Prediction of Toxicity and Pharmacological Potential of Selected Spice Compounds

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ABSTRACT

The use of computational tools in the prediction of ADME/Tox properties of compounds is growing rapidly in drug discovery as the benefits they provide in high throughput and early application in drug design are realized. Numerous examples exist of drugs that have had to be withdrawn, because of unacceptable toxicity, in clinical trials and even after reaching the market. In this study phytochemicals from selected spices were used to predict their rodent carcinogenicity, mutagenicity, PPB and BBB. Out of 108 compounds analysed, we found that only five compounds as non-mutagenic and non-carcinogenic and all the remaining were toxic in a pharmacological perspective. The five non-toxic compounds are alpha-zingiberene, delphinidin, laurotetanine, malabaricone-B and malabaricone-C. The PPB values of alpha-zingiberene, delphinidin and laurotetanine are in the <90% range (57.58, 88.41, 52.59, respectively) indicating that the three compounds were weakly bound to plasma proteins and the other two (malabaricone-B and malabaricone-C) strongly binds to plasma protein. The identification of delphinidin as a naturally occurring inhibitor of VEGF (vascular endothelial growth factor) receptors suggests that this molecule possesses important antiangiogenic properties that may be helpful for the prevention and treatment of cancer. The healing activity of malabaricone B and malabaricone C, the major antioxidant constituents of Myristaceae family, against indomethacin-induced gastric ulceration in mice has been studied. Though spices are well known for their antioxidant, antimicrobial, antinflammatory properties etc., this study clearly indicates the plethora of carcinogenic behaviour of spice compounds.

Categories and Subject Descriptors

A.0 [General]: Conference proceedings

General Terms

Experimentation.

Keywords

Phytochemicals, ADME/T prediction, Rodent carcinogenicity, Mutagenicity, Plasma-Protein Binding and Blood–Brain Barrier Penetration

1. INTRODUCTION

The use of computational tools in the prediction of ADME/Tox properties of compounds is growing rapidly in drug discovery as the benefits they provide in high throughput and early application in drug design are realized. There is an increasing range of models available (QSAR, SAR, etc), as model builders have advanced from the "first generation" models, which were predominantly focused towards solubility, absorption and metabolism, to include models of other optimization factors such as HERG, glucoronyl transferase and drug transport proteins. The search for new lead compounds which has concentrated on the required activity, with considerations of bioavailability and toxicity being left until later in the development process. Numerous examples exist of drugs that have had to be withdrawn, because of unacceptable toxicity, in clinical trials and even after reaching the market. If as many as possible of these expensive failures can be identified and eliminated early in the drug discovery process, there is considerable scope for improving the efficiency and cost-effectiveness of the industry. The maxim 'Fail early, fail fast, fail cheaply' is now firmly embedded in the minds of all drug discovery research managers. Since a typical drug takes 10–12 years and costs up to US$500 million to reach the market, it is clearly important to discover potential toxicity as soon as possible. Nowadays the advent of cheminformatics tools and accuracy in predicting the toxicity *in silico*, the cost is reduced drastically. Numerous *in vitro* methods have been used in the drug selection process for assessing the intestinal absorption of drug candidates. Among them, Caco-2 cell model [13] and MDCK (Madin-Darby canine kidney) cell [11] model has been recommended as a reliable *in vitro* model for the prediction of oral drug absorption. Additionally, *in silico* HIA (Human Intestinal Absorption) model and skin permeability model can predict and identify potential drug for oral delivery and transdermal delivery. In distribution, BBB (Blood Brain Barrier) penetration can give information of therapeutic drug in the central nervous system (CNS), plasma protein binding model in its disposition and efficacy. In an increasingly time and cost-conscious industry, the early estimation of the BBB permeation of drug candidates is vital in prioritizing compounds for further development. In the case of CNS-targeted drugs, signs of good BBB permeation will be sought; conversely, for systematically targeted drugs, minimal BBB permeation will help reduce the likelihood of CNS side-effects, such as the sedative effect observed in early generation

anti-histamines [21]. For this reason, there has been great interest in recent years in the computational prediction of BBB permeation, which offers the possibility of assessing compounds even prior to synthesis. As in ADME, toxicity grabs the most attention from pharmaceutical companies and lead developers. The *in silico* toxicity prediction will have greater importance in early drug discovery since 30% of drug candidates fail owing to these issues. In this paper we have analyzed 108 selected spice compounds for their carcinogenic, mutagenic, PPB and BBB penetration level.

Spices are dried seed, fruit, root, bark or vegetative substance used in nutritionally insignificant quantities as a food additive for the purpose of flavoring by killing or preventing the growth of harmful bacteria [6]. Culinary herbs and their essential oils have been used extensively for many years in food products, perfumery, and dental and oral products due to their different medicinal properties [19]. There are reports that clove, cinnamon, bishop's weed, chilli, horse raddish, cumin, tamarind, black cumin, pomegranate seed, nutmeg, garlic, onion tejpat, cellary cambodge have potent antimicrobial activity against *Bacillus subtillis*, *Esherichia coli* and *Saccharomyces cerevisiae* [9]. Since the 1960s experience in medicinal chemistry has shown that the rigorous application of quantitative structure activity relationship (QSAR) methods to homogeneous classes of chemicals inducing the same type of biological activity permits that formulation of efficient quantitative models. These QSAR models contribute both to the elucidation of the action mechanisms and to the prediction of the biological activity of yet untested chemicals. The use of QSAR methods has been exported from medicinal chemistry, where they presently constitute a basic building block in the design of new drugs, to the study of biological activities, including toxicity.

1.1 Rodent Carcinogenicity

The objectives of carcinogenicity studies are to identify a tumorigenic potential in animals and to assess the relevant risk in humans. Any cause for concern derived from laboratory investigations, animal toxicology studies, and data in humans may lead to a need for carcinogenicity studies. The practice of requiring carcinogenicity studies in rodents was instituted for pharmaceuticals that were expected to be administered regularly over a substantial part of a patient's lifetime. The carcinogens can be classified into: (i) genotoxic carcinogens, which damage DNA (mutation is one of the first steps in the development of cancer as a result of these chemicals) and (ii) epigenetic carcinogens, which do not bind covalently to DNA, do not directly cause DNA damage, and are usually negative in the mutagenicity assays [22]. Computational methods can be useful when existing experimental data are insufficient, unreliable, unavailable, or inconsistent between studies. This approach reduces animal testing, facilitates the review process and also has applicability for the toxicological evaluation of chemically identified individual components of botanical mixtures, or chemicals of natural origin that have not been subjected to in vivo testing.

1.2 Mutagenicity

Mutagenic toxicity, the capacity of a substance to cause genetic mutations, is of high public concern because it has a close relationship with carcinogenicity and other health problems [5]. In experiments, mutagenic toxicity can be assessed by various test systems. In drug/ pesticide discovery, identification of compounds which cannot be candidates, because of their carcinogenicity or mutagenicity as early as possible, even before they are synthesized, is imperative. Many computational models based on structure, mutagenicity relationships had been developed [3],[23].

1.3 Plasma-Protein Binding (PPB)

It is generally assumed that only the free drug can cross membranes and bind to the intended molecular target [18] and it is therefore important to estimate the fraction of drug bound to plasma proteins. Drugs can bind to a variety of particles in the blood, including red blood cells, leukocytes and platelets, in addition to proteins such as albumin (particularly acidic drugs), α-1-acid glycoproteins (basic drugs), lipoproteins (neutral and basic drugs), erythrocytes and α,β,γ-globulins. There have been relatively few attempts at modeling plasma protein binding (PPB), and most of those reported have focused on human serum albumin (HSA), which is the most abundant protein in plasma, although certainly not the only one responsible for PPB.

1.4 Blood-Brain Barrier Penetration (BBB)

Blood brain barrier prevents the entry of toxic blood molecules into the central nervous system, while allowing the circulation of adequate amounts of arterial blood through brain tissue. But the drugs that act in the CNS need to cross the blood–brain barrier (BBB) to reach their molecular target. By contrast, for drugs with a peripheral target, little or no BBB penetration might be required in order to avoid CNS side effects. Equally important is that peripherally acting drugs do not penetrate the cerebrospinal fluid. It is desirable to determine whether a compound will penetrate and distribute within the CNS with the requisite pharmacokinetic and pharmacodynamic performance required for a CNS target or if it will be excluded from the CNS. As a result, a variety of *in vivo* and *in vitro* methods for assessing CNS penetration have been developed and applied to advancing drug candidates with the desired properties. However, such methods are inevitably resource-intensive and they are of course retrospective in their application, requiring the existence of synthesized compounds. *In silico* prediction methods address this limitation, supporting the prospective design and selection of candidate structures prior to synthesis. A variety of models for the prediction of uptake into the brain have been developed [20], [7-10], [12], [15-16]. 'Rule-of-five'-like recommendations regarding the molecular parameters that contribute to the ability of molecules to cross the BBB have been made to aid BBB-penetration predictions [20], for example, molecules with a molecular mass of <450 Da or with PSA <100 Å are more likely to penetrate the BBB. Most of the early predictive models are based on a multiple linear regression approach and many use physicochemical properties [1]. One example of such a model is based on the combination of only three descriptors, namely the calculated octanol / water partition coefficient, the number of hydrogen bond acceptors in an aqueous medium and the polar surface area [8]. Since the structure of molecules contains all the information needed to predict the partition between blood and brain fluids, models have been developed that identify the important characteristics resulting in BBB permeation.

2. MATERIALS AND METHODS

The structural data for the study were collected from PubChem - National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/sites/ entrez?db=pccompound) to predict the rodent carcinogenicity, mutagenicity, PPB and BBB of selected phytochemicals from Allspice, Black pepper, Cinnamon, Clove, Garcinia and Nutmeg. A total of 108 compounds were collected. Majority of the compounds' biological activity had been predicted earlier. The online ADME/T predicting server facility, PreADMET (http://preadmet.bmdrc.org/) was used to calculate the above said parameters. The server predicts a compound's mutagenicity to *Salmonella strains* TA98, TA100 and TA1535, which are often used in Ames test [2] and the result can be calculated both with consideration of metabolite (metabolic activation by rat liver 10% homogenate, +S9) and without consideration of metabolite (no metabolic activation, -S9). The actual value of the prediction result is "positive" or "negative". The carcinogenicity was predicted based on the result from its model, which is built from the data of NTP (National Toxicology Program) and US FDA, which are the results of the *in vivo* carcinogenicity tests of mice and rats for two years. PPB of a drug influences not only on the drug's action but also its disposition and efficacy. PPB represents the binding ability of the molecule to the plasma protein in percentage. Strongly bound chemicals will give the value >90% and weakly bound, <90%. BBB data, BB(C_{brain}/C_{blood}) represents the absorption to CNS. The absorption is classified into three, high (value >2.0), medium (<1.0 value >2.0) and low (value <1.0).

3. RESULTS AND DISCUSSION

The utilization of plant materials to protect field crops and stored commodities against insect attack has a long history. Numerous therapeutic activities and toxicities were detected in volatile or non-volatile compounds of spices. A large body of evidence has accumulated to demonstrate the promising potential of medicinal plants used in various traditional, complementary and alternative systems. Several Indian medicinal plants have been studied for pharmacological activity in recent years. Here we have collected and predicted their PPB, BBB, mutagenicity and carcinogenicity using PreADMET server (**Table 1**).

Table 1. Predicted values for carcinogenicity, mutagenicity, PPB and BBB of 108 compounds from selected spices.

Chemical Compound	Spices	Ames test	Carcinogenicity (Mouse)	Carcinogenicity (Rat)	PPB (%)	BBB
(E)-alpha-Bergamotene	N	Non-mutagen	+	+	100	13.136
(E)-beta-Farnesene	C	Non-mutagen	+	+	100	21.70
(E)-beta-Ocimene	C	Mutagen	+	+	100	8.863
(E)-Cinnamyl-acetate	C	Mutagen	-	+	83.04	2.115
(E)-Methyl-cinnamate	C	Mutagen	-	-	66.96	1.461
(Z)-beta-Ocimene	C	Mutagen	+	+	100	8.863
(Z)-Hexenyl-benzoate	A,B	Mutagen	+	-	90.53	0.524
1,8-Cineole(eucalyptol)	B	Mutagen	+	+	100	1.467
1-Terpinen-4-ol	B,N	Mutagen	+	-	100	5.54
2,3 Diethyl-5-methyl pyrazine	B	Mutagen	-	-	97.26	1.612
2-Phenylethanol	C	Mutagen	+	-	6.66	1.478
2-Phenylethyl-propionate	C	Mutagen	-	-	68.21	1.404
6-Methyl-5-hepten-2-one	C	Mutagen	-	-	37.79	0.832
alpha-Amorphene	CL,B	Mutagen	+	+	100	13.291
alpha-cis-Bergamontene	B	Non-mutagen	+	+	100	13.136
alpha-Curcumene	B,	Mutagen	+	-	100	14.594
alpha-Humulene	A,B, C,CL,N	Non-mutagen	+	+	100	14.222
alpha-Phellandrene	A,B,C,N	Mutagen	+	+	100	7.170
alpha-Santalene	A,B	Non-mutagen	-	+	62.13	10.962
alpha-Selinene	A,B	Mutagen	-	+	100	13.989
alpha-Terpinene	A,B,C,N	Mutagen	+	+	100	8.037
alpha-Terpineol	A,C,CL,N	Mutagen	-	-	23.42	5.119
alpha-Thujene	A,C,N	Mutagen	-	+	100	5.533
alpha-trans-Bergamontene	B	Non-mutagen	+	+	100	13.136
alpha-Zingiberene	B,CL	Non-mutagen	-	-	57.58	0.035
Astragalin	B,CL	Mutagen	+	-	91.41	1.065
beta-Bisabolene	B,N	Mutagen	-	+	100	15.064
beta-Bisabolol	B	Non-mutagen	+	-	100	10.709
beta-Elemene	A,B,C	Mutagen	-	+	100	13.436

beta-Phellandrene	A,B,N	Mutagen	-	+	91.20	7.41
beta-Pinene	A,B,CL,N	Mutagen	-	+	100	5.756
beta-Sesquiphellandrene	N	Mutagen	+	+	100	20.193
beta-Sitosterol	A,C,N	Non-mutagen	+	-	100	19.888
Bicyclogermacrene	C	Non-mutagen	+	+	100	13.136
Biflorin	CL	Mutagen	+	-	100	8.120
Borneol	B,N	Mutagen	-	+	100	3.806
Bulnesol	B	Mutagen	-	-	85.10	9.556
Camphene	B,N	Mutagen	-	+	100	5.756
Camphor	B,N	Mutagen	-	+	100	0.867
Carvacrol	B	Mutagen	-	-	100	6.388
Caryophyllene oxide	B	Mutagen	+	+	90.85	3.752
cis-beta-ocimene	B	Mutagen	+	+	100	8.863
cis-carveol	B	Mutagen	+	-	57.95	5.079
cis-linalol-oxide	C	Mutagen	+	+	20.75	1.338
cis-sabinene hydrate	B	Mutagen	-	+	35.94	3.616
Citirc acid	G	Mutagen	-	+	4.22	0.065
Cubenol	B,CL	Mutagen	+	-	100	9.577
Cuminaldehyde	B,CL	Mutagen	-	-	93.44	1.128
Dehydrodiisoeugenol	N	Mutagen	-	+	99.69	1.911
Delphinidin	B,N	Non-mutagen	-	-	88.41	0.052
delta-3-Carene	B	Mutagen	-	+	100	5.533
delta-Cadinene	B	Mutagen	+	+	100	13.926
delta-Carene	CL	Mutagen	-	+	100	5.533
delta-Elemene	B	Mutagen	-	+	100	13.255
Dihydrocarveol	B	Mutagen	-	-	34.04	5.645
Dihydrocarvone	B	Mutagen	-	+	49.40	1.086
Elemicin	B,N	Mutagen	+	+	96.78	1.206
Estragole	C	Mutagen	+	-	100	1.512
Fenchone	C,CL	Mutagen	-	+	100	1.058
Gambogic-acid	G	Non-mutagen	+	-	91.35	0.14
gamma-decalactone	CL	Mutagen	-	+	100	13.472
gamma-terpinene	B,N	Mutagen	+	+	100	8.037
Garcinol	G	Mutagen	+	-	100	7.603
Geraniol	B,N	Mutagen	+	-	100	6.741
Germacrene D	B,N	Mutagen	+	+	100	14.518
Glyceryl-trimyristate	B,N	Non-mutagen	+	+	100	14.090
Hedycaryol	B	Non-mutagen	-	+	98.34	16.314
iso Caryophyllene	B	Mutagen	-	+	100	13.319
iso Elemicin	B	Mutagen	+	+	87.76	1.23
iso Borneol	B	Mutagen	-	+	100	3.806
iso obtusilactone	CL	Non-mutagen	+	+	100	6.121
iso obtusilactone-a	CL	Non-mutagen	+	+	100	9.828
Kaempferol	CL	Mutagen	-	+	89.61	0.286
Laurotetanine	CL	Non-mutagen	-	-	52.59	1.001
Ledene	A,B	Non-mutagen	-	+	100	11.147
Limonene	A,C,N	Mutagen	-	+	100	8.278
Linalool	A,C,CL,N	Mutagen	-	+	100	6.125

Malabaricone-b	N	Non-mutagen	-	-	100	3.648
Malabaricone-c	N	Non-mutagen	-	-	100	1.716
Methyl carvacrol	B	Mutagen	+	-	100	2.393
Methyl eugenol	A,B	Mutagen	+	+	100	1.109
Methyl heptanoate	B	Mutagen	+	+	100	0.825
Myrcene	A,B,C,N	Mutagen	-	+	100	9.102
Myristicin	N	Mutagen	+	+	96.98	1.299
Nerol	B,C,N	Mutagen	+	-	100	6.741
n-nonadecane	B	Non-mutagen	-	+	100	25.521
n-tridecane	B	Non-mutagen	-	+	100	22.434
o-methoxycinnamaldehyde	C	Mutagen	-	+	83.09	1.556
p-coumaric-acid	C	Mutagen	-	+	63.06	0.695
p-cymene	A,B,C,N	Mutagen	+	-	100	4.97
Phellandral	B	Mutagen	+	-	100	1.523
Piperidine	B	Mutagen	+	+	86.77	1.382
Piperitone	C,B	Mutagen	+	+	100	1.410
Piperonal	B	Mutagen	-	+	41.85	1.644
P-methyl acetophenone	B	Mutagen	-	-	25.34	1.226
Sabinene	A,B,N,C	Mutagen	-	+	60.97	5.756
Safrole	B,N,C	Mutagen	+	+	100	1.117
Spathulenol	A	Mutagen	+	+	82.89	6.966
Succinic-acid	G	Mutagen	-	+	76.78	0.241
Tartaric-acid	G	Mutagen	-	+	28	0.247
Terpinolene	B,N	Mutagen	+	+	93.16	8.904
T-muurolol	A,B	Mutagen	-	-	100	9.219
trans-Anethole	A,B	Mutagen	+	-	89.24	1.470
trans-Carveol	B	Mutagen	+	-	57.95	5.079
trans-Piperitol	N	Mutagen	+	-	100	5.900
trans-Sabinene hydrate	B	Mutagen	-	+	35.94	3.616
Viridifloral	B	Non-mutagen	-	+	100	7.566
Zingiberene	B	Mutagen	+	+	100	15.195

Note: '+' indicates the presence and '-' indicates the absence of carcinogenicity.

Spices Name: A= Allspice, B=Black pepper, C= Cinnamon, CL= Clove, G= Garcinia, N= Nutmeg.

Of the above 108 compounds, five were non-mutagenic and non-carcinogenic, all others were toxic; either mutagenic or carcinogenic. The five non-toxic compounds are alpha-zingiberene, delphinidin, laurotetanine, malabaricone-B and malabaricone-C. Also the PPB values of alpha-zingiberene, delphinidin and laurotetanine are in the range of <90% (57.58, 88.41, 52.59, respectively) indicating that the three compounds were weakly bound to plasma proteins and the other two (malabaricone-B and malabaricone-C), strongly binds to plasma protein. By comparing the value of blood-brain barrier permeation of the five compounds with the already mentioned range, the absorbtivity – whether highly absorbed/ medium/ poorly absorbed – by the central nervous system can be assessed. The identification of delphinidin as a naturally occurring inhibitor of VEGF (vascular endothelial growth factor) receptors suggests that this molecule possesses important antiangiogenic properties that may be helpful for the prevention and treatment of cancer [14]. The healing activity of Malabaricone B and Malabaricone C, the major antioxidant constituents of Myristaceae family, against indomethacin-induced gastric ulceration in mice had reported recently [4].

This study was intended to make predictions on toxicity and pharmacological potential of the compounds; however, the accuracy of the predictions for human cancer risk remains unknown. Therefore the predictions made by the above approach may serve only as an aid in human health risk assessment with the knowledge that the prediction is not a direct estimate of human carcinogenic risk for a single organic molecule. In the context of a new methodology for evaluating carcinogenic risk to naturally occurring dietary chemicals, the predictions in this study should be used to estimate rodent carcinogenic potential within appropriate safety and risk analysis paradigms for assessing human health-based effects, and as a decision support tool so that priorities for further testing may be set.

4. CONCLUSION

The ultimate goal of computational research into ADMET especially, the plasma protein binding affinity, blood brain

barrier penetration is to be able to identify compounds liable to failure at a later stage before they are even synthesized, resulting in even greater efficiency. The relevance of the results obtained from the selected spice compounds for the assessment of carcinogenicity and mutagenicity revealed the risk of consumption of high amounts of natural products, certain food additives and dietary constituents. Even though spices are well known for their antioxidant, antimicrobial, antinflammatory properties etc., this study indicates the plethora of carcinogenic behavior of spice compounds. Further studies are required to elucidate the mode of action to confirm the carcinogenic and mutagenic potential of the compounds, which can in future be avoided for further research in drug discovery.

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