Pharmacokinetics Prediction and Drugability Assessment of Diphenylheptanoids from Turmeric (*Curcuma longa* L)

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Abstract: Cheminformatics approaches are currently not employed in any of the spices to study the medicinal properties traditionally attributed to them. The aim of this study is to find the most efficacious molecule which do not have toxic effects but at the same time have desired pharmacokinetic profile. In the present study of the class 'diphenylheptanoids' from turmeric, cheminformatics methods were employed to predict properties such as physicochemical properties, Absorption, Distribution, Metabolism, Toxicity (mutagenicity, rodent carcinogenicity and human hepatotoxicity). These studies confirmed that curcumin and its derivatives cause dose-dependent hepatotoxicity. The results of these studies indicate that, in contrast to curcumin, few other compounds in turmeric such as compounds (**8**) and (**9**) [refer text], exhibit better activities and are drugable and do not have any side-effects.

Key Words: Turmeric, diphenylheptanoids, drugability, ADMET, pharmacokinetics, hepatotoxicity, mutagenicity, rodent carcinogenicity.

1. INTRODUCTION

Turmeric (*Curcuma longa* Linn. Syn *C.domestica* Valeton.) is extensively used as a spice, food preservative and colouring material commonly used in the Indian subcontinent. Traditionally many medicinal properties are attributed to this spice. Since the time of Ayurveda (1900 BC) numerous therapeutic activities have been assigned to turmeric for a wide variety of diseases and conditions, including those of the skin, pulmonary, and gastrointestinal systems, aches, pains, wounds, sprains, and liver disorders [1, 2].

Drug discovery projects experience very high failure rates. Absorption, Distribution, Metabolism, Excretion and Toxicity (ADME/Tox) factors are cited in 70% of these failures, so the early identification of problematic candidates has understandably become one of the highest priorities in the pharmaceutical industry [3]. This lack of knowledge usually results in poorly designed experiments that are not data productive and in many cases, have to be repeated when the drug candidate shows unexpected toxicity, low and variable delivery, instability or solubility problems, or unacceptable pharmacokinetic and drug metabolism profiles.

By going back to nature, one could overcome these failure rates and it is as an invaluable source of inspiration for drug discovery. Spices possess several efficacious compounds that are absorbed, distributed to the correct area, metabolized and excreted effectively. Scientific evidence underpins the pharmacological activity of several herbs which possess a number of novel therapeutic drug leads [4]. The slowness of conventional methods for investigation of plants limits enthusiasm in using them in the pharmaceutical industry [5]. The global market for herbal products may be around US$5 trillion by 2050 [6]. The properties of drug-like molecules are well studied and cover a wide range of sizes and physicochemical properties [7, 8]. Early identification is commonly facilitated through the use of *in vitro* ADME/Tox screens or with the aid of *in silico* models. As more data become available, computer-based predictions have become more reliable, so *in silico* ADME/Tox models are an increasingly important element of the drug discovery paradigm [3].

"Fail early and fail fast" is the current paradigm that the pharmaceutical industry has adopted widely. Removing non-drug-like compounds from the drug discovery lifecycle in the early stages can lead to tremendous savings of resources [9]. Structure information is increasingly used in the drug design process and has contributed significantly to the discovery of several marketed drugs [10-13]. Furthermore, it is well known that *in silico* approaches are comparatively cheaper than *in vivo* and *in vitro* screenings. In the present study ADME/Tox screens for 'diphenylheptanoids' is discussed in detail.

2. RESULTS AND DISCUSSION

The present study is focused on the physicochemical properties, pharmacokinetics and drugability prediction of the analysis data set (refer Table **1**).

2.1. Physicochemical Property Prediction

2.1.1. Solubility

Aqueous solubility was predicted for the analysis data set. The compounds (**1**), (**2**) and (**8**) were (refer Table **1**) having solubility level 2 (i.e. value -6.0 to -4.0) represents low-solubility at lower end of 95% of drugs, rest of the compounds of the analysis data set were having solubility level 3 (i.e. value -4.0 to -2.0) represents slight-solubility. However, exceptions will always exist such as substrates for transporters and natural products [14]. Solubility predictions based on

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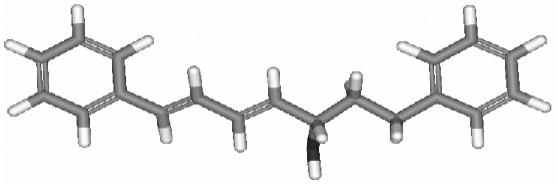
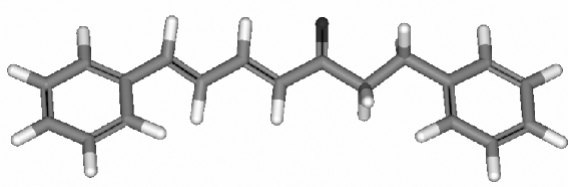
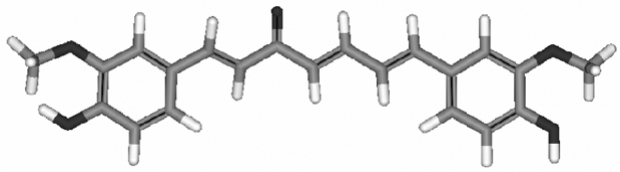
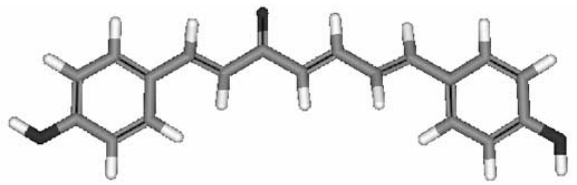
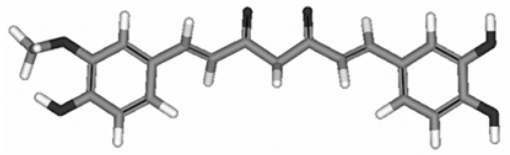
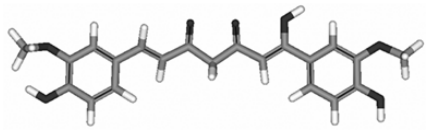
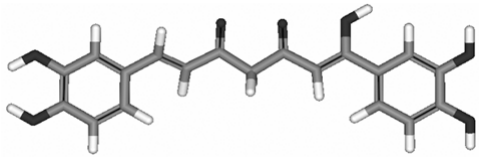
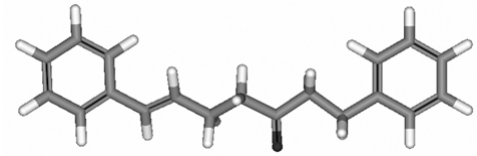
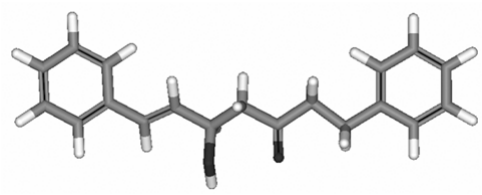
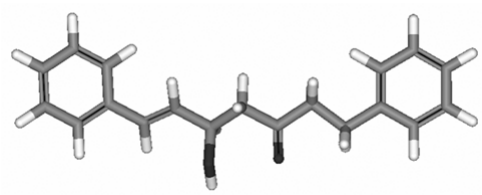
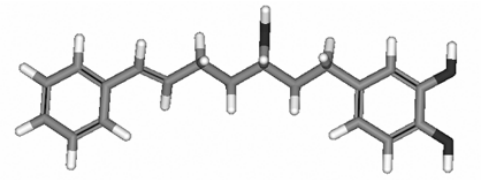
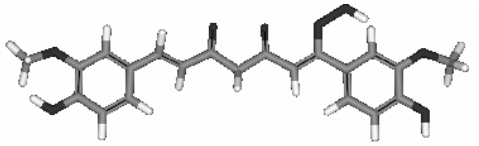
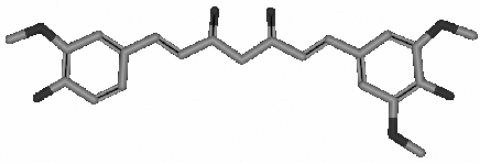
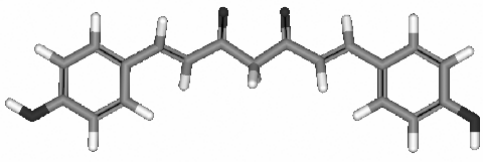
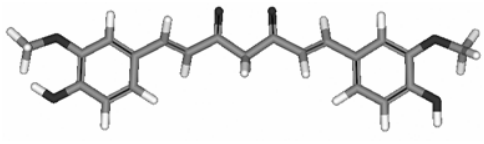
1573-4064/09 $55.00+.00

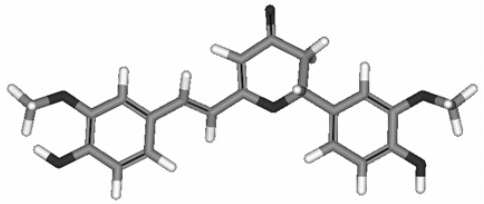
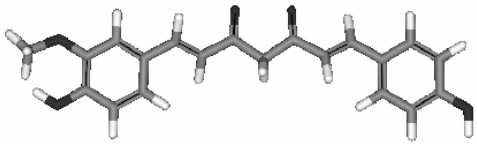
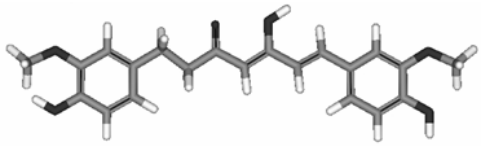
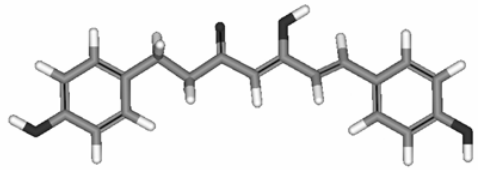
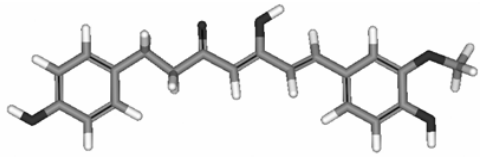
Table 1. The Chemical Compounds of Class 'Diphenylheptanoids' from Turmeric

No	Chemical compounds
1	 (1E,3E)-1,7-diphenyl-1,3-heptadien-5-ol
2	 (1E, 3E)-1,7-diphenyl-1,3-heptadien-5-one
3	 (1E, 4E, 6E)-1,7-Bis-(4-hydroxy-3-methoxyphenyl)-1,4,6-heptatrien-3-one
4	 (1E, 4E, 6E)-1,7-bis-(4-hydroxyphenyl)-1,4,6-heptatrien-3-one
5	 (1E, 6E)-1-(4-Hydroxy-3-methoxyphenyl)-7-(3,4-dihydroxy phenyl) -1,6-heptadiene-3,5-dione
6	 (E)-1,7-Bis-(4-hydroxy- 3-methoxyphenyl)- 1-heptene-3,5-dione
7	 (E)-1,7-Bis-(4-hydroxyphenyl)-1-hepten-3,5-dione
8	 (E)-1,7-diphenyl-1-hepten-5-one

(Table 1. Contd….)

No	Chemical compounds
9	 (E)-1,7-diphenyl-3-hydroxy-1-hepten-5-one
10	 (E)-5-Hydroxy-7-(4-hydroxyphenyl)-1-phenyl-1-heptene
11	 (E)-7-(3,4-dihydroxyphenyl)-5-hydroxy-1-phenyl-1-heptene
12	 (E)-7-Hydroxy-1,7-bis-(4-hydroxy-3-methoxyphenyl)-1-heptene-3,5-dione
13	 5'-methoxycurcumin
14	 bis-demethoxycurcumin
15	 Curcumin

(Table 1. Contd….)

No	Chemical compounds
16	 Cyclocurcumin
17	 Demethoxycurcumin
18	 Dihydrocurcumin
19	 tetrahydro-bis-demethoxycurcumin
20	 Tetrahydrodemethoxycurcumin

Artificial Neural Network (ANN) suggests that the compounds (**7**), (**14**) and (**19**) were having good water solubility, 81.67, 85.18 and 87.64 (in mg/l) respectively. ANN based buffer solubility predicted that the compounds (**5**), (**7**) and (**13**) were having buffer solubility 17900.80, 61389.50 and 21918.60 mg/l respectively.

2.1.2. Lipinski's Rule of Five (RO5)

All the compounds were checked for their oral bioavailability using Lipinski's scoring functions [7]. Each compound (refer Table **1**) satisfied all the four parameters that are less than or equal to the cut-off values originally proposed by Lipinski. Molecular weights of the compounds were in the range of 200 to 400. The lower molecular weight (MW) compounds can 'squeeze' through small gaps between the cells of the gut wall.

2.2. Absorption Prediction

2.2.1. Caco-2 Cell

For predicting Caco-2 cell permeability, chemical structures at pH 7.4 were applied, because Caco-2 cell permeability and MDCK cell permeability are measured at about pH 7.4. ANN based Caco-2 cell ($x10^6$ cm/sec) monolayer absorption model predicted that all compounds was having medium level of permeability.

2.2.2. Madin-Darby Canine Kidney (MDCK) Cell Permeability

MDCK cell based ANN descriptor for permeability screening MDCK ($x10^6$ cm/sec) showed that the compounds (**4**), (**6**), (**7**), (**10**), (**12**), (**14**), (**16**), and (**19**) were having low

permeability (refer Table **1**). The rest of the compounds were having medium permeability.

2.2.3. Skin Permeability

The compounds (**1**), (**2**), (**3**), (**4**), (**8**), (**9**) and (**10**) (refer Table **1**) were having better skin permeability, 0.0886968, 0.0700416, 0.0101176, 0.021869, 0.0696751, 0.0285722 and 0.0490199 (cm/hour) respectively in comparison with others especially with Curcumin (**15**) and its derivatives. The compounds (**7**) and (**12**), were having lower skin permeability, 0.000434927 and 0.000887979 (cm/hour) respectively.

2.2.4. Human Intestinal Absorption (HIA) Prediction

Human Intestinal Absorption (HIA) chart Fig. (**1**), shows that the three compounds (**1**) (**2**) and (**8**) (refer Table **1**) were having 100% HIA absorption as predicted by ANN. Since all the compounds were between 70 to 100%, it confirms that they are well absorbed compounds.

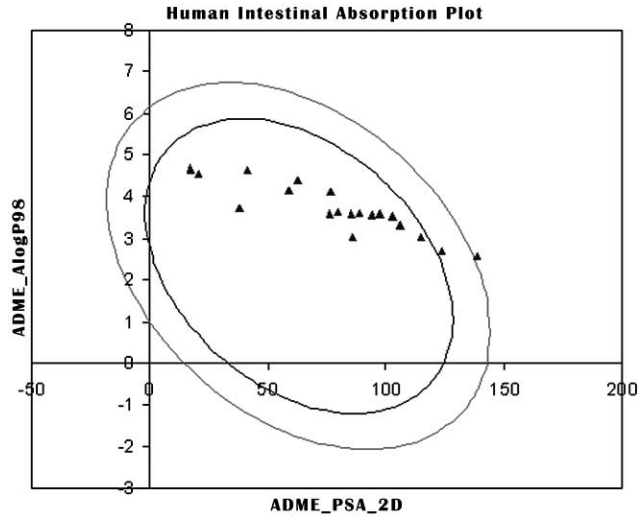


Fig. (1). Human Intestinal Absorption (HIA) prediction for the analysis dataset. Fast polar surface area (FPSA) and A Log P98 (atom-based Log P) are set as the x and y axes. Ellipses are also drawn to show 95% and 99% confidence regions for absorption level.

2.3. Distribution Prediction

Whether attempting to assess the ability of discovery leads for a CNS indication to reach the site of action or evaluating the potential toxicity of leads that should not be extensively delivered to the brain is a necessary first assessment.

2.3.1. Blood-Brain Barrier (BBB) Prediction

The predicted BBB penetration levels indicated that (**1**), (**2**) and (**8**) were having level '0' (i.e. Log BbR < -0.52) BbR (the level of blood-brain penetration). The compounds, (**5**), (**6**), (**7**), (**12**), (**13**) and (**18**) were at level '4', these compounds showed low and undefined levels and lie outside 99% ellipsis Fig. (**2**). The compounds (**9**), (**10**) and (**11**) were having very high penetration levels. Whereas (**3**), (**14**), (**17**), (**19**) and (**20**) were having medium level of penetration. ANN based BBB prediction showed that the compounds (**3**), (**5**), (**6**), (**7**), (**12**), (**13**), (**14**), (**15**), (**16**), (**17**), (**18**) and (**20**) were found to be CNS inactive (C brain/ C blood) < 1.0), whereas the rest of the compounds showed they are CNS active (>1.0).

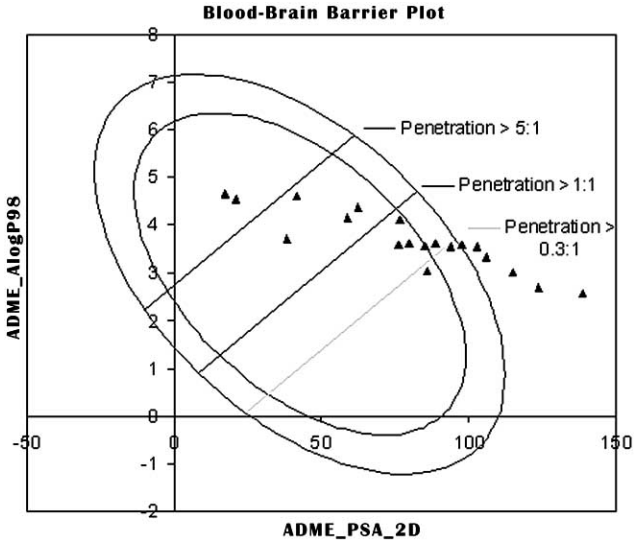


Fig. (2). Blood-Brain Barrier (BBB) prediction for the analysis dataset. Fast polar surface area (FPSA) and A Log P98 (atom-based Log P) are set as the x and y axes. Ellipses are also drawn to show 95% and 99% confidence regions for penetration level. Parallel lines inside the 99% ellipse indicate constant levels of LogBbR (the level of blood-brain penetration).

2.3.2. Plasma Protein Binding (PPB) Prediction

The binding of drugs to plasma proteins is often a major factor in drug distribution. Serum protein binding is an important determinant not only of drug action, but also of drug disposition. It is widely accepted that the net effect of a drug is related to the exposure of a patient to the unbound concentration of drug in plasma rather than total concentration of administered drug [15, 16]. Drug–protein complexes in plasma also serve as drug reservoirs to supply free drug. Thus plasma drug–protein binding can act to prolong the duration of drug action [17, 18]. The plasma protein binding model predicts whether a compound is likely to be highly bound to carrier proteins in the blood. Plasma protein binding studies indicated that the compounds (**8**), (**9**), (**10**) and (**16**) were having high binding at a level of 95% or greater. Whereas the compounds (**5**), (**6**), (**7**), (**12**), (**13**), (**15**), (**17**), (**18**) and (**20**) were in level 0 (binding is <90%). ANN prediction showed that all of the compounds were between 82 to 100% PPB, hence they belongs to "chemicals strongly bound" category.

2.4. Metabolism Prediction

2.4.1. Cytochrome P450 2D6 (CYP2D6) Enzyme Inhibition Prediction

CYP2D6 enzyme is involved in metabolizing many chemicals including amphetamines and phenethylamines. But some metabolites bind to the CYP2D6 enzyme and form an inhibitory complex which stops the enzyme from functioning normally for over a week [19]. If two or more drugs

that compete for the same P450 are administered concomitantly, and one has a significant affinity for the P450, whether as a substrate or inhibitor, the metabolism of the victim drug may be inhibited causing its plasma levels to rise which may lead to undesirable toxic effects. Early identification of compound-CYP P450 interaction is critical as it enables us to design out this liability early in the lead optimization process. The CYP2D6 enzyme inhibition studies showed that the compounds (**8**), (**11**) and (**16**) were likely to inhibit CYP2D6 enzyme (ADME.CYP2D6.Prob >0.5), the rest of the compounds were non-inhibitors.

2.5. Toxicity Prediction

Consistent with the U.S. Food and Drug Administration (FDA) Critical Path Initiative, predictive toxicology software programs employing quantitative structure-activity relationship (QSAR) models are currently under evaluation for regulatory risk assessment and scientific decision support for highly sensitive endpoints such as carcinogenicity, mutagenicity etc.

2.5.1. Mutagenicity Prediction (Ames Test)

The Ames test [20] is used world-wide as an initial screen to determine the mutagenic potential of new chemicals and drugs. The test is also used for submission of data to regulatory agencies for registration or acceptance of many chemicals, including drugs and biocides. International guidelines have been developed for use by corporations and testing laboratories to ensure uniformity of testing procedures. Hence the mutagenicity was predicted and found that the following compounds were non-mutagens (**3**), (**4**), (**5**), (**6**), (**10**), (**11**), (**12**), (**13**), (**14**), (**15**) and (**17**). Whereas Cyclocurcumin (**16**) and rest other compounds were found to be mutagen.

2.5.2. Rodent Carcinogenicity Prediction

Valerio *et al.* [21] studied and evaluated the carcinogenic potential of small, organic, naturally occurring chemicals found in the human diet and found that the *in silico* QSAR analysis was capable of identifying the rodent carcinogenic potential of naturally occurring organic molecules with a high degree of sensitivity. Similarly, our analysis of diphenylheptanoids showed that the compounds (**8**), (**9**), (**10**), (**11**) and (**19**) were not having carcinogenicity on Mouse and Rat. The rest of the compounds were carcinogenic on Mouse and Rat (refer Table **2**).

2.5.3. Human Hepatotoxicity Prediction

Human hepatotoxicity prediction showed (**2**), (**8**) and (**9**), these three compounds were found to be non-toxic, the rest of the compounds (including Curcumin (**15**) and its derivatives) were likely to cause dose-dependent liver injuries (ADME.Hepatotox.Prob >0.5). This finding can be correlated with the laboratory findings that turmeric extracts can be toxic to the liver when taken in high doses or for a prolonged period of time [22, 23]. For this reason, turmeric products should probably be avoided by individuals with liver diseases, heavy drinkers, and those who take prescription medications that are hard on the liver. It may cause skin problems and also cause stomach ulcers if used for a long time [24].

Table 2. The Following Serial Numbers Corresponds to the Compounds Listed in Table 1. '+' Indicates the Presence, '-' Indicates the Absence of Carcinogenicity

S. No	Mouse	Rat
1	+	-
2	+	+
3	+	+
4	+	+
5	-	+
6	-	+
7	-	+
8	-	-
9	-	-
10	-	-
11	-	-
12	+	+
13	+	+
14	-	+
15	-	+
16	+	+
17	-	+
18	-	+
19	-	-
20	-	+

From these screens based on ADME properties the following compounds (**9**), (**8**) and (**2**) were identified and have the drug-like attributes considered necessary for successful development and the rest of the leads from the analysis data set have undesirable properties or demerits that would be suggestive of potential development problems.

(E)-1,7-diphenyl-3-hydroxy-1-hepten-5-one (Compound (**9**)) had good (slightly soluble to soluble) (level 3) BBB penetration (-0.52 <logBbR< 0.00) was at level '1' represents high penetration level, non-inhibitor of CYP2D6 (Prob. 0.465347), non-hepatotoxic (hepatotoxicity probability = 0.450331) and protein binding level '2'(>=95%). HIA level is out of 95% confidence region of absorption level. ANN based water solubility (42.36 mg/l), Buffer solubility (459.57), Caco-2 cell (40.36 x 10-6 cm/sec), MDCK (55.63 x 10^6 cm/sec), 96.32% HIA Absorption, CNS Active, PPB (95.11 %).

(E)-1,7-diphenyl-1-hepten-5-one (Compound (**8**)) had low solubility (level 2), BBB penetration (logBbR < -0.52) was at level '0' represents very high penetration level, an inhibitor of CYP2D6 (Prob. 0.673267), non-hepatotoxic (he-

patotoxicity probability = 0.364238) and protein binding level '2'(>=95%). ANN based water solubility (10.82 mg/l), Buffer solubility (119.00), Caco-2 cell (56.51 x 10-6 cm/sec), MDCK (71.538 x 10^6 cm/sec), 100% HIA Absorption, CNS Active, PPB (98.38%).

(**1E, 3E)-1,7-diphenyl-1,3-heptadien-5-one** (Compound (**2**)) had low solubility (level 2), BBB penetration (logBbR < -0.52) was at level '0' represents very high penetration level, non-inhibitor of CYP2D6, non-hepatotoxic (hepatotoxicity probability = 0.357616) and the PPB level '1'(>=90%). ANN based water solubility (13.20 mg/l), Buffer solubility (327.07), Caco-2 cell (56.50 x 10-6 cm/sec), MDCK (71.73 x10^6 cm/sec), 100% HIA Absorption, CNS Active, PPB (95.98%).

From the above three selected compounds based on toxicity prediction the compounds (**E)-1,7-diphenyl-3-hydroxy-1-hepten-5-one** (Compound (**9**)) and (**E)-1,7-diphenyl-1-hepten-5-one** (Compound (**8**)) were predicted as drugable and the activity prediction of these compounds were given in the Table**s 3** & **4**. Leukotriene C4 antagonist is a common activity for both of the drugable compounds, for a drug to exert an effect in the brain, it must penetrate and pass through the blood-brain barrier. For travel across the barrier by a passive diffusion process, a molecule must have a markedly lipophilic solvent partition coefficient [17]. This can be exactly correlated with the predicted BBB permeability for both the compounds.

Table 3. 13 Possible Activities at Pa > 70% for the Compound (E)-1,7-diphenyl-3-hydroxy-1-hepten-5-one

Pa	Activity
0,827	Pulmonary hypertension treatment
0,779	3-Oxoadipate enol-lactonase inhibitor
0,769	Cholesterol synthesis inhibitor
0,764	Phosphoenolpyruvate-protein phosphotransferase inhibitor
0,742	Styrene-oxide isomerase inhibitor
0,737	(-)-(4S)-limonene synthase inhibitor
0,731	Fibrinolytic
0,722	Leukotriene C4 antagonist
0,717	Vasodilator, peripheral
0,711	CYP4A11 substrate
0,708	Antiinflammatory, intestinal
0,703	Pyruvate decarboxylase inhibitor
0,700	D-xylulose reductase inhibitor

3. CONCLUSION

The aim of this study was to find the most efficacious molecule of the class 'diphenylheptanoids' from turmeric is achieved using *in silico* tools. This resulted in identifying a couple of drugable compounds, (**E)-1,7-diphenyl-3-hydroxy-1-hepten-5-one** and (**E)-1,7-diphenyl-1-hepten-5-one,** they are non-carcinogenic and non-hepatotoxic but at the same time have desired pharmacokinetic profile. Discovery leads that do not have the characteristics necessary to become therapeutic agents have been identified early and prevented (i.e., the fail early, fail fast approach) from entering the development process. The result of these analyses will allow the preclinical development studies to be designed and conducted in a timely, cost-efficient manner and thus most likely allow the candidate to have an earlier entry into the clinic.

Table 4. 11 Possible Activities at Pa > 70% for the Compound (E)-1,7-diphenyl-1-hepten-5-one

Pa	Activity
0,905	Mucomembranous protector (Antiulcerative)
0,898	3-Oxoadipate enol-lactonase inhibitor
0,884	Sigma receptor agonist
0,874	(-)-(4S)-limonene synthase inhibitor
0,834	Leukotriene C4 antagonist (Antiasthmatic/Antidepressant / Psychotropic)
0,797	Vanillyl-alcohol oxidase inhibitor
0,799	Carminative
0,783	Leukotriene C antagonist (Antiallergic)
0,762	Prostaglandin E1 antagonist (Analgesic, non-opioid)
0,731	Trans-pentaprenyltranstransferase inhibitor
0,730	Amyotrophic lateral sclerosis treatment

4. EXPERIMENTAL SECTION

The chemical structures of the class 'diphenylheptanoids' from turmeric were collected from the NCBI PubChem database (Table **1**) and were drawn by using ACD/Chemsketch. The drawn structures were used to calculate chemical descriptors, drugability and the ADME/Tox properties derived from chemical structures using DS Accord for Excel Ver 6.0 [Accelrys, Inc., Release 6.0, (2006), San Diego.] and PreADMET Ver 1.0 (http://preadmet.bmdrc.org/preadmet) respectively.

4.1. Physicochemical Property Prediction

4.1.1. Aqueous Solubility

Aqueous solubility was predicted for the analysis data set by using the modules ADME.AQ.SOL.LOG (The base 10 logarithm of the molar solubility as predicted by the regression) and ADME.AQ.SOL.LOG.LEV (Categorical solubility level).

4.1.2. Lipinski's Rule of Five (RO5)

The compounds were checked for their oral bioavailability using Lipinski's scoring functions [7]. The scoring function used to assess drugability of the compounds is shown in Table **5**.

Table 5. Lipinski's Rule of Five

Parameter	Cut-Off Values
Log P	≤ 5
H-Bond Donors	≤ 5
H-Bond Acceptors	≤ 10
Molecular Weight	≤ 500

4.2. Absorption Prediction

4.2.1. Caco-2

For determining the potential intestinal absorption and secretion of discovery leads, the most commonly used *in vitro* assay system employs Caco-2 cells, which were originally derived from a human colorectal carcinoma a well-characterized *in vitro* model of intestinal drug absorption [25].

4.2.2. Madin-Darby Canine Kidney (MDCK)

MDCK is a cell line derived from the kidney used as a possible tool for assessing the membrane permeability properties of early drug discovery compounds [25]. MDCK is a cell based ANN descriptor for permeability screening MDCK ($x10^6$ cm/sec).

4.2.3. Skin Permeability

An ANN model was used to check the ability of the leads to penetrate through the skin and thus to reach systemic circulation. *In vitro* skin permeability was predicted and the result is given as log*Kp*. *Kp* (cm/hour) is defined as: Kp= Km*D/h. where *Km* is distribution coefficient between stratum corneum and vehicle, and *D* is average diffusion coefficient (cm^2/h), and *h* is thickness of skin (cm). This model predicts the leads that have delivery across the skin so as to find an alternate route of administration and thus may have lower exposure to potential organs or tissues.

4.2.4. Human Intestinal Absorption

In the Human Intestinal Absorption (HIA) chart, fast polar surface area (FPSA) and AlogP98 are set as the x and y axes. Ellipses are also drawn to show 95% and 99% confidence regions for absorption level.

4.3. Distribution Prediction

4.3.1. Blood-Brain Barrier (BBB)

BBB penetration levels were predicted by using fast polar surface area (2D.FPSA), Atom-based logP from Fast Desc (A.LogP98) and the Mahalanobis distance (T2) for the compound in the ADME_PSA_2D, ADME_AlogP98 plane. This distance is referenced from the center of the region of chemical space defined by known brain penetrating compounds. Base 10 logarithm of (brain conc) / (blood conc) as predicted by a robust (least-median-of-squares) regression derived from literature *in vivo* brain penetration data. The regression is based on ADME.BBB.Log and ADME.FST. AlogP98. The categorical level is shown in Table **6**.

Table 6. ADME.BBB.Log.Lev (Categorical Level)

Level	Value	Meaning
0	Very high	Brain-Blood ratio > 5:1
1	High	Brain-Blood ratio between 1:1 and 5:1
2	Medium	Brain-Blood ratio between 0.3:1 and 1:1
3	Low	Brain-Blood ratio less than 0.3:1
4	Undefined	Outside 99% confidence ellipse

4.3.2. Plasma Protein Binding

The plasma protein binding model predicts whether a compound is likely to be highly bound to carrier proteins in the blood. It makes this decision based on AlogP98 and 1D similarities to 2 sets of "marker" molecules. One set of marker is used to flag binding at a level of 90% or greater and the other set is used to flag binding at a level of 95% or greater.

4.4. Metabolism Prediction

4.4.1. Cytochrome P450 2D6 (CYP2D6) Enzyme Inhibition

ADMET_CYP2D6 model predicts CYP2D6 enzyme inhibition using 2D chemical structure as an input. The model was developed from known CYP2D6 inhibition data on a diverse set of 100 compounds. An ensemble of recursive partitioning trees was trained against 2D descriptors and 1D similarity data. The model classifies compounds as either 0 or 1 for non-inhibitor or inhibitor respectively and provides an average class-value estimate of confidence.

4.5. Toxicity Prediction

4.5.1. Ames Mutagenicity Assay

Mortelmans and Zeiger [20] described a short-term bacterial mutation assay caused by chemical substances. According to the data set of National Toxicology Program (NTP), the built biological model for toxicity prediction includes 3 strains: TA98, TA100, and TA1535.

4.5.2. Rodent Carcinogenicity

The Predictive Toxicology Challenge (PTC) was initiated for the development of advanced technology for predictive toxicology models. We have used computational models for carcinogenicity prediction created by Helma and Kramer [26] with the data set of both National Toxicology Program (NTP) [27] and Food and Drug Administration (FDA).

4.5.3. Hepatotoxicity

The hepatotoxicity model predicts potential organ toxicity for a wide range of structurally diverse compounds. The model was developed from literature data of 382 compounds known to exhibit liver toxicity (positive dose-dependent hepatocellular, cholestatic, neoplastic, etc.) or trigger dose-related elevated aminotransferase levels in more than 10% of humans. An ensemble of recursive partition trees were trained against AlogP98 and 1D similarity data. Accuracy against 54

compounds reserved for testing was 80%. The model classifies compounds as either 0 or 1, meaning either "non-toxic" or "toxic", and provides an average-class-value estimate of confidence.

4.6. Activity Prediction

The screened compounds were also checked for their possible biological activities by using PASS (Prediction of Activity Spectra for Substances), which predicts more than 300 biological activities and biochemical mechanisms on the basis of the structural formula of a substance [28] (Availability: http://www.ibmh.msk.su/PASS). Activity Scoring: The scoring functions used to check the biologically active substances are given below.

If the predicted activity (Pa) > 0.7, the substance is very likely to exhibit the activity in experiment and the chance of the substance to be the analogue of a known pharmaceutical agent is also very high. If 0.5<Pa<0.7, the substance is likely to exhibit the activity in experiment and the probability is less, and the substance is unlike to be a known pharmaceutical agent. If Pa <0.5, the substance is unlikely to exhibit the activity in experiment. However, if the presence of this activity is confirmed in the experiment, the substance might have a new chemical activity.

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