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Article

Conformational Analysis, Experimental and GIAO-DFT ^{13}C NMR Chemical Shift Calculation on 2'-Hydroxy-3,4,5-trimethoxy-chalcone

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In this paper we investigated the ability of the GIAO-mPW1PW91/6-31G(d)//mPW1PW91/6-31G(d) level of theory to predict the ^{13}C nuclear magnetic resonance (NMR) chemical shifts of the 2'-hydroxy-3,4,5-trimethoxy-chalcone molecule. Two different approaches were used. First: the absolute shieldings σ for all carbon atoms in each geometrically optimized conformers of the 2'-hydroxy-3,4,5-trimethoxy-chalcone molecule were calculated at the GIAO-mPW1PW91/6-31G(d)//mPW1PW91/6-31G(d) level of theory. This approach is further used to generate weighted average values for each atom considering the previously obtained conformational distribution. Second: only the σ for the lowest energetic conformer will be taken to account. The robustness of the method was evaluated for two other chalcones: (*E*)-1-(4-hydroxy-3-methoxyphenyl)-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)prop-2-en-1-one and (*E*)-1-(4-aminophenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one, corroborating the ability of the method in chemical shift prevision. Although, both approaches were able to reproduce the chemical shifts of the 2'-hydroxy-3,4,5-trimethoxy-chalcone, significant differences in the calculated values for C-4 and methoxy carbons were observed. The best results were obtained using the second approach (II).

Keywords: chalcone, 2'-hydroxy-3,4,5-trimethoxy-chalcone, GIAO-NMR, scaling factor, NMR spectroscopy

Introduction

Chalcones (1,3-diaryl-2-propen-1-ones) are important intermediates for the synthesis of compounds such as flavonoids, isoflavonoids and their derivatives.[1] Besides having a physiological role in plants, flavonoids have also been reported to have a wide variety of biological activities, including antioxidant,[2-6] antibacterial,[7,8] anticancer,[9,10] antiangiogenic ones.[11] The growing interest in these compounds and their potential use in medicinal applications is indicated by the increasing number of publications concerning the synthesis and biological evaluation of chalcone analogues.[1-11] Their properties are related, among other factors, to its great conformational freedom as well as to the several patterns of substitution of A and B rings.[12] Thus, the correct structural determination and the knowledge about three-dimensional (3D) atomic structure of chalcones are crucial to understand their properties. In this context, the complete assignments of ^{13}C nuclear magnetic resonance (NMR) chemical shifts for chalcones, even using computation methodology, can help in identifying the chemical structures of new chalcone derivatives. The 2'-hydroxy-3,4,5-trimethoxy-chalcone (Figure 1) has shown *in vitro* inhibitory effects on PGE2 (prostaglandin E2) production from RAW 264.7 cells induced by LPS (lipopolysaccharide).[13] At 10 μM 2'-hydroxychalcones proved strong effect to inhibit the PGE2 production (102.3%). However, these compounds also indicated the effect on cell viability with potential for anti-inflammation and anticancer activity.[13]

Our research group has worked with quantum mechanics using computational tools to better understand the interpretation of the ^{13}C NMR chemical shift experimental data. Recently, we published the applicability of the empirical equations for conversion of quantum mechanically calculated chemical shielding tensors into chemical shift values.[14-16] In these papers, it was pointed out the importance of a linear conversion

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formula in order to achieve great ^{13}C NMR chemical shift experimental data reproduction and prediction. In this context, the goal of this work was to investigate the ability of the scaling factor protocol at the GIAO-mPW1PW91/6-31G(d)//mPW1PW91/6-31G(d) level of theory to predict the ^{13}C NMR chemical shifts (δ) of the 2'-hydroxy-3,4,5-trimethoxy-chalcone molecule (Figure 1). Moreover, two different approaches for determining the δ of the 2'-hydroxy-3,4,5-trimethoxy-chalcone were compared.

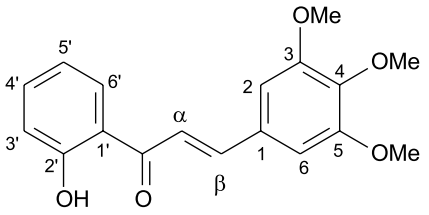

Figure 1. Structure of the 2'-hydroxy-3,4,5-trimethoxy-chalcone.

Methodology

Chemicals

The 2'-hydroxy-3,4,5-trimethoxy-chalcone was obtained from Sigma-Aldrich Chemical Co.

NMR system and operating conditions

NMR analyses were acquired on a Bruker Avance III 11.75 Tesla spectrometer at 298 K using a 5 mm triple resonance broadband inverse (TBI) probehead. The spectra were obtained at 125.77 MHz for ^{13}C using $CDCl_3$ as the solvent. The ^{13}C spectra (Figure S1, Supplementary Information (SI)) were acquired with spectral window of 37,878 Hz, 32,768 digitalized points and accumulation of 3,926 FIDs. The heteronuclear single-quantum correlation (HSQC, Figure S2, SI) and heteronuclear multiple-bond correlation (HMBC, Figure S3, SI) experiments were acquired with a spectral window of 10,000 and 37,731 Hz for ^{1}H and ^{13}C, respectively. The phase and baseline were corrected with the TopSpin software (version 3.2 Bruker BioSpin). NMR assignments are based on ^{1}H, ^{13}C and ^{1}H-^{13}C HSQC/HMBC experiments. ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.85 (s, OH), 7.93 (dd, *J* 8.1, 1.6 Hz, H-6'), 7.84 (d, *J* 15.4 Hz, H-β), 7.53 (d, *J* 15.4 Hz, H-α), 7.50 (ddd, *J* 8.4, 7.2, 1.6 Hz, H-4'), 7.03 (dd, *J* 8.4, 1.2 Hz, H-3'), 6.95 (ddd, *J* 8.1, 7.2, 1.2 Hz, H-5'), 6.88 (s, H-2/H-6), 3.93 (s, OMe-3/OMe-5), 3.92 (s, OMe-4); HMBC/HSQC crosspeaks: OMe-3/OMe-5 (C-2, C-3, C-5, C-6), OMe-4 (C-4), H-2/H-6 (C-1, C-3, C-4, C-5, C-β), H-β (C-1, C-2, C-6, C-α, C=O), H-α (C-1, C=O), H-3' (C-1', C-2', C-5', C=O), H-4' (C-2', C-3', C-5', C-6'), H-5' (C-2', C-3', C4', C-6'), H-6' (C-1', C-2', C-4', C-5', C=O).

Computational details

The ^{13}C NMR chemical shifts of the three chalcones were calculated with two different approaches. In the first one, the absolute shieldings (σ) of all carbon atoms in each geometrically optimized conformer of molecule were calculated using the GIAO (gauge-independent atomic orbital) approximation at the mPW1PW91/6-31G(d)//mPW1PW91/6-31G(d) (NMR//optimization) level of theory, and further used to generate weighted average values for each atom considering the previously obtained conformational distribution, σ_{aver}. In the second one, only the σ for the lowest energetic conformer was taken to account, σ_{lowe}. Molecular mechanics calculations were performed using the Spartan'08 modeling software,[17] whereas DFT (density functional theory) calculations were performed using the Gaussian 09 W software package.[18] Solvent effects were not taken into account in any calculation.

Statistical validation

In order to perform a statistical validation of our results the mean deviation (MD) and the root mean square deviation (RMSD) errors (in ppm) were calculated.

Results and Discussion

A randomized conformational search of the 2'-hydroxy-3,4,5-trimethoxy-chalcone molecule was performed (Figure 2) using the Monte Carlo (MC) method with a search limit of 200 structures. Merck molecular force field (MMFF) as implemented in the Spartan'08 software package, considering an initial energy cutoff of 10 kcal mol^{-1}, was employed.

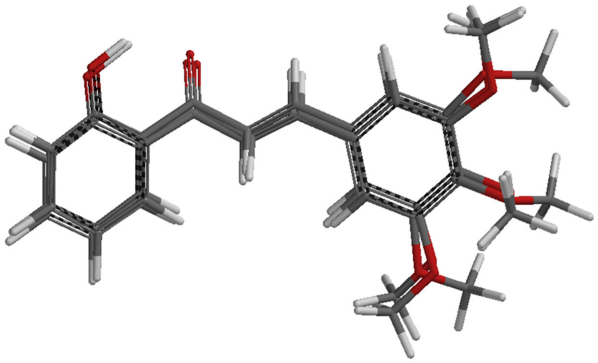

Figure 2. Superposition of the three lowest energy conformations of the 2'-hydroxy-3,4,5-trimethoxy-chalcone molecule.

The 28 more significant conformations of 2'-hydroxy-3,4,5-trimethoxy-chalcone molecule were saved, which are responsible for more than 99.99% of the total Boltzmann population in the first 10 kcal mol^{-1}. This was followed by single-point energy calculations at the B3LYP/6-31G(d) and level of theory. The 21 more significant conformations within the range of 0.0-5.0 kcal mol^{-1} were selected by energy minimization calculations carried out at the mPW1PW91/6-31G(d) level of theory. The relevant results are given in Table 1. Frequency calculations carried out at the mPW1PW91/6-31G(d) level of theory confirmed the optimized geometries to be local minima and delivered values of free energy at 298 K and 1 atm. In this step, the three most significant conformations within the range of 0.0-3.0 kcal mol^{-1} were selected (Figure 3).

In the approach (I), for each optimized conformer geometry, the ^{13}C atomic chemical shielding tensors (σ) were computed at the mPW1PW91/6-31G(d)//mPW1PW91/6-31G(d) level of theory. Isotropic atomic chemical shifts δ in units of ppm were computed as differences between the atomic isotropic shielding of the solutes and corresponding reference atoms in tetramethylsilane (TMS). Thus, the population-averaged chemical shifts for the selected conformers were computed assuming Boltzmann statistics, according to equation 1, based on mPW1PW91/6-31G(d) free energies. Finally, the ^{13}C NMR chemical shifts were

Table 1. DFT, thermo chemical parameter and DFT population for the 28 more significant conformations of 2'-hydroxy-3,4,5-trimethoxy-chalcone molecule

Conformer	ΔE^a / (kcal mol^{-1})	Bolt. dist.^{b} / %	ΔE^c / (kcal mol^{-1})	Bolt. dist.^{d} / %	ΔE^e / (kcal mol^{-1})	Bolt. dist.^{f} / %	ΔG^g / (kcal mol^{-1})	Bolt. dist.^{h} / %
001	0.00	34.6	0.00	40.8	0.00	79.2	0.00	80.9
002	0.09	29.7	0.09	35.1	1.01	12.3	1.06	11.4
003	0.62	12.1	0.62	14.2	1.21	8.5	1.27	7.7
004	0.67	11.2	1.30	4.5	2.60	–	–	–
005	1.30	3.8	2.22	1	3.00	–	–	–
006	1.31	3.8	2.30	0.8	4.01	–	–	–
007	2.22	0.8	2.35	0.8	4.05	–	–	–
008	2.30	0.7	2.39	0.7	4.23	–	–	–
009	2.35	0.7	2.68	0.4	4.31	–	–	–
010	2.39	0.6	2.74	0.4	–	–	–	–
011	2.68	0.4	2.85	0.3	–	–	–	–
012	2.70	0.2	2.89	0.3	–	–	–	–
013	2.74	0.3	3.38	0.1	–	–	–	–
014	2.74	0.2	3.48	0.1	–	–	–	–
015	2.85	0.3	3.50	0.1	–	–	–	–
016	2.89	0.1	3.65	0.1	–	–	–	–
017	3.38	0.1	3.73	0.1	–	–	–	–
018	3.48	0.1	3.76	0.1	–	–	–	–
019	3.50	0.1	4.42	0	–	–	–	–
020	3.63	0.1	4.47	0	–	–	–	–
021	3.65	0.1	4.99	0	–	–	–	–
022	3.73	0.1	–	–	–	–	–	–
023	3.76	0.1	–	–	–	–	–	–
024	4.42	0	–	–	–	–	–	–
025	4.47	0	–	–	–	–	–	–
026	4.99	0	–	–	–	–	–	–
027	5.52	0	–	–	–	–	–	–
028	6.87	0	–	–	–	–	–	–

[a]Relative MMFF energy of conformers obtained from Monte Carlo analysis; [b]MMFF Boltzmann population of the conformers; [c]relative B3LYP/6-31G(d) single point energy of conformers obtained from Monte Carlo analysis; [d]B3LYP/6-31(g) single point energy Boltzmann population of the conformers; [e]relative mPW1PW91/6-31G(d) energy minimization of the conformers; [f]mPW1PW91/6-31G(d) energy minimization Boltzmann population of the conformers; [g]relative mPW1PW91/6-31G(d) sum of electronic and free energy of the conformers; [h]mPW1PW91/6-31G(d) Boltzmann population calculated from ΔG values of the conformers.

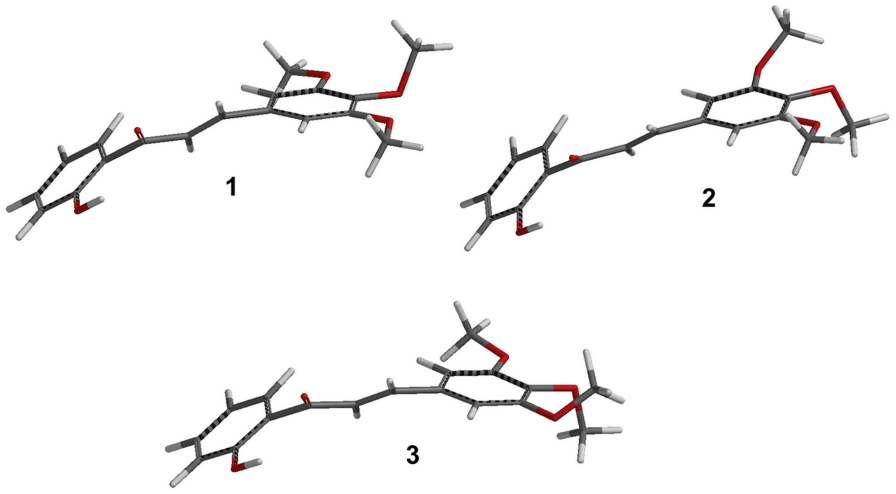

Figure 3. DFT-optimized structures of the three more stable conformers, 1, 2 and 3, respectively, of 2'-hydroxy-3,4,5-trimethoxy-chalcone. For relative energies and abundances of the conformers, see Table 1.

scaled according to Costa *et al*. protocols.[15]

$$\langle\delta\rangle = \sum_i \frac{\delta_i e^{-\Delta E_i/kT}}{\sum_i e^{-\Delta E_i/kT}} \quad (1)$$

ΔE_i is the relative energy of the i^{th} conformer to the lowest energy, k is the Boltzmann constant, and the temperature T is set to 298 K. In the approach (II), only the lowest-energetic conformer was used to obtain the scaled chemical shifts.

Figure 3 shows the three most significant conformations conformers of 2'-hydroxy-3,4,5-trimethoxy-chalcone according to the geometry optimization calculations carried out at the mPW1PW91/6-31G(d) level of theory. The two more significant factors that determine the stability of the 2'-hydroxy-3,4,5-trimethoxy-chalcone conformers are apparently the intramolecular hydrogen bond between the OH and C=O and steric effects of methoxyl groups in the B-ring.

Table 2 shows the calculated (δ_{calc}), scaled (δ_{scal}) and experimental (δ_{exp}) ^{13}C NMR chemical shifts of the 2'-hydroxy-3,4,5-trimethoxy-chalcone as well as the differences (≠) among them. The data comparison

Table 2. Calculated (scaled) and differences between experimental ^{13}C NMR chemical shifts (in ppm) in the 2'-hydroxy-3,4,5-trimethoxy-chalcone relative to TMS, for approaches (I) and (II)

Nuclei	δ_{exp} / ppm	Approach (I)		Approach (II)	
		δ_{calc}[a] (δ_{scal})[b] / ppm	≠ δ_{calc} (δ_{scal}) / ppm	δ_{calc}[a] (δ_{scal})[b] / ppm	≠ δ_{calc} (δ_{scal}) / ppm
C-1	130.07	124.46 (129.46)	–5.61 (–0.61)	124.18 (129.17)	–5.89 (–0.90)
C-2[c]	106.03	103.56 (107.52)	–2.47 (1.49)	101.93 (105.81)	4.10 (–0.22)
C-3	153.55	148.18 (154.37)	–5.37 (0.82)	148.05 (154.23)	–5.50 (0.68)
C-4	140.92	137.56 (143.22)	–3.36 (2.30)	136.44 (142.04)	–4.48 (1.12)
C-5	153.55	148.19 (155.38)	–5.41 (0.78)	147.90 (154.08)	–5.70 (–0.48)
C-6[c]	106.03	103.56 (107.52)	–2.47 (1.49)	101.93 (105.81)	4.10 (–0.22)
C-α	119.29	143.30 (149.24)	–2.34 (3.60)	143.27 (149.21)	–2.37 (3.57)
C-β	145.64	111.42 (115.77)	–7.88 (–3.53)	111.21 (115.55)	–8.09 (–3.75)
C-1'	120.05	183.25 (191.19)	–10.29 (–2.35)	183.22 (191.16)	–10.32 (–2.38)
C-2'	163.60	115.05 (119.58)	–4.95 (–0.42)	114.85 (119.37)	–5.15 (–0.63)
C-3'	118.65	159.43 (166.18)	–4.17 (2.58)	159.34 (166.08)	–4.26 (2.48)
C-4'	136.35	114.30 (118.79)	–4.35 (0.14)	114.09 (118.57)	–4.56 (–0.08)
C-5'	118.81	130.59 (135.90)	–5.76 (–0.45)	130.43 (135.73)	–5.92 (–0.62)
C-6'	129.61	111.37 (115.72)	–7.44 (–3.09)	111.17 (115.51)	–7.64 (–3.30)
C=O	193.54	124.40 (129.40)	–5.21 (–0.21)	124.24 (129.23)	–5.37 (–0.38)
OMe-3	56.29	54.25 (55.75)	–2.46 (–0.99)	52.98 (54.41)	–3.32 (–1.89)
OMe-4	61.03	57.26 (58.80)	–3.77 (–2.13)	57.42 (59.07)	–3.61 (–1.96)
OMe-5	56.29	53.84 (55.31)	–2.04 (–0.54)	52.96 (54.39)	–3.33 (–1.90)

[a]Calculated chemical shifts ($\delta_{calc} = \sigma_{TMS} - \sigma_{aver}$ or σ_{lowe}) obtained by TMS subtraction; [b]scaled chemical shifts ($\delta_{scal} = 1.05\delta_{calc} - 1.22$) obtained by the generated universal scaling factor;[15] [c]the δ_{calc} and δ_{scal} for C-2 and C-6 were arithmetically averaged.

Table 3. Statistical data illustrating the performance of two approaches for the GIAO calculation of ^{13}C NMR chemical shifts (δ, in ppm) for the 2'-hydroxy-3,4,5-trimethoxy-chalcone (a), (*E*)-1-(4-hydroxy-3-methoxyphenyl)-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)prop-2-en-1-one (b) and (*E*)-1-(4-aminophenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (c)

Chalcone	Approach (I)		Approach (II)	
	MD (RMSD)[a] / ppm	MD (RMSD)[b] / ppm	MD (RMSD)[a] / ppm	MD (RMSD)[b] / ppm
a	4.75 (5.20)	0.06 (1.90)	5.21 (5.54)	0.55 (1.89)
b	4.73 (5.75)	0.19 (1.89)	5.54 (6.26)	0.87 (1.56)
c	5.56 (6.63)	0.80 (2.87)	7.17 (7.82)	2.49 (3.54)

[a]Calculated chemical shifts (δ_{calc} = TMS − δ) obtained by TMS subtraction; [b]scaled chemical shifts (δ_{scal} = 1.05δ_{calc} − 1.22) obtained by the generated universal scaling factors.[15] MD: mean deviation; RMSD: root mean square deviation.

demonstrated a great agreement between experimental and calculated NMR chemical shifts.

The main differences between experimental and scaled chemical shifts, for both approaches, were observed in carbons C-4, OMe-3, OMe-4 and OMe-5. These differences are consistent with the three selected conformers (Figure 3) to obtain the chemical shifts at the approach (I). The reason is that they have different orientations of the OCH_3 group (atoms C-3, C-4 and C-5) with respect to the aromatic ring and their chemical shifts are systematically dependent on the orientation of the methoxyl group.

For evaluating the robustness of the method, the chemical shift for two other chalcones: (*E*)-1-(4-hydroxy-3-methoxyphenyl)-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) prop-2-en-1-one and (*E*)-1-(4-aminophenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (Figure S4, SI), were calculated. The results presented in Table 3 demonstrate an excellent predictive ability of the method. The experimental and theoretical calculation of the NMR data for these two chalcones are presented in the Supplementary Information.

Conclusions

In this work, we investigated the ability of the scaling factor protocol at the GIAO-mPW1PW91/6-31G(d)// mPW1PW91/6-31G(d) level of theory to predict the ^{13}C NMR chemical shifts of the 2'-hydroxy-3,4,5-trimethoxy-chalcone. The robustness of the method was evaluated for two other chalcones: (*E*)-1-(4-hydroxy-3-methoxyphenyl)-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) prop-2-en-1-one and (*E*)-1-(4-aminophenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one, corroborating the ability of the method in chemical shift prevision. After using the scale factor, the two approaches were able to correctly reproduce the chemical shifts, despite the differences in the conditions of the experimental measurements and computational predictions. Both approaches had similar performance. These findings suggest that, in this case, either the approach (I) or the approach (II) could be chosen and that further analysis of the methoxychalcones is therefore justified.

Supplementary Information

Supplementary information is available free of charge at http://jbcs.org.br as PDF file.

Acknowledgments

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References

1. Daskiewicz, J.-B.; Depeint, F.; Viornery, L.; Bayet, C.; Comte-Sarrazin, G.; Comte, G.; Gee, J. M.; Johnson, I. T.; Ndjoko, K.; Hostettmann, K.; Barron, D.; *J. Med. Chem.* **2005**, *48*, 2790.
2. Stevens, J. F.; Miranda, C. L.; Frei, B.; Buhler, D. R.; *Chem. Res. Toxicol.* **2003**, *16*, 1277.
3. Nishida, J.; Kawabata J.; *J. Biosci. Biotechnol. Biochem.* **2006**, *70*, 193.
4. Gacche, R. N.; Dhole, N. A.; Kamble, S. G.; Bandgar, B. P. J.; *Enzyme Inhib. Med. Chem.* **2007**, *23*, 28.
5. Vogel, S.; Ohmayer, S.; Brunner, G.; Heilmann, J.; *Bioorg. Med. Chem.* **2008**, *16*, 4286.
6. Jung, J.-C.; Jang, S.; Lee, Y.; Min, Y.; Lim, E.; Jung, H.; Oh, M.; Oh, S.; Jung, M.; *J. Med. Chem.* **2008**, *51*, 4054.
7. Sugamoto, K.; Kurogi, C.; Matsushita, Y.; Matsui, T.; *Tetrahedron Lett.* **2008**, *49*, 6639.
8. Avila, H. P.; Smania, E.; Monache, F. D.; Smania, A.; *Bioorg. Med. Chem*. **2008**, *16*, 9790.

9. Lawrence, N. J.; Patterson, R. P.; Ooi, L. L.; Cook, D.; Ducki, S.; *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5844.
10. Cabrera, M.; Simoens, M.; Falchi, G.; Lavaggi, M. L.; Piro, O. E.; Castellano, E. E.; Vidal, A.; Azqueta, A.; Monge, A.; de Cerain, A. L.; Sagrera, G.; Seoane, G.; Cerecettoand, H.; Gonzalez, M.; *Bioorg. Med. Chem.* **2007**, *15*, 3356.
11. Boumendjel, A.; Boccard, J.; Carrupt, P.-A.; Nicolle, E.; Blanc, M.; Geze, A.; Choisnard, L.; Wouessidjewe, D.; Matera, E.-L.; Dumontet, C.; *J. Med. Chem.* **2008**, *51*, 2307.
12. Larsen, M.; Kromann, H.; Kharazmi, A.; Nielsen, S. F.; *Bioorg. Med. Chem. Lett.* **2006**, *15*, 4858.
13. Tran, T.-D.; Park, H.; Kim, H. P.; Ecker, G. F.; Thai, K.-M.; *Bioorg. Med. Chem.* **2009**, *19*, 1650.
14. Costa, F. L. P.; de Albuquerque, A. C. F.; dos Santos Jr., F. M.; de Amorim, M. B.; *J. Phys. Org. Chem.* **2010**, *23*, 972.
15. Costa, F. L. P.; de Albuquerque, A. C. F.; Borges, R. M.; dos Santos Jr., F. M.; de Amorim, M. B.; *J. Comput. Theor. Nanosci.* **2014**, *11*, 219.
16. Costa, F. L. P.; de Albuquerque, A. C. F.; dos Santos Jr., F. M.; de Amorim, M. B.; *J. Comput. Theor. Nanosci.* **2015**, *12*, 2195.
17. *Spartan'08*, Wavefunction Inc., Irvine, California, USA, 2010.
18. Frisch, A. E.; Frisch, M. J.; Trucks, G.; *Gaussian 09 User's Reference*, Gaussian Inc., Pittsburgh, USA, 2009.

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