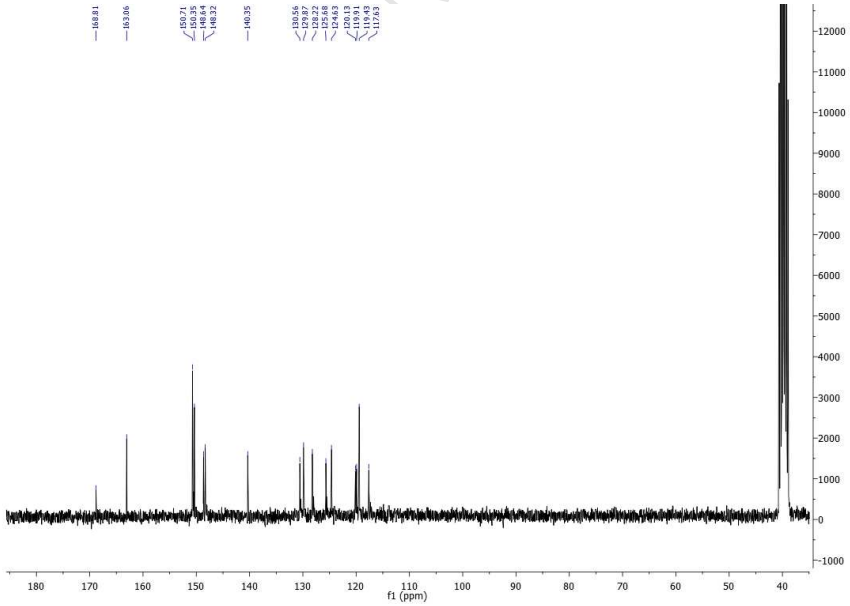
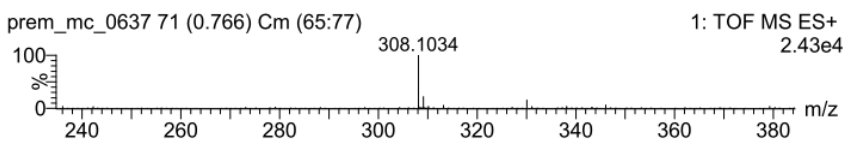
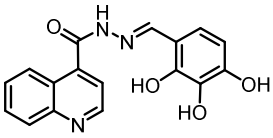
%
0
m/z

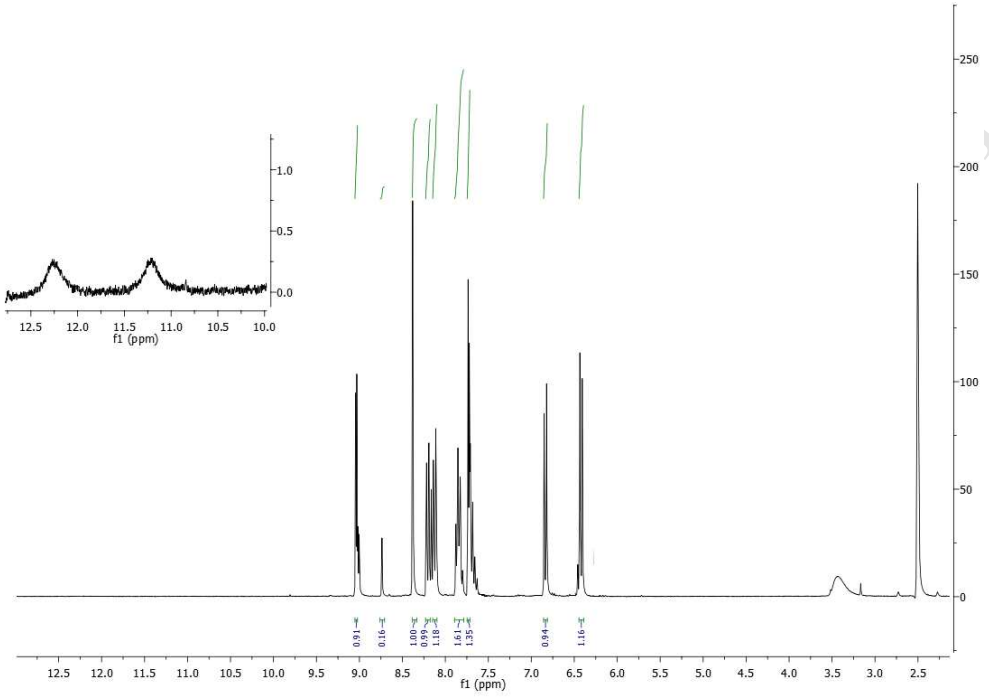

H
N
O
N
OH
HO
N

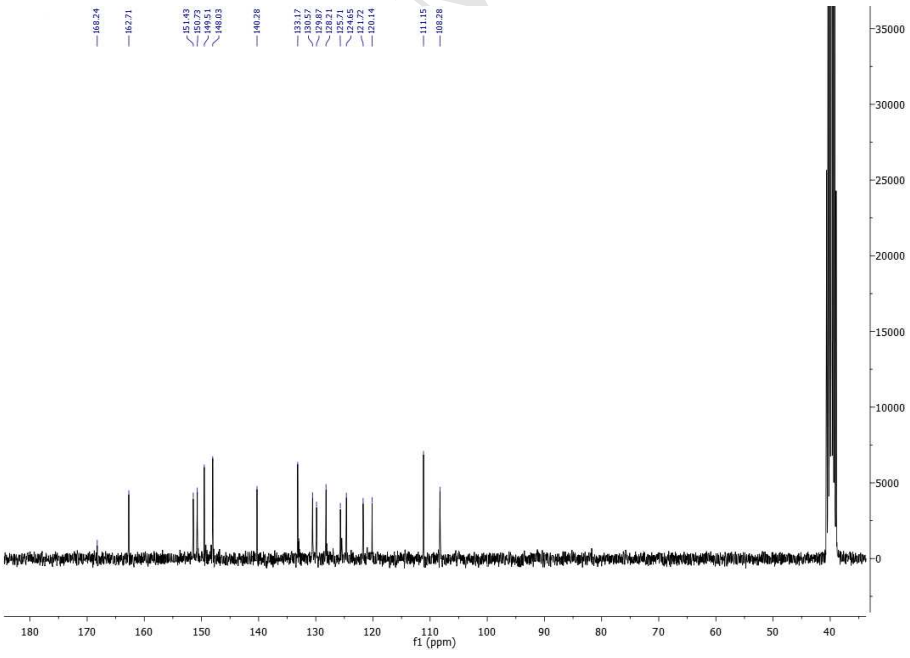

f1 (ppm)

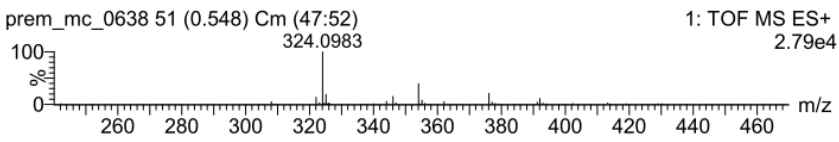

f1 (ppm)

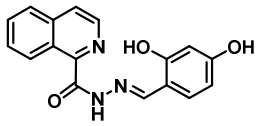

prem_mc_0637 71 (0.766) Cm (65:77)
1: TOF MS ES+
2.43e4
308.1034
100
%
0
m/z

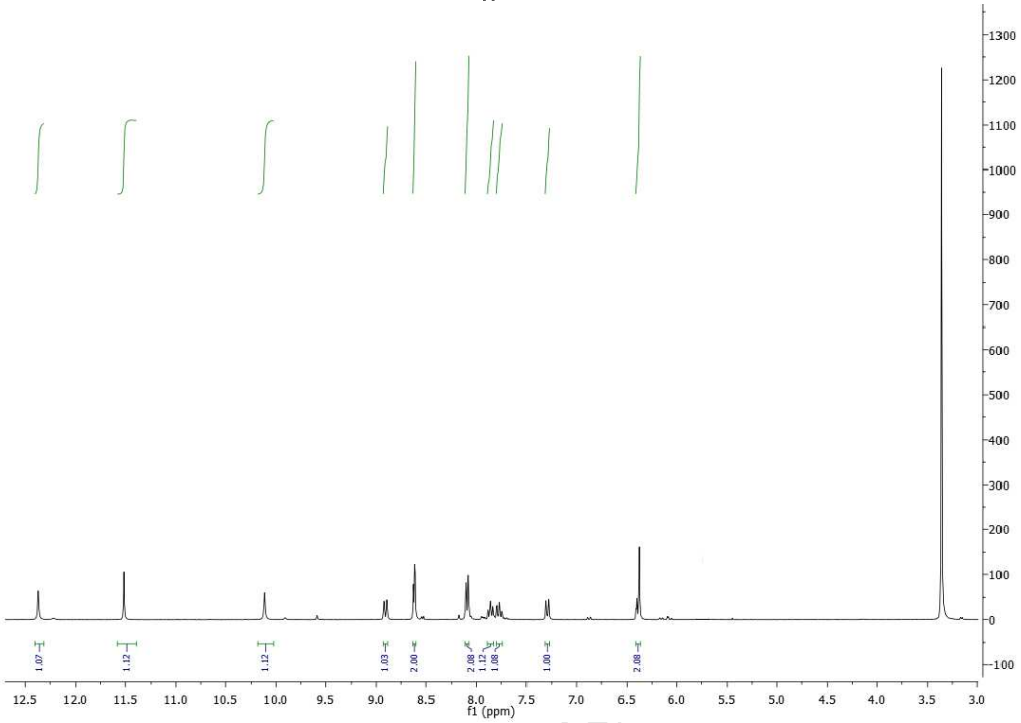

O
H
N
N
HO
OH
HO
N

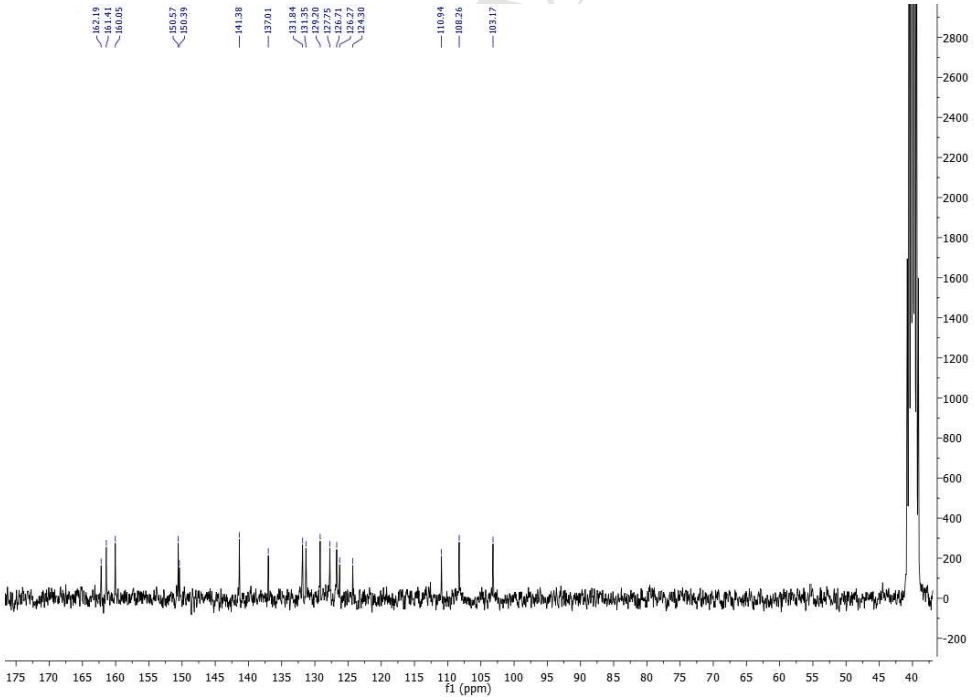

f1 (ppm)
0.91
0.16
1.00
0.99
1.18
1.61
1.35
0.94
1.16

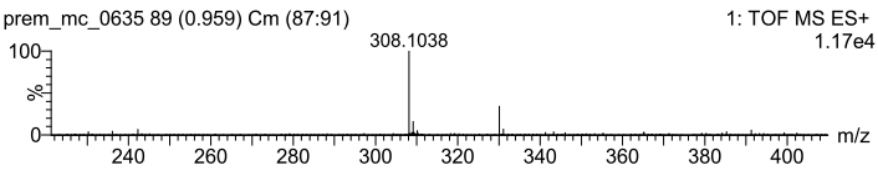

168.24
162.71
151.43
150.73
149.51
148.03
140.28
133.17
130.57
129.87
128.71
125.71
124.65
121.72
120.14
111.15
108.28
f1 (ppm)


prem_mc_0638 51 (0.548) Cm (47:52)
1: TOF MS ES+
2.79e4
324.0983
100
%
0
m/z


N
HO
OH
O
N
H
N


1.07
1.12
1.12
1.03
2.00
2.08
1.12
1.08
1.00
2.08
f1 (ppm)


162.19
161.41
160.05
150.57
150.39
141.38
137.01
131.94
131.35
129.20
127.75
126.71
126.27
124.50
110.94
108.26
103.17
f1 (ppm)


prem_mc_0635 89 (0.959) Cm (87:91)
1: TOF MS ES+
1.17e4
308.1038
100
%
0
240
260
280
300
320
340
360
380
400
m/z

Dose-response curve for some compounds: