

# Absorption, distribution and toxicity prediction of andrographolide and its derivatives as anti-HIV drugs

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## Abstract

*Andrographolide is a major bioactive labdane diterpenoidal compound of the medicinal plant Andrographis paniculata. Previous study concluded that Andrographis paniculata herb extract has been proven to exert anti-HIV activity and has also been proven with in silico methods that andrographolide can act as inhibitors with HIV-1 protease, a retroviral aspartyl protease (retro pepsin) that is essential for the life-cycle of HIV, the retrovirus that causes AIDS. The present study was intended to obtain detail information of the pharmacokinetic properties including absorption, distribution and toxicity of andrographolide and its derivatives using in silico methods. Pharmacokinetic properties, absorption as well as distribution prediction using parameters of HIA (Human Intestinal Absorption), permeability to Caco-2 cells and plasma protein binding (PPB) were studied using PreADMET. Toxicity was predicted by Toxtree package.*

*The result showed that andrographolide and its derivatives well absorbed in intestinal were strongly bound to plasma protein having medium permeability to Caco-2 cells. Based on computational scores, three andrographolide derivatives showed better absorption and distribution properties than andrographolide. Toxicity prediction of andrographolide and its derivatives showed no mutagenic or carcinogenic properties.*

**Keywords:** AIDS, andrographolide, andrographolide derivatives, HIV-1 protease, *in silico*.

## Introduction

Andrographolide is the major labdane diterpenoidal constituent of *Andrographis paniculata*<sup>1</sup>. The structure of andrographolide has been analyzed by X-ray crystallographic method. Its systematic name is 3-[2-[decahydro-6-hydroxy-5-(hydroxymethyl)-5, 8a-dimethyl-2-methylene-1-naphthalenyl] ethylidene] dihydro-4-hydroxy-2(3H)-furanone<sup>2</sup>. Andrographolide has an  $\alpha$ -alkylidene  $\gamma$ -butyrolactone, two olefin bonds at C-8 and C-12 and three hydroxyls at C-3, C-19 and C-14<sup>3</sup>. Its molecular formula is C<sub>20</sub>H<sub>30</sub>O<sub>5</sub>. This compound has many bioactivities including, anti-inflammatory, anticancer, hepatoprotective, antioxidant, antidiabetic,

antihyperlipidemic, antibacterial, antiviral and anti-malarial activities<sup>4-10</sup>.

HIV-1 protease inhibitors (PIs) have played a critical role in the success of highly active antiretroviral therapy for treatment of HIV-1 infected patients<sup>11-13</sup>. PIs have the highest intrinsic antiviral activity<sup>14</sup> and the only antiretroviral drugs that have been successfully used in monotherapy<sup>15</sup>. PIs are known to act by preventing cleavage of viral polyproteins into functional subunits thereby inhibiting maturation of the virus<sup>16</sup>. A recent study has suggested that in mediating their antiviral effects, PIs affect multiple distinct steps in the life-cycle of the virus including both entry and post-entry events explaining their remarkable potency in suppressing viral replication<sup>17</sup>.

Our previous *in silico* studies indicated that andrographolide interacted with two important aspartate residues (Asp25 and Asp29) in the binding pocket of HIV-1 protease, similarly as its hydroxybenzylidene derivatives. Therefore, andrographolide and its derivatives had potential to be developed as PIs for anti-HIV drugs<sup>18</sup>. In the drug discovery and development process, the discovery of drugs not only has good activity and bind selectively to target but also has the appropriate physico-chemical properties such as absorption and distribution properties<sup>19</sup> as well as the toxicity to reach the target site when delivered orally.

*In silico* approaches are being used today in drug discovery to assess the ADME (Absorption, Distribution, Metabolism, Excretion) and toxicity properties of compounds at the early stages of discovery/development. The need for early consideration of ADME properties is also increasingly urgent because of the implementation of combinatorial chemistry and high-throughput screening since this can generate vast numbers of potential lead compounds. In the present research, Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties of andrographolide as well as its derivatives were predicted using Pre-ADMET software<sup>20</sup> and the toxicity prediction was generated using Toxtree software<sup>21</sup>.

## Material and Methods

The PreADMET program was accessed at <http://preadmet.bmdrc.org/>. Andrographolide and eight structure modifications were used in this study (figure 1). The structure of all compounds were converted into molfile (\*.mol). The program automatically calculated the predictive absorption for Caco-2 cell, HIA (human

intestinal absorption) and plasma protein binding<sup>20</sup>. Predicting the toxicity properties was done using Toxtree free software and using Benigni/Bossa rule-based methods (for mutagenicity and carcinogenicity)<sup>22</sup>.

### Results and Discussion

The pharmacokinetic properties including absorption, distribution, metabolism and excretion of drug have an important role on its efficacy. Prediction of HIA is a major goal in the design, optimization and selection of candidates for development as oral drugs where the absorption of drug compounds in intestinal depends both on complex biological processes (including passive membrane penetration, active transport mechanisms and metabolism in the gastrointestinal tract).

In this study, the Pre-ADMET program was used to predict ADME of andrographolide and its derivatives. The aspect prediction of absorption properties included percentage human intestinal absorption (% HIA) and Caco-2 cell permeability. Caco-2 cells are derived from a human colon carcinoma and possess multiple drug transport cycles through the intestinal epithelium. The Caco-2 cells are widely used as an *in vitro* model for predicting human drug absorption while HIA is the sum of bioavailability and absorption evaluated from the ratio of excretion or cumulative excretion in urine, bile and feces.

The distribution properties were calculated using Pre-ADMET which will produce predictive plasma protein binding value. Those parameters are important because the degree of plasma protein binding of a drug has an important role on its disposition and the drug's efficacy<sup>20</sup>.

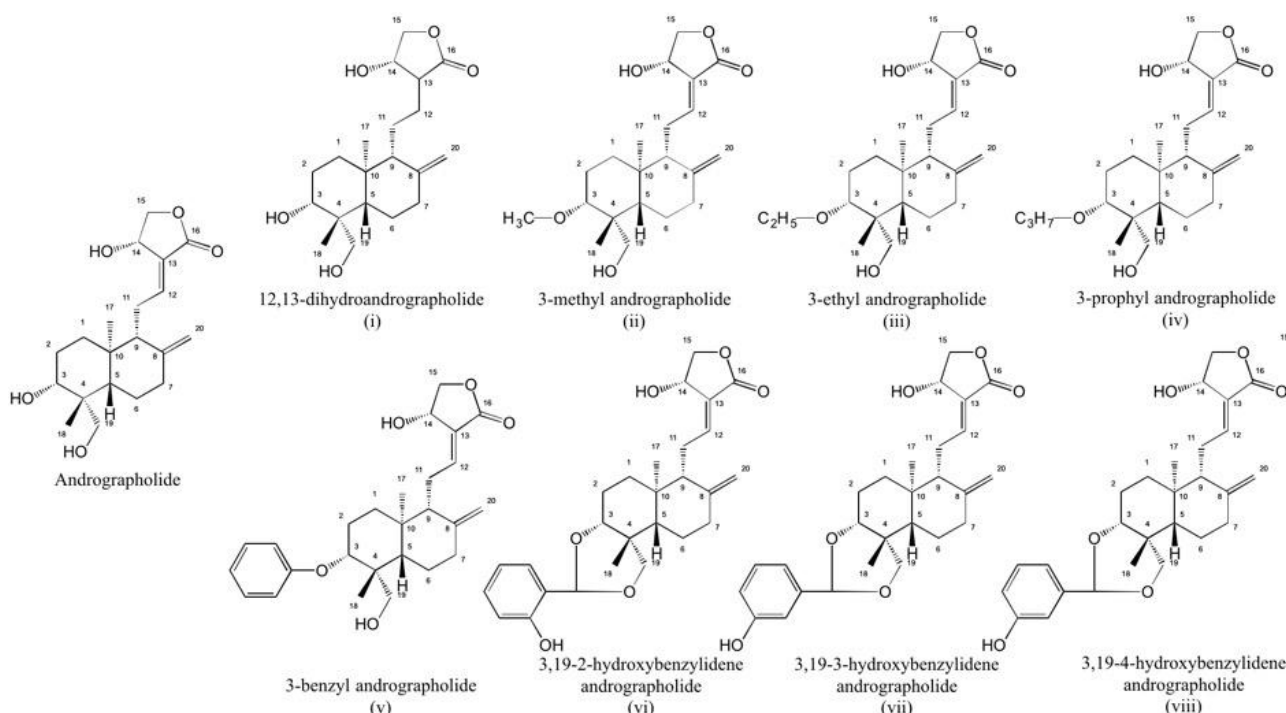
Based on the results as shown in table 1, all test compounds are to be well absorbed in the intestinal when the HIA values were between 70 - 100%. The HIA parameter was the sum of the bioavailability and absorption measured by the ratio of cumulative excretion or excretion in urine, bile and stool<sup>19</sup>. Value of % HIA was positively correlated with descriptors, the number of hydrogen bond acceptor groups, the logarithmic of octanol-water partition coefficient (LogP) and the logarithmic of solubility coefficient (LogS)<sup>23</sup>.

The permeability of andrographolide and its derivatives in Caco-2 cells was at intermediate levels between 4 - 70%, Caco-2 cells were colonic adenocarcinoma cells and play a role in the drug transport cycle through small bowel epithelial cells. The Caco-2 cell model was known to be reliable *in vitro* model for predicting oral absorption of the drug<sup>24</sup>. The permeability coefficient was expressed as a permeability of the Caco-2 monolayer cell culture, related to lipophilicity in addition to the hydrogen bonding capacity and charge. Permeability was a function of various physicochemical parameters namely: permeability = f (lipophilicity, molecular size, H-bond capacity, charge). The predicted permeability of Caco-2 for andrographolide derivatives was in line with that statement<sup>25</sup>.

Distribution parameters were predicted through plasma protein binding value (PPB) because these parameters were closely related to the ability of drug disposition and to provide efficacy<sup>24</sup>. Plasma protein binding (PPB) determines the fraction of available drugs in free form to be distributed to various tissues. Human plasma contains 70% protein with albumin (HAS, human albumin serum), alpha1-glycoprotein acid (AGP, alpha 1-acid glycoprotein) and lipoprotein as the main component.

**Table 1**  
**The results of absorption, distribution and toxicity prediction of andrographolide and its derivative**

Test Compounds	Absorption & Distribution			Toxicity	
	HIA (Human Intestinal Absorption)	Caco-2 Cells Permeability	PPB (Plasma Protein Binding)	Mutagenicity	Carcinogenicity
Andrographolide	87.6876	19.1341	94.8734	No Risk	No Risk
i	86.5997	19.1444	97.0300	No Risk	No Risk
ii	93.0386	20.3521	91.4487	No Risk	No Risk
iii	93.2810	20.6824	94.0451	No Risk	No Risk
iv	93.4766	21.9859	97.7455	No Risk	No Risk
v	94.4370	24.2593	98.1016	No Risk	No Risk
vi	94.4894	23.4408	94.4864	No Risk	No Risk
vii	94.4906	23.2361	96.0308	No Risk	No Risk
viii	94.9258	23.4402	94.9258	No Risk	No Risk



**Figure 1: Andrographolide and its derivatives**

Preferably, HAS was more readily linked to acidic or neutral drugs while AGP and lipoprotein are linked with basic medicines<sup>26</sup>. From the prediction of PPB, andrographolide and its derivatives had a high PPB value of > 90%, this was due to the high lipophilic of the compound so that its affinity in plasma protein was high<sup>26</sup>.

Prediction of toxicity test was made by Benigni/Bossa rules. Result showed that andrographolide and its derivatives were suspected not mutagenic and carcinogenic effects. This suggests that the andrographolide structure and all its derivative compounds were not reactive to cells that could cause DNA damage or mutation<sup>27</sup>.

## Conclusion

Andrographolide and its derivatives well absorbed in intestinal are strongly bound to plasma protein and having medium permeability to Caco-2 cells. Based on computational scores, three andrographolide derivatives (compounds v, vii and viii) showed better absorption and distribution properties than andrographolide as the lead compound. Toxicity prediction of andrographolide and its derivatives showed no mutagenic or carcinogenic properties.

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