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EVALUATION AND INHIBITORY MECHANISM ANALYSIS OF NATURAL COMPOUNDS AGAINTS DIHYDROOROTATE DEHYDROGENASE AS ANTI-CANCER AGENTS

Evaluasi dan Analisis Mekanisme Penghambatan Senyawa-senyawa Natural Terhadap Dihidroorotat Dehidrogenase Manusia Sebagai Agen Anti-kanker

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ABSTRACT

Cancer remains one of the deadliest diseases worldwide, and currently cancer treatment is facing several problems related to adverse effects and drug resistance. To address these problems, new prospective anticancer medications are required. Natural compounds, which have been extensively used in the drug research, including for the treatment of cancer, are emerging as viable candidates. This study aimed to evaluate 33 in-house natural compounds against dihydroorotate dehydrogenase (DHODH) enzyme, a viable target to develop anticancer agent, and to analyze the hit inhibitory mechanism against protein target. In the activity assay, atovaquone was the sole substance to have activity against DHODH, with an inhibition rate of 47.44% at 10 µM. However, discrepancies were shown in the molecular docking result, where atovaquone were identified as hits. Molecular dynamic analysis revealed that atovaquone initially bound to the active site before being forced to the outside due to cleavage of hydrogen bond between the ligand and responsible residue. This study clearly demonstrated the importance of molecular dynamic analysis to study inhibitory mechanism of compound against target protein that may be useful for further development.

***Keywords**: Anticancer, Drug discovery, Dihydroorotate dehydrogenase, Molecular docking, Molecular dynamic*

ABSTRAK

Kanker menjadi salah satu penyakit paling mematikan di dunia, dan saat ini penanganan kanker menghadapi beberapa masalah terkait efek samping pengobatan dan resistensi obat. Untuk mengatasi masalah ini, diperlukan obat antikanker yang baru. Senyawa alami, yang telah banyak digunakan dalam penelitian obat, termasuk untuk pengobatan kanker, muncul sebagai kandidat yang potensial. Penelitian ini bertujuan untuk menguji 33 senyawa alami yang telah diketahui aktivitasnya terhadap enzim dihidroorotat dehidrogenase (DHODH), target yang potensial untuk mengembangkan anti-kanker, dan untuk menganalisa mekanisme penghambatan senyawa terhadap target protein. Dalam uji aktivitas, atovaquone adalah satu-satunya senyawa yang memiliki aktivitas terhadap DHODH, dengan tingkat penghambatan 47.44% pada konsentrasi 10 µM. Namun, perbedaan ditunjukkan dalam hasil doking molekuler, di mana atovaquone diidentifikasi sebagai hit. Analisis dinamika molekular mengungkapkan bahwa di awal atovaquone

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berikatan dengan situs aktif sebelum terdorong ke luar karena terputusnya ikatan hidrogen antara ligan dan residu yang bertanggung jawab. Studi ini secara jelas mendemonstrasikan pentingnya analisis dinamika molekular untuk mempelajari mekanisme penghambatan senyawa terhadap protein target yang mungkin berguna untuk pengembangan lebih lanjut.

Kata kunci: Anti-kanker, Penemuan obat, Dihidroorotat dehydrogenase manusia, Doking molekular, Dinamika molekular

INTRODUCTION

Cancer is one of the leading causes of death in the world. Cancer is a type of disease that manifests as abnormal cell growth in any organ or tissue, which spreads to surrounding body parts and/or other organs after crossing normal cell-boundaries (World Health Organization 2022). In 2020, an estimated 19.3 million new cases of cancer and roughly 10 million cancer-related deaths were reported. Female breast and lung cancers dominated new cancer cases, with an estimation of 2.3 million (11.7%) and 2.2 million (11.4%) cases, respectively, while lung (18%) and colorectal (9,4%) cancers were the two most common causes of cancer death. In addition, the global cancer burden is expected to be 28.4 million cases in 2040, a 47% rise from 2020 (Sung et al. 2021).

The foundation of many cancer treatments today, regardless of the disease's stage, continues to be chemotherapy. This is despite the development of many other cancer treatment methods in recent years (Alfarouk et al. 2015). Chemotherapy is a pharmacological treatment that uses potent drugs to stop cancer cells from proliferating, dividing, and producing new cells. A number of malignancies can be treated with chemotherapy medications either alone or in combination (Cancer.Net 2022). Although it is an effective method for treating various cancers, chemotherapy has many issues related to its side effects and multidrug resistance (MDR). Toxicity in chemotherapy is affected by the selectivity and specificity problem of anticancer agents when targeting cancer tissue. Meanwhile the drug-resistance has also become a significant barrier limiting the therapeutic efficacy of chemotherapeutic drugs, which enables cancer to withstand chemotherapy (Dong and Mumper 2010; Alfarouk et al. 2015).Based on those problems above, it is critically necessary to find novel anticancer substances with improved cytotoxicity and activity for possible intervention. Dihydroorotate dehydrogenase (DHODH) has been widely used as a chemotherapeutic target for cancer. DHODH is a mitochondrial enzyme that is essential in the *de novo* pyrimidine biosynthesis pathway. This enzyme is an oxidoreductase that catalyzes two redox reaction, the conversion of dihydroorotate (DHO) to orotate (ORO), and the regeneration of flavin mononucleotide (FMN) (Reis et al. 2017; Madak et al. 2019). These pathways have a direct correlation to the cancer cell growth by producing the fundamental and essential substrate for DNA replication and protein synthesis during proliferation of the cancer cells (Evans and Guy 2004; Wang et al. 2021). In addition, inhibition of this target is also crucial for cell respiration due to ATP depletion and resulting in inhibition of cell proliferation (Mohamad Fairus et al. 2017). Extensive studies had been performed by focusing on blocking this target in order to develop drugs for various cancer, including small cell lung cancer (Li et al. 2019), breast cancer (Mohamad Fairus et al. 2017), bladder cancer (Cheng et al. 2020), colorectal cancer (Yamaguchi et al. 2019), and acute myeloid leukemia (Wu et al. 2018). Several substances have been found to be DHODH inhibitors to date; some of these are even presently undergoing clinical trials or have received FDA approval. These include brequinar (Maroun et al. 1993), leflunomide (Fragoso and Brooks 2015), BAY 2402234 (Christian et al. 2019), PTC299 (Cao et al. 2019), and ASLAN003 (Zhou et al. 2020).

Numerous compounds isolated from various natural resources demonstrated enormous anticancer activity, according to several research, such as microbes (Li et al.

2018; Ōmura et al. 2018), plants (He et al. 2018; Wu et al. 2018), marine organisms (Ahn et al. 2019; Shubina et al. 2019), and mangrove (Chen et al. 2018; Law et al. 2019). Those nature-derived compounds can operate as key building blocks for the creation of chemotherapeutic drugs due to their impressive structural variety and bioactive qualities (Pham et al. 2019). Moreover, several studies had revealed the potency of natural products for DHODH inhibitor. Wu et al. (2018) had specifically found isobavachalcone, a compound derived from traditional Chinese medicinal plant *Psoralea corylifolia*, which inhibits DHODH directly and triggers apoptosis of acute myeloid leukimia cells. Liu et al. (2020) also showed that piperine, isolated from black pepper, controlled T cell activation by pharmacologically inhibiting DHODH and preventing the synthesis of pyrimidines. In addition, another research has revealed a new compound originating from microbes that are capable of competitively inhibiting DHODH. Ascochlorin, a metabolite produced by fungus *Ascochyta viciae*, demonstrated significant immunosuppressive and anti-inflammatory properties both in vivo and in vitro via reversible DHODH inhibition (Shen et al. 2016). These emphasize the importance of natural compounds as source for anti-cancer drug discovery.

To explore more the potential of natural compounds as anticancer agent, particularly as inhibitor of DHODH activity, in this study, we assessed inhibitory activity of our *in-house* natural compound library against DHODH. The library composed from 33 natural compounds that showed various bioactivities, including anti-fungal, anti-tumor, anti-parasite, anti-inflamatory, anti-oxidant, etc. We also represented the *in-silico* studies to reveal protein-ligand interaction, bioactivity and pharmakokinetic properties of the hit. Moreover, the inhibitory mechanisms of the hits against protein target were predicted by using molecular dynamic simulation. Structurally important moiety of the compound that highly involved in inhibitory mechanism will also be discussed.

MATERIAL AND METHODS

Location and time

This study was conducted in Juni – November 2022 at the Biotechnology Laboratory, the National Research and Innovation Agency (BRIN), BJ Habibie Science and Technology Park, South Tangerang, Banten, Indonesia.

DHODH Inhibitory Activity Assay

DHODH recombinant enzyme was prepared as previously described (Inaoka et al. 2016). Each tested 33 in-house natural compound (Table 1) was added to 96-well plate containing 190 µL assay mix (100 mM HEPES pH 8, 150 mM NaCl, 10% (v/v) glycerol, 0.05% (w/v) triton X, 12 mM 2,6-dichloroindophenol (DCIP), 200 nM decylubiquinone, 20 nM DHODH recombinant enzyme) so the final concentration was 10 µM, then homogenized by shaking at 500-750 rpm for 30 s. Absorbance of mix solution was recorded at 600 nm, 25°C for 1 min in kinetic mode by a multiplate reader (Spectramax Paradigm, Molecular Devices, USA). Substrate L-DHO (dihydroorotate) was subsequently added to the mix solution (final concentration was 0.2 mM) then homogenized by shaking at 500-750 rpm for 30 s. Absorbance of the mix solution was read in a kinetic mode at 600 nm, 25°C. The inhibition activity was calculated as follows:

$$\%Inhibition = 100 - \frac{Absorbance\ (A_{600}) of\ Sample - Absorbance\ (A_{600}) of\ Positive\ Control}{Absorbance\ (A_{600}) of\ Negative\ Control}$$

Mix solution without addition of L-DHO and sample (replaced by water and DMSO, respectively) was regarded as positive control, while mix solution without sample (replaced by DMSO) was regarded as negative control.

Molecular Docking Simulation

The 3D X-ray diffraction structure of DHODH (PDB ID: 2FPY) were obtained from RCSB Protein Data Bank (https://www.rcsb.org/) (Burley et al. 2021). The quality of the protein structure were

examined using the Ramachandran plot using the Procheck tool (https://saves.mbi.ucla.edu/) (Laskowski et al. 1996). Subsequently, the protein crystal structure was prepared using UCSF Chimera v.1.16. Native inhibitor (3-({[3,5-difluoro-3'-(trifluoromethoxy)Biphenyl-4-Yl]amino}carbonyl)thiophene-2-carboxylic acid) were separated from the receptor and prepared using UCSF Chimera v.1.16 (Butt et al. 2020). Meanwhile, hit compound from activity assay was obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) (Kim et al. 2018) and was saved in 3D SDF format. Native inhibitor and hit compound were then merged into one file using Open Babel v.2.3.1 (Tran-Nguyen et al. 2022).

Validation of binding site was performed by re-docking of native ligand to the target protein using AutoDock Vina (Fradera and Babaoglu 2017) algorithm that was compiled in PyRx v.0.8 (Dallakyan and Olson 2015) in 20 replications. Vina Search Space (Centre X-Y-Z and Size/Dimensions X-Y-Z) was determined using amino acid residues that form the ubiquinone binding site as the basis, where the docking process will be carried out. Incorporation of these amino acid residues in the binding site was determined according to previous reports (Baumgartner et al. 2006; Miyazaki et al. 2018). The result were then analyzed using PyMOL v.2.5.2 Edu (Schrödinger, USA) and Ligplot+ v.2.2.5 (Verma et al. 2014). Performance of re-docking process was evaluated by RMSD (Root Mean Square Deviation) value between the superimposed ligand from the docking result and co-crystallized ligand <2Å and the catalytic residues involve in protein-ligand bound (Gln47 and Arg136 that involved in hydrogen bond).

Docking of tested compounds against the target protein was performed using the same software and parameters as used in the re-docking process. The Vina Search Space was determined based on residues that involved in the configuration of the active site (center X: 49.87614, Y: 41.07927, Z: -1.71916; size X: 23.45541, Y: 29.20333, Z: 21.20155). Docking simulations were executed at an exhaustiveness and number of mode of 8 and 9, respectively. The docking results were saved in CSV format. Visualization and analysis of the protein-ligand complexes, as well as 2D interaction between protein and ligand were carried out using PyMOL Molecular Graphic System v.2.5.2 Edu (Schrödinger, USA) and Ligplot+ v.2.2.5 software (Verma et al. 2014).

Molecular Dynamic

The molecular dynamic simulations were carried out using Yasara Dynamic (YASARA Biosciences, Austria). A cube water box was generated for the simulation cell, while the steepest descent minimization algorithm was used for energy minimization. AMBER14 was utilized as a force field. To neutralize the system, Na^+ and Cl^- ions were added. The following physiological conditions were applied into this system: temperature 298 K, pH 7.4, water density 0.997 g/L, and ion concentration 0.9% NaCl. Molecular dynamic simulation was performed for 150 ns and structural snapshot was taken for every 100 ps. Molecular dynamic simulations were examined based on RMSD (Root Mean Square Deviation) and RMSF (Root Mean Square Fluctuation) values, complexes' trajectories, and protein-ligand interactions.

Drug-likeness, Bioactivity, and ADMET Analysis

The SMILES notation of the hit compound was used for analysis of the drug-likeness, bioactivity and ADMET characteristics. The drug-likeness was evaluated using Lipinski's rules of five by the Swiss Institute of Bioinformatics: absorption, distribution, metabolism, and excretion test or SwissADME (http://www.swissadme.ch/index.php) (Wicaksono et al. 2022), while Way2Drug PASS Online website (http://www.way2drug.com/PASSOnline) was used for the prediction of biological activity (Druzhilovskiy et al. 2017). The ADMETLab 2.0 server (https://admetmesh.scbdd.com/service/evaluation/index) was used to tracked the adsorption, distribution, metabolism, excretion, and toxicity (Xiong et al. 2021), to determine the safety of our compound for consumption.

RESULT AND DISCUSSION

Inhibitory Activity of Natural Compounds Against DHODH

All 33 natural compounds were subjected to dihydroorotate dehydrogenase (DHODH) enzymatic reaction assay to examine their inhibitory activity. As shown in Figure 1, atovaquone showed inhibitory activity against DHODH as high as 47.44% at 10 µM, while the other tested compounds did not show any inhibitory activity at the same concentration. This result is similar to that of previous study (IC_{50} of 15 µM) (Knecht et al. 2000). We then further characterized atovaquone as anti-cancer agent.

Molecular Docking Simulation

Molecular docking was performed to analyze the protein-ligand binding and interaction of the hit as a test ligand and native inhibitor as a control against DHODH protein structure. Protein structure and active site validation was carried out before completing molecular docking. Protein structure was verified using Ramachandran plot analysis (Laskowski et al. 1993), and the result demonstrated that the protein structure employed in this study was valid and suitable for further analysis (Figure S1 and Table S1). Accordingly, the active site validation likewise produced a satisfying result, with RMSD values of 20 times re-docking procedures being less than the threshold line of 2.0 (Bell and Zhang 2019; Nguyen et al. 2020b) and the interacted protein residues with the ligand (Table S2) being identical to the results of the prior study by Baumgartner et al (2006).

Docking simulation revealed that atovaquone showed better binding affinity compared to that of native inhibitor with the value of -12.9 and -12.2, respectively. Moreover, atovaquone interacted with the protein at residues those are involved in ubiquinone active site. Atovaquone formed hydrogen bonds with residues Gln47 and Arg136 from protein, which was the same with that of native inhibitor (Baumgartner et al. 2006). On the other hand, atovaquone only inhibited DHODH by as much as 47,44% at 10 µM (Figure 1). Interestingly, although inhibitory activity of atovaquone was similar to the previous report (IC_{50} of 15 µM) (Knecht et al. 2000), this was much higher compared to that of the native inhibitor (IC_{50} of 2 nM), which showed lower binding affinity had in activity assay (Baumgartner et al. 2006). Thus, this result clearly demonstrated the distinct impact of molecular docking to the drug discovery process compared to that of activity assay.

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Molecular Dynamic Simulation

As described in above, binding affinity value of a ligand from molecular docking process does not directly reflect to its inhibitory activity. There are several considerations in molecular docking process that was took into account to decrease computational burden, including the use of simplified algorithms such as kept the protein in a rigid structure. We assumed that the disagreement of our docking result and activity assay result could also due to this circumstance. In order to clarify this assumption, we performed molecular dynamics simulation of protein-ligand complex structure to investigate the inhibitory mechanism of the protein by the ligand. Molecular dynamics simulation is a robust method to study macromolecules' dynamics by taking into account actual experimental conditions and it has been widely used for several purposes, including assessing the stability of ligand poses in the complex protein-ligand (Sakano et al. 2016; Liu et al. 2017), providing information about protein-ligand interactions which calculates the effect of solvent (Shen et al. 2013; Llanos et al. 2021), investigating the receptor's conformational changes upon ligand binding through molecular dynamic trajectories (Wang et al. 2013; Hirano et al. 2021). We compared the RMSD (root mean square deviation) and RMSF (root mean square fluctuation) values of protein-native ligand and protein-atovaquone complex structures from molecular dynamic simulation. Molecular dynamic of apo protein was also simulated under the same condition.

Molecular dynamics simulation of protein-native inhibitor revealed that the average RMSD value of the ligand movement was 1.112 Å with a constant pattern (not much fluctuated) (Figure 2), indicating that the native inhibitor bound firmly with the protein along the simulation period (150 ns). Trajectory result of this complex revealed

that the native inhibitor bond to active site (Figure 3) through hydrogen bonds between carboxyl group of the ligand and Gln47 and Arg136 of the protein (Figure 3b), indicating that the native inhibitor bond in the active site (ubiquinone binding site). This result was consistent with the previous study (Baumgartner et al. 2006). The hydrogen bond is not only important for binding, but also for transportation, adsorption, distribution, metabolization, and excretion of small molecules (Böhm and Schneider 2003). Other type of interactions, such as hydrophobic bond and π-interaction with several residues, also helped the ligand bind to the protein (Figure 3c). The RMSF value of Gln47 and Arg136 of protein-native inhibitor complex were decreased to 1.11 Å and 0.54 Å, respectively, while compared to that of the same residue in apo protein (2.04 Å and 0.91 Å, respectively) (Figure 4), suggested that respective residues were stabilized by the ligand binding. Both residues also showed to bind with the ligand consistently along the simulation (Figure S4a), indicating the importance of these residues in ligand binding.

We then examined the molecular dynamics of protein-atovaquone complex. The average initial RMSD value of atovaquone movement up to the first 43 ns was 1.15 Å (Figure 2), which is comparable with that of native inhibitor. The carbonyl group of the ligand formed hydrogen bonds with Gln47 and Arg136 within this period (Figure 5b). However, RMSD value of the ligand increased drastically to average 5.24 Å after 43 ns until the end of simulation (Figure 2). Hydrogen bond between the ligand and Arg136 was broken, while the hydrogen bond between Gln47 and the ligand was maintained during this period (Figure 5c). Trajectory observation of the ligand revealed that the ligand was protruded from the active site during this period (Figure 5c). Moreover, RMSF values of Gln47 and Arg136 of this complex were 1.87 Å and 1.51 Å, respectively. These values are higher than that of protein-native inhibitor, indicating that harsh fluctuation of respective residues was occurred during protein-atovaquone interaction.

Based on the molecular docking result, we identified that atovaquone as the most potent inhibitor of DHODH within the tested compounds. Although showed better binding affinity value, the inhibitory activity was not as high as that of native inhibitor. Based on molecular dynamics analysis result, hydrogen bond between carbonyl group of atovaquone and Arg136 was broken at 43 ns. In contrast, hydrogen bond between carboxyl group of the native inhibitor and Arg136 was maintained along the simulation, suggested the importance of carboxyl group for binding of the ligand in the active site. Therefore, conversion of carbonyl group of atovaquone into carboxyl group may increase the binding stability of the ligand to the active site, which may also increase the inhibitory activity. The importance of carboxyl group in the inhibitor of DHODH was also demonstrated in the previous report using Brequinar (Fritzson et al. 2010).

The disagreement between molecular docking result and activity assay has been widely experienced by many researchers. Recently, de Sousa *et al* conducted a molecular docking using Zinc15 database composed from 7070 compounds to identify inhibitor of *Plasmodium falciparum* β-haematin (de Sousa et al. 2020). Most of top 15 compounds with lowest binding affinity values (between -12.5 kcal/mol to -14.2 kcal/mol) showed high to moderate IC_{50} value (micromolar level) against the target. The issues might be related to deletion of water molecules during preparation of protein for the docking process, scoring function, and the use of rigid protein structure (Zoete et al. 2009; Pantsar and Poso 2018), which may lead to misprediction of binding affinity and binding pose.

Drug-likeness, Bioactivity, and ADMET Analysis

Analyzes were also carried out regarding the druglikeness, bioactivity and ADMET of Atovaquone to examine its pharmakokinetic properties and possible target. The fulfillment of the Lipinski requirements led to the identification of atovaquone for use as an oral medication. The result (Table 3)

showed atovaquone meets all standards in parameters molecular weight (MW < 500 g/mol), Moriguchi octanol-water partition coefficient (M log P < 4.15), hydrogen bond donors (< 5) and hydrogen bond acceptor (< 10) (Doak et al. 2014). Moreover, the bioavailability score shows that Atovaquone has a value of 0.86 (range 0 - 1) which means good pharmakokinetic properties (Bojarska et al. 2020). The synthetic accessibility property also showed a positive result with the value of 4.07 (range 1 – 10), indicating that atovaquone is generally simple to produce (Ertl and Schuffenhauer 2009).

The PASS Online software (http://www.way2drug.com/passonline) was also used to determine the probable cellular targets of Atovaquone. Due to our focus on finding anticancer agents, we were able to anticipate the anticancer activity in which some factors, such as antineoplastic, antioxidant, and angiogenesis inhibitor (Kapoor et al. 2022; Wicaksono et al. 2022) and also several types of cancer that related to DHODH activity: breast cancer (Mohamad Fairus et al. 2017), colorectal cancer (Yamaguchi et al. 2019; Kurth et al. 2021), acute myeloid leukemia (AML) (Wu et al. 2018), small cell lung cancer (Li et al. 2019), bladder cancer (Cheng et al. 2020), pancreatic cancer (Koundinya et al. 2018) etc, The result (Figure 6) showed that Atovaquone is indicated to have no bioactivity in the specified parameters, due all Pa values were less than 0.5 which meant the compound was very unlikely to be related to the biological activity of the substance tested. This may be related to the findings of molecular dynamics simulations, which indicate that atovaquone has a poor inhibitory mechanism because it has a low potential for bioactivity towards targets with a connection to the DHODH enzyme in their therapy.

The ADMET (Figure 7) data showed that Atovaquone some notes on its pharmacokinetic characteristics, especially on the toxicity parameters. Atovaquone may have harmful consequences on a number of variables, including carcinogenicity, AMES, oral acute toxicity, Human Ether-a-go-go-related Gene (hERG), especially on the Human hepatotoxicity (H-HT) and drug-induced liver injury (DILI), and this may be due to its structure.

CONCLUSION

In this study, we have discovered that atovaquone was the only compound that could inhibit the DHODH enzyme moderately through an activity assay on 33 natural compounds. However, this result did not correlate with the docking simulation, indicating that atovaquone might have promising activity against DHODH, due to its binding affinity score. Molecular dynamic analysis revealed the inhibitory mechanism of native ligand and atovaquone against DHODH where native ligand was stably bound to the active site, while atovaquone was driven out of the active site after being bound for only 43 ns of simulation as a result of the hydrogen bond breaking with the implicated residue, and that variation could be brought about by the two ligands' different chemical groups. Our result also demonstrated that molecular dynamic is a powerful tool for revealing ligand interactions in protein-ligand complexes and investigating their mode of action, which are beneficial for future research.

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REFERENCES

AdipoGen Life Sciences (2017) 1233B | CAS 34668-61-6. In: AdipoGen Life Sci. https://adipogen.com/ag-cn2-0112-1233b.html/. Accessed 7 Jul 2023

Ahn JH, Woo JH, Rho JR, Choi JH (2019) Anticancer activity of gukulenin a isolated from the marine sponge *Phorbas gukhulensis* in vitro and in vivo. Mar Drugs 17. https://doi.org/10.3390/md17020126

Alfarouk KO, Stock CM, Taylor S, Walsh M, Muddathir AK, Verduzco D, Bashir AHH, Mohammed OY, Elhassan GO, Harguindey S, Reshkin SJ, Ibrahim ME, Rauch C (2015) Resistance to cancer chemotherapy: Failure in drug response from ADME to P-gp. Cancer Cell Int 15:1–13. https://doi.org/10.1186/s12935-015-0221-1

Arivizhivendhan K V., Mahesh M, Boopathy R, Swarnalatha S, Regina Mary R, Sekaran G (2018) Antioxidant and antimicrobial activity of bioactive prodigiosin produces from Serratia marcescens using agricultural waste as a substrate. J Food Sci Technol 55:2661–2670. https://doi.org/10.1007/s13197-018-3188-9

Ataides D, Pamphile JA, Garcia A, Ribeiro MA dos S, Polonio JC, Sarragiotto MH, Clemente E (2018) Curvularin produced by endophytic *Cochliobolus* sp. G2-20 isolated from *Sapindus saponaria* L. and evaluation of biological activity. J Appl Pharm Sci 8:32–37. https://doi.org/10.7324/JAPS.2018.81204

Banks RM, Blanchflower SE, Everett JR, Manger BR, Reading C (1997) Novel anthelmintic metabolites from an *Aspergillus* species; the aspergillimides. J Antibiot (Tokyo) 50:840–846. https://doi.org/10.7164/antibiotics.50.840

Bansal R, Sherkhane PD, Oulkar D, Khan Z, Banerjee K, Mukherjee PK (2018) The viridin biosynthesis gene cluster of *Trichoderma virens* and its conservancy in the bat white-nose fungus *Pseudogymnoascus destructans* . ChemistrySelect 3:1289–1293. https://doi.org/10.1002/slct.201703035

Baumgartner R, Walloschek M, Kralik M, Gotschlich A, Tasler S, Mies J, Leban J (2006) Dual binding mode of a novel series of DHODH inhibitors. J Med Chem 49:1239–1247. https://doi.org/10.1021/jm0506975

Bell EW, Zhang Y (2019) DockRMSD: An open-source tool for atom mapping and RMSD calculation of symmetric molecules through graph isomorphism. J Cheminform 11. https://doi.org/10.1186/s13321-019-0362-7

Böhm H-J, Schneider G (2003) Protein-ligand interactions from molecular recognition to drug design. Wiley-VCH

Bojarska J, Remko M, Breza M, Madura ID, Kaczmarek K, Zabrocki J, Wolf WM (2020) A supramolecular approach to structure-based design with a focus on synthons hierarchy in ornithine-derived ligands: Review, synthesis, experimental and in silico studies. Molecules 25. https://doi.org/10.3390/molecules25051135

Bunbamrung N, Intaraudom C, Dramae A, Komwijit S, Laorob T, Khamsaeng S, Pittayakhajonwut P (2020) Antimicrobial, antimalarial and anticholinesterase substances from the marine-derived fungus *Aspergillus terreus* BCC51799. Tetrahedron 76:131496. https://doi.org/10.1016/j.tet.2020.131496

Burley SK, Bhikadiya C, Bi C, Bittrich S, Chen L, Crichlow G V., Christie CH, Dalenberg K, Di Costanzo L, Duarte JM, Dutta S, Feng Z, Ganesan S, Goodsell DS, Ghosh S, Green RK, Guranovic V, Guzenko D, Hudson BP, Lawson CL, Liang Y, Lowe R, Namkoong H, Peisach E, Persikova I, Randle C, Rose A, Rose Y, Sali A, Segura J, Sekharan M, Shao C, Tao YP, Voigt M, Westbrook JD, Young JY, Zardecki C, Zhuravleva M (2021) RCSB Protein Data Bank: Powerful new tools for exploring 3D structures of biological macromolecules for basic and applied research and education in fundamental biology, biomedicine, biotechnology, bioengineering and energy sciences. Nucleic Acids Res 49:D437–D451. https://doi.org/10.1093/nar/gkaa1038

Butt SS, Badshah Y, Shabbir M, Rafiq M (2020) Molecular Docking Using Chimera and Autodock Vina Software

for Nonbioinformaticians. JMIR Bioinforma Biotechnol 1:e14232. https://doi.org/10.2196/14232

Cancer.Net (2022) What is Chemotherapy? https://www.cancer.net/navigating-cancer-care/how-cancer-treated/chemotherapy/what-chemotherapy. Accessed 1 Jun 2023

Cao L, Weetall M, Trotta C, Cintron K, Ma J, Kim MJ, Furia B, Romfo C, Graci JD, Li W, Du J, Sheedy J, Hedrick J, Risher N, Yeh S, Qi H, Arasu T, Hwang S, Lennox W, Kong R, Petruska J, Moon YC, Babiak J, Davis TW, Jacobson A, Almstead NG, Branstrom A, Colacino JM, Peltz SW (2019) Targeting of hematologic malignancies with PTC299, a novel potent inhibitor of dihydroorotate dehydrogenase with favorable pharmaceutical properties. Mol Cancer Ther 18:3–16. https://doi.org/10.1158/1535-7163.MCT-18-0863

Chen C, Ye Y, Wang R, Zhang Y, Wu C, Debnath SC, Ma Z, Wang J, Wu M (2018) *Streptomyces nigra* sp. nov. Is a novel actinobacterium isolated from mangrove soil and exerts a potent antitumor activity in vitro. Front Microbiol 9:1–14. https://doi.org/10.3389/fmicb.2018.01587

Chen JW, Luo YL, Hwang MJ, Peng FC, Ling KH (1999) Territrem B, a tremorgenic mycotoxin that inhibits acetylcholinesterase with a noncovalent yet irreversible binding mechanism. J Biol Chem 274:34916–34923. https://doi.org/10.1074/jbc.274.49.34916

Cheng L, Wang H, Wang Z, Huang H, Zhuo D, Lin J (2020) Leflunomide inhibits proliferation and induces apoptosis via suppressing autophagy and PI3K/ akt signaling pathway in human bladder cancer cells. Drug Des Devel Ther 14:1897–1908. https://doi.org/10.2147/DDDT.S252626

Chiba T, Asami Y, Suga T, Watanabe Y, Nagai T, Momose F, Nonaka K, Iwatsuki M, Yamada H, Omura S, Shiomi K (2017) Herquline A, produced by *Penicillium herquei* FKI-7215, exhibits anti-influenza virus properties. Biosci Biotechnol Biochem 81:59–62. https://doi.org/10.1080/09168451.2016.1162084

Choi HY, Le DD, Kim WG (2022) Curvularin Isolated From *Phoma macrostoma* Is an Antagonist of RhlR Quorum Sensing in *Pseudomonas aeruginosa*. Front Microbiol 13. https://doi.org/10.3389/fmicb.2022.913882

Christian S, Merz C, Evans L, Gradl S, Seidel H, Friberg A, Eheim A, Lejeune P, Brzezinka K, Zimmermann K, Ferrara S, Meyer H, Lesche R, Stoeckigt D, Bauser M, Haegebarth A, Sykes DB, Scadden DT, Losman JA, Janzer A (2019) The novel dihydroorotate dehydrogenase (DHODH) inhibitor BAY 2402234 triggers differentiation and is effective in the treatment of myeloid malignancies. Leukemia 33:2403–2415. https://doi.org/10.1038/s41375-019-0461-5

Chung MC, Lee HJ, Chun HK, Kho YH (1998) Penicillide, a nonpeptide calpain inhibitor, produced by *Penicillium* sp. F60760. J Microbiol Biotechnol 8:188–190

Dallakyan S, Olson A (2015) Participation in global governance: Coordinating “the voices of those most affected by food insecurity.” Glob Food Secur Gov 1263:1–11. https://doi.org/10.1007/978-1-4939-2269-7

de Sousa ACC, Combrinck JM, Maepa K, Egan TJ (2020) Virtual screening as a tool to discover new β-haematin inhibitors with activity against malaria parasites. Sci Rep 10:3374. https://doi.org/10.1038/s41598-020-60221-0

Deflandre B, Stulanovic N, Planckaert S, Anderssen S, Bonometti B, Karim L, Coppieters W, Devreese B, Rigali S (2022) The virulome of *Streptomyces scabiei* in response to cello-oligosaccharide elicitors. Microb Genomics 8.

https://doi.org/10.1099/mgen.0.000760

Degenkolb T, Vilcinskas A (2016) Metabolites from nematophagous fungi and nematicidal natural products from fungi as an alternative for biological control. Part I: metabolites from nematophagous ascomycetes. Appl Microbiol Biotechnol 100:3799–3812. https://doi.org/10.1007/s00253-015-7233-6

Dickinson JM, Hanson JR, Hitchcock PB, Claydon N (1989) Structure and biosynthesis of harzianopyridone, an antifungal metabolite of *Trichoderma harzianum*. J Chem Soc Perkin Trans 1 1885–1887. https://doi.org/10.1039/p19890001885

Doak BC, Over B, Giordanetto F, Kihlberg J (2014) Oral druggable space beyond the rule of 5: Insights from drugs and clinical candidates. Chem Biol 21:1115–1142. https://doi.org/10.1016/j.chembiol.2014.08.013

Dong X, Mumper RJ (2010) Nanomedicinal strategies to treat multidrug-resistant tumors: Current progress. Nanomedicine 5:597–615. https://doi.org/10.2217/nnm.10.35

Dröse S, Altendorf K (1997) Bafilomycins and concanamycins as inhibitors of V-ATPases and P-ATPases. J Exp Biol 200:1–8. https://doi.org/10.1242/jeb.200.1.1

Druzhilovskiy DS, Rudik A V., Filimonov DA, Gloriozova TA, Lagunin AA, Dmitriev A V., Pogodin P V., Dubovskaya VI, Ivanov SM, Tarasova OA, Bezhentsev VM, Murtazalieva KA, Semin MI, Maiorov IS, Gaur AS, Sastry GN, Poroikov V V. (2017) Computational platform Way2Drug: from the prediction of biological activity to drug repurposing. Russ Chem Bull 66:1832–1841. https://doi.org/10.1007/s11172-017-1954-x

El-Bondkly AMA, Mervat MMM, Bassyouni RH (2012) Overproduction and biological activity of prodigiosin-like pigments from recombinant fusant of endophytic marine *Streptomyces* species. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 102:719–734. https://doi.org/10.1007/s10482-012-9772-5

Ertl P, Schuffenhauer A (2009) Estimation of synthetic accessibility score of drug-like molecules based on molecular complexity and fragment contributions. J Cheminform 1:1–11. https://doi.org/10.1186/1758-2946-1-8

Evans DR, Guy HI (2004) Mammalian pyrimidine biosynthesis: Fresh insights into an ancient pathway. J Biol Chem 279:33035–33038. https://doi.org/10.1074/jbc.R400007200

Evans G, White NH (1966) Radicicolin and radicicol, two new antibiotics produced by *Cylindrocarpon radicicola*. Trans Br Mycol Soc 49:563-IN7. https://doi.org/10.1016/s0007-1536(66)80004-9

Ezekiel CN, Kraak B, Sandoval-Denis M, Sulyok M, Oyedele OA, Ayeni KI, Makinde OM, Akinyemi OM, Krska R, Crous PW, Houbraken J (2020) Diversity and toxigenicity of fungi and description of *Fusarium madaense* sp. nov. From cereals, legumes and soils in north-central Nigeria. MycoKeys 67:95–124. https://doi.org/10.3897/MYCOKEYS.67.52716

Fradera X, Babaoglu K (2017) Overview of Methods and Strategies for Conducting Virtual Small Molecule Screening. Curr Protoc Chem Biol 9:196–212. https://doi.org/10.1002/cpch.27

Fragoso YD, Brooks JBB (2015) Leflunomide and teriflunomide: Altering the metabolism of pyrimidines for the treatment of autoimmune diseases. Expert Rev Clin Pharmacol 8:315–320. https://doi.org/10.1586/17512433.2015.1019343

Fritzson I, Svensson B, Al-Karadaghi S, Walse B, Wellmar U, Nilsson UJ, Da Graça Thrige D, Jönsson S (2010) Inhibition of human dhodh by 4-hydroxycoumarins, fenamic acids,

and n-(alkylcarbonyl)anthranilic acids identified by structure-guided fragment selection. ChemMedChem 5:608–617. https://doi.org/10.1002/cmdc.200900454

Gao X, Liu X, Shan W, Liu Q, Wang C, Zheng J, Yao H, Tang R, Zheng J (2018) Anti-malarial atovaquone exhibits anti-tumor effects by inducing DNA damage in hepatocellular carcinoma. Am J Cancer Res 8:1697–1711

Gaylarde C, Otlewska A, Cellikol-Aydi S, Skóra J, Sulyok M, Pielech-Przybylska K, Gillatt J, Beech I, Gutarowska B (2015) Interactions between fungi of standard paint test method BS3900. Int Biodeterior Biodegrad 104:411–418. https://doi.org/10.1016/j.ibiod.2015.07.010

He DL, Jin RY, Li HZ, Liu QY, Zhang ZJ (2018) Identification of a Novel Anticancer Oligopeptide from *Perilla frutescens* (L.) Britt. and Its Enhanced Anticancer Effect by Targeted Nanoparticles in Vitro. Int J Polym Sci 2018. https://doi.org/10.1155/2018/1782734

Hirano Y, Okimoto N, Fujita S, Taiji M (2021) Molecular dynamics study of conformational changes of tankyrase 2 binding subsites upon ligand binding. ACS Omega 6:17609–17620. https://doi.org/10.1021/acsomega.1c02159

Ibrahim SRM, Mohamed SGA, Altyar AE, Mohamed GA (2021) Natural Products of the Fungal Genus *Humicola* : Diversity, Biological Activity, and Industrial Importance. Curr Microbiol 78:2488–2509. https://doi.org/10.1007/s00284-021-02533-6

Inaoka DK, Iida M, Tabuchi T, Honma T, Lee N, Hashimoto S, Matsuoka S, Kuranaga T, Sato K, Shiba T, Sakamoto K, Balogun EO, Suzuki S, Nara T, Rocha JR da, Montanari CA, Tanaka A, Inoue M, Kita K, Harada S (2016) The Open Form Inducer Approach for Structure-Based Drug Design. PLoS One 11:e0167078. https://doi.org/10.1371/journal.pone.0167078

Isozaki S, Konishi H, Tanaka H, Yamamura C, Moriichi K, Ogawa N, Fujiya M (2022) Probiotic-derived heptelidic acid exerts antitumor effects on extraintestinal melanoma through glyceraldehyde-3-phosphate dehydrogenase activity control. BMC Microbiol 22:1–9. https://doi.org/10.1186/s12866-022-02530-0

Iwai Y, Kimura K, Takahashi Y, Hinotozawa K, Shimizu H, Tanaka H, Omura S (1983) OM-173,new nanaomycin-type antibiotics produced by a strain of *Streptomyces.* Taxonomy,production,isolation and biological properties. J Antibiot (Tokyo) 36:1268–1274. https://doi.org/10.7164/antibiotics.36.1268

Ji Y, Xin Z, He H, Gao S (2019) Total Synthesis of Viridin and Viridiol. J Am Chem Soc 141:16208–16212. https://doi.org/10.1021/jacs.9b08577

Kapoor R, Saini A, Sharma D (2022) Indispensable role of microbes in anticancer drugs and discovery trends. Appl Microbiol Biotechnol 106:4885–4906. https://doi.org/10.1007/s00253-022-12046-2

Karle IL (1996) Flexibility in peptide molecules and restraints imposed by hydrogen bonds, the AIB residue, and core inserts. Biopolymers 40:157–180. https://doi.org/10.1002/(SICI)1097-0282(1996)40:1<157::AID-BIP7>3.0.CO;2-V

Kasimin ME, Shamsuddin S, Molujin AM, Sabullah MK, Gansau JA, Jawan R (2022) Enterocin: Promising Biopreservative Produced by *Enterococcus* sp. Microorganisms 10. https://doi.org/10.3390/microorganisms10040684

Kato S, Motoyama T, Uramoto M, Nogawa T, Kamakura T, Osada H (2020) Induction of secondary metabolite production by hygromycin B and identification of the 1233A biosynthetic gene cluster with a self-

resistance gene. J Antibiot (Tokyo) 73:475–479. https://doi.org/10.1038/s41429-020-0295-4

Kehelpannala C, Kumar NS, Jayasinghe L, Araya H, Fujimoto Y (2018) Naphthoquinone Metabolites Produced by *Monacrosporium ambrosium* , the Ectosymbiotic Fungus of Tea Shot-Hole Borer, *Euwallacea fornicatus* , in Stems of Tea, *Camellia sinensis* . J Chem Ecol 44:95–101. https://doi.org/10.1007/s10886-017-0913-1

Kim S, Shoemaker BA, Bolton EE, Bryant SH (2018) Finding Potential Multitarget Ligands Using PubChem. In: Computational Chemogenomics. pp 63–91

Knecht W, Henseling J, Löffler M (2000) Kinetics of inhibition of human and rat dihydroorotate dehydrogenase by atovaquone, lawsone derivatives, brequinar sodium and polyporic acid. Chem Biol Interact 124:61–76. https://doi.org/10.1016/S0009-2797(99)00144-1

Koundinya M, Sudhalter J, Courjaud A, Lionne B, Touyer G, Bonnet L, Menguy I, Schreiber I, Perrault C, Vougier S, Benhamou B, Zhang B, He T, Gao Q, Gee P, Simard D, Castaldi MP, Tomlinson R, Reiling S, Barrague M, Newcombe R, Cao H, Wang Y, Sun F, Murtie J, Munson M, Yang E, Harper D, Bouaboula M, Pollard J, Grepin C, Garcia-Echeverria C, Cheng H, Adrian F, Winter C, Licht S, Cornella-Taracido I, Arrebola R, Morris A (2018) Dependence on the Pyrimidine Biosynthetic Enzyme DHODH Is a Synthetic Lethal Vulnerability in Mutant KRAS-Driven Cancers. Cell Chem Biol 25:705-717.e11. https://doi.org/10.1016/j.chembiol.2018.03.005

Kuncharoen N, Fukasawa W, Iwatsuki M, Mori M, Shiomi K, Tanasupawat S (2019) Characterisation of Two Polyketides from *Streptomyces* sp. SKH1-2 Isolated from Roots of Musa (ABB) cv. 'Kluai Sao Kratuep Ho.' Int Microbiol 22:451–459. https://doi.org/10.1007/s10123-019-00071-7

Kurth I, Yamaguchi N, Andreu-Agullo C, Tian HS, Sridhar S, Takeda S, Gonsalves FC, Loo JM, Barlas A, Manova-Todorova K, Busby R, Bendell JC, Strauss J, Fakih M, McRee AJ, Hendifar AE, Rosen LS, Cercek A, Wasserman R, Szarek M, Spector SL, Raza S, Tavazoie MF, Tavazoie SF (2021) Therapeutic targeting of SLC6A8 creatine transporter suppresses colon cancer progression and modulates human creatine levels. Sci Adv 7. https://doi.org/10.1126/sciadv.abi7511

Lapenda JC, Silva PA, Vicalvi MC, Sena KXFR, Nascimento SC (2015) Antimicrobial activity of prodigiosin isolated from *Serratia marcescens* UFPEDA 398. World J Microbiol Biotechnol 31:399–406. https://doi.org/10.1007/s11274-014-1793-y

Laskowski RA, MacArthur MW, Moss DS, Thornton JM (1993) PROCHECK: a program to check the stereochemical quality of protein structures. J Appl Crystallogr 26:283–291. https://doi.org/10.1107/s0021889892009944

Laskowski RA, Rullmann JAC, MacArthur MW, Kaptein R, Thornton JM (1996) AQUA and PROCHECK-NMR: Programs for checking the quality of protein structures solved by NMR. J Biomol NMR 8:477–486. https://doi.org/10.1007/BF00228148

Law JWF, Ser HL, Ab Mutalib NS, Saokaew S, Duangjai A, Khan TM, Chan KG, Goh BH, Lee LH (2019) *Streptomyces monashensis* sp. nov., a novel mangrove soil actinobacterium from East Malaysia with antioxidative potential. Sci Rep 9:1–18. https://doi.org/10.1038/s41598-019-39592-6

Leach BE, Calhoun KM, Johnson LE, Teeters CM, Jackson WG (1953) Chartreusin, a New Antibiotic Produced by *Streptomyces chartreusis*, a New Species. J Am

Chem Soc 75:4011–4012. https://doi.org/10.1021/ja01112a040

Li D, Liu J, Wang X, Kong D, Du W, Li H, Hse CY, Shupe T, Zhou D, Zhao K (2018) Biological potential and mechanism of prodigiosin from *Serratia marcescens* subsp. Lawsoniana in human choriocarcinoma and prostate cancer cell lines. Int J Mol Sci 19. https://doi.org/10.3390/ijms19113465

Li JY, Harper JK, Grant DM, Tombe BO, Bashyal B, Hess WM, Strobel GA (2001) Ambuic acid, a highly functionalized cyclohexenone with antifungal activity from *Pestalotiopsis* spp. and *Monochaetia* sp. Phytochemistry 56:463–468. https://doi.org/10.1016/S0031-9422(00)00408-8

Li L, Ng SR, Colón CI, Drapkin BJ, Hsu PP, Li Z, Nabel CS, Lewis CA, Romero R, Mercer KL, Bhutkar A, Phat S, Myers DT, Muzumdar MD, Westcott PMK, Beytagh MC, Farago AF, Heiden MGV, Dyson NJ, Jacks T (2019) Identification of DHODH as a therapeutic target in small cell lung cancer. Sci Transl Med 11. https://doi.org/10.1126/scitranslmed.aaw7852

Li T, Choi K, Jung B, Ji S, Kim D, Seo MW, Lee J, Lee SH (2022) Biochar inhibits ginseng root rot pathogens and increases soil microbiome diversity. Appl Soil Ecol 169:104229. https://doi.org/10.1016/j.apsoil.2021.104229

Li Y, Cheng W, Da Z, Hu F, Li C (2017) Analysis of radical scavenging active components in the fermented mycelia of *Ophiocordyceps formosana* . Mycology 8:276–285. https://doi.org/10.1080/21501203.2017.1383318

Liu K, Watanabe E, Kokubo H (2017) Exploring the stability of ligand binding modes to proteins by molecular dynamics simulations. J Comput Aided Mol Des 31:201–211. https://doi.org/10.1007/s10822-016-0005-2

Liu Y, Ding L, Fang F, He S (2019) Penicillilactone A, a novel antibacterial 7-membered lactone derivative from the sponge-associated fungus *Penicillium* sp. LS54. Nat Prod Res 33:2466–2470. https://doi.org/10.1080/14786419.2018.1452012

Liu Z, Hu Q, Wang W, Lu S, Wu D, Ze S, He J, Huang Y, Chen W, Xu Y, Lu W, Huang J (2020) Natural product piperine alleviates experimental allergic encephalomyelitis in mice by targeting dihydroorotate dehydrogenase. Biochem Pharmacol 177:114000. https://doi.org/10.1016/j.bcp.2020.114000

Llanos MA, Gantner ME, Rodriguez S, Alberca LN, Bellera CL, Talevi A, Gavernet L (2021) Strengths and Weaknesses of Docking Simulations in the SARS-CoV-2 Era: the Main Protease (Mpro) Case Study. J Chem Inf Model 61:3758–3770. https://doi.org/10.1021/acs.jcim.1c00404

Long L, Wang R, Chiang HY, Ding W, Li YX, Chen F, Qian PY (2021) Discovery of Antibiofilm Activity of Elasnin against Marine Biofilms and Its Application in the Marine Antifouling Coatings. Mar Drugs 19. https://doi.org/10.3390/MD19010019

Madak JT, Bankhead A, Cuthbertson CR, Showalter HD, Neamati N (2019) Revisiting the role of dihydroorotate dehydrogenase as a therapeutic target for cancer. Pharmacol Ther 195:111–131. https://doi.org/10.1016/j.pharmthera.2018.10.012

Mahmud F, Lai NS, How SE, Gansau JA, Mustaffa KMF, Leow CH, Osman H, Sidek HM, Embi N, Lee PC (2022) Bioactivities and Mode of Actions of Dibutyl Phthalates and Nocardamine from *Streptomyces* sp. H11809. Molecules 27:1–16. https://doi.org/10.3390/molecules27072292

Maroun J, Ruckdeschel J, Natale R, Morgan R, Dallaire B, Sisk R, Gyves J (1993) Multicenter phase II study of brequinar sodium in patients with advanced lung cancer. Cancer Chemother

Pharmacol 32:64–66. https://doi.org/10.1007/BF00685878

Maurya KK, Tripathi AD, Kumar D, Srivastava SK (2020) Production, purification and characterization of prodigiosin by Serratia nematodiphilia (NCIM 5606) using solid-state fermentation with various substrate. Ann Phytomedicine An Int J 9. https://doi.org/10.21276/ap.2020.9.2.30

Mc Namara L, Dolan SK, Walsh JMD, Stephens JC, Glare TR, Kavanagh K, Griffin CT (2019) Oosporein, an abundant metabolite in *Beauveria caledonica* , with a feedback induction mechanism and a role in insect virulence. Fungal Biol 123:601–610. https://doi.org/10.1016/j.funbio.2019.01.004

Miyazaki Y, Inaoka DK, Shiba T, Saimoto H, Sakura T, Amalia E, Kido Y, Sakai C, Nakamura M, Moore AL, Harada S, Kita K (2018) Selective cytotoxicity of dihydroorotate dehydrogenase inhibitors to human cancer cells under hypoxia and nutrient-deprived conditions. Front Pharmacol 9:1–13. https://doi.org/10.3389/fphar.2018.00997

Mohamad Fairus AK, Choudhary B, Hosahalli S, Kavitha N, Shatrah O (2017) Dihydroorotate dehydrogenase (DHODH) inhibitors affect ATP depletion, endogenous ROS and mediate S-phase arrest in breast cancer cells. Biochimie 135:154–163. https://doi.org/10.1016/j.biochi.2017.02.003

Mone NS, Bhagwat SA, Sharma D, Chaskar M, Patil RH, Zamboni P, Nawani NN, Satpute SK (2021) Naphthoquinones and their derivatives: Emerging trends in combating microbial pathogens. Coatings 11. https://doi.org/10.3390/coatings11040434

Moonjely S, Keyhani NO, Bidochka MJ (2018) Hydrophobins contribute to root colonization and stress responses in the rhizosphere-competent insect pathogenic fungus *Beauveria bassiana* . Microbiol (United Kingdom) 164:517–528. https://doi.org/10.1099/mic.0.000644

Nguyen HT, Pokhrel AR, Nguyen CT, Pham VTT, Dhakal D, Lim HN, Jung HJ, Kim TS, Yamaguchi T, Sohng JK (2020a) *Streptomyces* sp. VN1, a producer of diverse metabolites including non-natural furan-type anticancer compound. Sci Rep 10:1–14. https://doi.org/10.1038/s41598-020-58623-1

Nguyen NT, Nguyen TH, Pham TNH, Huy NT, Bay M Van, Pham MQ, Nam PC, Vu V V., Ngo ST (2020b) Autodock Vina Adopts More Accurate Binding Poses but Autodock4 Forms Better Binding Affinity. J Chem Inf Model 60:204–211. https://doi.org/10.1021/acs.jcim.9b00778

Nichea MJ, Palacios SA, Chiacchiera SM, Sulyok M, Krska R, Chulze SN, Torres AM, Ramirez ML (2015) Presence of multiple mycotoxins and other fungal metabolites in native grasses from a wetland ecosystem in Argentina intended for grazing cattle. Toxins (Basel) 7:3309–3329. https://doi.org/10.3390/toxins7083309

Nirma C, Eparvier V, Stien D (2015) Reactivation of antibiosis in the entomogenous fungus *Chrysoporthe* sp. SNB-CN74. J Antibiot (Tokyo) 68:586–590. https://doi.org/10.1038/ja.2015.36

Niu X (2017) Perspectives in Sustainable Nematode Management Through Pochonia chlamydosporia Applications for Root and Rhizosphere Health. Springer International Publishing, Cham

Nixon GL, Moss DM, Shone AE, Lalloo DG, Fisher N, O'neill PM, Ward SA, Biagini GA (2013) Antimalarial pharmacology and therapeutics of atovaquone. J Antimicrob Chemother 68:977–985. https://doi.org/10.1093/jac/dks504

Ohno H, Saheki T, Awaya J, Nakagawa A, Omura S (1978) Isolation and Characterization of Elasnin, a New Human Granulocyte Elastase Inhibitor Produced by a Strain of *Streptomyces*. J Antibiot (Tokyo) 31:1116–1123. https://doi.org/10.7164/antibiotics.31.1116

Ōmura S, Asami Y, Crump A (2018) Staurosporine: new lease of life for parent compound of today's novel and highly successful anti-cancer drugs. J Antibiot (Tokyo) 71:688–701. https://doi.org/10.1038/s41429-018-0029-z

Ōmura S, Crump A (2019) Lactacystin: first-in-class proteasome inhibitor still excelling and an exemplar for future antibiotic research. J Antibiot (Tokyo) 72:189–201. https://doi.org/10.1038/s41429-019-0141-8

Pakora GA, Mann S, Kone D, Buisson D (2021) Bioconversion of antifungal viridin to phytotoxin viridiol by environmental non-viridin producing microorganisms. Bioorg Chem 112:1–6. https://doi.org/10.1016/j.bioorg.2021.104959

Pantsar T, Poso A (2018) Binding affinity via docking: Fact and fiction. Molecules 23. https://doi.org/10.3390/molecules23081899

Pham J V., Yilma MA, Feliz A, Majid MT, Maffetone N, Walker JR, Kim E, Cho HJ, Reynolds JM, Song MC, Park SR, Yoon YJ (2019) A review of the microbial production of bioactive natural products and biologics. Front Microbiol 10:1–27. https://doi.org/10.3389/fmicb.2019.01404

Qiao X, Du R, Wang Y, Han Y, Zhou Z (2020) Purification, characterization and mode of action of enterocin, a novel bacteriocin produced by *Enterococcus faecium* TJUQ1. Int J Biol Macromol 144:151–159. https://doi.org/10.1016/j.ijbiomac.2019.12.090

Rangel-Grimaldo M, Macías-Rubalcava ML, González-Andrade M, Raja H, Figueroa M, Mata R (2020) α-Glucosidase and Protein Tyrosine Phosphatase 1B Inhibitors from *Malbranchea circinata* . J Nat Prod 83:675–683. https://doi.org/10.1021/acs.jnatprod.9b01108

Reis RAG, Calil FA, Feliciano PR, Pinheiro MP, Nonato MC (2017) The dihydroorotate dehydrogenases: Past and present. Arch Biochem Biophys 632:175–191. https://doi.org/10.1016/j.abb.2017.06.019

Riko R, Nakamura H, Shindo K (2014) Studies on pyranonigrins-isolation of pyranonigrin E and biosynthetic studies on pyranonigrin A. J Antibiot (Tokyo) 67:179–181. https://doi.org/10.1038/ja.2013.91

Rinderknecht H, Ward JL (1947) Studies on antibiotics; bacteriological activity and possible mode of action of certain non-nitrogenous natural and synthetic antibiotics. Biochem J 41:463–469. https://doi.org/10.1042/bj0410463

Rojas-Aedo JF, Gil-Durán C, Del-Cid A, Valdés N, Pamela Á, Vaca I, García-Rico RO, Levicán G, Tello M, Chávez R (2017) The biosynthetic gene cluster for andrastin A in *Penicillium roqueforti* . Front Microbiol 8:1–11. https://doi.org/10.3389/fmicb.2017.00813

Romsdahl J, Blachowicz A, Chiang YM, Venkateswaran K, Wang CCC (2020) Metabolomic Analysis of *Aspergillus niger* Isolated From the International Space Station Reveals Enhanced Production Levels of the Antioxidant Pyranonigrin A. Front Microbiol 11:1–13. https://doi.org/10.3389/fmicb.2020.00931

Rønnest MH, Raab MS, Anderhub S, Boesen S, Krämer A, Larsen TO, Clausen MH (2012) Disparate SAR data of griseofulvin analogues for the dermatophytes *Trichophyton mentagrophytes*, *T. rubrum*, and MDA-MB-231 cancer cells. J Med Chem 55:652–660. https://doi.org/10.1021/jm200835c

Roy K, Mukhopadhyay T, Reddy GCS, Desikan KR, Rupp RH, Ganguli BN (1988) Aranorosin, a novel antibiotic from *Pseudoarachiniotus roseus*. I. Taxonomy, fermentation, isolation, chemical and biological properties. J Antibiot (Tokyo) 41:1780–1784. https://doi.org/10.7164/antibiotics.41.

1780

Said G, Ahmad F (2022) Effects of salt concentration on the production of cytotoxic geodin from marine-derived fungus *Aspergillus* sp. Turkish J Biochem 47:399–402. https://doi.org/10.1515/tjb-2022-0058

Sakano T, Mahamood MI, Yamashita T, Fujitani H (2016) Molecular dynamics analysis to evaluate docking pose prediction. Biophys physicobiology 13:181–194. https://doi.org/10.2142/biophysico.13.0_181

Santamaría RI, Martínez-Carrasco A, de la Nieta RS, Torres-Vila LM, Bonal R, Martín J, Tormo R, Reyes F, Genilloud O, Díaz M (2020) Characterization of actinomycetes strains isolated from the intestinal tract and feces of the larvae of the longhorn beetle *Cerambyx welensii* . Microorganisms 8:1–14. https://doi.org/10.3390/microorganisms8122013

Sato S, Okusa N, Ogawa A, Ikenoue T, Seki T, Tsuji T (2005) Identification and preliminary SAR studies of (+)-geodin as a glucose uptake stimulator for rat adipocytes. J Antibiot (Tokyo) 58:583–589. https://doi.org/10.1038/ja.2005.79

Schaller MD (2001) Paxillin: A focal adhesion-associated adaptor protein. Oncogene 20:6459–6472. https://doi.org/10.1038/sj.onc.1204786

Shah AA, Shah AN, Bilal Tahir M, Abbas A, Javad S, Ali S, Rizwan M, Alotaibi SS, Kalaji HM, Telesinski A, Javed T, AbdElgawad H (2022) Harzianopyridone Supplementation Reduced Chromium Uptake and Enhanced Activity of Antioxidant Enzymes in *Vigna radiata* Seedlings Exposed to Chromium Toxicity. Front Plant Sci 13:1–12. https://doi.org/10.3389/fpls.2022.881561

Shen M, Yu H, Li Y, Li P, Pan P, Zhou S, Zhang L, Li S, Lee SMY, Hou T (2013) Discovery of Rho-kinase inhibitors by docking-based virtual screening. Mol Biosyst 9:1511–1521. https://doi.org/10.1039/c3mb00016h

Shen W, Ren X, Zhu J, Xu Y, Lin J, Li Y, Zhao F, Zheng H, Li R, Cui X, Zhang X, Lu X, Zheng Z (2016) Discovery of a new structural class of competitive hDHODH inhibitors with in vitro and in vivo anti-inflammatory, immunosuppressive effects. Eur J Pharmacol 791:205–212. https://doi.org/10.1016/j.ejphar.2016.09.004

Shinohara C, Chikanishi T, Nakashima S, Hashimoto A, Hamanaka A, Endo A, Hasumi K (2000) Enhancement of fibrinolytic activity of vascular endothelial cells by chaetoglobosin A, crinipellin B, geodin and triticone B. J Antibiot (Tokyo) 53:262–268. https://doi.org/10.7164/antibiotics.53.262

Shubina LK, Makarieva TN, von Amsberg G, Denisenko VA, Popov RS, Dyshlovoy SA (2019) Monanchoxymycalin C with anticancer properties, new analogue of crambescidin 800 from the marine sponge *Monanchora pulchra*. Nat Prod Res 33:1415–1422. https://doi.org/10.1080/14786419.2017.1419231

Skellam E (2022) Subcellular localization of fungal specialized metabolites. Fungal Biol Biotechnol 9:1–25. https://doi.org/10.1186/s40694-022-00140-z

Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F (2021) Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 71:209–249. https://doi.org/10.3322/caac.21660

Takase S, Kawai Y, Uchida I, Tanaka H, Aoki H (1985) Structure of amauromine, a new hypotensive vasodilator produced by *Amauroascus* sp. Tetrahedron 41:3037–3048. https://doi.org/10.1016/S0040-4020(01)96656-6

Taylor JT, Mukherjee PK, Puckhaber LS, Dixit K, Igumenova TI, Suh C, Horwitz BA, Kenerley CM (2020) Deletion of the *Trichoderma virens* NRPS, Tex7,

induces accumulation of the anti-cancer compound heptelidic acid. Biochem Biophys Res Commun 529:672–677. https://doi.org/10.1016/j.bbrc.2020.06.040

Tomoda H, Tabata N, Ito M, Omura S (1995) Amidepsines, Inhibitors of Diacylglycerol Acyltransferase Produced by *Humicola* sp. FO-2942. II. Structure Elucidation of Amidepsines A, B and C. J Antibiot (Tokyo) 48:942–947. https://doi.org/10.7164/antibiotics.48.942

Tran-Nguyen VK, Simeon S, Junaid M, Ballester PJ (2022) Structure-based virtual screening for PDL1 dimerizers: Evaluating generic scoring functions. Curr Res Struct Biol 4:206–210. https://doi.org/10.1016/j.crstbi.2022.06.002

Uchida R, Shiomi K, Inokoshi J, Tanaka H, Iwai Y, Omura S (1996) Andrastin D, Novel Protein Farnesyltransferase Inhibitor Produced by *Penicillium* sp. FO-3929. J Antibiot (Tokyo) 49:1278–1280. https://doi.org/10.7164/antibiotics.49.1278

Verma R, Agarwal M, Kumar Jatav V (2014) Computer-aided Screening of Therapeutic Ligands against KLF8 Protein (*Homo sapiens*). Int J Comput Bioinforma Silico Model 3:479–482

Volynkina IA, Zakalyukina Y V., Alferova VA, Belik AR, Yagoda DK, Nikandrova AA, Buyuklyan YA, Udalov A V., Golovin E V., Kryakvin MA, Lukianov DA, Biryukov M V., Sergiev P V., Dontsova OA, Osterman IA (2022) Mechanism-Based Approach to New Antibiotic Producers Screening among Actinomycetes in the Course of the Citizen Science Project. Antibiotics 11. https://doi.org/10.3390/antibiotics11091198

Wang W, Cui J, Ma H, Lu W, Huang J (2021) Targeting Pyrimidine Metabolism in the Era of Precision Cancer Medicine. Front Oncol 11:1–17. https://doi.org/10.3389/fonc.2021.684961

Wang YY, Li L, Chen TT, Chen WY, Xu YC (2013) Microsecond molecular dynamics simulation of Aβ42 and identification of a novel dual inhibitor of Aβ42 aggregation and BACE1 activity. Acta Pharmacol Sin 34:1243–1250. https://doi.org/10.1038/aps.2013.55

Wicaksono A, Raihandhany R, Zen TV, da Silva JAT, Agatha A, Cristy GP, Ramadhan ATK, Parikesit AA (2022) Screening Rafflesia and Sapria Metabolites Using a Bioinformatics Approach to Assess Their Potential as Drugs. Philipp J Sci 151:1771–1791. https://doi.org/10.56899/151.05.20

Wolf F, Leipoldt F, Kulik A, Wibberg D, Kalinowski J, Kaysser L (2018) Characterization of the Actinonin Biosynthetic Gene Cluster. ChemBioChem 19:1189–1195. https://doi.org/10.1002/cbic.201800116

World Health Organization (2022) Cancer. https://www.who.int/news-room/fact-sheets/detail/cancer#:~:text=The problem-,Cancer is a leading cause of death worldwide%2C accounting for,lung (2.21 million cases)%3B. Accessed 31 May 2023

Wu D, Wang W, Chen W, Lian F, Lang L, Huang Y, Xu Y, Zhang N, Chen Y, Liu M, Nussinov R, Cheng F, Lu W, Huang J (2018) Pharmacological inhibition of dihydroorotate dehydrogenase induces apoptosis and differentiation in acute myeloid leukemia cells. Haematologica 103:1472–1483. https://doi.org/10.3324/haematol.2018.188185

Xu Z, Jakobi K, Welzel K, Hertweck C (2005) Biosynthesis of the antitumor agent chartreusin involves the oxidative rearrangement of an anthracyclic polyketide. Chem Biol 12:579–588. https://doi.org/10.1016/j.chembiol.2005.04.017

Yamaguchi N, Weinberg EM, Nguyen A, Liberti M V., Goodarzi H, Janjigian YY, Paty PB, Saltz LB, Kingham TP, Loo J, de Stanchina E, Tavazoie SF (2019) PCK1 and DHODH drive colorectal

cancer liver metastatic colonization and hypoxic growth by promoting nucleotide synthesis. Elife 8:1–26. https://doi.org/10.7554/eLife.52135

Yamazaki H, Koyama N, Oura S, Tomoda H (2010) New rugulosins, Anti-MRSA antibiotics, produced by *Penicillium radicum* FKI-3765-2. Org Lett 12:1572–1575. https://doi.org/10.1021/ol100298h

Yan Y, Zang X, Jamieson CS, Lin HC, Houk KN, Zhou J, Tang Y (2020) Biosynthesis of the fungal glyceraldehyde-3-phosphate dehydrogenase inhibitor heptelidic acid and mechanism of self-resistance. Chem Sci 11:9554–9562. https://doi.org/10.1039/d0sc03805a

Yang N, Sun C (2016) The inhibition and resistance mechanisms of actinonin, isolated from marine *Streptomyces* sp. NHF165, against *Vibrio anguillarum*. Front Microbiol 7:1–11. https://doi.org/10.3389/fmicb.2016.01467

Yu H, Li X, Jia Y, Zhang D, Xu T (2021a) Isolation and total synthesis of penicimutans- and aranorosin-type natural products - A summary. Tetrahedron 91:132240. https://doi.org/10.1016/j.tet.2021.132240

Yu X, Gao Y, Frank M, Mándi A, Kurtán T, Müller WEG, Kalscheuer R, Guo Z, Zou K, Liu Z, Proksch P (2021b) Induction of ambuic acid derivatives by the endophytic fungus *Pestalotiopsis lespedezae* through an OSMAC approach. Tetrahedron 79:4–12. https://doi.org/10.1016/j.tet.2020.131876

Zhang L, Wang Z, Yuan X, Sui R, Falahati M (2021) Evaluation of heptelidic acid as a potential inhibitor for tau aggregation-induced Alzheimer's disease and associated neurotoxicity. Int J Biol Macromol 183:1155–1161. https://doi.org/10.1016/j.ijbiomac.2021.05.018

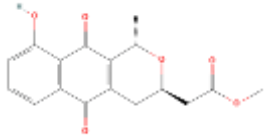
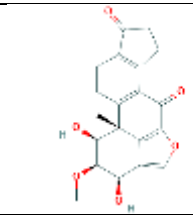
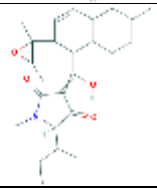
Zhang Q, Luan R, Li H, Liu Y, Liu P, Wang L, Li D, Wang M, Zou Q, Liu H, Matsuzaki K, Zhao F (2018) Anti-inflammatory action of ambuic acid, a natural product isolated from the solid culture of *Pestalotiopsis neglecta* , through blocking erk/jnk mitogen-activated protein kinase signaling pathway. Exp Ther Med 16:1538–1546. https://doi.org/10.3892/etm.2018.6294

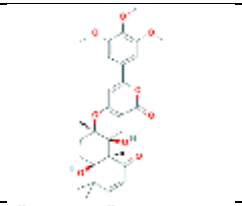
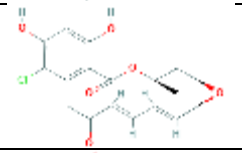
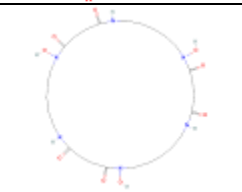
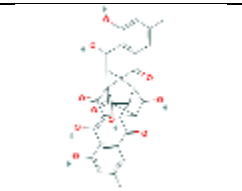
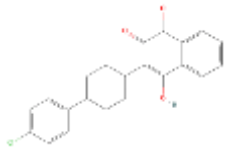
Zhou J, Quah JY, Ng Y, Chooi JY, Toh SHM, Lin B, Tan TZ, Hosoi H, Osato M, Seet Q, Lisa Ooi AG, Lindmark B, McHale M, Chng WJ (2020) ASLAN003, a potent dihydroorotate dehydrogenase inhibitor for differentiation of acute myeloid leukemia. Haematologica 105:2286–2297. https://doi.org/10.3324/haematol.2019.230482

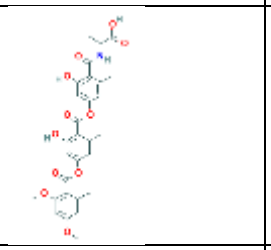
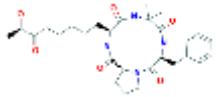
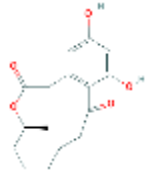
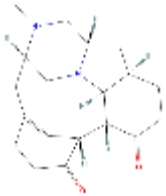
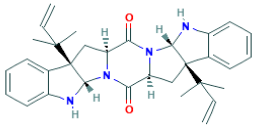
Zoete V, Grosdidier A, Michielin O (2009) Docking, virtual high throughput screening and in silico fragment-based drug design. J Cell Mol Med 13:238–248. https://doi.org/10.1111/j.1582-4934.2009.00665.x

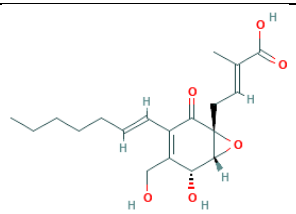
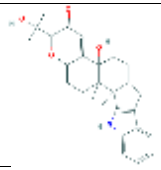
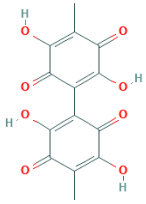
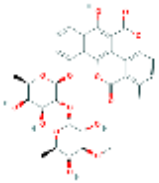
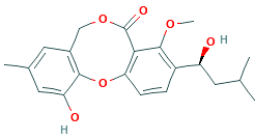
TABLES AND PICTURES

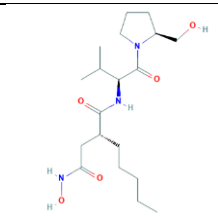
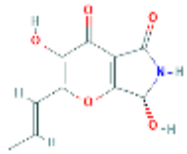
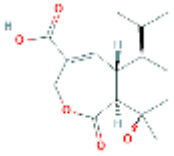
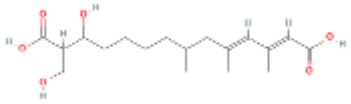
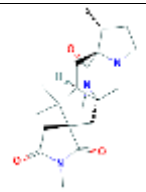
Table 1. List of compounds used in this study

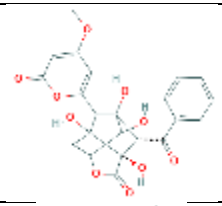
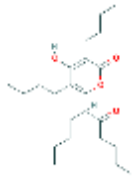
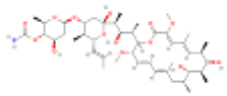
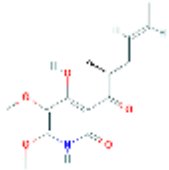
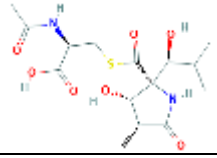
No.	Name	Pubchem ID	Chemical structure	Group	Molecular formula	Molecular weight	Origin	Activity
1	Prodigiosin	135455579		Pyrrole pigment	$C_{20}H_{25}N_3O$	323.4	*Serratia marcescens, Serratia nema-todiphilia* (Arivizhivendhan et al. 2018; Maurya et al. 2020)	Anticancer, antimicrobial, antifungal (El-Bondkly et al. 2012; Lapenda et al. 2015; Li et al. 2018)
2	OM-173 *α*A	12803368		Quinone	$C_{17}H_{16}O_6$	316.09	*Streptomyces* sp (Iwai et al. 1983)	Antimicrobial (Iwai et al. 1983)
3	Viridiol	5459246		Furanosteroid	$C_{20}H_{18}O_6$	354.4	*Trichoderma virens* (Bansal et al. 2018; Pakora et al. 2021)	Antifungal (Ji et al. 2019)
4	PF 1052 (Spylidone)	54687121		N-alkylpyrroli-dine	$C_{26}H_{39}NO_4$	429.6	*Ulocladium atrum* (Gaylarde et al. 2015)	Antibiotic (Gaylarde et al. 2015)

No.	Name	Pubchem ID	Chemical structure	Group	Molecular formula	Molecular weight	Origin	Activity
5	Territrem B	114734		Pyrone	$C_{29}H_{34}O_9$	526.6	*Aspergillus Terreus* (Bunbamrung et al. 2020)	Anti-AchE (Acetylcholin-esterase) (Chen et al. 1999)
6	Radicicol	6323491		Phenolic macrolide	$C_{18}H_{17}ClO_6$	364.8	*Cylindrocarpon de-structans* (Li et al. 2022)	Antibiotic and antifungal (Evans and White 1966)
7	Nocardamine	161532		Cyclic siderophore	$C_{27}H_{48}N_6O_9$	600.7	*Streptomyces* sp. (Santamaría et al. 2020; Mahmud et al. 2022)	Antimalarial, antimicro-bial (Santamaría et al. 2020; Mahmud et al. 2022)
8	Rugulosin/ rugulosin A	24776464		Anthraquinone dimers	$C_{30}H_{22}O_{10}$	542.5	*Chrysoporthe* sp, *Ophiocordyceps formosana*, *Penicil-lium* sp. (Nirma et al. 2015; Li et al. 2017; Liu et al. 2019)	Antimicrobial (Yamazaki et al. 2010)
9	Atovaquone	74989		Naphthoquinone	$C_{22}H_{19}ClO_3$	366.8	Derivative of Naphta-quinone, (from *Mon-acrosporium ambrosium, Plum-bago zeylanica, Caesalpinia sappan*) (Kehelpannala et al. 2018; Mone et al. 2021)	Antitumor, antimalarial (Nixon et al. 2013; Gao et al. 2018)

No.	Name	Pubchem ID	Chemical structure	Group	Molecular formula	Molecular weight	Origin	Activity
10	Amidepsine A	10698236		Depside	$C_{29}H_{29}NO_{11}$	567.5	*Humicola* sp. (Niu 2017; Ibrahim et al. 2021)	Antimicrobial, Anti-DGAT (Diacylglycerol Acyl-transferase) (Tomoda et al. 1995)
11	Dihydrochlamydocin	167994628		Peptide	$C_{28}H_{40}N_4O_6$	528.6	*Pochonia Chlamydosporia* (Degenkolb and Vilcinskas 2016)	Antibiotic (Karle 1996)
12	Curvularin	119418		Lactone macrolide	$C_{16}H_{20}O_5$	292.3	*Cochliobolus* sp., *Phoma macrostoma* (Ataides et al. 2018; Choi et al. 2022)	Antibacterial, antifungal, antivirulence (Ataides et al. 2018; Choi et al. 2022)
13	Herquline A	198537		Alkaloid	$C_{19}H_{26}N_2O_2$	314.4	*Penicillium herquei* (Chiba et al. 2017)	Anti-influenza virus (Chiba et al. 2017)
14	Amauromine	10369017		Alkaloid	$C_{32}H_{36}N_4O_2$	508.7	*Malbranchea circinate* (Rangel-Grimaldo et al. 2020)	Vasodilating activity (Takase et al. 1985)

No.	Name	Pubchem ID	Chemical structure	Group	Molecular formula	Molecular weight	Origin	Activity
15	Ambuic acid	11152290		Cyclohexenone	$C_{19}H_{26}O_6$	350.4	*Pestalotiopsis neglecta, Pestalotiopsis lespedezae* (Zhang et al. 2018; Yu et al. 2021b)	Anti-inflammatory, antifungal (Li et al. 2001; Zhang et al. 2018)
16	Paxilline	105008		Indole	$C_{27}H_{33}NO_4$	435.6	*Penicillium paxillin* (Skellam 2022)	A focal adhesion associated adaptor protein (Schaller 2001)
17	Oosporein	135426831		Quinone	$C_{14}H_{10}O_8$	306.22	*Beauveria bassiana, Beauveria caledonica* (Moonjely et al. 2018; Mc Namara et al. 2019)	Immunosuppressant-Pest control agent (Mc Namara et al. 2019)
18	Chartreusin	5281394		Naphthoquinone	$C_{32}H_{32}O_{14}$	640.6	*Streptomyces pseudovenezuelae, Streptomyces* sp. (Kuncharoen et al. 2019; Volynkina et al. 2022)	Antitumor, antibiotic (Leach et al. 1953; Xu et al. 2005)
19	Penicillide/ vermixocin A	124213		Lactone	$C_{21}H_{24}O_6$	372.4	*Penicillium* sp. (Nichea et al. 2015)	A nonpeptide calpain inhibitor (Chung et al. 1998)

No.	Name	Pubchem ID	Chemical structure	Group	Molecular formula	Molecular weight	Origin	Activity
20	Actinonin	443600		Hydroxamic acid, pseudo-peptide	$C_{19}H_{35}N_3O_5$	385.5	*Streptomyces* sp. (Yang and Sun 2016; Wolf et al. 2018)	Antimicrobial/ antibiotic (Wolf et al. 2018)
21	Pyranonigrin A	16756786		Pyranopyrrole	$C_{10}H_9NO_5$	223.18	*Aspergillus niger* (Riko et al. 2014; Romsdahl et al. 2020)	Antioxidant (Romsdahl et al. 2020)
22	Heptelidic acid/ koningic acid	10945834		Sesquiterpene lactone	$C_{15}H_{20}O_5$	280.32	*Trichoderma koningii, Trichoderma virens* (Yan et al. 2020; Zhang et al. 2021)	Glyceraldehyde-3-phosphate dehydrogen-ase inhibitor, tau aggregation-induced Alz-heimer's disease and as-sociated neurotoxicity in-hibitor, anticancer (Taylor et al. 2020; Yan et al. 2020; Zhang et al. 2021; Isozaki et al. 2022)
23	1233B	10405119		Hydrocarbon	$C_{18}H_{30}O_6$	342.43	*Fusarium* sp. (Kato et al. 2020)	Antibiotic, Antibacterial (AdipoGen Life Sciences 2017)
24	Aspergillimide/ asperparaline A	154701594		Paraher-quamide/ oxindole alkaloid	$C_{20}H_{29}N_3O_3$	359.5	*Aspergillus flavus* (Ezekiel et al. 2020)	Anthelmintic (Banks et al. 1997)

No.	Name	Pubchem ID	Chemical structure	Group	Molecular formula	Molecular weight	Origin	Activity
25	Enterocin	23654446		Polyketide	$C_{22}H_{20}O_{10}$	444.4	*Streptomyces, Enterococcus faecium, Enterococcus* sp. (Nguyen et al. 2020a; Qiao et al. 2020; Kasimin et al. 2022)	Antibacterial(Qiao et al. 2020; Kasimin et al. 2022)
26	Elasnin	54685126		Pyrone	$C_{24}H_{40}O_4$	392.6	*Streptomyces mobaraensis* (Long et al. 2021)	Human granulocyte elastase inhibitor, antibiofilm (Ohno et al. 1978; Long et al. 2021)
27	Concanamycin B	6440685		Macrolide	$C_{45}H_{73}NO_{14}$	852.1	*Streptomyces scabiei* (Deflandre et al. 2022)	V-ATPases and P-ATPases inhibitor (Dröse and Altendorf 1997)
28	Aranorosin	6444217		Cyclohexanone bisepoxide	$C_{23}H_{33}NO_6$	419.5	*Pseudoara chniotus roseus* (Yu et al. 2021a)	Antibiotic (Roy et al. 1988)
29	Harzianopyridone	54697782		Pyridine	$C_{14}H_{19}NO_5$	281.3	*Trichoderma harzianum* (Shah et al. 2022)	Antifungal, antioxidant enzyme and antioxidant metabolite stimulant, plan growth promoter, antimicrobial (Dickinson et al. 1989; Shah et al. 2022)
30	Lactacystin	6610292		*γ*-lactam	$C_{15}H_{24}N_2O_7S$	376.4	*Streptomyces lactacystinaeus* (Ōmura and Crump 2019)	Proteasome inhibitor (Ōmura and Crump 2019)

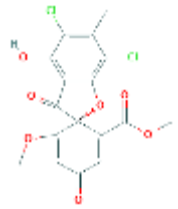
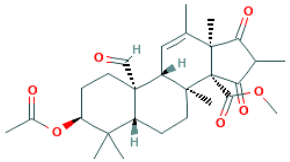
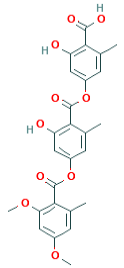
No.	Name	Pubchem ID	Chemical structure	Group	Molecular formula	Molecular weight	Origin	Activity
31	Geodin	216465		Hydroxyanthra-quinone	$C_{17}H_{12}Cl_2O_7$	399.2	*Aspergillus terreus*, *Aspergillus* sp. (Said and Ahmad 2022)	Antimicrobia, glucose stimulator for rat adipo-cytes, enhancement of fi-brinolytic activit, cytotoxic activity (Rinderknecht and Ward 1947; Shinohara et al. 2000; Sato et al. 2005; Rønnest et al. 2012)
32	Andrastin A	6712564		Steroid	$C_{28}H_{38}O_7$	486.6	*Penicillium roqueforti* (Rojas-Aedo et al. 2017)	Protein farnesyltransfer-ase Inhibitor/ antitumor (Uchida et al. 1996; Rojas-Aedo et al. 2017)
33	Amidepsine D	10391109		Depside	$C_{26}H_{24}O_{10}$	496.5	*Humicola* sp. (Niu 2017; Ibrahim et al. 2021)	Antimicrobial, Triacyl-glycerol inhibition, Di-acylglycerol acyltransfer-ase inhibitor (Tomoda et al. 1995)

Table 2. Molecular docking result of native ligand and atovaquone against DHODH

Compound	Binding affinity (kcal/mol)	Residue Involved
Native Inhibitor (3-({[3,5-difluoro-3'-(trifluorometh-oxy)biphenyl-4-yl]amino}car-bonyl)thiophene-2-carboxylic Acid)	-12.2	Tyr38, Leu42, Met43, Leu46, Gln47, Ala55, His56, Ala59, Leu67, Leu68, Val134, Arg136, Tyr356, Leu359, Thr360, Pro364
Atovaquone	-12.9	Tyr38, Met43, Leu46, Gln47, Ala55, His56, Ala59, Pro69, Phe98, Val134, Arg136, Tyr356, Leu359 Pro364

Note: Green, hydrogen bond; black, hydrophobic bond

Table 3. SwissADME output of atovaquone

Compound	Molecular weight (g/mol) ≤ 500 g/mol	Lipinski's Rule of Five ≤ 1 viola-tion	Bioavaila-bility score	Synthetic accessi-bility
Atovaquone	366.84	Yes; 0 violation	0.86	4.07

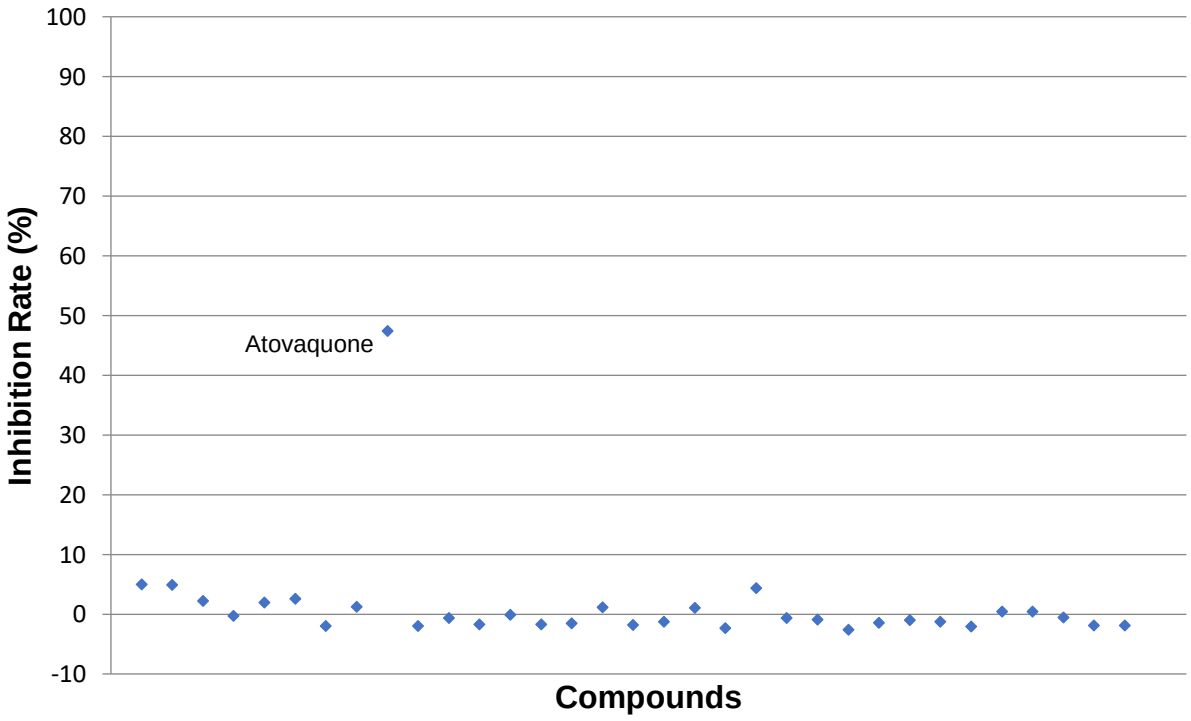


Figure 1. Inhibitory activity assay result of the tested compounds against DHODH

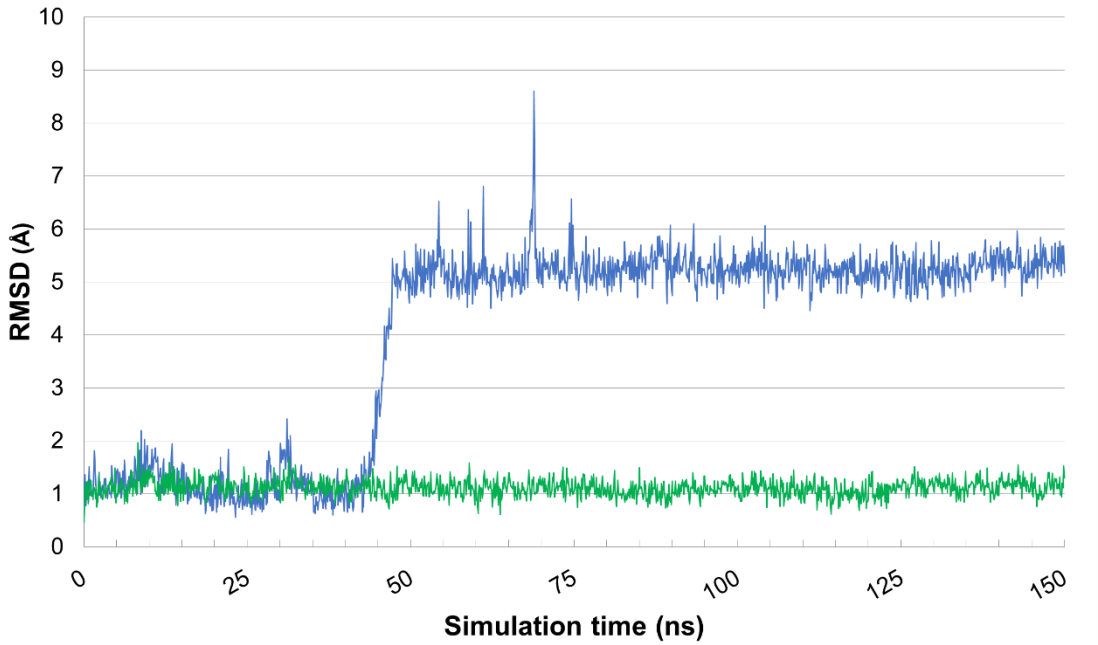


Figure 2. Root Mean Square Deviation (RMSD) of the movement of native inhibitor (green), and atovaquone (blue), in active site of DHODH

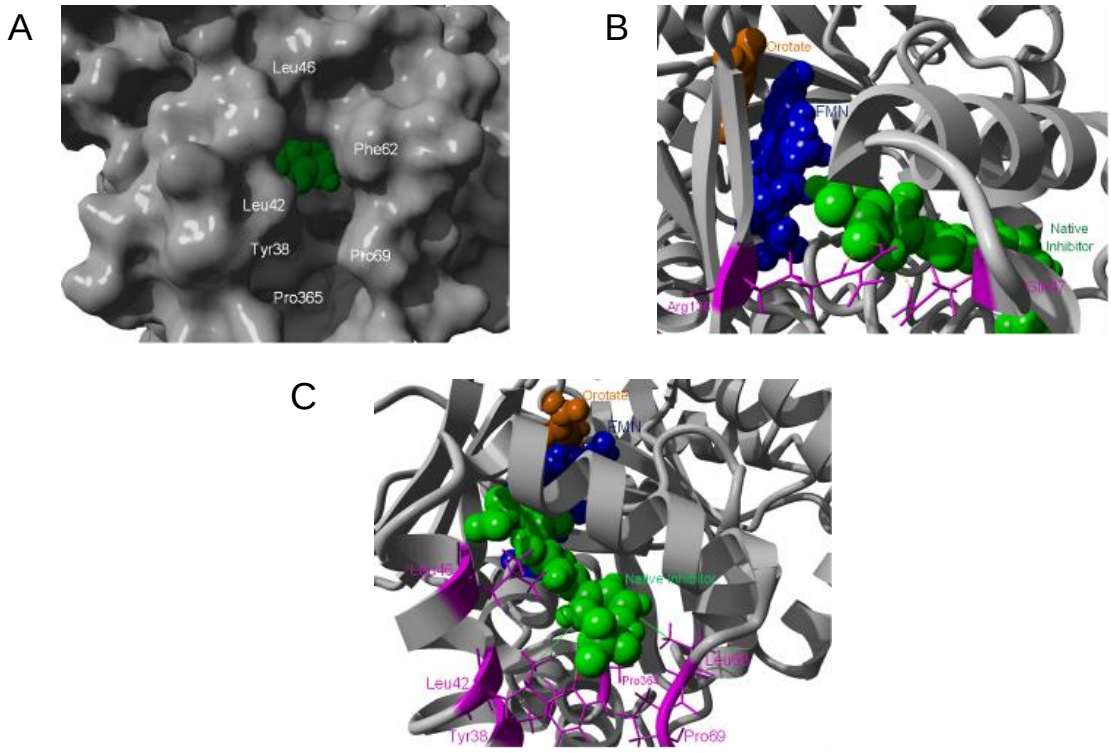


Figure 3. Graphical illustration of active site of complex DHODH-native inhibitor. Green, native inhibitor; A, surface of the active site; B, protein-ligand hydrogen bond interaction at 30 ns; C, other interactions inside the active site at 0 ns.

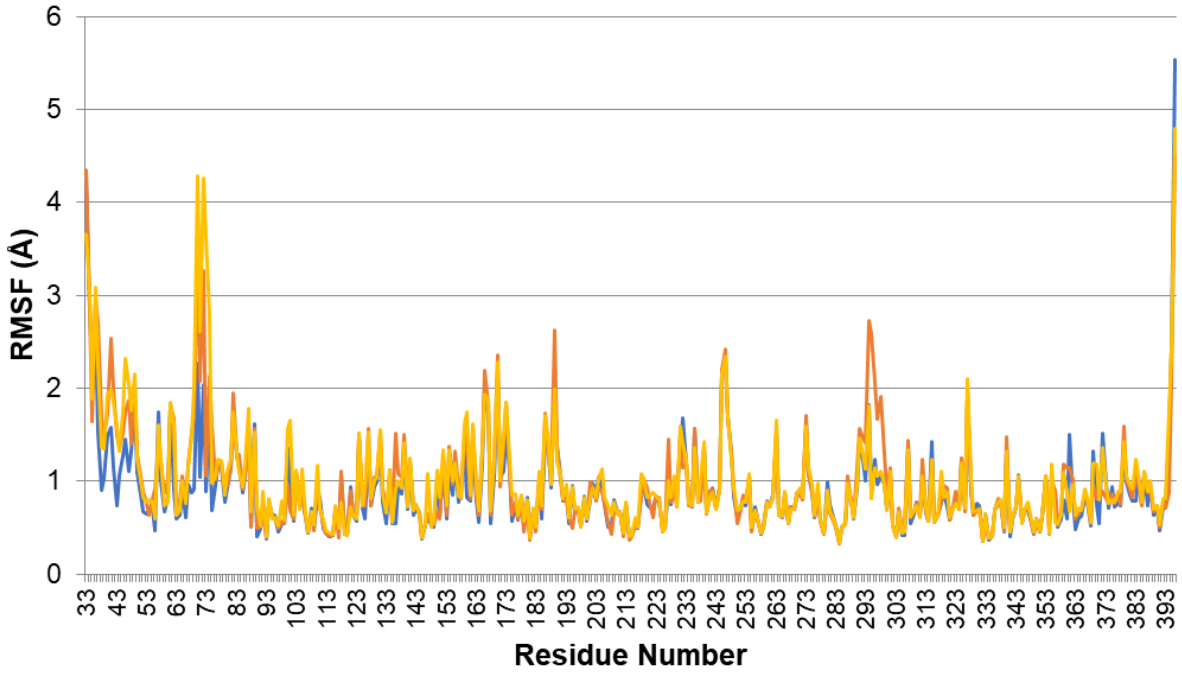


Figure 4. Root Mean Square Fluctuation (RMSF) of each residue in DHODH when interacted with native inhibitor (blue), and atoaquone (orange), Yellow line, RMSF for residues in apo protein.

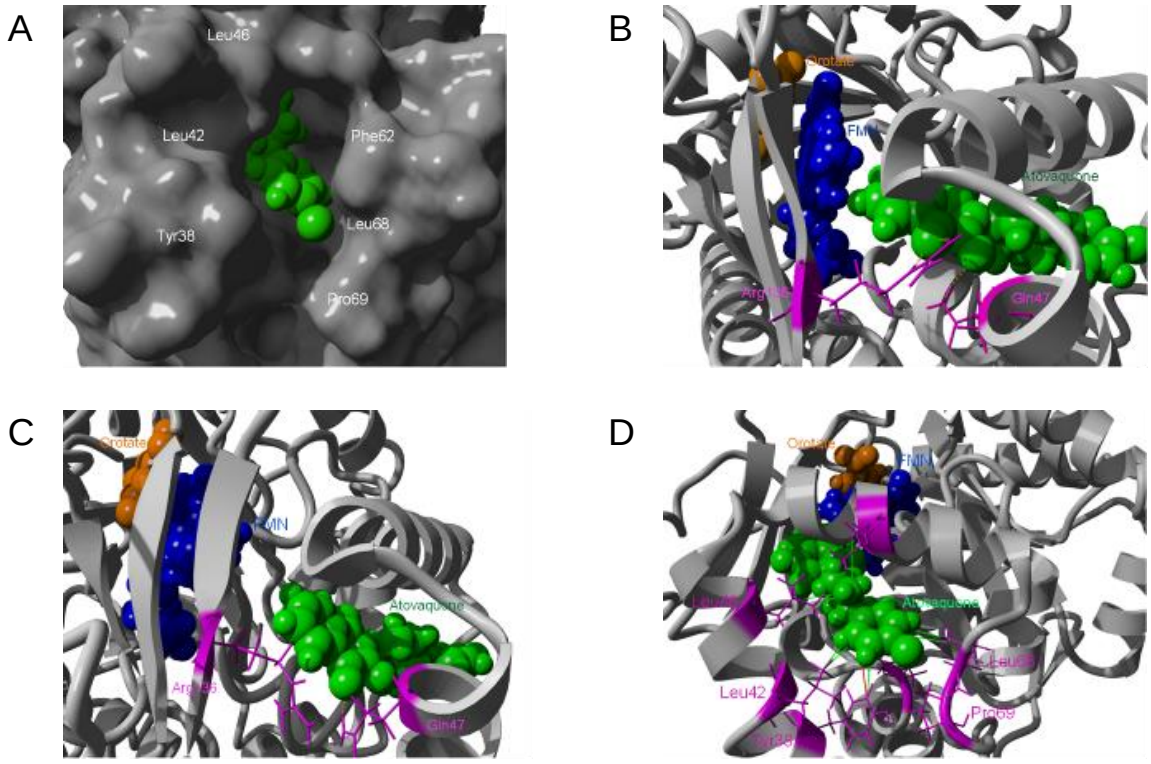


Figure 5. Graphical illustration of active site of complex DHODH-atovaquone. Green, atovaquone; A, surface of the active site; B, protein-ligand hydrogen bond interaction at 0 ns; C, protein-ligand hydrogen bond interaction at 45 ns; D, other interactions inside the active site at 0 ns.

Bioactivity Criteria	Pa	Pi
Anticarcinogenic	0.267	0.073
Antineoplastic	0.424	0.095
Antioxidant	0.216	0.047
Antineoplastic (breast cancer)	0.149	0.139
Angiogenesis inhibitor	-	-
Antineoplastic (non-small cell lung cancer)	-	-
Antineoplastic (colorectal cancer)	0.119	0.114
Antineoplastic (colon cancer)	0.11	0.109
Antineoplastic (non-Hodgkin's lymphoma)	0.338	0.172
Antineoplastic (lung cancer)	0.23	0.057
Antineoplastic (melanoma)	0.16	0.088
Antileukemic	-	-
Antineoplastic (multiple myeloma)	0.249	0.157

Figure 6. Bioactivity of anticancer prediction of atovaquone using Way2Drug (PASS) server

Absorption		
	Caco-2 Permeability	
	MDCK Permeability	
	Intestinal Absorption	
	Pgp-inhibitor	
	Pgp-substrate	
	Bioavailability (20%)	
	Bioavailability (30%)	
Distribution		
	PPB	
	VD	
	BBB	
	Fu	
Metabolism		
	CYP1A2 inhibitor	-
	CYP1A2 substrate	++
	CYP2C19 inhibitor	+
	CYP2C19 substrate	--
	CYP2C9 inhibitor	+
	CYP2C9 substrate	--
	CYP2D6 inhibitor	++
	CYP2D6 substrate	---
	CYP3A4 inhibitor	---
	CYP3A4 substrate	--

Excretion		
	Clearance	
Toxicity		
	hERG Blockers	
	H-HT	
	DILI	
	AMES Toxicity	
	Rat Oral Acute Toxicity	
	FDAMDD	
	Skin Sensitization	
	Carcinogencity	
	Eye Corrosion	
	Eye Irritation	
	Respiratory Toxicity	

Figure 7. ADMET analysis of Atoavquone. Green color represents desirable properties, yellow color represents probably not desirable and Red color represents non-desirable properties. Plasma protein binding (PPB); volume of distribution (Vd); blood brain barrier permeability (BBB), fraction unbound (Fu), hepatotoxicity (H-HT), Drug-induced Liver Injury (DILI), FDA maximum recommended daily dose (FDAMDD).