

Synthesis, Three-Dimensional Structure, Conformation and Correct Chemical Shift Assignment Determination of Pharmaceutical Molecules by NMR and Molecular Modeling

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This work includes the synthesis of phenanthrenequinone guanylhydrazone and phenanthro[9,10-*e*][1,2,4]triazin-3-amine to be tested as intercalate with DNA for treatment of cancer. The other synthesized product, 2-[(4-chlorophenylamino)methylene]malononitrile, was designed for future determination of its activity against leishmaniasis. A common problem about some articles on the literature is that some previously published compounds display error of their molecular structures. In this article it is shown the application of several procedures of nuclear magnetic resonance (NMR) to determine the complete molecular structure and the non questionable chemical shift assignment of the synthesized compounds, and also their analysis by molecular modeling to confirm the NMR results. To determine the capacity of pharmacological compounds to interact with biological targets is determined by docking. This work is to motivate the application of NMR and molecular modeling on organic synthesis, being a process that is very important for the study of the prepared compounds as interactions with biological targets by NMR.

Keywords: synthesis, phenanthrenequinone derivatives, NMR, molecular modeling, chemical shift determination, definitive molecular structure

Introduction

One of the most important information from compounds with biological activity is the determination of their complete three-dimensional and conformational structure, and also their definitive chemical shift assignment. This information is necessary to determine the interaction process of these pharmacological compounds with the biological targets, as enzymes, nucleic acids (DNA or RNA) and membrane receptors by nuclear magnetic resonance (NMR).

The molecular structure determination of pharmacological agents with biological targets is usually executed by X-ray diffraction,¹ but sometimes, when crystallization of the complex is not possible, this method is not available. On the other hand, when the ligand-target complex possess mass lower than 70 kD, it is possible to determine their complete structure and its dynamic condition by NMR.^{2,3} However, if their mass targets are more than 100 kD, the NMR method is generally not

available for their complete ligand-target complex structure determination, but in this case the NMR can be used to determine the interaction of the ligand with the target, especially discovering which areas of the ligand are the most effective for interacting sessions with the target,^{4,5} being this the most effective method to study the interaction of pharmacological ligands with any type of targets. In order to determine by NMR which is the target site of interaction with the ligand it is necessary to execute the ligand competition with the previously described inhibitors, which is a process that confirms its interaction with the specific regions of the target, especially with the active sites of the enzymes.⁶⁻⁹

The normal NMR interaction analysis of pharmacological agents with biological targets are usually executed using water as solvent, and for this type of analysis the NMR method requires the complete information of the conformational structure and the definitive chemical shift of these pharmacological agent, being a process that allows the determination of quality and interaction with this agent. These NMR methods are based on the ¹H chemical shift, relaxation times (T₁ and T₂), diffusion gradients and

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nuclear Overhauser effects (NOE).⁷⁻⁹ For example, one of the most NMR used methods for this type of interaction studies is the saturation transfer difference (STD).^{10,11}

Several compounds have been described in the literature with inappropriate chemical shifts and wrong structures, sometimes leading to their incorrect interaction with biological targets. Therefore, the complete structure determination and chemical shift assignment of new potential pharmacological agents is very important for the study for their biological activity and interaction with targets.

In this article are described the synthesis, the NMR methods used to determine the complete and definitive three-dimensional, conformational and chemical shift assignment of pharmacological agents, and the confirmation by molecular modeling. The docking study is also important to determine if these compounds can interact with some biological targets, especially with some specific enzymes. This process is very important for the study of interaction from these agents with biological targets.

Experimental

General experimental procedures

Solvents and reagents were obtained from commercial companies (Sigma-Aldrich, Synth and Merck). Melting points were determined on a Fisher-Johns apparatus. The infrared spectra (IR) were measured using a Shimadzu 21 spectrometer, with samples prepared in tablets of anhydrous potassium bromide (KBr). The thin layer chromatography analyses were conducted using Merck silica gel 60 F254 aluminum sheets. The NMR spectra were obtained using a Varian 600 NMR spectrometer, with 5 mm tubes and using the DMSO-*d*₆ as solvent at 25 °C. The solutions of these samples on the NMR tubes were used with 20 mg at 600 μL. The NMR spectra were ¹H, ¹³C, attached proton test (APT), heteronuclear single quantum coherence (gHSQC), heteronuclear multiple bond coherence (gHMBC), correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY) and gated decoupling ¹³C. The NMR frequency was 600 MHz for the ¹H and 150 MHz for the ¹³C. The molecular modeling calculations were executed with the Spartan' 10 and using density functional theory (DFT) with the B3LYP and the M06 methods by 6-311G* basis set.¹² These methods were used to determine the natural atomic charges (QNPA) for each atom, and the values of energy (kJ mol⁻¹), PSA (Å²), element volume (Å²) and the dipole moment (Debye), and the calculated chemical shift of hydrogens and carbons to confirm with the NMR results. The docking study was performed with AutoDock Vina.

Synthesis and spectra of phenanthrenequinone guanylhydrazone (**3**)

In a 100 mL round bottom flask were added a mixture of phenanthrenequinone (0.208 g, 1.0 mmol), aminoguanidine hydrochloride (0.132 g, 1.2 mmol), 20 mL ethanol 95% and a few drops of HCl 6 mol L⁻¹. The reaction was refluxed for 4 h. The solid product obtained after the solvent elimination by vacuum was washed with acetone and filtered to afford the pure solid. Orange solid; 93% yield; m.p.: 245-246 °C; IR (KBr) ν_{\max} / cm⁻¹ 3033, 1681, 1634, 1568, 1509, 1447, 1278, 1167, 1020, 757; ¹H NMR (600 MHz, DMSO-*d*₆) δ 14.28 (s, 1H, NH, H12), 8.89 (br s, 2H, NH₂, H14), 8.64 (br s, 2H, NH₂, H15), 8.48 (d, 1H, *J* 8.0 Hz, CH, H8), 8.27 (d, 1H, *J* 7.5 Hz, CH, H4), 8.18 (d, 1H, *J* 7.5 Hz, CH, H5), 8.13 (d, 1H, *J* 8.0 Hz, CH, H1), 7.88 (t, 1H, *J* 7.5 Hz, CH, H3), 7.61 (t, 1H, *J* 7.5 Hz, CH, H2), 7.60 (t, 1H, *J* 8.0 Hz, CH, H6), 7.50 (t, 1H, *J* 8.0 Hz, CH, H7); ¹³C NMR (150 MHz, DMSO-*d*₆) δ 181.9 (C, C10), 156.4 (C, C13), 139.3 (C, C4a), 136.3 (CH, C3), 134.9 (C, C9), 130.8 (CH, C6), 129.8 (C, C8a), 129.5 (C, C10a), 129.4 (C, C4b), 129.4 (CH, C7), 128.9 (CH, C2), 128.6 (CH, C1), 126.0 (CH, C8), 124.1 (CH, C4), 124.0 (CH, C6).

Synthesis and spectra of phenanthro[9,10-*e*][1,2,4]triazin-3-amine (**4**)

In a 100 mL round bottom flask were added a mixture of phenanthrenequinone guanylhydrazone (0.301 g, 1 mmol), 25 mL of distilled water and 15 drops of NH₄OH. The reaction was refluxed for 2 h. The solid obtained was filtered and washed with distilled water to afford pure solid product. Yellow solid; 95% yield; m.p.: 260-262 °C; IR (KBr) ν_{\max} / cm⁻¹ 3469, 3282, 3120, 3072, 1633, 1521, 1444, 1384, 1026, 765, 723; ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.07 (dd, 1H, *J* 7.1, 1.9 Hz, CH), 8.97 (d, 1H, *J* 7.8 Hz, CH), 8.73 (d, 1H, *J* 7.8 Hz, CH), 8.69 (dd, 1H, *J* 7.2, 1.5 Hz, CH), 7.89 (t, 1H, *J* 7.8 Hz, CH), 7.75 (t, 1H, *J* 7.8 Hz, CH), 7.74 (t, 1H, *J* 7.2 Hz, CH), 7.73 (t, 1H, *J* 7.1 Hz, CH), 7.59 (s, 2H, NH₂); ¹³C NMR (150 MHz, DMSO-*d*₆) δ 162.2 (C), 143.1 (C), 138.4 (C), 133.3 (C), 131.9 (CH), 128.4 (CH), 128.4 (CH), 128.2 (C), 127.9 (C), 127.7 (CH), 127.5 (C), 125.3 (CH), 123.5 (CH), 123.3 (CH), 120.0 (CH).

Synthesis and spectra of 2-[(4-chlorophenylamino)methylene]malononitrile (**5**)

In a 100 mL round-bottomed flask were added 0.500 g (3.94 mmol) 4-chloroaniline, followed by addition of ethoxymethylenemalononitrile 0.250 g (2.04 mmol) dissolved in 30 mL of ethanol 95% and was heated under reflux for 3 h. The precipitated product was filtered and washed with distilled water. Yellow solid; 76% yield; m.p. 252-254 °C; IR (KBr) ν_{\max} / cm⁻¹ 3449, 3294, 3225, 3024,

2368, 2214, 1659, 1589, 1489, 1335, 1180, 1095, 995, 818, 740, 548; ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 11.18 (s, 1H, NH), 8.51 (s, 1H, CH), 7.45 (d, 2H, J 9.1 Hz, 2 CH), 7.44 (d, 2H, J 9.1 Hz, 2 CH); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) δ 155.9 (CH), 138.2 (C), 129.3 (CH), 129.2 (C), 119.8 (CH), 116.3 (C), 114.0 (C), 52.4 (C).

Results and Discussion

The assignments of new potential pharmacological agents is normally executed with different one dimensional NMR spectra (1D), as ^1H , ^{13}C , ^{19}F and ^{15}N , and with some dimensional (2D) spectra, as COSY, TOCSY, HSQC and HMBC. In general, the 1D spectra afford the ppm chemical shift values of all atoms, but to confirm the correct assignment of all atoms is fundamental the use of the 2D spectra. The COSY affords the coupling between all hydrogen atoms, but sometimes this method affords difficult results, especially when some ^1H signals are with superposition or with very similar chemical shift.¹³ For this reason it is important the use of the TOCSY method, which indicates the hydrogen groups that are part of different coupling systems.¹⁴

The HSQC affords one single bond correlation between ^1H with ^{13}C , ^{19}F , ^{15}N and other atoms. This methodology confirms which hydrogens are directly bounded to the other atoms, being this information important to partial determination of the chemical shifts. The other important spectrum is the HMBC, which displays the correlation of ^1H with the other atoms with two, three and four bond correlations, being very important to determine the chemical shift and position of all atoms, especially the quaternary carbons. This method is very important for the obtention of the complete chemical shift of all atoms from different compounds. Therefore, these 2D spectra are very important to determine the molecular position of all atoms.¹⁵

Interestingly, sometimes the application of these methods does not allow the complete assignment of the analyzed agents, being necessary the application of other methods to determine the complete coupling constants, a process that usually confirms the complete and non questionable chemical shift assignment. There are some methods than can be applied for this type of information, especially IPAP-HSQMBC, which is measurement for

long-range heteronuclear coupling constants from spin-state selective multiplets.¹⁶ This method is very efficient, but sometimes requires several conditions for analysis. Other method, which was developed by our research group is the selective heteronuclear simultaneous short and long-range correlations (SHESSLOC),¹⁷ which is also appropriate. However, one of the simplest methods is the gated decoupling of ^{13}C NMR, which can also be applied to other atoms, as ^{15}N and ^{19}F , being an easy application and affording very good results for the small molecules. However, this method is usually not used by all specialists on NMR. In general terms, the gated decoupling of ^{13}C NMR is very important to determine the chemical shift of all quaternary carbons, a process that have been used to confirm the structure and chemical shift of pharmacological agents.¹⁸

In order to determine the three dimensional structure of molecules, despite the complete chemical shift assignment, it is also necessary the application of NOE. The NOE methods allow the calculation of dipolar coupling, a process that determines which hydrogens are close to other ones and the correlation distance without the participation of bonds. This methodology is very efficient to determine the diverse position of several hydrogens, allowing the confirmation of the three dimensional structure of these compounds. Another important procedure to confirm the three-dimensional structure of molecules is the application of molecular modeling. These NMR methodologies are described and applied for the non questionable assignment of molecules in this article.

The phenanthro[9,10-*e*][1,2,4]triazin-3-amine (**4**) was prepared with two reactions. Initially was selected phenanthrenequinone (**1**) to react with aminoguanidine hydrochloride (**2**), to form the phenanthrenequinone guanyldiazide (**3**), which was selected as an agent for decreasing the cancer radiotherapy and for treatment of Alzheimer's disease. Finally, the product **3** was transformed to phenanthro[9,10-*e*][1,2,4]triazin-3-amine (**4**) by cyclization, as shown in Figure 1, which was also selected as intercalate to DNA.

The compound **3** was previously tested as intercalate of DNA and with condition to decrease 50% of the cancer radiotherapy. For this reason compound **4** was designed as DNA intercalate with the possibility to decrease the dose

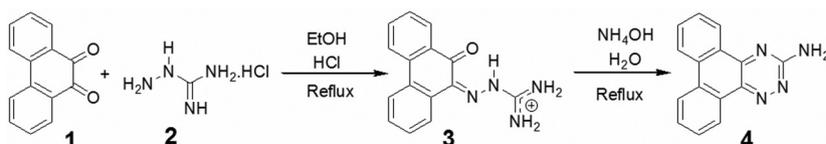


Figure 1. Synthesis of phenanthro[9,10-*e*][1,2,4]triazin-3-amine (**4**).

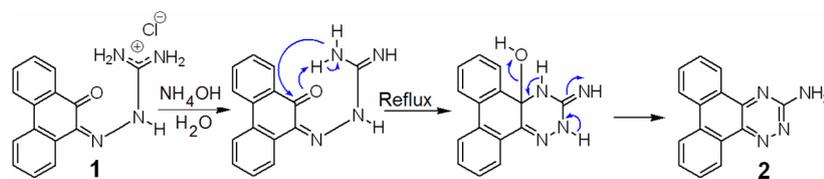


Figure 2. Mechanism of synthesis of phenanthro[9,10-*e*][1,2,4]triazin-3-amine (**4**).

Table 1. ^1H , COSY and TOCSY of phenanthro[9,10-*e*][1,2,4]triazin-3-amine (**4**)

δ_{H}	Integration	Mult.	$J_{\text{HH}} / \text{Hz}$	COSY ^a	TOCSY
9.07 (H12)	1H	dd	7.1, 1.9	7.73 , 7.74	9.07-8.69-7.74-7.75
8.97 (H5)	1H	d	7.8	7.75 , 7.89	8.97-8.73-7.89-7.75
8.73 (H8)	1H	d	7.8	8.69, 7.89	8.73-8.97-7.89-7.75
8.69 (H9)	1H	dd	7.2, 1.5	8.73, 7.74	8.69-9.07-7.74-7.75
7.89 (H13)	1H	t	7.8	8.73 , 7.75	7.89-8.97-8.73-7.75
7.75 (H6)	1H	t	7.8	8.97 , 7.89	7.75-8.97-8.73-7.89
7.74 (H10)	1H	t	7.2	8.69 , 7.73	7.74-9.07-8.69-7.73
7.73 (H11)	1H	t	7.1	9.07 , 7.74	7.73-9.07-8.69-7.74
7.59 (H13)	2H	s	–	–	–

^aThe COSY signals with bold are the most effective. COSY: correlation spectroscopy; TOCSY: total correlation spectroscopy.

of cancer radiotherapy. Therefore, the complete chemical shift assignment of this compound is fundamental to study its interaction with DNA.

The mechanism of cyclization of phenanthrenequinone guanylhydrazone (**3**) to prepare phenanthro[9,10-*e*][1,2,4]triazin-3-amine (**4**) is shown in Figure 2.

The initial NMR methods to obtain information for the chemical shift assignment of phenanthro[9,10-*e*][1,2,4]triazin-3-amine (**4**) were ^1H , COSY and TOCSY, which results are shown in Table 1. The ^{13}C , gHSQC and gHMBC results are shown in Table 2.

The ^1H NMR spectrum affords the chemical shift and multiplicity of all ^1H signals and the integration procedure allows the determination of the number of hydrogen atoms on each signal. These results, as shown on columns 1-4 of Table 1, indicate that the hydrogen signals of 8.69 to 9.07 ppm are hydrogens basically with only one other hydrogen at 3 bond distance, a process that allows $^3J_{\text{HH}}$ constants from 7.1 to 7.8 Hz. Interestingly, only two of these hydrogens also display coupling with other hydrogens at 4 bond distances with $^4J_{\text{HH}}$ of 1.5 and 1.9 Hz. This result indicates that the condition of the two benzyl rings containing hydrogens (A and B) are different, especially because the ligation condition of the aromatic ring containing the four nitrogen atoms with these two benzyl rings are different.

The COSY spectrum affords certain difficulty to determine the coupling of some hydrogen which display chemical shift very similar to other ones. In order to use better the COSY experiment is necessary to perform

Table 2. ^{13}C , HSQC and HMBC of phenanthro[9,10-*e*][1,2,4]triazin-3-amine (**4**)

δ_{C}	gHSQC	gHMBC ^a
162.2 (C3)	–	–
143.1 (C4a)	–	8.97, 8.73
138.4 (C12b)	–	9.07
133.3 (C8a)	–	8.97 , 8.73, 8.69, 7.89 , 7.75
131.9 (C7)	7.89	8.97, 8.73, 7.75
128.4 (C10)	7.74	9.07 , 8.69
128.4 (C11)	7.73	9.07, 8.69
128.2 (C8b)	–	9.07 , 7.74, 8.73, 8.69
127.9 (C12a)	–	9.07, 8.69, 7.73
127.7 (C6)	7.75	8.97, 7.89, 8.73
127.5 (C4b)	–	8.97, 8.73 , 7.75
125.3 (C5)	8.97	7.89 , 8.73, 7.75
123.5 (C8)	8.73	7.89, 7.75
123.3 (C9)	9.69	7.74, 7.73
122.0 (C12)	9.07	7.74 , 7.73

^aThe signals with bold are the strong correlations, in normal font are average and in italic are the lowest ones. HSQC: heteronuclear single quantum coherence; HMBC: heteronuclear multiple bond coherence.

the spectrum with lower expansion and selecting all the coupling hydrogens. However, the best methodology is the use of TOCSY spectrum, which indicates that the ^1H signals from 9.07, 8.69, 7.74 and 7.75 ppm are the hydrogens from one of the two aromatic rings and 8.97, 8.73, 7.89 and 7.75 ppm are from the other benzyl ring. This information

120.2, indicating that it is at three bonds from carbon 128.4, which is bounded to the hydrogen of 7.74 ppm, and with carbon 128.2 that is not bounded to any hydrogen. Interestingly, the 9.07 ppm signal to the other quaternary carbon, 138.44 ppm, is not very strong, but that must be due to a three bonding distance. The other interactions are with the CH carbon 128.4, which is bounded to the hydrogen of 7.73 ppm, and with another quaternary carbon, 127.9 ppm, being at two bonds from the 9.07 hydrogen.

The only problem is if the chemical shift of carbons 4a and 12b that could be exchanges, a process that could also exchange the position of atoms 1, 2, 3, 4 and 13, leading to structure B. The aromatic carbons bounded with C–N=N or C–N=C could be with chemical shift 143.1 and 138.4 on the structure B. However, there is the possibility that the aromatic carbons bounded to C–N=N and C–N=C could also be of 143.1 and 138.4 on the structure B.

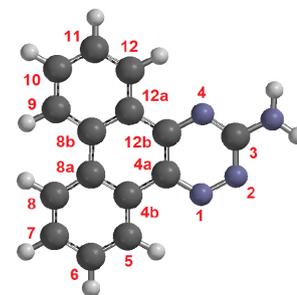
The possibility to confirm the structure of compound **4** is the study of the NMR spectra and comparison by molecular modeling, especially to determine which of the carbons 4a and 12b displays lower electronic density, a process that could indicate which carbon contains the highest chemical shift.

The structure calculation of compound **4** using density functional with B3LYP and 6-311G*, affords the electronic density calculated with natural form, the molecular energy (kJ mol⁻¹), polar surface area (PSA, Å²), element volume (Å³) and their dipole moment (Debye), as shown in Table 3.

When the calculated electronic density of one atom is a positive value, this indicates that the number of electrons on this atom is low, leading to higher chemical shift. On the other hand, if the electronic density is negative, the number of electrons is higher and affords low chemical shift. When these numbers are positive, if they contain grate value, their chemical shift is higher. If the numbers are negative, when the negative number is major, the chemical shift is very low. For this reason the electronic density values indicate the possible chemical shifts of the carbons. These results display that the electronic density of the carbon bounded with two nitrogen atoms (C–N=N–) is 0.104, and for the carbon bounded with only one nitrogen (C–N=C–) is 0.239. The major electronic density (0.239) is for a higher chemical shift, confirming that 4a is 143.1 ppm and 12b

Table 3. Molecular modeling calculation results of compound (**4**)

Energy / (kJ mol ⁻¹)	-0.3035
Area / Å ²	249.35
Volume / Å ³	242.79
PSA / Å ²	50.120
Dipole moment / Debye	2.53
Electronic charge	
C1	-0.166
C2	-0.186
C3	-0.187
C4	-0.176
C4a	-0.042
C4b	-0.013
C5	-0.181
C6	-0.171
C7	-0.196
C8	-0.070
C8a	-0.072
C9	0.239
C10	0.104
C10a	-0.044
C11	0.572



PSA: polar surface area.

is of 138.4 ppm, indicating that the correct assignment of compound **4** is the structure B shown in Figure 3.

In some cases the complete assignment of certain molecules requires the determination of the ⁿJ_{CH} in order to determine the position and chemical shift of some quaternary carbons. To determine the values of ⁿJ_{CH} several procedures can be used, such as IPAP-HSQMBC,¹⁶ and SHESSLOC,¹⁷ but the simple and very easy to apply this method is the gated decoupling of ¹³C NMR.¹⁸ One example of gated decoupling of ¹³C NMR application is on compound **5**, which was synthesized as shown in Figure 4.

The assignment of compound **5**, which was selected for treatment of leishmaniasis, is generally simple, but the only problem is the definition of the chemical shift and position of carbons C10 and C11, which are very similar. The complete NMR results of compound **5** are shown in Tables 4 and 5. This compound was selected as inhibitor of

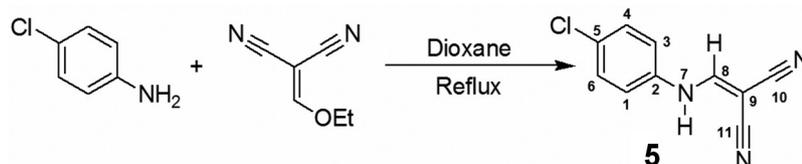


Figure 4. Synthesis of 2-[(4-chlorophenylamino)methylene]malononitrile (**5**).

the enzyme nucleoside hydrolases (NH) from *Leishmania donovani* and *Leishmania chagasi*, because we already discovered some of these agents.¹⁹

Table 4. ¹H, gHSQC and gHMBC of 2-[(4-chlorophenylamino)methylene]malononitrile (5)

δ_{H} (Int., mult., J Hz)	gHSQC	gHMBC ^a
11.18 (s, 1H, H7)	–	–
8.51 (s, 1H, H8)	155.9	138.2, 116.3, 114.1, 52.4
7.45 (d, 2H, 9.1, H2 and H6)	119.8	138.2, 129.3, 129.2
7.44 (d, 2H, 9.1, H3 and H5)	129.3	138.2 , 129.2, 119.8

^aThe signals with bold are the strong correlations, in normal font are average. HSQC: heteronuclear single quantum coherence; HMBC: heteronuclear multiple bond coherence.

Table 5. ¹³C, APT and gated decoupling of ¹³C of 2-[(4-chlorophenylamino)methylene]malononitrile (5)

δ_{C}	APT	Gated	
		Mult.	^a J_{CH} / Hz
155.9 (C8)	CH	d	176.9
138.2 (C1)	C	dt	6.9 and 4.6
129.3 (C3 and C5)	CH	dd	167.9 and 4.6
129.2 (C4)	C	tm	8.7
119.8 (C2 and C6)	CH	dd	165.4 and 5.8
116.3 (C10)	C	d	4.7
114.1 (C11)	C	d	10.4
52.4 (C9)	C	d	4.6

APT: attached proton test.

The hydrogen 8.51 ppm, which is a simple signal because it is not bounded to the benzyl ring and with other hydrogens, but it is coupled with the carbons 116.3 and 114.1 ppm. The coupling condition between 8.51 ppm with 116.3 and 114.1 ppm, because it is by three bonds, indicate that the respective ³ J_{CH} values depend on the conformation of the hydrogen with the two carbons, being at position *cis* of *trans*, as are shown in Figure 5.

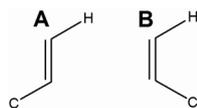


Figure 5. Conformation of three bond coupling of hydrogen with carbon.

The forms A and B from Figure 5 are linear *cis* and *trans*, respectively. The *trans* conformation allows the greater H–C coupling than the *cis* conformation. When the system is planar, the angle between C and H is of 180° in conformation A and of 0° in conformation B. Because the 116.3 and 114.1 ppm carbons are only strongly coupled

with H-8 (8.51 ppm) as shown by gHMBC, it confirms that these carbons are from the C≡N groups. On the other hand, the gated decoupling of ¹³C NMR affords all the ^a J_{CH} coupling constants, especially indicating that the ³ J_{CH} of the 116.3 and 114.1 ppm are 4.7 and 10.4 Hz, respectively. These results confirm that the carbon at the *trans* position about H-8 is the 114.1 ppm and the carbon at the *cis* position is the 116.3 ppm. Therefore, it is confirmed that the application of gated decoupling of ¹³C NMR is very important to obtain the complete and non questionable chemical shift of quaternary carbons. Since hydrogens 8.51 and 7.44 display strong interaction with the quaternary carbon of 138.2 ppm by gHMBC, this condition indicates that the hydrogen 7.44 is close to this carbon and far from the 8.51 ppm. As the 7.54 hydrogen interacts strongly with the quaternary carbon of 129.3 ppm, which is bounded to the Cl, this hydrogen is close to 8.51. The complete assignment of compound 4 is shown in Figure 6.

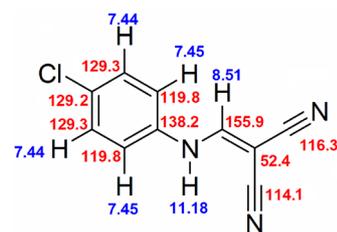
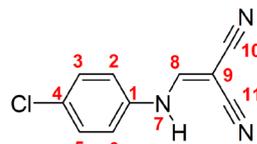


Figure 6. Complete assignment of 2-[(4-chlorophenylamino)methylene]malononitrile (5).

The molecular modeling calculation using density functional theory (DFT) with the B3LYP and the M06 method with 6-311-G* basis set, indicates the electronic atomic charges (QNPA) for each carbon, as shown in Table 6.

Table 6. Carbons molecular modeling calculation of electronic atomic charge from compound 5



Carbon	Electronic charge
C1	0.158
C2 and C6	-0.206 and -0.219
C3 and C5	-0.199 and -0.194
C4	-0.034
C8	0.172
C9	-0.370
C10	0.276
C11	0.285

The electronic condensation on each atom indicates their possible chemical shift. When the electronic concentration is major or lower, the chemical shift is lower or major, respectively. The carbons electronic charge from compound **5** are between -0.219 and $+0.285$, being the negative values with major electronic concentration, and the positive ones with lower concentration. In this case, the $C\equiv N$ carbon of major electronic charge display electronic charges of 0.276 of 116.3 ppm and the other one 0.285 with 114.1 ppm. The definitive chemical shift of these carbons is determined by all NMR methods, being also important the gated decoupling ^{13}C and molecular modeling.

The study of the potential capacity of these compounds to interact with the selected biological targets was executed by docking with AutoDock Vina. The interaction of compound **4** was selected to DNA. For this topic it was used the Drew-Dickerson-Dodecamer (DDD), and the docking

indicated that this compound is an appropriate intercalate to DNA, as shown in Figure 7.

This result indicates that compound **4** could be used as an agent to decrease the dose of cancer radiotherapy, in order to diminish the negative effects of radiotherapy to other human cells. This compound interacts better with the groups deoxydenosine 5'-monophosphate (A) and deoxythymidine 5'-nonophosphate (T) of DDD.

The docking study of compound **5** as inhibitor of the enzyme nucleoside hydrolase from *Leishmania donovani* (NH) is shown in Figure 8.

This result indicates that the CN groups of compound **5** interact with the amino acids Asp14, Thr128, Asn168 and the Ca^{2+} of this enzyme. On the other hand, the NH group interacts with Gly12 and the aromatic ring displays π -stacking with Phe167. For this reason, this compound could display activity against leishmaniasis.

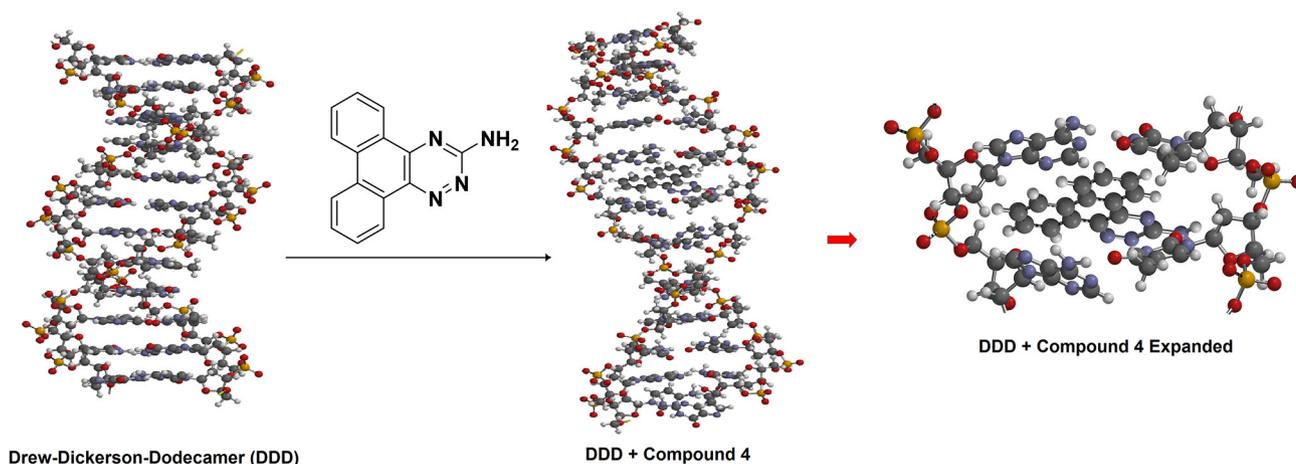


Figure 7. Docking of compound **4** with Drew-Dickerson-Dodecamer (DDD).

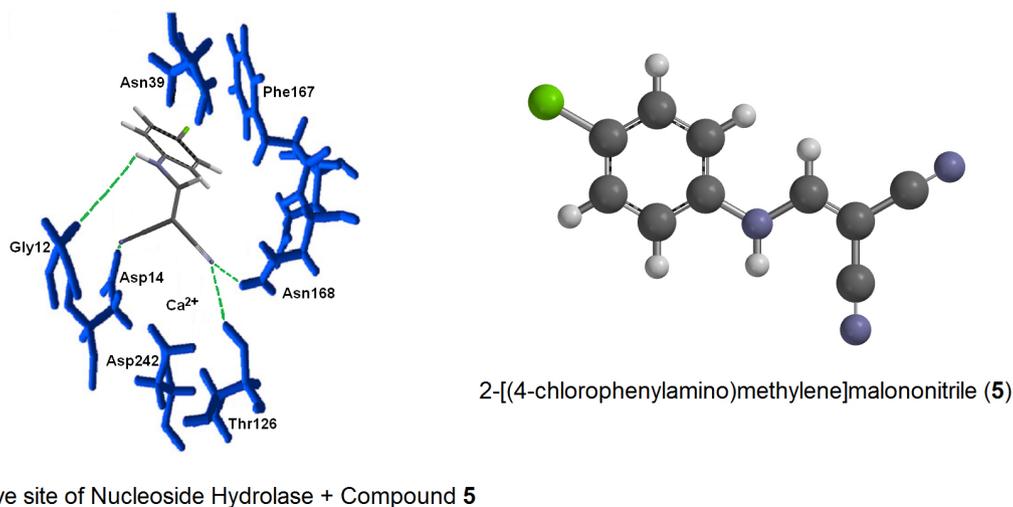


Figure 8. Docking study of the interaction of compound **5** with nucleoside hydrolase (NH).

The biological texts of these compounds will be performed on the future, and also with other similar compounds against cancer and *Leishmania*.

Conclusions

The determination of the non questionable and complete chemical shift assignment of potential pharmacological agents is very necessary to determine their condition of interaction with the biological targets involved on their biological activity. This information can be used for inhibition of enzymes and interaction with DNA, being a process to determine which areas of these pharmacological agents are interacting with the targets, a process that allows the design of new agents.

In general terms, the methods to definitely confirm the structure and chemical shift of all compounds must be executed by several NMR spectra and molecular modeling calculations, being these procedures very important to confirm the definitive structure of the obtained compounds prepared by synthesis. This condition is to motivate the complete structure and chemical shift assignment of all new synthesized compounds, in order to confirm their condition. This procedure is also confirmed by molecular modeling.

The use of docking is also very important to also determine the capacity of compounds to interact with biological targets, a process that also confirms their capacity as pharmacological agents.

In the future, the compound **4** will be tested as DNA intercalate and to determine the decrease of the cancer radiotherapy. This compound, being planar and with aromatic rings, it can interact with the elements of DNA by π -stacking, and the NH_2 group could interact with the $-\text{NH}$, $-\text{OH}$ and phosphate groups. The compound **5**, which was designed as inhibitor of the enzyme nucleoside hydrolases (NH) for *Leishmania*, being a process that its groups CN and NH could interact with the Ca^{2+} atoms of the active site of this enzyme, and the chlorinated benzyl group could also interact with some of the amino acids of this enzyme. The biological tests of these compounds will be executed on the future.

Supplementary Information

The supplementary data of NMR spectra ^1H , ^{13}C , gHSQC, gHMBC, COSY, gated decoupling ^{13}C and TOCSY, their molecular modeling structures and the NMR assignment by molecular modeling of compound **4** are available free of charge at <http://jbc.org.br> as a PDF file.

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References

1. Bunaciu, A. A.; Udristioiu, E. G.; Aboul-Enein, H. Y.; *Crit. Rev. Anal. Chem.* **2015**, *45*, 289.
2. Pervushin, K.; Riek, R.; Wider, G.; Wuthrich, K.; *Proc. Natl. Acad. Sci. U. S. A.* **1997**, *94*, 12366.
3. Wider, G.; Wuthrich, K.; *Curr. Opin. Struct. Biol.* **1999**, *9*, 594.
4. Rossi, C.; Donati, A.; Sansoni, M. R.; *Chem. Phys. Lett.* **1992**, *189*, 278.
5. Figueroa-Villar, J. D.; Tinoco, L. W.; *Curr. Top. Med. Chem.* **2009**, *9*, 811.
6. Coles, M.; Heller, M.; Kessler, K.; *Drug Discovery Today* **2003**, *8*, 803.
7. Soares, S. F. D. X.; Vieira, A. A.; Delfino, R. T.; Figueroa-Villar, J. D.; *Bioorg. Med. Chem.* **2013**, *21*, 5923.
8. Palmer, A. G.; *Acc. Chem. Res.* **2015**, *48*, 457.
9. Richards, M. R.; Brant, M. G.; Boulanger, M. J.; Cairo, C. W.; Wulff, J. E.; *MedChemComm* **2014**, *5*, 1483.
10. Viegas, A.; Manso, J.; Nobrega, F. L.; Cabrita, E. J.; *J. Chem. Educ.* **2011**, *88*, 990.
11. Cabeça, L. F.; Pomini, A. M.; Cruz, P. L. R.; Marsaioli, A. J.; *J. Braz. Chem. Soc.* **2011**, *4*, 702.
12. Shao, Y.; Molnar, L. F.; Jung, Y.; Kussmann, J.; Ochsenfeld, C.; Brown, S. T.; Gilbert, A. T. B.; Slipchenko, L. V.; Levchenko, S. V.; O'Neill, D. P.; DiStasio, J. R. A.; Lochan, R. C.; Wang, T.; Beran, G. J. O.; Besley, N. A.; Herbert, J. M.; Lin, C. Y.; Van Voorhis, T.; Chien, S. H.; Sodt, A.; Steele, R. P.; Rassolov, V. A.; Maslen, P. E.; Korambath, P. P.; Adamson, R. D.; Austin, B.; Baker, J.; Byrd, E. F. C.; Dachsel, H.; Doerksen, R. J.; Dreuw, A.; Dunietz, B. D.; Dutoi, A. D.; Furlani, T. R.; Gwaltney, S. R.; Heyden, A.; Hirata, S.; Hsu, C.-P.; Kedziora, G.; Khalliulin, R. Z.; Klunzinger, P.; Lee, A. M.; Lee, M. S.; Liang, W. Z.; Lotan, I.; Nair, N.; Peters, B.; Proynov, E. I.; Pieniazek, P. A.; Rhee, Y. M.; Ritchie, J.; Rosta, E.; Sherrill, C. D.; Simmonett, A. C.; Subotnik, J. E.; Woodcock III, H. L.; Zhang, W.; Bell, A. T.; Chakraborty, A. K.; Chipman, D. M.; Keil, F. J.; Warshel, A.; Hehre, W. J.; Schaefer, H. F.; Kong, J.; Krylov, A. I.; Gill, P. M. W.; Head-Gordon, M.; *Phys. Chem. Chem. Phys.* **2006**, *8*, 3172.
13. Kessler, H.; Muller, A.; Oschkinat, H.; *Magn. Reson. Chem.* **1985**, *23*, 844.
14. Wollborn, U.; Leibfritz, D.; *J. Magn. Reson.* **1991**, *3*, 653.
15. Parella, T.; Espinosa, J. F.; *Prog. Nucl. Magn. Reson. Spectrosc.* **2013**, *73*, 17.
16. Gil, S.; Espinosa, J. F.; Parella, T.; *J. Magn. Reson.* **2010**, *207*, 312.

17. Nascimento, C. J.; Figueroa-Villar, J. D.; *J. Magn. Reson.* **2007**, *187*, 126.
18. Figueroa-Villar, J. D.; *Appl. Magn. Reson.* **2015**, *46*, 607.
19. Rennó, M. N.; França, T. C. C.; Nico, D.; Palatnik-de-Sousa,

C. B.; Tinoco, L. W.; Figueroa-Villar, J. D.; *Eur. J. Med. Chem.* **2012**, *56*, 301.

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