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Research Article

Molecular Docking & *In Silico* ADME Analysis of 5-O-Methyl-11-O-Acetylalkannin

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Abstract

In the early phases of drug development, prediction of pharmacokinetic parameters including absorption, distribution, metabolism, and elimination (ADME), before synthesis may be helpful in the selection of candidates with less significant pharmacokinetic profiles. 5-O-methyl-11-O-acetylalkannin (MAA) is naphthoquinone isolated from the roots of *Alkanna* genus, *Alkanna cappadocica*. The aim of the present study is to predict the ***in silico*** ADME study of MAA using web tool called SwissADME. The 2D structure of the compound was drawn on Chemdraw Ultra version 8.0. The colored zone represents the optimal physicochemical region for oral bioavailability, according to the bioavailability radar, which takes into account the characteristics of flexibility, lipophilicity, saturation, size, polarity, and solubility. The pharmacokinetic properties were analyzed using the boiled egg model. The yolk-colored yellow component has a high chance of entering the brain, whereas the white portion has a high chance of being passively absorbed by the gastrointestinal tract. The study concluded that MAA did not violate the recommended ranges for Lipinski's rule of five, rotational bond count, and Topological Polar Surface Area (TPSA). MAA also showed moderate lipophilicity and good water solubility. It did not act as a substrate for P-glycoprotein. MAA displayed a potential to inhibit CYP1A2, CYP2C19, CYP2C9 and CYP3A4. Besides that, MAA exhibited no action against CYP2D6. It exhibited a uniform and good bioavailability score of 0.55 (55%) with high gastrointestinal (GI) absorption capabilities. Additionally, the Synthetic Accessibility score of the MAA represented easy-step reactions of synthesis. MAA does not violate any of the five drug-likeness parameters including Lipinski, Muegge, Ghose, Veber, and Egan rules. As a result, MAA could be considered a promising molecule in drug discovery. Moreover, the molecular docking analysis showed the formation of a stable protein ligand complex between TNFα and the ligand molecule.

Keywords: 5-O-methyl-11-O-acetylalkannin; *Alkanna cappadocica*; SwissADME analysis; therapeutic potential

1. Introduction

Computational pharmacology is a fast-developing field of study that uses databases and software to create and analyze molecular, biological, and medical data from various sources (Ekins, Mestres, and Testa 2007; Wu et al. 2020). Techniques for screening and identifying novel lead compounds in molecular libraries have been employed since the start of drug development (Daina, Michielin, and Zoete 2017; Dong et al. 2018; Schyman et al. 2017), enabling the concurrent enhancement of chemical efficacy and drug-like properties, thereby augmenting the drug candidate's standards (Al-Nour, Ibrahim, and Elsaman 2019). Even with limited access to physical samples, pharmacological compounds' design and development require early assessment of their pharmacokinetic properties, including absorption, distribution, metabolism, and excretion (ADME) (Daina, Michielin, and Zoete 2017; Arnott and Planey 2012). Due to the limited pharmacokinetics and toxicity profile of new drug-like molecules, about 11% of medicines approach the clinical development stage before

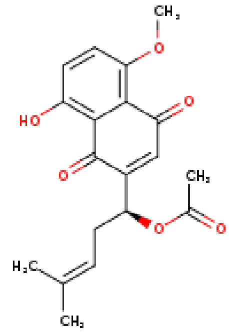

Figure 1: 2D structure of (*S*)-1-(8-hydroxy-5-methoxy-4-oxo-1,4-dihydronaphthalen-2-yl)-4-methylpent-3-enyl acetate

commercialization (Dong et al. 2018; Ghose et al. 2006; Ahmad et al. 2023). The high incidence of attrition in drug development is one of the major issues facing the pharmaceutical industry, which results in a greater interest in the use of computer-aided techniques for toxicity and pharmacokinetic profile prediction (Waring et al. 2015; Rauf et al. 2023). It is commonly acknowledged that these approaches provide a notable benefit over experimental methodologies in terms of time, human, and material resources while searching for new therapeutic compounds (Plewczynski et al. 2011; Azminah et al. 2019). Consequently, remarkable advancements have been made in computational chemistry and computer-aided drug design. These methods have been used to screen novel chemical species and their chemical properties (Pathak et al. 2017). Several online platforms including ADMETlab, Pro Tox-II, and SwissADME—have been developed to predict the ADMET properties of a drug candidate (Daina, Michielin, and Zoete 2017; Dong et al. 2018; Banerjee et al. 2018).

The SwissADME web service offers free access to a range of fast and accurate predictive models for physicochemical properties, pharmacokinetics, drug-likeness, and medicinal chemistry friendliness (Ahmad, Khan, Amin, Khalid, Behl, and Rahman 2021; Ahmad, Khan, and Serdaroğlu 2023). *In-vitro* ADME analysis is preferred because it is more effective, less costly, and provides correct data faster than *in-vivo* ADME assessment, which has been shown to be costly, time-consuming, and involving animal's life. The service also includes proprietary methods such as the BOILED-Egg, iLOGP, and Bioavailability Radar. Several input sources, multiple molecule computation, and the capacity to show, store, and share results worldwide or per molecule via user-friendly, interactive graphs are just a few of SwissADME's strong qualities. Moreover, SwissADME is integrated into the SwissDrugDesign workspace. A variety of CADD tools created by the Molecular Modelling Group of the SIB Swiss Institute of Bioinformatics are accessible with just one click e.g. biotarget prediction (SwissTargetPrediction), ligand-based virtual screening (Swiss Similarity), bioisosteric design (SwissBioisostere), molecular docking (SwissDock) or molecular mechanics (SwissParam) (Daina, Michielin, and Zoete 2017).

Molecular Docking is an *in-silico* technique which is used to assess and evaluate the molecular-level interactions between a ligand and a particular target receptor is called molecular docking. With various scoring functions, it allows ligands to be ranked and evaluated according to the affinity of the ligand with the target receptor which is mostly a protein (Huang and Zou 2010). Two key elements are required for ligands to bind favorably within the target's active site: (a) covering a large conformational space that takes into account

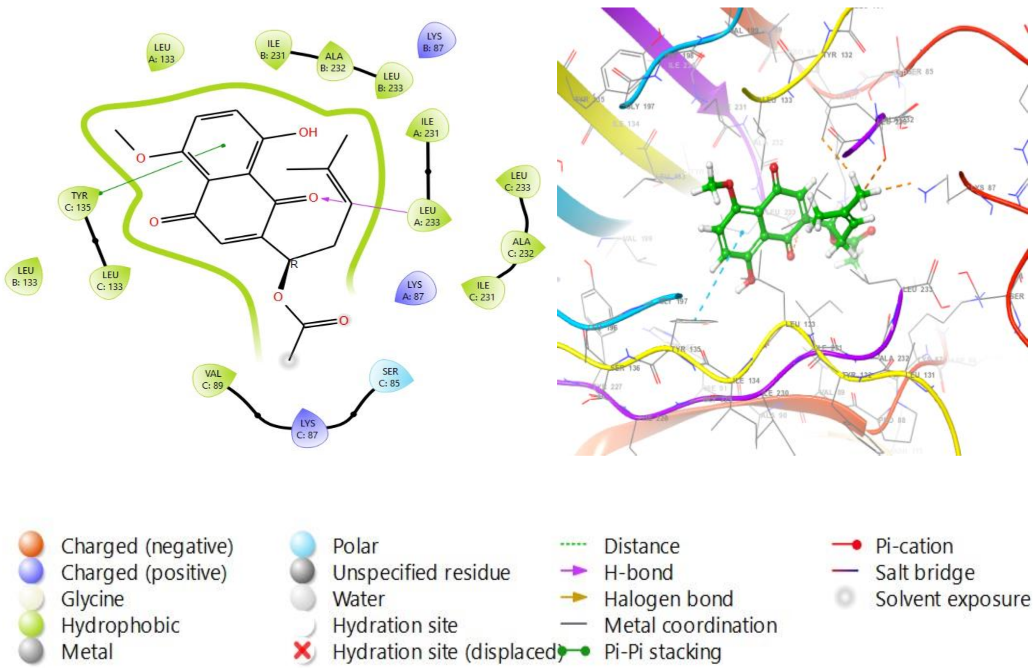

Figure 2: The ligand interaction diagram (2D and 3D) of TNF-α (7JRA) and 5-O-Methyl-11-O-acetylalkanin. The 3D LID also showed the orientation of the Ligand in the binding pocket

different binding poses; and (b) giving clear predictions of ligand binding affinity for each distinct binding pose (Kapetanovic 2008). Categorically, molecular docking can be subdivided into two major types namely: flexible-ligand search docking and flexible-protein docking. Three different strategies are usually used in flexible-ligand search docking to investigate the flexibility of the ligand: the simulation method, the stochastic method, and the systematic method (Sousa, Fernandes, and Ramos 2006). On the flip side, to account for protein flexibility, flexible-protein docking mostly uses Monte Carlo (MC) and molecular dynamic (MD) techniques (Oshiro, Kuntz, and Dixon 1995; Hart and Read 1992). Some important tools for molecular docking are GLIDE (Friesner et al. 2004), Autodock vina (Trott and Olson 2010), SwissDock (Grosdidier, Zoete, and Michielin 2011) and, Dock6 (Allen et al. 2015).

Alkannins are a type of naphthoquinone that contain two ketone moieties at C-1 and C-4. Hydroxynaphthoquinones are 5,8-dihydroxy derivatives of these molecules. Alkannin and shikonin, are the S and R enantiomers, respectively. These are the isohexenylnaphthazarine derivatives of hydroxynaphthoquinones and are found in the outer surface of the roots, as ester derivatives, of at least 150 species that belong to the genera *Alkanna*, *Lithospermum*, *Echium*, *Onosma*, *Anchusa*, and *Cynoglossum* of the family, *Boraginaceae*.

(Assimopoulou et al. 2009). 5-O-methyl-11-O-acetylalkannin (MAA) is naphthoquinone isolated from the roots of *Alkanna* genus, *Alkanna cappadocica.* The compound was tested against various cell line including HT-29, MDA-MB 231, PC-3, AU565, HepG2, LNCaP, MCF7, HeLa, SK-BR-3, DU 145, Saos, and Hep3B. The compound exhibited impressive cytotoxicity, with IC50 values ranging from 0.09 to 14.07μM and showed significantly more potent activity than positive controls doxorubicin and etoposide in 6 out of 12 cancer cell lines (Sevimli-Gur et al. 2010).

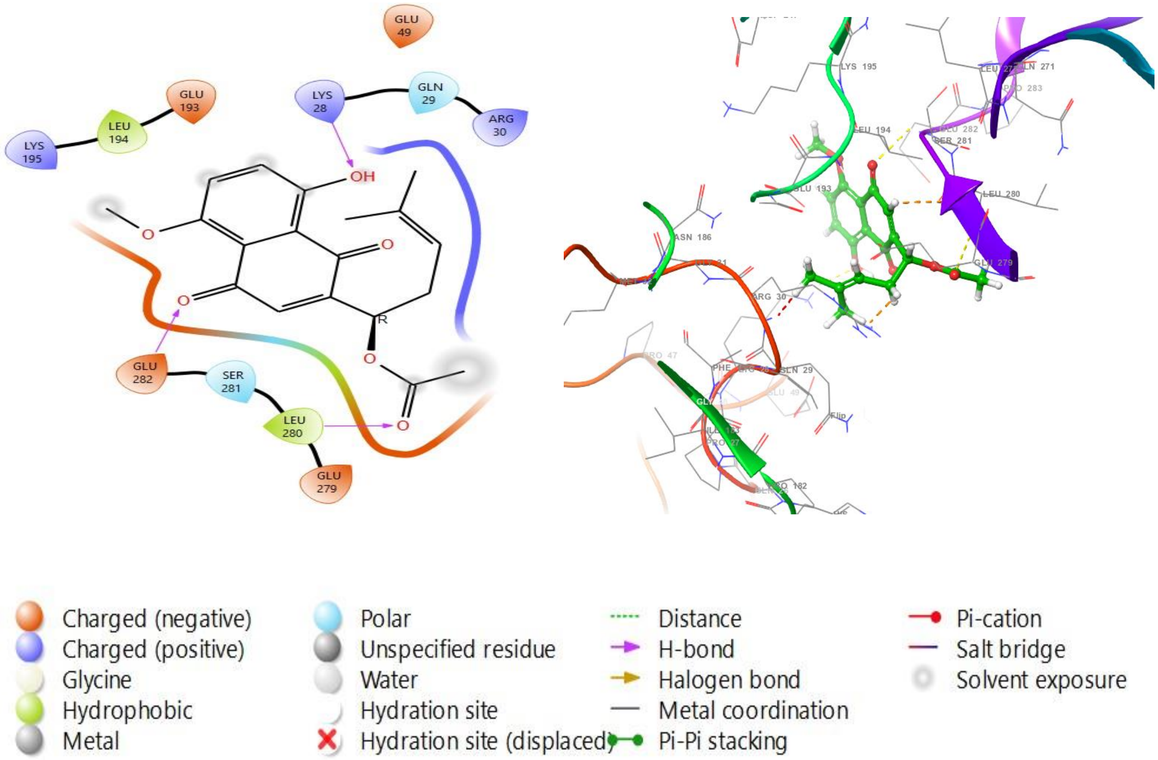

Figure 3: The ligand interaction diagram (2D and 3D) of NF-kB (1LE5) and 5-O-Methyl-11-O-acetylalkanin. The 3D LID also showed the orientation of the Ligand in the binding pocket

In the present study, ***in-silico*** pharmacokinetic (ADME) properties, drug-likeness and medicinal chemistry of 5-O-methyl-11-O-acetylalkannin (MAA) were examined using SwissADME.

2. Materials & Methods

The 2D structure of MAA was drawn on ChemDraw Ultra version 8.0. The structure was imported and the structure SMILES was entered. The Swiss ADME drug design study was run and the readings were noted down.

With a high resolution of 2.10 and 2.75 Å, the human TNF-α and NF-kB X-ray crystal structures (PDB ID: 7JRA and 1LE5 respectively) were extracted from the Protein Data Bank for this study (Rose et al. 2012). Using the Protein Preparation Wizard built into the Schrödinger interface, the protein was prepared to undergo docking analysis ***(Schrödinger 2023)***. A receptor grid for TNF-α was established centroid around the pre-docked ligand and a receptor grid for NF-kB was established with coordinates at 111.0 (x-axis), 39.0 (y-axis), and 29.0 (z-axis) during the preparation step. The scaling factor was additionally adjusted to 1.0 Å. The molecular docking investigation was carried out using a Glide *(Manual 2018)***.** Additionally, the extra precision (XP) docking method was employed in the molecular docking procedure for improved reliability and accuracy (Friesner et al. 2004). The ligand molecule was prepared by LigPrep in order to remove the steric clashes and maintain a minimum energy conformer *(Schrodinger 2023)***.**

3. Results

3.1. TNF-α (7JRA) and 5-O-methyl-11-O-acetylalkannin

The molecular docking yielded a stable complex between 7JRA and 5-O-Methyl-11-O-

Table 1: The Physicochemical property of MAA **calculated with the SwissADME database.**

Ligands	Molecular Formula	M.W g/mol)	Nha	nAHA	F. Csp³	nRB	nHBA	nHBD	MR	TPSA (A^2)
A	C19H20O6	344.36	25	6	0.32	6	6	1	92.02	89.90

Molecular weight: M.W, No. heavy atom: nHA, No. arom. heavy atom: nAHA, No. of sp^3 hybridized carbon out of total carbon count: F. Csp^3, No. rotatable bonds: nRB, No. H-bond acceptors: nHBA, No. H-bond donors: nHBD, Molar refractivity: MR, Topological Polar Surface Area: TPSA

Table 2: Lipophilicity and water solubility of 5-O-methyl-11-O-acetylalkannin

Ligands	Lipophilicity	Water Solubility					
	Consensus Log $P_{o/w}$	**Log S (ESOL)**	**Solubility Class**	**Log S (Ali)**	**Solubility Class**	**Log S (SILICOS-IT)**	**Solubility Class**
A	2.80	-3.84	Soluble	-4.87	Moderately Soluble	-3.95	Soluble

acetylalkanin. The XP docking score was -6.964KJ/mol. The docked complex showed two stable interactions. LEU233 formed a hydrogen bond one of the carbonyl groups of the ligand molecule. The bond distance was found to be 1.69Å which is an indication of a strong hydrogen bond interaction. Moreover, TYR135 showed a Pi-Pi interaction with the methoxy phenol ring of the ligand molecule. The bond distance was found to be 5.16Å which also is an indication of stable and strong interaction.

The 2D and 3D Ligand interaction diagram (LID) is shown below which shows a difference between the 2D and 3D orientation of the ligand molecule with the protein and is shown in Figure 2.

3.2. 5-O-Methyl-11-O-acetylalkanin and NF-kB (1LE5)

The molecular docking showed three important interactions between the ligand and the protein. All three interactions were found to be hydrogen bonds, which is again the strongest type of intermolecular interaction. However, the docking score (XP docking score) was found to be -1.567 KJ/mol which is an indication of very poor and unstable complex. However, the hydrogen bond distances were promising. LEU280 formed a hydrogen bond with the carbonyl of the ester moiety and with a bond distance of 2.09Å. GLU282 also showed a hydrogen bond with one of the carbonyl groups of the ligand molecule as shown in Fig.2. The bond distance was found to be 2.49Å. The final hydrogen bond was formed between LYS28 and the hydroxyl group of the methoxy phenol ring of the ligand molecule with a bond distance of 2.04Å. All of the hydrogen bond interactions were good but considering the XP docking scores the docked complex was deemed a little unstable. The 2D and 3D LID are shown in Figure 3.

3.3. Analysis of Physicochemical Properties

MAA did not violate Lipinski's rule of five. Values of Molecular weight, No of H-bond donors, Topological Polar Surface Area, Log P and number of hydrogen bond acceptors were 344.36, 1, 89.90, 2.80, and 6, respectively. The MAA exhibited 6 rotatable bonds. The molar refractivity of MAA was 92.02, as shown in table 1.

3.4. Lipophilicity and water solubility of 5-O-methyl-11-O-acetylalkannin

The log $P_{o/w}$ value of MAA was 2.80, which indicated its moderate lipophilicity. Concurrently, the Log S

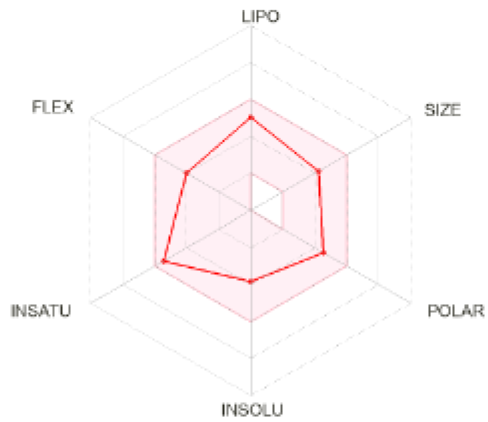

Figure 4: Schematic diagram of Bioavailability Radar for Drug likeness of a molecule (lipophilicity: XLOGP3 between-0.7 and+5.0, size: MW between 150 and 500 g/mol, polarity: TPSA between 20 and 130 A2, solubility: log S not higher than 6, saturation: fraction of carbons in the sp3 hybridization not less than 0.25, and flexibility: no more than 9 rotatable bonds)

Table 3: Pharmacokinetics of 5-O-methyl-11-O-acetylalkannin calculated with SwissADME database

Ligands	GI absorption	BBB permeant	P-gp substrate	CYP1A2 inhibitors	CYP2C19 inhibitors	CYP2C9 inhibitors	CYP2D6 inhibitors	CYP3A4 inhibitors	Log Kp (skin permeation) (cm/s)
A	High	No	No	Yes	Yes	Yes	No	Yes	-6.05

value signifies the aqueous solubility of compounds. MAA exhibited good water solubility, as shown in Table 2.

3.5. Pharmacokinetic Profile

The comprehensive evaluation performed via the SwissADME database unveiled noteworthy gastrointestinal (GI) absorption capabilities of a compound. The graphical representation of MAA in the form of a boiled egg graph is visually depicted in Figure 5. MAA did not exhibit the ability to permeate the blood-brain barrier. It was ascertained that it did not function as a substrate for P-glycoprotein. MAA displayed a potential to inhibit CYP1A2, CYP2C19, CYP2C9 and CYP3A4. Besides that, MAA exhibited no action against CYP2D6. MAA revealed a uniform bioavailability score of 0.55, signifying a consistent pattern in this regard, as shown in Table 3.

3.5.1. Drug Likeness

The MAA did not violate any of the five drug-likeness parameters including Lipinski, Muegge, Ghose, Veber, and Egan rules of drug-likeness, as shown in Table 4.

3.5.2. Medicinal Chemistry

The MAA exhibited 1 pan assay interference compounds (PAINS) alert. Brenk structural alert had identified one reactive group in MAA. MAA can serve as Lead. The SwissADME database has assigned the Synthetic Accessibility score of 3.94 to the MAA, which represents easy step reactions of synthesis, as shown in table 4.

4. Discussion

Rational drug development requires adherence to Lipinski's rule of five. Any medication molecule that disobeys even one of the guidelines could have poor absorption or low permeability (Pathak et al. 2017). Lipinski's Rule of Five (RO5) was first developed for orally active compounds. Four basic physicochemical characteristics were defined, including: molecular weight <500, hydrophobicity indicated by log P ≤ 5, H-bond donors ≤ 5, and H-bond acceptors ≤ 10. These characteristics have been shown to be associated with 90% of orally active drugs that have effectively moved forward to phase II clinical trials (Lipinski 2004). Molecular weight (MW), the count of hydrogen bond

Table 4: Drug likeness and Medicinal chemistry

Ligands	Drug likeness rules					Medicinal chemistry				
	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability Score	PAINS	Brenk	Lead likeness	Synthetic Accessibility
A	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55	1 alert: quinone_A	1 alert: isolated_alkene	Yes	3.94

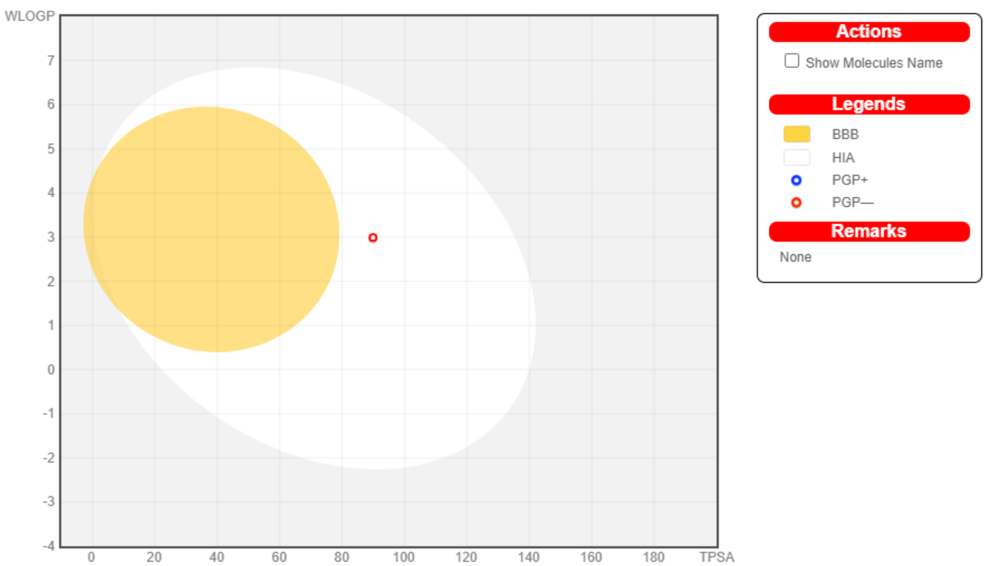

Figure 5: Boiled graph representations of 5-O-methyl-11-O-acetylalkannin.

acceptors (nHBA) and the count of hydrogen bond donors (nHBD) for MAA are within the required range.

The fraction of sp^3 carbon atoms in the total number of carbon atoms is known as Fsp^3. This illustrates the complexity of the molecular spatial structure and reflects the saturation of carbon. A reasonable and optimal value for Fsp^3 is ≥0.42, which is met by around 84% of pharmaceutical products on the market (Kombo et al. 2013). Sp^3 content must be raised within a range, though, as a higher Fsp^3 score does not imply improved performance and may instead make chemical synthesis more challenging (Gerlach et al. 2019). Natural products are a rich source of medications because synthetic products often contain a lower fraction of sp^3 (Jia et al. 2020).

The rotatable bond count is used as a' drug filter' which is associated with decreased rat oral bioavailability if the number of rotatable bonds is greater than 10 (Veber et al. 2002). The "rotatable bond filter's" mechanism is still unknown because its number does not correspond to the rate of in vivo clearance in rats. However because ligand affinity drops by an average of 0.5 kcal for every two rotatable bonds, the filter is justified from the perspective of in vitro screening (Andrews, Craik, and Martin 1984). Oral drugs exhibit a lower count of H-bond acceptors, donors, and rotatable bonds (Lipinski 2004; Ahmad, Khan, Amin, Khalid, Behl, and Ur Rahman 2021). The analyzed compound (MAA) manifests a rotational bond count of 6, fulfilling the required criteria.

The molecules with a TPSA of ≥140 Å^2 would be poorly absorbed with less than 10% fractional absorption, while those with a TPSA upto60 Å^2 would be well absorbed with greater than 90% fractional absorption (Clark 1999). MAA has TPSA values within the established limits and is predicted to have better absorption.

The Log Po/w, an essential parameter calculated utilizing SwissADME, constitutes an average of iLOGP, XLOGP3, WLOGP, MLOGP, and SILICOS-IT values, collectively referred to as consensus Log Po/w. This Log Po/w signifies the logarithm of the octanol/water partition coefficient, a pivotal metric. A higher log $P_{o/w}$ value indicates higher lipophilicity, and it depends upon polarity, molecular size, and hydrogen bonding (Bitew et al. 2021). The log $P_{o/w}$ value of MAA is 2.80, indicating its moderate lipophilicity.

The Log S value indicates the solubility of compounds in aqueous medium. In this context, the compound exhibits good water solubility. Understanding a drug molecule's pharmacokinetics (PK) is critical to achieving the desired pharmacological goals because every compound's pharmacokinetic parameter can have a significant impact on the drug's pharmacological profile. *P*-glycoprotein interactions have significant effect on the pharmacological profile of other drugs (Zhang et al. 2021). MAA does not function as a substrate for P-glycoprotein.

Enzymes known as cytochrome P450 are necessary for the metabolism of numerous drugs. Drugs have the ability to inhibit or induce cytochrome P450 enzymes, which can lead to clinically important drug-drug interactions that may result in unexpected adverse responses or therapeutic failures (Lynch and Price 2007).

MAA displays a potential to inhibit CYP1A2, CYP2C19, CYP2C9 and CYP3A4. Besides that, MAA exhibited no action against CYP2D6.

It's crucial to calculate permeability and bioavailability before moving forward for synthesis or other advanced tests. Therefore, a probability-based score is given to a drug candidate to have F > 10 % in rat (Martin 2005). The analyzed compound exhibits a uniform and good bioavailability score of 0.55 (55%). MAA does not violate any of the five drug-likeness parameters including Lipinski, Muegge, Ghose, Veber, and Egan rules of drug-likeness. The yellowish area on the boiled egg graph shows CNS penetration, and the white region displays human intestinal absorption. If the drug absorption is other than the oral route it will be represented in the gray area of the graph (Daina, Michielin, and Zoete 2017). MAA shows high gastrointestinal (GI) absorption capabilities. The compound does not exhibit the ability to permeate the blood-brain barrier.

During high-throughput screening (HTS), PAINS exhibit unrestricted behavior that results in false positive hits. They are linked to non-covalent interactions and protein reactivity, albeit the exact mechanism is unknown (Bolz, Adasme, and Schroeder 2021). MAA exhibited 1 PAINS alert including the 'quinone_A group'. The quinone group in the compound's chemical structure is responsible for the production of breaks in DNA strands. These breaks may be associated with the cytotoxic activity of the quinone agents (Begleiter and Blair 1984).

Brenk et al. identified 105 fragments, which are chemically reactive, hazardous, metabolically unstable, or likely to have poor pharmacokinetics. In SwissADME, a structural alert is made to identify such problematic fragments present in a given compound (Brenk et al. 2008). Brenk structural alert has identified 1 reactive group in MAA including 'isolated alkene', which suggested to improve before moving a drug to the next phase of development.

The lead likeness parameter indicates the ability of a compound to serve as 'lead' in the process of drug discovery (Ahmad, Khan, Amin, Khalid, Behl, and Ur Rahman 2021). MAA can serve as

Lead. The SwissADME database has assigned the Synthetic Accessibility score of 3.94 to the MAA, which represents easy step reactions of synthesis. The difficult synthetic approaches for those molecules having a score of 10 (Ahmad, Khan, Amin, Khalid, Behl, and Ur Rahman 2021).

5. Conclusion

The current study presents an ***in silico*** ADME evaluation of 5-O-methyl-11-O-acetylalkannin (MAA) using a web tool called SwissADME. MAA did not violate the recommended limits for Lipinski's rule of five, rotational bond count, and TPSA. MAA also exhibited moderate lipophilicity and good water solubility. It did not act as a substrate for P-gp and had a potential to inhibit CYP1A2, CYP2C19, CYP2C9, and CYP3A4. Besides that, MAA exhibited no action against CYP2D6. It displayed a uniform and good bioavailability score with high gastrointestinal (GI) absorption capabilities. Additionally, the Synthetic Accessibility score MAA represented easy step reactions of synthesis. MAA does not violate any of the five drug-likeness parameters including Lipinski, Muegge, Ghose, Veber, and Egan rules. As a result, MAA could be considered a promising molecule in drug discovery. Furthermore, by considering the docking scores and interaction between the proteins and the ligand molecule, the docked complex between TNF-α (7JRA) and 5-O-Methyl-11-O-acetylalkanin was deemed stronger and stable when compared with NF-kB (1LE5). Further, MD simulations and in-vitro investigation may shed light on the molecular interactions and authenticity of the interaction.

Conflict of Interest

The authors declare that they have no competing interests.

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Study Approval

NA

Consent Forms

NA.

Data Availability

All the data related to this manuscript are available with the authors**.**

Author Contributions

Main idea and conceptualization, and initial draft by HK, literature collection, and *in silico* analysis by HS & US, graphics, language, and grammar by HK, results analysis and proofreading by HS & US, final review editing, rebuttals, and final draft by HK.

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