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IN SILICO EVALUATION OF HYPERICIN AND PSEUDOHYPERICIN AS CANDIDATES FOR MONKEYPOX TREATMENT

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ABSTRACT

Monkeypox (Mpox) is a viral zoonotic and human-to-human disease with no specific drug or treatment protocol targeting the monkeypox virus (MPXV). In the MPXV life cycle, viral kinase phosphorylation plays a crucial role in early morphogenesis in the cytoplasm, making inhibition of MPXV kinase a potential therapeutic approach for controlling Mpox. *Hypericum sampsonii* contains several bioactive compounds, such as hypericin and pseudohypericin, which are known for their antiviral properties. In this study, a computational investigation of the physicochemical properties of hypericin and pseudohypericin revealed drug-like characteristics. Pharmacokinetic predictions indicated that hypericin and pseudohypericin are non-toxic to the central nervous system, hepatic system, and cardiac system. Molecular docking results indicated a strong binding affinity of hypericin/pseudohypericin with MPXV thymidylate kinase. As a result, these compounds are being considered as potential Mpox control candidates.

Keywords: Monkeypox virus, *Hypericum*, *Hypericum sampsonii*, hypericin, pseudohypericin, thymidylate kinase.

INTRODUCTION

Monkeypox (Mpox) is an infectious disease caused by the Monkeypox virus (MPXV) and capable of human-to-human transmission (Riopelle *et al.*, 2022). MPXV is classified under the Orthopoxvirus genus within the Poxviridae family, which also includes Vaccinia, Cowpox, and Smallpox viruses (Alkhalil *et al.*, 2009). The first confirmed case of MPXV infection was recorded in 1970 in the Demographic Republic of Congo (Breman *et al.*, 1980). In mid-2022, the World Health Organization

(WHO) declared the resurgence of Mpox, raising global concerns as numerous countries reported multiple cases confirming MPXV infections (Schnierle, 2022). By January 2024, Mpox had accounted for over 93,000 confirmed cases and 179 deaths across 117 countries, as reported by the WHO. This underscores the substantial threat Mpox poses to public health. Currently, no specific antiviral treatment for MPXV is available on the market (Kumari *et al.*, 2023). Therefore, developing novel molecular compunds capable of controlling MPXV is of pivotal significance in treating Mpox.

The previous study identified the A48R protein within the Orthopoxvirus genus as a promising candidate for drug design (Prichard, Kern, 2012). The A48R protein acts as a thymidylate kinase (TMPK), facilitating the phosphorylation of thymidine 5'-monophosphate to generate thymidine 5'-diphosphate (TYD). The thymidylate kinase protein of MPXV (MPXV-TMPK) is identified as a novel target protein that lacks a specific inhibitor (Pourhajibagher, Bahador, 2023). As a result, the exploration of new compounds capable of selectively binding to effectively MPXV-TMPK has the potential to control Mpox.

Hypericum sampsonii Hance, a member of the Hypericaceae family, is recognized as a medicinal plant in many countries (Sun *et al.*, 2023). It is predominantly found in Northern Vietnam, Eastern Myanmar, Northeast India, and China (Sun *et al.*, 2023). Previous studies have highlighted the diverse biological properties of *H. sampsonii*, including anti-inflammatory, antinociceptive, antioxidant activities, antimicrobial as well as antiviral effects (Sun *et al.*, 2023; Xie *et al.*, 2021). The chemical composition of *H. sampsonii* encompasses a range of metabolites, such as polycyclic polyprenylated acylphloroglucinols, benzophenones, flavonoids, xanthones, naphthodianthrones, anthraquinones, and aromatics (Sun *et al.*, 2023; Xie *et al.*, 2021). Naphthodianthrones contain highly biologically active substances, notably hypericin and pseudohypericin (Hongyan *et al.*, 2002). Hypericin can be extracted from flowers and fruits, while pseudohypericin is predominantly found in the aerial parts of *H. sampsonii* (Sun *et al.*, 2023). The antiviral properties of both compounds have been established in numerous studies, encompassing herpes simplex types 1 and 2, and HIV-1 (Barnes *et al.*, 2001; Zhang *et al.*, 2022). Hypericin is actively employed in the development of drugs or intermediate compounds for photodynamic therapy (PDT) and photodynamic diagnosis (PDD) (Jendželovská *et al.*, 2016; Yuan *et al.*, 2023). Due to their ability to inhibit protein kinases (Takahashi *et al.*, 1989), hypericin and pseudohypericin are the most promising compounds for blocking MPXV-TMPK activity.

MATERIALS AND METHODS

Building MPXV thymidylate kinase protein and validation model

The amino acid sequence of MPXV-TMPK was obtained from GenBank (Accession number YP_010377155.1). The structure of Vaccinia virus thymidylate kinase (PDB ID: 2V54) was used as the template for constructing MPXV-TMPK using the SWISS-MODEL (Waterhouse *et al.*, 2018). The homology protein model underwent validation the through Structure Assessment tool of the SWISS-MODEL server (Waterhouse *et al.*, 2018), ProSA (Wiederstein, Sippl, 2007), and ERRAT tools (Colovos, Yeates, 1993).

Target protein preparation

MPXV-TMPK was prepared using the Protein Preparation Wizard module within the Maestro software. This preparation involved assigning bond orders and incorporating missing hydrogen atoms. Subsequently, the orientations of hydrogen-bonded groups were optimized, and the overall structure was minimized using the OPLS4 force field (Lu *et al.*, 2021).

Ligands preparation

The chemical formulas of hypericin and pseudohypericin were determined in a previous study (Karioti, Bilia, 2010). The three-dimensional (3D) structures of hypericin and pseudohypericin were constructed and optimized using the OPLS4 force field through the Ligprep module.

***In silico* prediction of physicochemical and pharmacokinetics properties**

The physicochemical and pharmacokinetic properties of hypericin and pseudohypericin were assessed using the QikProp module. The physicochemical parameters included molecular weight **(MW)**, hydrogen-bond acceptor atoms **(HBA)**, hydrogen-bond donor atoms **(HBD)**, Octanol/water partition coefficient **(QPlog$P_{o/w}$)**, and polar surface area **(PSA)**. Pharmacokinetic predictions for the ligands encompassed **Volume**, Caco-2 cell permeability in nm/s **(QPPCaco)**, brain/blood partition coefficient **(QPlogBB)**, predicted IC_{50} value for blockage of HERG K^+ channels **(QPlogHERG)**, predicted aqueous solubility **(QplogS)** and human oral absorption **(%HOA)**. The pkCSM server (Pires *et al.*, 2015) was used to predict AMES toxicity, hepatic toxicity, and skin sensitisation of hypericin and pseudohypericin.

Molecular docking analysis

Molecular docking utilized TYD in the crystal structure as a template ligand to identify active residues of MPXV-TMPK. Autodock Vina version 1.2.5 (Eberhardt *et al.*, 2021) was employed to create complexes of hypericin and pseudohypericin with MPXV-TMPK. The dimensions of the grid box were set as follows: a box size of 60 for all coordinates centered at x = 9.176, y = 20.8, and z = 1.846. The ligand flexibility (*exhaustiveness*) was set to 32, and 9 models were generated. The model with the lowest docking score, as predicted by Autodock Vina, was selected as the most favored conformation. The PRODIGY server (Vangone *et al.*, 2019) was used to estimate the binding affinity of TYD, hypericin, and pseudohypericin with MPXV-TMPK. ChimeraX version 1.5 (Pettersen *et al.*, 2021) was used to illustrate the protein-ligand complex. The Ligand Interaction Diagram module of Maestro software determined the interaction of ligands with TMPK.

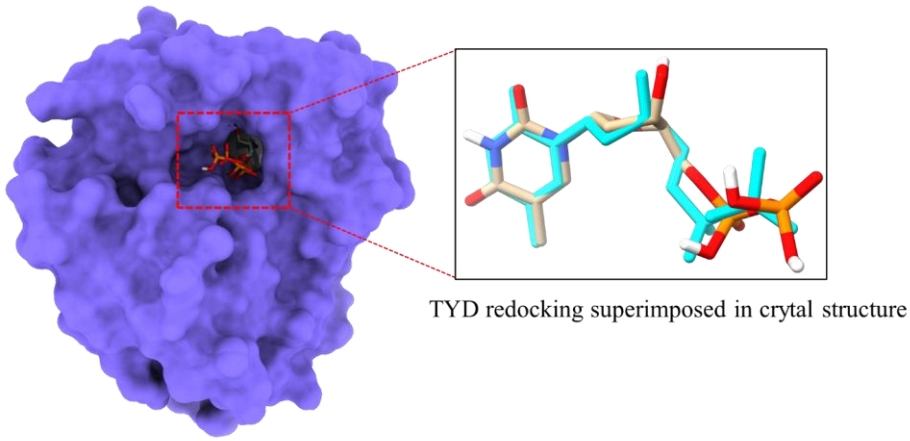

Figure 1. TYD is bound into the active site of MPXV-TMPK. TYD of crystal structure was colored in Cyan color and TYD redocked by Autodock Vina is present in Sand color.

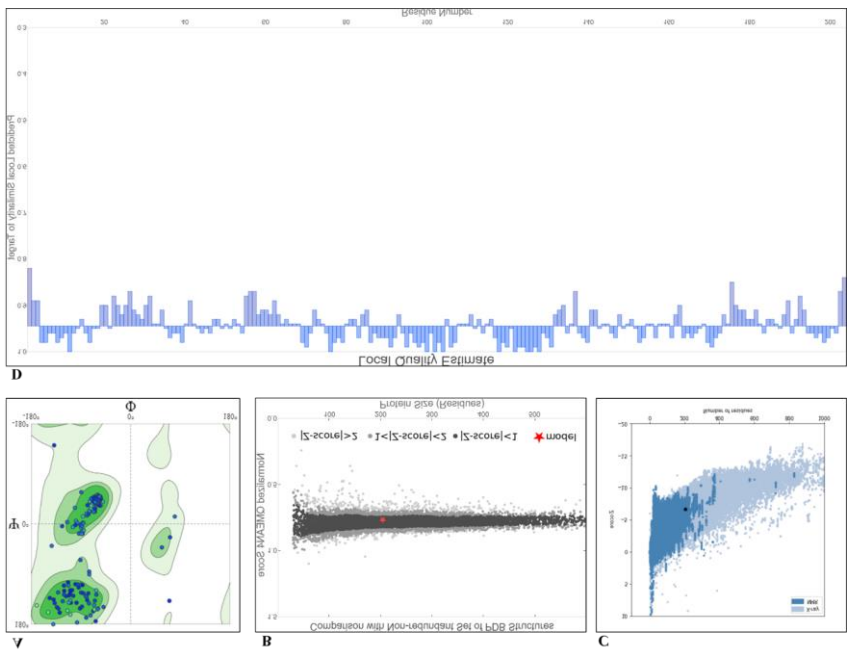

Figure 2. MPXV-TMPK validation parameters. **(A)** Ramachandran plot from SA server showing favored regions. The Z-score of MPXV-TMPK compared with similarly size protein by SA server **(B)** and **(C)** ProSA server. **(D)** Local Quality Estimate per residue of MPXV-TMPK by SA server.

RESULTS AND DISCUSSION

MPXV-TMPK protein model validation

The Ramachandran plot analysis from the Structure Assessment tool (Waterhouse *et al.*, 2018) revealed that MPXV-TMPK had 97.52% favored Ramachandran reagions (Figure 2A). The Overall Quality scores from ERRAT and SA servers were 97.4359 and 0.94 ± 0.06, respectively, indicating high-quality protein and validating the model's legitimacy. Structural predictions from SA and ProSA servers showed that MPXV-TMPK aligns with proteins of

similar sizes, with Z-scores < 1 (Figure 2B) and -6.68 (Figure 2C), respectively. The Predicted Local Quality Estimate from the SA server demonstrated that all TMPK residues scored above 0.8, confirming the absence of unusual amino acids in the structure. Therefore, the MPXV-TMPK model was considered reasonable and reliable as a target for hypericin and pseudohypericin.

In silico Prediction of Physicochemical and Pharmacokinetics Parameters

The primary objective of the physicochemical and pharmacokinetic investigation is to assess the drug-likeness of hypericin and pseudohypericin. Qikprop evaluation indicates that these compounds fall within accepted values (Table 1). Both compounds violated Lipinski's Rule of Five due to exceeding the molecular weight (MW) (> 500 g/mol) and PSA (> 140 Å²) values. However, approximately 50% of FDA-approved drugs are known to violate Lipinski's Rule or are not administered orally (Zackria *et al.*, 2022). It's worth noting that Lipinski's Rule has certain limitations and should be viewed as guidelines rather than strict rules (Zhang, Wilkinson, 2007).

Table 1. Predicted physicochemical parameters of hypericin and pseudohypericin[a].

	Hypericin	**Pseudohypericin**
MW (Accepted value: 130 – 725)	504.45	520.45
HBD (Accepted value: 0 – 6)	4	5
HBA (Accepted value: 2 – 20)	6.5	8.2
$QPlogP_{o/w}$ (Accepted value: −2 – 6.5)	2.13	1.169
PSA (Accepted value: 7 – 200)	164.58	186.61
Rule of five Lipinski (Violation) (Accepted value: 0 – 1)	2	2

[a]**MW:** molecular weight.; **HBA:** hydrogen-bond acceptor atoms.; **HBD:** hydrogen-bond donor atoms.; **QPlogPo/w:** predicted octanol/water partition coefficient; **PSA**: polar surface area.

The pharmacokinetics analysis predicted the properties of hypericin and pseudohypericin (Table 2). Generally, both hypericin and pseudohypericin exhibit good solubility in water (QplogS > -6.5). However, they showed low permeability across the gut–blood barrier (QPPCaco < 25), indicating inefficient oral absorption. Hypericin's %HOA fell within a medium range (> 25), while pseudohypericin had a low %HOA. These two compounds face challenges in absorption through the intestinal mucosa. Therefore, hypericin and pseudohypericin are not recommended for use as orally administered small-molecule drugs.

Hypericin exhibited a QplogBB value greater than -3, indicating it does not permeate the blood-brain barrier (BBB), whereas pseudohypericin reached -3.117, suggesting it barely permeates the BBB. Consequently, they are considered as impervious to the central nervous system (CNS) and are considered non-toxic to the CNS. Hypericin and pseudohypericin fall into a safe range for QPlogHERG (< -5), suggesting they do not pose cardiac toxicity. The pkCSM server reveals that these compounds did not induce hepatocellular toxicity or skin sensitization. In summary, based on Qikprop and pkCSM prediction, hypericin and pseudohypericin are considered promising candidates for small-molecule drug development.

Table 2. Predicted pharmacokinetic parameters of hypericin and pseudohypericin[b].

	Hypericin	**Pseudohypericin**
Volume (Accepted value: 500 – 2,000)	1250.906	1269.189
QplogS (Accepted value: −6.5 – 0.5)	-4.711	-4.012
QPlogHERG (Accepted value: < −5)	-5.039	-5.043
QPPCaco (Accepted value: < 25 poor, > 500 high)	13.123	5.201
QplogBB (Accepted value: −3 – 1.2)	-2.596	-3.117
%HOA (Accepted value: < 25 poor, > 80 high)	33.514	20.69
AMES toxicity	No	No
Hepatotoxicity	No	No
Skin Sensitisation	No	No

[b]**QPPCaco:** Caco-2 cell permeability in nm/s.; **QplogBB:** Brain/blood partition coefficient.; **QPlogHERG:** Predicted IC50 value for blockage of HERG K+ channels.; **QplogS:** Predicted aqueous solubility.; **% HOA:** Human oral absorption on 0 – 100% scale.

Molecular docking analysis

The residues of MPXV-TMPK involved in interactions with TYD have been identified in previous research, including D13, K14, S15, K17, T18, R41, F68, R72, and Y101 (Caillat *et al.*, 2008). Docking simulation results by Autodock Vina for hypericin and pseudohypericin are depicted in Figure 3. Notably, the docking positions of hypericin and pseudohypericin on MPXV-TMPK overlap with TYD (Figure 3), indicating that these two compounds can competitively bind to the active site of TMPK with TYD.

Hypericin exhibited the strongest binding affinity with MPXV-TMPK compared to TYD and pseudohypericin (Table 3). Protein-ligand interaction analysis reveals that hypericin and pseudohypericin bind to essential MPXV-TMPK residues located in the active site, including D13, K14, S15, K17, T18, R41, and Y101 (Table 3). These findings suggest that hypericin, in particular, binds more robustly to the active site of MPXV-TMPK, indicating potential inhibition of the target protein's activity. Based on these results, hypericin and pseudohypericin emerge as promising compounds for potential development in controlling Mpox.

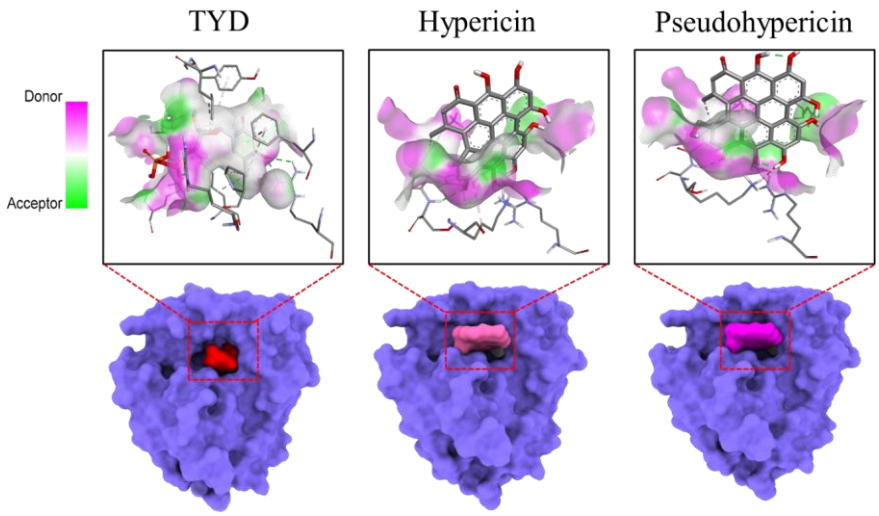

Figure 3. TYD, hypericin and pseudohypericin in complex with MPXV-TMPK.

Currently, Mpox remains under continuous monitoring by the WHO due to its approximately 6% mortality rate and the absence of an appropriate treatment protocol (according to the WHO). Thus, the ongoing exploration of potential ligands remains a top priority for future Mpox control. Previous research identified MPXV-TMPK as a target for controlling viral diseases through PDT (Pourhajibagher, Bahador, 2023). PDT involves the systemic or local administration of a non-toxic drug known as a photosensitizer (Karioti, Bilia, 2010). The photosensitizer tightly binds and accumulates in the target. Subsequently, the target is exposed to visible light, typically a red-light wavelength, in the presence of oxygen, resulting in the production of reactive oxygen species. This process damages the lipids, proteins, and nucleic acids of the virus without harming human tissue (Cieplik *et al.*, 2018; Namvar *et al.*, 2019; Vatansever *et al.*, 2013).

In this study, we successfully constructed a high-quality MPXV-TMPK model suitable for virtual screening. Hypericin and pseudohypericin, known for their antiviral activity and kinase inhibition properties (Sun *et al.*, 2023; Takahashi *et al.*, 1989; Xie *et al.*, 2021), were hypothesized as potential novel ligands for MPXV-TMPK inhibition. Molecular docking results showed stronger binding affinity of hypericin and pseudohypericin to the TMPK active site compared to TYD (Table 3). Property predictions indicated their suitability as small-molecular drug candidates (Table 1).

However, pharmacokinetic predictions suggest that despite their high solubility in water solvents, these compounds poorly absorb through the intestinal mucosa, limiting their efficacy for oral absorption (Table 2).

Table 3. Docking scores and binding affinity of TYD, hypericin, and pseudohypericin with MPXV-TMPK.

	Docking score (kcal.mol^{-1})	**Binding affinity (kcal.mol^{-1})**	**Residues interaction**
TYD	-8.139	-5.26	D13, K14, K17, T18, R41, F68, R72, S97, G98, Y101, A102.
Hypericin	-8.64	-5.42	L12, D13, K14, S15, G16, K17, T18, T19, N37, R41, L53, R92, R93, Y101, R137, E142,
Pseudohypericin	-8.283	-5.42	L12, D13, K14, S15, G16, K17, T18, T19, Q20, N37, R41, L53, D92, R93, R137, E142.

Decades ago, hypericin and pseudohypericin were used as photosensitizers in PDT to treat viral diseases and diagnose cancer (Karioti, Bilia, 2010). Although pseudohypericin shares structural similarities with hypericin, it is less effective and consequently less widespread (Karioti, Bilia, 2010). The mechanism by which hypericin permeates the mucosa remains unclear. However, substantial evidence suggests that hypericin accumulates in the cytoplasm (Agostinis *et al.*, 2002; Galanou *et al.*, 2008; Mikeš *et al.*, 2011). In the life cycle of the Poxviridae family, virion morphogenesis occurs in the cytoplasm (Prichard, Kern, 2012), allowing hypericin to tightly bind to the active site of MPXV-TMPK. Considering the crucial role of protein kinases in early morphogenesis and the regulation of the MPXV life cycle (Mercer, Traktman, 2005; Traktman *et al.*, 1995; Wang, Shuman, 1995), hypericin is highly suitable for controlling Mpox.

Our study is the first to investigate the bioavailability of hypericin in Mpox. Hypericinhas been successfully used in PDT in numerous clinical trials (Kacerovská *et al.*, 2008; Rook *et al.*, 2010) and has demonstrated safety in toxicity research (de Souza *et al.*, 2022), prompting high expectations for its efficacy against Mpox. As a characteristic compound of the *Hypericum* genus, hypericin is found in over 450 species distributed worldwide (Karioti, Bilia, 2010). This widespread availability of medicinal resources across various countries presents a promising opportunity for preventing Mpox outbreaks. Our *in silico* research contributes to identifying compounds from the *Hypericum* genus, laying the foundation for developing novel drug candidates to control Mpox.

CONCLUSION

Our research has successfully constructed a high-quality and applicable MPXV-TMPK protein model for future investigations. *In silico* predictions indicated that hypericin possesses suitable physicochemical and pharmacokinetic properties for development as a drug-like agent. Molecular docking results further demonstrate that hypericin and pseudohypericin exhibit a higher affinity for MPXV-TMPK than its natural ligand,

TYD. Therefore, hypericin emerges as a promising compound capable of inhibiting MPXV-TMPK and controlling Mpox.

ABBREVIATIONS

Mpox: Monkeypox desease.

MPXV: Monkeypox virus.

TMPK: Thymidylate kinase.

TYD: Thymidine 5'-diphosphate.

PDD: Photodynamic diagnosis.

PDT: Photodynamic therapy.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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REFERENCES

Agostinis P, Vantieghem A, Merlevede W, de Witte PAM (2002) Hypericin in cancer treatment: more light on the way. *Int J Biochem Cell Biol* 34: 221-241. https://doi.org/10.1016/S1357-2725(01)00126-1.

Alkhalil A, Strand S, Mucker E, Huggins JW, Jahrling PB, Ibrahim SM (2009) Inhibition of monkeypox virus replication by RNA interference. *Virol J* 6: 188. https://doi.org/10.1186/1743-422X-6-188.

Barnes J, Anderson LA, Phillipson JD (2001) St John's wort (*Hypericum perforatum L.*): a review of its chemistry, pharmacology and clinical properties. *J Pharm Pharmacol* 53: 583-600. https://doi.org/10.1211/0022357011775910.

Breman JG, Kalisa R, Steniowski MV, Zanotto E, Gromyko AI, Arita I (1980) Human monkeypox, 1970-79. *Bull World Health Organ* 58: 165-182.

Caillat C, Topalis D, Agrofoglio LA, Pochet S, Balzarini J, Deville-Bonne D, Meyer P (2008) Crystal structure of poxvirus thymidylate kinase: An unexpected dimerization has implications for antiviral therapy. *Proc Natl Acad Sci USA* 105: 16900-16905. https://doi.org/10.1073/pnas.0804525105.

Cieplik F, Deng D, Crielaard W, Buchalla W, Hellwig E, Al-Ahmad A, Maisch T (2018) Antimicrobial photodynamic therapy - what we know and what we don't. *Crit Rev Microbiol* 44: 571-589. https://doi.org/10.1080/1040841X.2018.1467876.

Colovos C, Yeates TO (1993) Verification of protein structures: patterns of nonbonded atomic interactions. *Protein Sci* 2: 1511-1519. https://doi.org/10.1002/pro.5560020916.

de Souza LM, de Sousa FD, Cruz RCR, Tavares DC, Francielli de Oliveira P (2022) Hypericin, a medicinal compound from St. John's Wort, inhibits genotoxicity induced by mutagenic agents in V79 cells. *Drug Chem Toxicol* 45: 1302-1307. https://doi.org/10.1080/01480545.2020.1822389.

Eberhardt J, Santos-Martins D, Tillack AF, Forli S (2021) AutoDock Vina 1.2.0: New Docking Methods, Expanded Force Field, and Python Bindings. *J Chem Inf Model* 61: 3891-3898. https://doi.org/10.1021/acs.jcim.1c00203.

Galanou MC, Theodossiou TA, Tsiourvas D, Sideratou Z, Paleos CM (2008) Interactive Transport, Subcellular Relocation and Enhanced Phototoxicity of Hypericin Encapsulated in Guanidinylated Liposomes via Molecular Recognition. *Photochem Photobiol* 84: 1073-1083. https://doi.org/10.1111/j.1751-1097.2008.00392.x.

Hongyan Z, Puhua Z, Gang PJNPR, Development (2002) Studies on chemical constituents of Hypericum sampsonii. *Nat Prod Res Dev* 14: 50-53.

Jendželovská Z, Jendželovský R, Kuchárová B, Fedoročko P (2016) Hypericin in the Light and in the Dark: Two Sides of the Same Coin. *Front Plant Sci* 7: 560. https://doi.org/10.3389/fpls.2016.00560.

Kacerovská D, Pizinger K, Majer F, Šmíd F (2008) Photodynamic Therapy of Nonmelanoma Skin Cancer with Topical Hypericum perforatum Extract-A Pilot Study. *Photochem Photobiol* 84: 779-785. https://doi.org/10.1111/j.1751-1097.2007.00260.x.

Karioti A, Bilia AR (2010) Hypericins as potential leads for new therapeutics. *Int J Mol Sci* 11: 562-594. https://doi.org/10.3390/ijms11020562.

Kumari S, Chakraborty S, Ahmad M, Kumar V, Tailor PB, Biswal BK (2023) Identification of probable inhibitors for the DNA polymerase of the Monkeypox virus through the virtual screening approach. *Int J Biol Macromol* 229: 515-528. https://doi.org/10.1016/j.ijbiomac.2022.12.252.

Lu C, Wu C, Ghoreishi D, Chen W, Wang L, Damm W, Ross GA, Dahlgren MK, Russell E, Von Bargen CD, Abel R, Friesner RA, Harder ED (2021) OPLS4: Improving Force Field Accuracy on Challenging Regimes of Chemical Space. *J Chem Theory Comput* 17: 4291-4300. https://doi.org/10.1021/acs.jctc.1c00302.

Mercer J, Traktman P (2005) Genetic and cell biological characterization of the vaccinia virus A30 and G7 phosphoproteins. *J Virol* 79: 7146-7161. https://doi.org/10.1128/JVI.79.11.7146-7161.2005.

Mikeš J, Hýžďalová M, Kočí L, Jendželovský R, Kovaľ J, Vaculová A, Hofmanová J, Kozubík A, Fedoročko P (2011) Lower sensitivity of FHC fetal colon epithelial cells to photodynamic therapy compared to HT-29 colon adenocarcinoma cells despite higher intracellular accumulation of hypericin. *Photochem Photobiol Sci* 10: 626-632. https://doi.org/10.1039/c0pp00359j.

Namvar MA, Vahedi M, Abdolsamadi HR, Mirzaei A, Mohammadi Y, Azizi Jalilian F (2019) Effect of photodynamic therapy by 810 and 940 nm diode laser on Herpes Simplex Virus 1: An in vitro study. *Photodiagnosis Photodyn Ther* 25: 87-91. https://doi.org/10.1016/j.pdpdt.2018.11.011.

Pettersen EF, Goddard TD, Huang CC, Meng EC, Couch GS, Croll TI, Morris JH, Ferrin TE (2021) UCSF ChimeraX: Structure visualization for researchers, educators, and developers. *Protein Sci* 30: 70-82. https://doi.org/10.1002/pro.3943.

Pires DEV, Blundell TL, Ascher DB (2015) pkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures. *J Med Chem* 58: 4066-4072. https://doi.org/10.1021/acs.jmedchem.5b00104.

Pourhajibagher M, Bahador A (2023) Virtual screening and computational simulation analysis of antimicrobial photodynamic therapy using propolis-benzofuran A to control of Monkeypox. *Photodiagnosis Photodyn Ther* 41: 103208. https://doi.org/10.1016/j.pdpdt.2022.103208.

Prichard MN, Kern ER (2012) Orthopoxvirus targets for the development of new antiviral agents. *Antiviral Res* 94: 111-125. https://doi.org/10.1016/j.antiviral.2012.02.012.

Riopelle JC, Munster VJ, Port JR (2022) Atypical and Unique Transmission of Monkeypox Virus during the 2022 Outbreak: An Overview of the Current State of Knowledge. *Viruses*. 14: 2012. https://doi.org/10.3390/v14092012.

Rook AH, Wood GS, Duvic M, Vonderheid EC, Tobia A, Cabana B (2010) A phase II placebo-controlled study of photodynamic therapy with topical hypericin and visible light irradiation in the treatment of cutaneous T-cell lymphoma and

psoriasis. *J Am Acad Dermatol* 63: 984-990. https://doi.org/10.1016/j.jaad.2010.02.039.

Schnierle BS (2022) Monkeypox Goes North: Ongoing Worldwide Monkeypox Infections in Humans. *Viruses* 14: 1874. https://doi.org/10.3390/v14091874.

Sun Z, Li Y, Zhong R, Li R (2023) Hypericum sampsonii Hance: a review of its botany, traditional uses, phytochemistry, biological activity, and safety. *Front Pharmacol* 14: 1247675. https://doi.org/10.3389/fphar.2023.1247675.

Takahashi I, Nakanishi S, Kobayashi E, Nakano H, Suzuki K, Tamaoki T (1989) Hypericin and pseudohypericin specifically inhibit protein kinase C: possible relation to their antiretroviral activity. *Biochem Biophys Res Commun* 165: 1207-1212. https://doi.org/10.1016/0006-291X(89)92730-7.

Traktman P, Caligiuri A, Jesty SA, Liu K, Sankar U (1995) Temperature-sensitive mutants with lesions in the vaccinia virus F10 kinase undergo arrest at the earliest stage of virion morphogenesis. *J Virol* 69: 6581-6587. https://doi.org/10.1128/jvi.69.10.6581-6587.1995.

Vangone A, Schaarschmidt J, Koukos P, Geng C, Citro N, Trellet ME, Xue LC, Bonvin AMJJ (2019) Large-scale prediction of binding affinity in protein-small ligand complexes: the PRODIGY-LIG web server. *Bioinformatics* 35: 1585-1587. https://doi.org/10.1093/bioinformatics/bty816.

Vatansever F, de Melo WC, Avci P, Vecchio D, Sadasivam M, Gupta A, Chandran R, Karimi M, Parizotto NA, Yin R, Tegos GP, Hamblin MR (2013) Antimicrobial strategies centered around reactive oxygen species--bactericidal antibiotics, photodynamic therapy, and beyond. *FEMS Microbiol Rev* 37: 955-989. https://doi.org/10.1111/1574-6976.12026.

Wang S, Shuman S (1995) Vaccinia virus morphogenesis is blocked by temperature-sensitive mutations in the F10 gene, which encodes protein kinase 2. *J Virol* 69: 6376-6388. https://doi.org/10.1128/jvi.69.10.6376-6388.1995.

Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, Heer FT, de Beer TAP, Rempfer C, Bordoli L, Lepore R, Schwede T (2018) SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res* 46: w296-w303. https://doi.org/10.1093/nar/gky427.

Wiederstein M, Sippl MJ (2007) ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. *Nucleic Acids Res.* 35: W407-410. https://doi.org/10.1093/nar/gkm290.

Xie M, Guo Y, Li F, Chen H, Lai X, Sui J, Jiang LJACMP (2021) Research progress on chemical components and pharmacological effects of Hypericum sampsonii and analysis and prediction of its quality markers. *Acta Chin Med Pharmacol*. 49: 40-44.

Yuan X, Yan F, Gao L-H, Ma Q-H, Wang J (2023) Hypericin as a potential drug for treating Alzheimer's disease and type 2 diabetes with a view to drug repositioning. *CNS Neurosci Ther* 29: 3307-3321. https://doi.org/10.1111/cns.14260.

Zackria AA, Pattabiraman R, Murthy TPK, Kumar SB, Mathew BB, Biju VG (2022) Computational screening of natural compounds from Salvia plebeia R. Br. for inhibition of SARS-CoV-2 main protease. *Vegetos* 35: 345-359. https://doi.org/10.1007/s42535-021-00304-z.

Zhang J, Gao L, Hu J, Wang C, Hagedoorn P-L, Li N, Zhou XJS, (2022) Hypericin: source, determination, separation, and properties. *Sep Purif Rev* 51: 1-10. https://doi.org/10.1080/15422119.2020.1797792.

Zhang MQ, Wilkinson B (2007) Drug discovery beyond the 'rule-of-five'. *Curr Opin Biotechnol* 18: 478-488. https://doi.org/10.1016/j.copbio.2007.10.005.