## This item is the archived peer-reviewed author-version of:

Conformational, vibrational and DFT studies of a newly synthesized arylpiperazine-based drug and evaluation of its reactivity towards the human GABA receptor

## Reference:

Onaw ole A.T., Al-Ahmadi A.F., Mary Y.S., Panicker C.Y., Ullah N., Armaković S., Armaković S.J., Van Alsenoy Christian, AI-Saadi A.A..- Conformational, vibrational and DFT studies of a new ly synthesized arylpiperazine-based drug and evaluation of its reactivity tow ards the human GABA receptor Journal of molecular structure - ISSN 0022-2860-1147(2017), p. 266-280
Full text (Publisher's DOI): https://doi.org/10.1016/J.MOLSTRUC.2017.06.107
To cite this reference: https://hdl.handle.net/10067/1441360151162165141

# Conformational, vibrational and DFT studies of a newly synthesized arylpiperazine-based drug and evaluation of its reactivity towards the human GABA receptor 

A. T. Onawole ${ }^{\text {a }}$, A. F. Al-Ahmadi ${ }^{\text {a }}$, Y.S. Mary ${ }^{\text {b }}$, C.Y. ${ }^{\text {Panicker }}{ }^{\text {b }}$, N. Ullah ${ }^{\text {a }}$, S. Armakovićc ${ }^{\text {c S. J. }}$ Armaković ${ }^{\text {d }}$, C.V. Alsenoy ${ }^{\mathrm{e}}$, A. A. Al-Saadi ${ }^{\text {a, }}{ }^{\text {, }}$

${ }^{\text {a }}$ Department of Chemistry, King Fahd University of Petroleum and Minerals, Dhahran 31261, Saudi Arabia
${ }^{\text {b }}$ Department of Physics, Fatima Mata National College, Kollam, Kerala, India
${ }^{\text {c }}$ University of Novi Sad, Faculty of Sciences, Department of Physics, Trg D. Obradovića 4, 21000 Novi Sad, Serbia
${ }^{\text {d }}$ University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg D. Obradovića 3, 21000 Novi Sad, Serbia
${ }^{\mathrm{e}}$ Department of Chemistry, University of Antwerp, Groenenborgerlaan 171, B-2020, Antwerp, Belgium

* Corresponding Author: asaadi@kfupm.edu.sa


#### Abstract

This study reports a computational assessment of important biochemical properties and vibrational assignments for the synthesized 1-(4-(3-methoxy-4-nitrophenyl)piperazin-1yl)ethanone (MNPE). MNPE is related to the commonly used arylpiperazine-based drugs that exhibit a wide range of pharmacological activities. The characterization of MNPE is based on the readily sighted $1363 \mathrm{~cm}^{-1}$ infrared band (associated with piperazine ring stretching), $1308 \mathrm{~cm}^{-}$ ${ }^{1}$ Raman line (associated with the phenyl ring breathing), $1242 \mathrm{~cm}^{-1}$ Raman line and $1092 \mathrm{~cm}^{-1}$ infrared band (both associated with C-N stretching) as key modes in its vibrational spectra. First principle calculations revealed that MNPE could exist in sixteen different plausible conformations, which were used as basis to understand the possible molecular docking mechanism of the molecule as an agonist in the human $\mathrm{GABA}_{\mathrm{A}}$ receptor. The best binding scenarios showed the presence of intramolecular hydrogen bonding in MNPE and was comparable with the most stable configuration. It was further evaluated for its reactivity properties by utilizing the concepts of Average Local Ionization Energies (ALIE) and Fukui functions. The autoxidation and hydrolysis degradation likelihood of MNPE estimated from the computed bond dissociation energies and radial distribution functions predicted that MNPE is to be readily biodegradable in aqueous solutions.


Keywords: Arylpiperazine; Human GABA $_{A}$ receptor; Molecular docking; Vibrational spectroscopy; Density functional theory.

## 1. Introduction

Arylpiperazines and its derivatives have demonstrated an alluring pharmacological framework that is found in different branded drugs. The inclusion of piperazine motif is a vital synthetic procedure in drug discovery. This is due to its solubility, proper alkalinity, and hydrogen bond formation capability. All these attributes in turn lead to its ease in the modification of molecular physicochemical properties [1, 2]. Moreover, arylpiperazine derivatives have found extensive applications in the pharmaceutical chemistry such as anticancer [3, 4], antiviral [5], antifungal [6], anti-oxidative [7], anti-histamine [8], anti-parasitic [9] psychotolytic [10] and as an agonist for the human GABA receptor [11]. Contrary, the frequent usage and improper disposal of this class of compounds can act as organic pollutants and pose threat to the environment especially in water and hence become toxic to aquatic organisms [12-14]. In general, arylpiperazine derivatives are quite stable and hence their degradation under normal conditions is very slow. This in turn make the conventional water purification methods unsuitable for efficient and economic removal of these compounds [15]. Therefore, considerable synthetic efforts have been devoted to design new arylpiperazines which are both medicinally effective and waterdegradable. In the light of above, we have synthesized an arylpiperazine derivative and have studied it computationally and spectroscopically as well as by means of bond dissociation energy (BDE), radial distribution function (RDF) and molecular docking approaches to gain some insights on its electronic properties and side effects.

Given the fact that the advanced oxidation is considered a widely used method in water purification technology [16], understanding the reactive properties of MNPE (Fig. 1) and the possible auto-oxidation locations within the molecule is essential. This in turn can be achieved by calculating BDEs during hydrogen abstraction. Thus, the BDE of the rest of the single acyclic bonds can be used for classification of bonds according to their strength and prediction of locations where degradation can occur. Considering the importance of water as solvent, molecular dynamics (MD) simulations can provide valuable information to ascertain the interaction with water molecules and different type of atoms of MNPE. In this case, RDF shall be particularly quite useful.

In addition, arylpiperazine derivatives are known as agonist to naturally occurring $\gamma$ aminobutyric acid (GABA) receptor, which in turn produces a synergistic anti-stress and anti-
anxiety effect. The $\mathrm{GABA}_{\mathrm{A}}$ receptor is one of the Ligand-gated Ion Channels (LICs) that are referred to as ionotropic receptors because they allow the passage of ions such as $\mathrm{Na}^{+}$and $\mathrm{K}^{+}$ through the membrane as a response to the stimulus effect when a ligand binds to them. [17-19]. Since most drugs often imitate the action of endogenous ligands [20a], the conformational analysis and the molecular docking study of can thus provide a deeper understanding of the mode of action by which drugs act on such a target protein.

## 2. Experimental

Melting Points were determined on a Büchi apparatus (Büchi Labortechnik AG, Switzerland) and are uncorrected. Elemental analysis was carried out on a Perkin Elmer Elemental Analyzer Series 11 Model 2400 (PerkinElmer Inc. USA). ${ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR spectra were measured in $\mathrm{CDCl}_{3}$ using TMS as internal standard on a JEOL JNM-LA 500 MHz spectrometer (JEOL USA Inc.). Analytical TLC was carried out on silica gel $60 \mathrm{~F}_{254}$ plates (E. Merck); column chromatography was carried out on silica gel (200-400 mesh, E. Merck). DR/Jasco FT-IR 6300 spectrometer employing KBr tablets was used to record the infrared spectrum (Fig. 2) of MNPE while a Bruker RFS 100/s, Germany was used to record the Raman spectrum (Fig. 3). The emission of Nd;YAG laser at the wavelength of 1064 nm was utilized for sample excitation with a maximal power of 150 mW .

### 2.1 Synthesis of MNPE (6)

To a suspension of compound 5 [20b] ( $1.05 \mathrm{~g}, 3 \mathrm{mmol}$ ) in $\mathrm{CH}_{2} \mathrm{Cl}_{2}(20 \mathrm{~mL})$ at $0{ }^{\circ} \mathrm{C}$ was added $\mathrm{Et}_{3} \mathrm{~N}(1.1 \mathrm{~mL}, 8 \mathrm{mmol})$ followed by the dropwise addition of acetyl chloride $(0.32 \mathrm{~mL}, 4.5$ mmol ). The mixture was stirred for three hours at room temperature until completion (TLC analysis). The reaction was quenched with saturated $\mathrm{NaHCO}_{3}(10 \mathrm{~mL})$ and the organic layer was separated, washed with $\mathrm{H}_{2} \mathrm{O}(10 \mathrm{~mL})$ and then dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$ followed by evaporation under reduced pressure to obtain a yellow solid, which was re-crystallized with ethanol to afford the title compound 6 as yellow crystalline solid. ${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ): $\delta 2.16$ ( $\mathrm{s}, 3 \mathrm{H}$, $\left.\mathrm{COCH}_{3}\right), 3.41(\mathrm{~m}, 4 \mathrm{H}$, piperazine H$)$, $3.68(2 \mathrm{H}$, t, piperazine H$), 3.80(2 \mathrm{H}, \mathrm{t}$, piperazine H$), 3.91$ $\left(\mathrm{s}, 3 \mathrm{H}, \mathrm{OCH}_{3}\right), 6.35(\mathrm{~d}, J=1.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2), 6.41(\mathrm{dd}, J=1.9,8.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-6), 8.01(\mathrm{~d}, J=8.6$ $\mathrm{Hz}, 1 \mathrm{H}, \mathrm{H}-5)$. Anal. Calcd for $\mathrm{C}_{13} \mathrm{H}_{17} \mathrm{~N}_{3} \mathrm{O}_{4}$ : C, 55.91 ; H, 6.14; N, 15.05\%. Found: C, 55.87; H, 6.17; N, 15.01.

## 3. Computational Details

An extensive conformational analysis was done for MNPE using Density Functional Theory (DFT) with Gaussian 09 program [21] at the Becke's three-parameter half-and-half model and the Lee-Yang-Parr connection useful (B3LYP) level of theory using both $6-311 \mathrm{G}(\mathrm{d}, \mathrm{p})$ and 6 $311++\mathrm{G}(\mathrm{d}, \mathrm{p})$ as the basis sets which are good for medium-size organic molecules. Sixteen possible structures (Fig. 4) of MNPE were determined, and their energies were computed (Table 1) $[22]$. The $6-311++G(\mathrm{~d}, \mathrm{p})$ basis set was employed to predict the geometrical parameters in details (Tables 2-5) and calculate the vibrational wavenumbers (Table 5) [23], [24] of the most stable conformer (Fig. 1) using a scaling factor of 0.9613 [25]in order to acheive a better correlation with experimental values, The assignments of the figured wavenumbers are supported by the atomic displacements visulized with GAUSSVIEW [26] and the Potential Energy Distribution (PED) values computed with the GAR2PED software [27].
Moreover, the Jaguar program 9.0 embedded in the Schrödinger Materials Science Suite [28] was utilized for DFT calculations of Average Local Ionization Energies (ALIE), Fukui capacities and BDE. For MD simulations of RDFs, the Desmond program [29]-[32] was utilized, likewise as executed in the Schrödinger Materials Science Suite. For all DFT estimations B3LYP exchange correlation functionals were employed [33]. . Concerning MD simulations, OPLS 2005 force field [34] was used. The system was modeled by placing one molecule of MNPE in a cubic box with $\sim 3000$ water molecules and treated with NPT (where N is constant particle number, P is pressure and T is temperature) ensemble class. The Pressure was set to 1.0325 bar while the temperature was set to be 300 K , with simulation time of 10 ns . The cut-off radius was set to 12 $\AA$, while the solvent description was done by employing the Simple Point Charge (SPC) model [35]. The molecular docking analysis was performed with the CLC Drug Discovery program [36] while the Moleman 2 [37] was used to validate the crystal structure of the human GABA $_{A}$ receptor which is the target protein in this study.

## 4 Results and Discussion

### 4.1 Synthesis of 1-(4-(3-methoxy-4-nitrophenyl)piperazin-1-yl)ethanone

The desired compound MNPE (6) was synthesized as outlined in scheme 1. In brief, Omethylation of phenol 1 with iodomethane in DMF rendered 2, which in turn was condensed
with 1-boc-piperazine $\mathbf{3}$ to produce coupled product 4. Acid induced removal of boc protection of $\mathbf{4}$ furnished salt $\mathbf{5}$, which was reacted with acetic anhydride in $\mathrm{CH}_{2} \mathrm{Cl}_{2}$ to yield the desired $\mathbf{6}$ in $44 \%$ overall yield from 1 [38].


Scheme 1. Synthesis of 1-(4-(3-methoxy-4-nitrophenyl)piperazin-1-yl)ethanone (MNPE)

### 4.2. Conformational Analysis

Exploring conformational properties of organic-based drugs is extremely useful in order to understand the structure-activity relationship. Detailed conformational analysis of the multi-rotor MNPE has led to 16 possible unique conformations depicted in Fig. 4. The variation of bond lengths, bond angles and dihedral angles among the stable forms are listed in Tables 2-4. Generally, the steric effect when the methoxy and nitro groups repel each other pushes the energy $1-2 \mathrm{kcal} / \mathrm{mol}$ to the higher side (Table 1). Consequently, the position of the methoxy with respect to the nitro groups is the main factor that influences the overall energy of the molecule. The average distance between $\mathrm{O}_{13}$ and $\mathrm{O}_{37}$ in A and B conformations is about $0.2 \AA$ shorter than in corresponding C and D conformations (Table 2). The internal rotation of the $\mathrm{OCH}_{3}$ group,
presented in Fig. 5(A), has shown that the eclipsing position of the $\mathrm{CH}_{3}$ group is 3-4 kcal higher in energy than when the methoxy and nitro group oxygens repel each other. On the other hand, the molecule seems more flexible flipping along the aromatic ring-piperazine ring linkage and the nitro group-aromatic ring linkage. The piperazine ring in its lowest energy form positons itself in the same plane of the benzene ring with about $42^{\circ}$ tilting angle. The stable position of the methoxy group in C and D configurations represents a metastable arrangement with $5 \mathrm{kcal} / \mathrm{mol}$ barrier (Fig. 5) to interchange to A and B conformations.
The conformer B4 is predicted to be the most stable form, and its detailed structural parameters are listed in Table S1. There is no significant difference between the results obtained by the B3LYP/6-311G(d,p) and B3LYP/6-311++G(d,p) basis sets in terms of bond angles and distances, yet some major dihedral angles, such as $\mathrm{O}_{37}-\mathrm{N}_{15}-\mathrm{C}_{10}-\mathrm{C}_{9}$ and $\mathrm{O}_{36}-\mathrm{N}_{15}-\mathrm{C}_{10}-\mathrm{C}_{11}$ (Table 4) showed a notable difference (up to $6^{\circ}$ in some cases) when these two different basis sets are utilized. This perception demonstrates the impact of the electronic properties of the nitro group when long-range repulsion parameters are considered in the basis set.

The molecular electrostatic potential (MEP) map (Fig 6a) indicates that the nitro and carbonyl groups demonstrate an electronegative region in the molecule whereas the electropositive region occurs at the piperazine ring vicinity. This map has given an insight into possible interactions between MNPE and the amino acid in the target protein. The HOMO (highest occupied molecular orbital) and LUMO (lowest unoccupied molecular orbital) energy values were calculated to be -7.83 eV and -4.55 eV , respectively, with an energy gap of 3.28 eV . Due to the relatively small gap in MNPE, the charge transfer process is predicted to be feasible and supports the docked pose of the compound in the molecular docking analysis, which will be discussed later in this script.

### 4.3 Geometrical Parameters for B4 Conformer

For the piperazine ring of the title compound, the bond lengths are computed as $\mathrm{N}_{4}-\mathrm{C}_{5}=$ $1.4586 \AA, \mathrm{~N}_{4}-\mathrm{C}_{3}=1.4567 \AA, \mathrm{C}_{3}-\mathrm{C}_{2}=1.5293 \AA, \mathrm{~N}_{1}-\mathrm{C}_{6}=1.4633 \AA, \mathrm{~N}_{1}-\mathrm{C}_{2}=1.4675 \AA, \mathrm{C}_{5}-\mathrm{C}_{6}=$ $1.5284 \AA$ and corresponding reported bond lengths are $1.465,1.463,1.514,1.458,1.471,1.511 \AA$ [39] and $1.4488,1.485,1.535,1.488,1.477,1.547 \AA$ [40]. The DFT calculations predict the bond angles within the piperazine ring $\mathrm{N}_{4}-\mathrm{C}_{3}-\mathrm{C}_{2}=110.4^{\circ}, \mathrm{N}_{4}-\mathrm{C}_{5}-\mathrm{C}_{6}=111.1^{\circ}, \mathrm{N}_{1}-\mathrm{C}_{6}-\mathrm{C}_{5}=110.9^{\circ}, \mathrm{N}_{1}-$ $\mathrm{C}_{2}-\mathrm{C}_{3}=111.3^{\circ}, \mathrm{C}_{6}-\mathrm{N}_{1}-\mathrm{C}_{2}=112.4^{\circ}$ whereas the corresponding reported values are 110.4, 113.1,
110.7, 109.7, $114.8^{\circ}$ [40] and $110.0,109.7,109.7,110.0,110.0^{\circ}$ [39].The dihedral angles of the piperazine ring, $\mathrm{C}_{5}-\mathrm{N}_{4}-\mathrm{C}_{3}-\mathrm{C}_{2}=-54.7^{\circ}, \mathrm{C}_{6}-\mathrm{N}_{1}-\mathrm{C}_{2}-\mathrm{C}_{3}=-55.1^{\circ}, \mathrm{N}_{4}-\mathrm{C}_{3}-\mathrm{C}_{2}-\mathrm{N}_{1}=53.9^{\circ}, \mathrm{C}_{2}-\mathrm{N}_{1}-\mathrm{C}_{6}-\mathrm{C}_{5}$ $=54.6^{\circ}, \mathrm{C}_{3}-\mathrm{N}_{4}-\mathrm{C}_{5}-\mathrm{C}_{6}=54.9^{\circ}, \mathrm{N}_{1}-\mathrm{C}_{6}-\mathrm{C}_{5}-\mathrm{N}_{4}=-53.6^{\circ}$ are in agreement with the reported values [41]. The C-O bond lengths of MNPE are $\mathrm{C}_{9}-\mathrm{O}_{13}=1.346 \AA$ and $\mathrm{C}_{14}-\mathrm{O}_{13}=1.424 \AA$ and the increase in bond length of $\mathrm{C}_{14}-\mathrm{O}_{13}$ is due to the noticeable hydrogen bonding experienced by the molecule as reported in literature [42], [43]. In the present case, the $\mathrm{C}=\mathrm{O}$ bond length is 1.2225 $\AA$ which is in agreement with literature [44]. The C-C bond lengths in the phenyl ring are in the range 1.3792-1.4118 $\AA$ and the bond lengths are somewhere in between the normal values for a single $(1.54 \AA)$ and a double $(1.33 \AA)$ bond [44]. For the title compound the N-O bond lengths are $1.2246 \AA$ and $1.2324 \AA$ which was in agreement with reported values [45].

The C-N-O angles are reported as $117.7^{\circ}$ and $117.5^{\circ}$ [45] where as for the title compound, the angles are $118.9^{\circ}$ and $117.0^{\circ}$. At $\mathrm{N}_{15}$ position, the bond angles are, $\mathrm{C}_{10}-\mathrm{N}_{15}-\mathrm{O}_{37}=$ $118.9^{\circ}, \mathrm{C}_{10}-\mathrm{N}_{15}-\mathrm{O}_{36}=117.0^{\circ}$ and $\mathrm{O}_{36}-\mathrm{N}_{15}-\mathrm{O}_{37}=124.1^{\circ}$ and this asymmetry in angles is due to the adjacent $\mathrm{OCH}_{3}$ group. At $\mathrm{C}_{10}$ position, the bond angle $\mathrm{C}_{9}-\mathrm{C}_{10}-\mathrm{C}_{15}$ increased by $2.9^{\circ}$ and $\mathrm{C}_{11}-\mathrm{C}_{10}-$ $\mathrm{N}_{15}$ is decreased by $2.5^{\circ}$ from $120^{\circ}$, which reveals the hydrogen bonding with the $\mathrm{H}_{31}$ atom. At $\mathrm{C}_{9}$ position, the bond angles are, $\mathrm{C}_{8}-\mathrm{C}_{9}-\mathrm{C}_{10}=118.5^{\circ}, \mathrm{C}_{8}-\mathrm{C}_{9}-\mathrm{O}_{13}=122.9^{\circ}$ and $\mathrm{C}_{10}-\mathrm{C}_{9}-\mathrm{O}_{13}=$ $118.5^{\circ}$ and the increase in $\mathrm{C}_{8}-\mathrm{C}_{9}-\mathrm{O}_{13}$ is due to the interaction between $\mathrm{CH}_{3}$ and $\mathrm{H}_{30}$ atom. At $\mathrm{C}_{7}$ position the bond angles are $\mathrm{C}_{8}-\mathrm{C}_{7}-\mathrm{C}_{12}=118.0^{\circ}, \mathrm{C}_{8}-\mathrm{C}_{7}-\mathrm{N}_{1}=121.4^{\circ}$ and $\mathrm{C}_{12}-\mathrm{C}_{7}-\mathrm{N}_{1}=120.5^{\circ}$ and the asymmetry in angles is due to the interaction between the phenyl ring and the $\mathrm{CH}_{2}$ groups of piperazine ring. Similarly, at $\mathrm{C}_{16}$ position, the bond angles are, $\mathrm{N}_{4}-\mathrm{C}_{16}-\mathrm{C}_{18}=117.5^{\circ}, \mathrm{N}_{4}-\mathrm{C}_{16}-\mathrm{O}_{17}$ $=121.5^{\circ}, \mathrm{C}_{18}-\mathrm{C}_{16}-\mathrm{O}_{17}=121.0^{\circ}$ and this asymmetry is due to the $\mathrm{CH}_{3}$ group and $\mathrm{CH}_{2}$ groups of piperazine ring. The methoxy group is slightly tilted from the phenyl ring as is evident from the torsion angles, $\mathrm{C}_{7}-\mathrm{C}_{8}-\mathrm{C}_{9}-\mathrm{O}_{13}=177.6^{\circ}, \mathrm{C}_{8}-\mathrm{C}_{9}-\mathrm{O}_{13}-\mathrm{C}_{14}=1.9^{\circ}, \mathrm{C}_{11}-\mathrm{C}_{10}-\mathrm{C}_{9}-\mathrm{O}_{13}=-175.9^{\circ}$ and $\mathrm{C}_{10}-$ $\mathrm{C}_{9}-\mathrm{O}_{13}-\mathrm{C}_{14}=178.8^{\circ}$ and the phenyl ring and piperazine ring are tilted from each other as is evident from the torsion angles, $\mathrm{C}_{8}-\mathrm{C}_{7}-\mathrm{N}_{1}-\mathrm{C}_{6}=-4.7^{\circ}, \mathrm{C}_{8}-\mathrm{C}_{7}-\mathrm{N}_{1}-\mathrm{C}_{2}=139.3^{\circ}, \mathrm{C}_{12}-\mathrm{C}_{7}-\mathrm{N}_{1}-\mathrm{C}_{2}=-$ $42.2^{\circ}$ and $\mathrm{C}_{12}-\mathrm{C}_{7}-\mathrm{N}_{1}-\mathrm{C}_{6}=173.7^{\circ}$. The $\mathrm{COCH}_{3}$ and the piperazine ring are tilted from each other as is evident from the torsion angles, $\mathrm{C}_{5}-\mathrm{N}_{4}-\mathrm{C}_{16}-\mathrm{O}_{17}=4.1^{\circ}, \mathrm{C}_{3}-\mathrm{N}_{4}-\mathrm{C}_{16}-\mathrm{O}_{17}=174.7^{\circ}, \mathrm{C}_{5}-\mathrm{N}_{4}-\mathrm{C}_{16}-$ $\mathrm{C}_{18}=-176.4^{\circ}$ and $\mathrm{C}_{3}-\mathrm{N}_{4}-\mathrm{C}_{16}-\mathrm{C}_{18}=-5.8^{\circ}$.

### 4.4 Molecular Reactivity

### 4.4.1 ALIE Surfaces and Fukui Functions

Two representative Average Local Ionization Energy (ALIE) surfaces of MNPE are depicted in Fig. 7a. ALIE is frequently used as a quantum molecular descriptor for the understanding of local reactivity properties of molecular systems. This quantity was introduced by Sjoberg et al. [46, 47], and it is useful when its values are mapped to the electron density surface. In that case the certain areas of the surface indicate the molecule locations where electrons are the least tightly bound, i.e. the most easily removed. Therefore these locations are the most prone to electrophilic attacks. ALIE is defined as followed:

$$
\begin{equation*}
I(r)=\sum_{i} \frac{\rho_{i}(\vec{r})\left|\varepsilon_{i}\right|}{\rho(\vec{r})} \tag{1}
\end{equation*}
$$

where $\rho_{i}(\vec{r})$ represents the electronic density of the $i$-th molecular orbital at the point $\vec{r}, \boldsymbol{\varepsilon}_{i}$ represents the orbital energy and $\rho(\vec{r})$ is the total electronic density function.

The results obtained for MNPE revealed that the locations at the benzene ring and $\mathrm{NO}_{2}$ group are prone to possible electrophilic attack. This implied that electrons in the regions near vicinity of carbon atoms $\mathrm{C}_{8}$ and $\mathrm{C}_{12}$ (belonging to benzene ring), nitrogen atom $\mathrm{N}_{1}$ (belonging to piperazine ring) and oxygen atoms of $\mathrm{NO}_{2}$ group are less tightly bound. At all these locations, the ALIE values were somewhat lower than $200 \mathrm{kcal} / \mathrm{mol}$, exhibiting possible reactive sites.

Besides MEP and ALIE analysis, values of Fukui functions (Fig. 7b) can also serve as indicators of possible reactive centers in the molecule. They provide insight on how electron density changes with the addition or removal of the charge. Two Fukui functions, $f^{+}$and $f^{-}$, were calculated with infinite difference approach as follows:

$$
\begin{align*}
& f^{+}=\frac{\left(\rho^{N+\delta}(r)-\rho^{N}(r)\right)}{\delta}  \tag{2}\\
& f^{-}=\frac{\left(\rho^{N-\delta}(r)-\rho^{N}(r)\right)}{\delta} \tag{3}
\end{align*}
$$

where $N$ is the number of electrons in the reference state of the molecule and $\delta$ represents the fraction of electron, which is set to be 0.01 [48].

According to the results presented in Fig.6a, purple region of Fukui $f^{+}$function is located at oxygen atoms of $\mathrm{NO}_{2}$ group, which indicated an increase in the electron density with the addition of charge. On the other side, the negative color was generated at the methyl group in the near vicinity of benzene ring and in the near vicinity of oxygen atom $\mathrm{O}_{13}$, which revealed these
locations as interesting possible reactive centers. It is evident that the regions indicated by Fukui functions corresponded to the regions where MEP surfaces have the highest or lowest values.

### 4.4.2 NBO Analysis

The natural bond orbitals (NBO) calculations were performed using NBO 3.1 program [49] as implemented in the Gaussian 09 [21] package, and various hyper-conjugative interaction parameters are given in tables 7 and 8 . The intra-molecular hyper-conjugative interactions in the title compound are: $\mathrm{C}_{7}-\mathrm{C}_{8}$ from $\mathrm{N}_{1}$ of $\mathrm{n}_{1}\left(\mathrm{~N}_{1}\right) \rightarrow \pi^{*}\left(\mathrm{C}_{7}-\mathrm{C}_{8}\right), \mathrm{C}_{16}-\mathrm{O}_{17}$ from $\mathrm{N}_{4}$ of $\mathrm{n}_{1}\left(\mathrm{~N}_{4}\right) \rightarrow \pi^{*}\left(\mathrm{C}_{16}-\right.$ $\left.\mathrm{O}_{17}\right), \mathrm{C}_{9}-\mathrm{C}_{10}$ from $\mathrm{O}_{13}$ of $\mathrm{n}_{2}\left(\mathrm{O}_{13}\right) \rightarrow \pi^{*}\left(\mathrm{C}_{9}-\mathrm{C}_{10}\right), \mathrm{N}_{4}-\mathrm{C}_{16}$ from $\mathrm{O}_{17}$ of $\mathrm{n}_{2}\left(\mathrm{O}_{17}\right) \rightarrow \sigma^{*}\left(\mathrm{~N}_{4}-\mathrm{C}_{16}\right), \mathrm{N}_{15}-\mathrm{O}_{37}$ from $\mathrm{O}_{36}$ of $\mathrm{n}_{3}\left(\mathrm{O}_{36}\right) \rightarrow \pi^{*}\left(\mathrm{~N}_{15}-\mathrm{O}_{37}\right), \mathrm{N}_{15}-\mathrm{O}_{36}$ from $\mathrm{O}_{37}$ of $\mathrm{n}_{2}\left(\mathrm{O}_{37}\right) \rightarrow \mathrm{\sigma}^{*}\left(\mathrm{~N}_{15}-\mathrm{O}_{36}\right)$ having electron densities, $0.42457,0.28120,0.46950,0.08444,0.62299,0.06304 \mathrm{e}$ and stabilization energies, $32.39,62.42,35.28,26.34,148.77,19.69 \mathrm{KJ} / \mathrm{mol}$.

From Table 7, the natural hybrid orbital with higher energies, considerable p-character (nearly $100 \%$ ) and low occupancy were predicted to be $\mathrm{n}_{2}\left(\mathrm{O}_{13}\right), \mathrm{n}_{2}\left(\mathrm{O}_{17}\right), \mathrm{n}_{3}\left(\mathrm{O}_{36}\right)$ and $\mathrm{n}_{2}\left(\mathrm{O}_{37}\right)$ with energies, $-0.31933,-0.24010,-0.25443$, _-0.26721a.u and p-characters, 99.99, 99.99, 99.84, $99.97 \%$ and occupation numbers, $1.81466,1.86423,1.46681,1.89783$, respectively. On the other hand, the orbitals with lower energies and high occupancy are: $\mathrm{n}_{1}\left(\mathrm{O}_{13}\right), \mathrm{n}_{1}\left(\mathrm{O}_{17}\right), \mathrm{n}_{1}\left(\mathrm{O}_{36}\right)$, $\mathrm{n}_{1}\left(\mathrm{O}_{37}\right)$ with energies, $-0.54441,-0.77681,-0.66876,-0.77363 \mathrm{a} . \mathrm{u}$ and p -characters, $62.67,41.68$, $24.97,25.40 \%$ and occupation numbers, $1.96232,1.97565,1.98127,1.98091$, respectively.

Thus, a nearly pure p-type lone pair orbital is predicted to participate in the electron donation to the $\mathrm{n}_{1}\left(\mathrm{~N}_{1}\right) \rightarrow \pi^{*}\left(\mathrm{C}_{7}-\mathrm{C}_{8}\right), \mathrm{n}_{1}\left(\mathrm{~N}_{4}\right) \rightarrow \pi^{*}\left(\mathrm{C}_{16}-\mathrm{O}_{17}\right), \mathrm{n}_{2}\left(\mathrm{O}_{13}\right) \rightarrow \pi^{*}\left(\mathrm{C}_{9}-\mathrm{C}_{10}\right), \mathrm{n}_{2}\left(\mathrm{O}_{17}\right) \rightarrow$ $\sigma^{*}\left(\mathrm{~N}_{4}-\mathrm{C}_{16}\right), \mathrm{n}_{3}\left(\mathrm{O}_{36}\right) \rightarrow \pi^{*}\left(\mathrm{~N}_{15}-\mathrm{O}_{37}\right)$, and $\mathrm{n}_{2}\left(\mathrm{O}_{37}\right) \rightarrow \sigma^{*}\left(\mathrm{~N}_{15}-\mathrm{O}_{36}\right)$ interactions in MNPE.

### 4.5 Degradation Properties

The degradation properties based on the autoxidation and hydrolysis processes of MNPE can be perceived with the help of computed Bond Dissociation Energy (BDE) values as obtained by DFT calculations [50,53]. BDE is a valuable tool for primary assessment of molecular sites wherein the mechanism of autoxidation could initiate. In addition, the efficiency of autoxidation process is related to the abstraction of hydrogen atom by peroxy radical from the parent drug molecule [50, 51]. It is well established that all peroxy radicals have similar BDE values (in the
range of $87 \mathrm{kcal} / \mathrm{mol}$ to $92 \mathrm{kcal} / \mathrm{mol}$ ). When the calculated BDE for hydrogen abstraction is within this range, corresponding molecule location is considered a candidate for the initiation of the autoxidation process. BDE values for peroxy radicals can be considered independent of the chemical surrounding [51,52]. When the BDE value of the hydrogen abstraction is in the interval of $75 \mathrm{kcal} / \mathrm{mol}$ to $85 \mathrm{kcal} / \mathrm{mol}$, then the molecule is considered to be highly prone to autoxidation mechanism [50], [53]. BDE values of all single acyclic bonds of the title molecule are presented in Fig. 8. All BDE values related to hydrogen abstraction are higher than 92 $\mathrm{kcal} / \mathrm{mol}$, thus it indicates that MNPE is highly stable in open air, in the presence of oxygen. On the other hand, two BDE values for the rest of the single acyclic bonds were predicted to have values of $\sim 66$ and $\sim 69 \mathrm{kcal} / \mathrm{mol}$, indicating that acetyl-phenyl linkage and $\mathrm{O}-\mathrm{CH}_{3}$ bond are dissociable and hence can be a potential site of degradation.

Furthermore, molecular areas that are likely to interact with water molecules are presented in Fig. 9 in the form of Radial Distribution Functions (RDFs), which was obtained from MD simulations. RDF, $g(r)$, is the probability of finding a particle in the distance $r$ from another particle [54]. When the obtained curve profile is sharp and when its peak is at a short distance, it indicates interactions between certain atoms and water molecules.

RDF curves shown in Fig. 9a indicate that the carbon atoms $\mathrm{C}_{10}, \mathrm{C}_{14}$ and $\mathrm{C}_{18}$ could have pronounced interactions with water molecules. Atoms $\mathrm{C}_{10}$ and $\mathrm{C}_{18}$ are very similar in terms of the highest $g(r)$ value, ranging between 1.3 and 1.5 , and its peak distance, which has a value of around $3.6 \AA$. On the other side carbon atom $\mathrm{C}_{14}$ shows the highest $g(r)$ value of 1.1 and a peak distance at around $4.7 \AA$. Moreover, RDFs of non-carbon atoms demonstrate that they are more apt to interact with water molecules (Fig. 9b). The shortest peak distances in these cases were calculated to be for $\mathrm{N}_{1}$ and $\mathrm{O}_{17}$ atoms, both less than $3 \AA$, with maximal $g(r)$ values of around 0.5 and 1.0 , respectively. $\mathrm{O}_{36}$ and $\mathrm{O}_{37}$ are very similar in terms of $g(r)$ value and peak distance, around 1.1 and $3.2 \AA$, respectively. Nitrogen atom $\mathrm{N}_{15}$ has scored the highest $g(r)$ value of almost 1.4 , while its peak distance is located at somewhat less than $4 \AA$. Nitrogen atom $N_{4}$ has maximal $g(r)$ value of around 1.2 with its peak distance located at around $4.7 \AA$. The facts that BDE values are the lowest for bonds that connect nitro and methoxy moieties to the benzene ring and that RDF curves show pronounced interactions for certain atoms belonging to these groups, indicate the role of these groups in terms of degradability of the title molecule.

### 4.6 MNPE Docking Analysis towards GABA Receptor

### 4.6.1 Protein Structure Validation

The validation of the structure of the target protein is an essential step in the drug discovery process [55]. The crystal structure of a human GABA receptor (PDB ID: 4COF) shown in Fig. 10a was done at a resolution of $2.97 \AA$ and was used as the target protein [17]. The target protein contains 5 possible binding sites, namely chains A, B, C, D and E. Each chain is consisted of about five $\alpha$-helices which are spiral in shape and about $8 \beta$-pleated sheets. Ramachandran plot (Fig. 10b) checked a total number of 1574 residues out of the 1665 amino residues present in the target protein, which is tantamount to about $95 \%$ of the total residues. The total number of core regions and outliers are 1546 and 28 respectively, which gave a rise to a percentage outlier of 1.8 \%. The Ramachandran plot uses percentage outlier to ascertain the validity of a protein structure. The recommended range for the percentage outlier for a valid protein structure is between 0 to 5 \% [56]. Hence, the target protein (PDB ID: 4COF) used for the molecular docking was valid.

### 4.6.2 Molecular Docking

The five binding sites of the benzamidine molecule, $\mathrm{A}, \mathrm{B}, \mathrm{C}, \mathrm{D}$ and E , complexed with the crystal structure of the target protein (PDB ID: 4COF) were used for docking analysis. Benzamidine is an agonist for the human GABA receptor [17] and hence its binding site was used as a reference for the molecular docking analysis. Table 8 lists the molecular docking results of MNPE and human GABA receptor. Binding site C which is the reference site for BEN C in the original crystal structure had the highest total docking score value which implies that MNPE exhibits the best binding affinity towards the target protein within this binding site. The total score value is the sum of the Hydrogen Bond (HB) score, the Steric Interaction (SI) score and the ligand penalty conformation. It was also predicted that the ligand in binding cite C has the lowest ligand conformation penalty value (7.910) and the most negative steric interaction score (-59.781) among the different binding sites. Four types of favorable interactions (Fig. 11) between MNPE and the target protein could be established. The favorable interactions between MNPE and the target protein included conventional hydrogen bonding with amino acids THR 202 and GLN 64, $\pi-\pi$ stacking and $\pi$-alkyl interactions with PHE 200, TYR 62 and TYR 205, and carbon hydrogen bond with ASP 43 and TYR 157 (Fig. 11).

### 4.6.3 Binding Mode Conformation

The molecular docking analysis involved the generation of five low energy conformational scenarios known as poses. Unlike regular conformations, a pose structure is dependent on the target protein. Each pose was screened against the target protein in each of the five binding sites, A thru E . The binding mode conformation of MNPE in a binding site C have shown the best binding affinity (Fig. 11). During molecular docking analysis, the molecule orientated itself such that there exist an intramolecular hydrogen bonding between one of the hydrogens of the piperazine ring and the oxygen of the carbonyl group. The comparison between the most stable conformer (B4) and the best binding pose from the molecular docking analysis is depicted in Fig. 12. It was evident that the docked pose and most stable conformer of MNPE were similar with respect to the position of the various functional groups except that the structure of the binding pose is contracted due to the presence of intramolecular hydrogen bonding along with its orientation of the flexible bonds in the molecule. The difference in the structure of the binding pose arises because in molecular docking the molecule would orientate itself such that it best-fit into the binding pocket of the target protein to ensure adequate binding.

### 4.7 Vibrational Assignments of the IR and Raman Spectra

Understanding the detailed infrared and Raman features of newly synthesized, biologically active molecules is essential for tracing purposes as well as gaining advanced structural comprehension. In order to further elaborate on the vibrational spectral properties of MNPE, the calculated scaled wavenumbers, observed IR, Raman bands and detailed vibrational assignments for MNPE were provided in table 5.

### 4.7.1 $\mathrm{NO}_{2}$ Modes

According to literature, the $\mathrm{NO}_{2}$ stretching modes are normally observed in the regions $1580 \pm 80$ (asymmetric) and $1330 \pm 30 \mathrm{~cm}^{-1}$ (symmetric) [57]. For the title compound, $\mathrm{NO}_{2}$ stretching modes are assigned at $1502,1310 \mathrm{~cm}^{-1}$ in the IR spectrum, $1492,1308 \mathrm{~cm}^{-1}$ in the Raman spectrum and at $1495,1307 \mathrm{~cm}^{-1}$ theoretically with PEDs 66 and $49 \%$. Both the modes possess a high IR intensity and the mode at $1307 \mathrm{~cm}^{-1}$ has a high Raman activity. Mary et al. reported the $\mathrm{NO}_{2}$ stretching modes at 1429, 1363 (DFT), 1435, 1368 (IR), $1435,1367 \mathrm{~cm}^{-1}$ (Raman) [58]. The $\mathrm{NO}_{2}$ deformation modes (scissoring, out-of-plane wagging, in-plane rocking
and torsion) are expected in the regions $855 \pm 40,760 \pm 30,540 \pm 30$ and $70 \pm 20 \mathrm{~cm}^{-1}$, respectively [57]..The bands at $844,808 \mathrm{~cm}^{-1}$ in the Raman spectrum and 842,807 and $549 \mathrm{~cm}^{-1}$ (DFT) are assigned as the deformation modes of the $\mathrm{NO}_{2}$ group for MNPE. These modes have $39,38,47 \%$ PEDs, and the first two modes possess a medium-strength IR and Raman intensities. The reported values of the $\mathrm{NO}_{2}$ deformations modes are: 778, 692, 533 (DFT), 773, 522 (IR), $773 \mathrm{~cm}^{-1}$ (Raman) [58] and at 800, 727, 534 (DFT), 809, 727, $524 \mathrm{~cm}^{-1}$ (experimentally) [59].

### 4.7.2 C - O - C and $\mathrm{C}=\mathrm{O}$ Modes

The C-O-C stretching vibrations are expected in the ranges, 1310-1110 and 1050-1010 $\mathrm{cm}^{-1}$ [57], [60]. In the present case the C-O-C stretching modes are assigned at $1240,1008 \mathrm{~cm}^{-1}$ theoretically with a PED of $40 \%$ and correspond experimentally to $1010 \mathrm{~cm}^{-1}$ in the IR and 1242 $\mathrm{cm}^{-1}$ in the Raman spectra. Mary et al. reported the C-O-C stretching modes at 1042, 1016, 982 $\mathrm{cm}^{-1}$ in the IR spectrum and at $1135,1132,1080,1046,1012,984 \mathrm{~cm}^{-1}$ theoretically [61]. Moreover, the $\mathrm{C}=\mathrm{O}$ stretching mode in MNPE is assigned at $1648 \mathrm{~cm}^{-1}$ in $\mathrm{IR}, 1649 \mathrm{~cm}^{-1}$ in Raman and $1655 \mathrm{~cm}^{-1}$ theoretically which is in agreement with the expected region of 1750-1600 $\mathrm{cm}^{-1}$ [57], [62], [63]. The carbonyl stretching mode is predicted to have minimum coupling from other modes with a PED of $75 \%$ and of high infrared and Raman intensities [57].

### 4.7.3 $\mathrm{CH}_{3}$ Modes

For the title compound, the $\mathrm{CH}_{3}$ stretching modes are assigned at $2977,2944 \mathrm{~cm}^{-1}$ in the infrared spectrum, $3032,2950,2900 \mathrm{~cm}^{-1}$ in the Raman spectrum and in the range 3030-2895 $\mathrm{cm}^{-1}$ theoretically. The deformation modes of the methyl groups of the title compound are normally highly coupled with other bending modes. In MNPE the infrared bands observed at 1430 and $1339 \mathrm{~cm}^{-1}$ and Raman lines observed at $1428,1159,970 \mathrm{~cm}^{-1}$ are assigned as $\mathrm{CH}_{3}$ deformations (Table 5). The corresponding theoretical values are in the range $1443-1338 \mathrm{~cm}^{-1}$ (scissoring) and 1161-967 $\mathrm{cm}^{-1}$ (rocking) [57].

### 4.7.4 C-N and C-C Modes

The C-N stretching modes are observed in the range $1300-1000 \mathrm{~cm}^{-1}$ [62], [63] and for the title compound these modes are assigned at $1178,1073 \mathrm{~cm}^{-1}$ in the IR spectrum, 1224, 1077 $\mathrm{cm}^{-1}$ in the Raman spectrum and at $1220,1185,1074 \mathrm{~cm}^{-1}$ theoretically with PEDs of 44,41 and
$36 \%$, respectively. El-Azab et al. [43] reported the C-N stretching modes at 1232, 1146, 1108, $966 \mathrm{~cm}^{-1}$ in the IR spectrum, $1232,1149,1108,967 \mathrm{~cm}^{-1}$ in the Raman spectrum and in the range $1235-965 \mathrm{~cm}^{-1}$ theoretically. For the title compound the C-C stretching mode is assigned at 904 $\mathrm{cm}^{-1}$ in the Raman spectrum and at $902 \mathrm{~cm}^{-1}$ theoretically as expected [63].

### 4.7.5 Piperazine Ring Modes

The $\mathrm{CH}_{2}$ stretching modes in the piperazine ring could be assigned to $3014,2977,2876$, $2820 \mathrm{~cm}^{-1}$ in the infrared and to 2999, 2981, 2885, $2843 \mathrm{~cm}^{-1}$ in the Raman spectra. El-Emam et al. [39] reported stretching $\mathrm{CH}_{2}$ vibrations in the piperazine ring in the range $3033-2834 \mathrm{~cm}^{-1}$. Renjith et al. reported the $\mathrm{CH}_{2}$ stretching modes in the piperazine ring at 2986, 2976, 2965, 2923 $\mathrm{cm}^{-1}$ (theoretical), 2990, $2923 \mathrm{~cm}^{-1}$ (IR) and 2988, $2924 \mathrm{~cm}^{-1}$ (Raman) [64]. The corresponding deformation modes could be assigned to $1363,1202,1178,1092,1036,829 \mathrm{~cm}^{-1}$ in the IR and to 1452, 1364, 1333, 1242, 1224, 1196, 1091, 1040, $829 \mathrm{~cm}^{-1}$ in the Raman spectra. According to H. L. Spell [65] the $\mathrm{CH}_{2}$ deformation modes associated with the piperazine ring are usually observed ass harp, well defined absorptions at $1380-1345 \mathrm{~cm}^{-1}, 125-1170 \mathrm{~cm}^{-1}$ and $1050-1025$ $\mathrm{cm}^{-1}$ regions in the infrared spectrum. It prompted us to assign the strong peak observed in the infrared spectrum of MNPE at $1363 \mathrm{~cm}^{-1}$ (Fig. 2) to the $\mathrm{CH}_{2}$ wagging mode of the piperazine ring in agreement with the DFT value ( $1361 \mathrm{~cm}^{-1}$ ). Furthermore, another intense band observed at $1036 \mathrm{~cm}^{-1}$ in the IR spectrum was assigned to the ring $\mathrm{CH}_{2}$ rocking vibration, in consistency with previously reported finding da Silva et al. [66]. According to H. L. Spell [65], this is one of the key bands useful for the detection of the presence of di-substituted piperazines.

Piperazine ring stretching modes in MNPE could be observed at 1131, 960, $697 \mathrm{~cm}^{-1}$ in the ir spectrum and at $1196,1018,954,904,701 \mathrm{~cm}^{-1}$ in the Raman spectrum. The PED analysis predicts these modes in the range $1193-699 \mathrm{~cm}^{-1}$. Renjith et al. reported the piperazine ring stretching modes $1099,985,930 \mathrm{~cm}^{-1}$ in the IR spectrum, 1100, $924,905 \mathrm{~cm}^{-1}$ in the Raman spectrum and $1159,1098,1020,982,924$ and $914 \mathrm{~cm}^{-1}$ theoretically [64]. The C-C stretching vibrations in the piperaizne ring were previously reported at 972 and $903 \mathrm{~cm}^{-1}$ [58] while the C N stretching modes were reported in the range $1154-756 \mathrm{~cm}^{-1}$ [58].

### 4.7.6 Phenyl Ring Modes

The C-H stretching modes of the phenyl ring are expected above $3000 \mathrm{~cm}^{-1}$ [57] and, for the title compound, the bands at $3118,3078 \mathrm{~cm}^{-1}$ (Raman) and 3120, 3094, $3081 \mathrm{~cm}^{-1}$ (DFT) are assigned as the $\mathrm{C}-\mathrm{H}$ stretching vibrations of the phenyl ring. The phenyl ring stretching modes are assigned at $1574,1550,1476,1288 \mathrm{~cm}^{-1}$ in the IR spectrum, $1577,1392 \mathrm{~cm}^{-1}$ in the Raman spectrum and at $1576,1553,1473,1391,1291 \mathrm{~cm}^{-1}$ theoretically which are expected in the region 1250-1600 $\mathrm{cm}^{-1}$ [57]. The ring breathing mode of the phenyl ring is assigned at $1010 \mathrm{~cm}^{-1}$ in the IR spectrum and at $1008 \mathrm{~cm}^{-1}$ theoretically as reported in literature [57], [67]. The reported values of the ring breathing mode of poly substituted phenyl ring at $978 \mathrm{~cm}^{-1}$ [43], $1025 \mathrm{~cm}^{-1}$ in the Raman spectrum and $1032 \mathrm{~cm}^{-1}$ theoretically [68]. The C-H in-plane and out-of-plane bendings of the phenyl ring are expected above and below $1000 \mathrm{~cm}^{-1}$ [57] and for the title compound, the bands at $1258,1073 \mathrm{~cm}^{-1}$ (IR), $1077 \mathrm{~cm}^{-1}$ (Raman), 1257, 1146, $1074 \mathrm{~cm}^{-1}$ (DFT) and $932,801 \mathrm{~cm}^{-1}$ (IR), $935,799,789 \mathrm{~cm}^{-1}$ (DFT) are assigned as the in-plane and out-of-plane CH deformation modes, respectively.

## 5. Conclusions

The synthesis and vibrational spectroscopic properties of 1-(4-(3-methoxy-4-nitrophenyl)piperazin-1-yl)ethanone (MNPE) have been accomplished. MEP studies revealed the most reactive sites in the molecule. The low HOMO-LUMO energy gap is associated with the high chemical reactivity and existence of charge transfer within the molecule. ALIE surfaces indicated that significant reactive centers, prone to electrophilic attacks, could be located at carbon atoms of the benzene ring, nitrogen atom of the piperazine moiety and oxygen atoms of the nitro group. Bond dissociation energy values of hydrogen abstraction indicated that MNPE is highly stable in open air and in the presence of oxygen, however, the calculated RDF curves revealed that MNPE is dissociable in aqueous systems. The molecular docking analysis showed that binding site C had the best binding affinity towards the GABA receptor

## Acknowledgment

Experimental and computational resources provided by King Fahd University of Petroleum and Minerals (KFUPM) to support this work are appreciated. Support received from Schrödinger Inc. is appreciated. Part of this study was conducted within the projects supported by the Ministry of

Education, Science and Technological Development of Serbia, grant numbers OI 171039 and TR 34019 (project period 2016-2019/20).

## References

[1] J. Cai, Y. Lu, L. Gan, Y. Zhang, and C. Zhou, "Recent advance in the research of piperazine-containing compounds as antimicrobial agents," Chin. J. Antibiot, 8 (2009) 3.
[2] L. L. Gan, Y. H. Lu, and Zhou C.H., "Advances in the research of piperazine compounds as enzyme inhibitors," Chin. J. Biochem. Pharm, vol. 30, no. 6, pp. 127-131, 2009.
[3] Y.-J. Shaw, Y.-T. Yang, J. B. Garrison, N. Kyprianou, and C.-S. Chen, "Pharmacological Exploitation of the $\alpha 1$-Adrenoreceptor Antagonist Doxazosin to Develop a Novel Class of Antitumor Agents That Block Intracellular Protein Kinase B/Akt Activation," J. Med. Chem., vol. 47, no. 18, pp. 4453-4462, 2004.
[4] J. Chen, M. Lu, Y. Jing, and J. Dong, "The synthesis of L-carvone and limonene derivatives with increased antiproliferative effect and activation of ERK pathway in prostate cancer cells," Bioorg. Med. Chem., 14 (2006) 6539-6547.
[5] S. Richter, C. Parolin, and M. Palumbo, "Antiviral properties of quinolone-based drugs," Drug Targets-Infectious, 4 (2004) 111-116.
[6] W. J. Watkins, L. Chong, A. Cho, R. Hilgenkamp, M. Ludwikow, N. Garizi, N. Iqbal, J. Barnard, R. Singh, D. Madsen, K. Lolans, O. Lomovskaya, U. Oza, P. Kumaraswamy, A. Blecken, S. Bai, D. J. Loury, D. C. Griffith, and M. N. Dudley, "Quinazolinone fungal efflux pump inhibitors. Part 3: (N-methyl)piperazine variants and pharmacokinetic optimization," Bioorg. Med. Chem. Lett., vol. 17, no. 10, pp. 2802-2806, May 2007.
[7] M. Kimura, T. Masuda, K. Yamada, N. Kawakatsu, N. Kubota, M. Mitani, K. Kishii, M. Inazu, Y. Kiuchi, K. Oguchi, and T. Namiki, "Antioxidative activities of novel diphenylalkyl piperazine derivatives with high affinities for the dopamine transporter," Bioorg. Med. Chem. Lett., vol. 14, no. 16, pp. 4287-4290, 2004.
[8] R. A. Smits, H. D. Lim, A. Hanzer, O. P. Zuiderveld, E. Guaita, M. Adami, G. Coruzzi, R. Leurs, and I. J. P. Esch, "Fragment Based Design of New H 4 Receptor-Ligands with Anti-inflammatory Properties in Vivo," J. Med. Chem., vol. 51, no. 8, pp. 2457-2467, 2008.
[9] W. Cunico, C. R. B. Gomes, M. Moreth, D. P. Manhanini, I. H. Figueiredo, C. Penido, M. G. M. O. Henriques, F. P. Varotti, and A. U. Krettli, "Synthesis and antimalarial activity of hydroxyethylpiperazine derivatives," Eur. J. Med. Chem., vol. 44, no. 3, pp. 13631368, 2009.
[10] J. Penjišević, V. Šukalović, D. Andrić, S. Kostić-Rajačić, V. Šoškić, and G. Roglić, "1-Cinnamyl-4-(2-methoxyphenyl)piperazines," Arch. Pharm. (Weinheim)., vol. 340, no. 9, pp. 456-465, 2007.
[11] D. S. Wishart, C. Knox, A. C. Guo, S. Shrivastava, M. Hassanali, P. Stothard, Z. Chang, and J. Woolsey, "DrugBank: a comprehensive resource for in silico drug discovery and exploration.," Nucleic Acids Res., vol. 34, no. Database issue, pp. D668-D672, 2006.
[12] A. Golubović, B. Abramović, M. Šćepanović, M. Grujić-Brojčin, S. Armaković, I.

Veljković, B. Babić, Z. Dohčević-Mitrović, and Z. V Popović, "Improved efficiency of sol-gel synthesized mesoporous anatase nanopowders in photocatalytic degradation of metoprolol," Mater. Res. Bull., vol. 48, no. 4, pp. 1363-1371, 2013.
[13] R. Ma, B. Wang, S. Lu, Y. Zhang, L. Yin, J. Huang, S. Deng, Y. Wang, and G. Yu, "Characterization of pharmaceutically active compounds in Dongting Lake, China: Occurrence, chiral profiling and environmental risk," Sci. Total Environ., vol. 557-558, pp. 268-275, Jul. 2016.
[14] S. J. Armaković, S. Armaković, N. L. Finčur, F. Šibul, D. Vione, J. P. Šetrajčić, and B. F. Abramović, "Influence of electron acceptors on the kinetics of metoprolol photocatalytic degradation in TiO 2 suspension. A combined experimental and theoretical study," RSC $A d v$, vol. 5, no. 67, pp. 54589-54604, 2015.
$[15] \quad$ D. Šojić, V. Despotović, D. Orčić, E. Szabó, E. Arany, S. Armaković, E. Illés, K. GajdaSchrantz, A. Dombi, T. Alapi, E. Sajben-Nagy, A. Palágyi, C. Vágvölgyi, L. Manczinger, L. Bjelica, and B. Abramović, "Degradation of thiamethoxam and metoprolol by UV, O3 and UV/O3 hybrid processes," J. Hydrol., vol. 472-473, pp. 314-327, 2012.
$[16]$ B. Abramović, S. Kler, D. Šojić, M. Laušević, T. Radović, and D. Vione, "Photocatalytic degradation of metoprolol tartrate in suspensions of two $\mathrm{TiO}_{2}$-based photocatalysts with different surface area. Identification of intermediates and proposal of degradation pathways," J. Hazard. Mater., vol. 198, pp. 123-132, 2011.
[17] P. S. Miller and A. R. Aricescu, "Crystal structure of a human GABAA receptor," Nature, 512 (2014) 270-275.
[18] P. R. Schofield, M. G. Darlison, N. Fujita, D. R. Burt, F. A. Stephenson, H. Rodriguez, L. M. Rhee, J. Ramachandran, V. Reale, T. A. Glencorse, P. H. Seeburg, and E. A. Barnard, "Sequence and functional expression of the GABAA receptor shows a ligand-gated receptor super-family," Nature, vol. 328, no. 6127, pp. 221-227, Jul. 1987.
[19] W. Sieghart, "GABAA receptors: ligand-gated $\mathrm{Cl}-$ ion channels modulated by multiple drug-binding sites," Trends Pharmacol. Sci., vol. 13, pp. 446-450, Jan. 1992.
$[20]$ a) A. J. Pawson, J. L. Sharman, H. E. Benson, E. Faccenda, S. P. H. Alexander, O. P. Buneman, A. P. Davenport, J. C. McGrath, J. A. Peters, C. Southan, M. Spedding, W. Yu, and A. J. Harmar, "The IUPHAR/BPS Guide to PHARMACOLOGY: An expert-driven knowledgebase of drug targets and their ligands," Nucleic Acids Res., vol. 42, no. D1, pp. 1098-1106, 2014. b) A. O. Ba-Salema, M. N. Shaikh, N. Ullah and M. Faiz, "Synthesis and magnetic relaxation properties of new Gd(III) complexes derived from DTPAbis(amide) conjugates of arylpiperazinyl amines," Inorg. Chem. Commun., vol. 56, pp. 57, 2015.
[21] G. E. S. M. J. Frisch, G. W. Trucks, H. B. Schlegel, B. M. M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, H. P. H. G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, M. H. A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, T. N. M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, J. Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, E. B. J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, J. N. K. N. Kudin, V. N. Staroverov, T. Keith, R. Kobayashi, J. T. K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. B. C. M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, R. E. S. V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, J. W. O. O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, G. A. V. R. L. Martin, K. Morokuma, V. G. Zakrzewski, A. D. D. P. Salvador, J. J. Dannenberg, S. Dapprich, J. C. O. Farkas, J. B. Foresman, J. V. Ortiz, and and D. J. Fox, "Gaussian 09, Revision D.01," Gaussian, Inc. Wallingford CT. 2013.
[22] A. Irfan, R. Jin, A. G. Al-Sehemi, and A. M. Asiri, "Quantum chemical study of the donor-bridge-acceptor triphenylamine based sensitizers," Spectrochim. Acta Part A Mol. Biomol. Spectrosc., vol. 110, pp. 60-66, 2013.
[23] A. Irfan, A. G. Al-Sehemi, and M. S. Al-Assiri, "The effect of donors-acceptors on the charge transfer properties and tuning of emitting color for thiophene, pyrimidine and oligoacene based compounds," J. Fluor. Chem., vol. 157, pp. 52-57, 2014.
[24] E. F. J.B. Foresman, Exploring Chemistry with Electronic Structure Methods: A Guide to Using Gaussian, no. 1 TS-CrossRef Metadata Search. Pittsburg: Gaussian Inc., 1996.
[25] A. A. Al-Saadi and J. Laane, "Ab initio and DFT calculations for the structure and vibrational spectra of cyclopentene and its isotopomers," J. Mol. Struct., vol. 830, no. 1-3, pp. 46-57, 2007.
[26] R. Dennington, T. Keith, and J. Millam, "GaussView." Semichem Inc, Shawnee Mission KS, 2009.
[27] C. V. A. J.M.L. Martin, "GAR2PED," vol. 44, no. 2. University of Antwerp, Belgium, 2007.
[28] A. D. Bochevarov, E. Harder, T. F. Hughes, J. R. Greenwood, D. A. Braden, D. M. Philipp, D. Rinaldo, M. D. Halls, J. Zhang, and R. A. Friesner, "Jaguar: A highperformance quantum chemistry software program with strengths in life and materials sciences," Int. J. Quantum Chem., vol. 113, no. 18, pp. 2110-2142, 2013.
[29] D. Shivakumar, J. Williams, Y. Wu, W. Damm, J. Shelley, and W. Sherman, "Prediction of Absolute Solvation Free Energies using Molecular Dynamics Free Energy Perturbation and the OPLS Force Field," J. Chem. Theory Comput., vol. 6, no. 5, pp. 1509-1519, 2010.
[30] Z. Guo, U. Mohanty, J. Noehre, T. K. Sawyer, W. Sherman, and G. Krilov, "Probing the $\alpha-$ Helical Structural Stability of Stapled p53 Peptides: Molecular Dynamics Simulations and Analysis," Chem. Biol. Drug Des., vol. 75, no. 4, pp. 348-359, Apr. 2010.
[31] K. J. Bowers, F. D. Sacerdoti, J. K. Salmon, Y. Shan, D. E. Shaw, E. Chow, H. Xu, R. O. Dror, M. P. Eastwood, B. A. Gregersen, J. L. Klepeis, I. Kolossvary, and M. A. Moraes, "Molecular dynamics---Scalable algorithms for molecular dynamics simulations on commodity clusters," in Proceedings of the 2006 ACMIIEEE conference on Supercomputing - SC '06, 2006, p. 84.
[32] D. E. Shaw Research, "Schrödinger Release 2015-4: Desmond Molecular Dynamics System." 2015.
[33] A. D. Becke, "Density-functional thermochemistry. III. The role of exact exchange," $J$. Chem. Phys., vol. 98, no. 7, pp. 5648-5652, 1993.
[34] J. L. Banks, H. S. Beard, Y. Cao, A. E. Cho, W. Damm, R. Farid, A. K. Felts, T. A. Halgren, D. T. Mainz, J. R. Maple, R. Murphy, D. M. Philipp, M. P. Repasky, L. Y. Zhang, B. J. Berne, R. A. Friesner, E. Gallicchio, and R. M. Levy, "Integrated Modeling Program, Applied Chemical Theory (IMPACT)," J. Comput. Chem., vol. 26, no. 16, pp. 1752-1780, 2005.
[35] H. J. C. Berendsen, J. P. M. Postma, W. F. van Gunsteren, and J. Hermans, "Interaction Models for Water in Relation to Protein Hydration," in Intermolecular Forces, vol. 14, B. Pullman, Ed. Dordrecht: Springer Netherlands, 1981, pp. 331-342.
[36] T. Knudsen, Bjarne Knudsen, "CLC Drug Discovery Workbench." pp. 1-367, 2016.
[37] G. J. Kleywegt and T. A. Jones, "Phi/Psi-chology," Structure, vol. 4, no. 12, pp. 13951400, 1996.
[38] I. P. Holmes, Y. Bergman, G. E. Lunniss, M. Nikac, N. Chol, C. F. Hemley, S. R. Walker,
R. C. Foitzik, D. Ganame, and R. Lessene, US 20130022594, 2013.
[39] A. A. El-Emam, A.-M. S. Al-Tamimi, K. A. Al-Rashood, H. N. Misra, V. Narayan, O. Prasad, and L. Sinha, "Structural and spectroscopic characterization of a novel potential chemotherapeutic agent 3-(1-adamantyl)-1-\{[4-(2-methoxyphenyl)piperazin-1yl]methyl $\}$-4-methyl-1H-1,2,4-triazole- $5(4 \mathrm{H})$-thione by first principle calculations," $J$. Mol. Struct., vol. 1022, pp. 49-60, 2012.
[40] K. S. Resmi, Y. S. Mary, H. T. Varghese, C. Y. Panicker, M. Pakosińska-Parys, and C. van Alsenoy, "Spectral investigations, DFT computations and molecular docking studies of 1,7,8,9-tetrachloro-10,10-dimethoxy-4-\{3-[4-(2-methylphenyl)piperazin-1-yl]propyl\}-4-azatricyclo[5.2.1.02,6]dec-8-ene-3,5-dione," J. Mol. Struct., vol. 1098, pp. 130-145, 2015.
[41] Z. Karczmarzy and W. Malinka, "Crystal Structure of 4,6-Dimethyl-2-[4-(2,3-dichlorophenyl)piperazin-1-ylmethyl]isothiazolo[5,4-b]pyridin-3(2H)-one," Anal. Sci. Xray Struct. Anal. Online, vol. 23, pp. x151-x152, 2007.
[42] Y. Sheena Mary, T. S. Yamuna, C. Y. Panicker, H. S. Yathirajan, M. S. Siddegowda, A. a Al-Saadi, C. Van Alsenoy, and J. A. War, "Vibrational spectroscopic studies and molecular docking of 10,10-Dimethylanthrone.," Spectrochim. Acta. A. Mol. Biomol. Spectrosc., vol. 135, pp. 652-61, 2015.
[43] A. S. El-Azab, Y. Sheena Mary, C. Yohannan Panicker, A. A.-M. Abdel-Aziz, M. A. ElSherbeny, and C. van Alsenoy, "DFT and experimental (FT-IR and FT-Raman) investigation of vibrational spectroscopy and molecular docking studies of 2-(4-oxo-3-phenethyl-3,4-dihydroquinazolin-2-ylthio)-N-(3,4,5-trimethoxyphenyl) acetamide," $J$. Mol. Struct., vol. 1113, pp. 133-145, 2016.
[44] Y. Sheena Mary, K. Raju, C. Y. Panicker, A. A. Al-Saadi, T. Thiemann, and C. van Alsenoy, "Molecular conformational analysis, vibrational spectra, NBO analysis and first hyperpolarizability of (2E)-3-phenylprop-2-enoic anhydride based on density functional theory calculations," Spectrochim. Acta Part A Mol. Biomol. Spectrosc., vol. 128, pp. 638-646, 2014.
[45] C. Y. Panicker, H. T. Varghese, P. S. Nayak, B. Narayana, B. K. Sarojini, H. K. Fun, J. A. War, S. K. Srivastava, and C. van Alsenoy, "Infrared spectrum, NBO, HOMO-LUMO, MEP and molecular docking studies (2E)-3-(3-nitrophenyl)-1-[4-piperidin-1-yl]prop-2-en-1-one," Spectrochim. Acta Part A Mol. Biomol. Spectrosc., vol. 148, pp. 18-28, 2015.
[46] P. Sjoberg, J. S. Murray, T. Brinck, and P. Politzer, "Average local ionization energies on the molecular surfaces of aromatic systems as guides to chemical reactivity," Can. J. Chem., vol. 68, no. 8, pp. 1440-1443, 1990.
[47] P. Politzer, F. Abu-Awwad, and J. S. Murray, "Comparison of density functional and Hartree-Fock average local ionization energies on molecular surfaces," Int. J. Quantum Chem., vol. 69, no. 4, pp. 607-613, 1998.
[48] A. Michalak, F. Proft, P. Geerlings, and R. F. Nalewajski, "Fukui Functions from the Relaxed Kohn-Sham Orbitals," J. Phys. Chem. A, vol. 103, no. 6, pp. 762-771, 1999.
[49] A. E. R. E.D. Glendening J.E. Carpenter, F.Weinhold, "NBO Version 3.1," vol. 81 TS-C, no. 32. Gaussian Inc., 2003.
[50] A. B. T. Andersson E. Evertsson, "Prediction of drug candidates sensitivity toward autoxidation:Computational estimation of $\mathrm{C}-\mathrm{H}$ dissociation energies of carbon centered radicals," J. Pharm. Sci., vol. 103, p. 1949-1955.
[51] P. Lienard, J. Gavartin, G. Boccardi, and M. Meunier, "Predicting drug substances
autoxidation," Pharm. Res., vol. 32, no. 1, pp. 300-310, 2015.
[52] Y.-R. Luo, Handbook of bond dissociation energies in organic compounds. Boca Raton, FL: CRC Press, 2003.
[53] J. S. Wright, H. Shadnia, and L. L. Chepelev, "Stability of carbon-centered radicals: Effect of functional groups on the energetics of addition of molecular oxygen," J. Comput. Chem., vol. 30, no. 7, pp. 1016-1026, May 2009.
[54] R. V. Vaz, J. R. B. Gomes, and C. M. Silva, "Molecular dynamics simulation of diffusion coefficients and structural properties of ketones in supercritical $\mathrm{CO}_{2}$ at infinite dilution," $J$. Supercrit. Fluids, vol. 107, pp. 630-638, Jan. 2016.
[55] M. E. Bunnage, A. M. Gilbert, L. H. Jones, and E. C. Hett, "Know your target, know your molecule," Nat. Chem. Biol., vol. 11, no. 6, pp. 368-372, 2015.
[56] G. J. Kleywegt and T. A. Jones, "Phi/Psi-chology: Ramachandran revisited," Structure, vol. 4, no. 12, pp. 1395-1400, 1996.
[57] N. P. G. Roeges, A guide to the complete interpretation of infrared spectra of organic structures. Wiley, 1994.
[58] Y. Sheena Mary, K. Raju, T. E. Bolelli, I. Yildiz, H. I. S. Nogueira, C. M. Granadeiro, and C. van Alseony, "FT-IR, FT-Raman, surface enhanced Raman scattering and computational study of 2-(p-fluorobenzyl)-6-nitrobenzoxazole," J. Mol. Struct., vol. 1012, pp. 22-30, 2012.
[59] H. T. V. C.Y. Panicker L. Usha Kumari, T. Ertan, I. Yildiz, C.M. Granadeiro, H.I.S. Nogueira, Y.S. Mary, "FT-IR, FT-Raman, SERS spectra and Computational calculations of 4-Ethyl-N-(2'-hydroxy-5'-nitrophenyl)benzamide," J. Raman Spectrosc., vol. 41, p. 381-390., 2010.
[60] B. Smith, Infrared spectral interpretation:A systematic approach, vol. 36 TS-C, no. 10. Washington, DC: CRC Press, 1999.
[61] Y. Sheena Mary, C. Yohannan Panicker, P. L. Anto, M. Sapnakumari, B. Narayana, and B. K. Sarojini, "Molecular structure, FT-IR, NBO, HOMO and LUMO, MEP and first order hyperpolarizability of (2E)-1-(2,4-Dichlorophenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one by HF and density functional methods," Spectrochim. Acta Part A Mol. Biomol. Spectrosc., vol. 135, pp. 81-92, 2015.
[62] L. H. D. N.B. Colthup S.E. Wiberly, Introduction to IR and Raman Spectroscopy. New York: Academic Press TS - CrossRef Metadata Search M4 - Citavi, 1990.
[63] R. M. (Robert M. Silverstein, F. X. Webster, D. J. Kiemle, and D. L. (David L. Bryce, Spectrometric identification of organic compounds. .
[64] R. Renjith, Y. Sheena Mary, C. Yohannan Panicker, H. T. Varghese, M. PakosinskaParys, C. van Alsenoy, and A. A. Al-Saadi, "Spectroscopic (FT-IR, FT-Raman) investigations and quantum chemical calculations of 1,7,8,9-tetrachloro-10,10-dimethoxy-4-\{3-4-(3-methox yphenyl)piperazin-1-ylpropy 1\}-4-azatricyclo5.2.1.0(2,6)dec-8-ene-3,5dione," Spectrochim. Acta. A. Mol. Biomol. Spectrosc., vol. 129, pp. 438-450, 2014.
[65] H. L. Spell, "Determination of piperazine rings in ethyleneamines, poly(ethyleneamine), and polyethylenimine by infrared spectrometry," Anal. Chem., vol. 41, no. 7, pp. 902-905, 2002.
[66] S. P. M.A.S. da Silva T. Francisco, F.O.N. Da Silva, A.A. Batista, J. Ellena, I.M.M. Varvalho, J.R. de Sousa, F.A. Dias-Filho, E. Longhinotti, I.C.N. Diogenes, "A novel and simple synthetic route for a piperazine derivative," J. Braz. Chem. Soc, vol. 21, pp. 17541759.
[67] G. Varsanyi, Assignments for Vibrational Spectra of Seven Hundred Benzene Derivatives, vol. 51 TS-C, no. 2. New York: Wiley, 1974.
[68] A. Raj, Y. Sheena Mary, C. Yohannan Panicker, H. T. Varghese, and K. Raju, "IR, Raman, SERS and computational study of 2-(benzylsulfanyl)-3,5-dinitrobenzoic acid," Spectrochim. Acta. A. Mol. Biomol. Spectrosc., vol. 113, pp. 28-36, 2013.

## LIST OF TABLES

Table 1. Conformations and relative energies of the title compound

| Conformation | Relative energy (kcal/mol) |  |
| :---: | :---: | :---: |
| Computational Method | B3LYP/6-311G(d,p) | B3LYP/6-311++G(d,p) |
| A1 | 0.127 | 0.137 |
| A2 | 0.107 | 0.126 |
| A3 | 0.434 | 0.474 |
| A4 | 0.174 | 0.188 |
| B1 | 0.283 | 0.298 |
| B2 | 0.286 | 0.319 |
| B3 | 0.258 | 0.29 |
| B4 | 0.000 | 0.000 |
| C1 | 1.271 | 1.591 |
| C2 | 1.5 | 1.837 |
| C3 | 1.627 | 1.975 |
| C4 | 0.89 | 1.236 |
| D1 | 1.205 | 1.523 |
| D2 | 1.475 | 1.811 |
| D3 | 1.645 | 1.995 |
| D4 | 0.972 | 1.318 |
|  |  |  |

Table 2. Bond lengths of selected atoms

| Models | $\mathrm{N}_{15}-\mathrm{O}_{37}$ | $\mathrm{~N}_{1}-\mathrm{C}_{6}$ | $\mathrm{C}_{9}-\mathrm{O}_{13}$ | $\mathrm{O}_{13}-\mathrm{O}_{37}$ | $\mathrm{~N}_{1}-\mathrm{C}_{7}$ | $\mathrm{~N}_{4}-\mathrm{C}_{16}$ | $\mathrm{C}_{16}-\mathrm{O}_{17}$ |
| :--- | ---: | ---: | ---: | ---: | ---: | ---: | ---: |
| A1 | 1.224 | 1.462 | 1.346 | 2.665 | 1.399 | 1.378 | 1.222 |
|  | $(1.223)$ | $(1.464)$ | $(1.346)$ | $(2.64)$ | $(1.398)$ | $(1.379)$ | $(1.22)$ |
| A2 | 1.225 | 1.461 | 1.345 | 2.644 | 1.398 | 1.377 | 1.222 |
|  | $(1.223)$ | $(1.464)$ | $(1.345)$ | $(2.622)$ | $(1.397)$ | $(1.379)$ | $(1.22)$ |
| A3 | 1.232 | 1.463 | 1.347 | 2.651 | 1.399 | 1.378 | 1.222 |
|  | $(1.23)$ | $(1.465)$ | $(1.346)$ | $(2.627)$ | $(1.398)$ | $(1.378)$ | $(1.219)$ |
| A4 | 1.232 | 1.46 | 1.346 | 2.657 | 1.397 | 1.378 | 1.222 |
|  | $(1.231)$ | $(1.463)$ | $(1.346$ | $(2.634)$ | $(1.396)$ | $(1.379)$ | $(1.219)$ |
| B1 | 1.224 | 1.462 | 1.347 | 2.666 | 1.399 | 1.378 | 1.222 |
|  | $(1.223$ | $(1.465)$ | $(1.347)$ | $(2.64)$ | $(1.399)$ | $(1.379)$ | $(1.219)$ |
| B2 | 1.225 | 1.461 | 1.346 | 2.643 | 1.397 | 1.378 | 1.222 |
|  | $(1.223$ | $(1.464$ | $(1.346$ | $(2.621)$ | $(1.397)$ | $(1.379)$ | $(1.219)$ |
| B3 | 1.232 | 1.462 | 1.346 | 2.651 | 1.398 | 1.378 | 1.222 |
|  | $(1.223$ | $(1.465)$ | $(1.346)$ | $(2.627)$ | $(1.398)$ | $(1.379)$ | $(1.22)$ |
| B4 | 1.232 | 1.46 | 1.346 | 2.657 | 1.397 | 1.377 | 1.222 |
|  | $(1.231)$ | $(1.463$ | $(1.345$ | $(2.635)$ | $(1.397)$ | $(1.379)$ | $(1.22)$ |
| C1 | 1.23 | 1.456 | 1.353 | 2.888 | 1.394 | 1.378 | 1.222 |
|  | $(1.229)$ | $(1.458)$ | $(1.354)$ | $(2.864)$ | $(1.393$ | $(1.379)$ | $(1.219)$ |
| C2 | 1.23 | 1.456 | 1.352 | 2.89 | 1.396 | 1.378 | 1.222 |
|  | $(1.229)$ | $(1.458)$ | $(1.353)$ | $(2.865)$ | $(1.395)$ | $(1.379)$ | $(1.22)$ |
| C3 | 1.23 | 1.456 | 1.354 | 2.882 | 1.393 | 1.378 | 1.222 |
|  | $(1.228)$ | $(1.458)$ | $(1.355)$ | $(2.858)$ | $(1.392)$ | $(1.379)$ | $(1.219)$ |
| C4 | 1.23 | 1.455 | 1.352 | 2.896 | 1.394 | 1.378 | 1.222 |
|  | $(1.228$ | $(1.457)$ | $(1.352)$ | $(2.877)$ | $(1.393)$ | $(1.379)$ | $(1.22)$ |
| D1 | 1.23 | 1.456 | 1.353 | 2.888 | 1.394 | 1.378 | 1.222 |
|  | $(1.229$ | $(1.458$ | $(1.354)$ | $(2.864)$ | $(1.393)$ | $(1.379)$ | $(1.219)$ |
| D2 | 1.23 | 1.456 | 1.353 | 2.888 | 1.396 | 1.378 | 1.222 |
|  | $(1.229)$ | $(1.458)$ | $(1.353)$ | $(2.864)$ | $(1.395)$ | $(1.379)$ | $(1.22)$ |
| D3 | 1.23 | 1.456 | 1.354 | 2.882 | 1.393 | 1.378 | 1.222 |
|  | $(1.228)$ | $(1.458)$ | $(1.354)$ | $(2.859)$ | $(1.392)$ | $(1.379)$ | $(1.219)$ |
| D4 | 1.23 | 1.455 | 1.352 | 2.897 | 1.394 | 1.378 | 1.222 |
|  | $(1.228)$ | $(1.457)$ | $(1.352)$ | $(2.877)$ | $(1.394$ | $(1.379)$ | $(1.22)$ |
|  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |

Table 3. Bond angles of selected atoms of the conformation

| Model | $\mathrm{O}_{1}-\mathrm{N}_{2}-\mathrm{O}_{3}$ | $\mathrm{O}_{3}-\mathrm{N}_{2}-\mathrm{C}_{4}$ | $\mathrm{C}_{8}-\mathrm{C}_{7}-\mathrm{N}_{10}$ | $\mathrm{C}_{15}-\mathrm{N}_{10}-\mathrm{C}_{11}$ | $\mathrm{C}_{12}-\mathrm{N}_{13}-\mathrm{C}_{16}$ | $\mathrm{O}_{17}-\mathrm{C}_{16}-\mathrm{C}_{18}$ |
| ---: | ---: | ---: | ---: | ---: | ---: | ---: |
| A1 | 124.2 | 117 | 122.1 | 112.1 | 119.6 | 121 |
|  | $(124.4)$ | $(116.9)$ | $(122.2)$ | $(112.2)$ | $(119.5)$ | $(121)$ |
| A2 | 124.1 | 117 | 120.5 | 112.3 | 119.9 | 121 |
|  | $(124.2)$ | $(117)$ | $(120.5)$ | $(112.4)$ | $(119.7)$ | $(121.1)$ |
| A3 | 124.2 | 118.8 | 120 | 112.1 | 119.7 | 121 |
|  | $(124.3)$ | $(118.8)$ | $(119.9)$ | $(112.1)$ | $(119.5)$ | $(121)$ |
| A4 | 124.1 | 118.8 | 121.5 | 112.3 | 119.7 | 121 |
|  | $(124.3)$ | $(118.8)$ | $(121.5)$ | $(112.4)$ | $(119.5)$ | $(121)$ |
| B1 | 124.3 | 117 | 122 | 112.1 | 125.9 | 121 |
|  | $(124.4)$ | $(116.9)$ | $(122.1)$ | $(112.2)$ | $(126.2)$ | $(121)$ |
| B2 | 124.1 | 117 | 120.3 | 112.2 | 125.9 | 121 |
|  | $(124.2)$ | $(116.9)$ | $(120.3)$ | $(112.3)$ | $(126.3)$ | $(121)$ |
| B3 | 124.2 | 118.8 | 119.9 | 112.1 | 125.9 | 121 |
|  | $(124.3)$ | $(118.8)$ | $(119.9)$ | $(112.2)$ | $(126.3)$ | $(121)$ |
| B4 | 124.1 | 118.9 | 121.4 | 112.4 | 126.2 | 121 |
|  | $(124.3)$ | $(118.8$ | $(121.4$ | $(112.4$ | $(126.5$ | $(121.1$ |
| C1 | 123.9 | 117.9 | 122.1 | 112.4 | 119.7 | 121 |
|  | $(124)$ | $(117.8)$ | $(122.2)$ | $(112.6)$ | $(119.5)$ | $(121)$ |
| C2 | 123.9 | 117.9 | 120.5 | 112.3 | 119.8 | 121 |
|  | $(124)$ | $(117.8)$ | $(120.5)$ | $(112.3)$ | $(119.6)$ | $(121)$ |
| C3 | 123.9 | 118.2 | 121.3 | 112.6 | 119.8 | 121 |
|  | $(124)$ | $(118.1)$ | $(121.3)$ | $(112.7)$ | $(119.6)$ | $(121.1)$ |
| C4 | 123.9 | 118.1 | 121.8 | 112.5 | 119.7 | 121 |
|  | $(124)$ | $(118.1)$ | $(121.8)$ | $(112.6)$ | $(119.6)$ | $(121)$ |
| D1 | 123.9 | 117.9 | 122 | 112.4 | 125.9 | 121 |
|  | $(124)$ | $(117.9)$ | $(122.1)$ | $(112.6)$ | $(126.2)$ | $(121.1)$ |
| D2 | 123.9 | 117.9 | 120.5 | 112.3 | 126.1 | 121 |
|  | $(124)$ | $(117.8)$ | $(120.5)$ | $(112.3)$ | $(126.4)$ | $(121.1)$ |
| D3 | 123.9 | 117.9 | 121.7 | 112.7 | 119.9 | 121 |
|  | $(124)$ | $(118.1)$ | $(121.2)$ | $(112.8)$ | $(126.5)$ | $(121.1))$ |
| D4 | 123.9 | 118.1 | 121.7 | 112.5 | 126.1 | 121 |
|  | $(124)$ | $(118.1)$ | $(121.7)$ | $(112.5)$ | $(126.4)$ | $(121)$ |
|  |  |  |  |  |  |  |

Table 4. Dihedral angles of some selected atoms in the 16 possible conformations

| Model | $\mathrm{O}_{37}-\mathrm{N}_{15}-\mathrm{C}_{10}-\mathrm{C}_{9}$ | $\mathrm{O}_{36}-\mathrm{N}_{15}-\mathrm{C}_{10}-\mathrm{C}_{11}$ | $\mathrm{C}_{8}-\mathrm{C}_{7}-\mathrm{N}_{1}-\mathrm{C}_{6}$ | $\mathrm{C}_{5}-\mathrm{N}_{4}-\mathrm{C}_{16}-\mathrm{C}_{18}$ |
| :---: | :---: | :---: | :---: | :---: |
| A1 | 23.37 | 23.9 | -6.6 | 6.59 |
|  | (27.43) | (28.65) | (-5.22) | (8.24) |
| A2 | 19.28 | 20.68 | 42.89 | 4.52 |
|  | (23.67) | (25.54) | (42.51) | (5.78) |
| A3 | 22.44 | 21.35 | 44.59 | 6.48 |
|  | (25.84) | (27.59) | (43.7) | (7.9) |
| A4 | 22.38 | 21.86 | -6.21 | 6.29 |
|  | (27.04) | (25.64) | (-5.07) | (7.85) |
| B1 | 23.55 | 24.14 | -6.76 | -175.8 |
|  | (27.71) | (28.97) | (-5.34) | (-175.26) |
| B2 | 19.48 | 20.85 | 42.84 | -175.95 |
|  | (23.84) | (25.7) | (42.52) | (-175.38) |
| B3 | 22.19 | 21.08 | 44.13 | -176.21 |
|  | (27.26) | (25.51) | (43.33) | (-175.46) |
| B4 | 22.34 | 21.84 | -5.87 | -176.34 |
|  | (27) | (25.61) | (-4.74) | (-176.40) |
| C1 | 26.62 | 22.96 | -4.8 | 6.86 |
|  | (29.64) | (26.43) | (-4.09) | -8.24 |
| C2 | 26.62 | 23.89 | 41.91 | 5.88 |
|  | (29.66) | (27.34) | (41.93) | (7.1) |
| C3 | 23.08 | 25.81 | 31.67 | 6.57 |
|  | (28.86) | (26.65) | (-1.61) | (-4.96) |
| C4 | 23.8 | 27.33 | -6.29 | 5.87 |
|  | (26.77) | (29.65) | (-5.51) | (7.22) |
| D1 | 26.67 | 23.03 | -4.83 | -175.52 |
|  | (29.65) | (26.45) | (-4.03) | (-175.11) |
| D2 | 26.56 | 23.81 | 40.98 | -176.5 |
|  | (29.58) | (23.81) | (40.8) | (-175.87) |
| D3 | 23.1 | 25.84 | 31.17 | -175.84 |
|  | (28.72) | (26.52) | (-2.22) | (-187.62) |
| D4 | 23.83 | 27.37 | -5.88 | -176.19 |
|  | (26.83) | (29.71) | (-5.08) | (-176.64) |

Table 5. Calculated scaled wave numbers, observed IR, Raman bands and assignments of the title compound

| B3LYP/6-311++G(d,p) (5D, 7F) |  |  | IR | Raman | Assignments $_{\mathrm{a}}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| $\underline{v\left(\mathrm{~cm}^{-1}\right)}$ | $\underline{\underline{I R}}$ | $\underline{\underline{R}}_{\text {A }}$ | $\underline{v\left(\mathrm{~cm}^{-1}\right)}$ | $\underline{v\left(\mathrm{~cm}^{-1}\right)}$ |  |
| 3120 | 1.69 | 44.38 |  |  | vCHI(99) |
| 3094 | 4.66 | 112.93 | - | 3118 | vCHI(99) |
| 3081 | 4.65 | 38.68 | - | - | vCHI(98) |
| 3030 | 6.99 | 86.1 | - | 3078 | vCH3 ${ }^{(99)}$ |
| 3024 | 13.8 | 124.33 | - | 3032 | $\mathrm{vCH}_{3}(91)$ |
| 3014 | 13.84 | 97.07 | - | - | $v \mathrm{CH}_{2}(97)$ |
| 3013 | 5.46 | 72.2 | 3014 | - | $\mathrm{vCH}_{2}(98)$ |
| 3011 | 8.77 | 42.83 | - | - | vCH2 $(95)$ |
| 2979 | 15.07 | 47.35 | - | 2999 | ${ }^{2} \mathrm{CH}_{2}(96)$ |
| 2976 | 10.16 | 43.68 | 2977 | 2981 | $\mathrm{vCH}_{3}(99)$ |
| 2956 | 29.15 | 42.82 | 2977 | - | $\mathrm{vCH}_{3}(100)$ |
| 2920 | 6.4 | 158.49 | 2944 | 2950 | $\mathrm{vCH}_{3}(100)$ |
| 2895 | 42.63 | 152.5 | - |  | $\mathrm{vCH}_{3}(100)$ |
| 2887 | 55.9 | 104.31 | - | 2900 | ${ }^{2} \mathrm{CH}_{2}(93)$ |
| 2877 | 42.61 | 71.25 | - | 2885 | $\mathrm{vCH}_{2}(92)$ |
| 2841 | 69.12 | 267.53 | 2876 | - | $\mathrm{vCH}_{2}(95)$ |
| 2838 | 26.23 | 25.36 | , | 2843 | $\mathrm{vCH}_{2}(95)$ |
| 1655 | 417.22 | 20.08 | 2820 | - | vC=O(75) |
| 1576 | 492.6 | 212.72 | 1648 | 1649 | $v \mathrm{PhI}(59), \delta \mathrm{CHI}(14)$ |
| 1553 | 252.25 | 41.74 | 1574 | 1577 | $v \mathrm{PhI}(41), \mathrm{vNO}_{2}(16)$ |
| 1495 | 175.89 | 31.98 | 1550 | - | $\mathrm{vNO}_{2}(66)$ |
| 1473 | 65.07 | 18.48 | 1502 | 1492 | $\delta \mathrm{CHI}(28), \mathrm{vPhI}(49)$ |
| 1448 | 6.46 | 20.87 | 1476 | - | $\delta \mathrm{CH}_{2}(83)$ |
| 1443 | 11.35 | 3.31 | - | 1452 | $\delta \mathrm{CH}_{2}(48), \delta \mathrm{CH}_{3}(37)$ |
| 1441 | 49.44 | 5.98 | - | - | $\delta \mathrm{CH}_{3}(56), \delta \mathrm{CH}_{2}(27)$ |
| 1438 | 9.44 | 11.6 | - | - | $\delta \mathrm{CH}_{3}(99)$ |
| 1437 | 20.68 | 3.64 | - | - | $\delta \mathrm{CH}_{2}(68), \delta \mathrm{CH}_{3}(23)$ |
| 1432 | 5.59 | 5.86 | - | - | $\delta \mathrm{CH}_{2}(84)$ |
| 1429 | 24.57 | 0.68 | - | - | $\delta \mathrm{CH}_{3}(56), \mathrm{\delta CH}_{2}(19)$ |
| 1427 | 5.64 | 10.67 | 1430 | 1428 | $\delta \mathrm{CH}_{3}(51), \delta \mathrm{CH}_{2}(39)$ |


| 1418 | 11.1 | 6.29 | - | - | $\delta \mathrm{CH}_{3}(78), 8 \mathrm{CH}_{2}(14)$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| 1396 | 272.94 | 4.57 | - | - | vCN(41), $\delta \mathrm{CH}_{2}(20), \mathrm{vPhII}(11)$ |
| 1391 | 51.48 | 25.46 | 1400 | - | $\delta \mathrm{CH}_{3}(22), \mathrm{vPhI}(52), \delta \mathrm{CHI}(19)$ |
| 1361 | 29.39 | 8.14 | - | 1392 | $\delta \mathrm{CH}_{2}(62)$ |
| 1355 | 15.77 | 6.35 | 1363 | 1364 | $\delta \mathrm{CH}_{2}(73)$ |
| 1343 | 21.43 | 2.26 | - | - | $\delta \mathrm{CH}_{2}(51), \mathrm{vCN}(11)$ |
| 1338 | 15.45 | 4.95 | 1339 | - | $\delta \mathrm{CH}_{3}(62), \delta \mathrm{CH}_{2}(11)$ |
| 1335 | 4.93 | 21.07 | 1339 | - | $\delta \mathrm{CH}_{2}(53), \delta \mathrm{CH}_{3}(17)$ |
| 1319 | 13.94 | 25.14 | - | 1333 | $\delta \mathrm{CH}_{2}(75)$ |
| 1307 | 369.53 | 605.65 | - | - | $\begin{aligned} & \mathrm{uNO}_{2}(49), v \mathrm{CN}(11), \mathrm{vPhI}(20), \\ & \delta \mathrm{CH} 2(10) \end{aligned}$ |
| 1291 | 212.78 | 91.72 | 1310 | 1308 | ${ }^{\text {vPhI }}$ (45), $\mathrm{vNO}_{2}$ (10) |
| 1261 | 75.88 | 11.49 | 1288 | - | $\delta \mathrm{CH}_{2}(53), \mathrm{vPhII}(17)$ |
| 1257 | 37.56 | 12.93 | - | - | $\delta \mathrm{CHI}(46), \mathrm{vPhI}(13)$ |
| 1240 | 282.66 | 168.39 | 1258 | - | $v \mathrm{CO}(40), \delta^{\text {CH}} \mathrm{H}_{2}(43)$ |
| 1220 | 268.29 | 63.25 | - | 1242 | $\delta \mathrm{CH}_{2}(39), \mathrm{vCN}(44)$ |
| 1198 | 46.22 | 18.12 | - | 1224 | $\delta \mathrm{CH}_{2}(41), \mathrm{vPhII}(14)$ |
| 1193 | 144.21 | 11.38 | 1202 | 1196 | $\delta \mathrm{CH}_{2}(48), \mathrm{vPhII}(45)$ |
| 1185 | 231.16 | 9.44 | - | 1196 | $\delta \mathrm{CH}_{2}(46), \mathrm{vCN}(41)$ |
| 1161 | 3.96 | 8.7 | 1178 | - | $\delta \mathrm{CH}_{3}(53), \delta \mathrm{CHI}(10), \mathrm{vPhI}(11)$ |
| 1146 | 6.14 | 7.25 | - | 1159 | $\delta \mathrm{CHI}(40), \delta \mathrm{CH}_{3}(19), \mathrm{vPhI}(15)$ |
| 1128 | 41.36 | 4.7 | - | - | vPhII(68) |
| 1119 | 0.76 | 2 | 1131 | - | $\delta \mathrm{CH}_{3}(94)$ |
| 1088 | 6.79 | 3.47 | - | - | $\delta \mathrm{CH}_{2}(40), \tau \mathrm{PhII}(10), \mathrm{vCN}(11)$ |
| 1074 | 112.68 | 77.08 | 1092 | 1091 | $v \mathrm{CN}(36), \delta \mathrm{CHI}(40), \mathrm{vPhI}(11)$ |
| 1034 | 8.76 | 5.27 | 1073 | 1077 | $\delta \mathrm{CH}_{2}(50), \delta \mathrm{CH}_{3}(10), \delta \mathrm{PhII}(10)$ |
| 1027 | 11.45 | 3.91 | 1036 | 1040 | $\delta \mathrm{CH}_{2}(56)$, vPhII(13) |
| 1020 | 26.42 | 54.01 | - | - | ${ }^{\text {vPhII }}$ (52), vCO(11) |
| 1009 | 2.46 | 0.67 | - | 1018 | $\delta \mathrm{CH}_{3}(76), \gamma \mathrm{C}=\mathrm{O}(18)$ |
| 1008 | 48.37 | 18.85 | - | - | vCO(40), vPhI(45) |
| 967 | 72.77 | 0.68 | 1010 | - | $\delta \mathrm{CH}_{3}(43), \mathrm{vPhII}(16)$ |
| 956 | 100.35 | 0.76 | - | 970 | ${ }^{\text {vPhII}}$ (56), $\delta \mathrm{PhI}(22)$ |
| 942 | 8.18 | 1.5 | 960 | 954 | ${ }^{\text {vPhII }}$ (53), $\delta \mathrm{PhI}(24)$ |
| 935 | 9.15 | 0.37 | - | - | $\gamma \mathrm{CHI}(86)$ |
| 902 | 5.74 | 2.35 | 932 | - | vCC(44), vPhII(44) |


| 842 | 12.39 | 24.42 | - | 904 | $\delta \mathrm{NO}_{2}(39), \delta \mathrm{PhI}(19), \mathrm{vPhII}(13)$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| 827 | 6.2 | 4.32 | - | 844 | $\delta \mathrm{CH}_{2}(62)$ |
| 807 | 17.69 | 14.09 | 829 | 829 | ¢ $\mathrm{NO}_{2}(38)$, vPhII(12) |
| 799 | 40.12 | 1 | - | 808 | $\gamma \mathrm{CHI}(51), \tau \mathrm{PhI}(21), \gamma \mathrm{CN}(11)$ |
| 789 | 1.93 | 5.3 | 801 | - | $\begin{aligned} & \gamma \mathrm{CHI}(54), \gamma \mathrm{CO}(10), \tau \mathrm{CN}(18), \\ & \tau \mathrm{PhI}(12) \end{aligned}$ |
| 733 | 12.62 | 2.57 | - | - | $\gamma \mathrm{CN}(54), \gamma \mathrm{CHI}(21), \tau \operatorname{PhI}(10)$ |
| 699 | 10.3 | 22.31 | 728 | 731 | vPhII(42), $\gamma \mathrm{CN}(12)$ |
| 692 | 4.53 | 3.28 | 697 | 701 | $\delta \mathrm{PhI}(40), \tau \operatorname{PhI}(25)$ |
| 680 | 8.5 | 8.97 | - | - | $\tau \operatorname{PhI}(41), \gamma \mathrm{CN}(12)$ |
| 646 | 15.24 | 3.83 | - | - | $\begin{aligned} & \gamma \mathrm{CN}(18), \delta \mathrm{PhI}(15), \gamma \mathrm{CO}(19), \\ & \delta \mathrm{PhII}(24) \end{aligned}$ |
| 627 | 8.13 | 1.7 | 651 | 650 | $\gamma \mathrm{CO}(26), \gamma \mathrm{CN}(15), \tau \mathrm{PhI}(15)$ |
| 594 | 6.65 | 4.37 | 620 | 620 | $\delta \mathrm{C}=\mathrm{O}(43), \delta \mathrm{PhII}(10), \delta \mathrm{CN}(10)$ |
| 578 | 9.37 | 3.56 | 592 | 596 | $\gamma \mathrm{C}=\mathrm{O}(20), \delta \mathrm{C}=\mathrm{O}(17), \delta \mathrm{PhII}(38)$ |
| 567 | 7.9 | 1.44 | - | - | $\gamma \mathrm{C}=\mathrm{O}(46), \delta \mathrm{CH}_{3}(31)$ |
| 549 | 4 | 7.1 | 566 | 567 | $\delta \mathrm{PhI}(25), \delta \mathrm{NO}_{2}(47)$ |
| 497 | 1.49 | 2.09 | - | - | $\delta \mathrm{CN}(25), \delta \mathrm{PhII}(24), \delta \mathrm{PhI}(18)$ |
| 461 | 0.22 | 0.91 | - | 501 | $\delta \mathrm{CN}(46), \delta \mathrm{PhII}(11), \tau \operatorname{PhI}(10)$ |
| 457 | 3.35 | 1.7 | - | 464 | $\delta \mathrm{PhII}(44), \delta \mathrm{CN}(10)$ |
| 452 | 9.7 | 7.8 | 457 | - | $\tau \operatorname{PhI}(44), \gamma \mathrm{CN}(25)$ |
| 416 | 2.42 | 5.7 |  | 444 | $\delta \mathrm{CN}(38), \tau \mathrm{PhI}(20)$ |
| 400 | 4.3 | 5.19 | 414 | 414 | $\delta \mathrm{CO}(39), \delta \mathrm{CN}(23)$ |
| 381 | 1.27 | 1.48 | - | - | $\tau \mathrm{PhII}(60)$ |
| 362 | 1.24 | 0.56 | - | - | $\delta \mathrm{PhII}(27), \delta \mathrm{PhI}(38), \delta \mathrm{CN}(15)$ |
| 305 | 1.32 | 7.66 | - | - | $\tau \mathrm{PhI}(20), \gamma \mathrm{CN}(27), \delta \mathrm{CN}(10)$ |
| 289 | 11.08 | 0.62 | - | 306 | $\tau \mathrm{PhII}(38), \delta \mathrm{CN}(39)$ |
| 273 | 20.06 | 4.46 | - | 286 | $\tau \mathrm{PhII}(41), \gamma \mathrm{CN}(12), \delta \mathrm{CN}(24)$ |
| 257 | 3.19 | 2.58 | - | - | $\tau \mathrm{CH}_{3}(46), \tau \mathrm{PhI}(26)$ |
| 232 | 4.81 | 1.11 | - | - | $\tau \mathrm{PhII}(22), \gamma \mathrm{CN}(16), \tau \mathrm{CH} 3(11)$ |
| 212 | 0.32 | 4.69 | - | - | $\delta \mathrm{PhI}(31), \delta \mathrm{PhII}(39)$ |
| 188 | 3.78 | 1.68 | - | - | $\tau \mathrm{PhI}(37), \tau \mathrm{CH}_{3}(26)$ |
| 183 | 1.13 | 1.24 | - | 190 | $\delta \mathrm{CN}(40), \tau \mathrm{PhI}(10), \tau \mathrm{PhII}(10)$ |
| 165 | 1.02 | 3.96 | - | - | $\delta \mathrm{CO}(31), \tau \mathrm{PhII}(23), \delta \mathrm{CN}(15)$ |
| 160 | 0.36 | 0.19 | - | 163 | $\delta \mathrm{CH}_{3}(74), \tau \mathrm{CH}_{3}(16)$ |
| 115 | 0.66 | 1.3 | - | - | $\tau \mathrm{PhI}(22), \gamma \mathrm{CN}(22), \tau \mathrm{PhII}(10)$ |
| 98 | 3.56 | 1.11 | - | - | $\tau \mathrm{C}=\mathrm{O}(54), \tau \mathrm{CH}_{3}(17)$ |
| 87 | 3.7 | 0.76 | - | 102 | $\tau \mathrm{CO}(61), \tau \mathrm{CH}_{3}(22)$ |


| 75 | 2.38 | 0.85 | - | - | $\gamma \mathrm{CN}(31), \delta \mathrm{CN}(23)$ |
| :--- | :--- | :---: | :---: | :---: | :--- |
| 51 | 3.26 | 3.53 | - | - | $\tau \mathrm{CN}(27), \gamma \mathrm{CN}(23), \tau \operatorname{PhI}(10)$ |
| 45 | 0.85 | 2.04 | - | - | $\tau \mathrm{CN}(53), \tau \operatorname{PhI}(13)$ |
| 34 | 5.36 | 0.84 | - | - | $\tau \mathrm{CN}(58)$ |
| 16 | 2.86 | 1.1 | - | - | $\gamma \mathrm{CN}(56), \tau \operatorname{PhII}(10), \tau \mathrm{C}=\mathrm{O}(10)$ |

${ }^{\text {a }}$ v-stretching; $\delta$-in-plane deformation; $\gamma$-out-of-plane deformation; $\tau$-torsion; PhI-phenyl ring; PhII-piperazine ring.

Table 6. Second-order perturbation theory analysis of Fock matrix in NBO basis corresponding to the intramolecular bonds of the title compound

| Donor(i) | Type | ED/e | Acceptor(j) | Type | ED/e | E(2) ${ }^{\text {a }}$ | E(j)-E(i) ${ }^{\text {b }}$ | F(i,j) ${ }^{\text {c }}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| C2-C3 | $\sigma$ | 1.984 | N1-C7 | $\sigma *$ | 0.029 | 2.480 | 1.080 | 0.046 |
| - | - | - | N4-C16 | $\sigma *$ | 0.084 | 2.360 | 1.100 | 0.046 |
| N4-C16 | $\sigma$ | 1.989 | C3-N4 | $\sigma *$ | 0.028 | 1.410 | 1.160 | 0.036 |
| - | - | - | N4-C5 | $\sigma *$ | 0.028 | 1.450 | 1.160 | 0.037 |
| C5-C6 | $\sigma$ | 1.983 | N1-C7 | $\sigma *$ | 0.029 | 2.720 | 1.070 | 0.048 |
| - | - | - | N4-C16 | $\sigma *$ | 0.084 | 1.930 | 1.090 | 0.042 |
| C7-C8 | $\sigma$ | 1.983 | N1-C2 | $\sigma *$ | 0.026 | 2.440 | 1.030 | 0.045 |
| - | - | - | N1-C7 | $\sigma *$ | 0.029 | 1.490 | 1.150 | 0.037 |
| - | - | - | C7-C12 | $\sigma *$ | 0.023 | 3.550 | 1.250 | 0.060 |
| - | - | - | C8-C9 | $\sigma *$ | 0.025 | 3.150 | 1.260 | 0.056 |
| - | - | - | C9-O13 | $\sigma *$ | 0.026 | 3.680 | 1.090 | 0.057 |
| - | $\pi$ | 1.669 | N1-C2 | $\sigma *$ | 0.026 | 1.360 | 0.590 | 0.027 |
| - | - | - | C7-C8 | $\pi *$ | 0.425 | 1.360 | 0.280 | 0.018 |
| - | - | - | C9-C10 | $\pi *$ | 0.470 | 27.600 | 0.270 | 0.080 |
| - | - | - | C11-C12 | $\pi *$ | 0.308 | 12.790 | 0.290 | 0.055 |
| C8-C9 | $\sigma$ | 1.975 | N1-C7 | $\sigma *$ | 0.029 | 3.590 | 1.170 | 0.058 |
| - | - | - | C7-C8 | $\sigma *$ | 0.022 | 3.470 | 1.270 | 0.059 |
| - | - | - | C9-C10 | $\sigma^{*}$ | 0.031 | 3.970 | 1.250 | 0.063 |
| - | - | - | C10-N15 | $\sigma *$ | 0.098 | 4.220 | 1.030 | 0.060 |
| C9-C10 | $\pi$ | 1.975 | C7-C8 | $\pi *$ | 0.425 | 13.940 | 0.280 | 0.056 |
| - | - | - | C9-C10 | $\pi *$ | 0.470 | 2.240 | 0.280 | 0.023 |
| - | - | - | C11-C12 | $\pi *$ | 0.308 | 25.160 | 0.300 | 0.079 |
| - | - | - | N15-037 | $\pi *$ | 0.623 | 23.880 | 0.170 | 0.060 |
| C10-N15 | $\sigma$ | 1.989 | C8-C9 | $\sigma *$ | 0.025 | 1.540 | 1.350 | 0.041 |
| - | - |  | C9-C10 | $\sigma *$ | 0.031 | 1.540 | 1.350 | 0.041 |
| - | - | - | C11-C12 | $\sigma *$ | 0.014 | 1.700 | 1.400 | 0.044 |
| N15-O37 | $\pi$ | 1.986 | C9-C10 | $\pi *$ | 0.470 | 3.240 | 0.450 | 0.039 |
| - | - |  | N15-O37 | $\pi *$ | 0.623 | 7.100 | 0.340 | 0.052 |
| C16-C18 | $\sigma$ | 1.987 | N4-C5 | $\sigma *$ | 0.028 | 4.040 | 0.980 | 0.056 |
| LPN1 | $\sigma$ | 1.763 | C2-C3 | $\sigma *$ | 0.022 | 1.990 | 0.630 | 0.033 |
| - | - | - | C5-C6 | $\sigma *$ | 0.019 | 1.910 | 0.640 | 0.033 |
| - | - | - | C7-C8 | $\sigma *$ | 0.022 | 1.880 | 0.830 | 0.037 |
| - | - | - | C7-C8 | $\pi *$ | 0.425 | 32.390 | 0.290 | 0.090 |
| LPN4 | $\sigma$ | 1.696 | C2-C3 | $\sigma *$ | 0.022 | 4.030 | 0.610 | 0.048 |
| - | - | - | C5-C6 | $\sigma *$ | 0.019 | 3.890 | 0.620 | 0.047 |
| - | - | - | C16-017 | $\pi *$ | 0.281 | 62.420 | 0.280 | 0.118 |
| LPO13 | $\sigma$ | 1.962 | C8-C9 | $\sigma *$ | 0.025 | 7.580 | 1.100 | 0.082 |


| - | $\pi$ | 1.815 | C9-C10 | $\pi^{*}$ | 0.470 | 35.280 | 0.330 | 0.104 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| LPO17 | $\sigma$ | 1.976 | N4-C16 | $\sigma^{*}$ | 0.084 | 1.970 | 1.130 | 0.043 |
| - | - | - | C16-C18 | $\sigma^{*}$ | 0.055 | 1.930 | 1.050 | 0.040 |
| - | $\pi$ | 1.864 | N4-C16 | $\sigma^{*}$ | 0.084 | 26.340 | 0.700 | 0.123 |
| - | - | - | C16-C18 | $\sigma^{*}$ | 0.055 | 20.040 | 0.620 | 0.101 |
| LPO36 | $\sigma$ | 1.981 | C10-N15 | $\sigma^{*}$ | 0.098 | 4.210 | 1.090 | 0.062 |
| - | - | - | N15-O37 | $\sigma^{*}$ | 0.060 | 2.410 | 1.220 | 0.049 |
| - | $\pi$ | 1.898 | C10-N15 | $\sigma^{*}$ | 0.098 | 12.580 | 0.580 | 0.076 |
| - | - | - | N15-O37 | $\sigma^{*}$ | 0.060 | 18.440 | 0.710 | 0.103 |
| - | - | - | N15-O37 | $\pi^{*}$ | 0.623 | 1.100 | 0.170 | 0.014 |
| - | $n$ | 1.467 | N15-O36 | $\sigma^{*}$ | 0.063 | 1.930 | 0.690 | 0.037 |
| - | - | - | N15-O37 | $\pi^{*}$ | 0.623 | 148.770 | 0.150 | 0.137 |
| LPO37 | $\sigma$ | 1.981 | C10-N15 | $\sigma^{*}$ | 0.098 | 4.280 | 1.080 | 0.062 |
| - | - | - | N15-O36 | $\sigma^{*}$ | 0.063 | 2.620 | 1.200 | 0.051 |
| - | $\pi$ | 1.898 | C10-N15 | $\sigma^{*}$ | 0.098 | 13.510 | 0.580 | 0.079 |
| - | - | - | N15-O36 | $\sigma^{*}$ | 0.063 | 19.690 | 0.700 | 0.106 |

${ }^{\mathrm{a}} \mathrm{E}(2)$ means energy of hyper-conjugative interactions (stabilization energy in $\mathrm{kJ} / \mathrm{mol}$ )
${ }^{\mathrm{b}}$ Energy difference (a.u) between donor and acceptor i and j NBO orbitals
${ }^{\mathrm{c}} \mathrm{F}(\mathrm{i}, \mathrm{j}$ ) is the Fock matrix elements (a.u) between i and j NBO orbitals

Table 7. NBO results showing the formation of Lewis and non-Lewis orbitals

| Bond(A-B) | ED/e ${ }^{\text {a }}$ | EDA\% | EDB\% | NBO | s\% | p\% |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| бX2-X3 | 1.984 | 49.99 | 50.01 | 0.7070( $\left.\mathrm{sp}^{2.61}\right) \mathrm{C}+$ | 27.70 | 72.30 |
| - | -0.634 | - | - | $0.7072\left(\mathrm{sp}^{2.60}\right) \mathrm{C}$ | 27.77 | 72.23 |
| бN4-X16 | 1.989 | 63.88 | 36.12 | $0.7993\left(\mathrm{sp}^{1.71}\right) \mathrm{N}+$ | 36.89 | 63.08 |
| - | -0.823 | - | - | 0.6010( $\left.\mathrm{sp}^{2.18}\right) \mathrm{C}$ | 31.36 | 68.64 |
| oX5-X6 | 1.983 | 49.27 | 50.73 | $0.7019\left(\mathrm{sp}^{2.61}\right) \mathrm{C}+$ | 27.67 | 72.33 |
| - | -0.627 | - | - | 0.7123( $\left.\mathrm{sp}^{2.58}\right) \mathrm{C}$ | 27.92 | 72.08 |
| бX7-X8 | 1.971 | 50.47 | 49.53 | $0.7104\left(\mathrm{sp}^{1.81}\right) \mathrm{C}+$ | 35.54 | 64.46 |
| - | -0.709 | - | - | $0.7038\left(\mathrm{sp}^{1.83}\right) \mathrm{C}$ | 35.38 | 64.62 |
| пX7-X8 | 1.669 | 42.12 | 57.88 | $0.6490\left(\mathrm{sp}^{99.99}\right) \mathrm{C}+$ | 0.01 | 99.99 |
| - | -0.265 | - | - | $0.7608\left(\mathrm{sp}^{1.00}\right) \mathrm{C}$ | 0.00 | 100.00 |
| бX8-X9 | 1.975 | 49.97 | 50.03 | $0.7069\left(\mathrm{sp}^{1.89}\right) \mathrm{C}+$ | 34.63 | 65.37 |
| - | -0.720 | - | - | 0.7073( $\left.\mathrm{sp}^{1.62}\right) \mathrm{C}$ | 38.22 | 61.78 |
| $\pi \mathrm{X} 9-\mathrm{X} 10$ | 1.625 | 41.03 | 58.97 | $0.6405\left(\mathrm{sp}^{1.00}\right) \mathrm{C}+$ | 0.01 | 99.99 |
| - | -0.269 | - | - | $0.7679\left(\mathrm{sp}^{1.00}\right) \mathrm{C}$ | 0.01 | 99.99 |
| бX10-N15 | 1.989 | 38.23 | 61.77 | $0.6183\left(\mathrm{sp}^{2.91}\right) \mathrm{C}+$ | 25.56 | 74.44 |
| - | -0.804 | - | - $\quad$ - | $0.7860\left(\mathrm{sp}^{1.76}\right) \mathrm{N}$ | 36.20 | 63.80 |
| $\pi \mathrm{N} 15-\mathrm{O} 37$ | 1.986 | 40.59 | 59.41 | $0.6371\left(\mathrm{sp}^{99.99}\right) \mathrm{N}+$ | 0.12 | 99.88 |
| - | -0.438 |  | - | $0.7708\left(\mathrm{sp}^{99.99}\right) \mathrm{O}$ | 0.26 | 99.74 |
| бX16-X18 | 1.987 | 48.68 | 51.32 | $0.6977\left(\mathrm{sp}^{1.76}\right) \mathrm{C}+$ | 36.26 | 63.74 |
| - | -0.640 | - | - | 0.7164(sp $\left.{ }^{2.76}\right) \mathrm{C}$ | 26.56 | 73.44 |
| n1N1 | 1.763 | - | - | sp ${ }^{15.22}$ | 6.16 | 93.84 |
| - | -0.275 |  |  |  |  |  |
| n1N4 | 1.696 | - | - | sp ${ }^{99.99}$ | 0.51 | 99.49 |
| - | -0.255 |  |  |  |  |  |
| n1O13 | 1.962 | - | - | sp ${ }^{1.68}$ | 37.33 | 62.67 |
|  | -0.544 |  |  |  |  |  |
| n2013 | 1.815 | - | - | $\mathrm{sp}^{1.00}$ | 0.01 | 99.99 |
| - | -0.319 |  |  |  |  |  |
| n1O17 | 1.976 | - | - | $\mathrm{sp}^{0.71}$ | 58.32 | 41.68 |
| - | -0.669 |  |  |  |  |  |
| n2017 | 1.864 | - | - | $\mathrm{sp}^{1.00}$ | 0.01 | 99.99 |
| - | -0.240 |  |  |  |  |  |


| n1O36 | 1.981 | - | - | $\mathrm{sp}^{0.33}$ | 75.03 | 24.97 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| - | -0.777 |  |  |  |  |  |
| n2O36 | 1.898 | - | - | sp ${ }^{99.99}$ | 0.05 | 99.95 |
| - | -0.269 |  |  |  |  |  |
| n3036 | 1.467 | - | - | sp ${ }^{99.99}$ | 0.16 | 99.84 |
| - | -0.254 |  |  |  |  |  |
| n1O37 | 1.981 | - | - | $\mathrm{sp}^{0.34}$ | 74.6 | 25.4 |
| - | -0.774 |  |  |  |  |  |
| n2O37 | 1.898 | - | - | sp ${ }^{99.99}$ | 0.03 | 99.97 |
| - | -0.267 |  |  |  |  |  |

${ }^{\text {a }}$ ED/e is expressed in a.u.

Table 8: The docking results of the title compound with the human GABA receptor.

| Binding <br> Sites | HB score | SI score | Ligand <br> conformation <br> penalty | Total <br> Score |
| :---: | :---: | :---: | :---: | :---: |
| A | -6.000 | -53.167 | 6.611 | -52.556 |
| B | -5.513 | -52.730 | 6.671 | -51.572 |
| C | -6.596 | -59.781 | 7.910 | -58.468 |
| D | -8.628 | -51.792 | 6.624 | -53.795 |
| E | -5.886 | -53.699 | 6.687 | -52.898 |



Figure 1. Molecular structure and atom numbering of MNPE.


Figure 2. The mid-infrared spectrum of MNPE.


Figure 3. The Raman spectrum of MNPE.



A3



Figure 4. Calculated stable conformations of MNPE.
A.

B.


Figure 5. (A) Potential energy scan of $\mathrm{C}_{8}-\mathrm{C}_{9}-\mathrm{O}_{13}-\mathrm{C}_{14}$. (B) Potential energy scan of $\mathrm{C}_{2}-\mathrm{N}_{1}-\mathrm{C}_{7}-\mathrm{C}_{8}$.


Figure 6. (A) Molecular Electrostatic Potential (MEP) map and (B) the LUMO (top) and HOMO (bottom) diagrams of MNPE.


### 198.95 ALIE [kcal/mol] 357.84



Figure 7. (A) Representations of the ALIE and (B) Fukui function surfaces of MNPE.


Figure 8. The BDE sites and values of MNPE. Values provided in red color are those for hydrogen abstraction, while blue color BDEs are for the rest of the single acyclic bonds


Figure 9. The plots showing the MNPE molecule areas that are prone to interactions with water molecules using RDF.


Figure 10. (A) The crystal structure of a human GABA receptor (PDB ID: 4COF). (B) The Ramachandran plot of the crystal structure of a human GABA receptor (PDB ID: 4COF).
A.

B.


Figure 11. (A) Docked conformation of the title compound in binding site C of GABA (PDB ID: 4COF). (B) The molecular interactions of MNPE and amino acids in binding site $C$ of GABA


Figure 12. (A) Docked conformation of the title compound in binding site $C$ showing the presence of intra-hydrogen bond (shown as yellow color). (B) The most stable conformation, B4 of the title compound

## HIGHLIGHTS

- A new bioactive arylpiperazine-based drug has been synthesized and characterized.
- Complete vibrational spectroscopic assignments have been performed.
- Average Local Ionization Energies (ALIE) and Fukui functions were calculated.
- Molecular docking predicted a potential anti-stress activity for the newly synthesized compound.

