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Vibrational spectroscopic analysis, molecular dynamics simulations and molecular docking study of 5-Nitro-2-phenoxyethyl benzimidazole

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Abstract

FT-IR and FT-Raman spectra of 5-nitro-2-phenoxyethylbenzimidazole were recorded and analyzed theoretically and experimentally. The splitting of N-H stretching mode in the IR spectrum with a red shift from the calculated value indicates the weakening of the NH bond. The theoretical calculations give the phenyl ring breathing modes at 999 cm⁻¹ for mono substituted benzene ring and at 1040 cm⁻¹ for tri-substituted benzene ring. The theoretical NMR chemical shifts are in agreement with the experimental chemical shifts. The most reactive sites for electrophilic and nucleophilic attack are predicted from the MEP analysis. HOMO of π nature is delocalized over the entire molecule whereas the LUMO is located over the complete molecule except mono-substituted phenyl ring and oxygen atom. Reactive sites of the title molecule have been located with the help of ALIE surfaces and Fukui functions. In order to determine locations prone to autoxidation and locations interesting for starting of

degradation, bond dissociation energies have been calculated for all single acyclic bonds. For the determination of atoms with pronounced interactions with water we have calculated radial distribution functions obtained after molecular dynamics simulations. The calculated first hyperpolarizability of the title compound is 58.03 times that of standard nonlinear optical material urea. The substrate binding site interactions of the title compound with Topo II enzyme is reported by using molecular docking study. Biological activity studies show that the title compound can be leaded for developing new anticancer agents.

Keywords: DFT; Benzimidazole; ALIE; RDF; BDE; Docking;

1. Introduction

Benzimidazole and its derivatives possess several pharmacological effects and biological activities and exhibit antifungal [1, 2], antimicrobial [3-5], antiviral [6], anticancer [7, 8], anti-parasitic [9], and anti-inflammatory [10] activities. A number of derivatives of benzimidazole show pharmaceutical activity [11, 12], have extensive uses in agriculture fields [13], synthetic precursor in new drug discovery [6, 14-19]. Mary et al. reported the vibrational spectroscopic investigations of certain benzimidazole derivatives [20, 21].

Since the activity of topoisomerases is essential for several cellular processes such as replication, transcription, and chromosome condensation, investigation of the inhibitory activities of eukaryotic topoisomerases is widely used in anticancer drug development. Topo II is the target for some of the most active anticancer drugs such as etoposide, teniposide and doxorubicin used in the treatment of human malignancies [22]. In 2004, we investigated the inhibitory activity of the title compound on eukaryotic DNA Topo II in cell free systems [23]. According to this study, 5-nitro-2-phenoxybenzimidazole showed Topo II inhibitory activities comparable to etoposide. In this study, it was compared the substrate binding site interactions of the title compound with Topo II enzyme by using molecular docking study.

Additionally, the title compound is investigated for their human DNA topoisomerase I and II α enzyme inhibition using relaxation assay and anti-proliferative activity against HeLa (human cervical cancer) and L929 (mouse fibroblast) cell lines using sulforhodamine B (SRB) assay in order to develop new anticancer compound.

Different chemical compounds used in pharmaceutical and agricultural fields are slowly entering and polluting the environment, especially endangering water resources. So far these types of compounds have been detected in all types of water, while conventional methods for their removal are not efficient and there is a constant need for development of efficient and economical methods for water purification [24-26]. Decomposition of

mentioned chemicals under natural conditions is very slow and one of the promising approaches for the improvement of degradation is employment of advanced oxidation processes [27].

In order to develop degradation techniques targeted towards special organic pollutants, it is necessary to understand reactive properties of molecules of interest. Detailed investigation and proposition of degradation pathway of molecules is expensive and tedious process. However, for these purposes principles of molecular modelling are particularly useful because they offer relatively inexpensive assessment of oxidative and hydrolysis properties, which are the fundamental mechanisms of degradation. Namely, determination of intra-molecular bonds prone to breaking with the lowest amounts of energy and locations prone to autoxidation can be performed by calculation of bond dissociation energies (BDE). On the other side, information about which atoms have the most pronounced interactions with water molecules can be obtained by calculation of radial distribution functions (RDF).

2. Experimental details

The FT-IR spectrum (Fig. S1-supporting information) was recorded using KBr pellets on a DR/Jasco FT-IR 6300 spectrometer. The FT-Raman spectrum (Fig. S2-supporting information) was obtained on a Bruker RFS 100/s, Germany. For excitation of the spectrum the emission of Nd:YAG laser was used, excitation wavelength 1064 nm, maximal power 150 mW, measurement on solid sample. The spectral resolution after apodization was 2 cm⁻¹. NMR spectra (¹H and ¹³C) for the compound were recorded on a 400MHz NMR Spectrometer (Bruker advance, Reinstetten, Germany) using deuteriated DMSO-d₆. The chemical shift values (ppm) and coupling constants (J) are given in δ and Hz respectively.

2.1 Synthesis of 5-Nitro-2-phenoxymethylbenzimidazole

A mixture of phenoxyacetic acid (10 mmol) and 4-nitro-2-phenyldiamine (10 mmol) was boiled under reflux with stirring for 2 h in 15 ml 6N HCl. At the end of the reaction period, the mixture was neutralized with excess of NaHCO₃. The collected precipitate washed with water, dried in vacuum, purified by flash chromatography, eluting with CHCl₃ and recrystallized [28, 29]. Mp: 190-191°C (Scheme 1).

Since the activity of topoisomerases is essential for several cellular processes such as replication, transcription, and chromosome condensation, investigation of the inhibitory activities of eukaryotic topoisomerases is widely used in anticancer drug development. Topo II is the target for some of the most active anticancer drugs such as etoposide, teniposide, and doxorubicin used in the treatment of human malignancies [22]. In 2004, we investigated the

inhibitory activity of title compound on eukaryotic DNA Topo II in cell free systems [23]. According to this study, 5-nitro-2-phenoxybenzimidazole showed Topo II inhibitory activities comparable to etoposide. In this study, it was compared the substrate binding site interactions of title compound with Topo II enzyme by using molecular docking study. It was found that there are four hydrogen bonds with GLY 478, GLY504 and DC8 base. Moreover, there are Van der Waals interactions between ARG 503, GLN 778 and DA12. It can be concluded, that 5-nitro-2-phenoxybenzimidazole can be lead compound for developing new anticancer drug.

2.2 Biological Assay

Materials

Human DNA topoisomerase I (hTopoI), reagents for electrophoresis, camptothecin, etoposide and the other chemicals were obtained from Sigma (Munich, Germany). Recombinant human DNA topoisomerase I α (hTopoII) was purchased from Amersham Pharmacia Biotech (Freiburg, Germany). Supercoiled pBR322 plasmid DNA and 6X loading buffer were purchased from Fermentas (Prizma Lab, Ankara, Turkey). DMSO (molecular biology grade) was obtained from Merck KgaA (Darmstadt, Germany). All the other chemicals were of analytical grade. All compounds were dissolved in DMSO and freshly prepared just before the experiments. DMSO compatibility tests were carried out for all experiments and 0.5% DMSO was used for relaxation and SRB assays.

Methods

Topo I Relaxation assay

Relaxation activity of DNA topoisomerase I was measured through the conversion of supercoiled pBR322 plasmid DNA to its relaxed form as described by Stewart and Champoux [30] with some modifications. Briefly, relaxation of 0.2 μ g of plasmid DNA by hTopo I (1U) in 10 μ l of reaction mixture (10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM PMSF, 1 mM β -mercaptoethanol) was obtained in the presence and absence of various concentrations (100, 200, 400 and 800 μ M) of the title compound. The enzyme and the title compound were preincubated for 5 min at 37°C in a reaction buffer. Reactions were started by addition of DNA. We used control wells which contained only plasmid, plasmid with the compound of interest without enzyme and plasmid with topoisomerase. After 30 min at 37°C, the reactions were terminated by 3 μ l stop solution which contained 1 μ l 1% SDS and 2 μ l of 6X loading buffer (0.09% bromophenol blue, 0.09% xylene cyanol FF, 60% glycerol and 60 mM EDTA). All samples were separated on 1% agarose gel electrophoresis with TEA buffer

(40 mM Tris, 20 mM glacial acetic acid, and 1 mM EDTA (pH 8.0)) at 45 V for 3 h at room temperature. At the end of the electrophoresis, gels were stained with ethidium bromide (1 µg/ml) and photographed under UV light. Band distributions were analyzed by a GDS 8000 Complete Gel Documentation and Analysis System (Gel Works 1D Intermediate, version 2.5; Ultra Violet Products). Camptothecin was used as the reference drug.

Topo II Relaxation Assay

Relaxation activity of human DNA topoisomerase I α was determined by measuring the conversion of supercoiled pBR322 plasmid DNA to its relaxed form [31, 32]. The reaction mixture contained 10 mM TrisHCl (pH 7.9), 50 mM NaCl, 50 mM KCl, 5 mM MgCl₂, 0.1 mM EDTA, 15 mL/mL bovine serum albumin (BSA), 1 mM ATP, 0.3 mM pBR322 plasmid DNA, 1 U of enzyme and different concentrations (100, 200 and 400 µM) of the title compound in a total volume 10 µl. The mixture was incubated for 1 h 37°C. After incubation period, 3 mL of loading buffer in electrophoresis buffer TAE (60 mM Tris, 30 mM acetic acid and 1.5 mM EDTA, pH 8.0) was added and mixture was subjected to electrophoresis on 1% agarose at 45 V for 3 h. After the electrophoresis gels were stained with ethidium bromide (1 mg/mL), photographed under light and band distribution was analyzed with a gel analysis system. The rate of formation of the newly formed bands was used as a measure of enzyme activity. Inhibitory activities were expressed as the micromolar concentration of test compound that caused 50% inhibition per unit of enzyme, under the assay conditions. Etoposide was used as the reference drug.

Analysis of data for relaxation assay

hTopo I and hTopoII enzyme activities were measured by the rate of different forms of DNA bands on agarose gel electrophoresis. Inhibition percentage were calculated by bands of intensity difference between control and compound applied wells. We analyzed supercoiled-relaxed DNA bands for catalytic inhibition. We assumed supercoiled DNA without enzyme well as a one hundred percent of enzyme was inhibited. We used direct proportion to calculate inhibition percentages of the remaining supercoiled DNA intensity that belonged to tested compounds in each well [33]. If inhibition was not obtained at any concentration of a tested compound it was assumed to have no inhibitory activity (NE) on human DNA topoisomerase I and I α .

Cell Cultures, Conditions, and Treatment

HeLa (human cervical cancer) and L929 (mouse fibroblast) cell lines were used in this study. The cell lines were kindly provided by Dr. Aylin Gurpinar (Department of Biology,

Faculty of Science, Hacettepe University, Turkey). Cells were routinely cultured in DMEM medium supplemented with 10% (v/v) fetal bovine serum (FBS), 2 mM L-glutamine, 50 U/ml penicillin, and 0.1 mg/ml streptomycin. The cells were grown in 25-cm² flasks at 37°C in a humidified atmosphere containing 5% CO₂.

The cell lines were stored in liquid nitrogen, and the 5th to 8th passages from the stock cultures were used for the experiments after recultivation. The media were changed every 2-3 days; when the cultures reached confluency, the cells were washed with Dulbecco's Phosphate Buffered Saline, detached with Trypsin/EDTA, centrifuged, and subcultured.

Sulforhodamine B (SRB) Cytotoxicity Assay

The antiproliferative SRB assay was performed to assess growth inhibition by a colorimetric assay which estimates cell number indirectly by staining total cellular protein with the dye SRB. The assay was carried out according to the method described by Vichai and Kirtikara [34]. Their method was optimized for the cytotoxic screening of the compounds to adherent cells in a 96-well format. Briefly, L929 and HeLa cells were seeded in 96-well microplates with 1×10^4 cells in 0.1 ml of DMEM medium supplemented with 10% FBS and routinely cultured in a humidified incubator (at 37°C in 5% CO₂) for 24 hours. After the compounds were added in serial concentrations (12.5, 25, 50, 100, 200 and 400 µM), the cells were incubated further for 48 hours. After the incubation, the cells were fixed by layering 100 µl of ice-cold 10% trichloroacetic acid on top of the growth medium. The cells were incubated at 4°C for 1 h. Afterwards, the plates were washed five times with cold water, the excess water was drained off, and the plates were left to dry in air. SRB stain (100 µl; 0.4% in 1% acetic acid) was added to each well and left in contact with the cells for 30 min. Later, they were washed with 1% acetic acid, rinsed four times until only dye adhering to the cells was left. The plates were dried, and 200 µl of 10 mM Tris base (pH 10.5) was added to each well to solubilise the dye. The plates were shaken gently for 20 min on a gyratory shaker, and the absorbance (OD) of each well was read on a plate reader at 510 nm. Cell survival was measured as the absorbance percentage compared with the control (non-treated cells). The IC₅₀ (50% inhibition concentrations) values were calculated with S-probit program and obtained by plotting the percentage of survival versus the concentrations.

3. Computational details

Using Gaussian09 software [35] and B3LYP/6-311++G(6D, 7F) basis set, the vibrational wavenumbers of the title compound are predicted and the theoretical wavenumbers are scaled by a scaling factor of 0.9613 [36]. At the optimized structure (Fig.1) of the title compound, no imaginary wavenumber modes were obtained. The vibrational assignments are done with the help of potential energy distribution analysis [37] and GaussView software [38]. In this work for molecular modelling of title molecule we have also used Schrödinger Materials Science Suite 2015-4 [39]. Beside other powerful tools, this modelling suite has Jaguar 9.0 program [40] for DFT calculations and Desmond program [41-44] for molecular dynamics simulations. Using Jaguar with B3LYP [45] exchange-correlation functional ALIE surfaces, Fukui functions and BDE have been calculated with 6-311++G(d,p), 6-31+G(d) and 6-311G(d,p) basis sets, respectively. BDE have been calculated for all single acyclic bonds, while intramolecular noncovalent interactions have been investigated using the method of Johnson [46, 47]. MD simulations have been conducted with OPLS 2005 force field [48] and within NPT ensemble. Pressure and temperature were set to 1.0325 bar and 300 K, respectively. Simulation time was 5 ns with cut off radius set to 12 Å. MD investigated system consisted of one title molecule placed in cubic box with ~3000 water molecules. Description of solvent was done by simple point charge (SPC) model [49].

4. Results and discussion

4.1 Geometrical parameters

The calculated geometrical parameters are given in Table S1 (supporting information). For the title compound the N-O bond lengths are 1.2698 and 1.2709 Å which are in agreement with reported values [50, 51]. The C-O bond lengths of the title compound are 1.4546 and 1.4039 Å which are in agreement with the reported values 1.4569 and 1.3956 Å [52]. For the title compound, the bond length, $C_{12}=N_{10} = 1.3211$ Å shows double bond character while, the bond lengths, $C_{12}-N_{11} = 1.3855$ Å, $C_6-N_{11} = 1.3853$ Å, $C_3-N_{29} = 1.4596$ Å, $C_5-N_{10} = 1.4071$ Å are shorter than the normal single C-N bond length of about 1.48 Å [52].

In the present case, the CNO bond angles are 118.5° while the reported values are 117.7, 117.5° [51] and 117.3 and 117.7° [50]. The C-C bond lengths in the phenyl ring lie in the range 1.3889-1.4229 Å for mono substituted phenyl ring and 1.3932-1.4013 Å for tri-substituted phenyl ring and for benzene the C-C bond length is 1.3993 Å [53].

4.2 IR and Raman spectra

The observed IR, Raman bands, calculated (scaled) wavenumbers and assignments are given in Table 1. In the present discussion, the mono-substituted phenyl, tri-substituted phenyl and imidazole rings are designated as PhI, PhII and PhIII, respectively.

The NO₂ stretching modes are in the region 1550-1300 cm⁻¹ region [54, 55]. The NO₂ stretching modes of the title compound are assigned at 1562 and 1369 cm⁻¹ theoretically while bands are observed at 1365 cm⁻¹ in the IR spectrum, 1369 cm⁻¹ in the Raman spectrum experimentally. The NO₂ deformations modes are expected in the following regions: scissoring modes 850 ± 60 cm⁻¹, wagging modes 740 ± 50 cm⁻¹, rocking modes 540 ± 70 cm⁻¹ [54] and in the present case, the NO₂ deformation modes are assigned at 776, 551 cm⁻¹ in the IR spectrum and at 772, 671, 548 cm⁻¹ theoretically. The reported values of NO₂ stretching modes are 1415, 1391, 1374, 1352 cm⁻¹ (DFT), 1398, 1347 cm⁻¹ (IR), 1387 cm⁻¹ (Raman) and deformation modes at 761, 668, 524, 458 cm⁻¹ (IR), 767, 683 cm⁻¹ (Raman), 772, 759, 699, 663, 522, 441 cm⁻¹ (DFT) [56].

Normally, the N-H modes are expected in the regions, 3400 ± 40 cm⁻¹ (stretching mode), 1510-1500, 1400-1300, 740-730 cm⁻¹ (deformation modes) [54, 57]. For the title compound, the PED analysis gives the NH modes at 3522, 1422, 663 cm⁻¹ theoretically. Experimentally bands are observed at 3568, 3302 cm⁻¹ in the IR spectrum and in the present case the stretching mode is split into a doublet in the IR spectrum with a difference of 266 cm⁻¹ and is red shifted by 220 cm⁻¹ from the calculated value, which indicates the weakening of the NH bond [50]. Mary et al. reported the NH modes at 3544, 1409, 669 cm⁻¹ (DFT), 3548 cm⁻¹ (IR) and at 660 cm⁻¹ (Raman) for a benzimidazole derivative [20].

According to literature, the C=N stretching modes [55, 58] are expected in the region 1650-1500 cm⁻¹. In the present case, these modes are observed at 1512 cm⁻¹ (IR), 1514 cm⁻¹ (Raman) and 1516 cm⁻¹ (DFT) and similar values are reported at 1520 cm⁻¹ with a band at 1518 cm⁻¹ in the IR spectrum [20] and at 1536 cm⁻¹ for 5-nitro-2-(4-nitrobenzyl)benzoxazole [56]. The C-N stretching modes of the title compound are assigned at 1238, 1046 cm⁻¹ in the IR spectrum, 1231, 1038 cm⁻¹ in the Raman spectrum and at 1230, 1176, 1040 cm⁻¹ theoretically as expected [55, 59]. For the title compound the C-O-C stretching modes are assigned at 1194 and 965 cm⁻¹ theoretically. Mary et al. Reported the C-O-C stretching modes at 1191, 1187, 988 and 993 cm⁻¹ theoretically [20, 21].

The CH₂ vibrations appear in the ranges, 2950-2850 cm⁻¹ (stretching), 1470-900 cm⁻¹ (deformations modes: scissoring, wagging, twisting and rocking) [54, 55, 59]. The bands at 2948, 1466, 1342 cm⁻¹ in the IR spectrum, 2945, 2865, 1466, 1346, 1012 cm⁻¹ in the Raman

spectrum and 2940, 2897, 1469, 1344, 1189, 1009 cm^{-1} theoretically are assigned as the stretching and deformation modes of the CH_2 group of the title compound.

For the title compound, the phenyl CH stretching modes are assigned at 3065, 3042 cm^{-1} in the IR spectrum, 3077 cm^{-1} in the Raman spectrum, 3081, 3075, 3066, 3051, 3042 cm^{-1} theoretically for the mono substituted phenyl ring and at 3102, 3065 cm^{-1} in the IR spectrum, 3111, 3105, 3066 cm^{-1} theoretically for the tri-substituted phenyl ring [54]. The phenyl ring stretching modes of the title compound are assigned at 1576 cm^{-1} in the IR spectrum for PhI and at 1596, 1555 cm^{-1} in the IR spectrum, 1593, 1548, 1390 cm^{-1} in the Raman spectrum for PhII. These modes are theoretically assigned in the ranges 1574-1286 cm^{-1} for PhI and 1593-1276 cm^{-1} for PhII [54]. The in-plane and out-of-plane phenyl CH bending modes are expected above and below 1000 cm^{-1} [54]. The in-plane CH deformation modes are assigned at 1158, 1073 cm^{-1} (IR), 1332, 1158, 1069, 1012 cm^{-1} (Raman), 1327, 1171, 1160, 1070, 1009 cm^{-1} (DFT) for PhI and at 1238, 1126, 1046 cm^{-1} (IR), 1231, 1128, 1038 cm^{-1} (Raman), 1230, 1124, 1040 cm^{-1} (DFT) for PhII. The out-of-plane CH modes are assigned at 946, 875, 742 cm^{-1} (IR), 946, 740 cm^{-1} (Raman), 964, 948, 878, 818, 745 cm^{-1} (DFT) for PhI and at 908, 812, 742 cm^{-1} (IR), 963, 912, 814 cm^{-1} (DFT) for PhII. The phenyl ring breathing mode of asymmetric tri-substituted benzene is expected in the regions: 500-600 cm^{-1} for light substituent, 1005-1100 cm^{-1} for heavy substituent and 600-750 cm^{-1} for mixed substituent and for mono substituted phenyl ring the ring breathing is expected near 1000 cm^{-1} [54, 60]. For the title compound, the ring breathing modes are assigned at 999 cm^{-1} for mono substituted benzene ring and at 1040 cm^{-1} for tri-substituted benzene ring theoretically as expected. The ring breathing mode of tri-substituted phenyl ring is reported at 1098 cm^{-1} in the IR and 1104 cm^{-1} (DFT) by Ulahannan et al [61]. Most of the vibrational modes are not pure but contains significant contributions from other modes also.

4.3 ^1H -NMR and ^{13}C -NMR spectra

Using B3LYP/GIAO model, the absolute isotropic chemical shielding was calculated [62] and relative chemical shifts were then estimated by using the corresponding TMS shielding: $\sigma_{\text{calc}}(\text{TMS})$ calculated previously at same theoretical level as in this article. Numerical values of chemical shift $\delta_{\text{calc}} = \sigma_{\text{calc}}(\text{TMS}) - \sigma_{\text{calc}}$ together with calculated values of $\sigma_{\text{calc}}(\text{TMS})$, are given in Table S2 (supporting information). The experimental values are: ^1H NMR (DMSO-d_6 , 400 MHz): $\delta = 8.45$ (d, 1H, $J = 2\text{Hz}$, H9), $\delta = 8.09\text{-}8.13$ (dd, 1H, $J = 9.2$; $J = 2.4$, H8), $\delta = 7.73\text{-}7.70$ (d, 1H, $J = 8.8$, H7), $\delta = 7.40\text{-}6.90$ (m, 5H, phenyl protons), $\delta = 5.20$ (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6 , 400 MHz): $\delta = 63.491$ (C14), 114.699 (C20, C22, C24),

117.747 (C1), 121.374 (C2, C4), 127.574 (C18, C19), 142.873 (C5, C6), 155.200 (C3), 157.699 (C12, C17) (Ar-C).

The protons of the mono-substituted and tri-substituted phenyl rings resonate in the ranges, 7.1165-7.8549 and 7.683-9.1476 ppm theoretically. The chemical shifts of the hydrogen atoms of the CH₂ groups is 5.7392 ppm and for hydrogen atom associated with the NH group, the predicted shift is 9.0368 ppm. The predicted shifts of carbon atoms are 110.7651-149.9161 ppm for tri-substituted phenyl ring and 111.929-157.9795 for mono-substituted phenyl ring. For C₁₄ and C₁₂ atoms the shifts are 70.5248 and 154.7909 ppm respectively. All these predicted shifts of the protons and carbons are in agreement with the experimental values.

4.4 Molecular Electrostatic Potential

Using Molecular electrostatic potential study, the reactive properties of a wide variety of chemical systems in both electrophilic and nucleophilic reactions, biological recognition processes and hydrogen bonding interactions can be explained [63]. The different values of the electrostatic potential at the surface are represented by different colours and potential decreases in the order red > orange > yellow > green > blue. The negative regions, red, orange and yellow of the MEP (Fig.S3-supporting information) are related to electrophilic reactivity. The maximum negative region is localized over the nitro group, mono-substituted phenyl ring and the nitrogen atom in the C=N group oxygen and the maximum positive region is localized on NH group indicating a possible site for nucleophilic attack.

4.5 ALIE surfaces and Fukui functions

Average local ionization energy (ALIE) was introduced by Sjöberg et al. [64, 65] in order to determine molecular reactive sites. This quantity indicates the areas where electrons are least tightly bound and therefore most easily removed. It is defined as following:

$$I(r) = \sum_i \frac{\rho_i(\vec{r})|\epsilon_i|}{\rho(\vec{r})}, \quad (1)$$

where $\rho_i(\vec{r})$ represents the electronic density of the i -th molecular orbital at the point $\vec{r}$, ϵ_i represents the orbital energy and $\rho(\vec{r})$ is the total electronic density function. When mapped to the electron density surface this quantum molecular descriptor is valuable indicator of locations where molecule is prone to the electrophilic attacks. Representative ALIE surface of title molecule is presented in Fig.S4 (Supporting information).

Beside benzene rings the red colour is located in the near vicinity of nitrogen atom N10, designating this molecular location to be possibly prone to electrophilic attacks. In the case of this molecule the red colour corresponds to the energy value of almost 204kcal/mol, i.e. this is the energy needed for the removal of electron. Title molecule has no intra-molecular non-covalent interactions.

Fukui functions are also often used in order to predict possible reactive sites of investigated molecule. With the help of this quantum molecular descriptor one is able to determine locations where electron density increases/decreases with addition/removal of charge. In the finite difference approach Fukui functions are defined as follows:

$$f^+ = \frac{(\rho^{N+\delta}(r) - \rho^N(r))}{\delta}, \quad (2)$$

$$f^- = \frac{(\rho^{N-\delta}(r) - \rho^N(r))}{\delta}, \quad (3)$$

where N is number of electrons in the reference state of the molecule and δ represents the fraction of electron, which is set to be 0.01 [66]. Fukui functions can be visualized by mapping their values to electron density surface. In such case, for Fukui f^+ function, positive values of surface (purple colour) indicate molecule locations where electron density increases with the addition of charge. On the other side, negative values (red colour) of Fukui f^- functions indicate molecule locations where electron density decreases with the removal of charge. Representative Fukui function surfaces are presented in Fig.S5 (Supporting information). Fukui f^+ function indicate that location in the near vicinity of hydrogen atom H13 gains electron density after the charge addition. This position is also coloured with red in the case of Fukui f^- function, together with nitrogen atom N11, designating them as locations where electron density decrease when charge is removed. Therefore, imidazole ring has interesting reactive properties from the aspect of Fukui functions.

4.6 Nonlinear Optical Properties

Nonlinear optical properties deal with the interaction of applied electromagnetic fields in various materials to produce new electromagnetic fields, changed in wavenumber, phase, or other physical properties [67-69]. The calculated first hyperpolarizability of the title compound is 7.544×10^{-30} e.s.u which is 58.03 times that of standard NLO material urea (0.13×10^{-30} e.s.u) [70]. The reported values of the hyperpolarizability of benzimidazole derivatives are 2.7×10^{-30} [20] and 2.34×10^{-30} [21]. The second hyperpolarizability value can give information about the physical properties of materials to study the origin of third

harmonic signals generated in chemical and biological structures [71-73]. The average second hyperpolarizability has been calculated by using the following expression.

$$\gamma_{av} = 1/5[\gamma_{xxxx} + \gamma_{yyyy} + \gamma_{zzzz} + 2\gamma_{xxyy} + 2\gamma_{xxzz} + 2\gamma_{yyzz}]$$

The amount of charge transfer within the molecule depends on the nature of the end group of the molecule and π -conjugated chain length in organic molecules enhances the magnitude of hyperpolarizability. The calculated value of γ_{av} for the title compound is -22.551×10^{-37} esu. The present investigation provides a new route to design high performance NLO materials.

4.7 Frontier Molecular Orbital analysis

Highest occupied molecular orbital and lowest unoccupied molecular orbital and their properties such as energy are used to explain several types of reactions and for predicting the most reactive position in conjugated systems [74-76]. The HOMO and LUMO plots of the title compound are shown in Fig. S6 (Supporting information). In the title compound, the HOMO of π nature is delocalized over the entire molecule whereas the LUMO is located over the complete molecule except mono-substituted phenyl ring and O27 atom. Using HOMO and LUMO orbital energies, the ionization energy and electron affinity can be expressed as: $I = -E_{HOMO}$, $A = -E_{LUMO}$ [77]. The hardness η and chemical potential μ are given the following relations $\eta = (I-A)/2$ and $\mu = -(I+A)/2$, where I and A are the first ionization potential and electron affinity of the chemical species [77]. For the title compound, $E_{HOMO} = -6.96$ eV, $E_{LUMO} = -3.09$ eV, energy gap = HOMO-LUMO = 3.87 eV, Ionization potential $I = 6.96$ eV, Electron affinity $A = 3.09$ eV, global hardness $\eta = 1.935$ eV, chemical potential $\mu = -5.025$ eV, global electrophilicity $= \mu^2/2\eta = 6.525$ eV.

4.8 Natural bond Orbital analysis

The natural bond orbital (NBO) calculations were performed using NBO 3.1 program [78] as implemented in the Gaussian09 package at the DFT/B3LYP level in order to understand various second-order interactions presented in Tables 2 and 3. The important inter-molecular hyper-conjugative interactions are: $N_{11}-C_{12}$ from N_{10} of $n_1(N_{10}) \rightarrow \sigma^*(N_{11}-C_{12})$, $N_{10}-C_{12}$ from N_{11} of $n_1(N_{11}) \rightarrow \pi^*(N_{10}-C_{12})$, $C_{17}-C_{18}$ from O_{27} of $n_2(O_{27}) \rightarrow \pi^*(C_{17}-C_{18})$, $N_{29}-O_{31}$ from O_{30} of $n_1(O_{30}) \rightarrow \pi^*(N_{29}-O_{31})$ and $N_{29}-O_{30}$ from O_{31} of $n_3(O_{31}) \rightarrow \pi^*(N_{29}-O_{30})$ with electron densities, 0.04602, 0.32907, 0.39133, 0.05671, 0.63187e and stabilization energies, 10.95, 48.57, 27.12, 19.20, 17.39 kJ/mol. The natural hybrid orbital with low occupation numbers, considerable p-characters and higher energy orbital are: $n_2(O_{27})$, $n_2(O_{30})$ and $n_3(O_{31})$ with energies, -0.33540, -0.28016, -0.26415 a.u and p-characters, 100.0, 99.87,

100.00% and low occupation numbers, 1.85971, 1.89858, 1.45254 and the orbital with lower energies, high occupation numbers are: $n_1(\text{O}_{27})$, $n_1(\text{O}_{30})$, $n_2(\text{O}_{31})$ with energies, -0.57486, -0.78613, -0.28090 a.u with p-characters, 62.09, 25.12, 99.85% and high occupation numbers, 1.96081, 1.98130, 1.89947.

4.9 Fukui indices

The Fukui function is a local reactivity descriptor that gives the preferred regions where a chemical species will change its density when the number of electrons is modified and it indicates the propensity of the electronic density to deform at a given position upon accepting or donating electrons [79-81]. Also, it is possible to define the corresponding condensed or atomic Fukui functions on the j^{th} atom site as,

$$f_j^- = q_j(\text{N}) - q_j(\text{N}-1)$$

$$f_j^+ = q_j(\text{N}+1) - q_j(\text{N})$$

$$f_j^0 = \frac{1}{2}[q_j(\text{N}+1) - q_j(\text{N}-1)]$$

For an electrophilic $f_j^-(r)$, nucleophilic or free radical attack $f_j^+(r)$, on the reference molecule, respectively. In these equations, q_j is the atomic charge (evaluated from Mulliken population analysis, electrostatic derived charge, etc.) at the j^{th} atomic site is the neutral (N), anionic (N + 1) or cationic (N-1) chemical species. Morell et al. [82] recently proposed a dual descriptor ($\Delta f(r)$), which is defined as the difference between the nucleophilic and electrophilic Fukui function and is given by,

$$\Delta f(r) = [f^+(r) - f^-(r)]$$

$\Delta f(r) < 0$, then the site is favoured for an electrophilic attack, whereas if $\Delta f(r) > 0$, then the site may be favoured for a nucleophilic attack. Under this situation, dual descriptors $\Delta f(r)$ provide a clear difference between nucleophilic and electrophilic attack at a particular site with their sign. From table S3 (supporting information), according to the condition for dual descriptor, nucleophilic sites for in our title compound are C2, C3, C5, N10, N11, H15, H16, C17, C18, C19, C20, H21, C22, H23, C24, H25, H26, O27, H28 (positive value i.e. $\Delta f(r) > 0$). Similarly the electrophilic attack sites are C1, C4, C6, H7, H8, H9, C12, H13, C14, N29, O30, O31 (negative value i.e. $\Delta f(r) < 0$). The behaviour of molecules as electrophiles/nucleophiles during reaction depends on the local behaviour of molecules.

4.10 Reactive properties based on autoxidation and hydrolysis

In order to determine molecule locations possibly prone to the autoxidation and bonds for which breaking the lowest amount of energy is needed, we have calculated BDEs for all single acyclic bonds. Taking into account the autoxidation process, BDEs for hydrogen abstraction indicate molecule sites that are prone to be oxidized. Rate in autoxidation process is related to the abstraction of hydrogen atom from the parent drug molecule by peroxy radical [83, 84]. Because all peroxy radicals have similar BDE values (in the range of 87 kcal/mol to 92 kcal/mol) it is clear that if the calculated BDE is in this range, corresponding molecule location is candidate for the start of autoxidation process. It is also important to notice that mentioned energy interval is practically independent of the chemical surrounding [83, 85]. Results presented in study of Wright et al. [83] state that range of BDE values from 75 kcal/mol to 85 kcal/mol indicates the highest risk of some molecule to be subjected to autoxidation [83], while BDE values below 70–75 kcal/mol are not appropriate for the degradation by autoxidation. Obtained results for BDEs of all single acyclic bonds have been presented in Fig.2 (BDEs for hydrogen abstraction are given in red colour, while BDEs for the rest of the single acyclic bonds are given in blue colour).

Results presented in Fig.2 indicate that autoxidation process could start with the hydrogen abstraction of hydrogen atoms H15 and H16 (denoted as bond 5 in Fig.2). The BDE value in this case is 87 kcal/mol and that value is on the lower border of BDE range for all peroxy radicals. In the same time this value is very close to the upper border value of 85 kcal/mol (for which is considered to be the most sensitive towards autoxidation according to the Wright et al.[86]). What is even more important bond 11, which is adjacent to the bond 5, has the lowest BDE of all calculated BDEs, somewhat higher than 69 kcal/mol, indicating that at these locations the degradation is most likely to occur.

Possible locations where degradation based on hydrolysis could start can be determined by calculation of RDF obtained after MD simulations. RDF, $g(r)$, is probability of finding a particle in the distance r from another particle [86]. The curve profile and peak distance of the calculated RDF indicate to what extent are significant interactions with water molecules. Namely, the atoms with sharp $g(r)$ peaks and short distances are considered to have significant interactions with water. RDFs of atoms with significant interactions are presented in Fig.3.

Results presented in Fig.3a indicate that five carbon atoms are having significant interactions with water molecules. These carbon atoms are C1, C3, C4, C12 and C14. Profiles

of these atoms differ and distance peak in all cases is above 3.5 Å, while peak $g(r)$ values vary from around 1.0 to 1.3. Regarding carbon atoms it is important to emphasize the RDF of atom C14 because this atom is shared by bonds 5 and 11, which are very important from the aspect of degradation and autoxidation. Interactions of water with this atom indicate that hydrolysis could compete to some extent with autoxidation mechanism. On the other side, non-carbon atoms have significantly more pronounced interactions with water molecules, especially in the case of hydrogen atom H13 which has peak distance at around 1.6 Å and peak $g(r)$ value of around 1.3. It is also important to notice that nitrogen atom N10, the one that is recognized by ALIE surface to be prone to electrophilic attacks, also has significant interactions with water molecules. Its RDF curve has peak distance at around 2.5 Å and peak $g(r)$ value of around 0.9. NO₂ group of title molecule also has significant interactions with water, as indicated by RDFs of nitrogen atom N29 and oxygen atom O31.

4.11 Molecular Docking Studies

DNA topoisomerase II regulates chromosome dynamics like condensation and segregation. Mammalian cells include two genetically and biochemically isozymes (IIA, IIB); which have highly conserved N-terminal ATP binding area and central core domains in both of types. But these isozymes show differences at their C-terminal (Fig.S7-supporting information). DNA topoisomerase IIB crystallized with DNA helix and Etoposide which is topoisomerase II inhibitor acts as an anticancer drug (Fig.S8-supporting information). The 3D crystal structure of Topo II was taken from Protein Data Bank (PDB ID: 3QX3). 3QX3 has a good resolution of 2.16 Å. The enzyme has an attached co-crystallized inhibitor and therefore has a well defined binding site. Molecular docking study was performed on Auto Dock Vina software [87]. 3QX3 was in dimer form so firstly one of the subunit B was removed and then the receptor was prepared by the cleaning of all heteroatom's (i.e., non-receptor atoms such as water, ions, co-crystallized ligand, etc.,) After that Geistenger charges and polar hydrogen's were assigned and receptor input file was prepared in PDBQT format for Auto-Dock-Vina by using the Auto-Dock Tools package. Ligand input file was also arranged in PDBQT format too. The docking area was defined around the ATP binding site, which was determined in crystal structure, by a grid box of 40Å×40Å×40Å using 0.375Å grid point spacing in Auto-Grid (Fig. S9-supporting information). The docking conformations of ligands in the binding sites of the receptor were searched with Lamarkian genetic Algorithm (LGA) in Autodock. Firstly the co-crystal inhibitor ligand of receptor (etoposide) was docked again for the confirmation of the docking protocol. The predicted conformation of docking

result was same with crystal structure in reliable RMSD values ($\sim 3\text{\AA}$) (Fig. 4, Table 4). Discovery Studio Client 3.5 (Fig. S10 – supporting information) was used for the analyzing of the detailed interactions. There are three hydrogen bonds with ASP479, ARG503 residues of receptor and DC8 base of DNA helix. There are two π - π interactions with ARG503 residue. Lastly there are Van der Waals interactions with GLU477, GLY504 residues. 5-Nitro-2-phenoxybenzimidazole was prepared for docking by minimizing its energy at B3LYP/6-311++G(d,p) (5D, 5F) level of theory. After then the title compound was docked with same protocol as given above and binding properties determined with Autodock (Fig. 5, Table S4 – supporting information). The binding affinity (~ -7 kcal/mol) and RMSD ($\sim 2\text{\AA}$) values of the title compound were found reliable. There is hydrogen bond with DC3 (third base of DNA sequence (C subunit)). There are some close contacts such as Van der Waals interactions with the DC4, DC14, DG16, DG17, DG18 bases of DNA helix. Also there is π - π interaction between benzene ring of ligand and LYS671 residue as seen in Fig. 6. Discovery Studio Client 3.5 (Fig. S11-supporting information) was used for the analyzing of the detailed interactions. The confirmation of interaction profile of the title compound was provided by this way. There are four hydrogen bonds with GLY478, GLY504 and DC8 base which was same like etoposide docking results. Lastly there are Van der Waals interactions between ARG503, GLN778 and DA12.

4.12 Biological Activity

Since the activity of topoisomerases is essential for several cellular processes such as replication, transcription, and chromosome condensation, investigation of the inhibitory activities of eukaryotic topoisomerases is widely used in anticancer drug development. Topo II is the target for some of the most active anticancer drugs such as etoposide, teniposide, and doxorubicin used in the treatment of human malignancies [22]. In 2004, we investigated the inhibitory activity of the title compound on eukaryotic DNA Topo II in cell free systems [23]. According to this study, 5-nitro-2-phenoxybenzimidazole showed Topo II inhibitory activities comparable to etoposide. In this study, the title compound is investigated for their human DNA topoisomerase I and II α enzyme inhibition using relaxation assay (Fig.S12-supporting information) and anti-proliferative activity against HeLa (human cervical cancer) and L929 (mouse fibroblast) cell lines using sulforhodamine B (SRB) assay. All the new biological data of the title compound are as shown in Table S5 (supporting information).

According to the results, while the title compound showed weak inhibitory activity against human topoisomerase I (hTopoI), it indicated moderate effect against human

topoisomerase II (hTopoII). However, the title compound was not found to be more active than the compared standard drugs camptothecin and etoposide. On the other hand, surprisingly the title compound ($IC_{50} = 19.4 \mu M$) indicated more effect than well known drugs etoposide ($IC_{50} = 22.87 \mu M$) and gossypol ($IC_{50} = 31.34 \mu M$). It can be concluded, that the title compound can be leaded for developing new anticancer agents. Additionally, it should be needed the further studies for the title compound.

5. Conclusion

The molecular structure, vibrational wavenumbers, NLO, MEP, NBO and frontier molecular orbital analyses of the title compound were reported. The NH stretching mode is split into a doublet in the IR spectrum with a difference of 266 cm^{-1} and is red shifted by 220 cm^{-1} from the calculated value, which indicates the weakening of the NH bond as reported in literature. The experimental and theoretical NMR chemical shifts are in agreement. From the MEP plot, it is clear that the maximum negative region is localized over the nitro group, mono-substituted phenyl ring and the nitrogen atom in the C=N group oxygen and the maximum positive region is localized on NH group indicating a possible site for nucleophilic attack. According to the molecular docking study, there are four hydrogen bonds with GLY 478, GLY504 and DC 8 base which was same like etoposide docking results and Van der Waals interactions between ARG 503, GLN 778 and DA12. According to the ALIE surfaces, beside benzene rings, nitrogen atom N10 is recognized as potentially prone to electrophilic attacks. On the other side possible reaction centres could be hydrogen atom H13 and nitrogen atom N11, belonging to imidazole ring, as determined by Fukui functions. BDE values indicate one position (bond denoted with number 5) to be considered as candidate for the start of autoxidation process. The BDE of hydrogen abstraction in this case is 87 kcal/mol . Besides, its adjacent bond (bond 11) has the lowest BDE of all calculated in this work, thus emphasizing the potential of this molecule location to be starting point in degradation. RDF curves indicate several atoms with pronounced interactions with water molecules. Among them RDF of carbon atom C14 is important as this atom is common for mentioned bonds 5 and 11, meaning that hydrolysis and autoxidation mechanisms could compete to some extent at this location. The most pronounced interactions with water has hydrogen atom H13 with peak distance at around $1.6 \text{ \AA}$. From the biological activity study, the title compound indicated more effect than well known drugs etoposide and gossypol and it can be leaded for developing new anticancer agents.

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Figure captions

- Fig.1 Optimized geometry of 5-Nitro-2-phenoxyethylbenzimidazole
- Fig.2 BDE of all single acyclic bonds of 5-Nitro-2-phenoxyethylbenzimidazole
- Fig.3 RDFs of a) carbon and b) non-carbon atoms with significant interactions with water
- Fig.4 Superposition of the predicted docking conformation and crystal structure
- Fig.5 Title compound with molecular surface which was prepared by MSMS module of AutoDock
- Fig.6 Receptor ligand interactions which were determined by AutoDock

Table 1

Calculated unscaled and scaled wave numbers, observed IR, Raman bands and assignments of 5-Nitro-2-phenoxyethylbenzimidazole

B3LYP/ 6-31++G(d) (5D, 7F)		IR		Raman		Assignments ^a
$\nu(\text{cm}^{-1})^b$	$\nu(\text{cm}^{-1})^c$	IR _I	R _A	$\nu(\text{cm}^{-1})$	$\nu(\text{cm}^{-1})$	-
3663	3522	121.01	156.49	3568	-	$\nu\text{NH}(99)$
				3302		
3236	3111	5.20	52.55	-	-	$\nu\text{CHII}(99)$
3230	3105	3.51	77.26	3102	-	$\nu\text{CHII}(95)$
3205	3081	1.31	293.54	-	3077	$\nu\text{CHI}(88)$
3199	3075	17.93	49.16	-	3077	$\nu\text{CHI}(94)$
3190	3066	35.53	61.18	3065	-	$\nu\text{CHI}(98)$
3189	3066	6.60	82.82	3065	-	$\nu\text{CHII}(95)$
3174	3051	10.27	127.35	-	-	$\nu\text{CHI}(98)$
3165	3042	2.27	40.47	3042	-	$\nu\text{CHI}(92)$
3059	2940	10.99	51.37	2948	2945	$\nu\text{CH}_2(100)$
3013	2897	10.95	282.34	-	2865	$\nu\text{CH}_2(100)$
1658	1593	81.36	195.74	1596	1593	$\nu\text{RII}(53)$, $\nu\text{CN}(11)$
1637	1574	62.59	11.27	1576	-	$\nu\text{RI}(55)$, $\delta\text{CHI}(22)$
1625	1562	46.42	55.41	-	-	$\nu\text{RI}(50)$, $\nu\text{NO}_2(43)$
1622	1559	5.61	74.74	1555	1548	$\nu\text{RII}(58)$, $\delta\text{CHII}(12)$

1577	1516	39.57	96.33	1512	1514	$\nu\text{C}=\text{N}(49)$, $\delta\text{NH}(19)$
1538	1478	37.63	5.89	-	-	$\nu\text{RI}(54)$, $\delta\text{CHI}(23)$
1528	1469	79.73	15.87	1466	1466	$\delta\text{CH}_2(67)$
1510	1452	10.36	11.70	-	-	$\nu\text{RII}(44)$, $\delta\text{CHII}(27)$
1500	1442	2.67	1.80	-	-	$\delta\text{CHI}(25)$, $\nu\text{RI}(43)$
1479	1422	21.66	26.49	1418	1419	$\delta\text{NH}(37)$, $\nu\text{RII}(32)$
1439	1384	37.20	31.71	-	1390	$\nu\text{RII}(43)$, $\delta\text{CHII}(23)$
1424	1369	243.71	9.60	1365	1369	$\nu\text{NO}_2(47)$, $\delta\text{CH}_2(21)$, $\nu\text{CN}(11)$
1398	1344	47.15	24.77	1342	1346	$\delta\text{CH}_2(44)$, $\nu\text{NO}_2(26)$, $\nu\text{RII}(16)$
1380	1327	7.97	1.43	-	1332	$\delta\text{CHI}(75)$
1371	1318	55.75	39.56	1312	1316	$\nu\text{CN}(23)$, $\nu\text{CN}(27)$, $\delta\text{CH}_2(24)$
1338	1286	1.79	3.41	-	-	$\nu\text{RI}(80)$
1328	1276	19.43	13.18	-	1274	$\nu\text{CN}(18)$, $\nu\text{RII}(28)$, $\delta\text{CHII}(46)$
1280	1230	146.55	423.86	1238	1231	$\delta\text{CHII}(42)$, $\nu\text{CN}(33)$, $\nu\text{RII}(13)$
1263	1214	320.35	521.01	-	1214	$\nu\text{NO}_2(22)$, $\nu\text{CN}(21)$, $\nu\text{RII}(19)$
1242	1194	487.18	4.03	-	-	$\nu\text{CO}(49)$, $\nu\text{RII}(14)$, $\delta\text{CHII}(18)$
1241	1193	22.19	29.58	-	-	$\delta\text{NH}(24)$, $\nu\text{CN}(17)$, $\nu\text{RII}(19)$
1237	1189	2.39	8.90	-	-	$\delta\text{CH}_2(97)$
1223	1176	23.35	11.87	1178	1180	$\nu\text{CN}(40)$, $\delta\text{NH}(27)$
1218	1171	21.58	2.96	-	-	$\delta\text{CHI}(48)$, $\nu\text{RI}(22)$
1207	1160	26.17	2.86	1158	1158	$\delta\text{CHI}(70)$, $\nu\text{RI}(10)$
1169	1124	47.47	23.56	1126	1128	$\delta\text{CHII}(55)$, $\nu\text{RII}(18)$

1113	1070	12.38	0.71	1073	1069	ν RI(41), δ CHI(38)
1081	1040	15.15	22.13	1046	1038	δ CHII(24), ν CN(28), ν RII(30)
1050	1009	26.81	8.20	-	1012	δ CHI(39), δ CH ₂ (45)
1049	1009	0.01	0.69	-	-	ν RI(64)
1039	999	45.51	1.83	995	996	ν RI(35), ν CO(32)
1024	984	3.12	112.80	-	-	δ RI(19), ν CN(17), δ RIII(23)
1004	965	27.72	4.63	-	-	ν CO(37), δ RIII(13), ν CN(10), ν RI(11)
1003	964	0.01	0.06	-	-	γ CHI(87)
1002	963	0.01	0.11	-	-	γ CHII(89)
986	948	0.11	0.07	946	946	γ CHI(91)
955	918	5.38	4.37	-	-	δ RII(37), ν CN(23)
949	912	28.65	0.11	908	-	γ CHII(83)
913	878	6.33	0.01	875	-	γ CHI(83)
862	829	4.49	2.48	832	831	ν RII(36), ν CN(12), γ CHI(22)
850	818	0.20	0.03	-	-	γ CHI(98)
847	814	49.87	0.07	812	-	γ CHII(85)
833	801	26.32	45.13	-	-	ν CO(25), δ CH ₂ (15), δ RI(18)
803	772	13.40	27.95	776	-	δ NO ₂ (38), δ RIII(29)
775	745	115.01	0.30	742	740	γ CHI(68), τ RI(15), γ CO(14)
741	712	8.02	9.82	-	-	δ RII(15), δ RIII(28), δ RI(21)
709	681	44.19	1.32	682	684	τ RIII(28), τ RII(27),

						$\gamma\text{NH}(14), \gamma\text{NO}_2(13)$
698	671	17.97	2.12	-	-	$\delta\text{NO}_2(36), \delta\text{RII}(24), \delta\text{RIII}(26)$
689	663	54.73	0.45	-	-	$\gamma\text{NH}(71), \tau\text{RIII}(11)$
686	660	10.00	0.40	-	-	$\tau\text{RI}(69), \gamma\text{CO}(29)$
675	649	7.59	0.53	647	-	$\tau\text{RII}(35), \gamma\text{NO}_2(29),$ $\tau\text{RIII}(15), \gamma\text{CN}(14)$
664	639	0.77	1.01	-	-	$\tau\text{RIII}(58), \gamma\text{CC}(19)$
645	620	0.63	4.98	621	618	$\delta\text{RI}(86)$
623	599	0.73	4.93	596	597	$\delta\text{RI}(37), \delta\text{RII}(28)$
570	548	4.75	5.35	551	-	$\delta\text{CC}(10), \delta\text{CN}(10),$ $\delta\text{NO}_2(34), \delta\text{RIII}(13)$
567	545	1.77	0.30	-	542	$\tau\text{RII}(61), \gamma\text{CN}(22)$
553	532	8.55	1.21	533	530	$\delta\text{NO}_2(38), \delta\text{RII}(23), \delta\text{RI}(13)$
516	496	8.72	0.37	500	-	$\tau\text{RI}(46), \gamma\text{CO}(47)$
500	480	10.12	0.25	-	-	$\delta\text{NO}_2(22), \delta\text{CO}(30), \delta\text{CC}(15)$
439	422	6.99	0.19	423	-	$\tau\text{RII}(60), \tau\text{RIII}(20), \gamma\text{CN}(10)$
424	408	0.01	0.07	-	-	$\tau\text{RI}(84)$
419	403	3.02	3.12	403	-	$\delta\text{CO}(37), \delta\text{NO}_2(12)$
363	349	2.32	1.21	-	351	$\delta\text{RII}(38), \delta\text{NO}_2(32)$
345	331	0.88	0.03	-	-	$\tau\text{RIII}(22), \gamma\text{CC}(21),$ $\gamma\text{CN}(19), \tau\text{RII}(12)$
278	267	5.17	0.16	-	-	$\tau\text{RII}(53), \tau\text{RIII}(12), \gamma\text{CN}(12)$

275	265	2.93	3.71	-	-	δ CN(26), δ CC(14), δ NO ₂ (28)
236	227	0.26	0.60	-	-	τ RI(59), γ CO(25)
177	170	3.81	2.70	-	172	γ CC(23), τ RII(18), γ CN(12), τ RIII(19)
169	163	0.88	2.05	-	-	δ CN(21), δ CH ₂ (18), δ CO(18)
163	157	1.42	0.20	-	-	δ CO(42), δ CN(19), δ CC(11)
100	96	3.34	0.46	-	103	τ CO(21), τ CH ₂ (18), τ RI(13), γ CN(11)
66	63	1.47	4.58	-	-	τ CO(65)
50	48	1.38	1.28	-	-	τ NO ₂ (78)
49	47	1.69	0.12	-	-	δ CH ₂ (30), δ CC(28), δ CO(25)
21	20	0.01	0.16	-	-	τ CO(59), γ CC(19)
14	13	4.83	0.89	-	-	τ CH ₂ (61), γ NH(16)

^a ν -stretching; δ -in-plane deformation; γ -out-of-plane deformation; τ -torsion; mono-substituted phenyl, tri-substituted phenyl and imidazole rings are designated as PhI, PhII and PhIII, respectively.

^bUnscaled wavenumbers

^cScaled wavenumbers

Table 2

Second-order perturbation theory analysis of Fock matrix in NBO basis corresponding to the intra-molecular bonds of the title compound

Donor(i)	Type	ED/e	Acceptor(j)	Type	ED/e	E(2) ^a	E(j)-E(i) ^b	F(i,j) ^c
C3-N29	σ	1.98968	C1-C2	σ^*	0.01287	1.51	1.38	0.041
C3-N29	σ	1.98968	C4-C5	σ^*	0.02266	1.55	1.38	0.041
C5-C6	π	1.56796	C1-C2	π^*	0.30046	17.09	0.28	0.064
C5-C6	π	1.56796	C3-C4	π^*	0.36323	22.07	0.28	0.071
N10-C12	π	1.86960	C5-C6	π^*	0.47979	18.38	0.33	0.077
N11-C12	σ	1.98657	C1-C6	σ^*	0.02144	5.28	1.38	0.076
C17-C18	π	1.67394	C19-C22	π^*	0.32550	17.07	0.30	0.063
C17-C18	π	1.67394	C20-C24	π^*	0.32731	21.13	0.30	0.071
N29-O30	π	1.98711	C3-C4	π^*	0.36323	3.25	0.46	0.038
LP N10	σ	1.92085	N11-C12	σ^*	0.04602	10.95	0.80	0.0084
LP N11	σ	1.60065	C5-C6	π^*	0.47979	35.75	0.29	0.093
LP N11	σ	1.60065	N10-C12	π^*	0.32907	48.57	0.28	0.107
LP O27	π	1.85971	C17-C18	π^*	0.39133	27.12	0.35	0.093
LP O30	π	1.89858	C3-N29	σ^*	0.10095	12.51	0.57	0.076
LP O30	π	1.89858	N29-O31	σ^*	0.05671	19.20	0.70	0.105
LP O31	π	1.89947	C3-N29	σ^*	0.10095	12.34	0.57	0.075
LP O31	π	1.89947	N29-O30	σ^*	0.05619	19.12	0.71	0.105
LP O31	n	1.45254	N29-O30	π^*	0.63187	161.41	0.14	0.138

^aE(2) means energy of hyper-conjugative interactions (stabilization energy in kJ/mol)

^bEnergy difference (a.u) between donor and acceptor i and j NBO orbitals

^cF(i,j) is the Fock matrix elements (a.u) between i and j NBO orbitals

Table 3

NBO results showing the formation of Lewis and non Lewis orbitals

Bond(A-B)	ED/e ^a	EDA%	EDB%	NBO	s%	p%
σ C3-N29	1.98968	50.28	49.72	0.7091(sp ^{1.80})C	35.73	64.27
-	-0.80639	-	-	+0.7051(sp ^{1.80})N	35.76	64.24
π C5-C6	1.56796	51.06	48.94	0.7145(sp ^{1.00})C	0.00	100.00
-	-0.26291	-	-	+0.6996(sp ^{1.00})C	0.00	100.00
π N10-C12	1.86960	59.78	40.22	0.7732(sp ^{1.00})N	0.00	100.00
-	-0.32242	-	-	+0.6342(sp ^{1.00})C	0.00	100.00
σ N11-C12	1.98657	62.68	37.32	0.7917(sp ^{1.94})N	33.95	66.95
-	-0.83029	-	-	+0.6109(sp ^{2.37})C	29.66	70.34
π C17-C18	1.67394	47.08	52.92	0.6861(sp ^{1.00})C	0.00	100.00
-	-0.26850	-	-	+0.7275(sp ^{1.00})C	0.00	100.00
π N29-O30	1.98711	39.63	60.37	0.6296(sp ^{1.00})N	0.00	100.00
-	-0.43955	-	-	+0.7770(sp ^{1.00})O	0.00	100.00
n1 N10	1.92085	-	-	sp ^{2.05}	32.73	67.27
-	-0.37193	-	-	-	-	-
n1 N11	1.60065	-	-	sp ^{1.00}	0.00	100.0
-	-0.28402	-	-	-	-	-
n1 O27	1.96081	-	-	sp ^{1.64}	37.91	62.09
-	-0.57486	-	-	-	-	-
n2 O27	1.85971	-	-	sp ^{1.00}	0.00	100.0
-	-0.33540	-	-	-	-	-
n1 O30	1.98130	-	-	sp ^{0.34}	74.88	25.12
-	-0.78613	-	-	-	-	-
n2 O30	1.89858	-	-	sp ^{99.99}	0.13	99.87
-	-0.28016	-	-	-	-	-
n1 O31	1.98127	-	-	sp ^{0.34}	74.90	25.10
-	-0.78668	-	-	-	-	-
n2 O31	1.89947	-	-	sp ^{99.99}	0.15	99.85

-	-0.28090	-	-	-	-	-
n3 O31	1.45254	-	-	sp ^{1.00}	0.00	100.0
-	-0.26415	-	-	-	-	-

^a ED/e is expressed in a.u.

Table 4

The binding affinity values of different poses of Etoposide predicted by AutodockVina

Mode	Affinity (kcal/mol)	Distance from best mode (Å)	
-	-	RMSD l.b.	RMSD u.b.
1	-11.0	0.000	0.000
2	-9.3	1.682	1.870
3	-8.5	3.939	8.687
4	-8.4	3.053	6.239
5	-8.2	3.977	8.760

Scheme 1: Synthetic pathway of 5-nitro-2-phenoxymethylbenzimidazole

Bond	BDE [kcal/mol]
1	118.41
2	120.51
3	121.55
4	110.54
5	87.05
6	117.18
7	117.47
8	118.67
9	71.98
10	102.51
11	69.25
12	95.31

Highlights

- Title compound has been characterized by IR, Raman and NMR spectroscopy
- NLO parameters are calculated and the computed hyperpolarizability is very large
- NBO analysis is carried out to study hyper conjugative interactions
- HOMO and LUMO study is used to determine the charge transfer within the molecule
- The substrate binding site interactions with Topo II enzyme is identified