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4-Nitrobenzoic Acid and Azaperone from *Thaumatococcus daniellii* Leaves as Potential Green Preservative Against Fungal Infestation of Cereals with Molecular Docking Studies

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Abstract
The pursuit of sustainable and eco-friendly preservatives against fungal infestation in cereals has prompted an investigation into the use of *Thaumatococcus daniellii* leaves, used traditionally as a food wrap for cooked cereal like corn pap. Previous studies have demonstrated that food stored with these leaves exhibited better preservation compared to non-wrapped food. Fresh leaves of *Thaumatococcus daniellii* were obtained from Ilorin metropolis in Nigeria. The leaves were dried, pulverized and extracted with methanol by means of soxhlet extraction. Gas chromatography-mass spectrometry (GC-MS) was used to characterize the crude extract. The qualitative phytochemical analysis of the leaves revealed the presence of saponins, glycosides, alkaloids, triterpenes, tannins, steroids, flavonoids, phenolics and terpenoids. The quantitative phytochemical profile of the leaves indicates a high proportion of phenolics, attributed to be responsible for antimicrobial activity. The gas chromatography-mass spectrometry analysis of the methanolic extract of the leaves revealed over 40 compounds. From the molecular docking, two hit compounds- 4-nitrobenzoic acid and azaperone showed a higher binding affinity with lanosterol-14α-demethylase than propiconazole, the standard antifungal drug. This implies a very promising antifungal activity of these compounds for further *in-vitro* and *in-vivo* studies.
***Keywords*: lanosterol-14α-demethylase, molecular docking, mycotoxins, pesticide residue, postharvest loss, propiconazole**

Introduction

The persistence of synthetic pesticides in the environment and the toxic residues left after their applications have become great causes for concern as being responsible for serious and chronic health problems such as cancer, nerve damage, skin diseases and birth defects. The use of synthetic pesticides poses great risk to non-target organisms because 98% of sprayed pesticides affect them directly or indirectly (Ali *et al*., 2021). In a study conducted in the United States of America, dichlorodiphenyltrichloroethane (DDT) was detected in the populace despite the ban on the use of DDT in the United States since 1972. Another important issue to contend with is pesticide poisoning. Globally, approximately 385 million cases of pesticide poisoning occur every year. In Nigeria pesticide poisonings occur regularly however, injuries and deaths are not well reported by government agencies. Pesticides contaminate water through infiltration, surface runoff and drift. They accumulate in the soil causing adverse effects on soil life, for a long period of time. Pesticide residues in food are hazardous to health. The European Union in 2015, placed a ban on the importation of dried beans and other Nigerian agricultural products containing levels of pesticide residues considered dangerous to human health (Pesticide Atlas, 2022). Exposure to these pesticides has been associated with adverse health outcomes such as cancers, neurological disorders, DNA damage, oxidative stress and other diseases (Curl *et al*., 2020). Farmers in desperation to combat post-harvest losses rely on the use of pesticides which are quite harmful to health.

Post-harvest losses are a significant problem affecting food security, especially in developing countries where a significant portion of the population relies on

agriculture for their livelihoods. A major threat to food sustainability is the menace of mycotoxins. Mycotoxins, the secondary metabolites produced by Penicillium, Fusarium and Trichoderma are considered very hazardous to health. They are frequently found in food and feed (Tola and Kebede, 2016). These contaminate feed and food such as legumes, cereals, oilseeds, milk, fruits and vegetables with high levels of moisture and nutrients (Marin *et al*., 2013; Gizachew *et al*., 2016). Mycotoxins are one of the major public health concerns because of their severe toxicity. Significant research work has been carried out on mycotoxins to determine their presence in different food commodities and the extent of their toxicity (Xu *et. al*., 2023). Mycotoxins constitute a danger to the economy and international trade as well as are responsible for irreversible health effects in living beings (WHO, 2006). Poor hygienic conditions during haulage and storage, moisture, heavy rains and high temperatures are conditions that favour mycotoxin growth (FAO, 1995). Mycotoxins are carcinogenic, cytotoxic, teratogenic, estrogenic, immunosuppressant and neurotoxic (Benkerroum, 2016).

The antifungal activity of *Thaumatococcus daniellii* is investigated in this study as an alternative to the use of synthetic pesticide. *T. daniellii* belongs to an African genera of Marantaceae. It is a perennial, understorey herb from the Zingiberales order of flowering plants (Chinedu *et al*., 2018) Locals in the Southern part of Nigeria where *Thaumatococcus daniellii* thrives, utilize its leaves in wrapping foods and its stalks to thatch houses. *Thaumatococcus daniellii* is now known globally as the natural source of thaumatin, a protein sweetener and taste modifier (Sotannde and Oluwadare, 2014). The outcome of different studies shows that medicinal plants have great potential as sources of new antimicrobial agents (Soma *et al*., 2010; Cédric *et al*., 2016, Ajayi and Olatunji, 2017). The antimicrobial properties of *T. daniellii* have been investigated. Hamid *et al*., (2017), Ukwubile *et al*., (2017) and Adeogun *et al*., (2016) have reported on the antimicrobial properties of *Thaumatococcus daniellii.* With this background in mind, this study aims to trace out the constituents present in the *T. daniellii* plant using GC-MS analysis and study the inhibition of these compounds against the antifungal drug target of interest by molecular docking method. It is expected from the outcome of this study that a green alternative to synthetic pesticides will be developed.

Materials and Methods

Collection and Identification of Plant Material

The leaves of *T. daniellii* were harvested from the Ilorin metropolis, Nigeria. The plant was authenticated by a taxonomist at the Department of Plant Biology, University of Ilorin, Kwara State, Nigeria and the voucher number was issued as UILH/001/1077.

Methanolic Extraction of Plant Material

The leaves were thoroughly rinsed with tap water to remove surface dirt and filthy particles. Distilled water was used to re-rinse them. They were dried under a shade at room temperature for the duration of 14 days. An electronic blender was used to grind the plant into a powdered form. 2000 mL of methanol was added to 200 g of the powdered plant sample and run through soxhlet extraction for 8 h. The extract was concentrated in a water bath at a temperature of 50 ^{o}C for five days. The extract was refrigerated and kept for further usage.

Qualitative Phytochemical Screening

Phytochemical analysis of the extract was done according to the method described by Evans (2009 and Aguru *et al*. (2017).

Quantitative Phytochemical Determination

Estimation of Total Phenolic Content

The total phenolic content of the sample was determined using the method of Makkar *et al*. (1997).

Estimation of Total Flavonoids

The aluminium chloride colourimetric assay was used to estimate the total flavonoid content as described by Talari *et al*. (2012).

Estimation of Saponins

The analysis of saponins was carried out using the spectrophotometric method of Brunner (1984). Percentage saponin was calculated using the formula:

% saponin =Absorbance of sample × Average gradient × Dilution factor/Weight of sample × 10,000

Determination of Alkaloids

The method outlined by Aguru *et al*. (2017) was employed in determining the total alkaloids content.

Determination of Tannins

The quantity of tannins was determined using the method of Ademoye *et al*. (2018). Calculation of percentage tannin was done using the formula:

% Tannin = Absorbance of sample × Average gradient × Dilution factor/ Weight of sample × 10,000

Estimation of Steroids

This was assayed by the method of Aguru *et al*. (2017). The absorbance was measured at 780 nm against the reagent blank. The result was calculated from a standard graph plotted and expressed as mg $100g^{-1}$ of the sample.

Determination of Triterpenes

The method outlined by Aguru *et al*. (2018) was used. Digital spectrophotometer was used in reading the absorbance of the sample and the standard concentrations of triterpenes at a wavelength of 510 nm. The percentage of triterpenes was calculated using the formula:

= Absorbance of sample × Average gradient × Dilution factor /Weight of sample × 10,000

Determination of Glycosides

The method of Aguru *et al*. (2018) was used in determining the percentage glycoside calculated as:

% Glycoside = Absorbance of sample × Average gradient × Dilution factor /Weight of sample × 10,000

Determination of Steroids

Percentage steroid was calculated using the formula:

% Steroid = Absorbance of sample × Average gradient × Dilution factor Weight of sample × 10,000

Gas Chromatography and Mass Spectrometry Analysis
GCMS analysis was done with a Hewlett-Packard 6890 gas chromatograph, operating at ionization energy, 70 eV. The injector temperature was operated at 260 °C with splitting ratio 1:30 and injector temperature 250 °C. An HP5MS column fused with phenyl methyl siloxane of length 30 m x 250 µm and film thickness 0.25 µm was used. The temperature of the column was maintained at 50 °C for five minutes and then increased to 240 °C at 5 °C min^{-1}. The carrier gas was helium-operated at a pressure of 100.2 kpa and a linear velocity of 46.5 cm s^{-1}. NIST mass spectral was used for the mass spectral survey. Some of the constituents observed from the GC-MS spectrum of the plant extract, are documented in Table 2.

Docking Analysis of the GCMS-derived Compounds against Selected Target Protein

Ligand Selection and Preparation
The isomeric smiles of six (5) phytochemicals, 4-(4-phenoxy-1H-pyrazol-3-yl) benzene-1,2,3-triol, N'-(2-chloroacetyl)-4-fluorobenzohydrazide, 1,4-Dihydro-pyridine-3-carboxylic acid,5-cyano-6-ethoxy-2-methyl-4-phenyl, 4-nitrobenzoic acid and azaperone present in *T. daniellii* were obtained from PubChem compounds database (https://pubchem.ncbi.nih.gov). Concatenation and conversion of the retrieved isomeric smiles were done to the MDF-SDF file format using OpenBabel. The MDF-SDF File format was converted from the two-dimensional form (2D form) to the three-dimensional form (3D-form) using Data Warrior. Conversion of the 3D form to PDBQT file format was done with PyRx. The PDBQT file format was taken through energy minimization using the optimization algorithm at force field mmff94 on PyRx.

Accession and Preparation of the Standard
The isomeric smile of the standard (propiconazole) present was obtained from the PubChem compounds database (https://pubchem.ncbi.nih.gov). The isomeric smile of the standard was converted to the MDF-SDF file format using OpenBabel. The MDF-SDF File format was converted from the two-dimensional form (2D-form) to the three-dimensional form (3D form) using Data Warrior, and the 3D-form was converted to the PDBQT file format with PyRx. The PDBQT file format was taken through energy minimization using the optimization algorithm at force field mmff94 on PyRx.

Accession and Preparation of the Protein Targets
The 3D crystal structure of the protein target, lanosterol-14α demethylase (PDB: 4LXJ) was retrieved from RCSB PDB (https://www.rcsb.org/pdb/home/home.do). The co-crystalized ligand, bound ligands and non-essential water molecules were removed from the retrieved protein targets using the Pymol tool and exported as a PDB file format.

Molecular Docking Analysis
Upon targets and ligands preparation, molecular docking analysis was performed using PyRxAutoDockVina. After the minimization processes, the grid box resolutions were centered at 22.0141 x 14.2175 x 19.8284 along the X, Y and Z axes at grid dimensions of 25 x 25 x 25°A to define the binding site of lanosterol-14α-demethylase. For each target, the standard and the compounds were docked within the same grid dimension and their resultant binding energies were compared. The molecular interactions between the targets and selected compounds were viewed with Discovery Studio Visualizer version 16.

Absorption Distribution Metabolism, Excretion, and Toxicity (ADMET) Study
The physicochemical properties (bioavailability radars) of the most effective inhibitors were analysed using the web server: http://www.swissadme.ch/index.php#; while the ADMET analysis was carried out using the web server: https://admet.scbdd.com/calcpre/index/.

Results

Phytochemical Screening
The plant extract was screened for the presence of phytochemicals. Tables 1 and 2 show the results of the phytochemical screening. Table 2 depicts the results of quantitative phytochemical screening of the plant. However, three metabolites; amino acid, anthocyanin and phlorotannin were not detected in the plant.

GC-MS analysis of leaf methanolic extract
Over 40 bioactive compounds were found in the methanolic leaf extract. Table 3 below shows the GC-MS results of some phytoconstituents in the *T. daniellii* plant.

Docking Analysis of the GC-MS-derived Compounds against lanosterol-14α-demethylase

Two-dimensional structures of the interactions of lanosterol-14α-demethylase with the hit compounds from T. daniellii and the standard ligand, propiconazole

Absorption, distribution, metabolism, excretion, and toxicity analysis of azaperone and 4-nitrobenzoic acid

The binding energies attained for the docking of the standard ligands (propiconazole) and the GC-MS-derived compounds against lanosterol-14α-demethylase are shown in Figure 3. The binding energy of propiconazole was -7.3 Kcal/mol, while the binding energies of the investigated compounds 1, 2, 3, 4 and 5 were -8.3, -7.3, -7.5, -9.0 and -8.9 Kcal/mol, respectively. Therefore, the investigated compounds with the highest binding energy were compounds 4 and 5. The two-dimensional structures of the interactions of propiconazole and the hit compounds (compounds 4 and 5) with the target protein (lanosterol-14α-demethylase) are shown in Figures 2, 6 and 7. A detailed analysis of these interactions is found in Tables 4, 8 and 9. In Table 4, the molecular interactions observed at the binding pocket of the target protein docked with propiconazole have ten hydrophobic bonds involving Ile-139 (2), Ile-471, Val-154, Val-311, Leu-307, Leu-147, Tyr-140 (2), and Tyr-126. In Table 8, the molecular interactions observed at the binding pocket of the target protein docked with compound 4 include one hydrogen bond; three hydrophobic interactions involving compound 4,

such as Phe-241, Phe-384, and Leu-95; and one other bond involving compound 4 with Met-509. In table 9, the molecular interactions observed at the binding pocket of the target protein docked with compound 5 include one hydrogen bond involving Pro-462; six hydrophobic interactions involving compound 5, such as Leu-380, Tyr-126, Pro-379, Cys-470, Ala-476, and Phe-463; and one other bond involving compound 5 with Cys-470.

The bioavailability radars of the top ligands, compounds 4 and 5 are shown in Figure 9. It was observed that compound 4 (4-nitrobenzoic acid) failed the saturated and polarity tests, while compound 5 (azaperone) failed none of the tests of the six parameters measured. The drug-likeness rules studied include those of Lipinski, Ghose, Oprea, Veber, and Verma. Although compound 4 violated one of Oprea's rules and one of Verma's rules, compound 5 violated none of these rules. However, both compounds violated none of Lipinski's, Ghose's, and Veber's (Table 10). The absorption parameters studied include Caco-2 Permeability (Papp), P-glycoprotein (Pgp)-inhibitor, Pgp-substrate, and human intestinal absorption. The Caco-2 permeability values, -4.453 for compound 4 and -4.742 for compound 5, were optimal. Similarly, it was observed that compound 4 was a non-inhibitor Pgp with a value of 0.262, while compound 5 was an inhibitor with a 0.892 value. However, both compounds were non-substrates of Pgp. The Pgp non-substrate values for compounds 4 and 5 were 0.002 and 0.308 respectively. In addition, the compounds yielded high human intestinal (HIA) absorption values of 0.621 and 0.799 respectively.

The distribution parameters studied were the blood-brain barrier (BBB), plasma protein binding (PPB), and volume distribution (VD). The compounds demonstrated a high ability to cross the BBB with values of 0.927 and 0.992 respectively for compounds 4 and 5; PPB values of 87.090 and 87.218; and VD values of -1.044 and 0.709, respectively (Table 10). The metabolism parameters studied include the CYP450 1A2 inhibitor and substrate, CYP450 3A4 inhibitor and substrate, CYP450 2C9 inhibitor and substrate, CYP450 2C19 inhibitor and substrate, and CYP450 2D6 inhibitor and substrate. It was observed that compound 4 was an inhibitor of CYP450 1A2 with a value of 0.663, while compound 5 was a non-inhibitor with a 0.446 value. Compound 4 was CYP450 1A2 non-substrate with a 0.356 value, while compound 5 had a 0.752 CYP450 1A2 substrate value. Meanwhile, both compounds were non-inhibitors of CYP450 3A4 with the values of 0.013 and 0.318, respectively, while compound 4 had 0.222 values for CYP450 3A4 non-substrate, compound 5 had 0.624 substrate. Furthermore, it was observed that both compounds were non-inhibitors of CYP450 2C9 with 0.076 and 0.153 values, and non-substrates for CYP450 2C9 with 0.353 and 0.260 values, respectively. It was observed also that both compounds were non-inhibitors of CYP450 2C19 with 0.357 and 0.304 values respectively, however, compound 4 had CYP450 2C19 non-substrate value of 0.312while compound 5 had a CYP450 2C19 substrate value of 0.562. Finally, it was observed that compound 4 was a non-inhibitor of CYP450 2D6 with a 0.183 value, while compound 5 was an inhibitor with a 0.754 value. As compound 4 was CYP450 2D6 non-substrate with a 0.287 value, compound 5 was CYP450 2D6 substrate with a 0.689 value. In predicting the rate of excretion of the compounds, the half-life ($T_{1/2}$) and clearance rate were studied. It was observed that the two compounds had low $T_{1/2}$ with 1.321 and 1.877 values, and low clearance rates with 0.551 and 1.611 values, respectively. The toxicity parameters studied include the hERG blockers (hERG), human hepatotoxicity (H-HT), Ames mutagenicity (AMES), and LD_{50} of acute toxicity (LD_{50}). It was observed that compound 4 was a non-blocker of hERG with a 0.301value, while compound 5 was a hERG blocker with a 0.976 value. It was observed also that compound 4 was non-hepatotoxic with a 0.418 value, compound 5 was hepatotoxic with a 0.780 value. However, as compound 4 was observed to be mutagenic with a 0.926 value, compound 5 was non-mutagenic with 0.200 value. Finally, compound 4 had an LD_{50} of 2.227, while that of compound 5 was 2.645 (Table 10).

Discussion

The analysis of the results of the phytochemical screening shows the amount of each secondary metabolite present in mg/100g of the plant sample. From the results, phenolics were found as the largest metabolites compared to other secondary metabolites (Table 2). Due to their bioactive properties, phenolic compounds are important natural molecules (Ogbunugafor *et al*., 2017). Phenols by their simple structure penetrate the microorganisms causing considerable damage to their cell metabolisms. The phytochemical results obtained for the plant extract tallies with the report of Ukwubile (2017). The result also corresponds to the work of Ayodeji *et al*., (2016). The GC-MS revealed over 40 compounds, 5 of which were hit compounds used in the molecular docking analysis. Azaperone, one of the phytoconstituents identified in the plant has also been reported in the methanolic extract of *Bridelia scandens* wild (Ravikumar *et al*., 2018). The modern developments in computational approaches have developed the basis for identification and design of pharmacologically active natural molecules with the objective of targeting proteins of interest. The naturally occurring compounds found in plants can be re-evaluated by computational approaches to prove their potential (Ali *et al*., 2023). Molecular docking analysis is a tool used to determine the interaction between the target protein and the ligand. The discovery of novel antimicrobials involves the target of proteins essential for microbial growth and survival. Lanosterol-14α-demethylase (CYP51A1), a fungal enzyme that is often the target of novel antimicrobials is an example of such an enzyme. CYP51A1 is the main enzyme involved in the synthesis of ergosterol, an important component of the fungal cell

membrane (Becher and Wirsel, 2012). The inhibition of lanosterol-14α-demethylase may cause an accumulation of 14α-methyl sterols on the fungal surface. This causes an alteration of the permeability and rigidity of the plasma membranes resulting in the arrest of fungal growth (Stana *et al*., 2017). In this study, 4-nitrobenzoic acid (compound 4), a constituent of *T. daniellii* inhibited CYP51A1 to a higher degree than all the other GC-MS-derived compounds and the most effective standard inhibitor (propiconazole) (Figure 8). The chemical interactions analyses revealed that there were three hydrophobic interactions and one hydrogen bond formed between compound 4 and the binding site of CYP51A1, while there were ten hydrophobic interactions formed between propiconazole and the binding site of CYP51A1 (Tables 8 and 4). Therefore, the high binding energy observed in the interaction of compound 4 with CYP51A1 can be due to the abundance of hydrophobic interactions. This assertion is solidified by the study of Shi *et al*. (2020), which outlines the role of hydrophobic interactions in the binding of triazoles (the most effective class of CYP51A1 inhibitors) to the binding site of CYP51A1. The authors stated clearly that the hydrophobic residues at the active site of CYP5A1 are necessary for inhibitors' anchoring. Thus, attributing the observed binding energy to the abundance of hydrophobic interactions seems rational.

The bioavailability radar gives firsthand information about the drug-likeness of a molecule (Daina *et al*., 2017). It accounts for six physicochemical properties: lipophilicity, size, polarity, solubility, flexibility, and saturation. It will be observed that compound 4 failed the saturated and polarity tests, being highly unsaturated and highly polar, while azaperone (compound 5) failed none of the tests for oral availability, being neither highly unsaturated nor polar (Figure 9). This may be an indication that the compounds possess a drug-like molecular nature. Lipophilicity relates directly with solubility, and a highly lipophilic compound is often highly insoluble (Box and Comer, 2008). Inhibition and induction of the CYP enzymes have been reported to probably be the common causes of most drug interactions (Hollenberg, 2002). Previous studies assert that a highly lipophilic substance has an increased likelihood of *in vitro* receptor promiscuity (Testa *et al*., 2000; Azzaoui *et al*., 2007; Bender *et al*., 2007; Leeson and Springthorpe, 2007; Peters *et al*., 2009; Gleeson *et al*., 2011; Lobo *et al*., 2020), and *in vivo* toxicity (Hughes *et al*., 2008; Price *et al*., 2009; Greene *et al*., 2010), as well as poor metabolic clearance (Arnott and Planey, 2012). Therefore, since compounds 4 and 5 neither failed the lipophilic test nor the solubility test, renders credence to both as a potential drug candidate (especially compound 5 which failed none of the tests). Although the fact that compound 4 failed the saturation and polarity tests is not as grave as the failure of the lipophilicity and solubility, nonetheless, it is worthy of consideration. It may imply that more fractions of the carbon atoms in compound 4 are probably sp or sp^2 hybridized than sp^3 hybridization, which may negatively affect the oral bioavailability of the drug (Daina *et al*., 2017). Nevertheless, a more polar drug usually encounters difficulty in passing through the phospholipid bilayer into the cell (Yusof and Segall, 2013).

Evaluation of the drug-likeness of the compounds were done according to certain rules (Table 10). As can be observed, compound 5 violated none of the Lipinski's, Ghose's, Opera's, Varma's, and Verber's rules, while compound 4 violated one each of the Oprea's and Varma's rules. This implies the drug-likeness of the compounds is inadequate and the compounds may require optimization. However, it is clear from the number of violations that compound 5 is a better drug candidate than compound 4. Additionally, some absorption parameters of the compounds were evaluated (Table 10). The Papp (Caco-2 Permeability) value of compound 4 was optimal, while compound 5 was low. This may imply that compound 4 would easily pass the human intestinal epithelial cell barrier into the portal circulation than compound 5. It may increase the chance of the compound reaching the target at an effective concentration for appropriate elicitation of action. The Papp (Caco-2 permeability) values of the compounds are corroborated by their high human intestinal absorption (HIA) values. A high human intestinal absorption value connotes better absorption from the intestinal tract upon oral administration, and it is often in tandem with the Papp values. Furthermore, the compounds were observed to be inhibitors and non-substrates of P-glycoprotein. P-glycoprotein acts as an efflux pump. It translocates a substrate from the intracellular to the extracellular compartment of the cell (Kim, 2002). Several vital therapeutic agents are substrates to P-glycoprotein, and their bioavailability is lowered by the action of P-glycoprotein (Srivalli and Lakshmi, 2012). The fact that the compounds are inhibitors of P-glycoprotein implies they can prevent the efflux of therapeutic agents from the intracellular milieu, thereby increasing their intracellular bioavailability. For the distribution parameter, the positive values of the blood-brain barrier (BBB) show that the compounds can act upon the central nervous system (CNS). The BBB serves as the main physical and enzymatic barrier separating brain tissues and the blood preventing the penetration of the CNS by undesirable substances and facilitating its penetration by neurotherapeutics (Misra *et al*., 2003; Abbot *et al*., 2010; Abbot, 2013). Therefore, the ability of the compounds to penetrate the CNS implies the brain and its components can be affected by the administration of the compounds because passage of drugs through the BBB might result to a serious harmful effect that can cause greater metabolic complications. The effect of the compounds on the cytochrome-P_{450} family of enzymes was investigated to assess their metabolism. The cytochrome-P_{450} family of enzymes comprises a large and diverse group whose primary function is in drug metabolism. It accounts for a high percentage of the total number of different metabolic reactions occurring *in vivo* (Guengrich, 2008). There has been reports of the

inhibition and induction of the cytochrome enzymes as the common reasons for most drug interactions (Hollenberg, 2002). They convert lipophilic and non-lipophilic substrates into more polar compounds to assist with their *in vivo* clearance (Arnott and Planey, 2012). Cytochrome-P_{450} induction is less likely to increase drug interactions through the formation of an active metabolite possibly leading to more efficacies, but inhibition of these enzymes can yield reactive metabolites causing toxicity (Muhammad *et al*., 2020). Specifically, CYP3A enzymes are important determinants in the therapeutic efficacy and toxicity of several drugs. The interactions at the CYP3A level are oftentimes the reason for pronounced drug-drug interactions (van Waterschoot and Schinkel, 2011). It was observed that cytochrome-P_{450} family of enzymes was inhibited by compound 4, but was not inhibited by compound 5 (Table 10). An inhibitor of the cytochrome-P_{450} family of enzymes hampers the biotransformation of drugs metabolized by them. Therefore, compound 4 may hamper the metabolism of a lot of drugs since the cytochrome-P_{450} metabolizes about 75% of the drugs that enter the body system. Furthermore, it was observed that the compounds were not substrates for many enzymes in the family. This may suggest that the compounds are largely metabolized by other means. The elimination parameters investigated revealed that both compounds had low half-life and clearance rate. Half-life is the time required for a compound to reduce to half of its initial value. Frequent dosing to maintain desired exposures, may be required for a low half-life. This may cause difficulties in achieving optimal efficacy, safety and patient compliance. A low half-life may require more (Smith *et al*., 2018). The toxicity parameters investigated revealed compound 5 is a hERG blocker, while compound 4 is not. This may imply that compound 5 might precipitate arrhythmias (irregular heartbeat) while compound 4 might not. Specifically, blockade of hERG K^+ channels reduces Ikr (rapidly activating delayed rectifier potassium current) and prolongs ventricular repolarization. The prolonged ventricular repolarization is reflected by an increased QT interval of the surface electrocardiogram, resulting in *Torsade de Pointes* (TdP) and sudden cardiac death (De Ponti *et al*., 2000; Raschi *et al*., 2009). Therefore, the optimization of compound 5 is of great importance should its therapeutic values be desired.

Compound 4 was revealed to be non-hepatotoxic, while compound 5 was hepatotoxic (Table 10).This may imply that compound 4 does not have the potential to harm the liver, while compound 5 could. Conversely, while compound 5 was non-mutagenic, compound 4 was mutagenic. Since mutagenicity is the ability of a compound to induce permanent changes in the genetic material of an organism, and most mutagenic compounds are carcinogenic, the potential mutagenicity of compound 4 calls also for its optimization. The negative value of volume distribution (VD) for compound 4 may indicate its higher distribution in plasma than in tissues. Compound 5 was non-hepatotoxic but mutagenic, while compound 4 was hepatotoxic but non-mutagenic. The mean lethal dose (LD_{50}) for compound 5 was 2.227 mg/kg, while that of compound 4 was 2.645 mg/kg.

Conclusion

The outcome of this study revealed that *T. daniellii* contains phytochemicals that have inhibitory properties against lanosterol-14α-demethylase. The most effective inhibitors in *T. daniellii* are4-nitrobenzoic acid (compound 4) and azaperone (compound 5). The result of this study is quite promising as isolating these two compounds can lead to the development of safe alternatives to harmful biopesticides used in postharvest management of durable food crops especially cereals. However, the ADMET study revealed that both compounds are laden with inadequacies that can hamper their therapeutic relevance. Therefore, further *in silico* studies (drug optimization) that will retain their therapeutic relevance but reduce undesirable features are necessary. Following optimization, experimental studies are recommended for validation of their possible application in post-harvest management.

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Conflict of Interests

The authors declare that there are no conflicts of interest.

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Figure 1: ***Thaumatococcus daniellii*** **plant**

Source: **Muanya (2018)**

Table 1. Qualitative Phytochemical Analysis of *T. daniellii* Extract

Phytochemicals	*T. daniellii*
Saponin	+
Tanin	+
Phenolics	+
Coumarin	+
Glycosides	+
Flavonoids	+
Terpenoids	+
Triterpenes	+
Anthocyanin	-
Amino acid	-
Phlorotannin	-
Alkaloids	+
Steroids	+

+ present - absent

Table 2. Quantitative Phytochemical Analysis of *T. daniellii*

Phytochemicals	*T. daniellii* Quantity (mg 100mg^{-1})
Glycosides	7.06 ± 0.03[a]
Alkaloids	36.91 ± 0.06[a]
Triterpenes	165.85 ± 0.23[a]
Tannins	12.17 ± 0.01[a]
Saponins	23.50 ± 0.02[a]
Steroids	183.27 ± 0.35[a]
Flavonoids	387.49 ± 0.73[a]
Phenolics	591.83 ± 0.20[a]
Terpenoids	42.49 ± 0.06[a]

Values represent the mean of duplicate determinations ± standard deviation (SD). Values with different letters are significantly different at $p < 0.05$

Table 3. GC-MS results of some Phytoconstituents in *T. daniellii* Plant

S/N	Compound	Formula	Molecular weight	Retention Time
1	m-Guaiacol	$C_7H_8O_2$	124.14g mol^{-1}	9.230
2	4-(4-phenoxy-1H-pyrazol-3-yl) benzene-1,2,3-triol	$C_{15}H_{12}N_2O_4$	284.27g mol^{-1}	12.652
3	Pentadecanoic acid, 14-methyl-ester	$C_{17}H_{34}O$	270.45 g mol^{-1}	14.241
4	n-Hexadecanoic acid	$C_{16}H_{32}O$	256.42 g mol^{-1}	19.770
5	Azaperone		327.17 gmol^{-1}	21.159
6	Oleic Acid	$C_{19}H_{22}FN_3O$	282.46 g mol^{-1}	21.553
7	N'-(2-chloroacetyl)-4-fluorobenzohydrazide		230.62g mol^{-1}	22.679
8	Bis(2-ethylhexyl) phthalate	$C_{18}H_{34}O_2$	390.56 g mol^{-1}	24.387
9	4-nitrobenzoic acid		167.12 g mol^{-1}	29.103
10	1,4-Dihydro-pyridine-3-carboxylic acid, 5-cyano-6-ethoxy-2-methyl-4-phenyl	$C_9H_8ClFN_2O_2$ $C_{24}H_{38}O_4$ $C_6H_4CO_2H$ $C_{16}H_{16}N_2O_3$	284.31g mol^{-1}	33.457

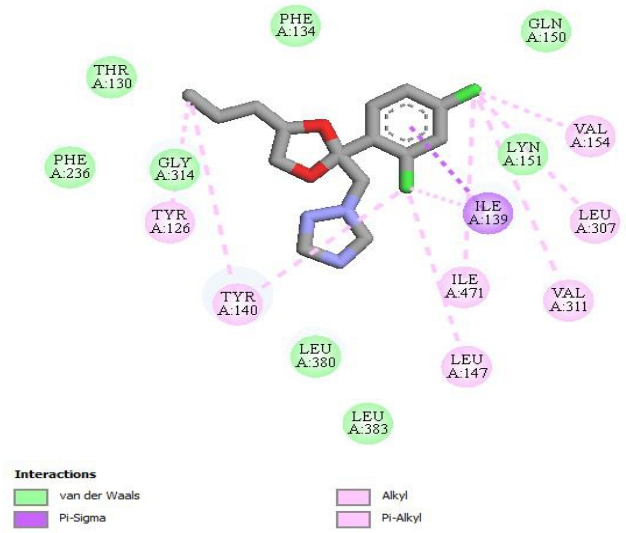


Figure 2. The two-dimensional structure of the interaction of propiconazole with the binding pocket of lanosterol-14α-demethylase

Table 4. Analysis of the interaction of propiconazole with the binding pocket of lanosterol-14α-demethylase

Name	Category	Types
A:ILE139:CD1 - N:Propiconazole	Hydrophobic	Pi-Sigma
N:Propiconazole:Cl - A:ILE139	Hydrophobic	Alkyl
N:Propiconazole:Cl - A:LEU147	Hydrophobic	Alkyl
N:Propiconazole:Cl - A:VAL154	Hydrophobic	Alkyl
N:Propiconazole:Cl - A:LEU307	Hydrophobic	Alkyl
N:Propiconazole:Cl - A:VAL311	Hydrophobic	Alkyl
N:Propiconazole:Cl - A:ILE471	Hydrophobic	Alkyl
A:TYR126 - N:Propiconazole:C	Hydrophobic	Pi-Alkyl
A:TYR140 - N:Propiconazole:C	Hydrophobic	Pi-Alkyl
A:TYR140 - N:Propiconazole:Cl	Hydrophobic	Pi-Alkyl

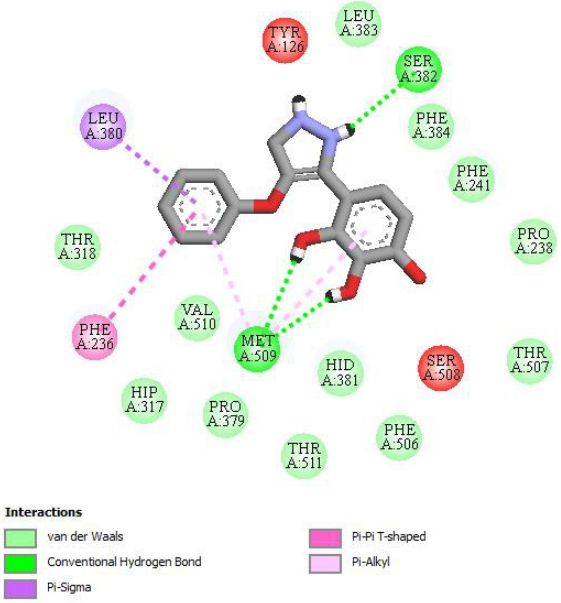


Figure 3. The two-dimensional structure of the interaction of compound 1 with the binding pocket of lanosterol-14α-demethylase

Table 5. Analysis of the two-dimensional structure of the interaction of compound 1 with the binding pocket of lanosterol-14α-demethylase

Name	Category	Types
N:Compound 1:H - A:SER382:O	Hydrogen Bond	Conventional Hydrogen Bond
N:Compound 1:H - A:MET509:O	Hydrogen Bond	Conventional Hydrogen Bond
N:Compound 1:H - A:MET509:O	Hydrogen Bond	Conventional Hydrogen Bond
A:LEU380:CD2 - N: Compound 1	Hydrophobic	Pi-Sigma
A:PHE236 - N: Compound 1	Hydrophobic	Pi-Pi T-shaped
N:Compound 1 - A:MET509	Hydrophobic	Pi-Alkyl
N:Compound 1 - A:MET509	Hydrophobic	Pi-Alkyl

4-(4-phenoxy-1H-pyrazol-3-yl)benzene-1,2,3-triol (Compound 1)

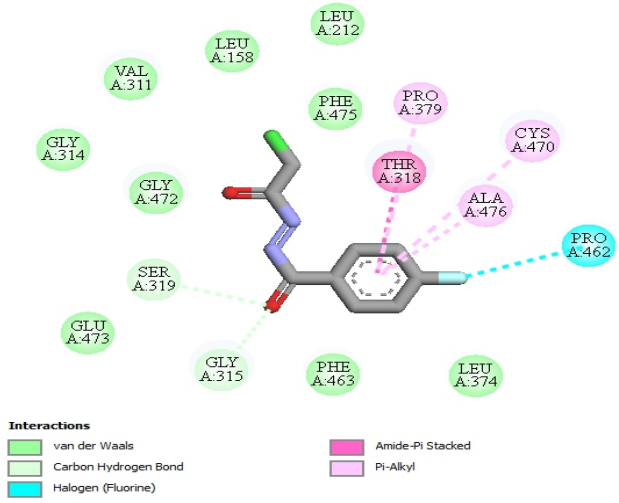


Figure 4. The two-dimensional structure of the interaction of compound 2 with the binding pocket of lanosterol-14α-demethylase

Table 6. Analysis of the interaction of compound 2 with the binding pocket of lanosterol-14α-demethylase

Name	Category	Types
A:GLY315:CA - N:Compound 2:O	Hydrogen Bond	Carbon Hydrogen Bond
A:SER319:CB - N:Compound 2:O	Hydrogen Bond	Carbon Hydrogen Bond
A:PRO462:O - N:Compound 2:F	Halogen	Halogen (Fluorine)
A:THR318:C,O;SER319:N - N:Compound 2	Hydrophobic	Amide-Pi Stacked
N:Compound 2 - A:PRO379	Hydrophobic	Pi-Alkyl
N:Compound 2 - A:CYS470	Hydrophobic	Pi-Alkyl
N:Compound 2 - A:ALA476	Hydrophobic	Pi-Alkyl

N'-(2-chloroacetyl)-4-fluorobenzohydrazide (Compound 2)

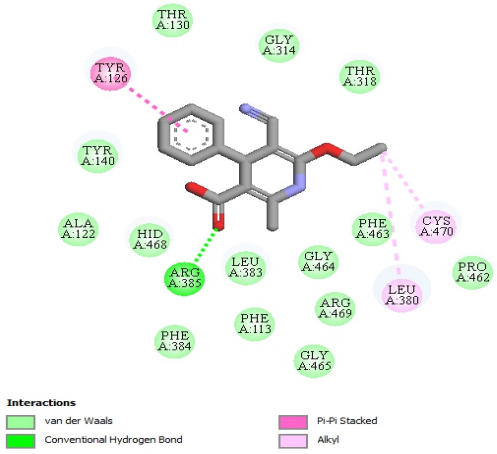


Figure 5. The two-dimensional structure of the interaction of compound 3 with the binding pocket of lanosterol-14α-demethylase

Table 7. Analysis of the interaction of compound 3 with the binding pocket of lanosterol-14α demethylase

Name	Category	Types
A:ARG385:HH12 - N:Compound 3:O	Hydrogen Bond	Conventional Hydrogen Bond
A:TYR126 - N:Compound 3	Hydrophobic	Pi-Pi Stacked
N::Compound 3 - A:LEU380	Hydrophobic	Alkyl
N:Compound 3:C - A:CYS470	Hydrophobic	Alkyl

1,4-Dihydro-pyridine-3-carboxylic acid, 5-cyano-6-ethoxy-2-methyl-4-phenyl (Compound 3)

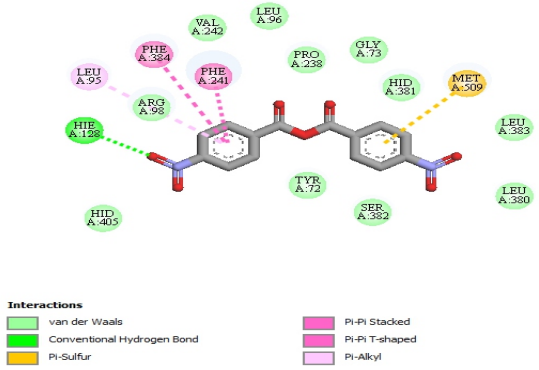


Figure 6. The two-dimensional structure of the interaction of compound 4 with the binding pocket of lanosterol-14α-demethylase

Table 8. Analysis of the interaction of compound 4 with the binding pocket of lanosterol-14α-demethylase

Name	Category	Types
A:HIE128:HE2 - N:Compound 4:O	Hydrogen Bond	Conventional Hydrogen Bond
A:MET509:SD - N:Compound 4	Other	Pi-Sulfur
A:PHE241 - N:Compound 4	Hydrophobic	Pi-Pi Stacked
A:PHE384 - N:Compound 4	Hydrophobic	Pi-Pi T-shaped
N:Compound 4 - A:LEU95	Hydrophobic	Pi-Alkyl

4-nitrobenzoic acid (Compound 4)

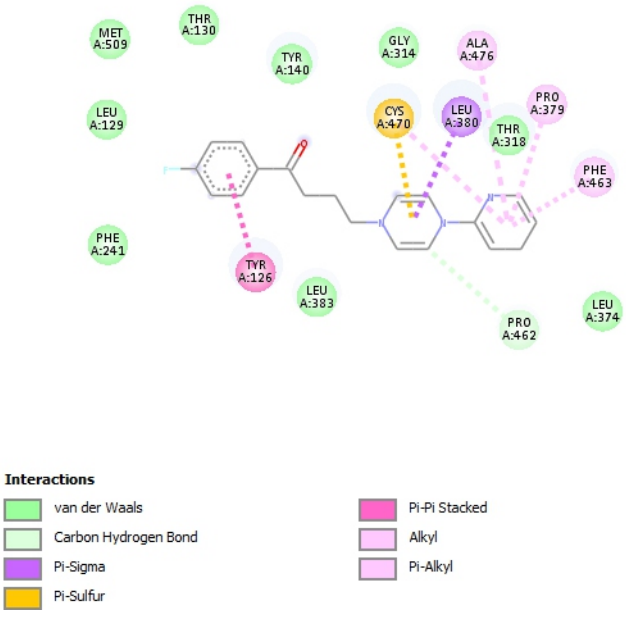


Figure 7. Two-dimensional structure of the interaction of compound 5 with the binding pocket of lanosterol-14α- demethylase

Table 9. Analysis of the interaction of compound 5 with the binding pocket of lanosterol-14α demethylase

Name	Category	Types
N: Compound 5:C - A:PRO462:O	Hydrogen Bond	Carbon Hydrogen Bond
A:LEU380:CD1 - N:Compound 5	Hydrophobic	Pi-Sigma
A:CYS470:SG - N:Compound 5	Other	Pi-Sulfur
A:TYR126 - N:Compound 5	Hydrophobic	Pi-Pi Stacked
A:PRO379 - N:Compound 5	Hydrophobic	Alkyl
A:CYS470 - N:Compound 5	Hydrophobic	Alkyl
A:ALA476 - N:Compound 5	Hydrophobic	Alkyl
A:PHE463 - N:Compound 5	Hydrophobic	Pi-Alkyl

Azaperone (Compound 5)

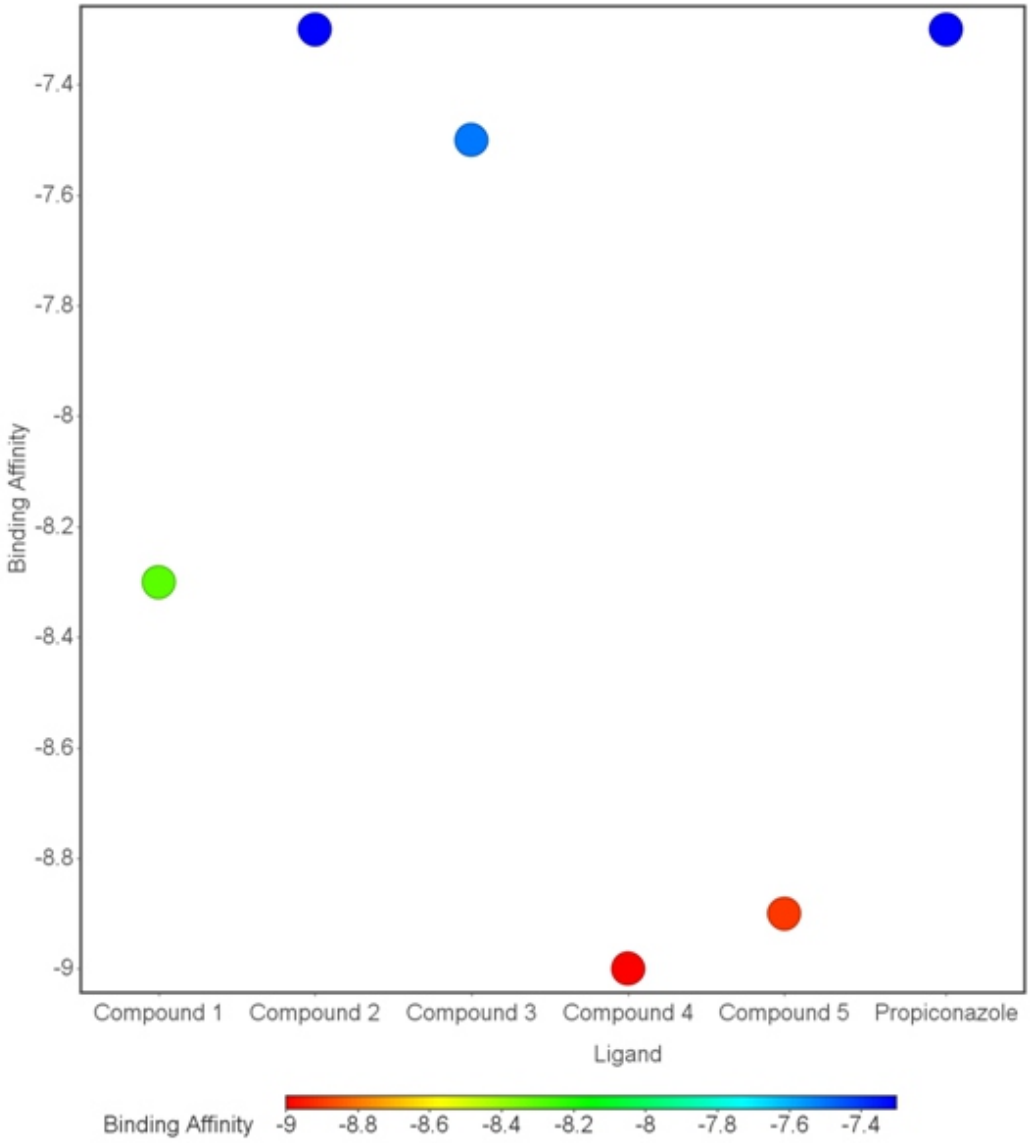


Figure 8. Binding affinities obtained from the docking analysis of lanosterol-14α-demethylase with propiconazole and hit compounds from *T. daniellii*

Legend:

4-(4-phenoxy-1H-pyrazol-3-yl)benzene-1,2,3-triol (Compound 1)

N'-(2-chloroacetyl)-4-fluorobenzohydrazide (Compound 2)

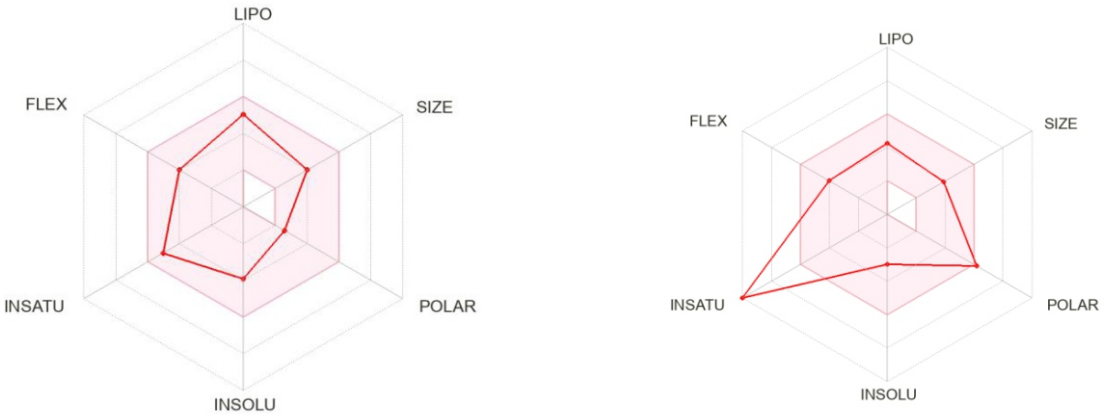
1,4-Dihydro-pyridine-3-carboxylic acid, 5-cyano-6-ethoxy-2-methyl-4-phenyl (Compound 3)

4-nitrobenzoic acid (Compound 4)

Azaperone (Compound 5)

Absorption, distribution, metabolism, excretion, and toxicity (ADMET) Study

Bioavailability estimation of the top ligands azaperone and 4-nitrobenzoic acid



Azaperone

4-nitrobenzoic acid

Figure 9. The bioavailability radars of azaperone and 4-nitrobenzoic acid

The colour space is the suitable physicochemical space for oral bioavailability

LIPO Lipophilicity: -0.7 < XLOGP3 < +5.0

SIZE: 150g/mol: < MW < 500g/mol

POLAR (Polarity): 20A ^{o2} < TPSA < 130 A^{o2}

INSOLU (insolubility): 0 < Log S (ESOL) < 6

INSATU (in saturation): 0.25 < Fraction Csp3 <1

FLEX (Flexibility): 0 < Num. rotatable bonds <

Table 10. Analysis of ADMET study for compounds 4 and 5

Parameter	Azaperone	4-nitrobenzoic acid
Druglikeness Evaluation		
Lipinski's Rule Violations	0 violation	0 violation
Ghose's Rule Violations	0 violation	0 violation
Oprea's Rule Violations	0 violation	1 violation
Veber's Rule Violations	0 violation	0 violation
Varma's Rule Violations	0 violation	1 violation
Absorption		
Papp (Caco-2 Permeability)	-4.742 (Low)	-4.453 (Optimal)
Pgp-inhibitor	0.892 (Inhibitor)	0.262 (Non-Inhibitor)
Pgp-substrate	0.308(Non-substrate)	0.002 (Non-substrate)
HIA (Human Intestinal Absorption)	0.799 (HIA+)	0.621 (HIA+)
F (20% Bioavailability)	0.717 (F20+)	0.464 (F20-)
F (30% Bioavailability)	0.715 (F30+)	0.676 (F30+)
Distribution		
BBB (Blood–Brain Barrier)	0.992 (BBB+)	0.927 (BBB+)
Plasma Protein Binding	87.218	87.090
Volume Distribution	0.709	-1.044
Metabolism		
CYP450 1A2 inhibitor	0.446 (Non-inhibitor)	0.663 (Inhibitor)
CYP450 1A2 substrate	0.752 (Substrate)	0.356 (Non-substrate)
CYP450 3A4 inhibitor	0.318 (Non-inhibitor)	0.013 (Non-inhibitor)
CYP450 3A4 substrate	0.624 (Substrate)	0.222 (Non-substrate)
CYP450 2C9 inhibitor	0.153 (Non-inhibitor)	0.076 (Non-Inhibitor)

CYP450 2C9 substrate	0.260 (Non-substrate)	0.353 (Non-substrate)
CYP450 2C19 inhibitor	0.304 (Non-inhibitor)	0.357 (Non-inhibitor)
CYP450 2C19 substrate	0.562 (Substrate)	0.312 (Non-substrate)
CYP450 2D6 inhibitor	0.754 (Inhibitor)	0.183 (Non-inhibitor)
CYP450 2D6 substrate	0.689 (Substrate)	0.287 (Non-substrate)
Excretion		
$T_{1/2}$ (Half-Life)	1.877 (Low)	1.321 (Low)
Clearance	1.611 (Low)	0.551 (Low)
Toxicity		
hERG (hERG Blockers)	0.976 (Blocker)	0.301 (Non-blocker)
H-HT (Human Hepatotoxicity)	0.780 (Hepatoxic)	0.418 (Non-hepatoxic)
AMES (Ames Mutagenicity)	0.200 (Non-mutagenic)	0.926 (Mutagenic)
SkinSen (Skin Sensitization)	0.437 (Non-sensitizer)	0.670 (Sensitizer)
LD50 (LD50 of Acute Toxicity)	2.645	2.227
DILI (Drug Induced Liver Injury)	0.448 (DILI-)	0.862 (DILI+)
FDAMDD (Maximum Recommended Daily Dose)	0.378 (FDAMDD-)	0.768 (FDAMDD+)