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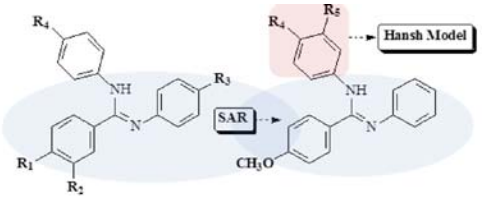

Synthesis, Antileishmanial Activity and Structure-Activity Relationship of 1-*N*-X-phenyl-3-*N*'-Y-phenyl-benzamidines

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Abstract

Two series of *N*,*N*'-diphenyl-benzamidines were synthesized as part of a study to search potential new drugs with antileishmanial activity. These compounds were obtained by anilides in PCl_5 halogenation reaction with generation in situ of the corresponding benzimidoyl chlorides, and subsequently treatment with adequate anilines. The series I showed expressive results of antileishmanial activity, highlighted the compounds **9a** with IC_{50} = 81.28 μM (log IC_{50} = 1.91 μM) against *L. chagasi*, **8e** with IC_{50} = 26.30 (log IC_{50} = 1.52 μM) against *L. braziliensis*. From the results obtained from SAR study (series I), the series II was planned from Craig 2-dimensional map, in which was possible the discovery of the potent compounds, **9v** and **9j** with IC_{50} = 12.60 μM (log IC_{50} = 1.10 μM) and 13.00 μM (log IC_{50} = 1.11 μM), respectively, against *L. amazonensis*. The results obtained from the SAR and QSAR studies indicated the best results when electron-donors groups in the ring attached to amidinic carbon, unlike when electron-withdrawing groups at the phenyl-*N* ring showing inhibitory activity increased. Furthermore, the QSAR model obtained indicated the hydrophobicity as a fundamental property for antileishmanial activity presented by these series.

Keywords*: N*,*N*'-diphenyl-benzamidines; *Leishmania amazonenses; Leishmania chagasi; Leishmania braziliensis;* QSAR; Hansch model.

1. Introduction

Leishmania parasites are small and intriguing organisms that cause leishmaniasis, and the symptoms of these diseases are skin sores that erupt weeks to months after the person affected is bitten by sandflies. Other consequences include fever, damage to the spleen and liver [1-2]. This disease is endemic in some geographical areas of world, where constitutes a serious public health problem [3].

Leishmania amazonensis has been isolated from patients with all the different clinical forms of the disease. The extracellular promastigote stage of this protozoan parasite is introduced into subcutaneous tissue in the human host during the bite of an infected sandfly vector. It is phagocytosed by a mononuclear phagocyte, after which it converts into the obligate intracellular amastigote form [3].

The World Health Organization considers leishmaniasis one of the most serious diseases worldwide caused by protozoan parasites. However, the control of this disease remains a problem; the available antileishmanial drugs still rely on the highly toxic pentavalent antimonials (meglumine antimoniate, Glucantime and sodium stibogluconate, Pentostam), which cause serious side effects and require long-term treatment [4].

Second-line drugs include pentamidine (**1**) and amphotericin B, but these drugs have not experienced widespread use because of toxicity and cost. Recently, the oral drug miltefosine (**2**), an alkylphosphocoline was approved for the treatment of human visceral *Leishmania* infections, but it present high cost and a long half-life (100 - 200 h) in humans and low therapeutic ratio, the characteristics that could encourage the development of resistance. In addition, the miltefosine is not suitable for use during pregnancy because of teratogenecity and also cause mild to severe gastrointestinal side effects (Figure 1) [5].

Please, insert the Figure 1

Since the chemotherapy against leishmaniasis is still inefficient, there is an urgent need for the development of new, efficient, and safe drugs for the treatment of this disease.

Amidines are much used in synthesis. In some cases for the preparation of acyclic compounds, but mostly for the synthesis of heterocyclic compounds such as aziridines, pyrroles, oxazoles, oxadiazoles, pyridines, pyrimidines, imidazoles and triazines [6]. They combine the properties of an azomethine-like and C-N single bond having some partial double bond character [7].

The amidines are important medical and biochemical agents. Their anti-inflammatory, antiviral, antifungal, antibacterial, antibiotic, antihypertensive and anesthetic activities have been reported [8]. We have previously demonstrated that *N*,*N*'-diphenyl-*p*-methoxy-benzamidine (**3**) was effective against *Leishmania amazonensis* promastigotas and axenic amastigotes and *Trypanosoma evansi* trypomastigotes. Besides it was the most effective derivative in the parasite-macrophage interaction [9-11].

Then, considering the previous results of compound **3** against trypanosomatid parasites and the importance of the discovery of new compounds for leishmaniasis treatment, our research group describes the synthesis and the *L. amazonensis* inhibitory activity of two new series of amidine derivatives. The series I was based in the methoxy moiety importance considering the compound **3,** the most active in the *N*,*N*'-diphenyl-*p*-X-benzamidine series [9-11] led us to prepare 8 derivatives with mono, di and tri-methoxy substituents on *para* and *meta* position in the *N*-phenyl groups. The results of antileishmania assays supplied a Structure-Activity Relationship (SAR) study and the Craig Graphic [12] allowed the synthesis of series II with 14 derivatives (Figure 2).

Please, insert the Figure 2

2. Chemistry

The route adapted [8] for the synthesis of the two series is outlined in Scheme 1. Initially the benzanilides (**6**) were prepared from of the corresponding aniline (**4)** and the appropriately substituted acid chlorides (**5**) supplying excellent yields (85-90%). The benzanilides (**6**) were purified by ethanol recrystallization.

After, the benzanilides were converted to benzimidoyl chlorides, *in situ,* by treatment with halogenating reagent (PCl_5) under reflux in dry toluene for 8h, that subsequently reacts with aniline dissolved in dry toluene to furnish the compounds **8a-v** in good yields (60-85%). The reactions were monitored by thin layer chromatography (TLC) and the benzamidinic chlorides (**8a-v**) were purified from acetone. Following, **8a-v** were treated with aqueous solution of $NaHCO_3$ (5%, m/v) submitted to $CHCl_3$ extraction affording the target compounds **9a-h** (series I) and **9i-v** (series II).

The formation of the benzimidoyl chloride *in situ* using dry toluene [13-14] demonstrated to be a more effective way than solvent-free [8, 15] to obtain *N*,*N*'-diphenyl-benzamidine derivatives, because it generated in high purity (it was verified by TLC). This small change was significant to affording the target compounds with high degree of purity (≥ 95%, CG-MS). The compounds that were obtained with less than 95% of purity were recrystallized from methanol afforded needle crystals.

Please, insert the Scheme

The infrared spectra show the disappearance of the benzanilide (**6**) ν (C=O) band at 1646-1655 cm^{-1} and the new ν (C=N) band at 1587-1632 cm^{-1} resulted from benzamidine evidence in agreement with the literature [15].

The values of ^{1}H and ^{13}C NMR spectra (supplementary data) permitted the full characterization of all compounds. The ^{13}C NMR chemical shifts indicated peak at 154-158 ppm corresponding to C=N. As the expected the central carbon atom in benzamidines resonates at higher field than in the corresponding benzanilides (164-165 ppm) and benzamidinium cations (161-163 ppm).

The structures of compounds **9b** and **9o** were determined by single crystal X-ray diffraction. Asymmetric unit of **9o** contains two independent molecules, which are different rotamers (**A** and **B**) with different methoxy group position (Figure 3).

The solid-state structure reveals a noncentrosymmetric molecule, with an *E* configuration around the C=N double bond. The crystal structures **9b**, **9o**-rotamer **A** and **9o** -rotamer **B**, respectively, shows lengthened C=N (1.282, 1.283 and 1.281) and shortened C-N (1.371, 1.364 and 1.372) bonds, a feature of n-π conjugation [15], an important characteristic of this moiety. In the crystal structures, molecules are linked *via* N-H….O interactions. It is already known that the difference between the C-N and C=N distances is related to the degree of delocalization in the N-C=N skeleton. An interesting fact that can be observed in these results was the electronic delocalization also depends of conformation, the compound **9o**-rotamer A, 0.081Å and **9o**-rotamer B, 0.091 Å.

Please, insert the Figure 3.

3. Results and Discussion

3.1. Antileishmanial Assays

The 50 % growth inhibitory activity value, IC_{50}, of each compound (**9a-v**) was determined using *L. amazonensis* in the evolutive form of promastigotes. For compounds **9a, 9e** and **9f**, and its salt forms (**8a, 8e** and **8f**), the *L. braziliensis* and *L. chagasi* inhibitory activities were evaluated. The remaining parasites were counted in a Naubauer chamber, and the IC_{50} /24h values were determined by linear regression, relating percentage and log of drug concentration in μg/mL and μM, as shown in Tables 1, 2 and 3.

The compound with one methoxy moiety (**9a**) and two methoxy moieties (**9e)** in phenyl group attached at amidinic carbon, and with two methoxy moieties (**9f)** in phenyl groups linked to *N* and *N´* atoms were evaluated against promastigotas of *L. amazonenesis*, *L. braziliensis* and *L. chagasi*, and also against macrophages in order to evaluate the toxicity (Table 1). The results for **9a**, in neutral form, IC_{50} = 1.91 μM presented the best antileishmanial activity against *L. chagasi*, in similar potency of the pentamidine (IC_{50} = 1.76 μM), reference drug, already assayed against *L. braziliensis* the compound **9e**, both neutral (IC_{50} = 1.52 μM) and salt form **8e** (IC_{50} =1.42 μM) presented comparable values with the pentamidine (IC_{50} = 1.36 μM). However, the results against *L. amazonensis* to **8f (**IC_{50} = 1.62 μM) presented the best antileishmanial activity (pentamidine, IC_{50} = 1.05 μM). Interesting, the macrophage toxicity presented of **9a** and **9f** was the 15% and 0%, respectively, while the pentamidine presented 100%, at 320 μg/mL of concentration. This SAR study made possible to verify that the substitutions on two *N*-phenyl rings do not

increase the antileishmanial activity against *L. amazonensis* and furthermore cause toxicity increase (Table 1). Then faced this data, this series I (**9a-h**) was proposed and evaluated against *L. amazonensis*. The results obtained demonstrated that the **9a** and **9h** highlighted among the compounds substituted with methoxy groups presenting values of IC_{50} = 1.85 μM and IC_{50} = 1.60 μM, respectively (Table 2).

Please insert the Tables 1 and 2

From the results obtained of SAR study, the new series II was planned, with methoxy group linked in the *para* position of the phenyl ring attached to amidinic carbon, because as it can be seen, the compound **9a** presented low toxicity, as well as the compound **9f**. Thus, it was selected the appropriate substituent groups with a wide range of both lipophilicity (π) and Hammett electronic (σ) parameters from 2-dimensional map proposed by Craig [12].

The compounds of series II were assayed against *L. amazonensis*, the results are showed in the Table 3. The most active compounds were the **9j** (R_3 = H; R_5= Cl; IC_{50} = 1.11 μM); **9m** (R_3 = Br; R_5 = H; IC_{50} = 1.34 μM); **9o** (R_3 = H; R_5 = NO_2; IC_{50} = 1.22 μM) and highlighted the compound **9v** (R_3 = Cl; R_5 = NO_2; IC_{50} = 1.10 μM) that presented inhibitory activity close to reference drug, pentamidine (IC_{50} = 1.05 μM). The results obtained from series II indicated that unlike the series I, when the best results were observed to electron donors moieties attached to benzamidine ring (methoxy group), the inhibitory activity increased when electron-withdrawing groups at the phenyl-*N* ring.

Please, insert the Table 3.

3.2. Hansch Model

To try to understand the intrinsic multivariate nature of the results of the antileishmanial activity presented by compounds, it decided to calculate the electronic, steric and lipophilicity properties represented for polarizability (POLZ), superficial tension (ST), volume molar (VM), molar refractivity (MR) and log *P* descriptors (Table 4). All these parameters were calculated from the ACDLabs software package (version 12.0), because Spessard [17] demonstrated that this is a good software to simulate these parameters. From the results was established the Hansh model [18-19].

Please, insert the Table 4.

The models of 2D-QSAR were obtained from of multiple linear regression (MLR) utilized BuildQSAR software [20].

After to analyze several models, it was possible to indicate a model with statistics parameters expressive, so much in the quality of the adjustment of the data in the model (r^2 = 0.85; F = 24.90) as in the predictability of this (q^2 = 0.74), according to the works of Erikssons *et al* [21] (Equation 1).

Equation 01.

$$\log 1/IC_{50} = 1.04\ (\pm 0.29)\log P^2 - 10.80\ (\pm 3.23)\log P - 0.04\ (\pm 0.02)MR + 29.04\ (\pm 9.98)$$

$$n = 18;\ r^2 = 0.85;\ s = 0.26;\ F = 24.90;\ q^2 = 0.74$$

The 2D-QSAR model (Equation 01) demonstrates a parabolic relationship with log *P*, and the linear dependence of MR. The lipophilicity presents larger importance that the refractivity molar due to your largest coefficient. The model indicates that the N-phenyl-N'-phenyl-benzamidines reaches good results of antileishmanial activity with molecules

with small volume and it lowers polarity, since the refractivity molar is an ambiguous descriptor, that combines as much volume effect as of polarizability, according to Verma & Hansch [22]. The proposed model presented 4 outliers, the compounds **9a**, **9k**, **9n** and **9o**.

4. Conclusions

The new series of *N*,*N'*-diphenyl-4-methoxybenzamidine derivatives showed important antileishmanial activity, especially when compared with the licensed drug, pentamidine, highlighted the compounds **9a** with IC_{50} = 81.28 μM against *L. chagasi*, **8e** with IC_{50} = 26.30 μM against *L. braziliensis*, and **9v** and **9j** with IC_{50} = 12.60 and 13.00 μM respectively, against *L. amazonensis*. Also, it was observed that the inhibitory activity increase when electron-withdrawing groups are attached on the *N*-phenyl ring, furthermore, the QSAR model obtained were satisfactory showing that the hydrophobicity is the fundamental property for antileishmanial activity. The results of the antileishmanial activity presented by *N*,*N'*-diphenyl-benzamidines indicated a promising new class of leishmanicidal drugs.

5. Experimental Section

5.1. Chemistry

5.1.1. Reagents and Instruments

Melting points were determined on a Kofler hot plate apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 1600 FTIR spectrophotometer using potassium bromide tablets. ^{1}H and ^{13}C NMR spectra were obtained in a Brucker Avance-II

400 or Avance-III 500 spectrometers, with tetramethysilane as the internal reference, in DMSO-d_6 as solvent; the chemical shifts were reported in ppm. Mass spectrums were recorded using Saturn GC-MS - CP-SIL8CB (30m x 25mm x 25mm). Reactions were monitored on Merck silica gel 60 F254 aluminum sheets. TLC spots were visualized by inspection of plates under UV light (254 and 365 mm). All commercial reagents were obtained from Aldrich or Across Co. and used without any further purification, only aniline was distillated.

*5.1.2. N-R_2-phenyl-R,R_1-benzamides (**6a-d**)*

These compounds were prepared and characterized according to the literature [23-27].

*5.1.3. General Procedure for the Preparation of the N-R_2-phenyl-N'-R_3,R_4-phenyl-R,R_1-benzamidine (**9a-v**).*

A mixture of *N*-R_2-phenyl-R,R_1-benzamide (**6a-d**) (2.25 mmol), and phosphorous pentachloride (PCl_5) (2.25 mmol) was refluxed in dry toluene for 8 h under N_2 atmosphere furnished, *in situ*, the benzimidoyl chlorides. Subsequently, was added drop wise to mixture the corresponding aniline (**4**) (2.25 mmol) in dry toluene reflux. After, the mixture was stirred for 3 h; was cooled and the precipitate was filtered afforded the corresponding benzamidinic chlorides (**8a-v**). The **8a-v** were sequentially washed with acetone (5 x 5 mL), and neutralized with $NaHCO_3$ 5% (m/v) under stirred at room temperature for 4 h. Finally, the mixture was extracted with $CHCl_3$, dried and concentrated to afford the target compounds (**9a-v**) purified by recrystallization from ethanol.

*5.1.3.1. N,N'-Diphenyl-4-methoxybenzamidine (**9a**).* Yield 87%; mp 122-124 °C (118-120 °C [8]); IR (KBr): ν 3312, 2927, 2835, 1627, 1591, 1533, 1252, 1030, 789 cm^{-1}; ^1H NMR

(DMSO-d_6): δ 9.10 (s, 1H), 7.84 (d, *J* = 8.0 Hz, 2H), 7.26 (d, *J* = 8.0 Hz, 1H), 7.21 (d, *J* = 8.0 Hz, 2H), 7.04 (t, *J* = 8.0 and 4.0 Hz, 2H), 6.95 (t, *J* = 8.0 and 4.0 Hz, 1H), 6.84 (t, *J* = 8.0 Hz, 2H), 6.75 (t, *J* = 8.0 and 4.0 Hz, 1H), 6.57 (t, *J* = 4.0 Hz, 2H), 3.71 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 159.5, 154.4, 150.8, 141.4, 130.5, 128.3, 126.9, 122.1, 121.7, 119.5, 113.5, 55.1. MS, m/z (%): 302 ($M^{+\cdot}$, 13), 286 (1), 210 (100), 77 (15), 51 (9).

*5.3.1.2. N,N'-Diphenyl-3-methoxybenzamidine (**9b**).* Yield 65%; mp 210-213 °C; IR (KBr): ν 3349, 2839, 1625, 1590, 1528, 1325, 1269, 1022 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 9.21 (s, 1H), 7.88 (sl, 2H), 7.21 (dd, *J* = 6.0 Hz, 3H), 7.06 (d, *J* = 8.0 Hz, 3H), 6.88 (t, *J* = 8.0 Hz, 4H), 6.61 (d, *J* = 8.0 Hz, 2H), 3.65 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 158.7, 154.3, 136.0, 129.1, 128.3, 121.8, 121.1, 119.6, 114.7, 114.5, 55.0. MS, m/z (%): 302 ($M^{+\cdot}$, 100), 287 (1), 210 (55), 75 (5), 51 (3). Anal. Calcd. for $C_{20}H_{18}N_2O$: C, 79.44, H, 6.00, N, 9.26. Found: C, 80.02, H, 5.90, N, 9.30.

*5.3.1.3. N-(4-methoxyphenyl)-N'-phenyl-benzamidine (**9c**).* Yield 80%; mp 110-111 °C (114 °C [27]); IR (KBr): ν 2935, 2839, 1632, 1513, 1242, 1026, 730, 639 cm^{-1}; ^{1}H NMR (DMSO-d-$_6$): δ 9.09 (s, 0.5H), 9.03 (s, 0.5 H), 7.76 (d, *J* = 8.0 Hz, 2H), 7.28 (d, *J* = 8.0 Hz, 3H), 7.01 (t, *J* = 8.0 and 4.0 Hz, 1H), 6.85 (d, *J* = 4.0 Hz, 1H), 7.2 (d, *J* = 4.0 Hz, 1H), 6.60 (s, 1H), 6.60 (s, 1H), 6.55 (s, 1H), 6.49 (s, 1H), 3.71 (s, 1.7H), 3.60 (s, 1.3H); ^{13}C NMR (DMSO-d_6): δ 161.9, 158.1, 136.2, 133.0, 132.3, 130.5, 128.6, 126.8, 125.0, 122.2, 114.2, 55.4. MS, m/z (%): 302 ($M^{+\cdot}$, 50), 286 (1), 180 (75), 77 (32), 51 (16).

*5.3.1.4. N-(4-methoxyphenyl)-N'-phenyl-3-methoxybenzamidine (**9d**).* Yield 70%; mp 127-128 °C; IR (KBr): ν 3349, 2948, 2834, 1634, 1593, 1541, 1335, 1240, 1034 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 9.05 (s, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.77 (d, *J* = 6.0 Hz, 1H), 7.20 (m, *J* =8.0 Hz, 2H), 7.03 (m, *J* = 8.0 Hz, 2H), 6.84 (m, *J* = 8.0 Hz, 5H), 6.58 (m, *J* = 4.0 Hz, 2H), 3.71 (s, 3H), 3.64 (sl, 3H); ^{13}C NMR (DMSO-d_6): δ 158.7, 154.4, 136.2, 129.2, 128.3, 122.9, 122.2 121.2, 119.2, 114.6, 55.0. MS, m/z (%): 332 ($M^{+\cdot}$, 63), 240 (100), 91 (1), 77 (28), 51 (15). Anal. Calcd. for $C_{21}H_{20}N_2O_2$: C, 75.88, H, 6.06, N, 8.43. Found: C, 76.05, H, 5.91, N, 8.60.

*5.3.1.5. N-(4-methoxyphenyl)-N'-phenyl-4-methoxybenzamidine (**9e**).* Yield 65%; mp 121-122 °C (124°C [28]) ; IR (KBr): ν 3334, 3037, 2950, 2833, 1626, 1592, 1533, 1505, 1244, 1030, 832 cm^{-1}, ^{1}H NMR (DMSO-d_6): δ 8.98 (s, 0.56H), 8.93 (s, 0.44H), 7.84 (d, *J* = 10.0 Hz, 1H), 7.75 (d, *J* = 5.0 Hz, 1H), 7.24 (d, *J* = 5.0 Hz, 1H), 7.19 (d, *J* = 10.0 Hz, 2H), 7.02 (t, *J* = 10.0 and 5.0 Hz, 1H), 6.93 (t, *J* = 10.0 and 5.0 Hz, 0.5H), 6.84 (sl, 3H), 6.73 (sl, 0.5H), 6.61 (d, *J* = 10.0 Hz, 1H), 6.55 (d, *J* = 10.0 Hz, 1H), 6.49 (d, J = 10.0 Hz, 1H), 3.72 (s, 5H), 3.62 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 159.4, 154.2, 134.0, 130.7 128.2, 127.1, 122.8, 122.2, 121.0, 120.5, 119.3, 113.6 113.4, 113.3, 55.12. MS, m/z (%): 332 ($M^{+\cdot}$, 47), 240 (100), 211 (90), 77 (21), 51 (11).

*5.3.1.6. N-(4-methoxyphenyl)-N'-(4-methoxyphenyl)-benzamidine **(9f)**.* Yield 60%; mp 119-120 °C (125 °C [28]); IR (KBr): ν 3352, 3055, 2933, 2834, 1625, 1505, 1241, 1032, 831 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 8.92 (s, 1H), 7.78 (d, *J* = 10.0 Hz, 2H), 7.30 (t, *J* = 5.0 Hz, 3H), 7.25 (dd, *J* = 5.0 Hz, 2H), 6.84 (d, *J* = 5.0 Hz, 2H), 6.59 (d, *J* = 10.0 Hz, 2H), 6.45 (d,

J = 5.0 Hz, 2H), 3.71 (s, 3H), 3.59 (s, 3H); ^{13}C NMR (DMSO-d_6): 162.4, 157.8, 136.0, 132.3, 130.3, 128.6, 126.8, 121.1, 113.9, 55.4. MS, m/z (%): 332 ($M^{+\cdot}$, 33), 210 (100), 77 (10), 51 (5).

*5.3.1.7. N-(4-methoxypheny)-N'-(4-methoxyphenyl)-4-methoxybenzamidine (**9g**).* Yield 70%; mp 110-113 °C (105 °C [28]); IR (KBr): ν 3414, 3065, 2837, 1606, 1551, 1512, 1458, 1253, 1027 cm^{-1}; ^{1}H NMR (DMSO-d_6): 7.62 (d, *J* = 8.0 Hz, 2H), 7.15 (d, *J* = 8.8 Hz, 4H), 6.82 (d, *J* = 8.6 Hz, 4H), 6.70 (sl, 2H); 3.69 (sl, 6H), 3.63 (sl, 3H); ^{13}C NMR (DMSO-d_6): δ 161.9, 157.7, 132.7, 128.3, 126.6, 119.8, 114.1, 55.7, 55.4. MS, m/z (%): 362 ($M^{+\cdot}$, 100), 241 (100), 226 (30), 197 (10), 123 (15), 77 (27).

*5.3.1.8. N-(4-methoxyphenyl)-N'-phenyl-3,4-methylenedioxybenzamidine (**9h**).* Yield 80%; mp 146-148 °C; IR (KBr): ν 3349, 2964, 2840, 1633, 1592, 1533, 1348, 1245, 1037 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 8.95 (sl, 1H), 7.78 (sl, 1H), 7.15 (sl, 3H), 6.82 (t, *J* = 8.0 Hz, 6H), 6.57 (sl, 2H), 5.99 (s, 2H), 3.67 (sl, 3H); ^{13}C NMR (DMSO-d_6): δ 155.1, 147.5, 146.7, 128.7, 128,3, 123,2, 107.9, 113,5, 101.5, 55.0. MS, m/z (%): 346 ($M^{+\cdot}$, 55), 254 (93), 224 (100), 91 (1), 77 (22), 51 (13). Anal. Calcd. for $C_{21}H_{18}N_2O_3$: C, 72.82, H, 5.24, N, 8.09. Found: C, 72.30, H, 5.54, N, 8.30.

*5.3.1.9. N-(4-chorophenyl)-N'-phenyl-4-methoxybenzamidine (**9i**).* Yield 55%; mp 168-170 °C; IR (KBr): ν 3124, 2963, 2839, 1620, 1591, 1534, 1340, 1252, 1030 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 9.24 (d, 1H), 7.86 (dd, *J* = 6.0 Hz, 2H), 7.22 (m, *J* = 8.0 Hz, 3H), 7.07 (d, *J* = 10.0 Hz, 2H), 6.89 (d, *J* = 6.0 Hz, 4H), 6.57 (d, *J* = 8.0 Hz, 2H), 3.72 (s, 3H); ^{13}C NMR

(DMSO-d_6): δ 159.7, 152.11, 149.9, 136.9, 130.5, 128.3, 128.2, 1256.6, 123.8, 122.0, 119.6, 120.9, 113.4, 55.1. MS, m/z (%): 337 ($M^{+\cdot}$, 100), 301 (2), 210 (92), 92 (3), 77 (6), 51 (5). Anal. Calcd. for $C_{20}H_{17}ClN_2O$: C, 71.32, H, 5.09, N, 8.32. Found: C, 70.80, H, 5.25, N, 8.48.

*5.3.1.10. N-(3-chorophenyl)-N'-phenyl-4-methoxybenzamidine (**9j**).* Yield 67%; mp 215-218 °C; IR (KBr): ν 3422, 2841, 1586, 1512, 1327, 1257, 1025 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.29 (d, 1H), 7.66 (m, *J* = 6.0 Hz, 2H), 7.24 (m, *J* = 6.0 Hz, 4H), 7.03 (m, *J* = 6.0 Hz, 3H), 6.87 (m, *J* = 10.0 Hz, 3H), 6.82 (m, *J* = 4.0 Hz, 1H), 3.72 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 162.1, 158.1, 152.1, 149.3, 135.0, 131.9, 130.7, 129.6, 128,0, 123.8, 122.9, 121.3, 114.5, 55.7. MS, m/z (%): 337 ($M^{+\cdot}$, 100), 301 (2), 210 (85), 92 (3), 77 (6), 51(5). Anal. Calcd. for $C_{20}H_{17}ClN_2O$: C, 71.32, H, 5.09, N, 8.32. Found: C, 71.40, H, 5.05, N, 8.55.

*5.3.1.11. N-(4-fluorophenyl)-N'-phenyl-4-methoxybenzamidine (**9k**).* Yield 89%; mp 149-150 °C; IR (KBr): ν 3300, 2932, 2843, 1627, 1596, 1535, 1335, 1254, 1031 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.15 (s, 1H), 7.80 (m, *J* = 8.0 Hz, 2H), 7.22 (d, *J* = 8.0 Hz, 4H), 7.07 (d, *J* = 10.0 Hz, 2H), 6.88 (m, *J* = 8.0 Hz, 3H), 6.57 (d, *J* = 8.0 Hz, 2H), 3.72 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 159.6, 154.8, 150.8, 147.4, 141.4, 130.5, 128.3, 123.4, 122.2, 121.9, 120.9, 115.0, 114.6, 113.4, 55.1. MS, m/z (%): 320 ($M^{+\cdot}$, 20), 305 (5), 210 (85), 91 (1), 77 (20), 51 (9). Anal. Calcd. for $C_{20}H_{17}FN_2O$: C, 74.98, H, 5.35, N, 8.74. Found: C, 74.71, H, 5.23, N, 8.33.

*5.3.1.12. N-(3-fluorophenyl)-N'-phenyl-4-methoxybenzamidine (**9l**).* Yield 85%; mp 95-97 °C; IR (KBr): ν 3329, 2960, 2839, 1628, 1597, 1531, 1336, 1251, 1031 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 9.27 (d, 1H), 7.87 (m, *J* = 6.0 Hz, 2H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.25 (dd, *J* = 6.0 Hz, 3H), 7.06 (m, *J* = 6.0 Hz, 2H), 6.83 (d, *J* = 8.0 Hz, 3H), 6.59 (d, *J* = 8.0 Hz, 1H), 6.39 (d, *J* = 8.0 Hz, 1H), 3.72 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 164.6, 159.8, 154.6, 153.4, 150.5, 141.2, 130.9, 129.8, 128.5, 126.7, 122.9, 119.6, 115.4, 112.5, 107.8, 55.3. MS, m/z (%): 320 ($M^{+\cdot}$, 20), 304 (1), 210 (68), 91 (2), 77 (20), 51 (8). Anal. Calcd. for $C_{20}H_{17}FN_2O$: C, 74.98, H, 5.35, N, 8.74. Found: C, 75.80, H, 5.44, N, 8.28.

*5.3.1.13. N-(4-bromophenyl)-N'-phenyl-4-methoxybenzamidine (**9m**).* Yield 65%; mp 175-178 °C; IR (KBr): ν 3120, 2963, 2838, 1618, 1591, 1534, 1341, 1251, 1030 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 9.23 (d, 1H), 7.87 (d, *J* = 8.0 Hz, 2H), 7.43 (d, *J* = 8.0 Hz, 1H), 7.22 (m, *J* = 8.0 Hz, 4H), 7.05 (d, *J* = 8.0 Hz, 2H), 6.88 (d, *J* = 8.0 Hz, 2H), 6.54 (t, *J* = 8.0 Hz, 2H), 3.73 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 159.7, 154.9, 150.3, 141.2, 140.8, 131.1, 130.5, 128.3, 126.6, 124.4, 122.6, 122.0, 121.3, 119.8, 113.3, 55.2. MS, m/z (%): 381 ($M^{+\cdot}$, 50), 350 (1), 210 (100), 91 (5), 77 (15), 51 (5). Anal. Calcd. for $C_{20}H_{17}BrN_2O$: C, 63.00, H, 4.49, N, 7.35. Found: C, 62.85, H, 4.55, N, 7.77.

*5.3.1.14. N-(3-methoxyphenyl)-N'-phenyl-4-methoxybenzamidine (**9n**).* Yield 65%; oil; IR (KBr): ν 3384, 2932, 2842, 1603, 1266, 1023 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.75 (d, *J* = 8.0 Hz, 2H), 7.28 (m, *J* = 10.0 Hz, 8H), 6.77 (d, *J* = 8.0 Hz, 3H), 3.88 (s, 3H), 3.78 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 161.7, 138.1, 135.9, 133.1, 131, 130.1, 126.8, 125.2, 121.9, 115.3, 114.1, 111.0, 55.9, 55.24. MS, m/z (%): 332 ($M^{+\cdot}$, 20), 317 (1), 210 (80), 91 (3), 77 (27),

51 (10). Anal. Calcd. for $C_{21}H_{20}N_2O_2$: C, 75.88, H, 6.06, N, 8.43. Found: C, 74.11, H, 5.94, N, 8.50.

*5.3.1.15. N-(4-nitrophenyl)-N'-phenyl-4-methoxybenzamidine (**9o**)*. Yield 60%; mp 150-153 °C; IR (KBr): ν 3375, 2930, 2838, 1625, 1582, 1527, 1322, 1251, 1028 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.72 (d, 1H), 8.19 (d, *J* = 8.0 Hz, 1H), 8.07 (d, *J* = 8.0 Hz, 1H), 7.95 (d, *J* = 8.0 Hz, 1H), 7.75 (d, *J* = 8.0 Hz, 1H), 7.28 (m, *J* = 4.0 Hz, 4H), 6.90 (d, *J* = 8.0 Hz, 2H), 7.05 (m, *J* = 8.0 Hz, 1H), 6.73 (d, *J* = 8.0 Hz, 1H), 6.63 (d, *J* = 8.0 Hz, 1H), 3.73 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 159.9, 158.0, 149.9, 140.6, 130.8, 128.5, 124.6, 122.8, 122.6, 122.0, 120.2, 113.7, 113.7, 55.27. MS, m/z (%): 347 ($M^{+\cdot}$, 10), 300 (1), 210 (30), 91 (1), 77 (6), 51 (6). Anal. Calcd. for $C_{20}H_{17}N_3O_3$: C, 69.15, H, 4.93, N, 12.10. Found: C, 68.77, H, 4.85, N, 11.86.

*5.3.1.16. N-(3-nitrophenyl)-N'-phenyl-4-methoxybenzamidine (**9p**)*. Yield 76%; oil; IR (KBr): ν 3410, 2923, 2850, 1602, 1527, 1349, 1260, 1031 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.52 (d, 1H), 7.82 (d, *J* = 8.0 Hz, 1H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.27 (m, *J* = 8.0 Hz, 4H), 7.03 (m, *J* = 8.0 Hz, 3H), 6.90 (m, *J* = 8.0 Hz, 3H), 6.63 (d, *J* = 6.0 Hz, 1H), 3.72 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 160.0, 156.0, 152.4, 140.8, 147.9, 150.1, 130.8, 129.5, 128.4, 122.5, 122.2, 120.0, 115.5, 113.6, 55.2. MS, m/z (%): 303 (30), 210 (100), 91 (1), 77 (20), ,51 (10). Anal. Calcd. for $C_{20}H_{17}N_3O_3$: C, 69.15, H, 4.93, N, 12.10. Found: C, 68.85, H, 4.87, N, 11.82.

*5.3.1.17. N-(4-hydroxyphenyl)-N'-phenyl-4-methoxybenzamidine (**9q**).* Yield 80%; mp 160-162 °C; IR (KBr): ν 3202, 2923, 2852, 1626, 1605, 1536, 1361, 1236, 1028 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.66 (d, *J* = 2.0 Hz, 2H), 7.10 (m, *J* = 6.0 Hz, 10H), 6.80 (d, *J* = 8.0 Hz, 2H), 3.83 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 154.6, 152.3, 130.7, 128.4, 127.1, 122.2, 121.2, 120.9, 115.0, 55.1. MS, m/z (%): 318 ($M^{+\cdot}$, 30), 226 (100), 210 (100), 91 (2), 77 (26), 51 (20). Anal. Calcd. for $C_{20}H_{18}N_2O_2$: C, 75.45, H, 5.70, N, 8.80. Found: C, 75.05, H, 5.58, N, 8.76.

*5.3.1.18. N-(3-hydroxyphenyl)-N'-phenyl-4-methoxybenzamidine (**9r**).* Yield 75%; mp 190-192 °C; IR (KBr): ν 3118, 2918, 1604, 1536, 1347, 1266, 1025 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.34 (dd, *J* = 8.0 Hz, 2H), 7.17 (t, *J* = 8.0 Hz, 3H), 7.01 (d, *J* = 8.0 Hz, 2H), 6.88 (m, *J* = 8.0 Hz, 5H), 6.35 (dd, *J* = 8.0 Hz, 1H), 3.77 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 157.6, 154.7, 154.6, 151.6, 131.8, 130.1, 129.5, 122.8, 115.1, 114.3, 113.1, 111.1, 55.7. MS, m/z (%): 318 ($M^{+\cdot}$, 20), 226 (100), 210 (90), 91 (3), 77 (18), 51 (10). Anal. Calcd. for $C_{20}H_{18}N_2O_2$: C, 75.45, H, 5.70, N, 8.80. Found: C, 74.80, H, 5.62, N, 8.23.

*5.3.1.19. N-(4-methylphenyl)-N'-phenyl-4-methoxybenzamidine (**9s**).* Yield 60%; mp 140-142 °C; IR (KBr): ν 3290, 2926, 2857, 1624, 1591, 1535, 1336, 1250, 1031 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 9.09 (sl, 1H), 7.77 (dd, *J* = 8.0 Hz, 2H), 7.20 (d, *J* = 8.0 Hz, 2H), 7.05 (d, *J* = 10.0 Hz, 2H), 6.85 (d, *J* = 12.0 Hz, 5H), 6.65 (d, *J* = 6.0 Hz, 2H), 371 (s, 3H), 2.18 (d, 3H); ^{13}C NMR (DMSO-d_6): δ 159.5, 154.3, 151.0, 141.5, 139.0, 130.5, 128.7, 128.3, 127.0, 122.1, 119.6, 113.3, 55.1. MS, m/z (%): 316 ($M^{+\cdot}$, 20), 224, (100), 210 (85), 91 (5), 77

(20), 51 (6). Anal. Calcd. for $C_{21}H_{20}N_2O$: C, 79.72, H, 6.37, N, 8.85. Found: C, 78.67, H, 6.33, N, 8.54.

*5.3.1.20. N-(3-methylphenyl)-N'-phenyl-4-methoxybenzamidine (**9t**)*. Yield 86%; mp 234-236 °C; IR (KBr): ν 3391, 2851, 1603, 1547, 1334, 1263, 1024 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.02 (sl, 1H), 7.83 (d, *J* = 12.0 Hz, 1H), 7.63 (d, *J* = 10.0 Hz, 1H), 7.22 (m, *J* = 8.0 Hz, 3H), 7.10 (m, *J* = 8.0 Hz, 1H), 6.85 (m, *J* = 6.0 Hz, 4H), 6.56 (d, *J* =6.0 Hz, 2H), 6.32 (m, *J* = 8.0 Hz, 1H), 3.71 (s, 3H), 2.18 (sl, 3H); ^{13}C NMR (DMSO-d_6): 159.6, 154.2, 150.7, 141.4, 137.3, 128.3, 128.1, 122.9, 122.2, 121.6, 122.5, 119.1, 116.8, 113.32, 55.15, 30.7. MS, m/z (%): 316 ($M^{+\cdot}$, 25), 224, (100), 210 (80), 91 (12), 77 (15), 51 (8). Anal. Calcd. for $C_{21}H_{20}N_2O$: C, 79.72, H, 6.37, N, 8.85. Found: C, 78.87, H, 6.24, N, 8.77.

*5.3.1.21. N-(4-sulfonylaminophenyl)-N'-phenyl-4-methoxybenzamidine (**9u**)*. Yield 60%; mp 159-161 °C; IR (KBr): ν 3199, 2913, 1602, 1552, 1337, 1262, 1014 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.67 (s, 0.2H), 9.43 (s, 0.6H), 8.73 (s, 0.3H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.25 (d, *J* = 8.0 Hz, 2H), 7.21 (d, *J* = 4.0 Hz, 1H), 7.05 (t, *J* = 8.0 Hz, 1H), 6.97 (d, *J* = 8.0 Hz, 1H). 6.90 (d, *J* = 10.0 Hz, 2H), 6.83 (d, *J* = 8.0 Hz, 1H), 6.78 (dd, *J* = 8.0 and 4.0 Hz, 1H), 6.58 (d, *J* = 8.0 Hz, 2H), 371 (s, 2.2H), 3.68 (0.7H); ^{13}C NMR (DMSO-d_6): δ 162.24, 151.2, 143.5, 129.5, 132, 127.6, 123.9, 122.6, 114.6, 55.2. MS, m/z (%): 301, (13), 210 (100), 91 (1), 77 (18), 51 (10). Anal. Calcd. for $C_{20}H_{19}N_3O_3S$: C, 62.98, H, 5.02, N, 11.02. Found: C, 61.76, H, 4.89, N, 11.20.

*5.3.1.22. N-(4-choro-3-nitrophenyl)-N'-phenyl-4-methoxybenzamidine (**9v**).* Yield 50%; mp 128-130 °C; IR (KBr): ν 3418, 2920, 2849, 1603, 1537, 1336, 1265, 1026 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 9.45 (s, 1H), 8.00 (d, *J* = 6.0 Hz, 2H), 7.39 (m, 7H), 6.86 (d, *J* = 6.0 Hz, 2H), 6.65 (s, 1H), 3.75 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 160.1, 154.4, 151.3, 147.1, 140.7, 131.4, 130.8, 128.4, 127.6, 125.8, 122.6, 120.1, 115.7, 113.7, 55.2. MS, m/z (%): 381 ($M^{+\cdot}$, 15), 289, (100), 210 (37), 91 (1), 77 (13), 51 (6). Anal. Calcd. for $C_{21}H_{16}ClN_3O_3$: C, 62.91, H, 4.22, N, 11.01. Found: C, 61.89, H, 4.15, N, 10.92.

5.2. Biology.

5.2.1. Parasite Culture. *L. amazonensis* promastigotes MHOM/BR/77/LTB0016 strain were grown at 25 °C in LIT medium supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS). Cells were harvested in the late log phase, resuspended in fresh medium, counted in Neubauer's chamber, and adjusted to a final concentration of 4 x 10^6/mL. This strain has been characterized by molecular and immunological techniques [9].

5.2.2. Antileishmanial Assays. The assays were carried out in 96-well plates in volume of 180 μL/well. The drugs were added to a parasite culture in a concentration range from 160 to 5 μg/mL, solubilized in DMSO (the highest percentage used was 1.6%, v/v, which was not hazardous to the parasites). After 24 h incubation, the remaining parasites were counted and the percentage of inhibition was calculated, comparing to the controls (DMSO without the drugs and with the parasites alone). The IC_{50} values were determined by linear regression from these percentages of inhibition using statistical error limits up to 10%. All

tests were done in triplicate and pentamidine isethionate (May & Baker Lab., England) was used as reference drug.

5.3. Molecular Descriptors. The molecular structures were drawn by ACD/ChemSketch software (ACDLabs software package, version 12.0) and log *P*, polarizability, superficial tension, volume molar and molar refractivity and log *P* descriptors were calculated for each compound.

5.4. QSAR Analysis. QSAR models were derived by multiple regression analyses that were performed using the BuildQSAR program to determine the coefficients of the correlation equations. In all equations in this paper, the numbers in parentheses represent the 95% confidence intervals of the coefficients, n is number of data points, r is the correlation coefficient, s is the standard deviation, q^2 is the cross-validated and F is Fisher value; measures for the statistical significance.

5.5. X-ray diffraction Analyses. A crystal of each sample was selected from representative crystalline mass. The intensity data for compounds **9b** and **9o** were collected with an Enraf-Nonius CAD4 diffractometer, at room temperature, with graphite-monochromated Mo Kα radiation. The unit cell parameters were determined on the setting angles of 25 centered reflections. All data were corrected for Lorentz and polarization effects. The structure was solved by direct methods and refined by full-matrix least-squares methods using SIR97 [29] and SHELXL97 [30] programs, respectively. All non-hydrogen atoms were refined anisotropically. H atoms attached to C atoms were placed at their idealized positions, with

C-H distances and U_{eq} values taken from the default settings of the refinement program. H atoms of the secondary amine groups were located from Fourier difference maps and treated as free atoms. ORTEP plots and cif validate procedure were performed by using PLATON software [31]. Further data of crystallographic analysis for compounds **9b** and **9o** are summarized in Table S1 (supplementary data). Crystallographic data (without structure factors) have been deposited at the Cambridge Crystallographic Data Centre (CCDC) as supplementary publication no. CCDC-837978 and CCDC-837979. Copies of the data may be obtained free of charge from the CCDC (12 Union Road, Cambridge CB2 1EZ, UK; Tel.: +44-1223-336408; Fax: +44-1223-336003; e-mail: deposit@ccdc.cam.ac.uk; http://www.ccdc.cam.ac.uk).

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Appendix. Supplementary data

Supplementary data related to this article can be found at http…

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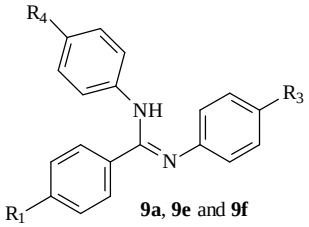
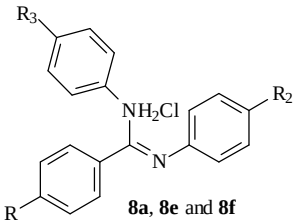
Table 1. IC_{50} values for compounds **9a, 9e** and **9f** neutral and salts forms (**8a**, **8e** and **8f)** assayed against *L. amazonesis*, *L. brazilensis and L. chagasi* Promastigotes and macrophage toxicity.



9a, **9e** and **9f**

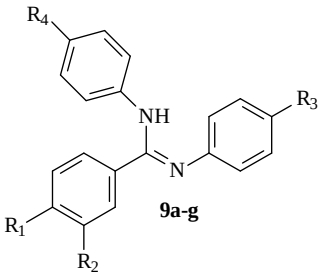


8a, **8e** and **8f**

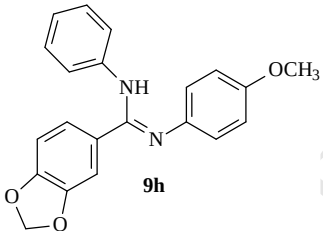
Compound	Substituents			*L. amazonensis*		*L. braziliensis*		*L. chagasi*		Toxicity (%)
	R	R_2	R_3	IC_{50} (μg/mL)	IC_{50} (μM)	IC_{50} (μg/mL)	IC_{50} (μM)	IC_{50} (μg/mL)	IC_{50} (μM)	
9a	OCH_3	H	H	1.34 ± 0.18	1.85 ± 0.25	1.48 ± 0.04	1.90 ± 0.14	1.41 ± 0.05	1.91 ± 0.66	No toxic
9e	OCH_3	OCH_3	H	2.33 ± 0.15	2.80 ± 0.19	1.05 ± 0.21	1.52 ± 0.31	1.48 ± 0.09	1.95 ± 0.12	15
9f	H	OCH_3	OCH_3	1.77 ± 0.11	2.24 ± 0.14	1.77 ± 0.66	2.25 ± 0.14	1.75 ±0.067	2.25 ± 0.25	100
8a[a]	OCH_3	H	H	1.43 ± 0.07	1.90 ± 0.10	1.52 ± 0.11	2.05 ± 0.06	2.25 ± 0.17	2.76 0.21	-
8e[a]	OCH_3	OCH_3	-	1.39 ± 0.27	1.82 ± 0.35	0.99 ± 0.36	1.42 ± 0.52	1.61± 0.12	2.04 ± 0.16	-
8f[a]	H	OCH_3	OCH_3	1.19 ± 0.31	1.62 ± 0.42	1.70 ± 0.16	2.13 ± 0.20	> 2.5	>3.0	-
Pentamidine[b]	-	-	-	0.82 ± 0.19	1.05 ± 0.25	1.12 ± 0.11	1.36 ± 0.14	1.53 ± 0.11	1.76 ± 0.12	100

[a] benzamidine hydrochorides ; [b] Reference drug

Table 2. IC_{50} values for compounds **9a-h (**series I**)** assayed against *L. amazonensis* promastigotes.



9a-g

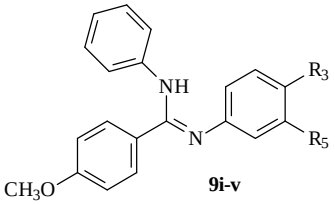


9h

Comp	Substituent				*L. amazonensis*	
	R_1	R_2	R_3	R_4	IC_{50} (μg/L)	IC_{50} (μM)
9a	OCH_3	H	H	H	1.34 ± 0.18	1.85 ± 0.25
9b	H	OCH_3	H	H	2.0 ± 0.11	2.56 ± 0.14
9c	H	H	OCH_3	H	2.28 ± 0.21	2.80 ± 0.26
9d	H	OCH_3	OCH_3	H	2.10 ± 0.01	2.57 ± 0.020
9e	OCH_3	H	OCH_3	H	2.33 ± 0.16	2.80 ± 0.19
9f	H	H	OCH_3	OCH_3	1.77 ± 0.11	2.24 ± 0.14
9g	OCH_3	H	OCH_3	OCH_3	> 2.50	> 3.0
9h	OCH_2O	OCH_2O	OCH_3	H	1.13 ± 0.08	1.60 ± 0.11
Pent.[a]	-	-	-	-	-	1.05 ± 0.25

[a]Pentamidine: reference drug

Table 3. IC_{50} values for compounds **9i-v (**series II**)** assayed against *L. amazonensis* promastigotes.



9i-v

Compound	Substituents		*L. amazonensis*	
	R_3	**R_5**	IC_{50} (μg/ml)	IC_{50} (μM)
9i	Cl	H	1.43 ± 0.43	1.90 ± 0.57
9j	H	Cl	0.64 ± 0.0	1.11 ± 0.0
9k	F	H	0.94 ± 0,0	1.43 ± 0.0
9l	H	F	1.13 ± 0,0	1.62 ± 0.0
9m	Br	H	0.92 ± 0.0	1.34 ± 0.0
9n	H	OCH_3	1.20 ± 0.12	1.68 ± 0.17
9o	NO_2	H	0.76 ± 0.11	1.22 ± 0.18
9p	H	NO_2	2.13 ± 0.17	2.59 ± 0.2
9q	OH	H	2.01 ± 0.27	2.51 ± 0.34
9r	H	OH	2.15 ± 0.076	2.65 ± 0.09
9s	CH_3	H	1.92 ± 0.21	2.42 ± 0.27
9t	H	CH_3	1.92 ± 0.32	2.42 ± 0.41
9u	SO_2NH_2	H	1.37 ± 0.11	1.79 ± 0.14
9v	Cl	NO_2	0.69 ± 0.0	1.10 ± 0,0
Pentamidine[a]	-	-	0.82 ± 0.19	1.05 ± 0.25

[a]Pentamidine: reference drug.

Table 04. Table of descriptors; lipophilicity, log *P*, molecular refractivity (MR), molecular volume (MV), polarizability (POLZ) and superficial tension (ST).

Compound	log *P*[a]	MR[a]	MV[a]	POLZ[a]	ST[a]
9a	5.28	94.38	284.0	37.41	39.7
9b	5.28	94.38	284	37.41	39.7
9c	5.07	94.38	284.0	37.41	39.7
9d	5.23	100.19	305.7	39.72	38.9
9e	5.23	100.19	305.7	39.72	38.9
9f	4.89	100.19	305.7	39.72	38.9
9g	5.05	106.41	327.3	42.02	38.1
9h	4.48	93.28	265.7	36.97	44.12
9i	6.27	98.98	293.3	39.24	40.9
9j	6.31	98.98	293.3	39.24	40.9
9k	5.73	94.25	286.9	37.36	38.2
9l	5.77	94.25	286.9	37.36	382
9m	6.45	101.94	296.5	40.41	42.4
9n	5.44	100.19	305.7	39.72	38.9
9o	5.74	100.04	289.3	39.66	47.3
9p	5.67	100.04	289.3	39.66	47.3
9q	4.54	95.23	281.2	37.75	42.6
9r	4.93	95.23	281.2	37.75	42.6
9s	5.74	98.8	299.2	37.17	38.3
9t	5.74	98.8	299.2	39.17	38.3
9u	4.09	106.05	301.0	42.04	50.2
9v	6.45	106.64	298.6	41.48	48.4

[a]ACDLabs software package version 12.0

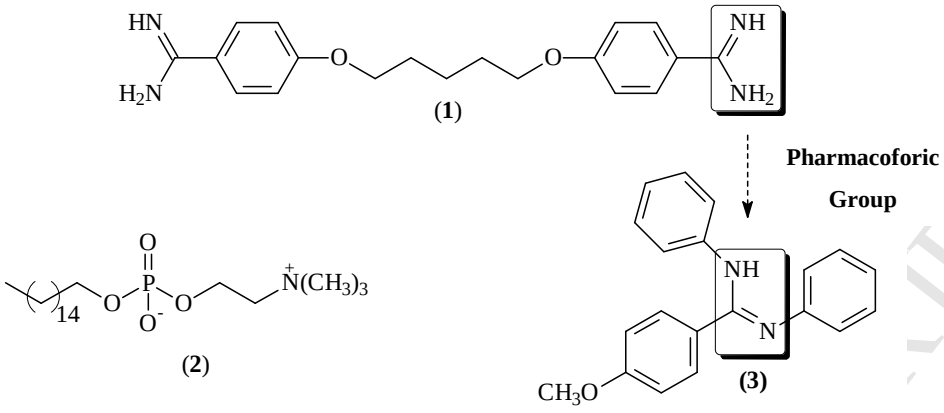


Figure 1. Drugs **1** and **2** and compound **3** with antileishmanial activity.

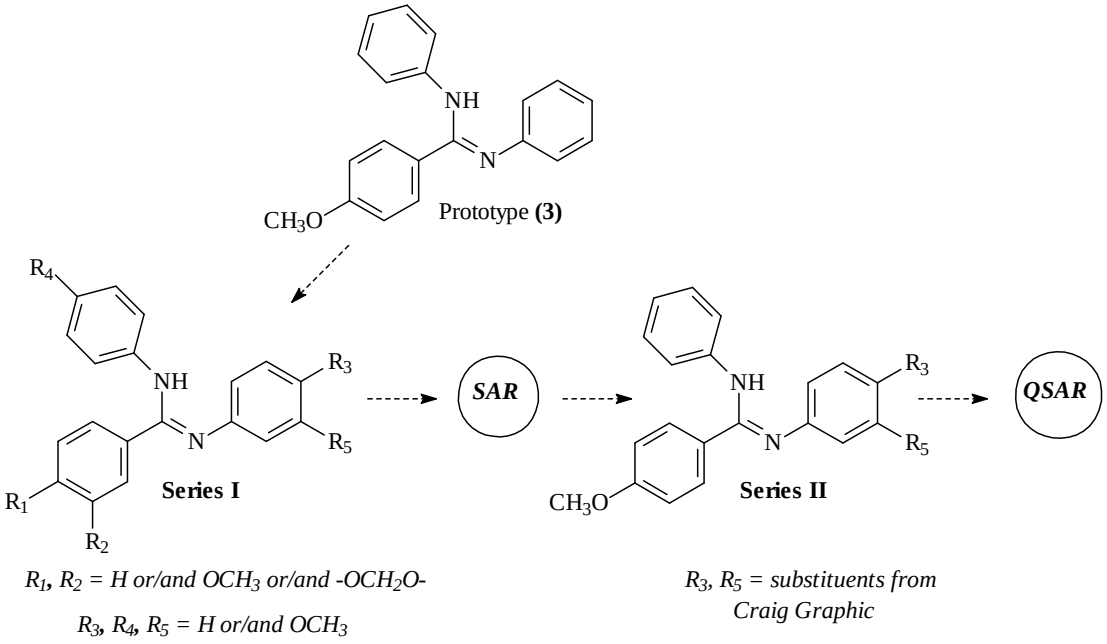
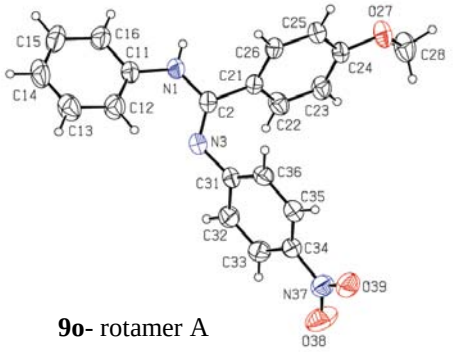
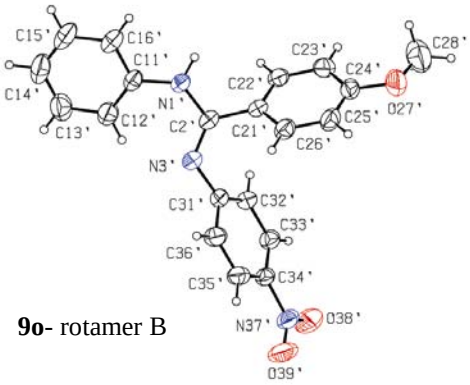


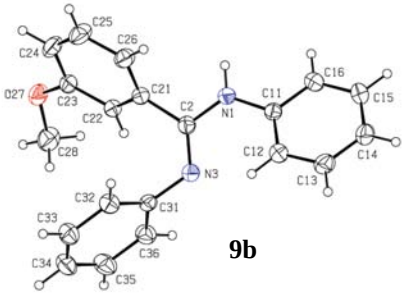
Figure 2. Plannig of *N,N'*-diphenyl-4-methoxybenzamidine derivatives.



9o- rotamer A



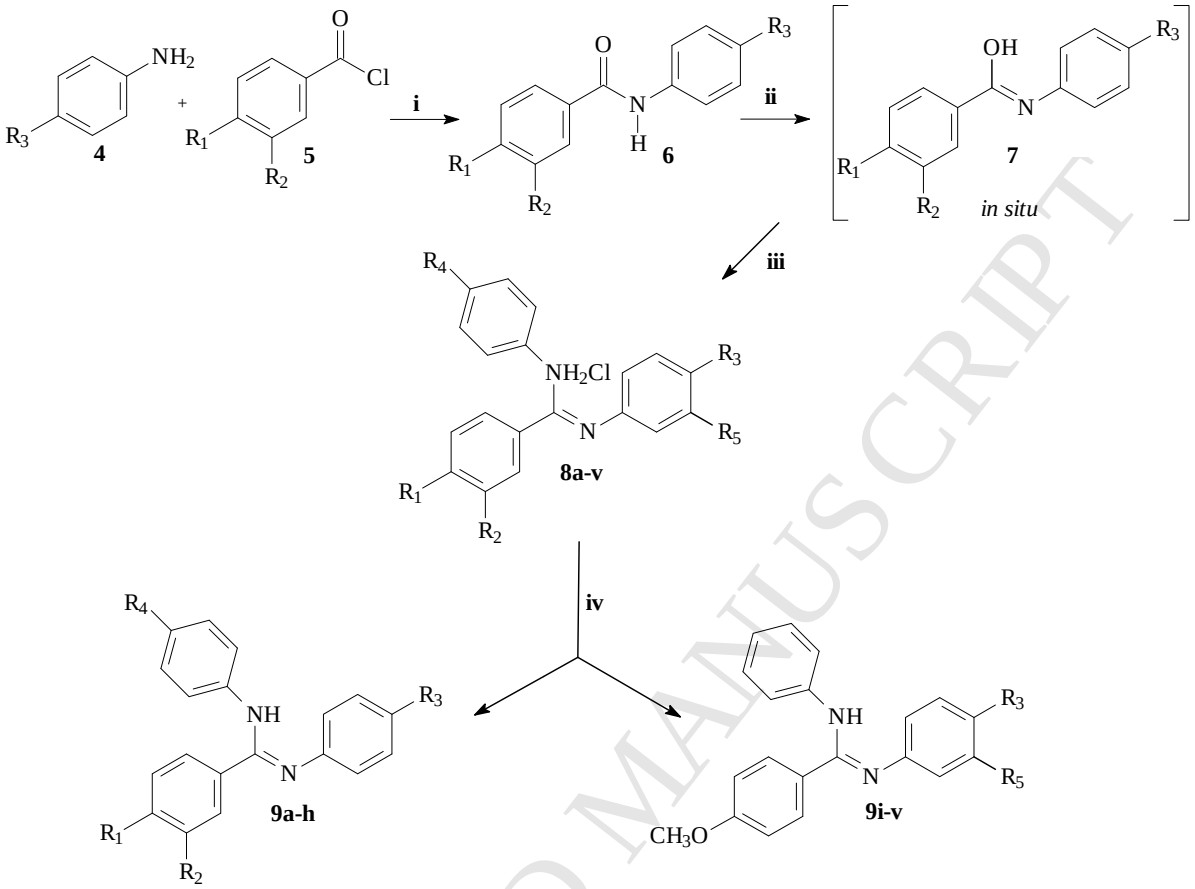
9o- rotamer B



9b

Figure 3. Molecular structure **9b** and **9o**-rotamer A and **9o**-rotamer B.

Scheme 1[a]



Series I

9a = **3** $R_1 = OCH_3$, R_2, R_3, R_4 = H

9b* $R_2 = OCH_3$; R_1, R_3, R_4 = H

9c $R_3 = OCH_3$; R_1, R_2, R_4 = H

9d* R_2, $R_3 = OCH_3$; R_1, R_4 = H

9e R_1, $R_3 = OCH_3$; R_2, R_4 = H

9f R_3, $R_4 = OCH_3$; R_1, R_2 = H

9g R_1, R_3, $R_4 = OCH_3$; R_1 = H

9h* R_1, $R_2 = OCH_2O$; $R_3 = OCH_3$ R_4 = H

Series II

9i*	R_3 = Cl; R_5 = H	**9p***	R_5 = H; $R_5 = NO_2$
9j*	R_3 = H; R_5 = Cl	**9q***	R_5 = OH; R_5 = H
9k*	R_3 = F; R_5 = H	**9r***	R_5 = H; R_5 = OH
9l*	R_3 = H; R_5 = F	**9s***	$R_5 = CH_3$; R_5 = H
9m*	R_3 = Br; R_5 = H	**9t***	R_5 = H; $R_5 = CH_3$
9n*	R_3 = H; $R_5 = OCH_3$	**9u***	$R_5 = SO_2NH_2$;R_5 = H
9o*	$R_3 = NO_2$; R_5 = H	**9v***	R_5 = Cl; $R_5 = NO_2$

*New compounds

[a]Reagents and conditions: (i) NaOH 10 % (m/v), 45 min; (ii) PCl_5; dry toluene, 8 h.; (iii) *p*-R_3-aniline in dry toluene, reflux, 6 h; (iv) 5% aq. $NaHCO_3$, 4 h, CH_2Cl_3 extraction.

Highlights

1. Antileishmania activity evaluation of new *N,N'*-substituted benzamidines series
2. QSAR study involving the hydrophobicity and electronic parameters
3. Synthesis and characterization of *N,N'*-substituted benzamidines series

Supplementary data

Synthesis, Antileishmanial Activity and Structure-Activity Relationship of 1-*N*-X-phenyl-3-*N*'-Y-phenyl-benzamidines

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28 ^{13}C NMR spectrum of **9d**..... S30
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31 ^{13}C NMR spectrum of **9g**..... S33
32 ^{13}C NMR spectrum of **9h**..... S34
33 ^{13}C NMR spectrum of **9i**..... S35
34 ^{13}C NMR spectrum of **9j**..... S36
35 ^{13}C NMR spectrum of **9k**..... S37
36 ^{1}H NMR spectrum of **9l**..... S38
37 ^{13}C NMR spectrum of **9m**..... S39
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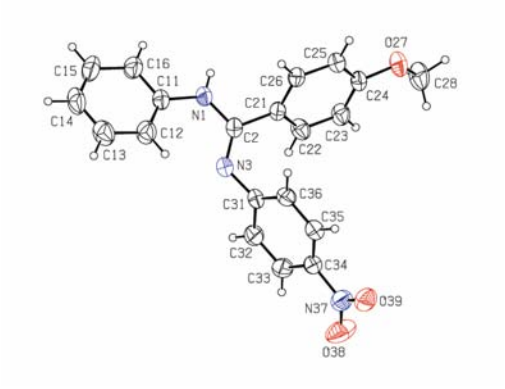
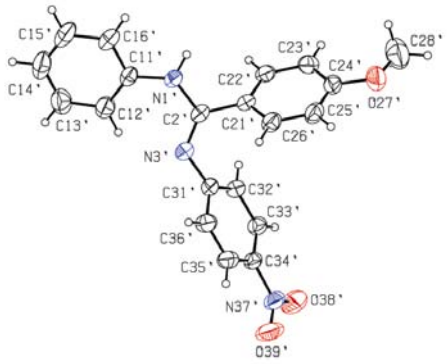
X-ray crystal data



9o- rotamer A



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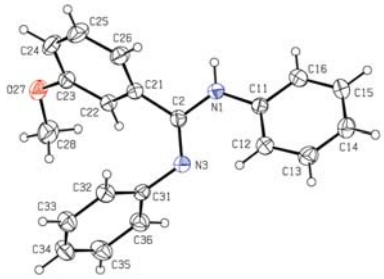

9b

Table S1. Crystal data and structure refinement for compounds **9b** and **9o**.

	Compound **9b**	Compound **9o**
Empirical formula	$C_{20}H_{18}N_2O$	$C_{20}H_{17}N_3O_3$
Formula weight	302.36	347.37
Temperature (K)	293(2)	293(2)
Wavelength (Å)	0.71069	0.71069
Crystal system	Monoclinic	Triclinic
Space group	$P2_1/c$	$P\bar{1}$
Unit cell dimensions		
A (Å)	9.5968(19)	10.4554(15)
b (Å)	14.2860(9)	11.4787(16)
c (Å)	12.0218(7)	16.6234(18)
α (°)		78.403(9)
β (°)	103.998(9)	73.919(9)
γ (°)		70.702(9)
Volume ($Å^3$)	1599.2(4)	1796.0(4)
Z	4	4
Density (cal.) (Mg/m^3)	1.256	1.285
μ (mm^{-1})	0.078	0.088
F(000)	640	728
Crystal size (mm^3)	0.50 x 0.36 x 0.36	0.46 x 0.30 x 0.26
Theta range (°)	2.19 to 25.07	1.28 to 25.07
Reflections collected	2986	6593
Independent reflections	2841 [R(int) = 0.0125]	6356 [R(int) = 0.0233]
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data / restraints / parameters	2841 / 0 / 214	6356 / 0 / 479
Goodness-of-fit on F^2	1.046	1.045
Final R indices [$I>2\sigma(I)$]	R1 = 0.0377, wR2 = 0.0958	R1 = 0.0534, wR2 = 0.1382
R indices (all data)	R1 = 0.0668, wR2 = 0.1065	R1 = 0.1481, wR2 = 0.1677
Extinction coefficient	0.0213(18)	
Largest diff. peak and hole ($e.Å^{-3}$)	0.161 and -0.159	0.296 and -0.170

25 Oct 2007

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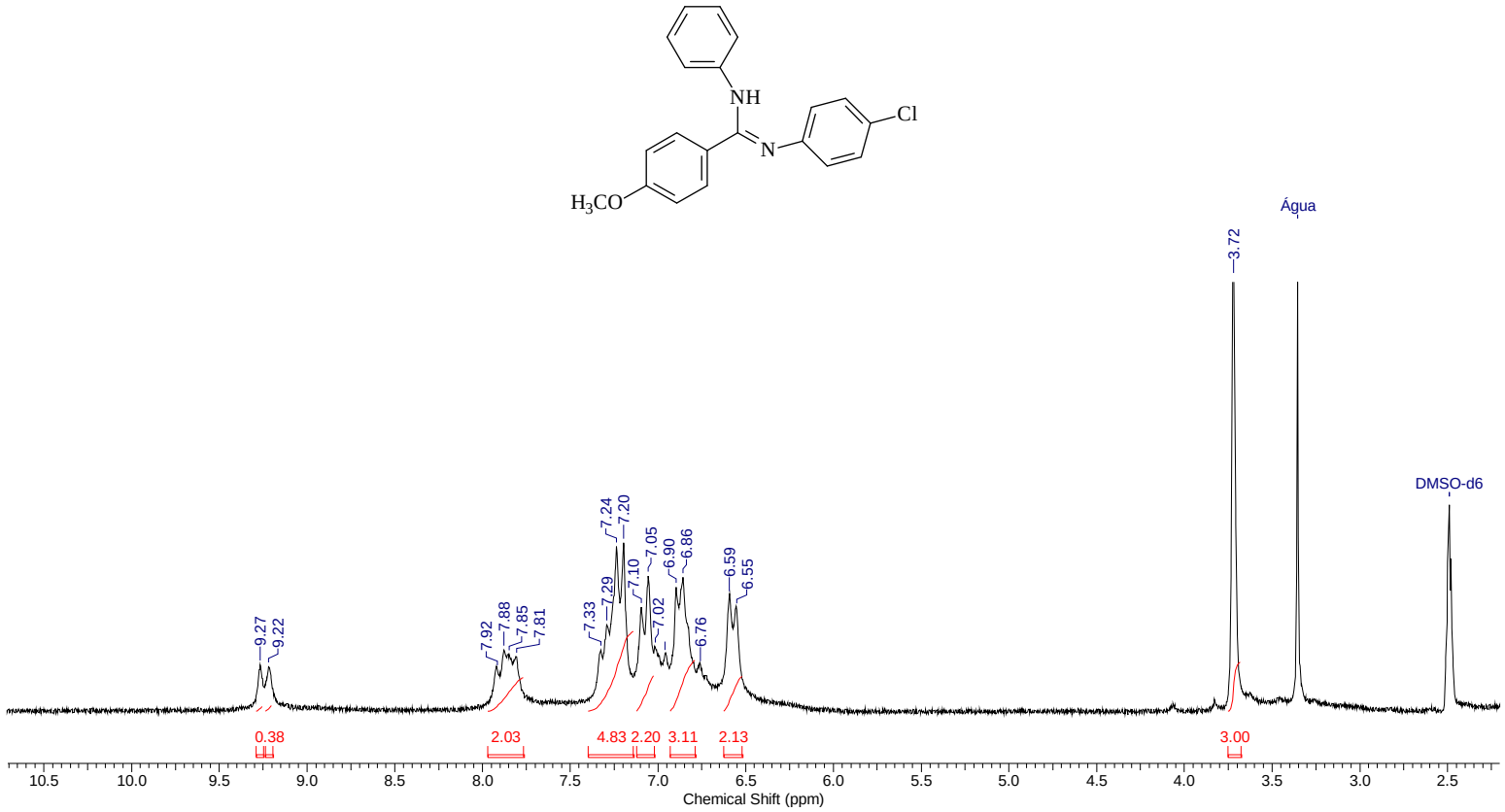

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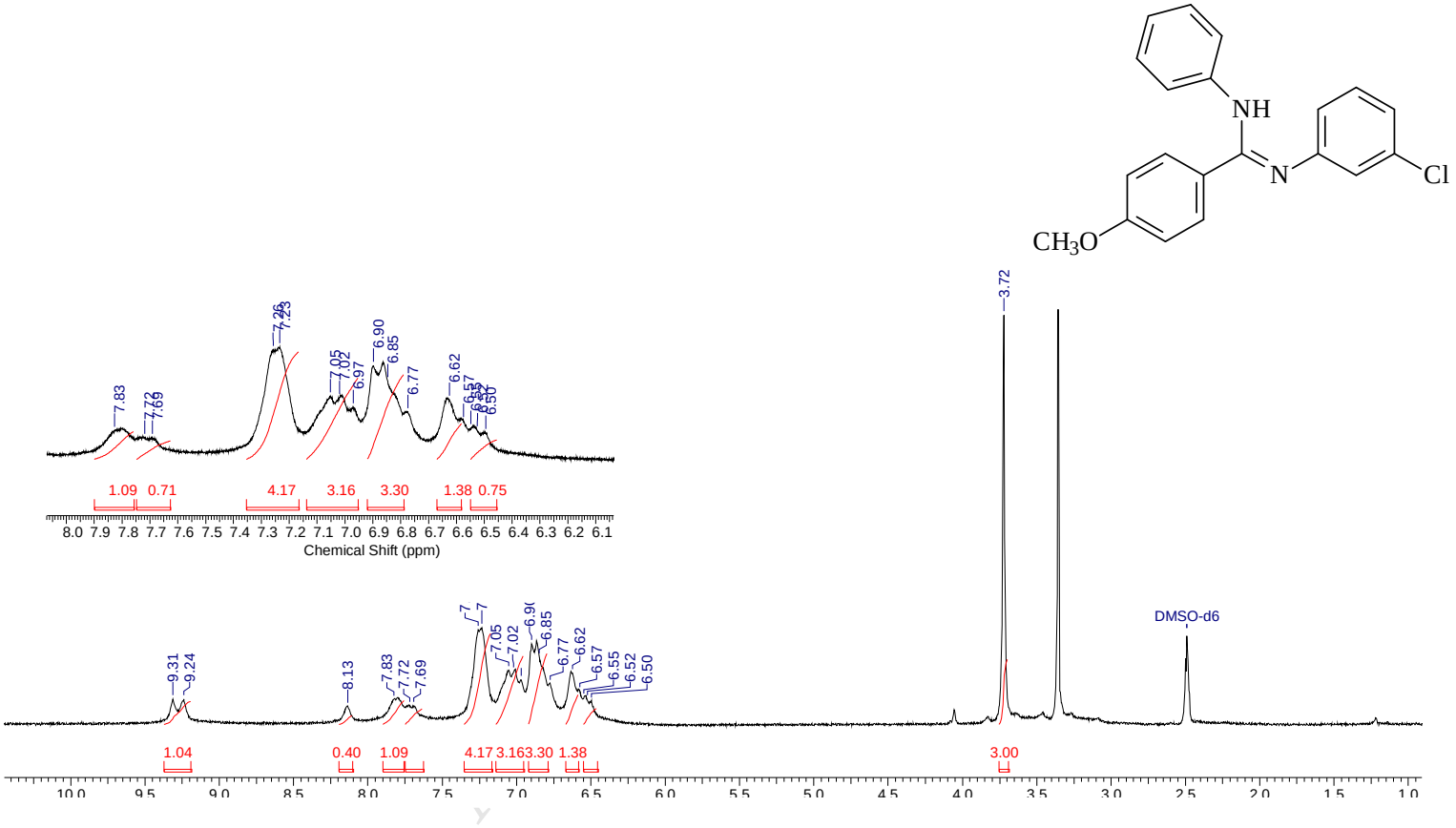

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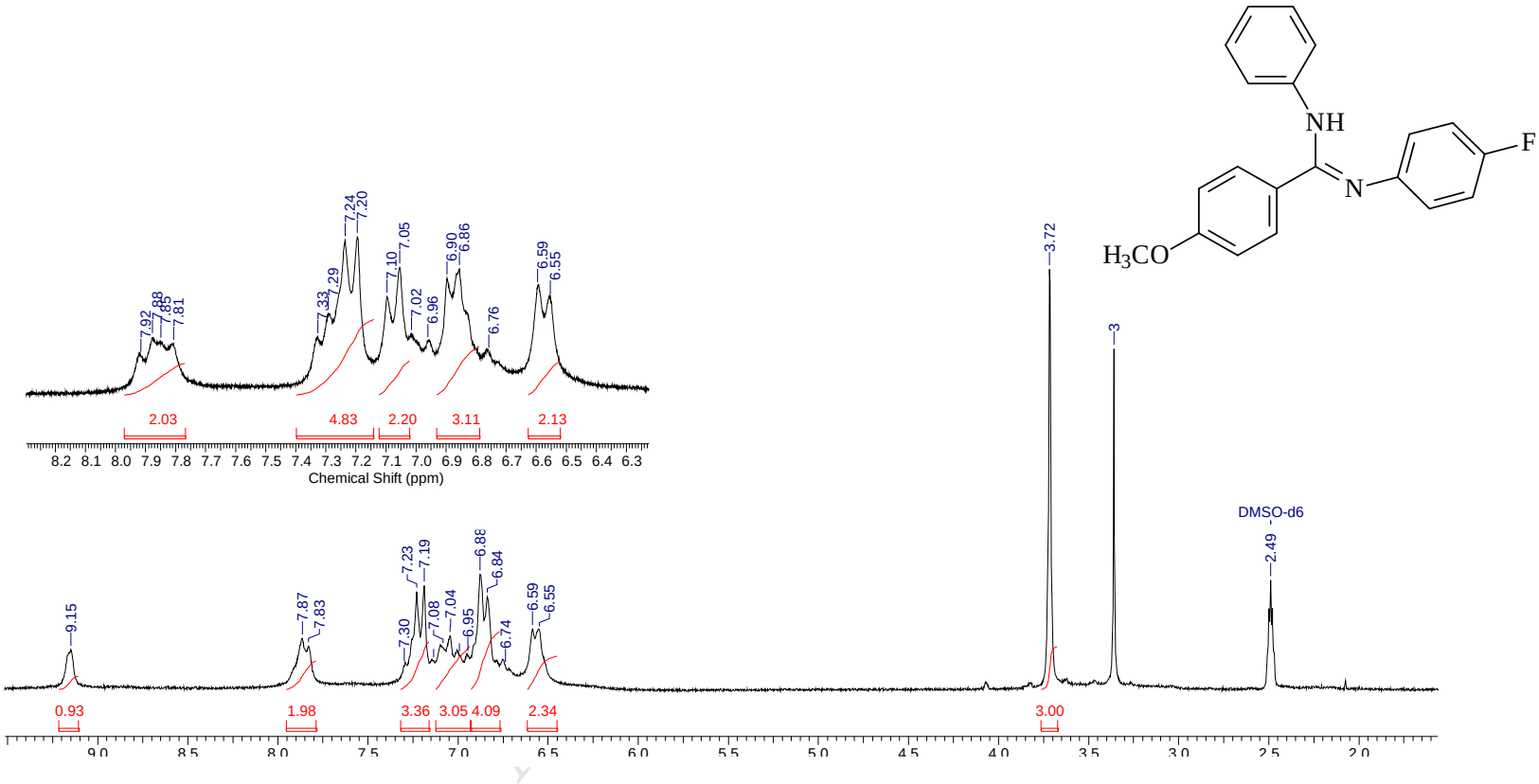

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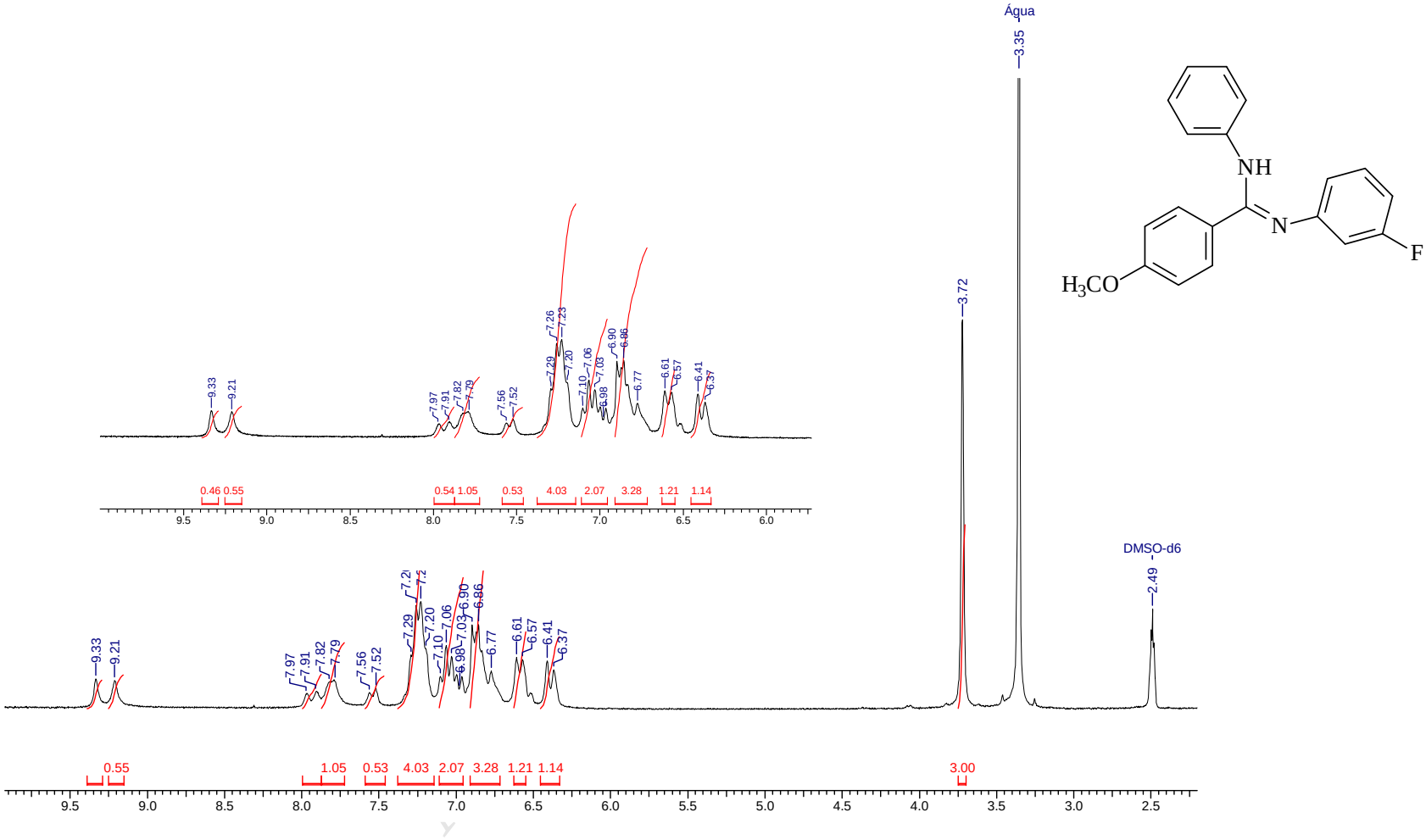
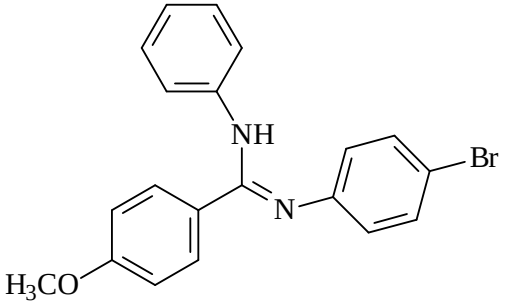
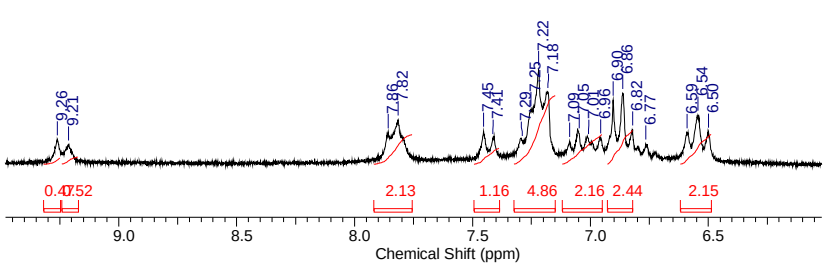
Frequency (MHz)	200.13	***Nucleus***	1H	***Number of Transients***	32	***Original Points Count***	4096	***Points Count***	8192
Sweep Width (Hz)	2032.52			***Temperature (grad C)***	24.000		



25 Oct 2007

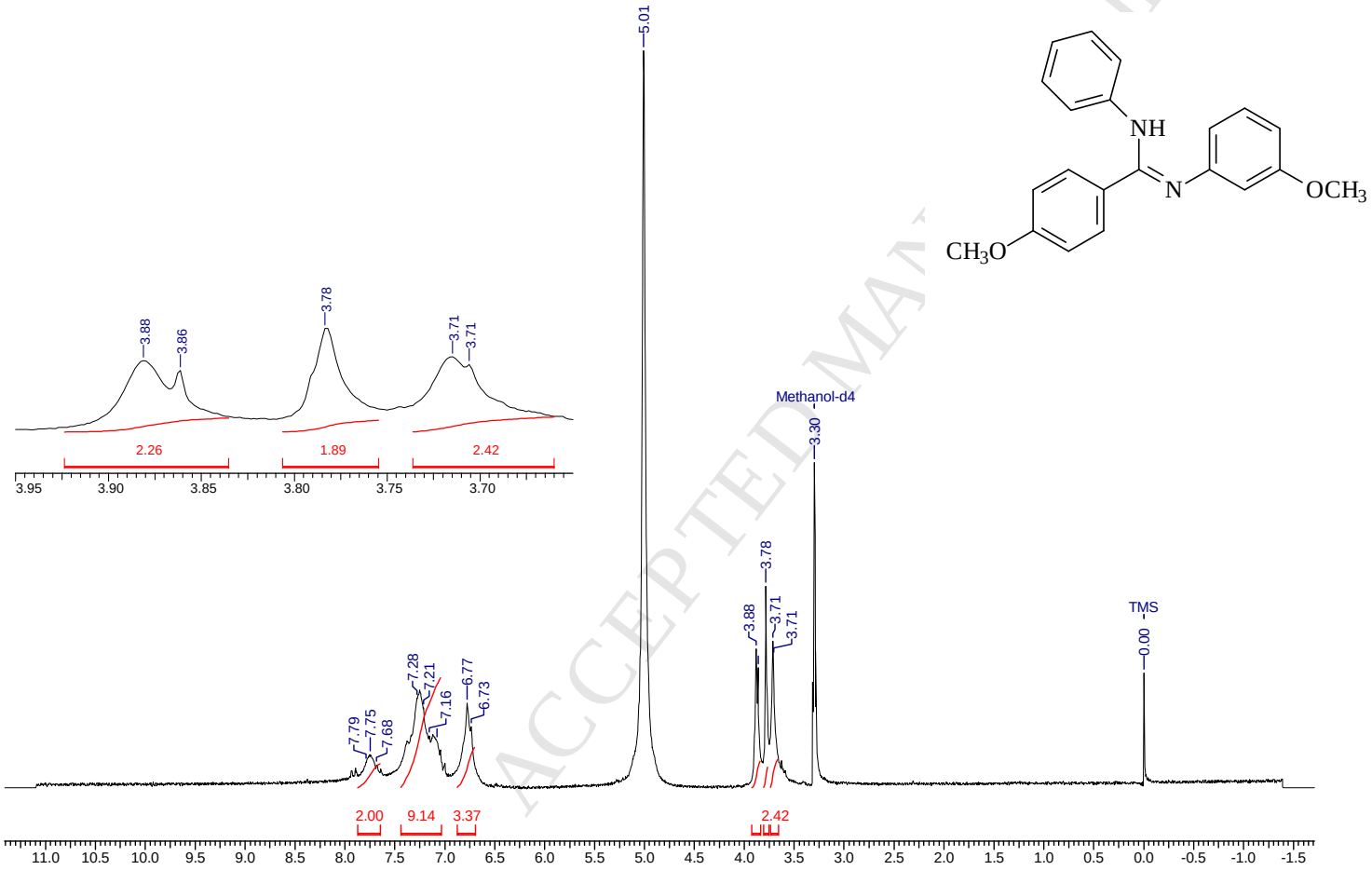
Acquisition Time (sec)	7.9299	***Date***	24 Oct 2007 13:38:26			***File Name***	E:\69\pdata\1_069001r
Frequency (MHz)	200.13	***Nucleus***	1H	***Number of Transients***	16	***Original Points Count***	32768
Pulse Sequence	zg30	***Solvent***	DMSO-D6	***Sweep Width (Hz)***	4132.23	***Temperature (degree C)***	27.000





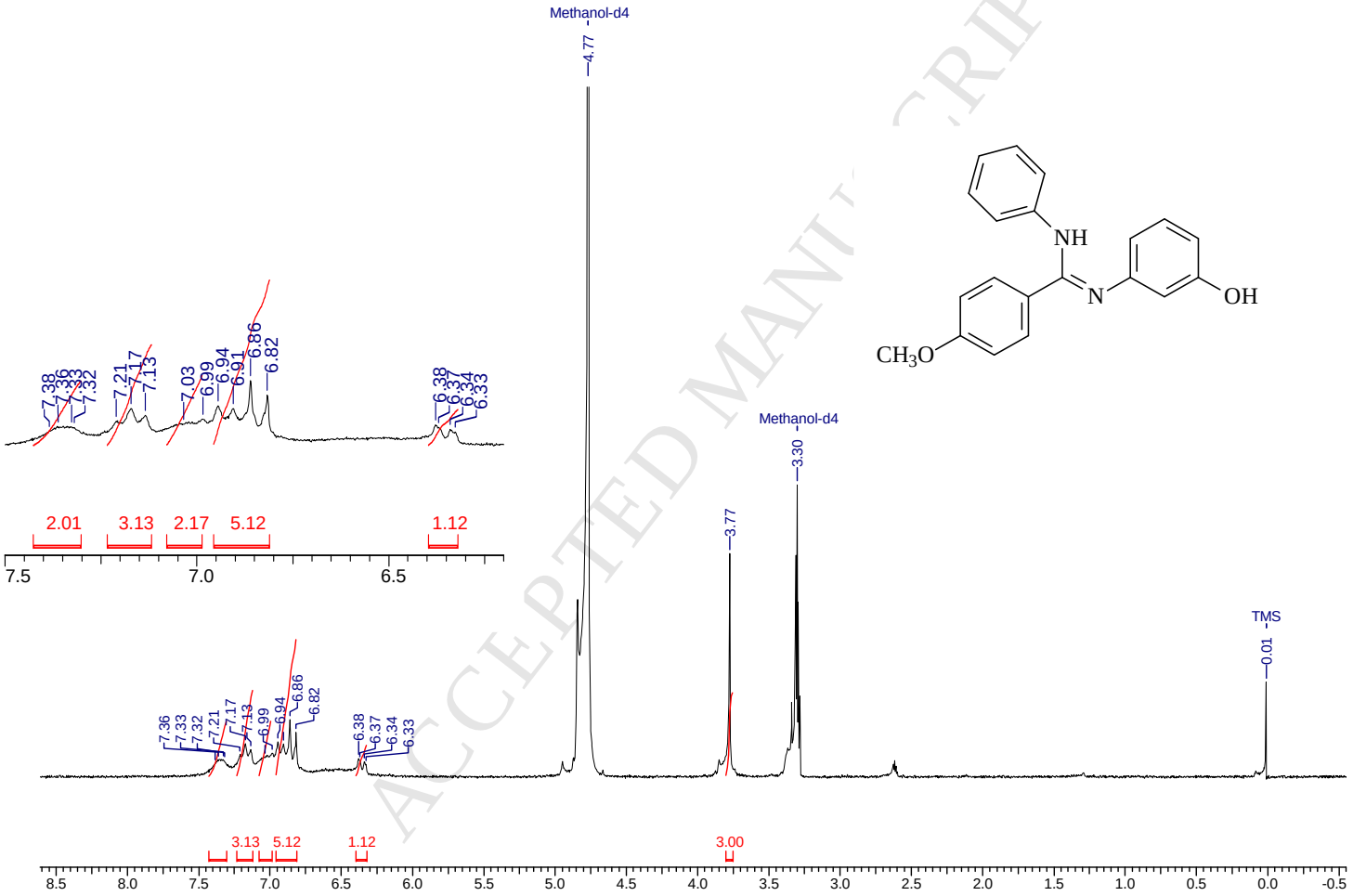
19 Sep 2006

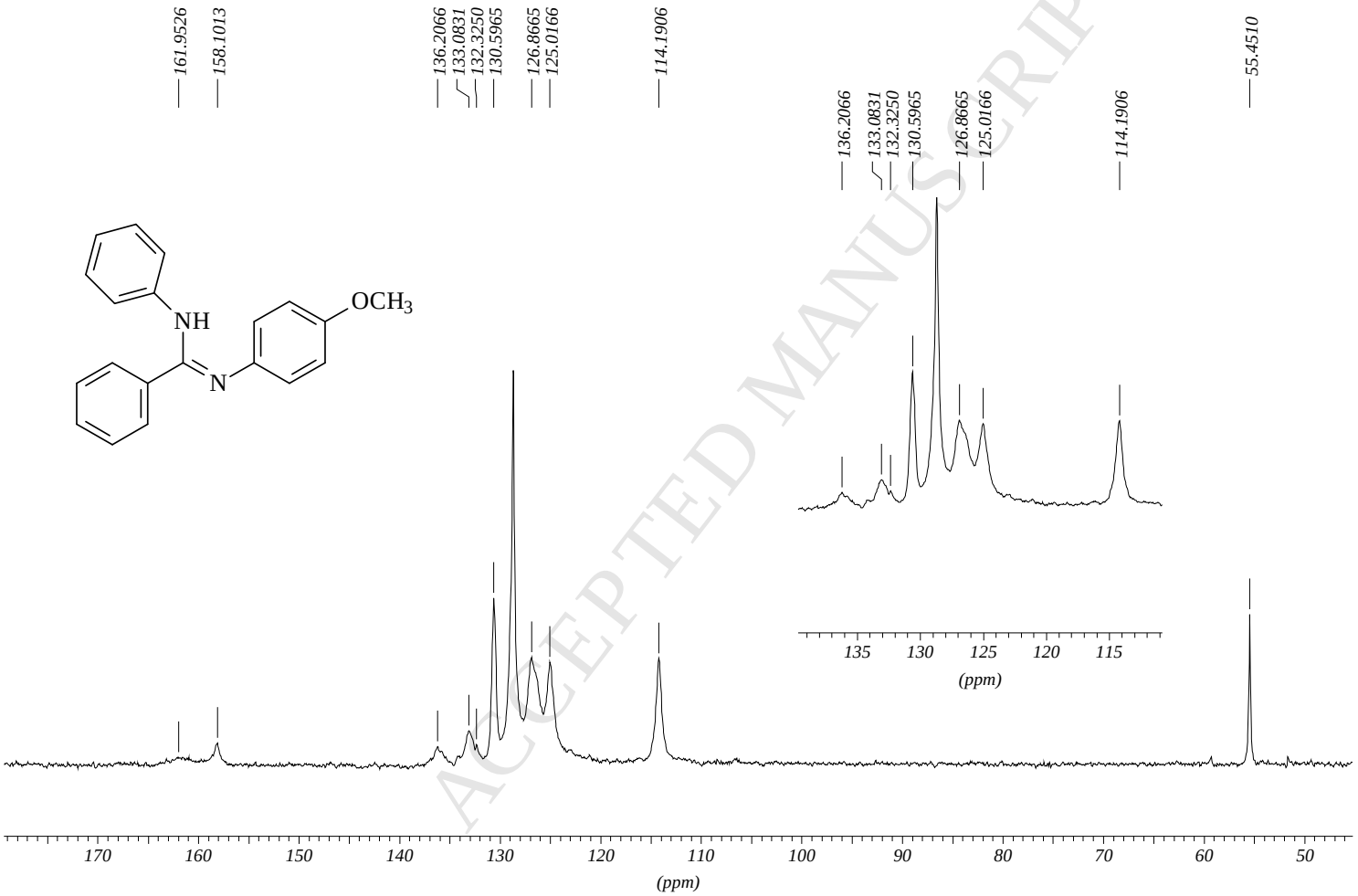
Acquisition Time (sec)	1.6384	***Comment***	CE103OCH			***Date***	18/11/15 22:45:39
Frequency (MHz)	200.13	***Nucleus***	1H	***Number of Transients***	32	***Original Points Count***	4096
Sweep Width (Hz)	2500.00			***Temperature (grad C)***	24.000		



19 Sep 2006

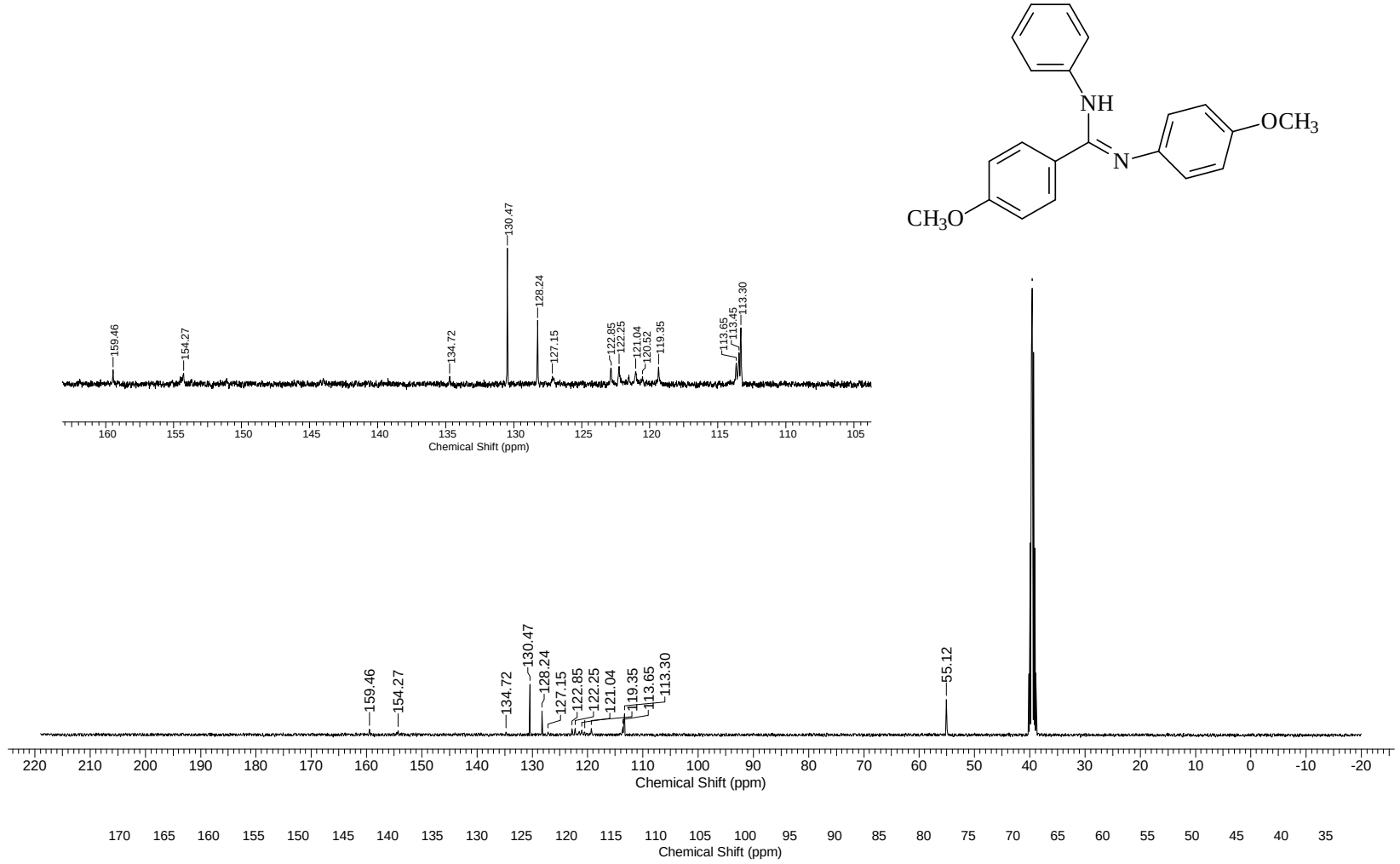
Acquisition Time (sec)	1.6056	***Comment***	CE103OH			***Date***	18/11/15 03:36:36
Frequency (MHz)	200.13	***Nucleus***	1H	***Number of Transients***	16	***Original Points Count***	4096
Sweep Width (Hz)	2551.02			***Temperature (grad C)***	24.000		



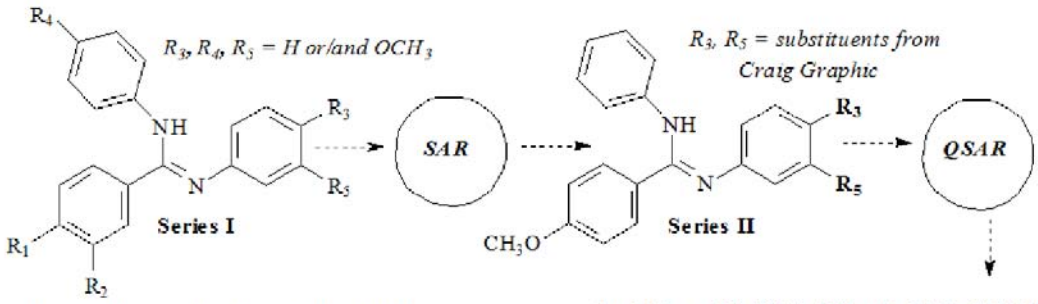


22 Feb 2013

Acquisition Time (sec)	1.3631	***Comment***	AMOSTRA: CE101-d (13C_CPD) - 400MHZ SOLVENTE: DMSO				
Date	21 Feb 2013 12:56:32			***File Name***	D:\claudio_029001r		
Frequency (MHz)	100.61	***Nucleus***	13C	***Number of Transients***	1024	***Original Points Count***	32768
Points Count	32768	***Pulse Sequence***	zgpg30	***Solvent***	DMSO-D6	***Sweep Width (Hz)***	24038.46
Temperature (degree C)	27.000						



New class of leishmanicidal compounds



R_1, R_2 = *H or/and* OCH_3 *or/and* $-OCH_2O-$

$\log 1/IC_{50} = 1.04\ (\pm 0.29)\log P - 10.80\ (\pm 3.23)\log P - 0.04\ (\pm 0.02)MR + 29.04\ (\pm 9.98)$