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1,2,4-triazole derivatives with morpholine; DFT study and Antileishmanial activity

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ABSTRACT

1,2,4-triazole derivatives with morpholine; 4-((3-methylthiophene-2-yl)methylenamino)-1-((4-(3-methylthiophene-2-yl)methylene amino)-1-(morpholinomethyl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazole-3-yl)methyl)-3-(thiophene-2-ylmethyl)-1H-1,2,4-triazole-5(4H)-one (**I**) and 1-((1-(morpholinomethyl)-4-(5-nitrothiophene-2-yl)methyleneamino)-5-thioxo-4,5-dihydro-1H-1,2,4-triazole-3-yl)methyl)-4-((5-nitrothiophene-2-yl)methyleneamino)-3-(thiophene-2-ylmethyl)-1H-1,2,4-triazole-5(4H)-one (**II**), were optimized by DFT method with 6-311G(d,p) basis set and, structural and spectral parameters were determined. In vitro antileishmanial activities of compounds were performed against *Leishmania infantum* promastigots by microdilution broth assay with Alamar Blue Dye. Amphotericin B was used as standard drug. The results show that both compounds are antiparasitic and especially compound **II** has considerable antileishmanial activity due to the Minimal Inhibitory Concentration (MIC) value of 312 µg/mL.

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1. Introduction

1,2,4-Triazole derivatives have a wide spectrum of biological activities including antifungal, antibacterial, antitumor, antioxidant, anti-inflammatory, antiproliferative and antitubercular [1,2]. Schiff bases derived from various heterocycles possess some biological activities. In particular, they show antifungal, antibacterial, antitubercular, anticancer, antioxidant, anti-inflammatory, pesticidal antimalarials, anticonvulsant properties [3]. N-functionalized morpholines have found to possess diverse pharmacological activities such as antimicrobial, platelet aggregation inhibitory, anticancer, antiemetic, antitumor, antiproliferative, and bronchodilator activity [4,5]. It has been reported that, compounds both triazole and morpholine nucleus have antimicrobial activity [6,7].

Leishmaniasis, as a disease worldwide, commonly in tropical and subtropical regions and sickened about 20 million humans is presumed one of most important diseases, which cause serious health problems [8,9]. Most of the antileishmanial medicaments used for the treatment are extremely toxic and in most of the cases resistance to drugs emerges [10,11].

In this study, 1,2,4-triazole derivatives with morpholine (**I** and **II**) were optimized using the density functional theory (DFT) method with 6-311G(d,p) basis set. Vibrational frequencies, chemical shift values were also determined for both of compounds. Antileishmanial activities of compounds **I** and **II** against to *Leishmania infantum* promastigotes in the axenic culture were tested by the microdilution broth method with the Alamar blue dye. That the development of new medicines, which have very low side effects and can be effective in treatment, is of great importance in terms of the quality of life of the patients. This study is indication of both the antileishmanial activity of 1,2,4-triazole derivatives with morpholine and the effect of functional groups on the antiparasitic properties.

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2. Experimental and computational method

2.1. Synthesis

1,2,4-triazole derivatives with morpholine; 4-((3-methyl thiophene-2-yl)methylenamino)-1-((4-(3-methylthiophene-2-yl)methyleneamino)-1-(morpholinomethyl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazole-3-yl)methyl)-3-(thiophene-2-ylmethyl)-1H-1,2,4-triazole-5(4H)-one (**I**) and 1-((1-(morpholinomethyl)-4-(5-nitrothiophene-2-yl)methyleneamino)-5-thioxo-4,5-dihydro-1H-1,2,4-triazole-3-yl)methyl)-4-((5-nitrothiophene-2-yl)methylene amino)-3-(thiophene-2-ylmethyl)-1H-1,2,4-triazole-5(4H)-one (**II**) were published in literature [12]. The structures of the compounds **I** and **II** are showed in Scheme 1.

Scheme 1. The structures of 1,2,4-triazole derivatives with morpholine (**I** and **II**).

2.2. Computational details

Theoretical study was performed by using Gaussian 03 [13] software. Optimized geometries, the vibration frequencies and chemical shift values of compounds **I** and **II** were calculated by DFT/B3LYP [14,15] method with 6-311G(d,p) basis set. All geometries were visualised by using GaussView 4.1 [16] software package. ^1H and ^{13}C NMR chemical shifts were calculated by means of standart GIAO approach [17,18].

3. Pharmacology

3.1. In vitro Antileishmanial Activity test

Leishmania infantum promastigotes cultures were prepared in RPMI-1640 medium enriched with fetal bovine serum (FBS) and containing penicillin and streptomycin antibiotics (100.000 units and 10 mg/mL, respectively). The stock solutions of compounds **I** and **II** dissolved by DMSO/H₂O (10%) were prepared as 40 mg/mL by adding RPMI-1640 medium containing 10% FBS but not antibiotics. Antileishmanial activity test was carried out by repeating the procedures of our previous study [19]. The dilution concentrations of the compounds were from 20 mg/mL to 39 $\mu\text{g/mL}$. Amphotericin B was used as standard drug. The results were evaluated at the end of the 1st, 2nd and 3rd day.

4. Results and discussion

4.1. Geometry Optimizations

Both compound **I** and **II** have 3 thiophene, 2 triazole and 1 morpholine rings. In compound **I**, methyl groups bonded to two thiophene rings, whereas nitro groups are bonded instead of methyl groups in the compound **II**. The structures of these molecules were optimized by using DFT with 6-311G(d,p) basis set. Optimized structures are shown in Fig. 1 and their structural parameters are given in Table 1. According to obtained data, the lengths of single bonded C-O and C-N bonds in morpholine rings of compound **I** and **II** vary between 1.415 - 1.434 Å and between 1.456 - 1.464 Å respectively. On the other hand, the bond

lengths in literature are 1.410(4)/1.418(3), 1.415(3)/1.420(3) and 1.416(3)/1.419(3) Å for C–O bonds and 1.457(3)/1.459(3), 1.462(2)/1.467(2) and 1.459(2)/1.473(2) Å for C–N bonds [20–22]. The N–C–C–O torsion angles in the morpholine ring vary between -38.63 and -44.72° . These angles vary between $-40.0(2)$ and $60.0(2)^\circ$ in literature [20–23]. The double bonded C=N bond lengths in triazole rings of both structures are 1.298 and 1.297 Å and the lengths of single bonded N–N in the same rings are 1.379/1.381 and 1.367 Å. If compared, similar structures having triazole rings in literature have C=N and N–N bond lengths 1.288(3)/1.304(2) and 1.371(3)/1.381(2) Å [24,25] respectively. The single bonded S–C bond lengths in thiophene rings, to which methyl and nitro groups are bonded vary between 1.719 and 1.757 Å, whereas these vary between 1.7214(19) and 1.743(2) Å in literature [20–22]. The bond lengths of both structures are compatible to bond lengths of the single crystal structures in literature, that have morpholine, triazole and thiophene rings. The compatibility with the values in literature proves the conformability of the selected method and the correctness of the selected basis set for the optimization of both structures. Determination of spectral parameters in the correct way is the usual result of the appropriate selections.

Fig. 1. The molecular structures of 1,2,4-triazole derivatives with morpholine (**a**; compound **I** and **b**; compound **II**) obtained by DFT/B3LYP/6-311G(d,p) method.

Table 1. Some optimized parameters obtained DFT methods with 6-311G(d,p) basis sets.

Compound I		Compound II	
Bond Lengths (Å)		Bond Lengths (Å)	
C39–O49	1.434	C37–O47	1.433
C41–N48	1.464	C24–N25	1.366
C26–N27	1.368	C24–S27	1.667
C26–S29	1.669	C6–O13	1.215
N30–C59	1.289	N7–C8	1.287
C59–C60	1.450	C69–S4	1.729
C8–O15	1.217	N60–O61	1.227
N9–C10	1.291	N60–O62	1.224
C21–S19	1.734	N66–O67	1.228
S62–C64	1.719	N66–O68	1.225

S62–C60	1.757	S56–C54	1.745
C4–S5	1.754	S56–C63	1.725
S5–C1	1.720	S4–C3	1.743
Bond Angles (°)		Bond Angles (°)	
C39–O49–C40	113.5	C37–O47–C38	113.2
N48–C37–N27	113.6	N46–C35–N25	113.2
N27–C26–S29	128.4	N25–C24–S27	128.9
N28–N30–C59	120.5	N26–N28–C53	118.2
C24–C34–N12	114.6	C22–C32–N10	112.9
N14–N9–C10	117.9	N12–N7–C8	119.0
N9–C10–C4	121.2	N7–C8–C3	118.6
		O61–N60–O62	125.7
		O67–N66–O68	125.6
Torsion Angles (°)		Torsion Angles (°)	
N48–C37–N27–C26	-84.7	N46–C35–N25–C24	-83.7
N28–N30–C59–C60	-7.9	N26–N28–C53–C54	-10.2
N25–C24–C34–N12	81.8	N23–C22–C32–N10	85.5
N14–N9–C10–C4	179.4	N12–N7–C8–C3	-179.4

4.2. Vibrational spectra and NMR spectra

Harmonic vibrational frequencies (scaled with 0.9619 [26]), GIAO ^1H and ^{13}C chemical shift values (regarding TMS) of compounds **I** and **II** by employing DFT/B3LYP/6-311G(d,p) method and experimental results obtained from literature [12] are presented in Tables 2 and 3, respectively. Calculated vibrations and chemical shift values are generally consistent with experimental data. FT-IR theoretical data given in Table 2 were obtained in gas phase, but experimental spectrum was recorded in solid phase, so deviations from experimental data observed in C=N band for compound **I** and, NO₂, C=N and C-O-C bands for compound **II** can be explained by intermolecular dipole-dipole interactions at solid phase. In addition, Table 3 shows that the theoretical NMR data are agreement with experimental ones.

Table 2. The experimental and calculated vibrational frequencies (cm^{-1}).

COMPOUND I			COMPOUND II	
Assignments	Experimental*	Calculated	Experimental*	Calculated
ν_{as} (NO ₂)		-----	1591	1537
ν_{s} (NO ₂)		----	1443	1309
ν (C=O)	1701	1717	1706	1724
ν (C=N)	1675, 1596	1588-1555	1651, 1591	1593-1557
ν_{s} (C–O–C)	1114	1095	1263	1095
ν (C–N)	1070	1034	1044	1033

ν , Stretching; s, symmetric; as, asymmetric.

*Referred [12]

Table 3. ^1H and ^{13}C isotropic chemical shifts (with respect to TMS, all values in ppm) for compounds **I** and **II** in DMSO- d_6 .

COMPOUND I			COMPOUND II		
Atom	Experimental*	Calculated	Atom	Experimental*	Calculated
C1	131.72	140.02	C1	133.26	134.97
C2	131.05	136.43	C2	126.01	127.42
C3	145.04	146.90	C3	143.05	156.70
C4	137.11	139.37	C6	149.56	153.00
C8	149.92	153.56	C8	147.25	145.81

C10	148.21	149.46	C9	146.33	151.33
C11	145.39	150.87	C14	25.85	27.69
C16	25.89	27.54	C15	136.79	146.79
C17	137.11	147.83	C16	127.39	129.32
C18	125.89	128.57	C18	127.39	130.56
C20	127.15	130.30	C19	130.67	132.48
C21	127.26	132.46	C22	145.02	150.20
C24	146.71	151.82	C24	162.74	168.12
C26	163.57	169.40	C32	40.54	38.85
C34	40.61	38.30	C35	66.67	73.77
C37	69.23	73.06	C36	50.64	46.07
C38	50.70	45.93	C37	65.36	70.29
C39	66.52	70.55	C38	65.36	60.63
C40	66.52	60.86	C39	50.64	53.16
C41	50.70	53.22	C53	160.10	160.46
C55	14.29	19.56	C54	150.05	152.27
C59	159.30	159.64	C55	134.72	134.26
C60	143.96	142.76	C57	134.72	134.42
C61	131.67	148.92	C63	153.05	168.03
C63	132.94	135.86	C69	153.05	165.96
C64	132.94	143.67	H5	6.92-7.93,m,7H	8.08
C67	14.29	16.61	H20	6.92-7.93,m,7H	7.05
H6	7.72,s,2H	7.75	H21	6.92-7.93,m,7H	7.34
H7	6.89-7.00,m,4H	7.22	H29	4.25,s,2H	3.56
H22	6.89-7.00,m,4H	7.10	H30	4.25,s,2H	4.11
H23	6.89-7.00,m,4H	7.40	H31	9.88,s,2H	9.94
H31	4.16,s,2H	3.81	H33	4.92-5.27,m,4H	5.25
H32	4.16,s,2H	4.21	H34	4.92-5.27,m,4H	4.89
H33	9.84,s,1H	10.26	H40	2.71,s,4H	3.46
H35	5.04-5.12,m,4H	5.23	H41	3.54,s,4H	3.83
H36	5.04-5.12,m,4H	5.06	H42	3.54,s,4H	3.54
H42	2.67,s,4H	3.49	H43	2.71,s,4H	2.76
H43	3.54,s,4H	3.83	H44	4.92-5.27,m,4H	5.13
H44	3.54,s,4H	3.52	H45	4.92-5.27,m,4H	4.32
H45	2.67,s,4H	2.78	H48	3.54,s,4H	4.02
H46	5.04-5.12,m,4H	5.30	H49	2.71,s,4H	2.92
H47	5.04-5.12,m,4H	4.23	H50	3.54,s,4H	3.84
H50	3.54,s,4H	3.99	H51	2.71,s,4H	3.44
H51	2.67,s,4H	3.01	H52	6.92-7.93,m,7H	6.88
H52	3.54,s,4H	3.91	H58	6.92-7.93,m,7H	7.94
H53	2.67,s,4H	3.53	H59	9.88,s,2H	8.69
H54	7.32,s,1H	6.99	H64	6.92-7.93,m,7H	7.04
H56	2.30,s, 6H	2.56	H65	6.92-7.93,m,7H	7.78
H57	2.30,s, 6H	2.67			
H58	2.30,s, 6H	2.47			
H65	6.89-7.00,m,4H	7.02			
H66	7.72,s,2H	7.91			
H68	2.30,s, 6H	1.91			
H69	2.30,s, 6H	2.64			
H70	2.30,s, 6H	1.01			
H71	9.72,s,1H	8.59			

*Referred [12]

4.3. The results of Antileishmanial Activity in vitro

In antileishmanial activity study, compounds **I** and **II** were tested against to the axenic standard MON-183 *Leishmania infantum* promastigotes. In tests, It was evaluated that changing the color from blue to pink in well was proliferation of parasites, while the unchanged color wasn't proliferation. Obtained results can be seen in Fig. 2. According to these results, the antileishmanial activity of compound **II** (MIC = 312 $\mu\text{g} / \text{ml}$) is significantly higher than that of compound **I** (MIC = 5000 $\mu\text{g} / \text{ml}$). As considering molecular structures of compounds, the reason can be attributed to the presence of the nitro groups in compound **II**. In our previous study, the MIC value for the 4-amino-1,2,4-triazole derivative containing the NH_2 group was determined as 625 $\mu\text{g}/\text{ml}$ [19]. As taken together with this study, it may be advisable to consider 1,2,4-triazole derivatives containing nitro and amino groups in antiparasitic drugs development studies. The MIC value of amphotericin B used as standard drug is <39 $\mu\text{g} / \text{ml}$. The antiparasitic effect of compound **II** is promising.

Fig. 2. The in vitro antileishmanial activity of compounds **I** and **II** against to the standard *Leishmania infantum* promastigotes (N.C.:negative control, P.C.:positive control).

5. Conclusions

Theoretical study for published compounds **I** and **II** were performed by the density functional theory (DFT) method with 6-311G(d,p) basis set. Structural parameters and spectroscopic parameters (for FT-IR and NMR) are determined. The theoretical spectral data are consistent with the experimental ones. For this reason, it can be said that the optimized parameters correctly reflect the structures of compounds **I** and **II**. The antileishmanial activities of compounds **I** and **II** were investigated by using microdilution broth method with Alamar blue dye, against to the axenic standard MON-183 *Leishmania infantum* promastigotes. The results indicate that as probably due to the presence of nitro group, the antileishmanial activity of compound **II** (MIC = 312 $\mu\text{g}/\text{mL}$) is significantly high according to that of compound **I** (MIC = 5000 $\mu\text{g}/\text{mL}$) and, as comparing with the minimal inhibitor concentration (MIC) value of Amphotericin B (MIC = <39 $\mu\text{g}/\text{ml}$), it can be stated that compound **II** has considerable antiparasitic activity.

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