

DFT Calculations Of Nitrophenyl Psoralen, A New Inhibiting Compound As Part Of Treatment Could Become Available To Reduce The Paralysis Induced By Botulinum

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Abstract – In the given work, the structural properties of the Nitrophenyl psoralen compound were calculated within the Density Functional Theory (DFT) method using B3LYP functional and 6-311G(d,p) as the basis set. As result, the good agreement between the theoretical and the experimental geometrical parameters (bond lengths and angles). The energy of the HOMO orbital is about -0.244 eV and the energy level of the LUMO orbital is about -0.106 eV. The HOMO-LUMO energy gap of the title compound is equal to -0.138 eV. Additionally, the frontier molecular orbitals properties (FMOs), as well as the molecular electrostatic potential (MEP) were performed and discussed.

Keywords – Nitrophenyl psoralen, DFT, Botulinum.

I. INTRODUCTION

Botulinum neurotoxins (BoNT, seven different serotypes: A, B, C, D, E, F, and G) [1] are produced by the anaerobic, gram-positive bacterial species *Clostridium botulinum*. BoNT is synthesized by the bacterium as a 150kDa polypeptide (called as holotoxin), which consists of a 50 kDa light chain, and a 100 kDa heavy chain domains linked by a disulfide bond. Among the seven known serotypes of BoNT, BoNT serotypes A, B, E, and F are known to be toxic to humans. With a lethal dose for humans is 0.1-1 ng per kg body weight and the oral dose is 1 µg per kg [2]. Recently, botulism by BoNT/B has become a serious problem in Vietnam. The beginning of September 2020, cases of BoNT/B poisoning occurred with signs, such as: muscle weakness, difficulty breathing, difficulty speaking after eating canned vegetarian food. Currently, the only drug used to detoxify Botulinum poisoning patients is Antitoxin botulinum. The drug is only effective within 7 days since the manifestation of poisoning, while the poisoned patients in Vietnam are already in the late stage. It was too far from the time when the drug was most effective. Therefore, the process of diagnosing and treating patients has many difficulties in terms of time and cost of treatment when importing antitoxin botulinum antidote with quite expensive cost. Therefore, the development of countermeasures and therapeutics against treat pre-or postexposure BoNT intoxication is a high-priority research area for public health because of its extreme toxicity. Extensive research has focused on designing antagonists that block the catalytic activity of BoNT, including studies to discover and develop small molecular weight inhibitors of BoNT.

For the past 10 years, many compounds have been developed that inhibit Botulinum neurotoxin, such as: The first compound is Benzoquinones [3], Benzoquinones are found during bacterial fermentation and can be easily identified by ultraviolet (λ_{max} 243, 280, 440 nm) [21] and its characteristic infrared spectroscopy. Benzoquinones (BQ) compounds acted as irreversible

inhibitors that presumably covalently modify cysteine 165 of BoNT/A LC. Although most BQ derivatives were highly reactive toward glutathione *in vitro*, a few compounds such as natural product naphthazarin displayed low thiol reactivity and good BoNT/A inhibition. The second compound is 8-hydroxyquinoline (quinolin-8-ol) has also been found to be a promising BoNT/A light chain (LC) inhibitor scaffold [4], 8-hydroxyquinoline (quinolin-8-ol) as a potential inhibitor scaffold for preventing the deadly neurochemical effects of the toxin. Quinolinols are known chelators that can disrupt the BoNT/A metalloprotease zinc-containing active site, thus impeding its proteolysis of the endogenous protein substrate, synaptosomal-associated protein 25 (SNAP-25). By use of this information, the structure–activity relationship (SAR) of the quinolinol-5-sulfonamide scaffold was explored through preparation of a crude sulfonamide library and evaluation of the library in a BoNT/A LC enzymatic assay. Potency optimization of the sulfonamide hit compounds was undertaken as informed by docking studies, granting a lead compound with a submicromolar K_i . These quinolinol analogues demonstrated inhibitory activity in a cell-based model for SNAP-25 cleavage and an *ex vivo* assay for BoNT/A-mediated muscle paralysis. The third compound is Picolinic acids as β -exosite inhibitors of botulinum neurotoxin A LC [5], in searching for more stable scaffolds containing the same pharmacophores, identified picolinic acid (PA) as a potential alternative core molecule that was amenable to library preparation. As an indication of its safety and *in vivo* compatibility, PA is found endogenously in mammals. In developing small-molecule inhibitors of botulinum neurotoxin serotype A light chain (BoNT/A LC), substituted picolinic acids were identified. Extensive investigation into the SAR of the picolinic acid scaffold revealed 5-(1-butyl-4-chloro-1H-indol-2-yl) picolinic acid (CBIP), which possessed low micromolar activity against BoNT/A.

Moreover, Targeted 8-hydroxyquinoline fragment based small molecule drug discovery against neglected botulinum neurotoxin type F (Bremer, 2017). Three course studies were performed involving *in silico*, *in vitro* and *in vivo* cascade to screen 8-HQ small molecule inhibitors against BoNT/F intoxication. ~800 molecules obtained from open repositories were screened *in silico* and commercially obtained twenty-four 8-HQ derived small molecule inhibitors were evaluated against rBoNT/F light chain through fluorescence thermal shift (FTS) assay. Selected compounds were further evaluated through endopeptidase assay. Further binding affinity analysis was done through surface plasmon resonance (SPR) based Proteon™ XPR 36 system. Finally, the *in vivo* efficacy of these compounds was evaluated in mice model. Three compounds NSC1011, NSC1014 and NSC84094 were found to be highly inhibitory after screening of 8-HQ compounds through FTS assay and endopeptidase assay. SPR based protein-small molecule interaction studies showed highest affinity binding of NSC1014 (K_D : $5.58E-06$) with BoNT/F-LC. NSC1011, NSC1014, and NSC84094 displayed IC_{50} of 30.47 ± 6.24 , 14.91 ± 2.49 and 17.39 ± 2.74 μM , respectively, in endopeptidase assay. NSC1011 and NSC1014 displayed marked extension of survival time in mice model.

Recently, a new report about the inhibition of BoNT/A light chain (LC) by nitrophenyl psoralen (NPP) one natural compound, was published [6]. They screened 300 small natural compounds and their analogues extracted from Indian plants for their activity against BoNT serotype A as well as its light chain using biochemical and cellular assays. Nitrophenyl psoralen was indentified to be a specific inhibitor of LCA with an *in vitro* 50% inhibitory concentration (IC_{50}) value of 4.74 ± 0.03 μM . However, to the best of our knowledge, no theoretical study of Nitrophenyl psoralen has been published in the literature so far. Therefore, we present the results of the structural properties and the inhibitory capacity of this compound on the base Density Functional Theory (DFT) calculated. DFT calculations involving frontier molecular orbitals (FMOs), molecular electrostatic potential (MEP) surfaces, chemical reactivity descriptors were also calculated and discussed.

II. MATERIALS AND METHOD

The quantum chemical calculations on Nitrophenyl psoralen (NPP) were performed using density functional theory (DFT) with Becke's [7] three-parameter hybrid model, Lee, Yang and Parr's [8] correlation functional under 6-311G(d,p) basis set as implemented in the GAUSSIAN 16 suite of program [9]. The optimized molecular structure, HOMO and LUMO surfaces have been visualized using GaussView 6 program [10]. The molecular electrostatic potential (MEP) [11] diagram of NPP was evaluated at B3LYP/6-311G(d,p). Additionally, the density of state (DOS) plot was obtained using the GaussSum program [12].

III. RESULTS AND DISCUSSION

3.1. Geometrical analysis

The structure of Nitrophenyl psoralen was optimized to global minima using DFT (B3LYP) method with 6-311G(d,p) basis sets by the Gaussian program package. The results are displayed in Figure 1. The data of the theoretical DFT calculations for Electronic

Energy, Dipole Moment, Polarizability, Thermal Energy, Heat Capacity, and Entropy of Nitrophenyl psoralen are summarized in Table 1.

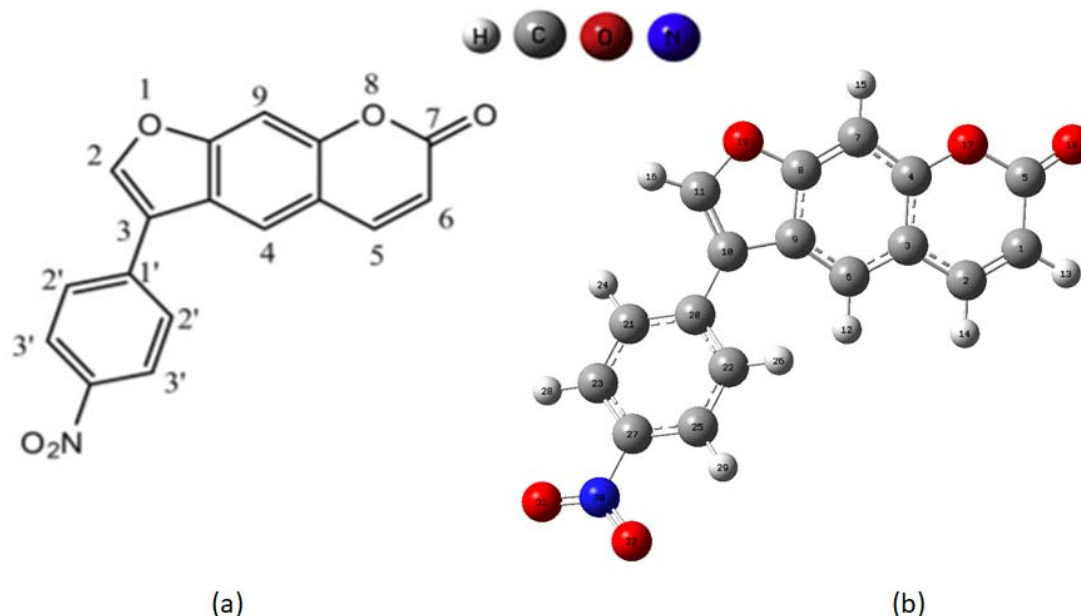


Figure 1. (a) 2D structure was redrawn by using ChemDraw (<http://www.cambridgesoft.com/>). and (b) optimized geometrical structure of Nitrophenyl Psoralen.

The results in Table 1 showed that the Nitrophenyl psoralen total dipole moment is about 1.560 Debye. Elevated dipole moment enhances the hydrogen bond formation, nonbonding interactions, binding affinity, and polar nature of a molecule [13], [14]. As Table 1 shows, the polarizability value is 226.105 a.u. These value show that the polarizability of the molecular depends on how susceptibility of the molecular system electron could be affected by approaching a charge. Moreover, it depends on the complexity of the compounds as well as the size of the molecular structure [15].

Table 1. The values of thermodynamic parameters of Nitrophenyl psoralen by DFT method with B3LYP/6-311G(d,p) basis set.

Parameter	Nitrophenyl psoralen	Unit
Electronic Energy	-1084.272	Hartree
Total Dipole Moment	1.560	Debye
Polarizability (α)	226.105	a.u
E (Thermal)	152.635	Kcal/mol
Heat Capacity (Cv)	66.848	Cal/mol-kelvin
Entropy (S)	135.331	Cal/mol-kelvin

3.2. Frontier Molecular Orbitals (FMOs)

The frontier molecular orbitals (FMOs) are formed mainly by the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO), which important parameters that affect the bioactivity of chemical entities. FMO analysis shows that the HOMO orbital of the Botulinum molecule mainly centralized on double bonds ($-C=C-$) of phenyl ring, and oxygen atoms in nitro and furan groups, whereas the LUMO orbital focuses on dounle bonds ($-C=C-$) of psoralen ring (Figure 2). The energy gap between HOMO and LUMO of title molecule is -0.138 eV. However, the smaller the difference between the two energy values LUMO and HOMO, the higher the reactivity of the molecular and can be very promising for nonlinear optical (NLO)

applications [16]. Besides, the value of E_{HOMO} and E_{LUMO} also provides chemical properties through the ionization potential, electron affinity, global hardnesses, electronegativity, softness and electrophilicity values that will be discussed in the following chemical reactivity part. DOS plots show the change of the energy gaps of the title molecule (see Figure 2).

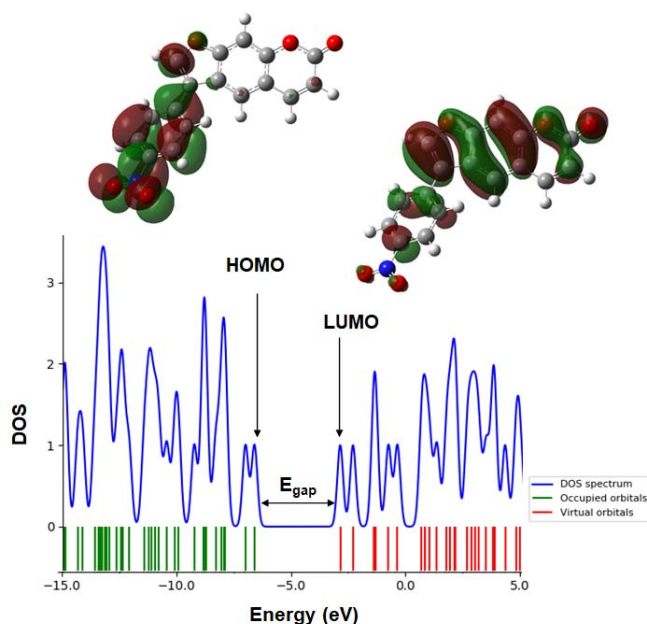


Figure 2. HOMO, LUMO orbitals and DOS plots of Nitrophenyl Psoralen

The electronic properties of title molecule such as the highest occupied molecular orbital energy (E_{HOMO}) and lowest unoccupied molecular orbital energy (E_{LUMO}), energy gap between LUMO and HOMO ($\Delta E_{\text{gap}} = E_{\text{HOMO}} - E_{\text{LUMO}}$), ionization potential ($I = -E_{\text{HOMO}}$), electron affinity ($A = -E_{\text{LUMO}}$), global hardnesses (η), global softness (S), electrophilicity (ω) have been calculated. The results of the calculations are summarized in Table 2.

Table 2. Frontier molecular orbital energies (in eV unit) and global reactivity descriptors of Nitrophenyl psoralen by the B3LYP-6-311G(d,p) level of theory.

E_{HOMO}	E_{LUMO}	ΔE_{gap}	$I = -E_{\text{HOMO}}$	$A = -E_{\text{LUMO}}$	$\eta = (I-A)/2^a$	$S = 1/\eta^b$	$\omega = \mu^2/2\eta^c$
-0.244	-0.106	-0.138	0.244	0.106	0.069	14.493	0.222

^{a,b,c} Taken from Ref. [17, 18]

Ionization potential is a descriptor of the chemical reactivity of atoms and molecules. The obtained results from Table 2 show that the value of the ionization potential of the Nitrophenyl psoralen molecular is about 0.244 eV. The smaller ionization energy indicates higher reactivity of the investigated molecular [19]. The value of the global hardnesses and global softness seen in Table 2. This value is an important property to measure the stability and reactivity of the compound. Hence, the higher values of global softness and lower values of the global hardnesses are *ca.* 0.069 and 14.493 eV, respectively. It is more reactive to be better corrosion inhibitors. In addition, the electrophilicity (ω) value has been calculated. The results of electrophilicity shown in Table 1, which has a value (0.222) obtained with the B3LYP-6-311G(d,p) level of theory.

3.3. Molecular Electrostatic Potential (MEP)

To validate the possibility of the reactivity of the compound as inhibitors, the molecular electrostatic potential is a useful feature to be studied. The importance of the MEP gives an indication about the molecular size and shape of the positive, negative as well as the neutral electrostatic potential and is very useful in research of physicochemical property relationships with the molecular structure of the compounds. Moreover, the molecular electrostatic potential is a useful tool to estimate the reactivity of compounds toward electrophilic and nucleophilic attacks are represented by various colors. Red color represents the maximum negative area in

which favorable site for an electrophilic reactivity while the blue color indicates the maximum a positive area in which favorable site for nucleophilic reactivity and green color represents zero potential areas [16].

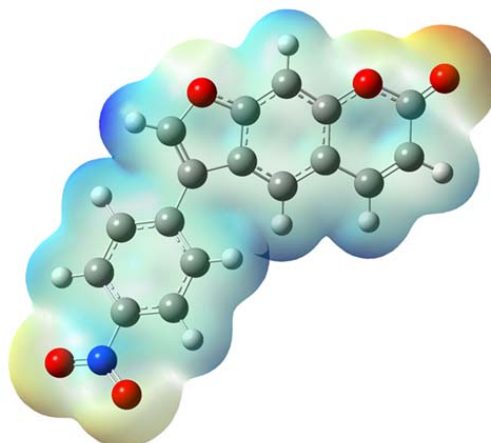


Figure 3. Molecular Electrostatic Potential (MEP) plot of title compound calculated at the B3LYP/6-311G(d,p) level at an isovalue electron density of 0.004 atomic units.

In this work, the MEP for the title molecule was calculated by the same method under the same base sets and depicted in Figure 3. The different values of the electrostatic potential at the surface are represented by different colors. As can be seen in the Figure 3, the negative regions (red color) are mainly near the oxygen atoms of nitro ($-\text{NO}_2$) and carbonyl ($-\text{C}=\text{O}$) groups; the positive (blue color) is around the hydrogen atom. These active sites are evidence of the biological activity of NPP.

IV. CONCLUSION

Quantum chemical calculation on the molecular structure of Nitrophenyl psoralen has been investigated. The geometry optimization and frontier molecular orbitals (FMOs), molecular electrostatic potential (MEP) surfaces, chemical reactivity descriptors were obtained by theoretical calculations based on DFT method using B3LYP functionals with 6-311G(d,p) basis set. The lower value of HOMO and LUMO energy gap describes the stability and biological activity of Nitrophenyl psoralen compound. The MEP drawing showed that there are enough sites for nucleophilic and electrophilic interaction in the molecules, which the negative regions are mainly near the oxygen atoms of nitro ($-\text{NO}_2$) and carbonyl ($-\text{C}=\text{O}$) groups and the positive is around the hydrogen atom. These active sites are evidence of the biological activity of Nitrophenyl psoralen. The results of this study are considered a valuable document recommended for the development of drugs for the treatment of Botulinum poisoning.

LIST OF ABBREVIATIONS

NPP - Nitrophenyl psoralen
DFT - Density Functional Theory
MEP - Molecular Electrostatic Potential
HOMO - Highest Occupied Molecular Orbital
LUMO - Lowest Unoccupied Molecular Orbital
DOS - Density of State
NLO - Nonlinear Optical

COMPETING INTERESTS STATEMENT

Authors declare no conflicts of interest regarding the publication of this article.

AUTHOR'S CONTRIBUTIONS

T.T.Trung and N.T.Thom designed the method, accomplished the data analysis, and writing the manuscript. The author T.T.Trung edited and revised the final manuscript. All authors have read and agreed to the published version of the manuscript.

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