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Original Article

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In silico studies concerning the cytotoxic potential and the inhibition of cytochrome P450 of some bioactive compounds present in *Asphodeline lutea* root extracts

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Abstract

Background and Aims: The roots of *Asphodeline lutea* are traditionally consumed in the diet of people from Mediterranean countries. The methanol root extracts of *A. lutea* have been proven to possess cytotoxic activity against the MCF-7 and MCF-10A cell lines. The goal of our investigation was to determine the physicochemical and pharmacokinetic properties, possible inhibition against CYP450 isoenzymes, and probable cytotoxic effect of some bioactive components isolated from *A. lutea* roots using *in silico* methods.

Methods: The Absorption, distribution, metabolism, and excretion (ADME) profiles were determined using the freely available SwissADME server. To increase the robustness of the SwissADME results, further calculations with the QikProp module in Maestro were carried out. The docking studies were carried out with Glide. The Induced-fit docking (IFD) and Molecular Mechanics-Generalized Born Surface Area (MM/GBSA) modules in Maestro were applied for recalculations. The anticancer activities were predicted by the online server CLC-Pred.

Results: The *in silico* ADME studies identified chrysophanol and helminthosporin as suitable for future evaluations considering their optimal pharmacokinetic profiles. The former compounds adhere to all of Lipinski's rule of five for drug likeness. The docking studies identified helminthosporin as a potential inhibitor of CYP1A2 and a weak CYP3A4 inhibitor. Chrysophanol, helminthosporin, asphodelin and 10, 7'-bichrysophanol are expected to manifest a strong cytotoxic effect against A2780cisR cell line. They exerted a strong to modest cytotoxic effect against HOP-18, RCC4 and M19-MEL cell lines. 1,5,8-trihydroxy-3-methylanthraquinone exhibited modest action against GIST430 cell line, while 10, 7'-bichrysophanol had moderate activity against A2780 and SW1990 cell lines.

Conclusion: Our findings justify the future *in vitro* and *in vivo* studies of *A. lutea* plant extracts as potential anticancer agents.

Keywords Asphodeline lutea · Cytotoxicity · In silico · Molecular docking

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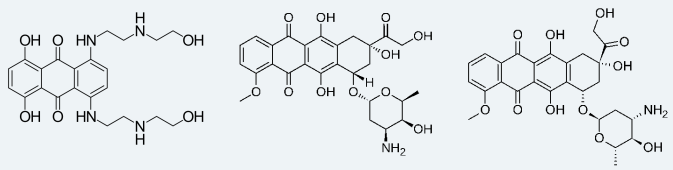
INTRODUCTION

Natural products are valuable sources for new medicines and prospective lead compounds for further modification in drug development. The important contribution of natural products in drug discovery due to their diverse structures and the intricate carbon skeletons. Since natural secondary metabolites have been elaborated within living systems, they often showed more "drug-likeness and biological friendliness than totally synthetic molecules" (Lahlou, 2013). Natural products and their structural analogs have historically been a major input to pharmacotherapy, especially for cancer and infectious diseases (Atanasov et al., 2021).

The Asphodeline lutea Rchb. (Asphodelaceae) plant is widespread in southeastern Europe, northern Africa, the Caucasus and the Levant compared to the most other endemic to Turkey *Asphodeline* species. *Asphodeline lutea* is from the same family as the *Aloe vera* plant, which is well known for its valuable ethnomedicinal applications. It has been proven in pre-clinical and clinical investigations that *Aloe vera* exhibits anticancer activities because of some lead bioactive components such as anthraquinones, polysaccharides, and chromones (Majumder et al., 2019). The roots, shoots, and flowers of *A. lutea* are traditionally consumed in the Mediterranean diet. The methanol root extracts of *A. lutea* have been proven to possess cytotoxic activity against the MCF-7 and MCF-10A cell lines (Lazarova et al., 2015). They are a rich source of phenolic compounds, especially 1,8-dihydroxyanthraquinones based on chrysophanol units (Lazarova et al., 2014; Lazarova et al., 2015). Our previous investigation led to the isolation of chrysophanol, helminthosporin, asphodelin and 10,7'-bichrysophanol as the major anthraquinones present in *the A. lutea* methanolic root extract (Todorova et al., 2010). Anthraquinones are privileged chemical scaffolds that have been applied in various therapeutics, including anticancer agents. There are various marketed anticancer drugs such as mitoxantrone, doxorubicin, and epirubicin with anthraquinone-based rings (Tikhomirov et al., 2018) (Table 1). Unfortunately, cancer cells can acquire multidrug resistance to conventional anticancer drugs. The search for new prospective anticancer agents is the main goal of the modern science of this field. Natural plant products provide the main source of novel, more effective and less toxic anticancer drugs (Tilaoui et al., 2021).

The emergence of drug resistance defines the development of new anticancer agents. The overall conformation of the anthraquinone ring system has been investigated extensively with respect to its anticancer effects. Importantly, the planarity of the former structure contributes to the embedment of some anthroquinones in the DNA double helix, thus acting as DNA intercalators. Thus, the scientific interest in the design/synthesis or isolation of novel anthraquinones with potential anticancer effects is increasing, considering the need for novel anticarcinogenic therapeutic options (Malik et al., 2021).

Table 1. Chemical structures of anthraquinones with anticancer properties.



Mitoxantrone Doxorubicin Epirubicin

A recent report demonstrated that more than 90% of the drug failures in the drug development phases were connected to not ideal pharmacokinetic profiles, which includes a lack of pharmacological effect, enhanced toxicity, and poor drug-like properties (Sun et al., 2022). Importantly, the absorption, distribution, metabolism, and excretion (ADME) of the drugs are associated with around 40% of the drug failure that leads to an unproductive investment of resources both financial and productive (Durán-Iturbide et al., 2020). Thus, early evaluation of the ADME profiles of the hit/lead molecules is essential in the drug discovery process.

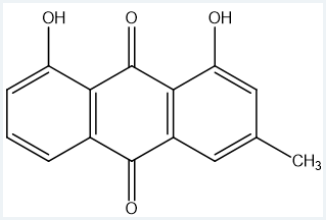
Based on research on anthraquinones as a suitable source for drug candidates and the requirement for a comprehensive *in silico* ADME profile, the present study aimed to determine the physicochemical and pharmacokinetic properties and probable cytotoxic effects of chrysophanol, helminthosporin, asphodelin and 10,7'-bichrysophanol isolated from *A. lutea* roots using *in silico* methods (Table 2).

MATERIAL AND METHODS

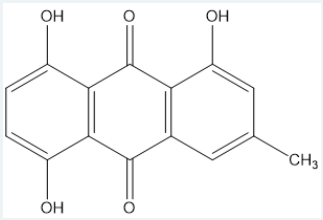
In silico ADME Studies

TheSwissADME freely available web-application was used to determine the ADME profiles of the anthraquinones chrysophanol, helminthosporin, asphodelin and 10,7'-bichrysophanol through fast yet robust predictive models for physicochemical properties, pharmacokinetics, druglikeness and medicinal chemistry friendliness. A physics-based model for lipophilicity (iLOGP) and an intuitive graphical classification model for gastrointestinal absorption and brain access (BOILED-Egg) were applied (Daina et al., 2017). To increase the robustness of the SwissADME results, further calculations with the QikProp module in Schrödinger (Schrödinger Release 2024-1: QikProp, Schrödinger, LLC, New York, NY, 2024.) were carried out.

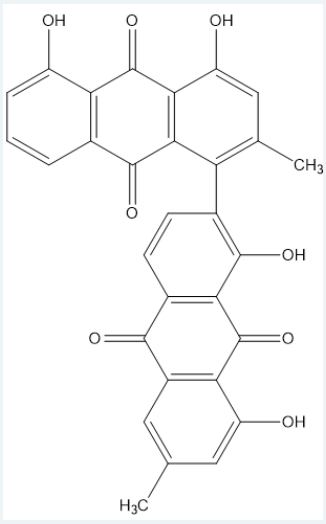
Table 2. Chemical structures of chrysophanol, helminthosporin, asphodelin, and 10,7′-bichrysophanol isolated from *A. lutea* roots.



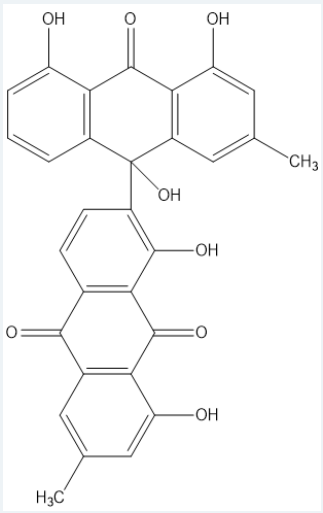
Chrysophanol



Helminthosporin



Asphodelin



10,7'-bichrysophanol

Molecular Docking Studies

Preparation of the Crystal Structures

The crystal structures of the three CYP isoforms were retrieved from the protein data bank (PDB): CYP1A2 (2HI4) (Marechal et al., 2006), CYP2C9 (5W0C) (Liu et al., 2017), and CYP3A4 (2V0M) (Ekroos & Sjögren, 2006). In each X-ray structure, a co-crystallised ligand was situated, which was applied for generating the grid space. For the preparation of the crystal structures, a module in Maestro was used - "Protein Preparation". Applying the former hydrogen bonds were generated, water molecules that were not situated in the active sites of the CYP isoforms were removed, and the states were fixed at physiological pH. The final protein structures were minimised by an Optimized Potentials for Liquid Simulations 4 (OPLS4) force field.

Preparation of the Structures

The structures of the examined anthraquinones were converted to the corresponding 3D structures with Ligprep (Schrödinger Release 2024-1: LigPrep, Schrödinger, LLC, New York, NY, 2024.). Applying Ligprep hydrogen bonds were added and ionisation states were generated at physiological pH. Furthermore, the geometric structures of the derivatives were optimised using OPLS4 force field.

Docking Simulations

Glide was used for the docking calculations. To increase the robustness of the *in silico* calculations, we applied the most precise docking function of Glide-XP docking. Furthermore, the obtained docking poses were explored with the Induced-fit docking (IFD) mode. The IFD examines the protein's side chains as fully flexible, which leads to exhaustive sampling. Finally, Molecular Mechanics-Generalized Born Surface Area (MM/GBSA) calculations were included to increase the reliability of the *in silico* calculations and to determine the binding free energies of the complexes.

Cytotoxicity Studies

CLC-Pred (Cell Line Cytotoxicity Predictor) online server was used for *the in silico* prediction of the cytotoxic action of anthraquinones under investigation in non-transformed and cancer cell lines. CLC-Pred predicts the cytotoxicity of a chemical substance to evaluate the appropriateness of the chemical compounds' inclusion in further *in vitro* and *in vivo* experiments. Analysis was performed using Prediction of Activity Spectra for Substances (PASS) technology and the training set received on the basis of information for cytotoxic properties retrieved from ChEMBLdb. Simplified molecular-input line-entry system (SMILES) were used for inputting the structure of the investigated compounds. The BC CLC-Pred web tool was used to predict IC_{50} and IG_{50} values quantitatively and qualitatively for nine breast cancer cell lines (Lagunin et al., 2018).

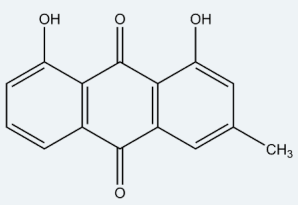
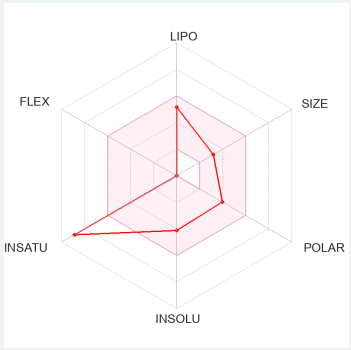
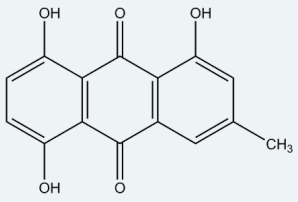
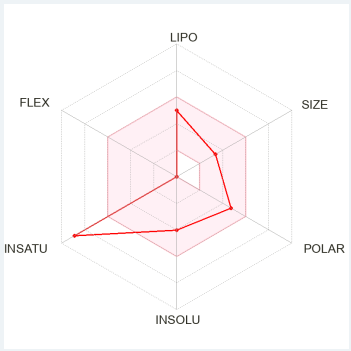
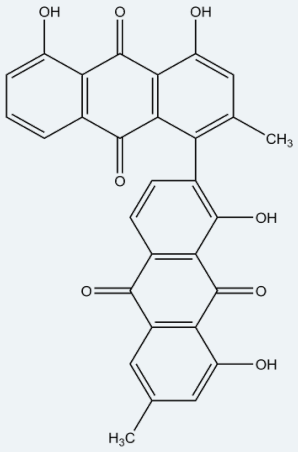
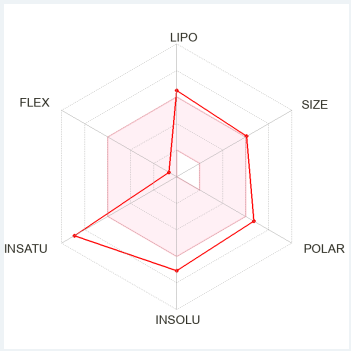
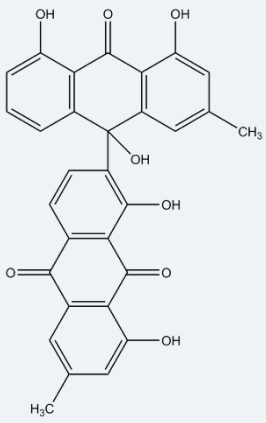
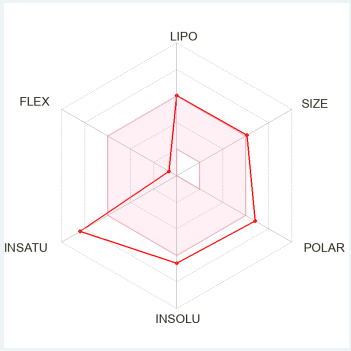
RESULTS AND DISCUSSION

In silico ADME Studies

The key parameters that describe the pharmacokinetic behaviour of a drug are the log of the octanol/water partition coefficient (logP), the negative log of the acid dissociation constant (pKa), human intestinal permeability (measured using Caco-2 cells), Blood–Brain Barrier (BBB) penetration, Cytochrome P450 inhibition, oral acute toxicity, and the ability to induce cancer (Dulsat et al., 2023). Thus, the aforementioned properties of the four anthraquinones (Table 3) were examined through *in silico* evaluations.

The Lipinski's rules of five state that, substances that are drug candidates should correspond to the following parameters: molecular weight less than 500 dl, no more than 5 hydrogen bond donors, and no more than 10 hydrogen bond acceptors. Moreover, the log P should be in range of −0.4 to +5.6. Chrysophanol and helminthosporin corre-

Table 3. Chemical structures and bioavailability radar of the examined anthraquinones.

 **Chrysophanol**		
 **Helminthosporin**		
 **Asphodelin**		
 **10, 7′-bichrysophanol**		

sponded to Lipinski's rules of five for druglikeness, while asphodeline and 10,7'-bichrysophanol adhered to Lipinski's rule of five with one violation. The molecular weights of the two compounds were above 500 Da (506.46 g/mol for asphodeline and 508.48 for 10,7'-bichrysophanol). The physicochemical properties of the anthraquinones chrysophanol and helminthosporin on one hand and bianthraquinones asphodeline and 10,7'-bichrysophanol on the other hand were very similar. The bianthraquinones were more polar than the anthraquinones. Chrysophanol and helminthosporin were moderately soluble in water, whereas asphodeline and 10,7'-bichrysophanol possessed poor solubility in water. Generally, the hydrofobic properties of all compounds were confirmed by positive values of Log P. The presence of –OH groups in all the discussed compounds is a prerequisite for forming hydrogen bonds with water molecules, but does not guarantee that they will dissolve in water. The nonpolar anthracene cores and the attached methyl groups interfere with the water molecules' ability to form hydrogen bonds among themselves. Chrysophanol and helminthosporin possess one anthracene unit and one methyl group, whereas asphodelin and 10,7'-bichrysophanol have two anthracene units and two methyl groups. Therefore, the larger the nonpolar part of the molecules, the less soluble the compound is in water. The four compounds had the same insaturation data. The definition of the topological polar surface area (TPSA) of a molecule is the sum over all polar atoms or molecules, mainly O and N, and their attached hydrogen atoms. The TPSA values of asphodelin and 10,7'-bichrysophanol were higher than those of chrysophanol and helminthosporin because the bianthraquinones contained more hydroxyl and keto groups. The TPSA values of asphodelin and 10,7'-bichrysophanol were found to be 149.20 and 152.36, respectively, using SwissADME. Molecules with a polar surface area of greater than 140 Å² tend to be poor at permeating cell membranes (Pajouhesh & Lenz, 2005) (Table 4). The results showed that all the studied molecules could not cross the BBB except for chrysophanol. The gastrointestinal absorption of chrysophanol and helminthosporium was high, while it was low for bianthraquinones.

An additional module incorporated in Maestro - QikProp was used to confirm the data from SwissADME (Table 5). The observed results demonstrated that most of the calculated results with SwissADME correspond to the data from QikProp.

Interactions with the CYP450 Isoforms

The CYP450 enzymes (57 isoforms) participate in the metabolism of various medications; therefore, the interactions of the drug-candidates with the former isoforms could prevent unwanted drug-drug interactions (Sansen et al., 2007). The

Table 4. Physicochemical properties of the test compounds calculated by SwissADME

	MW (g/mol)	Log (S)	Num-rotatable bonds	Num -H bond acceptors	Num -H bond donors	TPSA (A^2)	Consensus Log P
Chrysophanol	254.24	· 4.11	0	4	2	74.60	2.38
Helminthosporin	270.24	–4.02	0	5	3	94.83	2.06
Asphodeline	506.46	–7.07	1	8	4	149.20	4.06
10,7'-bichrysophanol	508.48	–6.59	1	8	5	152.36	3.70

MW - Molecular weight
TPSA-Topological polar surface area
logS - log (solubility measured in mol/l)
log P - partition coefficient

Table 5. Physicochemical properties calculated by QikProp (Schrodinger)

	MW (g/mol)	Log (S)	Num-rotatable bonds	Num -H bond acceptors	Num -H bond donors	TPSA (A^2)	QPlogP
Chrysophanol	254.242	–2.860	0	4	2	83.452	1.781
Helminthosporin	270.241	–2.996	0	5	3	111.905	1.736
Asphodeline	506.467	–6.038	1	8	4	158.509	3.319
10,7'-bichrysophanol	508.483	–6.97	1	8	5	172.712	3.981

MW - Molecular weight
TPSA-Topological polar surface area
logS - log (solubility measured in mol/l)
log P - partition coefficient

problems related to the inhibition of cytochrome P450 are of great interest because the plasma levels of subsequently taken pharmacologically active compounds could drastically increase. Unwanted interactions of CYP450 inhibitors with medicines used as anticancer agents have been reported (Kivistö et al., 1995), which highlights the need for initial *in silico* and *in vitro* testing. Nowadays, the robustness of the *in silico* studies for identifying if a drug is a CYP450 inhibitor or a substrate has increased considering the introduction of feasible hardware resources. Thus, the SwissADME and molecular docking calculations were applied.

The SwissADME calculations demonstrated that chrysophanol and helminthosporin have probable CYP1A2 and CYP3A4 inhibitor activity. Helminthosporin, asphodeline and 10,7'-bichrysophanol expressed the possible inhibition of the CYP2C9 enzyme. To further explore the interactions between the titled anthraquinone derivatives and the main CYP450 isoforms, molecular docking was performed. The molecular docking calculations were accomplished with the licenced software Glide. The latter program provides good correlation with the experimental results in similar studies (Ridhwan et al., 2022). However, various conformations of the active sites of the CYP450 isoforms have been previously described (Yim et al., 2020), which leads to the addition calculations with fully flexible active amino acids. Nearly 90% success rate after the implementation of the Induced fit docking (IFD) against various CYP isoforms was observed (Ridhwan et al., 2022; Kim et al., 2010), which determined the utilisation of IFD simulations in the current work. The docking protocols were validated in our previous study through redocking (Angelov et al., 2022). After conducting self-docking simulations of all co-crystallised ligands it was found that the root mean square deviation (RMSD) values were under 1 Å which validated the docking protocols of CYP1A2 (PDB:2HI4), CYP2C9 (PDB:5W0C) and CYP3A4 (PDB:2V0M). Subsequent docking calculations of the title compounds in the active sites of the aforementioned enzymes were carried out. The results are provided in Table 6.

The calculated IFD and MM/GBSA scores of helminthosporin were comparable to the results of the applied standard CYP1A2 inhibitor**,** Alpha-naphthoflavone.

In the active site of CYP1A2 (PDB: 2HI4), two ligands (chrysophanol and helminthosporin) showed IFD scores of 8.935 and 9.356 kcal/mol, respectively. The scores obtained with the IFD mode provide more robust results considering the ability of the former module to examine the active amino residues as fully flexible during the calculations. Moreover, the scores obtained after the use of the induced flexible docking were recalculated by applying MM/GBSA. The enhanced hardware efficiency, with the improved force field algorithms, allows the introduction of binding free energy calculations through the MM/GBSA method. The former method has been included in many papers regarding drug optimisation and drug design

Table 6. IFD and MM/GBSA scores of 11b in various CYP450 isoforms

Compound	CYP1A2 (PDB: **2HI4**)		CYP2C9 (PDB: **5W0C**)		CYP3A4 (PDB: **2V0M**)	
	IFD (kcal/mol)	MM/GBSA (kcal/mol)	IFD (kcal/mol)	MM/GBSA (kcal/mol)	IFD (kcal/mol)	MM/GBSA (kcal/mol)
Chrysophanol	−8.935	−55.31	−6.131	−29.43	−7.783	−24.57
Helminthosporin	−9.356	−81.27	−7.588	−31.86	−7.681	−47.21
Asphodeline	n/a	n/a	n/a	n/a	−6.697	−32.82
10,7'-bichrysophanol	n/a	n/a	−7.189	−27.14	n/a	n/a
Alpha-naphthoflavone	−11.241	−89.435	-	-	-	-
*9W6	-	-	−9.02	−58.36	-	-
Ketoconazole	-	-	-	-	−12.194	−53.54

n/a – not available (the docking algorithm did not return any score, which corresponds to the unavailability of the ligand to fit into the grid space)
*9W6 - Ethyl {2-[([1,3]thiazolo[4,5-C]pyridine-2-Carbonyl)amino]thiophene-3-Carbonyl}carbamate
IFD - Induced Fit Docking scores
MM/GBSA - Molecular Mechanics-Generalized Born Surface Area

(Wang et al. 2019). The binding free energy of helminthosporin, recalculated with the MM/GBSA, was comparable to the co-crystallised compound. Thus, helminthosporin could be theoretically accessed as a good CYP1A2 inhibitor. Notably, the MM/GBSA energies should be prioritised since good correlation between *the in vitro* experimental and *in silico* MM/GBSA data was detected by our research group (Angelov et al., 2022).

The IFD in CYP2C9 showed that three ligands—chrysophanol, helminthosporin and asphodeline—have good binding affinities; however, a rescoring with the reliable MM/GBSA, displayed low values of the complexes. Thus, low inhibition towards CYP2C9 is expected. In CYP3A4 (PDB: 2V0M), helminthosporin demonstrated the formation of the most stable interactions with the active amino acids of the protein. The former ligand showed an MM/GBSA value of −47.21 kcal/mol, which is close to the results of the applied active CYP3A4 inhibitor, ketoconazole. Overall, helminthosporin is a theoretical CYP1A2 and CYP3A4 inhibitor that should be validated by *in vitro* experiments.

For further examination of the intermolecular interactions between the best-scored inhibitor helminthosporin and the active sites of CYP1A2 and CYP3A4, 2D and 3D visualisation and analysis were carried out (Figure 1).

The optimal distance between the haem ion and the docked compound is 6 Å (Bonomo et al., 2017). Using the former criteria, the localisation of helminthosporin was in close vicinity to the hem group (5.69Å) and thus the docking results were with higher robustness. Interestingly, in the active site of CYP3A4, helminthosporin was situated at a distance of 9.40Å from the iron of the het fragment, which determines its suboptimal conformation and low chance of inhibiting the former enzyme. The visualised interactions of CYP3A4 with helminthosporin demonstrated that the main stabilising bonds are hydrogen. ALA370 and ARG105 interacted with the two hydroxyl moieties of helminthosporin. A hydrophobic interaction with ALA370 was observed in a recent study by our research group when choline was placed in the active site of CYP3A4 (Mateev et al., 2022). Moreover, several active amino acids were involved in the hydrophobic interactions with the examined molecule. For instance, PHE57, PRO107, PHR108, MET371, and LEU492 formed the hydrophobic pocket in which helminthosporin was situated.

In CYP1A2, several amino acids were involved in the stabilisation of the ligand-protein complex. ASP320 participated in the formation of a hydrogen bond (1.85Å) with a hydroxyl group from the helminthosporin. A recent study showed that ASP320 is involved in the stabilisation of the CYP1A2 enzyme-ligand complex (Mateev et al., 2022). A water-mediated hydrogen bond (2.35Å) was also created between a carbonyl group and GLY316, which underlines the importance of including the active water molecules in the docking simulations in CYP1A2. PHE226 participated in stabilisation (4.00Å) with one of the aromatic rings of the helminthosporin. Interestingly, several polar interactions with the active amino residues THR118, SER122, THR124, THR223, ASN257, ASN312, THR321 and THR498 were observed. Moreover, helminthosporin was involved in hydrophobic interactions with ILE117, PHE125, VAL227, PHE256, LEU382, and LEU497.

The docking studies identified helminthosporin as a potential inhibitor of CYP1A2 and a weak CYP3A4 inhibitor. Some authors supposed that the main factors for CYP450 inhibition are electrostatic and hydrophobic interactions and lipophilicity using QSAR analysis. The inhibition of CYP1A2 could be pharmacologically beneficial because CYP1A2 is involved in the activation of procarcinogen (Appiah-Opong et al., 2008).

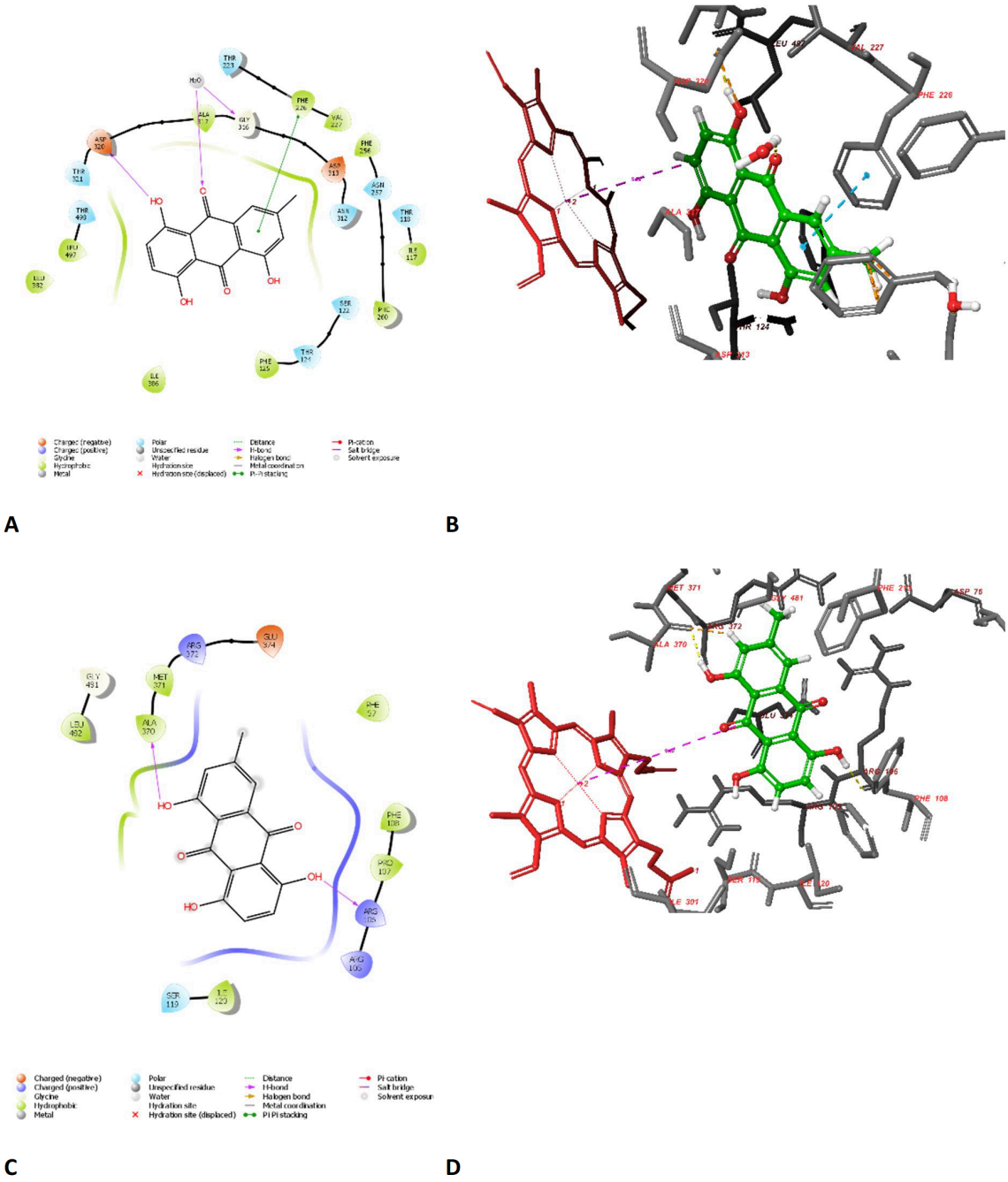


Figure 1. Visualised interactions of helminthosporin in the active sites of CYP1A2 (PDB: 2HI4) and CYP3A4 (PDB: 2V0M) after using the IFD and MM/GBSA in silico modes. The active conformations were provided in both the 2D (A and C) and 3D (B and D) forms. The enzymes are depicted in grey and the active amino acids are displayed, the hem group is in red, and ligand - helminthosporin , is presented as green sticks.

Cytotoxicity Profile

In the our previous investigation, root extracts of *A. lutea* were studied and proved for their ability to regulate the growth of the breast cancer cell line MCF-7 and the noncancerous breast epithelial cell line MCF-10A via System xCELLigence for real-time analysis of cell proliferation (Lazarova et al., 2015) . These findings inspired us to search for individual compounds isolated from plants possessing this or other types of cytotoxicity. Hydroxyanthraquinones are one of the most abundant components of the herb. The structure–activity relationships (SARs) for cytotoxicity against MCF-7 cells showed that chrysophanol, aloe-emodin and emodin, which have the 1- and 8-OH groups, possessed an effect. $-CH_3$ at position 3 increased the activity, whereas $-CH_2OH$ and -OH groups at position 6 decreased the activity (aloe-emodin, inactive) (Yuenyongsawad et al., 2014). In this context, the study was followed by the *in silico* evaluation of the cytotoxicity profile of chrysophanol, helminthosporin, asphodelin and 10,7'-bichrysophanol on cell lines via the CLC-Pred server. The results are provided in (Table 7).

Table 7. Cytotoxic activity of the test compounds against tumor cells applying the web tool CLC-Pred

	A2780cisR Cisplatin-resistant ovarian carcinoma P_a; P_i	HOP-18 Non-small-cell lung carcinoma P_a; P_i	RCC4 Clear cell renal cell carcinoma P_a; P_i	M19-MEL Melanoma P_a; P_i	GIST430 Gastrointestinal stromal tumor P_a; P_i	A2780 Ovarian carcinoma P_a; P_i	SW1990Pancreatic adenocarcinoma P_a; P_i
Helminthosporin	0.911; 0.004	0.724; 0.002	0.594; 0.004	0.592; 0.009	0.503; 0.033	0.147; 0.123	0.338; 0.068
Chrysophanol	0.912; 0.004	0.726; 0.002	0.58; 0.004	0.581; 0.010	0.477; 0.042	-	0.32; 0.079
10,7'-bichrysophanol	0.885; 0.008	0.678; 0.002	0.426; 0.016	0.598	0.326; 0.009	0.528; 0.012	0.524; 0.009
Asphodeline	0.888; 0.007	0.649; 0.003	0.475; 0.01	0.564; 0.013	0.373; 0.106	-	0.371; 0.046

Chrysophanol, helminthosporin, asphodelin, and 10,7'-bichrysophanol are expected to manifest a strong cytotoxic effect against the cisplatin-resistant ovarian carcinoma (A2780cisR) cell line with the probability of being active compounds (Pa) = 0.912, 0.911, 0.888 and 0.885, respectively. They exerted a strong to modest cytotoxic effect against non-small cell lung carcinoma (HOP-18), clear cell renal cell carcinoma (RCC4) and melanoma (M19-MEL) with Pa = 0.726, 0.724, 0.649 and 0.678 for the HOP-18 cell line; Pa = 0.58, 0.594, 0.475 and 0.426 for the RCC4 cell line; and 0.581, 0.592, 0.564 and 0.598 for the M19-MEL cell line. 1,5,8-Trihydroxy-3-methylanthraquinone exhibited modest action against gastrointestinal stromal tumor (GIST430) cell line, while 10, 7'-bichrysophanol had moderate activity against the ovarian carcinoma (A2780) and pancreatic adenocarcinoma (SW1990) cell lines.

CONCLUSION

The conducted study determined the theoretical pharmacokinetic profiles, CYP450 inhibition capacities, and probable cytotoxic effects of our anthraquinones isolated from *A. lutea* roots. Chrysophanol and helminthosporin were identified as suitable for further *in vitro* evaluation considering their optimal ADME profiles. Notably, the former compounds adhere to all of Lipinski's rules of five for druglikeness. Moreover, it was noticed that chrysophanol and helminthosporin have probable CYP1A2 and CYP3A4 inhibitor capacities, which were further examined by molecular docking studies. IFD and MM/GBSA studies were included, considering the enhanced reliability of the former modules. Overall, it was found that helminthosporin is a theoretical CYP1A2 and weak CYP3A4 inhibitor considering the odd active conformation, which should be validated by *in vitro* experiments. The cytotoxic studies demonstrated that all of the examined anthraquinones are probable cytotoxic agents against cisplatin-resistant ovarian carcinoma (A2780cisR). Future *in vitro* and *in vivo* studies should be carried out to validate the observed results.

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