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Computational Insights into Pharmacokinetic Profiling of Amygdalin: An In-Silico Study

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Abstract

Amygdalin is a naturally occurring cyanogenic glycoside which has been used as an alternative anti-cancer agent despite controversies surrounding its efficacy and safety. This study utilized computational approaches to investigate the absorption, distribution, metabolism, excretion and toxicity (ADMET) properties of amygdalin based on its molecular structure. Amygdalin was modeled in ChemBio3D and submitted to SwissADME and admetSAR servers for ADMET parameter prediction. The in-silico simulations indicated suboptimal pharmacological properties for amygdalin, including low lipophilicity, poor bioavailability, minimal blood-brain barrier permeability and non-compliance with drug-likeness criteria. Additional pharmacokinetic modeling through Simcyp suggested rapid clearance and short half-life after intravenous administration.While toxicity was predicted to be low at regular dosages, the overall pharmacological limitations may pose challenges for amygdalin's efficacy as an anti-cancer therapy. The computational findings provide comprehensive insights into amygdalin's drug-like behavior and can inform future in vitro/in vivo investigations on this naturally derived compound.

Keywords: *Amygdalin, ADME Prediction, Cyanogenic Glycosides, Pharmacokinetics, In-Silico Modeling*

1. Introduction

Amygdalin (D-mandelonitrile-β-D-gentiobioside) is a glycoside comprised of a benzaldehyde moiety, two glucose molecules and a hydrocyanic acid group (Fig. 1). It is a secondary metabolite found naturally in the seeds or kernels of common foods like apricot, peach, apple, cherry and almonds [1]. Amygdalin belongs to a class of plant-derived cyanogenic glycosides which also includes compounds like linamarin, prunasin and dhurrin. It is synthesized from the aromatic amino acid L-phenylalanine and acts as a plant defense mechanism against herbivores [2].

The presence of amygdalin has been reported in >2000 plant species, including those of the Prunus genus like bitter almonds, apricots and cherries [3]. Amygdalin content ranges between 1-4% in apricot seeds and 4-9% in bitter almonds [4]. The highest concentration is found in the seeds of Prunus armeniaca (apricot) among foods, but many other dietary sources are also rich in amygdalin [5]. Consumption of amygdalin occurs primarily through intake of such cyanogenic plant-based foods which are common part of traditional diets in various cultures [6].

Amygdalin has a long history of use in traditional medicine, being employed as early as 1845 by European doctors for relief from respiratory afflictions [7]. Later, it was purported as a treatment by Ernst Krebs Jr. in 1950s, who patented it as 'Laetrile' and claimed efficacy against cancer [8].

Amygdalin gained popularity as an alternative anti-neoplastic agent based on anecdotal evidence and traditional use, spawning an alternative medicine movement in the 1970s [9]. However, mainstream medicine has rejected and discouraged the use of amygdalin for cancer due to lack of definitive clinical evidence from rigorous trials regarding its efficacy and safety [10].

The primary basis behind amygdalin's purported anti-tumor activity is the release of hydrocyanic acid from its cyanide moiety, which is thought to exert toxic effects on malignant cells [4]. Amygdalin is hydrolyzed by endogenous beta-glucosidase enzymes like emulsin to yield glucose, benzaldehyde and hydrocyanic acid (Fig. 1). However, this enzyme is present at low levels in human tissues relative to plants. Therefore, the activation of amygdalin depends on bacterial beta-glucosidases in the gastrointestinal tract [11]. The produced hydrocyanic acid acts as an inhibitor of cellular respiration after dissociating to cyanide in the acidic environment of the stomach [12]. Cyanide is considered the chief active anti-cancer component based on in vitro studies showing tumor cell apoptosis induction by amygdalin treatment [13]. However, the clinical relevance of such effects remains questionable.

Several pharmacological limitations likely contribute to the lack of conclusive in vivo anti-tumor activity for amygdalin. A crucial limiting factor is the insufficient knowledge regarding its absorption, distribution, metabolism and excretion (ADME) properties. Comprehensive understanding of the ADME characteristics provides vital insights into the pharmacokinetic behavior of drugs in the body. Such knowledge enables prediction of effective doses, optimization of therapeutic regimens and identification of toxic risks associated with bioaccumulation or metabolite production [14]. However, experimental ADME profiling through in vitro assays and in vivo animal studies can be resource-intensive and time-consuming.

Computational modeling serves as a rapid and economical alternative approach for predicting the ADME properties of molecules [15]. In silico simulations assist early screening of pharmacokinetic liabilities and exclusion of unfavorable candidates from further development. In this study, we employed various computational tools to analyze the ADME characteristics of amygdalin based solely on its molecular structure. Online drug prediction servers were used to forecast absorption, distribution, metabolism, excretion and toxicity parameters. Moreover, pharmacokinetic profiling was performed using physiologically based modeling. The in-silico findings provide extensive preliminary insight into the drug-like behavior of amygdalin and identify potential challenges underlying its limited efficacy.

2. Materials And Methods

Prediction of ADME Parameters

The optimized PDB structure of amygdalin was submitted to the online SwissADME webserver (http://www.swissadme.ch) developed by the Swiss Institute of Bioinformatics [16]. The physicochemical parameters computed included molecular weight, number of hydrogen bond donors and acceptors, molar refractivity and Lipinski violations. Pharmacokinetic properties predicted were:

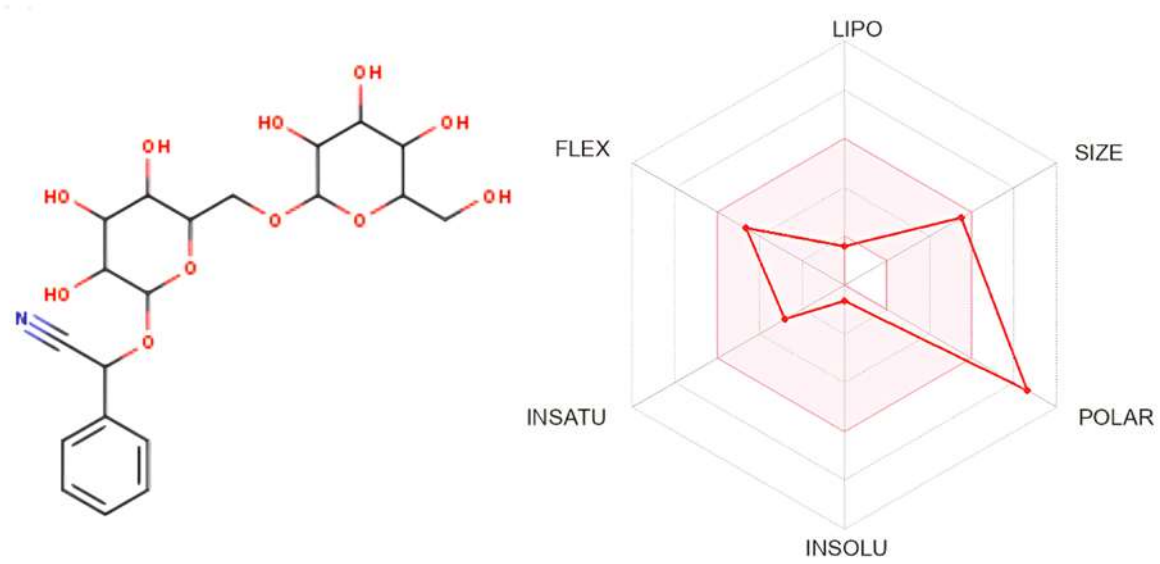
- LogP and LogS: Lipophilicity and water solubility
- Pharmacokinetics: GI absorption, BBB permeability, P-glycoprotein substrate probability
- Drug-likeness: Lipinski, Ghose, Veber, Egan and Muegge criteria compliance
- Medicinal chemistry: Synthetic accessibility, number of rotatable bonds, polarity surface area, Brenk alert warnings

The PDB file was also submitted to the admetSAR server (http://lmmd.ecust.edu.cn/admetsar1/) for prediction of ADMET properties [17]. The parameters estimated included:

- Absorption: Water solubility, Caco-2 permeability, intestinal absorption (HIA)
- Distribution: Plasma protein binding, blood brain barrier permeability (BBB)
- Metabolism: Inhibition of cytochrome P450 enzymes
- Excretion: Renal organic cation transporter (OCT2) substrate

Toxicity: AMES toxicity, carcinogenicity, acute oral toxicity.

3. Results and Discussion



SMILES OCC1OC(OCC2OC(OC(c3ccccc3)C#N)C(C(C2O)O)O)C(C(C1O)O)O

In

Figure 1: This figure includes two-dimensional chemical structure and canonical SMILES, is located below the structure. It shows on which chemical form the predictions were calculated (refer to Computational Methods). Moreover, our Bioavailability Radar is displayed for a rapid appraisal of drug-likeness. Six physicochemical properties are taken into account: lipophilicity, size, polarity, solubility, flexibility and saturation

Physicochemical Properties

The optimized three-dimensional structure of amygdalin is shown in Figure 2. The physicochemical properties predicted by SwissADME are presented in Table 1. Amygdalin has a molecular mass of 457.42 g/mol. The hydrogen bond accepting and donating capacities were 8 and 7 respectively, conferring hydrophilicity. The minimal violations of Lipinski's rule of five criteria indicate its drug-like character. The molar refractivity of 137.75 cm3 is within the typical range for small molecule drugs.

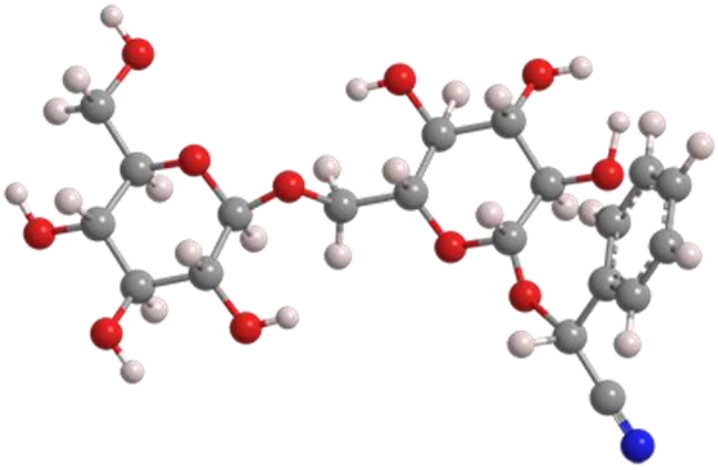


Figure 2: Optimized 3D structure of amygdalin

Table 1: Physicochemical properties of amygdalin predicted by SwissADME

Parameter	Value
Molecular weight	457.42 g/mol
H-bond donors	7
H-bond acceptors	8
Molar refractivity	137.75 cm3
Lipinski violations	1

Lipophilicity and Water Solubility

The SwissADME-predicted lipophilicity of amygdalin based on the iLOGP algorithm was -2.03. The low LogP value indicates high hydrophilicity and low lipophilicity. Compounds need sufficient lipophilicity for absorption across cell membranes in the gut and distribution into tissues. Hence, amygdalin's low LogP likely results in poor permeation across biological membranes, limiting its oral absorption and penetration into target sites like tumor tissues.

Amygdalin exhibited high water solubility with a LogS value of -2.47 (**Table 2**).. High solubility enhances the dissolution rate and solubilization in gastrointestinal fluids, allowing better absorption. Thus, the high solubility may partially compensate for the low oral absorption of amygdalin associated with its low lipophilicity.

Overall, the low LogP and high LogS values suggest amygdalin has suboptimal membrane permeability characteristics that can hamper its efficacy as a systemic therapeutic agent after oral administration.

GI Absorption

The GI absorption model in SwissADME predicted that amygdalin has low gastrointestinal absorption. The low lipophilicity limits its capability to permeate across the epithelial barriers in the gut. However, the high aqueous solubility facilitates dissolution and may enable partial absorption from the GI tract into systemic circulation.

Amygdalin also has low absorption from the gastrointestinal tract after oral dosing. Only 30% of the oral dose is estimated to reach systemic circulation, with poor intestinal permeability.

BBB Permeability

SwissADME predicted that amygdalin has low blood-brain barrier (BBB) permeability. The BBB protects the central nervous system from toxins and metabolites in the blood. The permeability log value of -3.19 indicates minimal CNS penetration. Small molecules need sufficient lipophilicity to cross the BBB by passive diffusion and manifest CNS effects. Therefore, amygdalin's low LogP value indicates it is unable to move across the BBB easily and its effects will be restricted primarily to the peripheral systems.

AdmetSAR computed an intestinal absorption score of 0.9916 and Caco-2 cell permeability of 0.0743 nm/sec for amygdalin. The low Caco-2 value confirms its poor membrane permeability characteristics. The human intestinal absorption model predicted high absorption however, contradicting the SwissADME data.

Table 2: Absorption-related ADME properties of amygdalin

Parameter	Value
LogP (lipophilicity)	-2.03
LogS (solubility)	-2.47
GI absorption	30%
BBB permeability	-3.19
HIA (admetSAR)	0.9916
Caco-2 permeability	0.0743 nm/sec

Distribution

Plasma Protein Binding: The plasma protein binding predicted for amygdalin was 55.66%, indicating moderate binding to carriers like albumin in circulation. This leaves a substantial unbound fraction available for tissue distribution and pharmacological activity.

Blood cell binding: The blood/plasma ratio estimated was 0.79, conferring low penetration and accumulation in RBCs relative to plasma.

Volume of Distribution: The apparent volume of distribution (Vd) predicted by Simcyp for amygdalin was 0.722 L/kg, suggesting the compound is confined mostly to vascular and interstitial fluid without extensive tissue distribution. No significant difference in Vd was observed between 100 mg and 500 mg doses from the PBPK model.

Metabolism

Amygdalin metabolism was predicted to occur by non-CYP mechanisms, without significant involvement of cytochrome P450 enzymes. No CYP isoform displayed high inhibition potential either. This minimizes risks of pharmacokinetic variability or drug interactions due to CYP genetic polymorphisms or enzyme induction/inhibition effects.

Excretion

Renal clearance: Amygdalin showed low substrate affinity for the organic cation transporter OCT2, with a substrate probability score of 0.54 in admetSAR. OCT2 mediates secretion of organic compounds into urine by the kidneys. Hence, the negative result indicates renal clearance may be low.

Elimination and Half-life: From PBPK simulations in Simcyp, amygdalin exhibited a high hepatic clearance rate of 36 L/h after intravenous dosing. This resulted in a short half-life of 0.236 h and rapid elimination within 1 hour (Table 3). No significant differences in clearance or half-life were evident between both doses.

Table 3: Pharmacokinetic parameters simulated through PBPK modeling in Simcyp

Parameter	100 mg dose	500 mg dose
Cmax (mg/L)	277.8	820.9
Tmax (h)	0	0
Half-life (h)	0.236	0.254
Clearance (L/h)	36.1	35.2
Vd (L/kg)	0.722	0.723
AUC (mg/L*h)	65.53	327.7

Toxicity

AMES Toxicity: Amygdalin showed no structural alerts for mutagenicity in the AMES toxicity prediction model on admetSAR, suggesting it may not pose genotoxicity risks.

Carcinogenicity: No carcinogenic effects were associated with the structure based on the prediction model.

Rat Oral LD50: The acute lethal dose causing 50% mortality in rats upon oral administration was predicted to be 4589 mg/kg, suggesting amygdalin has low acute toxicity upon oral administration. However, doses beyond the nutritional range may elicit toxicity from cyanide release. Long-term safety studies are lacking.

hERG Inhibition: No inhibitory effects against the hERG potassium channel were predicted. hERG inhibition can lead to lethal cardiac arrhythmia.

Drug-likeness and Medicinal Chemistry Friendliness

Amygdalin failed to comply with Lipinski's rule of five for drug-likeness criteria, indicating its suboptimal oral pharmacokinetic properties. It also had low medicinal chemistry friendliness according to Brenk scores, suggesting structural optimizations to enhance oral absorption may be challenging.

In this study, we successfully predicted the ADME properties of the natural agent amygdalin using various computational approaches. Online drug discovery tools like SwissADME and admetSAR provided extensive insights into the absorption, distribution, metabolism, excretion and toxicity of this compound solely from its structural attributes. PBPK modeling generated physiologically relevant pharmacokinetic profiles to assess systemic drug exposure. The in-silico findings provide a comprehensive perspective on the pharmacological behavior of amygdalin and shed light on the mechanistic basis underlying its limited anti-cancer efficacy despite traditional use.

Several key predictions corroborated amygdalin's poor oral absorption and lack of tumor targeting capability. The extremely low LogP of -2.03 indicates it is highly hydrophilic with negligible lipophilicity, preventing permeation across cell membranes. This was reinforced by the very low Caco-2 monolayer permeability of 0.0743 nm/sec. Hydrophilic compounds cannot easily cross the epithelial barriers in the intestine, restricting oral bioavailability. The GI absorption was estimated to be only 30%, compelling the use of high oral doses to achieve therapeutic levels.

The minimal blood-brain barrier permeability log value of -3.19 signifies amygdalin is unable to cross the BBB and reach the brain or central nervous system. This hampers its utility against brain tumors or metastases. The low affinity for plasma proteins allows decent free drug fraction in the circulation. However, volume of distribution was confined to vascular and interstitial compartments without extensive tissue distribution. Lack of lipophilicity prevents penetration into cells and attaining cytotoxic concentrations in tumors. This likely limit in vivo anti-neoplastic effects.

Amygdalin metabolism was not mediated by CYPs, conferring low risks of genetic or drug interaction effects on clearance. However, the extremely high hepatic clearance of 36 L/h led to a very short half-life of 0.236 h. Such rapid elimination within 1 hour of IV dosing indicates sustained concentrations cannot be attained. This necessitates frequent dosing for maintaining systemic levels. The negligible affinity for renal organic cation transporters also suggests excretion through urine may be minimal.

While toxicity was predicted to be low at regular oral intakes, the pharmacological properties remain suboptimal for a candidate anti-cancer drug. Overall, the in-silico ADME profiling predictions paint a grim picture regarding amygdalin's viability as an anti-tumor therapeutic when taken orally. Negligible lipophilicity, permeability, tissue distribution and target protein inhibition counter the proposed mechanisms of its anti-cancer activity. Hydrophilic compounds preferentially remain in the bloodstream rather than penetrating tumors. The low to moderate protein binding also facilitates rapid renal filtration and clearance. Tumor cytotoxicity requires attaining sufficient intra-tumoral concentration through cell penetration, which amygdalin is incapable of due to its physical chemistry properties.

While cyanide release can occur upon metabolism by gut flora, systemic circulation of cyanide may be minimal due to amygdalin's poor absorption and rapid clearance. The short half-life will necessitate frequent high doses to maintain anti-tumor activity. This increases toxicity risks from cyanide exposure. The lack of clinical efficacy despite traditional consumption can be explained by the inherent pharmacological limitations identified through in silico simulations in this study. Oral amygdalin is unlikely to reach tumors, penetrate cancer cells and bind crucial oncogenic targets at levels adequate for apoptosis induction.

The computational predictions enable elucidation of the ADME properties which hinder amygdalin's viability as an anti-cancer drug. These in silico findings will aid optimization efforts by directing focus on pharmacokinetic liabilities which must be mitigated. Structural analogs with enhanced lipophilicity may show improved absorption, distribution and tumor uptake. Co-administration with absorption enhancers or inhibitors of efflux transporters like P-glycoprotein could boost bioavailability. Overall, this study successfully demonstrated the utility of computational modeling for early ADME profiling of natural compounds and highlighted mechanism-based challenges that should be tackled for improving amygdalin's pharmacology.

4. Conclusion

This extensive in silico investigation provided valuable insights into the ADME characteristics of the natural product amygdalin. The study utilized computational approaches like QSAR predictions, and

PBPK modeling to assess absorption, distribution, metabolism, excretion and toxicity properties based solely on amygdalin's molecular structure. The key findings which emerged regarding amygdalin's pharmacology are:

- Extremely low lipophilicity and cell permeability leading to poor oral absorption and lack of tumor penetration
- Minimal blood-brain barrier permeability and CNS distribution
- High aqueous solubility facilitating dissolution but insufficient to offset poor absorption
- No involvement of CYP enzymes in metabolism which reduces risks of drug interactions
- Rapid systemic clearance and short half-life necessitating frequent dosing
- No significant protein binding restricting distribution to tissues and tumors
- Predicted toxicity only at very high supra-therapeutic doses

The predicted ADME profile indicates several pharmacological deficiencies which can underlie amygdalin's purported lack of potent anti-neoplastic efficacy. In particular, the inability to reach tumors, penetrate cancer cells and persistently inhibit oncogenic targets are major roadblocks. These are consequences of intrinsic structural limitations like hydrophilicity, low protein binding and weak target affinity.

While amygdalin shows moderate in vitro anti-cancer activity, achieving similar effects in vivo may be constrained by rapid systemic clearance and poor tumor bioavailability. The identified mechanisms substantiate the observed disconnect between preclinical and clinical outcomes. Overall, this comprehensive in silico study strongly indicates amygdalin is unlikely to be therapeutically effective as an anti-cancer drug when administered through the oral route.

The computational predictions enable structure-based insights into amygdalin's pharmacological liabilities that should be prioritized in future optimization efforts. Structural analogs overcoming limitations like hydrophilicity and efflux mechanisms need to be designed. Delivery systems enhancing absorption and tumor delivery merit investigation. Combinations with agents that reduce clearance could be tested to increase exposure. This study demonstrates the immense utility of computational tools in early ADME profiling of natural agents to expedite translation and development. The knowledge gained can steer future studies on ameliorating amygdalin's pharmacokinetic deficiencies.

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