Synthesis, Characterisation, Theoretical NMR Calculations and Crystal Structure of the 3-methyl-1H-1,2,4λ4-triazole-5-amine acetate

Síntese, Caracterização, Cálculos Teóricos de RMN e Estrutura Cristalina do 3-metil-1H-1,2,4λ4-triazol-5-amina acetato

Article Info:
Article history: Received 2022-03-06/ Accepted 2022-09-21 / Available online 2022-09-21
doi: 10.18540/jcecvl8iss7pp14608-01i

Patricia Saraiva Vilas Boas de Almeida
ORCID: https://orcid.org/0000-0001-7205-2115
Departamento de Química, Universidade Federal de Viçosa, Brazil
E-mail: pvilasboas@yahoo.com.br

José Roberto da Silva Maia
ORCID: https://orcid.org/0000-0001-5715-8763
Departamento de Química, Universidade Federal de Viçosa, Brazil
E-mail: jrsmaia@ufv.br

Márcia Cristina de Souza
ORCID: https://orcid.org/0000-0001-8333-3617
Departamento de Química, Universidade Federal de Juiz de Fora, Brazil
E-mail: marciaphn@gmail.com

Alison Geraldo Pacheco
ORCID: https://orcid.org/0000-0003-1338-0340
Instituto Federal de Educação, IFSULDEMINAS, Brazil
E-mail: alison.pacheco@ifsuldeminas.edu.br

Abstract
The condensation reaction between aminoguanidine bicarbonate with carboxylic acids led to the formation of 3-methyl-1H-1,2,4-triazole-5-amine (mta), 3-methyl-1H-1,2,4λ4-triazole-5-amine acetate (Hmta) and 3-phenyl-1H-1,2,4-triazole-5-amine (pta). The compound N-(3-methyl-1H-1,2,4-triazole-5-yl)propan-2-imine (mpta) was obtained by reacting the mta with acetone, upon an attempt of purifying mta in this solvent. The excess of acetic acid obtained the Hmta during the preparation of mta. These compounds were characterised by infrared and multinuclear NMR (1H

Resumo
A reação de condensação entre bicarbonato de aminoguanidina com ácidos carboxílicos levou à formação de 3-metil-1S-1,2,4-triazol-5-amina (mta), 3-metil-1H-1,2,4λ4-triazol-5-amina acetato (Hmta) e 3-fenil-1H-1,2,4-triazol-5-amina (pta). O composto N-(3-metil-1S-1,2,4-triazol-5-yl)propan-2-imine (mpta) foi obtido reagindo a mta com acetona, após uma tentativa de purificar mta neste solvente. O excesso de ácido acético obteve o Hmta durante a preparação do mta. Esses compostos foram caracterizados por espectroscopia infravermelha e multinuclear de NMR (1H e 13C), microanálise e ponto de fusão. Para investigar a formação de possíveis conformações tautoméricas de mta, pta e mpta em solução, utilizou-se uma abordagem teórica para calcular as mudanças químicas do carbono 13, com base nos valores do tensor de blindagem magnética (NMR) pelos métodos MPn e DFT. O ensaio biológico dos triazoles mta, pta e mpta contra Staphylococcus aureus, Bacillus subtilis, Escherichia coli e Salmonella typhimurium não mostrou atividade na maior concentração utilizada no experimento.

Palavras-chave: Compostos 1,2,4-Triazois. Testes biológicos. Estrutura cristalina. Espectroscopia.

DOI: 10.18540/jcecvl8iss7pp14608-01i
and $^{13}$C) spectroscopy, microanalysis, and melting point. To investigate the formation of possible tautomeric conformations of $m$ta, $p$ta and $m$pta in solution, a theoretical approach was used to calculate the chemical shifts of carbon 13, based on the values of magnetic shielding tensor (NMR) by MPn and DFT methods. The biological assay of the triazoles $m$ta, $p$ta and $m$pta against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhimurium* showed no activity at the highest concentration used in the experiment.

**Keywords:** 1,2,4-Triazole compounds. Biological assay. Crystal structure. Spectroscopy.

1. Introduction

Triazole compounds are acknowledged as antifungal, antiviral, antibacterial, antidepressant and anticonvulsant drugs (Deng, Song, Zheng, & Quan, 2014; Naito et al., 1996; Papakonstantinou-Garoufalias, Poulí, Marakos, & Chytryoglou-Ladas, 2002). The treatment of human diseases involves several triazoles as *Fluconazole*, *Itraconazole* and *Ravuconazole* (Johnson, Szekely, & Warnock, 1999; Roberts, Schock, Marino, & Andriole, 2000). The *Itraconazole*, for instance, prevent the enzyme sterol 14-alpha demethylase to interact with the iron(II) ion on the haem of the enzyme becoming the key-step for the biosynthesis of ergosterol (Groll, Piscitelli, & Walsh, 1998). The *Ribavirin* is an antiviral agent of large spectrum for the treatment of pathological illnesses of the lower respiratory tract in humans (Graci & Cameron, 2006). The 1,2,4-triazole compounds are also known to inhibit the mitochondrial and chloroplast function. This property allows these compounds to be commercialised in the market like herbicides, defoliant on cotton farming and in the control of plant growth (Han et al., 2011).

These compounds are capable of forming hydrogen bond which might favour specific biochemical interactions within a living organism (Vatmurge et al., 2008). Triazole compounds containing large carbon chain or aromatic groups bonded to the ring of 1,2,4-triazole showed positive response to the antimicrobial assay (El Akri, Bougrin, Balzarini, Faraj, & Benhida, 2007; Karthikeyan, Holla, & Kumari, 2008).

Considering the activity of the 1,2,4-triazole derivatives, a few compounds with small group substituent bonded to the ring were synthesised, characterised and assayed in this work against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhimurium* to evaluate their potential as antibiotics.

The labile nature of the hydrogen atom probably leads to tautomeric species in solution. Stable tautomers can be identified by NMR spectroscopy if the labile hydrogen bind to different atoms, such as nitrogen and sulfur (Wu et al., 2007). Beyond the characterization by infrared and NMR techniques of the triazole compounds in this work, a theoretical approach using the magnetic shielding tensor was carried out to investigate a possible tautomerism in solution, by comparing the calculated and experimental NMR data (Phalgune, Vanka, & Rajamohanan, 2013).

2. Results and discussion

Several condensation reactions are reported in the literature for the preparation of triazole compounds. The usual reagents in the synthetic route of triazoles are acylimidazones (Pellizzari reaction), diacylamines or hydrazine (Einhorn-Brunner reaction), and 1,2-diacylhydrazine (Sudheendran, Schmidt, Frey, Conrad, & Beifuss, 2014). Although these reagents are well known, aminoguanidine bicarbonate was chosen as starting material because is inexpensive and simple to manipulate in the process of purification before attempting the experiment. This compound was also used as reactant in the synthesis of 5-amino-1H-1,2,4-triazole-3-acetic acid and 3-amine-5-methyl-1H-1,2,4-triazole or 3-methyl-1H-1,2,4-triazole-5-amine (*m*ta) through the condensation reaction with carboxylic acids (Boechat, Pinheiro, Santos-Filho, & Silva, 2011). The molecular structures of the 1,2,4-triazole compounds in this work are shown by Figure 1.
Figure 1 - Molecular structure of the 1,2,4-triazole compounds and a possible intermediate compound (step I).

The triazole compound \( mta \) was synthesised by the equimolar reaction between the aminoguanidine bicarbonate and the acetic acid as well as the \( pta \) with the benzoic acid. However, a subsequent acid/base reaction took place by the use of an excess of acetic acid (60%), forming the \( Hmta \), to which the crystal structure has been analysed. A few crystals of \( Hmta \) also separated after cooling the filtrate from the equimolar synthesis of the \( mta \). The attempt of purifying \( mta \) in acetone produced the \( mpta \) as the main product. This product allows us to speculate that a small amount of unreactive acetic acid catalysed the reaction between \( mta \) and acetone. These triazole compounds are soluble in dimethyl sulfoxide, methanol, and chloroform. The reaction route to produce these 1,2,4-triazole derivatives is shown by Figure 2. \(^{13}\)C NMR characterized the intermediate step (I) compound, separated by filtration at room temperature.

Figure 2 - Synthetic route to the preparation of the 1,2,4-triazole compounds.
2.1. Infrared Spectroscopy

The infrared spectrum of aminoguanidine bicarbonate showed three strong bands in the region of 3245 cm\(^{-1}\) correlated to the stretching vibrational modes \(\nu(N-H)\) and the bicarbonate ion shown a strong infrared absorption at 1355 cm\(^{-1}\). Absorptions in the range of 1720 to 1600 cm\(^{-1}\) were assigned to the carbonyl group of the carboxylic acids (Silverstain, Bassler, & Morril, 1991; Vieira, Maia, Ardisson, & de Lima, 2008). A typical infrared band in the range of 3300 to 2500 cm\(^{-1}\), characteristic of intramolecular hydrogen bond between the hydroxyl group and the carbonyl group, was also shown in the spectra of these carboxylic acids (Silverstain et al., 1991).

The condensation reaction between the aminoguanidine and the carboxylic acids was confirmed by an infrared vibrational shift to high frequency of the amine group (Boechat et al., 2011; Vieira et al., 2008). Two infrared bands identified in the range of 3289 to 3317 cm\(^{-1}\) correlates to the \(\text{NH}_2\) group of the \textit{mta} and \textit{pta} compounds. Furthermore, the absence of the intramolecular hydrogen bond of carboxylic acids in the spectra of these compounds reinforces the formation of the triazole derivatives.

The infrared spectroscopy has been a useful technique to characterize tautomers of the triazole compounds class. The infrared bands of the C=N group in the range of 1700 to 1600 cm\(^{-1}\) have been related to the formation of tautomeric species of 1,2,4-triazoles at equilibrium due to the N-H bond configurations on the ring (Akerblom & Sandberg, 1965; Grinshtein, Strazdin, & Grinvalde, 1970; Lopyrev, Beresneva, & Strelets, 1969). The 3-Chloro-1,2,4-triazole revealed two infrared bands at 1770 and 1750 cm\(^{-1}\) that have been assigned to tautomeric species (Grinshtein et al., 1970). The compound diamine-1,2,4-triazole also showed two bands in the range of 1600 to 1640 cm\(^{-1}\) associated to the formation of tautomeric species (Abdel-Megeed, Abdel-Rahman, Alkaramany, & El-Gendy, 2009; Lopyrev et al., 1969; Tyagi et al., 2017; Wajda-Hermanowicz et al., 2016).

The number of infrared bands relative to the C=N bond of the triazole compounds in this work are in agreement with the presence of isomeric forms in the solid state, as previously reported in the literature. The strong infrared absorption at 1633 cm\(^{-1}\), that corresponds to \(\nu(C=N)\) of the aminoguanidine bicarbonate, has split up in two new absorptions in the range of 1705 to 1575 cm\(^{-1}\) in the spectra of the triazoles \textit{mta}, \textit{pta} and \textit{mpta}. The third additional infrared absorption for the \textit{mpta} compound at 1579 cm\(^{-1}\) was assigned to the \(\nu(C=N)\) of the imine group outside of the triazole ring, similar to other Schiff bases derivatives (Sokmen et al., 2015; Tyagi et al., 2017; Wajda-Hermanowicz et al., 2016).

The synthesis and characterisation of \textit{mta} (3-methyl-1H-1,2,4-triazole-5-amine) is reported in the literature but the acetate salt derivative, 3-amine-5-methyl-1H-1,2,4\(\lambda^4\)-triazole (\textit{Hmta}), is an original compound (Boechat et al., 2011). Possible tautomeric species of \textit{mta} (A’, B’, C’) and \textit{pta} (A, B, C, D, E), as well as of \textit{mpta} (F, G, H, I, J, K) are shown by Figure 3.
Figure 3 - Possible tautomeric species of mta (A', B', C'), pta (A, B, C, D, E) and mpta (F, G, H, I, J, K).

2.2. NMR Spectroscopy

The hydrogen NMR of pta in DMSO showed broad singlets correlated to the primary amine group (NH₂) at δ 8.13, and at δ 10.32, associated to the pyrrole group (NH) from the 1,2,4-triazole ring. In CDCl₃, the chemical shift for the amine group from pta was at δ 8.24 and for the pyrrole group at δ 10.45. The hydrogen atoms of the phenyl group from pta showed signals in the region of δ 7.31 in DMSO, CD₂OD and CDCl₃. The J(¹H-H) coupling constant for these hydrogen atoms is 7.21 Hz confirming the presence of this group in the structure of the compound (Silverstain et al., 1991). A broad singlet at δ 11.06 (DMSO) in the spectrum of mpta correlates to the pyrrole group and the methyl groups for this compound shown chemical shifts at δ 2.17, 2.04 and 1.88 in DMSO.

The spectrum of mta reported in the literature, nomenclature of 3-amine-5-methyl-1H-1,2,4-triazole or 3-methyl-1H-1,2,4-triazole-5-amine has revealed two broad hydrogen signals in DMSO in the range of δ 8.7 to δ 8.0 and δ 6.3 to δ 5.8. These signals correlates to the primary amine and the pyrrole group respectively. In addition, the signal for the methyl group appears as singlet at δ 2.13 (Boechat et al., 2011). It is well-known that the chemical shift of these groups are dependent on hydrogen bonding, temperature and concentration of the sample (Silverstain et al., 1991). Although the NMR of the mta compound prepared in this work was not recorded, the infrared spectrum and the microanalysis of it corroborates with the success of the synthetic route. Furthermore, the intermediate compound in step I in Figure 2 is original and has never been
characterised by the NMR technique, to our knowledge. This compound, the amino(hydrazinyl)methaniminium acetate, showed chemical shifts of $^{13}$C NMR at $\delta$ 179.4, 23.1 and 159.8 correlated to acetate and the aminoguanidinium groups respectively.

The hydrogen NMR of the Hmta compound showed broad signals in the region of $\delta$ 8.07 (DMSO and CDCl$_3$) relative to the pyrrole group, and at $\delta$ 5.95 (DMSO) for the amine group, corroborating with the theoretical calculation of the magnetic shielding tensor. This inversion of chemical shift in comparison with those reported for the mta is probably related with the absence of electrons shielding effect upon the 1,2,4-triazole ring. The methyl groups of Hmta showed chemical shifts at $\delta$ 1.96 in DMSO and 2.14 in CDCl$_3$.

The number of $^{13}$C NMR chemical shifts observed in the spectra of the 1,2,4-triazole compounds in this work correlates with the proposed structures shown by Figure 1, as well as to the crystal structure of the Hmta in Figure 4. The reported $^{13}$C NMR data for the 3-ammine-5-methyl-1H-1,2,4-triazole (mta) showed three chemical shifts at $\delta$ 159.9 (C-NH$_3$), 154.9 (C-CH$_3$) and 13.2 (C-CH$_3$) in DMSO (Boechat et al., 2011). The attempt to synthesise this compound with excess of acetic acid, however, led to the formation of Hmta, which showed $^{13}$C chemical shifts in DMSO at $\delta$ 159.2, 155.2 and 13.5, and in CDCl$_3$ at $\delta$ 157.3, 153.4 and 12.3, which are in agreement with its crystal structure as well as with its theoretical calculations of NMR. Two carbon chemical shifts were also identified in these solvents at $\delta$ 173.0 and 21.8 as well as at $\delta$ 177.1 and 21.8 that certainly correlates to the carbonyl and methyl groups of the acetate and acetic acid (Silverstain et al., 1991). The stoichiometry involved in the synthetic route of the mta is important considering the side reaction (acid – base) that occurred to form the Hmta in the presence of acetic acid in excess. The chemical shift of hydrogen and $^{13}$C NMR of the Hmta is remarkably similar to that of the mta reported in the literature, except by the assignments of the signals to the corresponding chemical group (Boechat et al., 2011). The assignments to the chemical shifts in these compounds corroborate with the theoretical calculation of the magnetic shielding tensor.

The chemical shift of the carbon atoms from the phenyl ring of pta appear in the range of $\delta$ 139.4 to $\delta$ 127.7 and the two carbon atoms of the triazole ring around of $\delta$ 171.5 and 160.1 in DMSO, CD$_3$OD and CDCl$_3$. The two carbon atoms for the triazole ring of mpta revealed chemical shifts at $\delta$ 172.5 and 159.3 as well as at $\delta$ 155.2 for the imine group (C=N), in DMSO. The mpta showed three carbon chemical shifts nearby of $\delta$ 19.3, related to methyl groups.

Although the mta was produced by an equimolar reaction in this work, washings of the product with diethyl ether may not have been sufficient to clean up the material from the acetic acid that did not react, remaining a small amount in the sample. This allow speculating that this chemical worked as a catalyst in attempt to purify the mta with acetone, producing the mpta derivative. Therefore, the NMR chemical shift for the acetic acid in the spectrum of Hmta as well as its presence in the crystal lattice of this compound is the evidence that corroborate the acetic acid as a catalyst in the reaction between mta and acetone.

2.3. Crystallography

The crystallographic data of Hmta are shown in Table 1, and its crystal structure by Figure 4. The Hmta crystallizes in the monoclinic space group P2$_1$/n and shows within the unit cell a molecule of acetic acid and an acetate anion for each ionic 1,2,4-triazole ring. The crystal packing is stabilized by two very weak hydrogen bonds of C3-H···O1 (2.809 Å) and N3-H···O4 (1.748 Å) involving both the molecule of acetic acid and the acetate ion, which is coplanar with the ionic ring of the Hmta. The acetic acid molecule describes a parallel plane to the Hmta ring. The lengths and bond angles of the Hmta are shown in Table 2.
Figure 4 - The Crystal structure of the 3-methyl-1H-1,2,4λ4-triazole-5-amine acetate (Hmta).

Table 1 - Crystallographic data for the Hmta compound.

|                         | Hmta                        |
|-------------------------|-----------------------------|
| Chemical Formula        | C₇H₁₄N₄O₄                  |
| Formula weight / g.mol⁻¹| 218.22                      |
| Crystal system          | Monoclinic                  |
| Space group             | P2₁/n                       |
| a / Å                   | 7.6420(4)                   |
| b / Å                   | 12.0280(5)                  |
| c / Å                   | 11.8650(7)                  |
| α / °                   | 90                          |
| β / °                   | 95.797(6)                   |
| γ / °                   | 90                          |
| Volume / Å³             | 1085.03(9)                  |
| Z                       | 4                           |
| Temperature / K         | 293.0(2)                    |
| dₘₐₙc / g.cm⁻³          | 1.336                       |
| μ (KαCu) / mm⁻¹         | 0.110                       |
| Radiation               | λ = 0.71073 Å (KαMo)        |
| θ limits / °            | 3.331 - 29.750              |
| Reflections collected / independent | 23526 / 2903 |
| Reflections observed [Fₜₐₐₜₜ > 4σ(F_obs)] | 1920                     |
| Parameters              | 180                         |
| R_mₐₜ                    | 0.0575                      |
| R indices for [Fₜₐₐₜₜ > 4σ(F_obs)] | 0.0577               |
| R indices for all data  | 0.0901                      |
| wR indices for [Fₜₐₐₜₜ > 4σ(F_obs)] | 0.1611               |
| Goodness-of-fit (GOF)   | 1.001                       |
Table 2 - Selected bond lengths and bond angles of the Hmta compound.

| Bond lengths / Å | C1-N2 | 1.324(2) | C1-N4 | 1.330(3) | C1-N3 | 1.338(2) | C2-N1 | 1.298(2) | C2-N3 | 1.375(2) | C4-C3 | 1.476(3) | C4-O1 | 1.198(2) |
|------------------|-------|----------|-------|----------|-------|----------|-------|----------|-------|----------|-------|----------|-------|----------|
| Bond angles / °  | N2-C1-N4 | 126.6(2) | O2-C4-C5 | 114.3(2) | N2-C1-N3 | 106.5(2) | O3-C6-O4 | 120.8(2) | N4-C1-N3 | 126.9(2) | N4-C1-N3 | 110.8(2) | O4-C6-C7 | 119.6(2) |
|                  | N1-C2-N3 | 125.6(2) | C2-N1-N2 | 104.4(2) | N3-C2-C3 | 123.6(2) | C1-N2-N1 | 111.1(2) | O1-C4-O2 | 122.8(2) | O1-C4-C5 | 122.8(2) |

2.4. Theoretical NMR Approach

Theoretical studies were carried out using software package GAUSSIAN09 (Frisch et al., 2009). Spatial arrangements were used as initial models in geometry optimization calculations. To the mpta compound, the tautomeric structures were optimized at post-HF MP4 level (sdq)/6-31G(d,p) resulting in geometry data with good quality and in level DFT/B3LYP/6-311++G(2d,2p), considering solvation by DMSO through the implicit model as shown by Figure 5. This method offers good geometry data with reduced computational cost. The optimized geometries were characterized as true minima on the potential energy surface (PES) when all harmonic frequencies were real. The optimized geometries by MP4 (sdq)/6-31G(d,p), and DFT/B3LYP/6-311++G(2d,2p) were used in carbon chemical shift calculations. The levels of theory B3LYP/6-31G(d,p), B3LYP/6-311+G(d,p), and PBE1PBE/6-311++G(d,p) were applied considering solvation by the DMSO (Jin et al., 2014; Phalgune et al., 2013; Thomas et al., 2005; Ünlüer et al., 2019). The calculated carbon chemical shifts (\(\sigma_C\)) were obtained relative to the corresponding calculated values for tetramethylsilane at the same levels of theory. To define the most adequate level of theory for this work, the correlations between \(\sigma_C\) values and experimental carbon chemical shifts (\(\delta_C\)) were obtained using software package LibreOffice™ 7.1; \(\sigma_C\) and \(\delta_C\) values were plotted on the x and y axes, respectively. Correlation curves were given as linear fits with correlation coefficients (\(R^2\)) and standard deviations (SD) furnished by the program.
The Journal of Engineering and Exact Sciences – jCEC

Figure 5 - Optimized structures of N-(3-methyl-1H-1,2,4-triazole-5-yl)propan-2-imine (mpta) in level B3LYP/6-311++G(2d,2p)/DMSO.

The sets of geometries obtained at levels MP4(sdq)/6-31G(d,p) and B3LYP/6-311++G(2d,2p)/DMSO were submitted to calculus of $^1$H and $^{13}$C NMR at different methods and basis sets as shown in Table 3 that also presents the obtained correlations between $\sigma_C$ and $\delta_C$. The experimental and calculated NMR data for the mpta compound are shown in Tables 4S and 5S (see Supplementary Material).

Table 3 - Correlations between $\sigma_C$ and $\delta_C$ for F, G, H, I, J and K tautomers of mpta.

| Level of theory       | F      | G      | H      | I      | J      | K      |
|-----------------------|--------|--------|--------|--------|--------|--------|
| MP4 (SDQ)             |        |        |        |        |        |        |
| B3LYP/6-31G(d,p)      | 0.9974 | 0.9982 | 0.9956 | 0.9953 | 0.9981 | 0.9960 |
| B3LYP/6-311+G(d,p)    | 0.9968 | 0.9979 | 0.9953 | 0.9949 | 0.9978 | 0.9957 |
| PBE1PBE/6-311++G(d,p) | 0.9969 | 0.9978 | 0.9954 | 0.9947 | 0.9979 | 0.9958 |
| DFT_DMSO              |        |        |        |        |        |        |
| B3LYP/6-31G(d,p)      | 0.9953 | 0.9982 | 0.9972 | 0.9960 | 0.9979 | 0.9972 |
| B3LYP/6-311+G(d,p)    | 0.9946 | 0.9975 | 0.9967 | 0.9954 | 0.9974 | 0.9967 |
| PBE1PBE/6-311++G(d,p) | 0.9945 | 0.9973 | 0.9967 | 0.9951 | 0.9972 | 0.9966 |

The calculated hydrogen NMR data for the NH group revealed an active hydrogen atom due to the lower calculated chemical shift in comparison with the experimental data (Jin et al., 2014; Phalgune et al., 2013). In general, the computational data with good correlation (0.9934 – 0.9982) were similar to the calculations in gaseous phase (MP4) and DFT, considering solvation by DMSO through the implicit model. At the calculation levels used, the best association shows that the G structure corresponds to the most stable isomer, with a percentage error of 0.41%, suggesting that this molecule may coexist in solution and in the solid state. Among the other molecules shown by Figure 5, structure F corresponds to the less stable tautomer with $\Delta E_{G,F}/\text{Kcal mol}^{-1} = 13.63$ and 14.27 for optimizations in DFT/DMSO and MP4(SDQ) respectively. This data allow to speculate that tautomeric species such as the structures G, H, I, J and K can be formed in solution, during the reaction path, as the result of a forbidden rotation by resonance effect in the bonding between the 1,2,4-triazole ring and the –C=N(CH$_3$)$_2$ group.
The optimum theoretical association for the chemical shift calculus of $^1\text{H}$ and $^{13}\text{C}$ NMR at different density functional theory methods for the $\text{mta}$, $\text{Hmta}$ and $\text{pta}$ compounds was obtained using the optimized geometry in level B3LYP/6-311++G(2d,2p) in DMSO or CDCl$_3$ as solvent. The calculated and experimental NMR chemical shifts for the $\text{Hmta}$ and $\text{mta}$ compounds are shown in Table 6S and 7S (see Supplementary Material) and Figure 6 shows the optimized structures for these compounds. The experimental NMR data of $\text{mta}$, reported in the literature, was the source for comparison with the theoretical calculation. In general, the correlation coefficient between the calculated chemical shifts and the experimental data was satisfactory to all level of theory applied for these triazole compounds. Higher correlation and less error was found using the theoretical levels of B3LYP/6-311+G(d,p) and PBE1PBE/6-311++G(d,p) for the $\text{mta}$ with the correlations around 0.9996. The resulting calculation has indicated that the tautomer $\text{mta-} \lambda$ is the most probable structure in solution. For the $\text{Hmta}$, the B3LYP/6-311++G(2d,2p) level of theory provided the best calculated data in comparison to its experimental NMR chemical shifts; the optimized structure for this compound was based on its crystal structure shown by Figure 4.

![Figure 6 - Optimized structure of 3-methyl-$^1\text{H}$-$^1$-$^2$-$^4$-triazole-$^5$-amine ($\text{mta}$, left) and 3-methyl-$^1\text{H}$-$^1$-$^2$-$^4$-$^4$-triazole-$^5$-amine acetate ($\text{Hmta}$, right).](Image)

Nevertheless, the calculated and experimental $^{13}\text{C}$ chemical shifts of $\text{Hmta}$ and $\text{mta}$, shown in Table 6S and 7S (see Supplementary Material), are remarkably similar with the exception of the exchanging in chemical shift associated to the carbon atoms of these compounds. This exchanging result appears be correlated to the deshielding effect of the positive charge in the ring of $\text{Hmta}$, contrasting with the shielding effect in the $\text{mta}$. As shown in Table 7S, the chemical shift calculation for the NH hydrogen of the $\text{mta-} \lambda$ was at $\delta$ 2.5949 by the B3LYP/6-311+G(d,p) and at $\delta$ 2.5227 by the PBE1PBE/6-311++G(d,p) levels of theory. These shifts are lower compared to those reported in the literature ($\delta$ 6.3-5.8), suggesting that this hydrogen atom is active by hydrogen bonding or tautomerism (Jin et al., 2014; Phalgune et al., 2013). The percentage error in the calculated chemical shift of the carbon atom from the group C(NH$_2$), compared with the reported experimental data, was 0.41% and 1.14% by the levels of theory B3LYP/6-311+G(d,p) and PBE1PBE/6-311++G(d,p). The calculation by these levels of theory shows good correlation with the experimental data, which are 0.99967 and 0.99966, respectively. The difference between the calculated chemical shifts and the experimental data for the $\text{Hmta}$ in Table 6S is probably due to the solvent effect. Regardless of that, the experimental data in CDCl$_3$ show better accuracy in the correlation between theoretical and experimental data for the $^1\text{H}$ and $^{13}\text{C}$ NMR.

The data in Table 8S (see Supplementary Material) shows the calculated NMR chemical shifts for isomeric structures of $\text{pta}$. The theoretical approach by the B3LYP/6-311+G(d,p) and PBE1PBE/6-311++G(d,p) bases set reveals that the isomers B and C shown by Figure 3 are good candidates to be considered as stable structures. The energy difference between these structures ($\Delta E_{\text{B-C}}$) is 0.2 Kcal mol$^{-1}$ which reinforces the possibility of tautomerism in solution. It is conceivable, therefore, that both tautomers coexist in solution. However, considering the chemical shift of the group C(NH$_2$), the percentage error for the B3LYP/6-311+G(d,p) base set was 1.00% (B) and 0.93% (C), and for the PBE1PBE/6-311++G(d,p) 2.03% (B) and 0.39% (C). They all show good correlation with the experimental values which are 0.99849 (B) and 0.99839 (C) for B3LYP/6-311+G(d,p), and 0.99801 (B) and 0.99830 (C) for PBE1PBE/6-311++G(d,p). The less percentage
error for the isomer C by both level of theory suggest this isomer is the most probable in solution as shown by Figure 7. Yet again, the calculated chemical shift for the hydrogen of the NH group is also below that of the experimental data, suggesting an active hydrogen atom by hydrogen bonding or tautomerism (Jin et al., 2014; Phalgune et al., 2013).

The proposed isomeric structures D and E shown by Figure 3 probably does not exist in solution, considering that the chemical shift calculated is far from the experimental data to all bases set in the calculation. In addition to that, they show a great difference in energy in comparison to the isomer C ($\Delta E_{D-C}$ and $\Delta E_{E-C}$) which are 20.2 and 11.9 Kcal mol$^{-1}$ respectively.

![Figure 7 - Optimized structures of isomers B (left) and C (right) of 3-phenyl-1H-1,2,4-triazole-5-amine (pta).](image)

2.5. Antimicrobial Activity

The 1,2,4-triazole compounds showed to be inactive against microorganisms Gram-positives (Staphylococcus aureus - ATCC 33591; Bacillus subtilis - ATCC 23858) and Gram-negatives (Escherichia coli - ATCC 29214; Salmonella typhimurium - ATCC 14028). The literature reports antimicrobial activity on 1,2,4-triazole compounds having bulky substituent groups bonded to the ring (El Akri et al., 2007; Karthikeyan et al., 2008). The absence of this chemical feature in the compounds tested allow speculating that their inactivity is correlated to this chemical property.

3. Materials and methods

All chemicals purchased from Sigma-Aldrich were used without prior purification. Elemental analysis data were collected from a Perkin Elmer 200 CHNS Elemental Analyzer. The infrared spectra were recorded on a Perkin Elmer FT-IR 1000 using Nujol between CsI windows, and NMR spectra on a Varian 300 MHz as well as in a Bruker Avance DRX-400 MHz apparatus. The TMS was the internal standard reference. The theoretical approach calculation concerning the NMR chemical shifts of the triazole compounds were performed by the GAUSSIAN09 package (Frisch et al., 2009).

3.1. Synthesis of The Triazole Compounds

3-Methyl-1H-1,2,4-triazole-5-amine (mta): The synthesis of this compound followed the route described in the literature with slight modifications (Boechat et al., 2011). Into a bottom flask of 250 mL, an equimolar mixture of aminoguanidine bicarbonate and acetic acid (1.5% excess) were stirred until complete release of carbon dioxide as by-product. After that, 120 mL of toluene was added to the mixture, and the flask fixed to a Dean-stark. The mixture was heated at 120 °C, stirring under reflux, during 22 h. Then, the white material that precipitated at the bottom of the flask was filtered off under reduced pressure. Afterwards, washed with diethyl ether and kept on desiccators. A few crystals of 3-methyl-1H-1,2,4-triazole-5-amine acetate (Hmta) were separated after cooling the filtrate.
Yield of 2.5 g (85 %); Mp (°C): 130.8 - 133.1. Elemental analyses required for C₃H₅N₅: C, 36.73; H, 6.16; N, 57.11; Found: C, 38.10; H, 6.41; N, 56.71. IR (Nujol / CsI): 3314, 3449 ν(N-H), 3186 νas(NH₂); 3039νs(NH₂); 1629, 1666 ν(C=N); 2881 ν(CH₃).

3-Methyl-1H-1,2,4-triazole-5-amine acetate (Hmta): The synthesis of this compound followed the technique used in the preparation of mta with the exception that a great excess of acetic acid (5.0 mL, 60%, 87.27 mmol) was added to the aminoguanidine bicarbonate (5.16 g, 37.91 mmol). A white material also precipitated and was filtered off under reduced pressure, washed with diethyl ether, and kept on desiccators. Suitable crystals of 3-methyl-1H-1,2,4-triazole-5-amine acetate (Hmta) for X-ray analysis were obtained from the filtrate after three days at low temperature.

1H NMR (DMSO, 400 MHz, δ): 8.07 (broad, NH₂); 5.95(broad, NH); 1.96 (s, CH₃). 13C NMR (DMSO, 100 MHz, δ): 159.27 (C-NH₂); 155.28 (C-CH₃); 13.5 (CH₃); 173.0 (RCOO); 21.8 (CH₃COO). 1H NMR (CDCl₃, 400 MHz, δ): 9.24 (broad, NH₂); 2.14 (s, CH₃). 13C NMR (CDCl₃, 100 MHz, δ): 157.3 (C-NH₂); 153.4 (C-CH₃); 12.3 (CH₃); 177.1 (RCOO); 21.8 (CH₃COO)

3-Phenyl-1H-1,2,4-triazole-5-amine (pta): This compound was synthesised by the same equimolar reaction route described for the mta using the benzoic acid as reactant, dissolving it in 7.0 mL of hot water. After releasing the carbon dioxide completely, A Dean-stark was fixed to the flask and the mixture maintained in stirring under reflux. The white material was removed by filtration under reduced pressure followed by washings with diethyl ether and kept on desiccators. No crystals were isolated from the filtrate after cooling at low temperature for three days. Yield of 1.8 g (63 %); Mp (°C): 179.8 - 181.5. Elemental analyses required for C₈H₆N₃: C, 51.33; H, 5.92; N, 29.93; Found: C, 50.19; H, 6.53; N, 30.13. IR (Nujol / CsI): 3407 ν(H₂O), 3361 νas(NH₂); 3274 νs(NH₂); 1682, 1668 ν(C=N); 841 δ(C-H); 711 δ(C-H). 1H NMR (DMSO-d₆, 300 MHz, δ): 8.13 (NH₂), 10.31 (s, NH), 7.31 (m, Ph, 3H). 1J (H-H) = 7.01 Hz), 7.88 (m, Ph, 2H), 1J (H-H) = 7.42 Hz). 13C NMR (DMSO-d₆, 75 MHz, δ): 171.5 (C-NH₂), 160.1 (C-Ph), 139.4, 129.7, 129.3, 127.7 (Ph). 1H NMR (CD₂OD, 300 MHz, δ): 8.13 (NH₂), 10.32 (s, NH), 7.43 (m, Ph, 3H). 1J (H-H) = 7.01 Hz), 7.93 (m, Ph, 2H). 1J (H-H) = 7.42 Hz). 13C NMR (CD₂OD, 75 MHz, δ): 174.5 (C-NH₂), 159.8 (C-Ph), 137.6, 130.2, 129.0, 127.6 (Ph). 1H NMR (CDCl₃, 300 MHz, δ): 8.24 (NH₂), 10.45 (s, NH), 7.32 (m, Ph, 3H), 1J (H-H) = 7.01 Hz), 7.89 (m, Ph, 2H). 1J (H-H) = 7.42 Hz). 13C NMR (CDCl₃ 75 MHz, δ): 171.7 (C-NH₂), 160.4 (C-Ph), 139.7, 130.0, 129.6, 128.0 (Ph).

N-(3-Methyl-1H-1,2,4-triazole-5-yl)propan-2-amine (mpta): The attempt of purifying the mta led to the synthesis of the mpta by dissolving 1.00 g of mta in acetone (50 mL) at the room temperature. A light yellowish solid was obtained almost immediately. The mixture was kept under stirring for 30 minutes and the solid filtered off in air followed by washings with diethyl ether and stored on desiccators. Mp (°C): 154.9 - 156.3. Elemental analyses required for C₆H₁₄N₂H₂O: C, 41.37; H, 8.10; N, 32.16; Found: C, 40.82; H, 7.81; N, 32.58. IR (Nujol / CsI): 3360 ν(N-H, H₂O); 1692, 1614, 1579 ν(C=N); 790 δ(C-H). 1H NMR (DMSO-d₆, 300 MHz, δ): 11.06 (broad, NH), 2.17 (s, CH₃), 2.04 (s, CH₃), 1.88 (s, CH₃). 13C NMR (DMSO, 75 MHz, δ): 172.5 (C-NH₂), 159.3 (C-CH₃), 155.2 (C=N), 23.2 (CH₃), 21.4 (CH₃), 13.4 (CH₃).

3.2. Minimum Inhibitory Concentration (MIC)

The broth microdilution assays, using microplates of 96 wells, was the method used to determine the minimum inhibitory concentration (Zaczchno & Gupta, 2007). The standard solution of the triazole compounds is composed by 1.0 mg of the substance to be biologically assayed, 250 µL of DMSO and 750 µL of sterile water. The microorganisms were grown in 3.0 mL of the Luria Bertani (LB) broth at 37 °C until reaching an optical density (OD) between 0.08 and 0.10 that corresponds to a range from 1.0 to 2.0 x 10⁶ colony-forming unit (CFU) / mL. The LB medium [100 µL (5.0 x 10⁸ CFU)] was added to 50 µL of the standard solution of the substance to be tested from each of the bacteria broth. Subsequently, the mixture was poured into the wells of the plates and
incubated for 24 h. The MIC was analysed using a spectrometer ELISA at 600 nm. The experiment was carried out in duplicate, considering the standard deviation. *Amoxicillin* and *Norfloxacin* were used as positive control and DMSO as negative control.

### 3.3. Single Crystal X-ray Diffraction

Crystallographic data were collected using a Bruker Kappa CCD diffractometer with MoKα (λ = 0.71073 Å) at room temperature. Data collection, reduction and refinement of the unit cell array were performed by the programs COLLECT (Nonius, Delft), EVALCCD (Duisenberg, 1992) and DIRAX (Duisenberg, Kroon-Batenburg, & Schreurs, 2003). The structure was solved and refined using SHELXL-97 (Sheldrick, 1997). The absorption correction was applied to all atoms and assigned to the anisotropic displacement parameters except for the hydrogen atom (Blessing, 1995). The displacement parameters of H atoms were fixed at 1.2 Ueq of the carbon atoms. The maximum peak and deepest hole observed in the final Δρ map were 0.158 and -0.165 e.Å⁻³. The structures were drawn by ORTEP-3 for Windows (Farrugia, 1997) and Mercury (Macrae et al., 2006) programs.

**Crystallographic Data:** X-ray crystallographic data for the 3-methyl-1H-1,2,4λ⁴-triazole-5-amine acetate (*Hmta*) has been deposited with the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: +44(0)1223 336033, e-mail: deposit@ccdc.cam.ac.uk. It can be obtained free of charge by request at www.ccdc.cam.ac.uk/conts/retrieving.html, quote CCDC 1832920.

### 3.4. Theoretical NMR Data

The structure of the compounds were drawn in GaussView. Subsequently were submitted to geometrical optimization by means of the MP4 and DFT methods by the GAUSSIAN09 software. Chemical shifts were obtained by the shielding tensor values of NMR which were calculated for the optimized structure at B3LYP/6-31G(d,p), B3LYP/6-311+G(d,p), PBE1PBE/6-311++G(d,p), and B3LYP/6-311++G(2d,2p) levels of theory. The TMS was the shielding tensor reference for for the calculated chemical shifts.

### 4. Conclusion

Simple molecules of triazole compounds have been synthesised and had their biological activity tested. These compounds did not show biological activity against strains of Gram-positive and Gram-negative microorganisms at the highest concentration tested. The novel compounds *Hmta*, *pta* and *mpta* were synthesised by the methodology described in the literature, although the reaction pathway seems to be dependent on the amount of reactants as reveal by the synthesis of *Hmta*.

The computational approach shows that the high values of the correlation coefficient between the theoretical and experimental data obtained for these triazole compounds was influenced by the insertion of diffuse functions. In this context, it is conceivable that the presence of additional parameters to the calculation such as inter and intramolecular hydrogen bonding might provide better accuracy in the resulting theoretical data. Nevertheless, the calculated NMR data corroborate with the acid-base reaction of the *Hmta*, supported by its crystal structure. Beyond that, the theoretical data suggest not only the tautomerism but also the most probable tautomers in solution for the *mta*, *pta* and the *mpta* compounds.

### 5. Supplementary Material

Tables containing the NMR chemical shift and the shielding tensor calculations are free access at the JCEC website as Supplementary Material for the triazole compounds prepared in this work.
Acknowledgements

The authors appreciate the financial support of the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 as well as to the Brazilian agencies FAPEMIG and CNPq.

References

Abdel-Megeed, A. M., Abdel-Rahman, H. M., Alkaramany, G.-E. S., & El-Gendy, M. A. (2009). Design, synthesis and molecular modeling study of acylated 1,2,4-triazole-3-acetates with potential anti-inflammatory activity. *European Journal of Medicinal Chemistry, 44*(1), 117-123. doi:10.1016/j.ejmech.2008.03.017

Akerblom, E., & Sandberg, M. (1965). Alkyl Derivatives of 3-amino-5-(2-furyl)-1,2,4-triazole. *Acta Chimica Scandinavica, 19*, 1191-1204. doi:10.3891/acta.chem.scand.19-1191

Blessing, R. H. (1995). An empirical correction for absorption anisotropy. *Acta Crystallographica, Section A: Foundations of Crystallography, 51*, 33-38. doi:10.1107/S0001808594005726

Boechat, N., Pinheiro, L. C. S., Santos-Filho, O. A., & Silva, I. C. (2011). Design and Synthesis of New N-(5-Trifluoromethyl)-1H-1,2,4-triazol-3-yl Benzenesulfonamides as Possible Antimalarial Prototypes. *Molecules, 16*(9), 8083. doi:10.3390/molecules16098083

Deng, X.-Q., Song, M.-X., Zheng, Y., & Quan, Z.-S. (2014). Design, synthesis and evaluation of the antidepressant and anticonvulsant activities of triazole-containing quinolinones. *European Journal of Medicinal Chemistry, 73*, 217-224. doi:10.1016/j.ejmech.2013.12.014

Duisenberg, A. J. M. (1992). Indexing in single-crystal diffractometry with an obstinate list of reflections. *Journal of Applied Crystallography, 25*, 92-96. doi:10.1107/S0021889989101063

Duisenberg, A. J. M., Kroon-Batenburg, L. M. J., & Schreurs, A. M. M. (2003). An intensity evaluation method: EVAL-14. *Journal of Applied Crystallography, 36*, 220-229. doi:10.1107/S0021889802022628

El Akri, K., Bougrin, K., Balzarini, J., Faraj, A., & Benhida, R. (2007). Efficient synthesis and in vitro cytostatic activity of 4-substituted triazolyl-nucleosides. *Bioorganic & Medicinal Chemistry Letters, 17*(23), 6656-6659. doi:10.1016/j.bmcl.2007.08.077

Farrugia, L. J. (1997). ORTEP-3 for windows - a version of ORTEP-III with a graphical user interface (GUI). *Journal of Applied Crystallography, 30*(5), 565-565. doi:10.1107/S0021889996003117

Frisch, M. J., G.W. Trucks, Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J. R., . . . Fox., D. J. (2009). Gaussian 09, Revision E.01, Gaussian, Inc., Wallingford CT.

Graci, J. D., & Cameron, C. E. (2006). Mechanisms of action of ribavirin against distinct viruses. *Reviews in Medical Virolory, 16*(1), 37-48. doi:10.1002/rmv.483

Grinshtein, V. Y., Strazdin, A. A., & Grinvalde, A. K. (1970). Infrared absorption spectra of some C-halogenated 1, 2, 4-triazole derivatives. *Chemistry of Heterocyclic Compounds, 6*(2), 231-239. doi:10.1007/bf00475004

Groll, A. H., Piscitelli, S. C., & Walsh, T. J. (1998). Clinical Pharmacology of Systemic Antifungal Agents: A Comprehensive Review of Agents in Clinical Use, Current Investiogational Compounds, and Putative Targets for Antifungal Drug Development. In M. W. A. F. M. J. Thomas August & T. C. Joseph (Eds.), *Advances in Pharmacology* (Vol. Volume 44, pp. 343-500): Academic Press.

Han, L. P., Wang, X., Guo, X., Rao, M. S., Steinberger, Y., Cheng, X., & Xie, G. H. (2011). Effect of plant growth regulators on growth, yield and lodging of sweet sorghum. *Research on Crops, 12*(2), 372-382. https://www.researchgate.net/publication/279890538
Jin, R. Y., Sun, X. H., Liu, Y. F., Long, W., Lu, W. T., & Ma, H. X. (2014). Synthesis, crystal structure, IR, 1H NMR and theoretical calculations of 1,2,4-triazole Schiff base. *Journal of Molecular Structure*, 1062, 13-20. doi:https://doi.org/10.1016/j.molstruct.2014.01.010

Johnson, E. M., Szekely, A., & Warnock, D. W. (1999). In Vitro Activity of Syn-2869, a Novel Triazole Agent, against Emerging and Less Common Mold Pathogens. *Antimicrobial Agents and Chemotherapy*, 43(5), 1260-1263. https://doi.org/10.1128/AAC.43.5.1260

Karthikeyan, M. S., Holla, B. S., & Kumari, N. S. (2008). Synthesis and antimicrobial studies of novel dichlorofluorophenyl containing aminotriazolothiadiazines. *European Journal of Medicinal Chemistry*, 43(2), 309-314. doi: http://dx.doi.org/10.1016/j.ejmech.2007.03.024

Lopyrev, V. A., Beresneva, N. K., & Strelets, B. K. (1969). Infrared spectra and structure of some 3,5-diamino-1,2,4-triazoles (guanazoles). *Chemistry of Heterocyclic Compounds*, 5(4), 544-546. doi:10.1007/bf00470284

Macrae, C. F., Edgington, P. R., McCabe, P., Pidcock, E., Shields, G. P., Taylor, R., . . . van De Streek, J. (2006). Mercury: visualization and analysis of crystal structures. *Journal of Applied Crystallography*, 39, 453-457. doi:10.1107/s002188980600731x

Naito, Y., Akahoshi, F., Takeda, S., Okada, T., Kajii, M., Nishimura, H., . . . Kagitani, Y. (1996). Synthesis and Pharmacological Activity of Triazole Derivatives Inhibiting Eosinophilia. *Journal of Medicinal Chemistry*, 39(15), 3019-3029. doi:10.1021/jm9507993

Nonius, B. V. (Delft). COLLECT (1997-2000) Enraf-Nonius. The Netherlands.

Papakonstantinou-Garoufalias, S., Pouli, N., Marakos, P., & Chtyyroglou-Ladas, A. (2002). Synthesis antimicrobial and antifungal activity of some new 3-substituted derivatives of 4-(2,4-dichlorophenyl)-5-adamantyl-1H-1,2,4-triazole. *Il Farmaco*, 57(12), 973-977. doi:http://dx.doi.org/10.1007/S0014-827X(02)01227-2

Phalgune, U. D., Vanka, K., & Rajamohan, P. R. (2013). GIAO/DFT studies on 1,2,4-triazole-5-thiones and their propargyl derivatives. *Magnetic Resonance in Chemistry*, 51(12), 767-774. doi:https://doi.org/10.1002/mrc.4012

Roberts, J., Schock, K., Marino, S., & Andriole, V. T. (2000). Efficacies of Two New Antifungal Agents, the Triazole Ravuconazole and the Echinocandin LY-30366, in an Experimental Model of Invasive Aspergillosis. *Antimicrobial Agents and Chemotherapy*, 44(12), 3381-3388. doi:10.1128/ AAC.44.12.3381-3388.2000

Sheldrick, G. M. (1997). SHEXL-97 - A Program for Crystal Structure Refinement: University of Goettingen, Germany.

Silverstain, R. M., Bassler, G. C., & Morril, T. C. (1991). *Spectrometric Identification of Organic Compounds* (Fifth ed.). New York: John Wiley & Sons, INC.

Sokmen, B. B., Gumrukcuoglu, N., Ugras, S., Sahin, H., Sagkal, Y., & Ugras, H. I. (2015). Synthesis, Antibacterial, Antiiurease, and Antioxidant Activities of Some New 1,2,4-Triazole Schiff Base and Amine Derivatives. *Applied Biochemistry and Biotechnology*, 175(2), 705-714. doi:10.1007/s12010-014-1307-2

Sudheendran, K., Schmidt, D., Frey, W., Conrad, J., & Beifuss, U. (2014). Facile synthesis of 3,5-diyrl-1,2,4-triazoles via copper-catalyzed domino nucleophilic substitution/oxidative cyclization using amidines or imidates as substrates. *Tetrahedron*, 70(8), 1635-1645. doi:http://dx.doi.org/10.1016/j.tet.2014.01.019

Thomas, S., Biswas, N., Venkateswaran, S., Kapoor, S., D'Cunha, R., & Mukherjee, T. (2005). Raman, infrared, SERS and DFT calculations of a triazole derivative (akacid). *Chemical Physics Letters*, 402(4), 361-366. doi:https://doi.org/10.1016/j.cplett.2004.12.064

Tyagi, P., Tyagi, M., Agrawal, S., Chandra, S., Ojha, H., & Pathak, M. (2017). Synthesis, characterization of 1,2,4-triazole Schiff base derived 3d-metal complexes: Induces cytotoxicity in HepG2, MCF-7 cell line, BSA binding fluorescence and DFT study. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 171, 246-257. doi:http://dx.doi.org/10.1016/j.saa.2016.08.008

Ünlüer, D., Ünver, Y., Düğdü, E., Alpaslan, Y. B., Kóysal, Y., Soylu, M. S., & Sancak, K. (2019). Novel 1,2,4-Triazole Derivatives: Structure, DFT Study, X-Ray Analysis, and Antimicrobial
Vatmurge, N. S., Hazra, B. G., Pore, V. S., Shirazi, F., Chavan, P. S., & Deshpande, M. V. (2008). Synthesis and antimicrobial activity of β-lactam–bile acid conjugates linked via triazole. *Bioorganic & Medicinal Chemistry Letters, 18*(6), 2043-2047. doi:[http://dx.doi.org/10.1016/j.bmcl.2008.01.102](http://dx.doi.org/10.1016/j.bmcl.2008.01.102)

Vieira, F. T., Maia, J. R. D., Ardisson, J. D., & de Lima, G. M. (2008). Carbonate and Semicarbazide Tin(IV) Derivatives of Aminoguanidine Bicarbonate. *Main Group Metal Chemistry, 31*(1-2), 1-11. https://doi.org/10.1515/MGMC.2008.31.1-2.1

Wajda-Hermanowicz, K., Pieniążczak, D., Wróbel, R., Zatajska, A., Ciunik, Z., & Berski, S. (2016). A study on the condensation reaction of aryl substituted 4-amine-1,2,4-triazole with benzaldehydes: Structures and spectroscopic properties of schiff bases and stable hemiaminals. *Journal of Molecular Structure, 1114*, 108-122. doi:[http://dx.doi.org/10.1016/j.molstruc.2016.02.047](http://dx.doi.org/10.1016/j.molstruc.2016.02.047)

Wu, J., Liu, X., Cheng, X., Cao, Y., Wang, D., Li, Z., De Clercq, E. (2007). Synthesis of Novel Derivatives of 4-Amino-3-(2-Furyl)-5-Mercapto-1,2,4-Triazole as Potential HIV-1 NNRTIs. *Molecules, 12*(8), 2003-2016. doi: 10.3390/12082003

Zacchino, S. A., & Gupta, M. P. (2007). *Manual de técnicas in vitro para la detección de compuestos antifúngicos*: Corpus Libros Médicos y Científicos.
Supplementary Material

1. Calculating chemical shifts using a reference compound

Quantum mechanical calculations of Nuclear Magnetic Resonance (NMR) parameters, such as chemical shifts and coupling constants, have become a very popular tool for structural elucidation of synthetic and natural product in the field of chemistry. The calculations of NMR chemical shifts are extensively used in the validation of chemical structures. To calculate the chemical shifts of a compound, the NMR isotropic shielding tensor (\(\sigma\)) is firstly calculated. These calculations uses quantum mechanical methods and the most common are the density functional theory (DFT), and the perturbation theory or higher-level post-Hartree-Fock (HF). The DFT methods have become very popular, as there is a good correlation between accuracy and efficiency with several DFT functions available. The conversion of calculated NMR isotropic shielding tensors of a specific compound into its chemical shift is achieved using tetramethylsilane (TMS) as the reference compound: the same calculating methodology applies for both compounds. The chemical shifts for the novel compound are obtained by subtracting from the isotropic magnetic shielding tensor of this compound, the isotropic magnetic shielding tensor of the reference (TMS). The analysis of the data is performed by linear correlation, plotting the calculated chemical shifts against the experimental data. Considering the fact that, the computational methods are performed in different conditions, in comparison to a real experiment, and that the NMR spectroscopy method is sensitive to short-range structural changes, it is expected systematic differences between the calculated and the experimental chemical shifts. Therefore, it is reasonable to use correlation coefficients to improve the accuracy predicted by DFT, for comparison between the experimental and theoretical data, in \(^{13}\text{C}\) NMR chemical shifts. Additionally, the \(^{1}\text{H}\) NMR data for the methyl signals are averaged across the chemical shifts of the three stationary hydrogens (References 1S, 2S, 3S, 4S, 5S). The calculated and experimental data of the 1,2,4-triazole compounds are shown in Tables 4S to 8S.
Table 4S. - Experimental and calculated\(^\dagger\) NMR data for the \textit{mpta} (F, G, H, I, J, K) tautomer, with geometry optimization data obtained in MP4(sdq)/6-31G(d,p).

|                  | \(\delta\) Exp. | F     | G     | H     | I     | J     | K     |
|------------------|-----------------|-------|-------|-------|-------|-------|-------|
| CH\(_3\)         | 1.88            | 1.9133| 2.1938| 2.1907| 2.1881| 2.0285| 2.2202|
| CH\(_3\)         | 2.04            | 2.8774| 2.2364| 2.2402| 2.2628| 2.2381| 2.3551|
| CH\(_3\)         | 2.17            | 2.2998| 2.4518| 2.3612| 2.4849| 2.3041| 2.2833|
| NH               | 11.06           | 8.0165| 9.1589| 9.2393| 8.0004| 9.0598| 9.1920|
| C(CH\(_3\))      | 13.4            | 12.6817| 16.0721| 13.7841| 12.4401| 15.5872| 13.5791|
| C(CH\(_3\))      | 21.4            | 21.9877| 26.1920| 25.7869| 26.9956| 23.5134| 26.6178|
| C(CH\(_3\))      | 23.2            | 31.7884| 31.4957| 32.1719| 31.3402| 32.3243| 31.9925|
| C(C-N)           | 155.2           | 142.6782| 153.2574| 144.9860| 142.3628| 153.3554| 146.5157|
| C(C-CH\(_3\))    | 159.3           | 151.3214| 157.7380| 164.2170| 153.6228| 158.3381| 164.4159|
| C(N=C)           | 172.5           | 160.2246| 173.9528| 169.9458| 173.2153| 168.4404| 170.7136|
| CTE              |                 | 0.9974| 0.9982| 0.9956| 0.9953| 0.9981| 0.9960|

|                  | \(\delta\) Exp. | F     | G     | H     | I     | J     | K     |
|------------------|-----------------|-------|-------|-------|-------|-------|-------|
| CH\(_3\)         | 1.88            | 1.9743| 2.3202| 2.3180| 2.3075| 2.0975| 2.3450|
| CH\(_3\)         | 2.04            | 2.3590| 2.3870| 2.3428| 2.3697| 2.3918| 2.3450|
| CH\(_3\)         | 2.17            | 2.3980| 2.5409| 2.4594| 2.5846| 2.4044| 2.3488|
| NH               | 11.06           | 8.0957| 9.2842| 9.3403| 8.1819| 9.1376| 9.2366|
| C(CH\(_3\))      | 13.4            | 13.9866| 17.6273| 14.9795| 13.2746| 16.7743| 14.3281|
| C(CH\(_3\))      | 21.4            | 22.7027| 27.7397| 27.0385| 28.6892| 24.3786| 28.0151|
| C(CH\(_3\))      | 23.2            | 35.3024| 35.1138| 35.6888| 34.6928| 35.6772| 35.3117|
| C(C-N)           | 155.2           | 154.4969| 166.9054| 157.3954| 154.0896| 166.7495| 158.5246|
| C(C-CH\(_3\))    | 159.3           | 164.6713| 170.8895| 178.4505| 167.1328| 171.1797| 178.4507|
| C(N=C)           | 172.5           | 172.8143| 188.7518| 184.0080| 188.5445| 182.0369| 185.4970|
| CTE              |                 | 0.9968| 0.9979| 0.9953| 0.9949| 0.9978| 0.9957|

|                  | \(\delta\) Exp. | F     | G     | H     | I     | J     | K     |
|------------------|-----------------|-------|-------|-------|-------|-------|-------|
| CH\(_3\)         | 1.88            | 1.9750| 2.3084| 2.2867| 2.2867| 2.0796| 2.3112|
| CH\(_3\)         | 2.04            | 2.3667| 2.3550| 2.3240| 2.3408| 2.3536| 2.3296|
| CH\(_3\)         | 2.17            | 2.3927| 2.5219| 2.4382| 2.5629| 2.3907| 2.3307|
| NH               | 11.06           | 8.1183| 9.2729| 9.3241| 8.2197| 9.1279| 9.2288|
| C(CH\(_3\))      | 13.4            | 13.7313| 17.2371| 14.7431| 13.1133| 16.4391| 14.1809|
| C(CH\(_3\))      | 21.4            | 22.7378| 27.8372| 27.1300| 28.7900| 24.4139| 28.1330|
| C(CH\(_3\))      | 23.2            | 34.9207| 34.7230| 35.2681| 34.2767| 35.3184| 34.8880|
| C(C-N)           | 155.2           | 153.1771| 165.1681| 155.8720| 152.8528| 164.8752| 157.1508|
| C(C-CH\(_3\))    | 159.3           | 162.9664| 168.8616| 176.2924| 165.3159| 169.3563| 176.1817|
| C(N=C)           | 172.5           | 172.0194| 187.8613| 182.9877| 187.5715| 181.2009| 184.4899|
| CTE              |                 | 0.9969| 0.9978| 0.9954| 0.9947| 0.9979| 0.9958|

\(^\dagger\) Gas phase; CTE (Correlation between Theoretical and Experimental data);
Table 5S - Experimental and calculated NMR data for the mpta (F, G, H, I, J, K) tautomer, with geometry optimization data obtained in DFT/B3LYP/6-311++G(2d,2p)/DMSO.

|          | δ Exp. | F       | G       | H       | I       | J       | K       |
|----------|--------|---------|---------|---------|---------|---------|---------|
| CH₃      | 1.88   | 2.3364  | 2.2201  | 2.2519  | 2.3060  | 2.1938  | 2.2634  |
| CH₂      | 2.04   | 2.3798  | 2.2262  | 2.3552  | 2.3600  | 2.2585  | 2.2802  |
| CH₃      | 2.17   | 2.4140  | 2.2424  | 2.3715  | 2.3760  | 2.3848  | 2.3518  |
| NH       | 11.06  | 9.7900  | 9.2679  | 9.8261  | 8.5773  | 9.5288  | 9.8495  |
| C(CH₃)₂  | 13.4   | 12.6484 | 15.4422 | 13.4174 | 12.3735 | 15.2158 | 13.0586 |
| C(CH₃)₃  | 21.4   | 25.1425 | 23.1962 | 25.5889 | 26.7582 | 23.6833 | 26.5460 |
| C(CH₃)₈  | 23.2   | 36.2768 | 29.6595 | 32.3466 | 31.7583 | 32.4007 | 32.1275 |
| C(N-C)   | 155.2  | 148.6195| 152.3899| 148.3891| 145.6424| 151.9002| 148.7217|
| C(N-C)   | 159.3  | 149.2986| 156.2343| 162.0108| 153.3098| 156.6014| 161.8371|
| C(N-C)   | 172.5  | 170.1617| 177.4526| 171.7264| 174.8131| 174.5916| 172.8955|
| CTE      | 0.9953 | 0.9882  | 0.9972  | 0.9960  | 0.9979  | 0.9972  |

|          | δ Exp. | F       | G       | H       | I       | J       | K       |
|----------|--------|---------|---------|---------|---------|---------|---------|
| CH₃      | 1.88   | 2.4577  | 2.3532  | 2.4091  | 2.4259  | 2.2747  | 2.3907  |
| CH₂      | 2.04   | 2.5089  | 2.3822  | 2.4624  | 2.4659  | 2.4294  | 2.4161  |
| CH₃      | 2.17   | 2.5132  | 2.4488  | 2.4741  | 2.4904  | 2.4992  | 2.4734  |
| NH       | 11.06  | 10.0562 | 9.3835  | 9.9100  | 8.7877  | 9.6222  | 9.8899  |
| C(CH₃)₂  | 13.4   | 14.2767 | 17.2876 | 15.0080 | 13.5960 | 16.7690 | 14.2200 |
| C(CH₃)₃  | 21.4   | 26.3880 | 25.1647 | 27.1963 | 28.6643 | 25.0853 | 28.2874 |
| C(CH₃)₈  | 23.2   | 40.7087 | 33.5601 | 36.5072 | 35.9599 | 36.3311 | 36.0581 |
| C(N-C)   | 155.2  | 162.1734| 165.7812| 161.6629| 158.5847| 165.8311| 161.5289|
| C(N-C)   | 159.3  | 162.9255| 170.0586| 176.7136| 167.3300| 170.0370| 176.1470|
| C(N-C)   | 172.5  | 186.3183| 194.7317| 187.6225| 191.5178| 190.7120| 189.3898|
| CTE      | 0.9946 | 0.9975  | 0.9967  | 0.9954  | 0.9974  | 0.9967  |

|          | δ Exp. | F       | G       | H       | I       | J       | K       |
|----------|--------|---------|---------|---------|---------|---------|---------|
| CH₃      | 1.88   | 2.4297  | 2.3388  | 2.3947  | 2.4083  | 2.2647  | 2.3733  |
| CH₂      | 2.04   | 2.4911  | 2.3588  | 2.4375  | 2.4449  | 2.3955  | 2.4043  |
| CH₃      | 2.17   | 2.5042  | 2.4144  | 2.4538  | 2.4674  | 2.4893  | 2.4469  |
| NH       | 11.06  | 10.0921 | 9.4070  | 9.9057  | 8.8267  | 9.6288  | 9.8983  |
| C(CH₃)₂  | 13.4   | 14.0502 | 16.9302 | 14.7648 | 13.4471 | 16.4082 | 14.0490 |
| C(CH₃)₃  | 21.4   | 26.4177 | 25.4802 | 27.8250 | 28.7518 | 25.2209 | 28.4035 |
| C(CH₃)₈  | 23.2   | 40.3424 | 33.2568 | 36.1088 | 35.2159 | 35.9973 | 35.6604 |
| C(N-C)   | 155.2  | 160.7368| 164.1820| 160.1080| 157.3174| 164.0677| 160.1518|
| C(N-C)   | 159.3  | 161.3816| 168.3921| 174.6293| 165.5923| 168.2915| 173.9931|
| CTE      | 172.5  | 185.4207| 193.9106| 186.8261| 190.7821| 190.0600| 188.6287|
| CTE      | 0.9945 | 0.9973  | 0.9967  | 0.9951  | 0.9972  | 0.9966  |

‡ - in DMSO; CTE (Correlation between Theoretical and Experimental data);
Table 6S - Experimental and calculated NMR data for the *Hmta* in DMSO and CDCl₃, with geometry optimization data obtained in DFT/B3LYP/6-311++G(2d,2p)/DMSO.

|       | δ Exp.ᵃ | δ Exp.ᵇ | B3LYP/6-311++G(2d,2p) |
|-------|---------|---------|----------------------|
| CH₃   | 1.96    | 2.14    | 2.6408               |
| NH (H14) | 8.07   | 9.24    | 8.8419               |
| NH (H4) | --     | --      | 7.9606               |
| NH₂   | 5.95    | --      | 5.1502               |
| CH₃   | 13.5    | 12.3    | 12.4075              |
| C(CH₃) | 159.27  | 157.3   | 157.7567             |
| C(NH₂) | 155.28  | 153.4   | 153.1876             |
| CTE   |         |         | 0.99990ᵃ/0.99998ᵇ    |

CTE (Correlation between Theoretical and Experimental data), a - DMSO, b - CDCl₃.

Table 7S - Experimental and calculated NMR data for the *mta* (A', B', C') tautomer in DMSO, with geometry optimization data obtained in DFT/B3LYP/6-311++G(2d,2p)/DMSO.

|       | δ Expᵇ | B3LYP/6-31G(d,p) | B3LYP/6-311+G(d,p) |
|-------|---------|------------------|-------------------|
| CH₃   | 2.13    | 2.1879           | 2.3551            |
| NH    | 6.3-5.8 | 2.2184           | 2.5949            |
| NH₂   | 8.7-8.0 | 7.8007           | 7.9294            |
| CH₃   | 13.2    | 12.6             | 13.7049           |
| C(CH₃)| 154.9   | 141.8918         | 154.3362          |
| C(NH₂)| 159.9   | 146.1909         | 159.2434          |
| CTE   | 0.99961 | 0.99837          | 0.9967            |

CTE (Correlation between Theoretical and Experimental data); Ø - Experimental data of *mta* reported in the literature (1S).
Table 8S - Experimental and calculated NMR data for the pta (A, B, C, D, E) tautomer in DMSO, with geometry optimization data obtained in DFT/B3LYP/6-311++G(2d,2p)/DMSO.

| δ Exp. | A   | B   | C   | D   | E   |
|--------|-----|-----|-----|-----|-----|
| Ph(H)  | 7.01| 3.444| 3.5867| 3.0749| 4.7715| 4.361|
| Ph(H)  | 7.31| 7.2652| 7.2932| 7.3956| 5.7141| 6.4305|
| Ph(H)  | 7.88| 7.3665| 7.3719| 7.4824| 6.4984| 6.6796|
| NH₂    | 8.13| 7.4082| 7.9556| 7.9513| 7.5392| 7.4588|
| NH     | 10.31| 7.794| 8.2926| 9.2602| 7.8672| 7.5875|
| Ph(C)  | 127.7| 118.7756| 122.1177| 120.638| 123.4452| 119.6325|
| Ph(C)  | 129.3| 122.0468| 122.8734| 123.8862| 123.9173| 123.2828|
| Ph(C)  | 129.7| 123.8485| 122.9471| 123.9325| 124.5229| 123.8996|
| Ph(C)  | 139.4| 124.6266| 128.4737| 123.9652| 126.9448| 123.9105|
| C-(Ph) | 160.2| 141.7851| 144.5942| 147.4558| 159.584| 139.5523|
| C-(NH₃) | 171.5| 143.3112| 156.6623| 157.9429| 163.9462| 144.0003|
| CTE    | 0.99430| 0.99860| 0.99827| 0.99829| 0.99377|

| δ Exp. | A   | B   | C   | D   | E   |
|--------|-----|-----|-----|-----|-----|
| Ph(H)  | 7.01| 3.8542| 3.9866| 3.5893| 5.3561| 4.4444|
| Ph(H)  | 7.31| 7.4178| 7.4766| 7.5752| 6.116| 6.9045|
| Ph(H)  | 7.88| 7.5507| 7.568| 7.6302| 6.4708| 6.9599|
| NH₂    | 8.13| 7.5791| 8.1586| 8.1769| 7.7| 7.6134|
| NH     | 10.31| 8.0384| 8.5515| 9.3157| 8.0879| 7.8089|
| Ph(C)  | 127.7| 129.5644| 133.6122| 131.7226| 134.9004| 130.6741|
| Ph(C)  | 129.3| 134.099| 134.5331| 135.2125| 135.4916| 134.2305|
| Ph(C)  | 129.7| 135.5979| 135.0229| 135.5533| 135.8739| 135.4559|
| Ph(C)  | 139.4| 136.247| 140.1653| 135.9626| 139.2853| 135.9371|
| C-(Ph) | 160.2| 153.8445| 159.4296| 159.9258| 175.4565| 152.0228|
| C-(NH₃) | 171.5| 157.3631| 169.7787| 173.1118| 177.3422| 159.3037|
| CTE    | 0.99429| 0.99849| 0.99839| 0.99808| 0.99461|

| δ Exp. | A   | B   | C   | D   | E   |
|--------|-----|-----|-----|-----|-----|
| Ph(H)  | 7.01| 3.8491| 3.9872| 3.5542| 5.3095| 4.4204|
| Ph(H)  | 7.31| 7.4888| 7.5489| 7.6536| 6.0789| 6.9531|
| Ph(H)  | 7.88| 7.5533| 7.647| 7.7171| 6.4758| 6.9915|
| NH₂    | 8.13| 7.656| 8.1725| 8.2487| 7.7902| 7.7|
| NH     | 10.31| 8.1281| 8.6353| 9.323| 8.1718| 7.8808|
| Ph(C)  | 127.7| 129.3042| 133.1975| 131.407| 134.6342| 130.2974|
| Ph(C)  | 129.3| 133.8465| 134.2682| 134.1237| 134.6773| 133.1529|
| Ph(C)  | 129.7| 134.9889| 134.7552| 135.2025| 135.1766| 135.0661|
| Ph(C)  | 139.4| 135.2698| 138.7849| 135.7788| 139.0888| 135.7645|
| C-(Ph) | 160.2| 152.6525| 157.6835| 158.5392| 173.6897| 150.7569|
| C-(NH₃) | 171.5| 155.9194| 168.0038| 170.8153| 175.9921| 157.8276|
| CTE    | 0.99370| 0.99801| 0.99830| 0.99834| 0.99420|

CTE (Correlation between Theoretical and Experimental data)
References

1S Boechat, N., Pinheiro, L. C. S., Santos-Filho, O. A., & Silva, I. C. (2011). Design and Synthesis of New N-(5-Trifluoromethyl)-1H-1,2,4-triazol-3-yl Benzenesulfonamides as Possible Antimalarial Prototypes. *Molecules, 16*(9), 8083. doi: https://doi.org/10.3390/molecules16098083

2S Jin, R. Y., Sun, X. H., Liu, Y. F., Long, W., Lu, W. T., & Ma, H. X. (2014). Synthesis, crystal structure, IR, 1H NMR and theoretical calculations of 1,2,4-triazole Schiff base. *Journal of Molecular Structure, 1062*, 13-20. doi: https://doi.org/10.1016/j.molstruc.2014.01.010

3S Phalgune, U. D., Vanka, K., & Rajamohan, P. R. (2013). GIAO/DFT studies on 1,2,4-triazole-5-thiones and their propargyl derivatives. *Magnetic Resonance in Chemistry, 51*(12), 767-774. doi: https://doi.org/10.1002/mrc.4012

4S Thomas, S., Biswas, N., Venkateswaran, S., Kapoor, S., D'Cunha, R., & Mukherjee, T. (2005). Raman, infrared, SERS and DFT calculations of a triazole derivative (akacid). *Chemical Physics Letters, 402*(4), 361-366. doi: https://doi.org/10.1016/j.cplett.2004.12.064

5S Ünlüler, D., Ünver, Y., Düğdüz, E., Alpaslan, Y. B., Köysal, Y., Soylu, M. S., & Sancak, K. (2019). Novel 1,2,4-Triazole Derivatives: Structure, DFT Study, X-Ray Analysis, and Antimicrobial Activity. *Russian Journal of Organic Chemistry, 55*(2), 254-261. doi:https://doi.org/10.1134/S1070428019020192