Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2017, 9(19):9-13
(http://www.derpharmachemica.com/archive.html)

In silico Investigation of Physicochemical, Pharmacokinetic and Toxicological Properties of Hispolon

Mohammad Firoz Khan[1*], Sharmin Aktar[1], Ridwan Bin Rashid[1], Mohammad A Rashid[2]

[1]*Department of Pharmacy, Computational Chemistry and Bioinformatics Laboratory, State University of Bangladesh, Dhaka-1205, Bangladesh*

[2]*Department of Pharmaceutical Chemistry, University of Dhaka, Dhaka-1000, Bangladesh*

ABSTACT

Hispolon is a natural phenolic compound possessing diverse biological and pharmacological activity. The purpose of the study was to explore the physicochemical, pharmacokinetic and toxicological properties and to correlate the calculated physicochemical properties with the absorption and distribution profile of hispolon. The physicochemical properties such as acid Dissociation Constant (pKa), Partition Coefficient (logP), Distribution Coefficient (logD), Aqueous Solubility (logS) and Isoelectric Point (pI) of hispolon over the pH range of 0.0-14.0 at 298 K were calculated using MarvinSketch software. The pharmacokinetic and toxicological properties were calculated using online server PreADMET. The calculated physicochemical properties demonstrate that the unionized form of hispolon predominates over the pH of 0.0-8.5 and ionization increases with increasing pH of the solution leading to increase in aqueous solubility and decrease in distribution coefficient. Hispolon showed good Human Intestinal Absorption (HIA) and moderate permeability through Caco-2 and Maden Darby Canine Kidney (MDCK) cell model. Further, hispolon also exhibited moderate CNS activity due to moderate permeability through BBB. However, hispolon acts as an inhibitor of CYP2C19, CYP2C9 and CYP3A4 in phase I reaction and a substrate for UDP-glucuronosyl Transferase (UGT) and sulfotransferase (SULT) in phase II reaction. The toxicological study revealed that hispolon is a mutagenic compound and also showed positive prediction in carcinogenicity test of rat model i.e. non-carcinogenic. Our computed properties may be of assistance for the development of analytical method to assay hispolon or to develop hispolon derivatives with better pharmacokinetic and toxicological profile.

Keywords: Hispolon, Physicochemical properties, Pharmacokinetics, Toxicological properties

INTRODUCTION

Hispolon, 6-(3,4-Dihydroxyphenyl)-4-hydroxyhexa-3,5-dien-2-one (Figure 1) is a natural phenolic type bioactive compound first found in *Inonotus hispidus* in 1996 [1]. It is a yellow pigment and was isolated from *Phellinus linteus* [2,3] and *Phellinus igniarius* [4]. Hispolon possesses diverse biological activities including antioxidant [5], hepatoprotective [6], anti-inflammatory [7], antiproliferative [5], antimetastatic [8], immunomodulatory and antiviral effects [1,9]. Hispolon also inhibits chemilumescence response of human mononuclear cells and suppress nitrogen-induced proliferation of spleen lymphocytes of mice [10].

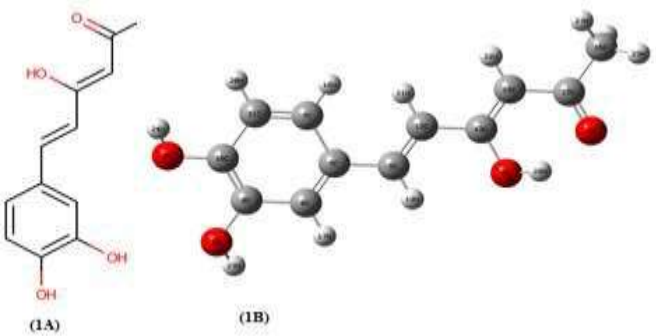

Figure 1: Two (1A) and three (1B) dimensional structure of hispolon

Literature survey revealed that no report on the physicochemical, pharmacokinetic and toxicological properties of hispolon has been reported. In this study, we computationally investigated (a) Physicochemical properties such as acid Dissociation Constant (pKa), Partition Coefficient (logP), distribution coefficient (logD), aqueous solubility (logS), (b) pharmacokinetic properties like Human Intestinal Absorption (HIA), cellular permeability using Caco-2 and Maden Darby Canine Kidney (MDCK) cell models, Skin Permeability (P_{Skin}), Plasma Protein Binding (PPB), penetration of the Blood Brain Barrier (BBB) and (c) toxicological properties including mutagenicity, carcinogenicity, acute algae toxicity and acute daphina toxicity. The purpose of this study was to explore the physicochemical, pharmacokinetic and toxicological properties and to correlate the calculated physicochemical properties with the absorption and distribution profile of hispolon. These computed properties may be of assistance for the development of analytical method [11] to assay hispolon or to develop hispolon derivatives with better pharmacokinetic and toxicological profile.

METHODOLOGY

Theoretical investigations of physicochemical properties

The acid dissociation constant, partition coefficient, distribution coefficient, aqueous solubility and isoelectric point of hispolon over the pH range of 0.0-14.0 at 298 K were calculated using MarvinSketch 15.06.29 [12]. The consensus method was applied to calculate partition and distribution coefficient of the three molecules.

Theoretical investigations of pharmacokinetic and toxicological properties

The pharmacokinetic and toxicological properties were calculated using online server PreADMET [13]. The pharmacokinetic properties such as HIA, *in vitro* cellular permeability using Caco-2 and MDCK cell models, P_{Skin}, PPB and penetration of the BBB were calculated. However, to evaluate toxicological properties the mutagenicity and carcinogenicity of hispolon were virtually investigated.

RESULTS AND DISCUSSION

Calculation of acid dissociation constant (pKa)

The calculation of pKa revealed that hispolon has seven ionized species (Hisp_2-Hisp_8) along with the unionized form (Hisp_1) (Figure 2). It is clear from Figure 2 Hisp_1 predominates in solution having pH of 0.0 to about 8.5. However, the abundance of ionized form (Hisp_2 to Hisp_8) of hispolon increases with increasing pH of the solution after pH 8.5.

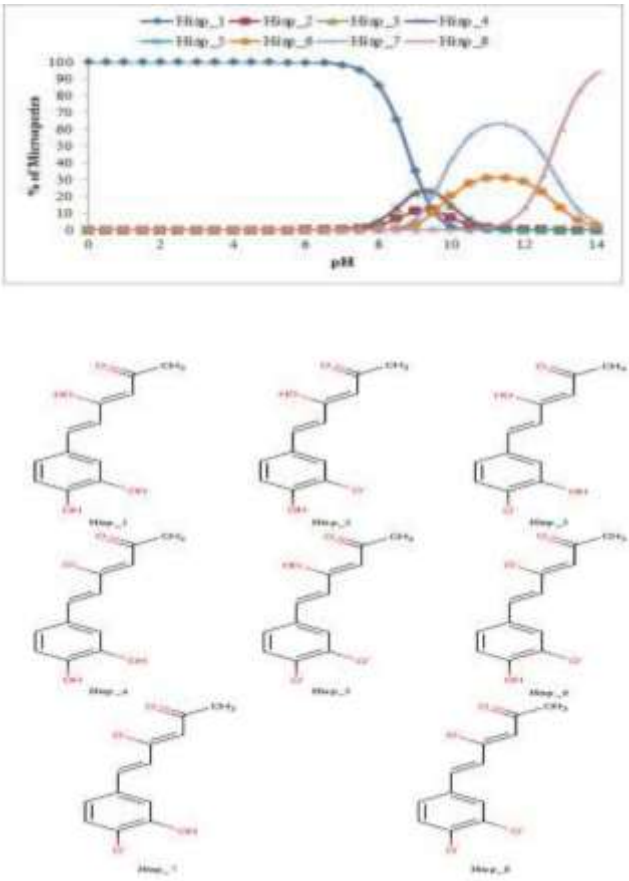

Figure 2: Microspecies distribution (%) of hispolon

Partition coefficient (logP)

The logP was calculated at 0.1 M electrolyte concentration using consensus and ChemAxon method. The logP value (Table 1) demonstrates that hispolon is lipophilic i.e., has higher affinity to *n*-octanol suggesting that it will pass plasma membrane easily.

Table 1: Partition coefficient of hispolon

Method	logP
Consensus	1.71
ChemAxon	1.89

Distribution coefficient (logD)

The logD was calculated at electrolyte concentration of 0.1 M using consensus method implemented in MarvinSketch. The plot of logD *vs.* pH of hispolon has been presented in Figure 3. From pH 0.0-9.0, the logD value of hispolon was over 1 suggesting it has higher solubility in *n*-octanol over this pH range and above that pH range logD value sharply decreases with increasing pH due to increasing ionization of hispolon.

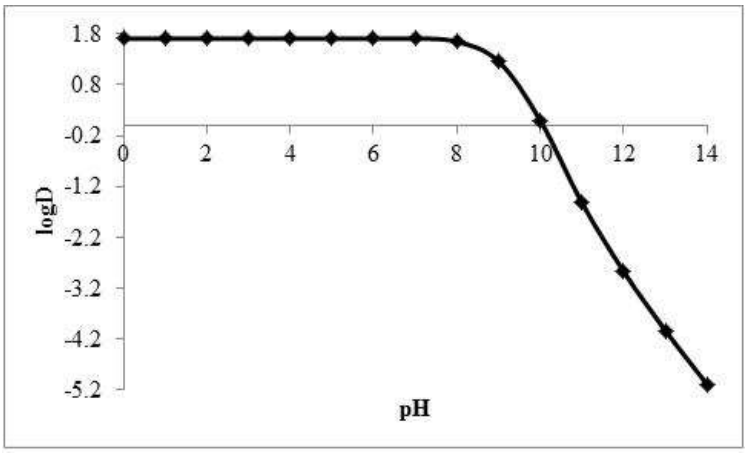

Figure 3: Distribution coefficient (logD) of hispolon

Aqueous solubility (logS)

The logS of hispolon have been presented in Table 2 and Figure 4. From Table 2 and Figure 4 it is clear that hispolon is slightly soluble, sparingly soluble and soluble to freely soluble over the pH range of 0.0-8.7, 8.8-9.4 and 9.5-14.0, respectively. However, the intrinsic solubility i.e., the solubility of unionized form of hispolon was found to be 4.39 mg/ml.

Table 2: Solubility of hispolon in water over the pH of 0.0-14.0

Name of drugs	Molecular weight	pH range	Solubility in water			Remarks [14]
			logS	mol/l	mg/ml	
Hispolon	220.22	0.0 to 8.7	-1.7 to -1.4	0.020 to 0.040	4.39 to 8.77	Slightly soluble
		8.8 to 9.4	-1.3 to -0.8	0.050 to 0.158	11.04 to 34.90	Sparingly soluble
		9.5 to 14.0	-0.7 to 0.3	0.200 to 1.995	43.94 to 439.40	Soluble to freely soluble

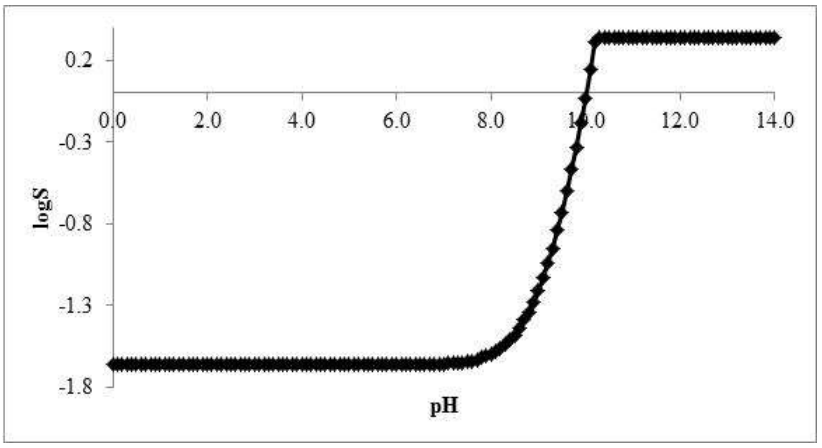

Figure 4: Solubility (logS) of hispolon in water

Isoelectric point (pI)

The pI is the pH where the molecule has no net charge. A plot of charge *vs*. pH has been presented in Figure 5. Hispolon possesses no isoelectric point but the compound achieves negative charges from being neutral with increasing pH of the solution (Figure 5). Table 3 indicates that from pH 0.0-6.0 hispolon is neutral and possesses negative charge over the pH of 6.5-14.0.

Table 3: Variation of charge over the pH of 0.0-14.0

Name of compound	pH range	Charge
Hispolon	0.0 to 6.0	0.0
	6.5 to 14.0	-0.01 to -2.98

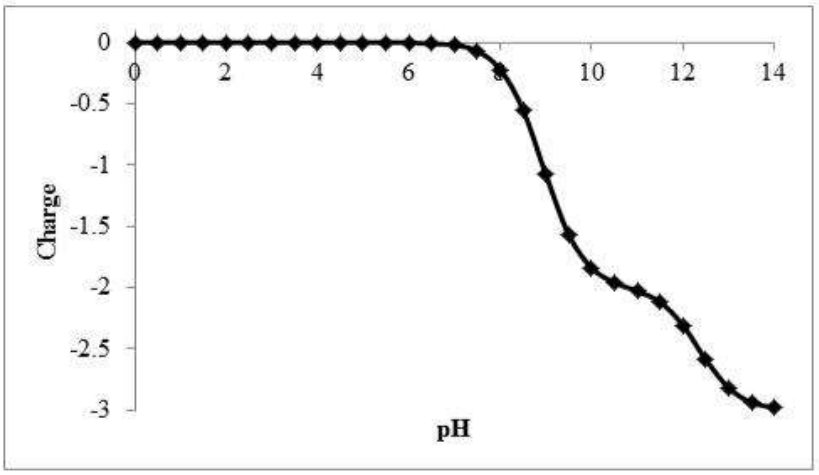

Figure 5: Variation of charge over the pH of 0.0-14.0

Pharmacokinetic study

The pharmacokinetic study such as absorption, distribution and metabolism of hispolon was performed using online server PreADMET [13]. The calculated absorption, distribution and metabolism parameters are presented in Table 4. The absorption characteristics of drugs is evaluated using Caco-2 cell model, Madin-Darby canine kidney (MDCK) cell model, skin permeability and human intestinal absorption (HIA). The calculated HIA of hispolon (Table 4) was 84.38% indicating well absorption through the intestinal cell [15]. The permeability of hispolon through Caco-2 cell (P_{Caco-2}) and MDCK (P_{MDCK}) were 15.59 and 60.77, respectively suggesting moderate permeability of hispolon [16]. The skin permeability (P_{Skin}) of pharmaceuticals, cosmetics and agrochemicals is a crucial parameter for transdermal administration of drugs and for the risk assessment of chemical products that come into contact with the skin accidentally [17]. The calculated P_{Skin} of hispolon was -3.35 indicating least permeability through the skin.

The distribution properties were evaluated in terms of PPB and brain to blood partition coefficient (C_{brain}/C_{blood}). The calculated value of PPB and C_{brain}/C_{blood} were 84.8% and 0.133, respectively proposing that hispolon weakly binds plasma protein and moderate amount of hispolon will pass the BBB
[18] i.e., hispolon may exert moderate CNS activity (Table 4). The computed metabolism of hispolon (Table 4) revealed that it is an inhibitor of CYP2C19, CYP2C9 and CYP3A4 in phase I reaction and also acts as a substrate for UDP-glucuronosyl transferase (UGT) and sulfotransferase (SULT) in phase II reaction.

Table 4: Absorption, distribution and metabolism properties for hispolon

Absorption				**Distribution**	
HIA (%)	**P_{Caco-2} (nm/s)**	**P_{MDCK} (nm/s)**	**P_{Skin}**	**PPB (%)**	**C_{brain}/C_{blood}**
84.38	15.59	60.77	-3.35	84.8	0.133
Metabolism					
Phase I		**Phase II**			
Enzyme	Inhibitor/Substrate	Enzyme	Substrate/Non-substrate		
Cytochrome P450 2C19 (Inhibition)	Inhibitor	UGT	Substrate		
Cytochrome P450 2C9 (Inhibition)	Inhibitor	SULT	Substrate		
Cytochrome P450 2D6 (substrate)	Non				
Cytochrome P450 3A4 (Inhibition)	Inhibitor				
Cytochrome P450 3A4 (substrate)	Non				

Toxicological study

Table 5 shows the results of the toxicological properties of mutagenicity (Ames test) and carcinogenicity (mouse and rat) for hispolon. One of the important steps for the discovery of new drugs is the evaluation of toxicity of drug candidates. This means that the toxicity study is very important for new compounds. The Ames test is widely used and accepted test to evaluate the mutagenicity of a chemical agents. In this test, various strains of *Salmonella typhimurium* bacterium with mutations in genes involved in histidine synthesis were used and the test compound is considered to be mutagenic if it enables the mutated bacterium to grow histidine-exempt medium i.e., if it stimulates the reversion process [19]. The hispolon was virtually screened to evaluate its mutagenicity. In this *in silico* investigation hispolon exhibited positive prediction, meaning that hispolon is a mutagenic compound.

Table 5: Toxicological properties of hispolon

Mutagenicity (Ames test)	**Carcinogenicity in rat**
Mutagenic	Non-carcinogenic

In carcinogenicity study, the PreADMET server was utilized to predict the carcinogenicity of chemical agent. This server is constructed from the data of the National Toxicology Program (NTP) and the USA/FDA, which are the results of *in vivo* carcinogenicity tests for mice and rats for 2 years. Hispolon displayed positive prediction of carcinogenicity in rat model suggesting it does not exhibit carcinogenic activity.

CONCLUSION

The calculated physicochemical properties demonstrates that the unionized form of hispolon predominates over the pH of 0.0-8.5 and ionization

increases with increasing pH of the solution leading to increase in aqueous solubility and decrease in distribution coefficient. The hispolon will remain unionized at the physiological pH (7.4). This is why, hispolon showed good HIA and moderate permeability through Caco-2 and MDCK cell model. Further, hispolon also exhibited moderate CNS activity due to moderate permeability through BBB. However, hispolon acts as an inhibitor of CYP2C19, CYP2C9 and CYP3A4 in phase I reaction and a substrate for UGT and SULT in phase II reaction. The toxicological study revealed that hispolon is a mutagenic compound and also showed positive prediction in carcinogenicity test of rat model.

REFERENCES

[1] N.A.A. Ali, R. Jansen, H. Pilgrim, K. Liberra, U. Lindequist, *Phytochemistry.*, **1996**, 41, 927-929.
[2] Y.C. Chen, H.Y. Chang, J.S. Deng, J.J. Chen, S.S. Huang, I.H. Lin, W.L. Kuo, W. Chao, G.J. Huang, *Am. J. Chin. Med*., **2013**, 41(6), 1439-1457.
[3] P. Toopmuang, C. Khamchum, C. Punsuvon, *Sci. Asia.*, **2014**, 40, 141-144.
[4] S. Mo, S. Wang, G. Zhou, Y. Yang, Y. Li, X. Chen, J. Shi, *J. Nat. Prod*., **2004**, 67, 823-828.
[5] G.J. Huang, J.S. Deng, S.S. Huang, M.L. Hu, *J. Agric. Food. Chem*., **2011**, 59, 7104-7113.
[6] G.J. Huang, J.S. Deng, C.S. Chiu, J.C. Liao, W.T. Hsieh, M.J. Sheu, C.H. Wu, *Evid. Based. Complement. Alternat. Med*., **2012**, 480714.
[7] H.Y. Chang, M.J. Sheu, C.H. Yang, T.C. Lu, Y.S. Chang, W.H. Peng, S.S. Huang, G.J. Huang, *Evid. Based. Complement. Alternat. Med*., **2011**, 478246.
[8] G.J. Huang, C.M. Yang, Y.S. Chang, S. Amagaya, H.C. Wang, W.C. Hou, S.S. Huang, M.L. Hu, *J. Agric. Food. Chem*. **2010**, 58, 9468-9475.
[9] N.A.A. Ali, R.A.A. Mothana, A. Lesnau, H. Pilgrim, U. Lindequist, *Fitoterapia.*, **2003**, 74 483-485.
[10] N.A. Ali, J. Lüdtke, H. Pilgrim, U. Lindequist, *Pharmazie*., **1996**, 51(19), 667-670.
[11] M.F. Khan, S.A. Rita, M.S. Kayser, M.S. Islam, S.Asad, R. Bin Rashid, M.A. Bari, M.M. Rahman, D.A.A. Al Aman, N.I. Setu, R. Banoo, M.A. Rashid, *Front. Chem*., **2017**, 5, 27.
[12] http://www.chemaxon.com.
[13] https://preadmet.bmdrc.kr/.
[14] P.J. Sinko, 6th Ed., Lippincott, **2010**, 183.
[15] S. Yee, *Pharm. Res*., **1996**, 14(6), 763-766.
[16] M. Yazdanian, S.L. Glynn, J.L. Wright, A. Hawi, *Pharm. Res*., **1998**, 15(9), 1490-1494.
[17] S. Singh, J. Singh, *Med. Res. Rev*., **1993**, 13(5), 569-621.
[18] M. Xiao-lei, C. Cheng, Y. Jie, *Acta. Pharm. Sinica*., **2005**, 26(4), 500-512.
[19] K. Mortelmans, E. Zeiger, *Mutat. Res*., **2000**, 455(1-2), 29-60.