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# MANAGING RIFAMPICIN RESISTANCE IN TUBERCULOSIS UTILIZING VIRTUAL SCREENING TECHNIQUES

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**ABSTRACT**

Belonging to the class of Rifamycins, a semisynthetic antibiotic, Rifampicin is one of the major first-line anti-tuberculosis drugs utilized for treating tuberculosis. Recent researches suggest that the advent of mutations in the rpoB gene in the causative agent Mycobacterium tuberculosis appears to have resulted in the development of acquired resistance against Rifampicin action. Thus, this makes the development of effective anti-tuberculosis drugs necessary for treating rifampicin-resistant tuberculosis. With the help of virtual screening, molecular docking, a group of lead molecules was discovered in this study. With the assistance of the Pubchem database, virtual screening was performed with Rifampicin as the query and using molecular docking, the data was reduced. Physicochemical and structural parameters of the lead compounds were predicted and calculated using Molinspiration, as well as docking scores were predicted with the help of PatchDock. The Lipinski rule of five was used to assess the bioavailability of lead compounds. The OSIRIS program was utilized to examine the screened lead molecules for toxicity profiles, drug-likeness, drug score, and other physic-chemical characteristics of the drugs. The findings of this study show that CID: 265237, a phytochemical derived from the plants belonging to the *Withania somnifera* might be a promising candidate that could be of potential to assist patients with tuberculosis overcome treatment resistance. Our study correlates well with the experimental findings as well.

***Keywords*** **:** Tuberculosis, Rifampicin, Mutation, Virtual screening, Molecular docking.

## Introduction

One of the most infectious and deadliest diseases around the globe is Tuberculosis (TB), the causative agent of which is the bacteria Mycobacterium tuberculosis (Mtb). Tuberculosis largely affects the lungs, yet can likewise infect different organs or parts of an individual's body (Smith, 2003). Common manifestations of dynamic TB are a continuous cough along with body fluid that consists of blood, fever, as well as loss of weight. When individuals that are infected with Mtb in their lungs either cough, spit, or even talk, or sneeze, the disease spread onto the next via the air (Nicas *et al.*, 2005). An X-ray of the chest helps determine TB (Konstantinos, 2010). Treatment requires the utilization of different anti- microbials over a significant stretch of time. Data from the World Health Organization (WHO) displays that a sum of 1.5 million deaths occurred in the year 2020 due to TB (including 214,000 ones with HIV). Moreover, tuberculosis is curable and preventable.

The causative agent and thus the fundamental driver of TB, Mycobacterium tuberculosis (Mtb) is a small, vigorous, non-motile bacillus that starts the infection when it arrives in the lungs (particularly at the alveolar air sacs), where it attacks as well as reproduces inside alveolar macrophages, particularly inside the endosomes (Queval *et al.*, 2017). Then, the bacterium is identified by the macrophages as non-native which then endeavour to get rid of it through the process of phagocytosis. When this happens, the bacterium gets enveloped by the macrophage and are briefly kept away in a phagosome (a film bound vesicle) which then combines with a lysosome thereby creating a phagolysosome. The cell endeavors in the phagolysosome to execute the bacterium by making use of reactive oxygen species and corrosive. Nonetheless, Mtb is protected from these dangerous elements by a thick waxy mycolic corrosive container. Mtb has the ability to proliferate inside the macrophage, eventually killing the immune cell.

Several anti-microbial drugs have been used in the treatment of TB like first-line drugs which include isoniazid (INH), rifampicin (RIF), ethambutol (EMB), etc and certain second-line drugs like Fluoroquinolones including ofloxacin (OFX), levofloxacin (LEV), etc, Linezolid, delamanid, bedaquiline (new ones), and injectables like Kanamycin (KAN), amikacin (AMK) and others (Rendon *et al.*, 2016).

Rifampicin is one of these antibiotics, belonging to the class of Rifamycins,a semisynthetic antibiotic produced from Amycolatopsis rifamycinica (previously known as Amycolatopsis mediterranei and Streptomyces mediterranei) that is widely used. It's a macrocyclic antibiotic with a lot of action against mycobacteria that is often used in TB therapy in conjunction with other drugs because it inhibits DNA-dependent RNA polymerase (Kumar, 2017). However, during the most recent couple of many years, the frequency

of this microbial infections has expanded drastically and so, ceaseless deployment of antimicrobial medications in treating this diseases has driven to the development of resistance among the strains (called drug resistance which is defined as the decrease in viability of medications like an antimicrobial or an antineoplastic in treating a sickness or condition). Thus, emerges rifampicin-resistant (RR), a type of drug resistance which is a human-made problem and emerging due to improper utilisation of anti-TB drugs through erroneous remedy by medical services suppliers, low-quality medications, and patients halting therapy rashly, poor management of TB which possess a threat to control of TB (Trivedi and Desai, 1988). It includes every type of drug resistance involving rifampicin, like mono-, poly-, multidrug resistance (MDR), and extensive drug resistance (XDR) (Manson *et al.*, 2017).

The action of rifampicin in *M. tuberculosis* has been assumed to have the mycobacterial RNA polymerase as the target for over 20 years (Blanchard, 1996). The majority of rifampicin-resistant strains of Mtb isolated clinically comprise of mutations in a gene that codes for the RNA polymerase's β-subunit which is the *rpoB* gene as a result of which it undergoes structural changes, lowering its affinity for the drug in TB and leading to the emergence of resistance. Most rifampicin resistance-causing mutations in the mycobacterial rpoB gene are single nucleotide changes resulting in single amino acid substitutions (93%) while the rest are found to be insertions (3%) and deletions (4%) (Blanchard, 1996).

According to analyses, mutations in the "hot-spot region" of the rpoB gene (at the 81-bp spanning codons 507–533) are identified in around 96 per cent of M. tuberculosis strains obtained that exhibit rifampicin resistance. Rifampicin resistance-determining region is the name given to this area. In most studies, mutations in the codons 516, 526, and 531 account for many mutations related to this form of resistance (Zaw *et al.*, 2018). However, outside this hot-spot area, some mutations (perhaps present in the protein's carboxy-terminal region) have been reported in certain instances (albeit they are less common) (Blanchard, 1996).

With the rise in TB drug resistance cases and a higher rate of mortality, it is critical to gain a better understanding of Mtb drug resistance, which will improve available techniques for rapid detection of drug resistance and aid in the exploration of new drug activity and development targets in order to develop a new and more effective anti-microbial drug against TB, particularly for the treatment of rifampicin-resistant TB. Computational techniques, (virtual screening (VS) particularly), have been found to be of great use for years to reduce the time period and cost for developing a new drug (Lin *et al.*, 2020). According to the evidence in the literature, VS approaches were effective in providing qualitative predictions in distinguishing active compounds from inactive ones. As a result, VS approach has been utilised in this study to address rifampicin resistance in TB. This study is expected to assist experimental biologists in identifying promising candidates for TB treatment.

## Materials and Methodology

### Dataset

With the help of the Protein Data Bank (PDB) (Bernstein, 1997), the three dimensional (3D) structure of native and mutant (S531L) RNA polymerase (RNAP) structures were obtained for the analysis. The corresponding PDB codes were 5UHC and 5UAL for the native and mutant (rifampicin-resistant) structures respectively. Rifampicin was employed as the study's small molecule, the SMILES string of which and of the lead molecules were obtained from PubChem database and submitted into the CORINA 3D structure generator for the 3D structure of the molecules' construction.

### Virtual screening

It is a computational technique utilised in drug discovery, virtual screening (VS) (Sousa *et al.*, 2010) is an important technique in computer-aided drug discovery (CADD), used to look through libraries of small molecules to distinguish those structures which are well on the way to bind to a drug target, regularly a protein receptor or enzyme. With the increase in the precision of the strategy, virtual screening has become a fundamental piece in the process of drug discovery.

Using PubChem database (Kim *et al.*, 2016) and rifampicin as a query, virtual screening was carried out. It is notable highlighting that the PubChem database has about 27 million distinct constructions of chemical compounds with compound ID (CID) obtained from almost 70 million depositions of substances with substance ID (SID). The PubChem database, which is open to the public, gives excellent opportunities for scientists to undertake the VS process (Kim, 2016). The PubChem database yielded several hits, which were then evaluated using molecular docking studies.

### ADME and toxicity

With the help of Lipinski's rule of five (Benet *et al.*, 2017), which comprises of four molecular properties that are logP (octanol-water partition coefficient), molecular weight (MW), the quantity of hydrogen bond acceptors, as well as that of hydrogen bond donors in a molecule, bioavailability of the lead compounds were evaluated. According to the rule, an orally active molecule with good membrane permeability should have a molecular weight of 500 daltons, calculated octanol-water partition coefficient, logP to not exceed 5, not more than 5 hydrogen bond donors, hydrogen bond acceptors to not exceed 10, and van der Waals bumps polar surface area (PSA) to be less than 120 $Å^2$.

The various physicochemical properties of all the lead compounds in this study have been calculated with the help of the Molinspiration program (Tariq, 2016) (https://www.molinspiration.com/cgi-bin/properties). The overall potential of a lead compound to be qualified as a potential drug lies in whether or not it is toxic in nature. Thus toxicity parameters like mutagenicity, tumorigenicity, and reproductive effects are the next most important to be taken into account in the analysis of lead compounds. As a matter of fact, the failure of most of the lead cases is reported due to toxicity. The toxicity of the lead compounds in this study was examined using the OSIRIS software (Nalini *et al.*, 2011) (https://www.organic-chemistry.org/prog/peo). 3300 traded pharmaceuticals and 15,000 commercially accessible compounds combined to produce almost 5300 different substructure pieces, yielding a comprehensive inventory of all available fragments and their therapeutic potential (Balakrishnan *et al.*, 2015). It also helped analyse the lead compounds' drug-likeness and drug

score (which integrates drug-likeliness, cLogP, logS, molecular weight, as well as toxicity risk to create a total number that reflects a compound's net likely to be qualified for a medicine) (Balakrishnan *et al.*, 2015).

**Molecular docking**

Getting to understand the bioactivity of the screened lead compounds necessitates a docking investigation for which, SMILES strings were first employed to build the three-dimensional structure of all the lead compounds. Following that, with the help of the Patch dock server (Schneidman-Duhovny *et al.*, 2005) (available at http:// bioinfo3d.cs.tau.ac.il/PatchDock/), the docking algorithm was carried out which is a geometry-based molecular docking computational technique (Morris and Lim-Wilby, 2008). The protein is represented by the PDB coordinate file, whereas the ligand molecule serves as the docking input parameter. The docked complexes were ranked based on the geometric matching score with the target proteins. The method has three governing steps: molecular shape representation, surface patch matching, and filtering and scoring. In this study, docking of Rifampicin and all the lead compounds was carried out with 5UHC and 5UAL (the native and mutant (rifampicin-resistant) structures respectively).

## Results and Discussion

**Virtual screening technique**

The current study began with a search of various scientific literature available witnessing the potential of various phytochemicals from plants rendering inhibition against the infection of Mtb as well as its resistant strains. The search led to a yield of about 59 compounds from about 26 medicinal plants. The PubChem database was then utilised for extracting these compounds which were used in the future study. Rifampicin was employed as a query chemical in this study. The bioavailability of rifampicin and the lead compounds was predicted with the help of the Molinspiration software. The characteristics of rifampicin were estimated using the Molinspiration program (Fig. 1) which was then used as a reference for screening the other lead compounds. Table 1 displays the outcome of this. The chart clearly shows that 19 compounds including rifampicin, CID: 36462, CID: 452548, CID: 13342, CID: 5978, CID: 6436208, CID: 36314, CID: 148124, CID: 11734982, CID: 12309402, CID: 5270628, CID: 92158, CID: 222284, CID: 5280794, CID: 108058, CID: 10906239, CID: 73611, CID: 119247, CID: 9549171, violated the rule of five. As a result, it can be concluded that the bioavailability of the remaining 41 compounds in our dataset was much higher.

These hits have been refined even further by limiting the number of rotatable links to ten in order to pass the oral bioavailability criterion according to which, the number of rotatable bonds should be less than ten (Veber *et al.*, 2002). Table 2 displays the final outcome. Table 2 shows that virtually all of the 41 compounds selected from the ADME study had a sufficient number of rotatable bonds, not exceeding 10. As a result of this finding, these compounds have the potential to be used as lead compounds. Toxicity, on the other hand, is an essential concern that should be considered for all lead compounds before they are chosen.

**Toxicity analysis**

Toxicological testing is a crucial phase in the creation of new drugs and in the improvement of current ones' therapeutic potential. In fact, the majority of compounds in the drug development process fail due to problems related to their pharmacokinetics and toxicity (Bugrim *et al*, 2004). These difficulties were addressed in the current study with the aid of the OSIRIS property explorer program. Parameters like clogP and logS can be used to evaluate a lead compound's pharmacokinetic properties. Table 3 shows the outcome from the OSIRIS property explorer program for all the lead compounds taken forward. The hydrophilicity of a compound is measured by clogP. Because of the compound's limited hydrophilicity, large logP values may result in poor absorption or permeation of the compound. It has been proven that compounds must have a logP value of less than 5.0 in order to have a fair chance of being properly absorbed. The logP values of all the 41 compounds in this study were determined to be within acceptable parameters, as shown in the table.

It is seen that a compound's absorption and distribution properties are generally influenced by its solubility. In reality, poor absorption of a drug could be due to its low solubility might result in its poor absorption (Coltescu *et al.*, 2020). LogS is a common solubility unit that corresponds to the 10-based logarithm of a molecule's solubility assessed in mol/L. A (anticipated) log S value larger than -4 is seen in more than 80% of drugs on the market. Table 3 reveals some of the lead compounds' solubility was found to be equal to that of common pharmaceuticals in order to fulfil the solubility standards, indicating that this might be deemed an acceptable drug for oral absorption.

**Drug likeness**

Drug likeness (Bickerton *et* al., 2012) is an important metric as potential drug-like molecules have favourable absorption, distribution, metabolism, excretion, and toxicity (ADMET). The drug-likeness of rifampicin and other virtually screened compounds in this investigation was determined with the help of the OSIRIS program. It is important to note that the drug-likeness value of all the 41 lead compounds met the acceptable standards.

**Drug score and toxicity**

When the 41 lead compounds are put through the mutagenicity evaluation system and compared to standard drugs used, the information in Table 3 demonstrates that 30 compounds should be non-mutagenic and non-tumorigenic. CID: 10607, CID: 6167, CID: 5280343, CID: 6683, CID: 6293, CID: 196978, CID: 3314, CID: 637511, CID: 400072, CID: 10205, and CID: 5464032 were shown to be mutagenic and tumorigenic after failing to pass via the Osiris program (demonstrated in Table 3). The lead compounds' overall drug scores (DS) were also evaluated and compared to that of rifampicin's. Drug likeness, miLogP, logS, molecular weight, and toxicity risks are all factored into the score (Brito, 2011). The DS might potentially be a useful criterion for determining if a compound has the potential to satisfy all of the standards for drug approval.

When compared to the standard drug rifampicin, the identified lead compounds showed moderate to excellent DS. A drug score similar to that of rifampicin was found in about 8 lead compounds in the dataset. About six compounds such

as CID: 10666, CID: 15558419, CID: 124049, CID: 24360, CID: 5281605, and CID: 667450, showed a drug score of 0.6 and above. As a result, 30 compounds were further studied.

### Molecular docking

In order to determine the binding affinity of the lead compounds with the target protein, a molecular docking program was utilised. To rule out false positives, docking analysis was carried out twice. Table 4 showcases the docking outcome. The docking score of the native-type Mtb transcription initiation complex-rifampicin complex was calculated to be 6502, while that for the mutant-type RNAP-rifampicin complex was calculated to be 6126. The lower docking score of the mutant complex demonstrates that the mutation (S531L) has a substantial impact on rifampicin binding to RNAP structures. It is thought that a prospective lead compound would have a higher docking score than rifampicin, the current drug molecule. As a result, using both normal and mutant systems, the docking scores for each of the 30 hits were looked at. It is worth noting that 1 hit from the dataset (CID: 265237) displayed significantly superior docking scores in both the native and mutant forms compared to rifampicin. The native-type Mtb transcription initiation complex-CID: 265237 complex had a docking score of 6550, while the mutant-type RNAP-CID: 265237 complex had a docking score of 6158. When compared to rifampicin, this data reveals that CID: 265237 has a stronger binding affinity not only with the native type but also with the mutant.

It's also important to emphasize that CID: 265237 showed better pharmacokinetic and pharmacodynamic outcomes than the other lead compounds investigated in our study (Fig. 2). A study of CID: 265237 (Withaferin A) reveals that it is a with anolide that has hydroxy groups at positions 4 and 27 and is 5,6:22,26-diepoxyergosta-2,24-diene-1, 26-dione (the 4β, 5β, 6β, 22R stereoisomer) (Sultana *et al.*, 2021). It is found mainly in the leaves and bark of *Withania somnifera* also known as Ashwagandha or sometimes referred to as Indian ginseng. It has cytotoxic properties.

## Conclusion

With the usage of the virtual screening technique, this study was able to address the rifampicin resistance in tuberculosis. CID: 265237 was revealed to be more drug-like after successfully passing through the pharmacokinetics and toxicology criteria. CID: 265237 was obtained from *Withania somnifera*. Among the lead compounds examined from the Pubchem database, CID: 265237 exhibits the highest binding affinity with both native and mutant type TB systems, according to the docking study. It's significant to stress that our findings are in line with existing experimental research (Periyakaruppan *et al.*, 2012; Sharma, 2022; Manivar and Shanuvas, 2018). The available evidence shows that Withaferin A obtained from *Withania somnifera* may have anti-tuberculosis properties. We anticipate that the data presented here will aid in the development of effective medications to treat drug-resistant tuberculosis. It's important to note that the results of this study are consistent with previous experimental research. It is anticipated that the data presented here will aid in the development of effective medications to treat rifampicin-resistant tuberculosis.

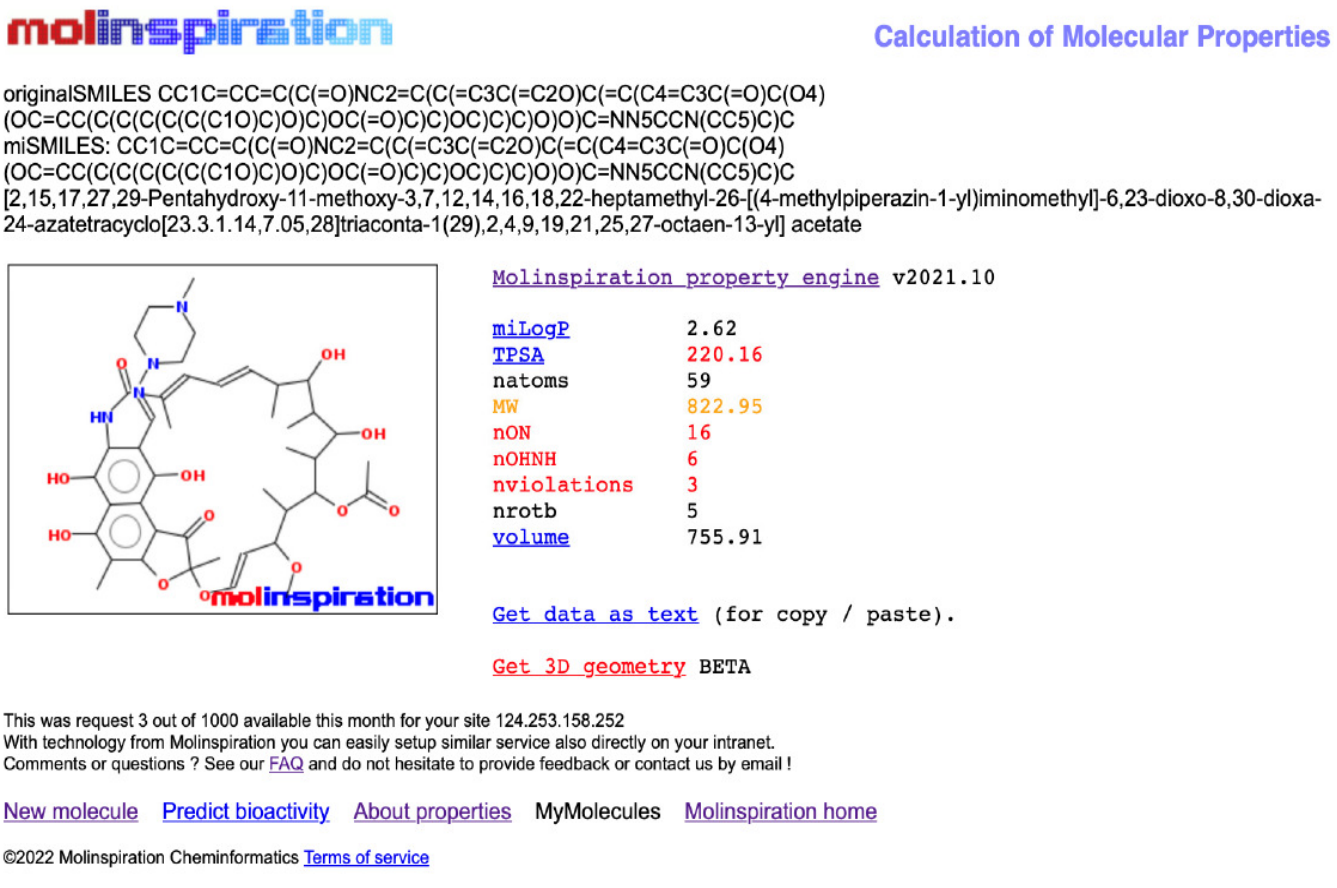


**Fig. 1:** Molecular properties of Rifampicin calculated using the Molinspiration program.

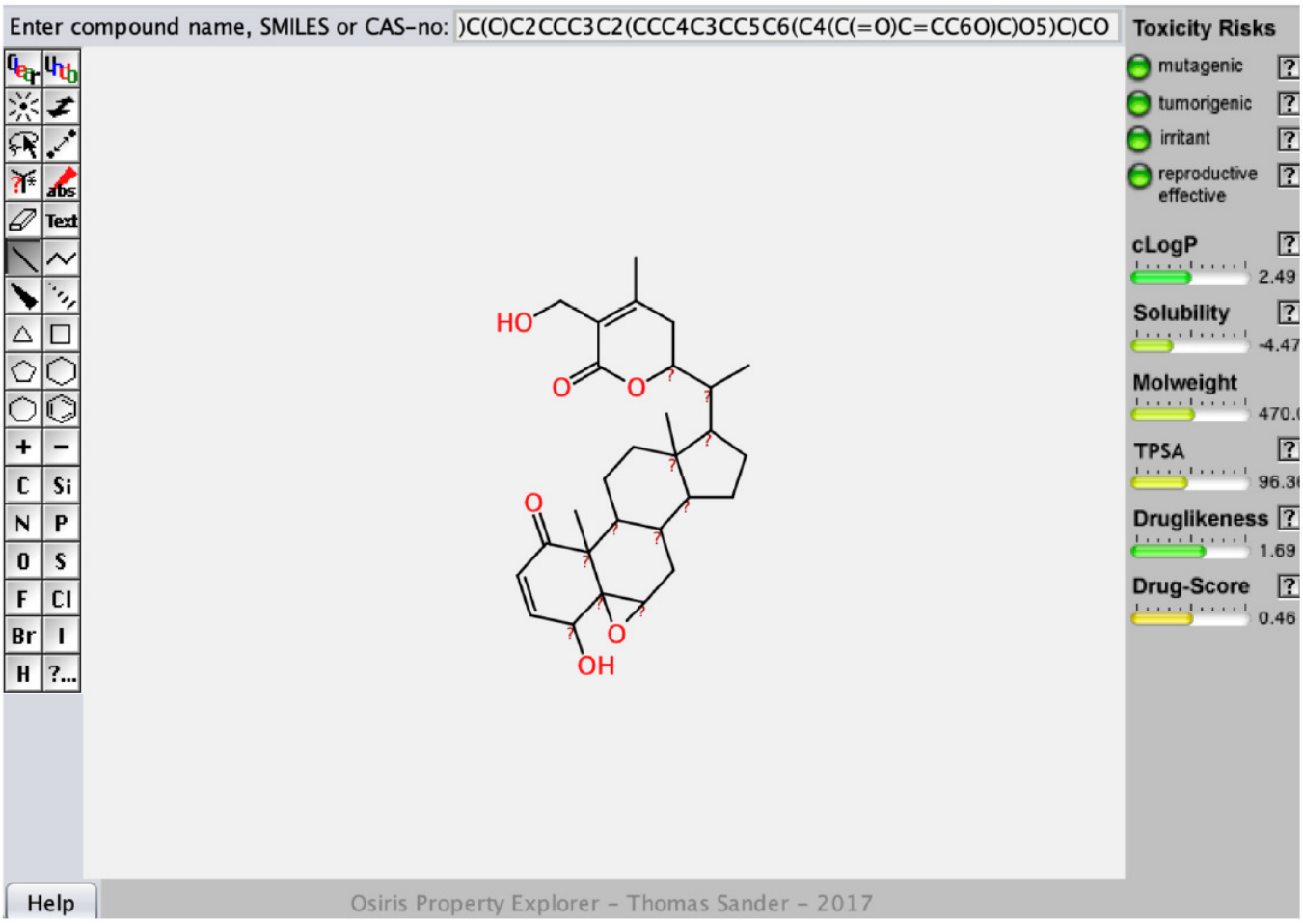


**Fig. 2:** Osiris property explorer displaying drug-likeness of CID: 265237.

**Table 1:** Molecular properties of rifampicin and lead compounds calculated using molinspiration.

| S.No. | Compound | miLogP | TPSA | MW | nON | nOHNH | nviolations | Volume |
|---|---|---|---|---|---|---|---|---|
| 1 | Rifampicin | 2.62 | 220.16 | 822.95 | 16 | 6 | 3 | 755.91 |
| 2 | CID:10607 | 1.32 | 92.70 | 414.41 | 8 | 1 | 0 | 354.43 |
| 3 | CID: 36462 | 0.70 | 160.86 | 588.56 | 13 | 3 | 2 | 493.51 |
| 4 | CID: 452548 | 1.81 | 160.86 | 656.66 | 13 | 3 | 2 | 539.07 |
| 5 | CID: 13342 | 5.56 | 154.11 | 810.99 | 13 | 3 | 3 | 744.65 |
| 6 | CID: 5978 | 4.95 | 171.18 | 824.97 | 14 | 3 | 2 | 747.07 |
| 7 | CID: 6167 | 1.10 | 83.11 | 399.44 | 7 | 1 | 0 | 364.15 |
| 8 | CID: 6436208 | 4.68 | 221.31 | 831.91 | 15 | 4 | 2 | 745.73 |
| 9 | CID: 36314 | 4.95 | 221.31 | 853.92 | 15 | 4 | 2 | 756.60 |
| 10 | CID: 148124 | 4.24 | 224.46 | 807.89 | 15 | 5 | 2 | 723.85 |
| 11 | CID: 11734982 | 3.73 | 148.83 | 658.86 | 10 | 3 | 1 | 614.86 |
| 12 | CID: 10666 | 0.02 | 55.03 | 164.16 | 4 | 0 | 0 | 147.36 |
| 13 | CID: 5318517 | 1.05 | 86.99 | 350.45 | 5 | 3 | 0 | 338.33 |
| 14 | CID: 11624161 | 1.72 | 66.76 | 334.46 | 4 | 2 | 0 | 330.29 |
| 15 | CID: 71589914 | 1.75 | 93.07 | 392.49 | 6 | 2 | 0 | 374.84 |
| 16 | CID: 11078630 | 1.05 | 86.99 | 350.45 | 5 | 3 | 0 | 338.33 |
| 17 | CID: 11666871 | 2.87 | 46.53 | 318.46 | 3 | 1 | 0 | 322.24 |
| 18 | CID: 6473762 | 2.68 | 70.67 | 332.44 | 4 | 2 | 0 | 324.10 |
| 19 | CID: 5708351 | 1.22 | 66.76 | 332.44 | 4 | 2 | 0 | 324.10 |
| 20 | CID: 969516 | 2.30 | 93.07 | 368.38 | 6 | 2 | 0 | 332.18 |
| 21 | CID: 442793 | 3.22 | 66.76 | 294.39 | 4 | 2 | 0 | 295.61 |
| 22 | CID: 5281775 | 3.32 | 76.00 | 356.42 | 5 | 2 | 0 | 336.19 |
| 23 | CID: 5281794 | 4.35 | 46.53 | 276.38 | 3 | 1 | 0 | 281.38 |
| 24 | CID: 31211 | 1.52 | 46.53 | 194.23 | 3 | 1 | 0 | 186.75 |
| 25 | CID: 265237 | 3.86 | 96.36 | 470.61 | 6 | 2 | 0 | 442.38 |
| 26 | CID: 12309402 | 7.56 | 40.46 | 444.74 | 2 | 2 | 1 | 474.95 |
| 27 | CID: 5270628 | 8.29 | 20.23 | 426.73 | 1 | 1 | 1 | 461.60 |

| S.No. | Compound | miLogP | TPSA | MW | nON | nOHNH | nviolations | Volume |
|---|---|---|---|---|---|---|---|---|
| 28 | CID: 92158 | 8.14 | 17.07 | 424.71 | 1 | 0 | 1 | 455.74 |
| 29 | CID: 15558419 | 2.64 | 58.56 | 297.35 | 4 | 2 | 0 | 281.45 |
| 30 | CID: 6450230 | 3.19 | 79.90 | 332.40 | 5 | 2 | 0 | 314.44 |
| 31 | CID: 222284 | 8.62 | 20.23 | 414.72 | 1 | 1 | 1 | 456.52 |
| 32 | CID: 5280794 | 7.87 | 20.23 | 412.70 | 1 | 1 | 1 | 450.33 |
| 33 | CID: 65036 | 2.06 | 17.07 | 162.28 | 1 | 0 | 0 | 145.51 |
| 34 | CID: 16590 | 2.63 | 0.00 | 146.28 | 0 | 0 | 0 | 137.96 |
| 35 | CID: 5280343 | 1.68 | 131.35 | 302.24 | 7 | 5 | 0 | 240.08 |
| 36 | CID: 108058 | 3.55 | 118.36 | 540.61 | 9 | 0 | 1 | 488.96 |
| 37 | CID: 12313376 | 1.94 | 92.06 | 466.53 | 7 | 0 | 0 | 417.03 |
| 38 | CID: 10906239 | 5.33 | 56.52 | 436.59 | 4 | 0 | 1 | 423.93 |
| 39 | CID: 188289 | 2.72 | 85.98 | 358.39 | 6 | 1 | 0 | 313.96 |
| 40 | CID: 73611 | 2.41 | 238.49 | 868.07 | 16 | 9 | 3 | 802.65 |
| 41 | CID: 119247 | 1.40 | 258.72 | 884.07 | 17 | 10 | 3 | 810.91 |
| 42 | CID: 9549171 | 1.60 | 240.69 | 868.07 | 16 | 9 | 3 | 802.63 |
| 43 | CID: 124062 | 3.72 | 74.60 | 254.24 | 4 | 2 | 0 | 215.17 |
| 44 | CID: 5281792 | 1.63 | 144.52 | 360.32 | 8 | 5 | 0 | 303.54 |
| 45 | CID: 6683 | 2.61 | 94.83 | 256.21 | 5 | 3 | 0 | 206.63 |
| 46 | CID: 6293 | 2.90 | 74.60 | 240.21 | 4 | 2 | 0 | 198.61 |
| 47 | CID: 196978 | 2.87 | 74.60 | 240.21 | 4 | 2 | 0 | 198.61 |
| 48 | CID: 3314 | 2.10 | 29.46 | 164.20 | 2 | 1 | 0 | 162.14 |
| 49 | CID: 124049 | 4.85 | 66.76 | 324.38 | 4 | 2 | 0 | 299.58 |
| 50 | CID: 24360 | 2.03 | 81.43 | 348.36 | 6 | 1 | 0 | 297.41 |
| 51 | CID: 637511 | 2.48 | 17.07 | 132.16 | 1 | 0 | 0 | 130.44 |
| 52 | CID: 400072 | 2.16 | 52.61 | 234.25 | 4 | 0 | 0 | 217.66 |
| 53 | CID: 160474 | 2.88 | 83.83 | 284.27 | 5 | 2 | 0 | 240.96 |
| 54 | CID: 5281605 | 2.68 | 90.89 | 270.24 | 5 | 3 | 0 | 224.05 |
| 55 | CID: 667450 | 1.31 | 43.38 | 246.31 | 3 | 0 | 0 | 232.17 |
| 56 | CID: 119093 | 0.90 | 93.07 | 346.38 | 6 | 2 | 0 | 314.38 |
| 57 | CID: 10205 | 1.78 | 54.37 | 188.18 | 3 | 1 | 0 | 163.16 |
| 58 | CID: 633024 | 3.31 | 108.74 | 374.35 | 6 | 2 | 0 | 313.69 |
| 59 | CID: 5464032 | -2.22 | 166.11 | 423.46 | 10 | 5 | 0 | 338.62 |
| 60 | CID: 5281545 | 0.00 | 71.08 | 290.27 | 6 | 0 | 0 | 242.36 |

**Table 2:** Details of the number of rotatable bonds.

| S.No. | Compound | nrotb | S.No. | Compound | nrotb |
|---|---|---|---|---|---|
| 1 | Rifampicin | 5 | 22 | CID: 5280343 | 1 |
| 2 | CID: 10607 | 4 | 23 | CID: 12313376 | 4 |
| 3 | CID: 6167 | 5 | 24 | CID: 188289 | 1 |
| 4 | CID: 10666 | 1 | 25 | CID: 124062 | 0 |
| 5 | CID: 5318517 | 3 | 26 | CID: 5281792 | 7 |
| 6 | CID: 11624161 | 4 | 27 | CID: 6683 | 0 |
| 7 | CID: 71589914 | 5 | 28 | CID: 6293 | 0 |
| 8 | CID: 11078630 | 3 | 29 | CID: 196978 | 0 |
| 9 | CID: 11666871 | 4 | 30 | CID: 3314 | 3 |
| 10 | CID: 6473762 | 3 | 31 | CID: 124049 | 3 |
| 11 | CID: 5708351 | 3 | 32 | CID: 24360 | 1 |
| 12 | CID: 969516 | 8 | 33 | CID: 637511 | 2 |
| 13 | CID: 442793 | 10 | 34 | CID: 400072 | 6 |
| 14 | CID: 5281775 | 9 | 35 | CID: 160474 | 2 |
| 15 | CID: 5281794 | 9 | 36 | CID: 5281605 | 1 |
| 16 | CID: 31211 | 4 | 37 | CID: 667450 | 0 |
| 17 | CID: 265237 | 3 | 38 | CID: 119093 | 2 |
| 18 | CID: 15558419 | 6 | 39 | CID: 10205 | 2 |
| 19 | CID: 6450230 | 7 | 40 | CID: 633024 | 2 |
| 20 | CID: 65036 | 5 | 41 | CID: 5464032 | 2 |
| 21 | CID: 16590 | 5 | 42 | CID: 5281545 | 2 |

**Table 3:** Physicochemical properties along with toxicity risks of rifampicin and lead compounds predicted using osiris property explorer.

| S. No. | Compound | Mutagenic | Tumorigenic | Reproductive effective | cLogP | Solubility | Drug likeness | Drug score |
|---|---|---|---|---|---|---|---|---|
| 1 | Rifampicin | No | No | No | 2.62 | -2.42 | -3.27 | 0.20 |
| 2 | CID: 10607 | No | No | Yes | 1.79 | -3.84 | 4.19 | 0.45 |
| 3 | CID: 6167 | No | No | Yes | 1.86 | -3.05 | 1.02 | 0.42 |
| 4 | CID: 10666 | No | No | No | -0.5 | -1.07 | -1.05 | 0.62 |
| 5 | CID: 5318517 | No | No | No | 1.88 | -2.95 | -4.59 | 0.43 |
| 6 | CID: 11624161 | No | No | No | 2.72 | -3.35 | -4.83 | 0.25 |
| 7 | CID: 71589914 | No | No | No | 2.37 | -3.36 | -4.37 | 0.4 |
| 8 | CID: 11078630 | No | No | No | 1.88 | -2.95 | -4.59 | 0.43 |
| 9 | CID: 11666871 | No | No | No | 3.58 | -3.75 | -10.97 | 0.23 |
| 10 | CID: 6473762 | No | No | No | 2.47 | -3.58 | -5.13 | 0.41 |
| 11 | CID: 5708351 | No | No | No | 2.47 | -3.12 | -4.9 | 0.26 |
| 12 | CID: 969516 | No | No | No | 2.95 | -3.62 | -3.95 | 0.4 |
| 13 | CID: 442793 | No | No | No | 3.56 | -3.25 | -7.78 | 0.4 |
| 14 | CID: 5281775 | No | No | No | 4.0 | -3.47 | -3.48 | 0.38 |
| 15 | CID: 5281794 | No | No | No | 4.33 | -3.42 | -14.4 | 0.37 |
| 16 | CID: 31211 | No | No | No | 1.86 | -2.03 | -2.22 | 0.31 |
| 17 | CID: 265237 | No | No | No | 2.49 | -4.47 | 1.69 | 0.46 |
| 18 | CID: 15558419 | No | No | No | 2.14 | -2.9 | 2.0 | 0.83 |
| 19 | CID: 6450230 | No | No | No | 3.25 | -3.51 | -4.54 | 0.24 |
| 20 | CID: 65036 | No | No | No | 1.84 | -1.22 | -6.13 | 0.48 |
| 21 | CID: 16590 | No | No | No | 2.93 | -2.71 | -4.7 | 0.45 |
| 22 | CID: 5280343 | Yes | Yes | No | 1.49 | -2.49 | 1.6 | 0.3 |
| 23 | CID: 12313376 | No | No | No | 2.77 | -4.45 | -3.04 | 0.2 |
| 24 | CID: 188289 | No | No | No | 0.97 | -3.28 | 1.35 | 0.46 |
| 25 | CID: 124062 | No | No | No | 2.69 | -4.48 | -3.81 | 0.19 |
| 26 | CID: 5281792 | No | No | No | 1.45 | -2.23 | -2.07 | 0.49 |
| 27 | CID: 6683 | Yes | Yes | No | 2.0 | -3.84 | -3.8 | 0.09 |
| 28 | CID: 6293 | Yes | No | No | 2.34 | -4.14 | -3.59 | 0.15 |
| 29 | CID: 196978 | Yes | No | No | 2.34 | -4.14 | -3.47 | 0.15 |
| 30 | CID: 3314 | Yes | Yes | No | 2.27 | -2.05 | -2.78 | 0.11 |
| 31 | CID: 124049 | No | No | No | 4.52 | -3.87 | 1.52 | 0.61 |
| 32 | CID: 24360 | No | No | No | 1.18 | -2.74 | 5.35 | 0.87 |
| 33 | CID: 637511 | No | Yes | No | 1.61 | -2.23 | -6.47 | 0.18 |
| 34 | CID: 400072 | No | Yes | Yes | 2.09 | -2.49 | -3.13 | 0.14 |
| 35 | CID: 160474 | No | No | No | 2.02 | -4.34 | -3.63 | 0.19 |
| 36 | CID: 5281605 | No | No | No | 2.34 | -2.86 | 0.75 | 0.75 |
| 37 | CID: 667450 | No | No | No | 1.85 | -2.7 | 1.81 | 0.85 |
| 38 | CID: 119093 | No | No | No | 1.35 | -2.71 | -6.8 | 0.26 |
| 39 | CID: 10205 | Yes | No | Yes | 1.53 | -2.67 | -1.19 | 0.21 |
| 40 | CID: 633024 | No | No | No | 3.07 | -5.81 | -1.35 | 0.34 |
| 41 | CID: 5464032 | No | No | Yes | -0.29 | -0.39 | -1.35 | 0.31 |
| 42 | CID: 5281545 | No | No | No | -0.21 | -1.93 | -2.74 | 0.5 |

**Table 4:** Docking score of Rifampicin and the lead compounds obtained from pubchem database against the target structure.

| S.No. | Compound | Score | |
|---|---|---|---|
| | | 5UHC | 5UAL |
| 1 | Rifampicin | 6502 | 6126 |
| 2 | CID: 10666 | 3372 | 3730 |
| 3 | CID: 5318517 | 5334 | 5214 |
| 4 | CID: 11624161 | 5474 | 5420 |
| 5 | CID: 71589914 | 6032 | 5904 |
| 6 | CID: 11078630 | 5334 | 5214 |
| 7 | CID: 11666871 | 5132 | 5148 |
| 8 | CID: 6473762 | 5350 | 5394 |
| 9 | CID: 5708351 | 5436 | 5452 |
| 10 | CID: 969516 | 6206 | 5704 |
| 11 | CID: 442793 | 5692 | 5338 |
| 12 | CID: 5281775 | 6290 | 6044 |
| 13 | CID: 5281794 | 5678 | 5448 |
| 14 | CID: 31211 | 4110 | 4208 |
| **15** | **CID: 265237** | **6550** | **6158** |
| 16 | CID: 15558419 | 5482 | 5178 |
| 17 | CID: 6450230 | 5702 | 6104 |
| 18 | CID: 65036 | 3518 | 3548 |
| 19 | CID: 16590 | 3466 | 3440 |
| 20 | CID: 12313376 | 6080 | 6004 |
| 21 | CID: 188289 | 5512 | 5092 |
| 22 | CID: 124062 | 4304 | 4304 |
| 23 | CID: 5281792 | 5454 | 5474 |
| 24 | CID: 124049 | 5582 | 5418 |
| 25 | CID: 24360 | 5598 | 5240 |
| 26 | CID: 160474 | 4780 | 4698 |
| 27 | CID: 5281605 | 4568 | 4530 |
| 28 | CID: 667450 | 4394 | 4462 |
| 29 | CID: 119093 | 5100 | 5344 |
| 30 | CID: 633024 | 5520 | 5422 |
| 31 | CID: 5281545 | 4756 | 4746 |

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