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Synthesis, experimental, theoretical characterization and biological activities of 4-ethyl-5-(2-hydroxyphenyl)-2*H*-1,2,4-triazole-3(4H)-thione

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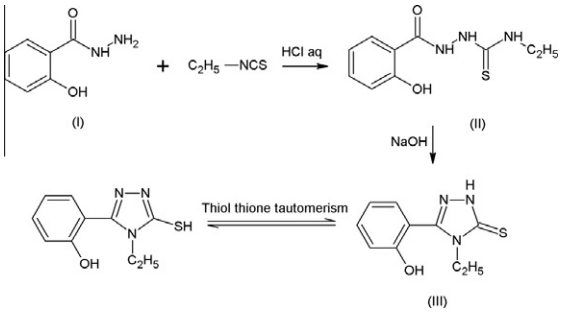
HIGHLIGHTS

- 4-Ethyl-5-(2-hydroxyphenyl)-2*H*-1,2,4-triazole-3(4H)-thione was prepared.
- Synthesis compound was confirmed by IR, NMR and X-ray single-crystal diffraction.
- Experimental parameters of title compound were compared with calculated parameters.
- The title compound has been tested in vitro for biological effects.

GRAPHICAL ABSTRACT



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ABSTRACT

This work presents the characterization of 4-ethyl-5-(2-hydroxyphenyl)-2*H*-1,2,4-triazole-3(4H)-thione (**III**) by quantum chemical calculations and spectral techniques. The molecular geometry, vibrational frequencies and gauge including atomic orbital (GIAO) ^{1}H and ^{13}C NMR chemical shift values of **III** in the ground state have been calculated using the density functional method (B3LYP) with the 6-31G(d) basis set. The calculated results show that the optimized geometry can well reproduce the crystal structure, and the theoretical vibrational frequencies and chemical shift values show good agreement with experimental values. To determine conformational flexibility, the molecular energy profile of the title compound was obtained by DFT calculations with respect to the selected torsion angle, which was varied from −180° to +180° in steps of 10°. The energetic behavior of **III** in solvent media was examined using the B3LYP method with the 6-31G(d) basis set by applying the Onsager and the polarizable continuum model (PCM). The predicted nonlinear optical properties of **III** are greater than ones of urea. In addition, DFT calculations of molecular electrostatic potentials and frontier molecular orbitals of **III** were carried out at the B3LYP/6-31G(d) level of theory. The title compound was screened for antibacterial, antifungal and antioxidant activities.

Introduction

1,2,4-triazole rings are typically planar 6π-electron aromatic systems, featuring an extensive chemistry [1,2]. 1,2,4-triazole and its derivatives represent one of the most biologically active classes of compounds, possessing a wide spectrum of activities, including anti-inflammatory [3,4], antiviral [5], analgesic [6], antimicrobial [7], anticonvulsant [8], anticancer [9], antioxidant [10], antitumoral [11] and antidepressant activity [12], the last usually being explored by the forced-swim test [13,14]. Furthermore, some of the complexes containing 1,2,4-triazole ligands have rather peculiar structures and specific magnetic properties [15–18].

4,5-Substituted products containing 1,2,4-triazole ring in their structure seem to be suitable candidates for further chemical modifications and might be of interest as pharmacologically active

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compounds and useful ligands in coordination chemistry [19]. Derivatives of 4-amino-5-substituted 1,2,4-triazole were synthesized by intramolecular cyclization of 1,4-disubstituted thiosemicarbazides [20]. In addition there are some studies on electronic structures and thiol-thione tautomeric equilibrium of heterocyclic thione derivatives [21–25]. Several heterocycles containing a thiadiazole or triazole moiety have been reported; [26–30] however, the synthesis of heterocyclic systems containing a phenol-substituted triazole ring has rarely been reported [31–33].

Density functional theory (DFT) has been one of the widely used theories in theoretical modeling during recent years. By means of the development of better exchange–correlation functionals, it has become possible to calculate many molecular properties which have accuracies that can be comparable to traditionally correlated *ab initio* methods, all these could be done with more favorable computational costs [34]. It has been figured out during the literature survey that in reproducing the experimental values in geometry, dipole moment, vibrational frequency, etc. DFT has a precise accuracy [35–39].

The aim of this study is to investigate the energetic and structural properties of the 1,2,4-triazole compound, 4-ethyl-5-(2-hydroxyphenyl)-2*H*-1,2,4-triazole-3(4H)-thione (Fig. 1), using density functional theory calculations. In this study, the optimized geometry, vibrational spectra and assignments, statistical energetic parameters, conformational analysis and nonlinear optical properties of **III** have been studied. These calculations are valuable for providing insight into molecular properties of 1,2,4-triazole compounds. Besides the characterization of the title compound, the biological activities of the **III**, such as antibacterial, antifungal and antioxidant activities, were investigated.

Experimental

Synthesis

For the synthesis of 1(2-hydroxybenzoyl)-4-ethyl thiosemicarbazide (**II**), a mixture of **I** (0.01 mol) and ethyl isothiocyanate (0.01 mol) in absolute ethanol was refluxed for 8 h. The solid material obtained on cooling was filtered, washed with diethyl ether, dried and crystallized from ethanol–dioxane (yield 65%, m.p. 489–491 K). IR (υ, cm^{-1}): 3495, 3317 (N—H, OH), 1668 (C=O), 1262 (C=S). For the synthesis of **III**, a stirred mixture of **II** (0.01 mol) and sodium hydroxide (40 mg, 0.01 mol, as a 2 *N* solution) was refluxed for 4 h. After cooling, the solution was acidified with HCl (37%) and the precipitate was filtered off. The precipitate was then crystallized from an ethanol–dioxane mixture (yield 85%, m.p. 527–531 K). IR (υ, cm^{-1}):3390, 3216(N—H, O—H), 1622 (C=N), 1535, 1260, 1050, 950 (N—C=S, amide **I**, **II**, **III** and **IV** bands); ^{1}H NMR (400 MHz, DMSO-d_6, 24C): δ 1.03 (t, J = 7.02, 3H, CH_3), 3.47 (q, J = 7.32, 2H, CH_2), 7.82–7.14 (m, 4H, Ar—H), 10.00 (s, 1H, OH), 13.80 (s, 1H, SH). Elemental analysis: C, 54.25; H, 5.05; N, 18.96.

Physical measurements

Melting points were determined on a Thomas Hoover melting point apparatus and uncorrected, but checked by differential scanning calorimeter (DSC). KBr pellets on a Perkin–Elmer Spectrum one FT-IR spectrophotometer was used in order to record FT-IR spectra of **III** in 4000–400 cm^{-1} region. Electronic spectral studies were conducted on a Shimadzu model UV-1700 spectrophotometer in the wavelength 1100–200 nm. The ^{1}H and ^{13}C spectra were taken on Bruker AC-400 NMR spectrometer operating at 400 MHz for ^{1}H—, 100 MHz for ^{13}C NMR. Elemental analyses were done on a LECO-CHNS-938. Compound was dissolved in DMSO-d_6 and chemical shifts were referenced to TMS (^{1}H and ^{13}C NMR). Starting chemicals were provided by Merck or Aldrich. The synthesis reaction of **III** is shown in Fig. 2.

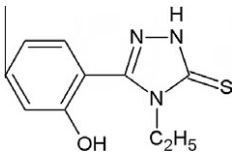

Fig. 1. Chemical diagram of 4-ethyl-5-(2-hydroxyphenyl)-2*H*-1,2,4-triazole-3(4H)-thione.

Antibacterial activity

The synthesized compound **III** was screened for their antibacterial activity against *Escherichia coli* (ATTC-25922), *Staphylococcus aureus* (ATTC-25923), *Pseudomonas aeruginosa* (ATCC-27853) and *Klebsiella pneumoniae* (recultured) bacterial strains by serial plate dilution method [40,41]. Serial dilutions of the drug in Muller–Hinton broth were taken in tubes and their pH was adjusted to 5.0 using phosphate buffer. Standardized suspension of the test bacterium was inoculated and incubated for 16–18 h at 37 °C. The minimum inhibitory concentration (MIC) was noted by observing the lowest concentration of the drug at which there was no visible growth. A number of antimicrobial disks are placed on the agar for the sole purpose of producing zones of inhibition in the bacterial lawn. Agar media were poured into each Petri dish. Excess of suspension was decanted and placing in incubator at 37 °C for 1 h dried the plates. Using an agar punch, wells were made on these seeded agar plates and minimum inhibitory concentrations of the test compounds in DMSO were added into each labeled well. A control was also prepared for the plates in the same way using DMSO as solvent. The Petri dishes were prepared in triplicate and maintained at 37 °C for 3–4 days. Antibacterial activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with Ciprofloxacin as standard [42,43]. Zone of inhibition was determined for title compound the results are summarized in Table 1.

Antifungal activity

Newly prepared compound was screened for their antifungal activity against *Aspergillus flavus* [NCIM No. 524], *Aspergillus fumigatus* [NCIM No. 902], *Penicillium marneffei* [recultured] and *Trichophyton mentagrophytes* [recultured] in DMSO by serial plate dilution method [44,45]. Agar media were prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting the pH to 5.7. Normal saline was used to make a suspension of spore of fungal strains for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Agar media of 20 mL were poured into each Petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37 °C for 1 h. Using an agar punch each labeled well were made on these seeded agar plates and MIC of the test compounds in DMSO were added into each labeled well. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dish were prepared in triplicate and maintained at 37 °C for 3–4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone. Activity of each compound was compared with Ciclopiroxolamine as standard. Zones of inhibition were determined for title compound the results are summarized in Table 2.

DPPH free radical scavenging activity

Free radical scavenging activity of the title compound was determined by measuring the change in the absorbance of