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Research Article

vNN model cross validation towards Accuracy, Sensitivity, Specificity and *kappa* performance measures of β-caryophyllene using a restricted-unrestricted applicability domain on Artificial Intelligence & Machine Learning approach based *in-silico* prediction of ADMET properties

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Abstract

Wide-reaching interest in use of plant derived secondary metabolites (PDSMs) has been growing world over. This implies that the beneficial effects of plant based natural products (PBNPs) being rediscovered and harnessed for the development of novel drugs; drug-leads and structural background for drug design. Survey of literature on the indigenous use of traditional medicinal knowledge bestows ethnopharmacological potentials of PBNPs that has inspired drug design and development through research in drug discovery; PBNPs provide baseline for the development of drug leads against various pharmacological targets. Studies indicate that *Cymbopogon martini* Essential Oil (CMEO) exhibits a wide range of biological activities (anticancer, antidiabetic, antibacterial, antifungal, antioxidant, hepatoprotective and insecticide) attributed to the presence of bio-active secondary metabolites (BASM). However, wide-spread application of CMEO is limited because of typical odor, color and taste. In the present study, *In-silico* Absorption, Distribution, Metabolism, Elimination, Toxicity (ADMET) studies of β-caryophyllene (BCP) from *Cymbopogon martinii* is envisaged. β-caryophyllene enhances wound healing through multiple routes therefore, Non AMES toxic, Non-carcinogens, and biodegradable nature of β-caryophyllene prospects this compound as considered as an apt lead for the development of drugs for the treatment of cuts and wounds besides other diseases viz., diabetics, cancer, metabolic inflammation and other long term complications associated with these diseases. In particular, BCP inhibits cytochrome P450 isoforms (CYP3A4) that metabolize xenobiotics and results in adverse effects. Pharmacokinetic properties and predicted score values of β-caryophyllene have highlighted that both pharmacological and toxicological aspects need to be further investigated before put to clinical trials.

Keywords: ADMET; β-caryophyllene; *Cymbopogon martinii;* Cardiotoxicity; Cytotoxicity; Drugability; Pharmacokinetics; Bioactivity; Essential Oil;

INTRODUCTION

Cymbopogon is widely distributed in tropical and subtropical regions of Africa, Asia and America. Genus *Cymbopogon* comprises of more than 144 species, and is well known for its essential oils[1-4]. Studies have led to the isolation of alkaloids, volatile and non-volatile terpenoids, flavonoids, carotenoids and tannins from *Cymbopogon* spp. *Cymbopogon martinii* native to India and China is cultivated for its aromatic essential oil. Popular as Palmarosa, this plant is known for its typical aroma and therapeutic application, commonly used as condiment and food preservative. CMEO BASMs are endowed with splendid pharmacological properties[5]. CMEO is of commercial importance and is being extensively used in perfumes, soaps, cosmetics and toiletry products[6]. CMEO is used as an effective insect repellent when applied on stored grain and beans[7], anti-helminthic against nematodes[8], antifungal[9] and mosquito repellent[10]. In Ayurvedic medicine, it is used to treat skin problems and relieve nerve pain. Reports indicate that immunomodulatory action of CMEO had been evaluated towards production of pro- and anti-inflammatory cytokines (TNF-α and IL-10) by human monocytes *in vitro*[11].

CMEO contains bioactive compounds of GRAS nature. Due to its overwhelming physiochemical and biological properties CMEO has been used in aromatherapy as volatile phyto-pharmaceuticals, flavor and fragrances agent in food and cosmetic industries, and more recently, natural preservatives/ additives in biopesticides[12,13]. CMEOs are concentrated form of liquid mixtures of volatile phyto-compounds with unique structural chemistry comprising of terpenoid and non-terpenoid hydrocarbons and their oxygenated derivatives, natural color, odor and flavor[14]. Furthermore, concern about the negative effect of synthetic chemicals as food additives warrants "GO" products with no or lesser side effects.

Interest in natural products as alternatives for synthetic additives is attributed to (a) their synergy with other preservation methods (b) generally regarded as safe, and (c) PBNPs are endowed with antioxidant, antidiabetic, antimutagenic, antitoxigenic and antibacterial properties[15,16] CMEO has proven fungicidal effect against pathogenic fungi and yeasts, including *Aspergillus* spp., *Candida albicans*, *Monilia sitophila* and *Trichophyton tonsurae*[16]. In Ayurvedic medicine - Charak gave the decoction of CM to treat abdominal, spleen, liver disorders, jaundice, and fever. In Sushruta, decoction of CM is prescribed in inflammation of throat, chest pain, indigestion, bronchitis, cough and asthma[17].

BCP possesses pharmacological activities ranging from pain killer to neurological/ metabolic disorders. Chemically, sesquiterpenoids, a group of PBSM consists of 15 carbon atom backbone with a wide array of chemical structures[18]. Among them, *β*-caryophyllene is the major component in *Cannabis sativa, Chenopodium ambrosioides, Cinnamomum* spp*., Citrus myrtfolia, Cymbopogon* spp*., Mentha piperita, Ocimum* spp*., Origanum vulgare, Piper nigrum, Rosmarinus officinalis, Salvia officinalis, Syzygium aromaticum, Thymus vulgaris, Zingiber officinale*[19]. BCP mainly occurs as *trans*-caryophyllene ((E)-BCP) mixed with small amount of isomers (Z)-*β*-caryophyllene ((Z)-BCP) and *α*-humulene (*α*-caryophyllene) and its oxidation derivative, *β*-caryophyllene oxide (BCPO).

BCP belongs to cannabinoid family and cannabinoid receptors CB1-R and CB2-R (metabotropic receptors) of G protein (protein binding GTP)-coupled receptors, are involved in the regulation of neurotransmitters responsible for energy balance, through metabolism and immune response[20]. Aforementioned receptors are bound and activated by endogenous anandamide. Both receptors are bound to proteins, act as mediators of cellular responses to biomolecules. Expression of CB2-Rs in CNS is increased in neurodegenerative pathologies, such as Parkinson's disease, Alzheimer's disease[21], amyotrophic lateral sclerosis (ALS), glioma, spinal/ brain injuries, stroke, ischemia, anxiety, depression, colitis, fibrosis and liver ischemia, atherosclerosis, osteoporosis, osteoarthritis, diabetes, obesity and cancer[18].

BCP is the subject of interest in recent past, quantum of research about the effects *in vitro* and in *vivo* on animals have been conducted, and thus experimental data about its biological properties have been verified. However, in-depth studies still remain a major prerequisite to translate the findings in animal models into promising pre-clinical and clinical trials on humans. Pre-clinical studies have revealed that BCP is a modulator of nervous system and exerts beneficial effects on neurodegenerative and inflammatory pathologies[18]. Further, it has been proved that BCP/ BCPO significantly influence microRNA that regulates gene expression, portraying the role of BCP/ BCPO as biomarkers of neuroinflammation[22].

In addition, BCP is involved in cell-to-cell communication, regulates functioning of blood–brain barrier (BBB), and modulates filament network/ repair brain injuries. BCP prevents apoptosis, by inhibiting the expression of Bax and caspase-3 and increasing Bcl-2 one[23]. It reduces phosphorylation of JNK (c-Jun N-terminal Kinase), that increases HO-1 (heme oxygenase-1) during pathological conditions. All these effects are related to CB2/Nrf2 pathway. BCP is cytoprotective towards CNS due to its modulation of redox state and reduce inflammation as chemotherapeutic agent[24]. BCP inhibits production of nitric oxide, hydrogen peroxide, TNF-α, IFN-γ, IL-17[25] thus reduce macrophage infiltration. BCP is useful in multiple sclerosis management, as demonstrated by murine model of multiple sclerosis (MS). BCP exhibits antidepressant properties by the activation of CB2-R[26].

BCP/ BCPO have shown cytotoxic activity against various cancer cell lines. In particular, BCP reduce proliferation of two colon cancer cell lines, HT-29/ HCT-116, and a pancreas cancer cell line, PANC-1. In the intestinal cancer cell line CaCo-2, BCP has not been able to exert a significant effect on cell growth, unlike *α*-humulene. BCP enhances effectiveness of antitumor drugs. In particular, it increases the activity of paclitaxel in lots of cell lines: MCF-7 (breast cancer), L-929 (mouse fibroblasts), DLD-1 (colon cancer)[27]. BCP according to OECD guideline 423 is a 5th category substance. No damage to the gastric mucosa has been observed, nor have changes in other internal organs (brain, heart, liver, lungs, spleen, and kidney) or in haematological parameters been reported. Moreover, Ames test has shown no mutagenicity, body weight decreased by 5% in female Swiss mice.

In fact, there exist Cytochrome P450-mediated pharmacokinetic interactions between herbs, food, and dietary supplements in cancer treatments and other metabolic disorders[28]. A significant inhibition by BCP has been reported against enzymes involved in metabolism and xenobiotic detoxification[18]. The extent of the inhibition of cytochrome P450 isoform CYP1A2 is greater in rat microsomes than in human, further BCP inhibits CYP3A4, a key cytochrome P450 isoform involved in metabolism of xenobiotics. Finally, terpenes exert a weak inhibition against CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1.

Owing to the growing importance of BCP, it is planned to evaluate the occurrence of this important endocannabinoid in the selected plant species. Therefore, CMEO was used as natural sources of BCP in order to provide the pharmaceutical, nutraceutical and aroma industries a summary of BCP from ADMET perspective with a view that ADMET profiling of BCP would pave way for the inclusion of BCP as food supplement and ensure compliance of similar botanicals in terms of quality and safety.

MATERIALS AND METHODS

Collection, Preparation and Analysis

Samples were collected from Alagar Hills, (Eastern Ghats), Madurai District, TamilNadu, INDIA during Nov 2021. The leaf samples were well preserved, taken to laboratory, identified by using flora[29,30] shade dried and processed as per the protocol previously described[30], however, with modifications in temperature and duration of processing of sample and GCMS was carried out.

ADMET Prediction

Drug-like features and Drug Score were evaluated using Molinspiration online tool (http://molinspiration.com/) while properties related to absorption, distribution, metabolism, excretion and toxicity (ADMET) were accessed using ADMET SAR2 web-server[31-33].

RESULTS AND DISCUSSION

Ethnobotanical studies coupled with the use of AI-driven solutions to enable pre-clinical drug discovery is growing within the pharmaceutical industry[34-51]. It has the potential to deliver across the drug discovery and development value chain, starting from target identification and reaching through clinical development. 2D, 3D structures, molecular and biological properties of β-caryophyllene in CMEO is given in Fig 1. Molecular and biological properties of β-caryophyllene depicts that the molecule contains 318.1 hydrogen atoms well within the optimal range of 100~600. Van der Waals volume of the molecule was estimated as 316.597 with a density value of 1.005. Number of hydrogen bond acceptors in the compounds was 5 whereas the Number of hydrogen bond donors was 0. Number of rotatable bonds and Number of rings in the molecule were 7 and 2 respectively. Toxic (mutagenic, toxicology, irritant, reproductive properties) risk assessment towards drugability/ drug score indicated that the compounds were neither mutagenic nor toxic to biological system. Performance measures of vNN models in 10-fold cross validation using a restricted/ unrestricted applicability domain is given in Table 1a, b. All the parameters studied were drugable and had good score for Druggability Properties of β-caryophyllene - Lipinski's rule of 5 violations` - 0; Veber rule - Good; Egan rule - Good; Oral PhysChem score (Traffic Lights) - 1; GSK's 4/400 score - Good; Pfizer's 3/75 score - Bad; Weighted quantitative estimate of drug-likeness (QEDw) score - 0.514; Solubility - 4277.90; Solubility Forecast Index – Good (Table 2). Pharmacokinetic properties, predicted score values of β-caryophyllene is given in Table 3.

Chemical kingdom	:	Organic compounds
Super class	:	Lipids and lipid-like molecules
Class	:	Prenol lipids
Subclass	:	Sesquiterpenoids
PubChem Identifier	:	5281515
ChEBI Identifier	:	10357
CAS Identifier	:	87-44-5
Canonical SMILES	:	C/C/1=CCCC(=C)[C@@H]2[C@@H](CC1)C(C2)(C)C
InChI Key	:	NPNUFJAVOOONJE-GFUGXAQUSA-N

ADMET properties of β-Caryophyllene

The implemented Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) prediction models, including their performance measures were predicted online.[33] The 15 models cover a diverse set of ADMET endpoints. Some of the models have already been published, including those for Maximum Recommended Therapeutic Dose (MRTD), chemical mutagenicity, human liver microsomal (HLM), Pgp inhibitor/ substrates.

Liver Toxicity - Drug-induced liver injury (DILI)

DILI has been one of the most commonly cited reasons for drug withdrawals from the market. This application predicts whether a compound could cause DILI. The dataset of 1,431 compounds was obtained from four sources used. This dataset contains both pharmaceuticals and non-pharmaceuticals; classified a compound as causing DILI if it was associated with a high risk of DILI and not if there was no such risk.

Cytotoxicity (HepG2)

Cytotoxicity is the degree to which a chemical causes damage to cells. Cytotoxicity prediction model was developed using *in vitro* data on toxicity against HepG2 cells for 6,000 structurally diverse compounds, collected from ChEMBL. In developing the model, compounds with an $IC_{50} \leq 10$ µM were considered in the *in vitro* assay as cytotoxic.

Human Liver Microsomal (HLM)

Human liver microsomal (HLM) stability assay is commonly used to identify and exclude compounds that are too rapidly metabolized. For a drug to achieve effective therapeutic concentrations in the body, it cannot be metabolized too rapidly by the liver. Compounds with a half-life of 30 min or longer in an HLM assay are considered as stable; otherwise they are considered unstable. HLM data was retrieved from the ChEMBL database, manually curated, and classified compounds as stable or unstable based on the reported half-life ($T_{1/2} > 30$ min was considered stable, and $T_{1/2} < 30$ min unstable. The final dataset contained 3,654 compounds. Of these, 2,313 compounds were classified as stable and 1,341 as unstable.

Cytochrome P450 enzyme (CYP) inhibition

CYPs constitute a superfamily of proteins that play an important role in the metabolism and detoxification of xenobiotics. *In vitro* data derived from five main drug-metabolizing CYPs - 1A2, 3A4, 2D6, 2C9, and 2C19 was used to develop CYP inhibition models. CYP inhibitors were retrieved from PubChem and classified a compound with an $IC_{50} \leq 10$ µM for an enzyme as an inhibitor of the enzyme. Predictions have been provided for the following enzymes: CYP1A2, CYP3A4, CYP2D6, CYP2C9, and CYP2C19 (Table 1).

Membrane Transporters - Blood-Brain Barrier (BBB)

Blood-brain barrier (BBB) is a highly selective barrier that separates the circulating blood from the central nervous system. vNN-based BBB model has been developed using 352 compounds whose BBB permeability values (log-BB) were obtained from the literature. Compounds were classified with log-BB values of less than −0.3 and greater than +0.3 as BBB non-permeable and permeable.

Pgp Substrates and Inhibitors

P-glycoprotein (Pgp) is an essential cell membrane protein that extracts many foreign substances from the cell. Cancer cells often overexpress Pgp, which increases the efflux of chemotherapeutic agents from the cell and prevents

treatment by reducing the effective intracellular concentrations of such agents—a phenomenon known as multidrug resistance. For this reason, identifying compounds that can either be transported out of the cell by Pgp (substrates) or impair Pgp function (inhibitors) is of great interest. Models were developed to predict both Pgp substrates and Pgp inhibitors. Pgp substrate dataset was collected with a dataset consisting of measurements of 422 substrates and 400 non-substrates. To generate a large Pgp inhibitor dataset, datasets were combined and duplicates were removed to form a combined dataset consisting of a training set of 1,319 inhibitors and 937 non-inhibitors.

hERG (Cardiotoxicity)

Human ether-à-go-go-related gene (hERG) codes for a potassium ion channel involved in the normal cardiac repolarization activity of the heart. Drug-induced blockade of hERG function can cause long QT syndrome, which may result in arrhythmia and death. As much as 282 known hERG blockers were retrieved from the literature and classified compounds with an IC_{50} cut-off value of 10 μM or less as blockers. A set of 404 compounds were collected with IC_{50} values greater than 10 μM from ChEMBL and classified them as non-blockers.

MMP (Mitochondrial Toxicity)

Given the fundamental role of mitochondria in cellular energetics and oxidative stress, mitochondrial dysfunction has been implicated in cancer, diabetes, neurodegenerative disorders, and cardiovascular diseases. Dataset of chemical-induced changes in mitochondrial membrane potential (MMP) was used based on the assumption that a compound that causes mitochondrial dysfunction is also likely to reduce the MMP. vNN-based MMP prediction model was developed using 6,261 compounds collected from a previous study that screened a library of 10,000 compounds (~8,300 unique chemicals) at 15 concentrations, each in triplicate, to measure changes in the MMP in HepG2 cells. Data depict that 913 compounds decreased the MMP, whereas 5,395 compounds had no effect.

Mutagenicity (Ames test):

Mutagens are chemicals that cause abnormal genetic mutations leading to cancer. A common way to assess a chemical's mutagenicity is the Ames test. The prediction model was developed, using a literature dataset of 6,512 compounds, of which 3,503 were Ames-positive. Details of the model and its performance rate indicate the molecule is Non-mutagenic (Table 3).

Maximum Recommended Therapeutic Dose (MRTD)

Maximum Recommended Therapeutic Dose (MRTD) is an estimated upper daily dose that is safe. A prediction model was built based on a dataset of MRTD values publically disclosed by FDA, mostly of single-day oral doses for an average adult with a body weight of 60 kg, for 1,220 compounds (most of which were small organic drugs). Organometallics were excluded, high-molecular weight polymers (>5,000 Da), nonorganic chemicals, mixtures of chemicals, and very small molecules (<100 Da). We used an external test set of 160 compounds that were collected by the FDA for validation. The total dataset for our model contained 1,185 compounds. The predicted MRTD value is 20 mg/day based upon an average adult weighing 60 kg.

β-Caryophyllene from CMEO is used in the treatment of colitis, osteoarthritis, diabetes, cerebral ischemia, anxiety and depression, liver fibrosis as the phytocompounds possess antitumor, antioxidant, anti-infectious, anti-inflammatory, and analgesic activities and effects on central nervous system, endocrine system, disorders such as cardiac remodeling after myocardial infarction, body weight changes, dyslipidemia, cerebral ischemia, hepato-nephrotoxicity, stress, and anxiety. Anti-inflammatory activity of CMEO has been attributed to the presence and synergistic activity of carnosol and carnosic, rosmarinic, ursolic, oleanolic, and micromeric acids (A)[18]. Based on the ADMET data this drug can be proposed for preclinical and clinical studies in different diseases and pathological conditions.

CONCLUSION

C. martini (Palmarosa) contains a large variety of bioactive molecules with great therapeutic potential. CMEO has been used in traditional medicine due to pharmacological potential of phytochemicals. However, development of new formulations containing other less common CMEO extracts is warranted through trials to establish the credentials of pharmacologically active phyto-compounds towards safety/ efficacy, in treating various pathological conditions including COVID-19 and other viral infections owing to modulation of immune-inflammatory responses and druggable nature of CMEO. In particular, BCP is a unique dietary molecule, with negligible toxicity, without of psychotropic effects, widely distributed in plants, with high degree of oral bioavailability, druggable properties and functionality. However, the use of BCP shall remain indecisive until in-silico observations could be experimentally validated.

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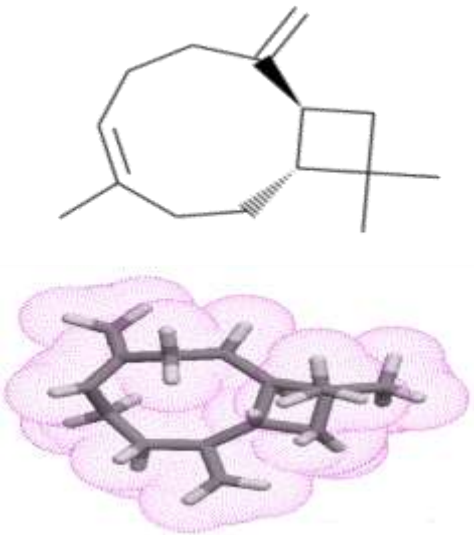

Molecular Properties	Calculated Values
miLogP	5.17
TPSA	0.00
natoms	15
MW	204.36
nON	0
nOHNH	0
nviolations	1
Total volume	229.95
Number of Rotatable Bonds	0
Number of Acceptors	0
Number of Donors	0
Total Surface Area	94.458
Biological Properties	**Calculated Score**
GPCR ligand	-0.34
Ion channel modulator	**0.28**
Kinase inhibitor	-0.78
Nuclear receptor ligand	0.13
Protease inhibitor	-0.60
Enzyme inhibitor	0.19

Figure 1: 2D, 3D structures, molecular and biological properties of β-caryophyllene

Table 1a: Summary of performance measures of vNN models in 10-fold cross validation using a restricted/ unrestricted applicability domain

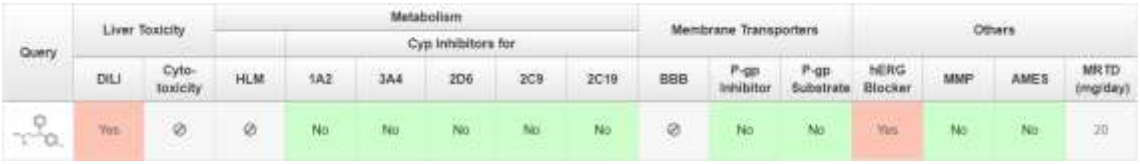

Table 1b: Performance measures of vNN models in 10-fold cross validation using a restricted/ unrestricted applicability domain

Model	Data[a]	d_0[b]	h[c]	Accuracy	Sensitivity	Specificity	*kappa*	R[d]	Coverage
DILI	1427	0.60	0.50	0.71	0.70	0.73	0.42		0.66
		1.00	0.20	0.67	0.62	0.72	0.34		1.00
Cytotox (hep2g)	6097	0.40	0.20	0.84	0.88	0.76	0.64		0.89
		1.00	0.20	0.84	0.73	0.89	0.62		1.00
HLM	3219	0.40	0.20	0.81	0.72	0.87	0.59		0.91
		1.00	0.20	0.81	0.70	0.87	0.57		1.00
CYP1A2	7558	0.50	0.20	0.90	0.70	0.95	0.66		0.75
		1.00	0.20	0.89	0.61	0.95	0.60		1.00
CYP2C9	8072	0.50	0.20	0.91	0.55	0.96	0.54		0.76
		1.00	0.20	0.90	0.44	0.96	0.46		1.00
CYP2C19	8155	0.55	0.20	0.87	0.64	0.93	0.58		0.76
		1.00	0.20	0.86	0.52	0.94	0.50		1.00
CYP2D6	7805	0.50	0.20	0.89	0.61	0.94	0.57		0.75
		1.00	0.20	0.88	0.52	0.95	0.51		1.00
CYP3A4	10373	0.50	0.20	0.88	0.76	0.92	0.68		0.78
		1.00	0.20	0.88	0.69	0.93	0.64		1.00
BBB	353	0.60	0.20	0.90	0.94	0.86	0.80		0.61
		1.00	0.10	0.82	0.88	0.75	0.64		1.00
Pgp Substrate	822	0.60	0.20	0.79	0.80	0.79	0.58		0.66
		1.00	0.20	0.73	0.73	0.74	0.47		1.00
Pgp Inhibitor	2304	0.50	0.20	0.85	0.91	0.73	0.66		0.76
		1.00	0.10	0.81	0.86	0.74	0.61		1.00
hERG	685	0.70	0.70	0.84	0.84	0.83	0.68		0.80
		1.00	0.20	0.82	0.82	0.83	0.64		1.00
MMP	6261	0.50	0.40	0.89	0.64	0.94	0.61		0.69
		1.00	0.20	0.87	0.52	0.94	0.50		1.00
AMES	6512	0.50	0.40	0.82	0.86	0.75	0.62		0.79
		1.00	0.20	0.79	0.82	0.75	0.57		1.00
MRTD[e]	1184	0.60	0.20					0.79	0.69
		1.00	0.20					0.74	1.00

[a]Number of compounds in the dataset; [b]Tanimoto-distance threshold value; [c]Smoothing factor; [d]Pearson's correlation coefficient; [e]Regression model.

Table 2: Summary of Physicochemical, Druggability, ADMET of β-caryophyllene

PROPERTY		VALUE
Molecular weight		204.36 g/mol
LogP		4.73
LogD		4.52
LogSw		-3.87
Number of stereocenters		2
Stereochemical complexity		0.133
Fsp3		0.733
Topological polar surface area		0.00 Å^2
Number of hydrogen bond donors		0
Number of hydrogen bond acceptors		0
Number of smallest set of smallest rings (SSSR)		1
Size of the biggest system ring		11
Number of rotatable bonds		0
Number of rigid bonds		13
Number of charged groups		0
Total charge of the compound		0
Number of carbon atoms		15
Number of heteroatoms		0
Number of heavy atoms		15
Ratio between the number of non-carbon atoms and carbon atoms		0
Lipinski's rule of 5 violations		0
Veber rule		Good
Egan rule		Good
Oral PhysChem score (Traffic Lights)		1
GSK's 4/400 score		Good
Pfizer's 3/75 score		Bad
Weighted quantitative estimate of drug-likeness (QEDw) score		0.514
Solubility		4277.90
Solubility Forecast Index		Good
Property	**Value**	**Probability**
Human Intestinal Absorption	HIA+	0.993
Blood Brain Barrier	BBB+	0.954
Caco-2 permeable	Caco2+	0.633
P-glycoprotein substrate	Substrate	0.578
P-glycoprotein inhibitor I	Non-inhibitor	0.599
P-glycoprotein inhibitor II	Inhibitor	0.669
CYP450 2C9 substrate	Non-substrate	0.900
CYP450 2D6 substrate	Non-substrate	0.839
CYP450 3A4 substrate	Substrate	0.578
CYP450 1A2 inhibitor	Non-inhibitor	0.670
CYP450 2C9 inhibitor	Non-inhibitor	0.625
CYP450 2D6 inhibitor	Non-inhibitor	0.928
CYP450 2C19 inhibitor	Non-inhibitor	0.596
CYP450 3A4 inhibitor	Non-inhibitor	0.867
CYP450 inhibitory promiscuity	Low CYP Inhibitory Promiscuity	0.843
Ames test	Non AMES toxic	0.917
Carcinogenicity	Non-carcinogens	0.686
Biodegradation	Ready biodegradable	0.573
Rat acute toxicity	1.435 LD_{50}, mol/kg	NA
hERG inhibition (predictor I)	Weak inhibitor	0.923
hERG inhibition (predictor II)	Non-inhibitor	0.854

Physicochemical properties were computed using FAF-Drugs4 (28961788); RDKit open-source cheminformatics platform. Druggabiity scoring schemes were computed using FAF-Drugs4 (28961788) and FAF-QED (28961788) open-source cheminformatics platform. ADMET were predicted using admetSAR (23092397) open-source tool.

Table 3: Pharmacokinetic properties, predicted score values of β-caryophyllene

PROPERTY	NAME OF THE MODEL	PREDICTED VALUE	REFERENCE UNIT
Absorption	Water solubility	-5.534	(log mol/L)
Absorption	Caco2 permeability	1.434	(log $P10^{-6}$ cm/s)
Absorption	Intestinal absorption (human)	95.304	(% Absorbed)
Absorption	Skin Permeability	-1.596	(log Kp)
Absorption	P-glycoprotein substrate	No	(Yes/No)
Absorption	P-glycoprotein I inhibitor	No	(Yes/No)
Absorption	P-glycoprotein II inhibitor	No	(Yes/No)
Distribution	VDss (human)	0.653	(log L/kg)
Distribution	Fraction unbound (human)	0.26	(Fu)
Distribution	BBB permeability	0.74	(log BB)
Distribution	CNS permeability	-2.182	(log PS)
Metabolism	CYP2D6 substrate	No	(Yes/No)
Metabolism	CYP3A4 substrate	No	(Yes/No)
Metabolism	CYP1A2 inhibitior	No	(Yes/No)
Metabolism	CYP2C19 inhibitior	No	(Yes/No)
Metabolism	CYP2C9 inhibitior	No	(Yes/No)
Metabolism	CYP2D6 inhibitior	No	(Yes/No)
Metabolism	CYP3A4 inhibitior	No	(Yes/No)
Excretion	Total Clearance	1.088	(log ml/min/kg)
Excretion	Renal OCT2 substrate	No	(Yes/No)
Toxicity	AMES toxicity	No	(Yes/No)
Toxicity	Max. tolerated dose (human)	0.489	(log mg/kg/day)
Toxicity	hERG I inhibitor	No	(Yes/No)
Toxicity	hERG II inhibitor	No	(Yes/No)
Toxicity	Oral Rat Acute Toxicity (LD_{50})	1.678	(mol/kg)
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	1.44	(log mg/kg_bw/day)
Toxicity	Hepatotoxicity	No	(Yes/No)
Toxicity	Skin Sensitisation	Yes	(Yes/No)
Toxicity	*T. pyriformis* toxicity	1.406	(log ug/L)
Toxicity	Minnow toxicity	0.445	(log mM)