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Research Article

In-silico ADMET profile of Ellagic Acid from *Syzygium cumini*: A Natural Biaryl Polyphenol with Therapeutic Potential to Overcome Diabetic Associated Vascular Complications

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Abstract

Plant Based Natural Products (PBNPs) are the primary source of natural antioxidants capable of neutralizing or eliminating harmful Reactive Oxygen Species (ROS). Oxidative stress contributes not only to the pathogenesis of type 2 diabetes (T2DM) but also to diabetic related vascular complications by lipid peroxidation. Oxidation induced DNA and protein damage leads to development of vascular complications like coronary heart disease, CVD, stroke, neuropathy, retinopathy, nephropathy, CKD, and other long term complications associated with diabetics. Likewise Multidrug resistance (MDR) is one of the major clinical challenges in cancer treatment and compromises the effectiveness of conventional anticancer chemotherapeutics. P-glycoprotein (P-gp) has been characterized as a major mechanism of MDR. Ellagic acid (EA) is a bioactive secondary metabolite widely distributed in vegetables and fruits (Strawberry, Grapes, Blackberry, Raspberry, Plums etc.) Chemically, EA is 2,3,7,8-tetrahydroxychromeno [5,4, -cde] chromene-5, 10-dione, a heterotetracyclic dimer of Gallic Acid (GA) molecules formed by oxidative aromatic coupling involving intramolecular lactonization. EA is associated with pharmacological activities such as anti-inflammatory, neuroprotective, cardio-protective, antioxidant, anti-mutagenic, multidrug resistance etc. EA has been marketed as a dietary supplement with claimed benefits against cancer, CVD, CKD and other metabolic disorders. However, pharmacological limitation of EA is attributed to its low solubility in water and reduced bioavailability. In the present study, bimolecular potential of EA has been bioprospected in the revised framework of ADMET pharmacoinformatics to further widen its biomedical applications.

Keywords: ADMET; Pharmacoinformatics; Ellagic Acid; Gallic Acid; *Syzygium cumini*; Alagarkovil Reserve Forest (ARF); Reactive Oxygen Species (ROS)

INTRODUCTION

Naturally, EA is present in fruits (pomegranates, persimmons, raspberries, black raspberries, wild strawberries, peaches, plums - Indian black plum), seeds (walnuts, almonds), and vegetables^{1,2}. Pharmacological attributes of EA includes antioxidant^{3,4,5}, anti-proliferative⁵, apoptotic^{5,6}, antimalarial⁷, anti-inflammatory⁸, anti-hepatotoxic^{9,10}, antitumor¹¹, anti-cholestatic, anti-fibrogenic and antiviral^{12,13}. EA has been reported for its potential neuroprotective, cytotoxic, anticancer, cardio-protective, anti-tubercular activity and is used in the management of metabolic syndrome. EA has been claimed to improve hepatic functions against toxic and pathological conditions. Derivatives of EA-urolithins and 4,4'-Di -O-methyl EA have been reported to inhibit colon cancer cell proliferation. EA has been reported to block activated pancreatic stellate cells, and exhibit apoptosis inducing activities¹.

EA belongs to the class of hydrolysable tannins (MW 302.194 g/mol; MF C₁₄H₆O₈) with melting point 350°C and is highly thermo-stable due to the presence of four lipophilic rings, four phenolic groups and two lactones (Fig. 1). EA is organic hetero-tetracyclic compound resulting from dimerization of GA by oxidative aromatic coupling with intramolecular lactonisation of carboxylic acid groups¹. EA is a cyclic ketone, (lactone) and a member of catechols - polyphenol, appears as cream-colored needles or yellow powder, odourless, sparingly soluble in water, soluble in alkalies, pyridine, insoluble in ether, when heated emits acrid smoke and irritating vapour^{1,14}.

Nevertheless a few reports on improving water-solubility and bioavailability of EA are available, a wide gap still exists from practical utility point of view. Currently, synthetic antioxidants are replaced by natural antioxidants as the former are reported to have carcinogenic properties. Plants

serve as primary source of natural antioxidant molecules capable of eliminating or neutralizing the harmful reactive oxygen species (ROS). Natural antioxidants (NAs) are free-radical scavengers, reduction agents, pro-oxidant metal complexes, singlet oxygen quenchers. NAs safeguard human body from free radicals and delay progression of chronic illnesses (Cancer, Heart Disease, Stroke) prevent lipid oxidative rancidity, boost plasma's antioxidant ability and natural immunity of the system^{1,14}.

Maintenance of good health is attributed to consumption of fruits, vegetables, herbs, seeds that contain a group of natural polyphenols classified as hydrolysable tannins (HT) viz., ellagitannins (ETs). ETs represent a diverse class of polyphenolic natural products with remarkable structural complexity. Members of ETs include glycosyl esters of EA and/or GA motifs. ETs that contain flavone motifs as part of the structure, referred to as flavano-ellagitannins¹⁵. EA, a component of ellagitannins, is a biaryl polyphenol where two GA motifs are oxidatively coupled via a carbon-carbon bond to join two aryl rings. Further, one or more hexahydroxydiphenolic acid (HHDP) units are ester-linked with a sugar molecule. ETs are rather not/ less absorbed in the gastrointestinal tract (GIT), when hydrolysed yields EA¹⁶. Due to water soluble nature, its metabolism in GIT is further complicated by the irreversible binding to cellular DNA and proteins. Hence, EA has a very low or sometime very poor bioavailability in the system. Additionally, gut bacteria are known to metabolize EA into urolithins that has a better bioavailability compared to EA. Rate of metabolism of EA by gut bacteria and levels of urolithins is attributed to discrepancies in outcomes observed from *in vitro* vs *in vivo* studies¹.

Metabolic inflammation plays a key role in the pathogenesis of diabetes associated complications (diabetic nephropathy, retinopathy, and neuropathy). T2DM is characterized by systemic inflammation, sustained elevation of circulating levels of pro-inflammatory cytokines (IL-6, IL-1 β , TNF- α), chemokine-evoked enhanced recruitment of inflammatory cells, activation of inflammatory response to NF- κ B and signaling of AMPK and PPAR- γ . On the other hand, treatment, cancer exhibits resistance to drugs, phenomenon referred to as multidrug resistance. MDR is attributed to (a) heightened DNA repair, (b) reduced drug uptake, (c) enhanced drug efflux, (d) mutation of drug targets, (e) altered inherent apoptotic process, and (f) increased drug metabolism¹⁶. Drug resistance has been attributed to increased levels of proteins such as mitogen-activated protein kinases (MAPKs), protein kinase B (PKB), and nuclear factor- κ B (NF- κ B), and overexpression of a type of ATP-binding cassette (ABC) transporter, referred to as P-glycoprotein (P-gp)¹⁷. Recently, EA has attracted researchers to develop drug leads to overcome MI and MDR¹⁸.

At the biochemical level, several mechanisms have been proposed to be associated with therapeutic action, including efficacy in normalizing lipid metabolism and lipidemic profile, regulating pro-inflammatory mediators, such as IL-6, IL-1 β , and TNF- α , upregulating nuclear factor erythroid 2-related factor 2 and inhibiting NF- κ B action¹⁹. EA exerts appreciable neuroprotective activity by its free radical-scavenging action, iron chelation, initiation of several cell signalling pathways, and alleviation of mitochondrial dysfunction¹⁹. Presence of poly-oxygenated aryl rings allows EA to quench free radicals, making it highly effective bioactive molecule with significant antioxidant and cytoprotective potential¹⁸. Physico-chemical and biological properties of EA have been extensively worked out and reviewed in literature^{1,14,20}, however, major pharmacological

limitation of EA is accredited to its low solubility that significantly reduces its bioavailability therefore in-depth study on absorption, distribution, metabolism, excretion, and toxicity (ADMET) is warranted.

MATERIALS AND METHODS

Syzygium cumini - botanical description

Trees, 6-20 m tall; branchlets grayish white when dry, terete; petiole 1-2 cm; leaf blade broad to narrowly elliptic, 6-12 \times 3.5-7 cm, leathery, abaxially slightly pale when dry, adaxially brownish green to blackish brown and slightly glossy when dry, both surfaces with small glands, secondary veins numerous, 1-2 mm apart, and gradually extending into margin, intra-marginal veins ca. 1 mm from margin; base broadly cuneate to rarely rounded, apex rounded to obtuse and with a short cusp; inflorescences axillary on flowering branches or occasionally terminal, paniculate cymes, to 11 cm; hypanthium obconic or long pyriform, ca. 4 mm or 7-8 mm; calyx lobes inconspicuous, 0.3-0.7 mm; petals 4, white or light purple, coherent, ovate and slightly rounded, ca. 2.5 mm; stamens 3-4 mm; style as long as stamens; fruit red to black, ellipsoid to pot-shaped, 1-2 cm, 1-seeded; persistent calyx tube 1-1.5 mm; fl. Feb-Mar or Apr-May; fr. Jun-Sep²¹⁻²⁵.

GC-MS Analysis

Leaf samples of *S. cumini*, were collected from Alagarkovil Reserve Forest (longitude/ latitude geographical coordinates 10.0748° N, 78.2131° E, Eastern Ghats) Dindigul District, Tamil Nadu, India. Phyto-components were identified using GC-MS detection system as described previously²⁶, however with modification, whereby portion of the extract was analysed directly by headspace sampling. GC-MS analysis was accomplished using an Agilent 7890A GC system set up with 5975C VL MSD (Agilent Technologies, CA, and USA). Capillary column used was DB-5MS (30 m \times 0.25 mm, film thickness of 0.25 μ m; J&W Scientific, CA, USA). Temperature program was set as: initial temperature 50°C held for 1 min, 5°C per min to 100°C, 9°C per min to 200°C held for 7.89 min, and the total run time was 30 min. The flow rate of helium as a carrier gas was 0.811851 mL/ min. MS system was performed in electron ionization (EI) mode with Selected Ion Monitoring (SIM). The ion source temperature and quadrupole temperature were set at 230°C and 150°C, respectively. Identification of phyto-components was performed by comparison of their retention times and mass with those of authentic standards spectra using computer searches in NIST 08.L and Wiley 7n.l libraries²⁷.

ADMET Predictions

Physicochemical properties were computed using FAF-Drugs4 (28961788)/ RDKit - open-source CIP. Selected phytocompounds were subjected to ADMET prediction using QikProp (version 4.3, Suite 2015-1; Schrödinger, LLC: New York, NY) and toxicity prediction using TOPKAT (Accelrys, Inc., USA). Qik-Prop develops and employs QSAR/QSPR models using partial least squares, principal component analysis and multiple linear regression to predict physico-chemically significant descriptors²⁸⁻³¹. Druggability scores were computed using FAF-Drugs4 (28961788)/ FAF-QED (28961788) - open-source CIP. The vNN-ADMET webserver was used to predict absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties and predict properties, such as cytotoxicity, mutagenicity, cardiotoxicity, drug-drug interactions, microsomal stability, and drug-induced liver injury³²⁻³⁵.

RESULTS AND DISCUSSION

Chemical kingdom	: Organic compounds
Super class	: Phenylpropanoids and polyketides
Class	: Tannins
Subclass	: Hydrolyzable tannins
PubChem Identifier	: 5281855
ChEBI Identifier	: 4775
CAS Identifier	: 476-66-4
Synonyms	: Ellagic Acid; Methyl Jasmonate
Canonical SMILES	: <chem>OC1CC2C(=O)OC3C4C2C(C1O)OC(=O)C4CC(C3O)O</chem>
InChI Key	: AFSDNFLWKVMVRB-UHFFFAOYSA-N

Physicochemical Properties

Despite the efficient pharmacodynamics and safety profile of EA, it suffers from the low bioavailability³⁶. Molecular weight of EA was calculated as 302.19 g/mol; LogP value was predicted as 1.31; LogD value was predicted as 0.53; LogSw value was predicted as -2.83. Number of stereo-centers was predicted as 0; Stereo-chemical complexity was predicted as 0.000; Fsp3 was predicted as 0.000; Topological polar surface area was calculated as 141.34 Å²; Number of hydrogen bond donors was calculated as 4; Number of hydrogen bond acceptors was calculated as 8; Number of smallest set of smallest rings (SSSR) was calculated as 1; Size of the biggest system ring was calculated as 16; Number of rotatable bond was calculated as 0; Number of rigid bond was calculated as 21; Number of charged group was calculated as 0; Total charge of the compound was calculated as 0; Number of carbon atoms was ascertained as 14; Number of heteroatoms was ascertained as 8; Number of heavy atoms was ascertained as 22; Ratio between the number of non-carbon atoms and the number of carbon atoms was ascertained³⁷ as 0.57 (Table 1).

Druggability Properties

In silico studies are expected to reduce the risk of late-stage attrition of drug development and to optimize screening and testing by looking at promising Druggability Properties³⁷. Lipinski's rule of 5 violations was predicted as 0; Veber rule was predicted as Good; Egan rule was predicted as Good; Oral PhysChem score (Traffic Lights) was predicted as 2; GSK's 4/400 score was predicted as Good; Pfizer's 3/75 score was predicted as Good; Weighted quantitative estimate of drug-likeness (QEDw) score was predicted as 0.245; Solubility of EA was predicted as 17843.03; Solubility Forecast Index of EA was predicted as Good (Table 2).

ADMET Properties

Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) prediction models, including performance measures were performed online³⁸ (Table 3). In the present study 15 models covered a diverse set of ADMET endpoints including Maximum Recommended Therapeutic Dose (MRTD),³⁹ chemical mutagenicity,³² human liver microsomal (HLM),³⁴ Pgp inhibitor/substrates,⁵ vNN model for cross validation of ADMET data for EA is provided in Table 4 a,b.

Liver Toxicity DILI

Drug-induced liver injury (DILI) is the most commonly cited reason for drug withdrawals from the market. This model predicts whether a compound could cause DILI. The dataset of 1427 compounds were obtained and used.⁴⁰ Dataset contained both pharmaceuticals and non-pharmaceuticals assuming 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 - 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 6,097 structurally diverse compounds, collected from ChEMBL considering compounds with an IC₅₀ ≤ 10 µM in the in vitro assay as cytotoxic.

Metabolism HLM

Human liver microsomal (HLM) stability assay is 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 is considered as stable; otherwise considered unstable. HLM data was retrieved from the ChEMBL database, manually curated, and classified as stable or unstable compounds based on the reported half-life (T_{1/2} > 30 min was considered stable, and T_{1/2} < 30 min as unstable. Analysis indicated that in the final dataset of 3219 compounds 2,313 were stable and 1,341 were unstable.³⁴

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 viz., 1A2, 3A4, 2D6, 2C9 and 2C19 were used to develop CYP inhibition models⁴¹. CYP inhibitors were retrieved from PubChem and classified a compound with an IC₅₀ ≤ 10 µM for an enzyme as an inhibitor [CYP1A2 (7558), CYP3A4 (8072), CYP2D6 (8155), CYP2C9 (7805), and CYP2C19 (10373)].

Membrane Transporters - BBB

Blood-brain barrier (BBB) is a highly selective barrier that separates the circulating blood from the central nervous system. A vNN-based BBB model was developed, using 353 compounds whose BBB permeability values (log BB) were obtained.⁴² Compounds were classified with log BB values of less than -0.3 and greater than +0.3 as BBB non-permeable and permeable⁴³ Fig. 2.

Pgp Substrates and Inhibitors

P-glycoprotein (Pgp) is cell membrane protein that extracts many foreign substances from the cell. Cancer cells often overexpress Pgp, which increases efflux of chemotherapeutic agents from cell and prevents treatment by reducing the effective intracellular concentrations of such agents. Hence, identifying compounds that can either be transported out of cell by Pgp (substrates) or impair Pgp function (inhibitors) is considered as significant aspect of drug lead. Models to predict both Pgp substrates and Pgp inhibitors were developed^{44,45} with a dataset consisting of 422 substrates and 400 non-substrates in combination^{46,47}. Analysis of the final dataset indicates that among the selected substrates 2304 were inhibitors and 822 were 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 causes long QT syndrome, which may result in arrhythmia and death. 282 known hERG blockers were retrieved and classified with an IC₅₀ cutoff value of 10 µM or less as blockers.⁴⁰ Analysis with set of 404 compounds with IC₅₀ values greater than 10 µM from ChEMBL 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. Large dataset of chemical-induced changes in mitochondrial membrane potential (MMP) was used based on the assumption that a compound that causes mitochondrial dysfunction is likely to reduce MMP. vNN-based MMP prediction model was developed, using 6,261 compounds from a library of 10,000 compounds (~8,300 unique chemicals) at 15 conc., each in triplicate, to measure changes in the MMP in HepG2 cells.⁴⁸ Data depicted that 913 compounds decreased MMP, whereas 5,395 compounds had no effect.

Mutagenicity (AMES Test)

Mutagens cause abnormal genetic mutations leading to cancer. A common way to assess a chemical's mutagenicity is Ames test³⁵. A prediction model was developed using a dataset of 6,512 compounds, of which 3,503 were Ames-positive.

Maximum Recommended Therapeutic Dose - MRTD

Maximum Recommended Therapeutic Dose (MRTD) is an estimated upper daily dose that is safe. A prediction model was developed based on a dataset of MRTD values publically disclosed by FDA of GRAS standard, mostly of single-day oral doses for an average adult with a body weight of 60 kg, for 1,220 compounds (most of which are small organic drugs), excluding organometallics, high-molecular weight polymers (>5,000 Da), nonorganic chemicals, mixtures of chemicals, and very small molecules (<100 Da) using external test set of 160 compounds collected by FDA for validation⁴⁹. The total dataset for model contained 1,184 compounds.³⁵ The predicted MRTD value is reported in mg/day unit based upon an average adult weighing 60 kg.

Further, Predicted Human Target Proteins (STITCH database) towards Ellagic Acid is provided in Table 5 and Cytoscape network Fig. 3. The major challenging issue in diabetes management is the prevention of diabetes associated complications that remain the leading cause of diabetes-related mortality. Inflammatory response is one of key driving forces to promote the pathogenesis of diabetes and its complications. Bioactive phytochemicals such as EA with significant anti-inflammatory benefits hold great promise for the treatment of diabetes^{50,51,52}.

CONCLUSION

Polyphenolic natural products represent a chemically unique class of molecules as potential antioxidants and anticancer agents. EA and its derivatives are plant based natural products with significant health benefits and holds potential for advanced biomedical applications. However, its production on industrial scale for biomedical applications from commercial point of view has been hampered by the lack of in-depth information on EA and its derivatives. In-

silico-ADMET prospecting and pharmacoinformatics is expected fulfil the lack of high-grade products with high bioavailability. Accordingly, QSAR knowledge base on structural and functional informatics of ETs and EA derivatives would be the first step towards a wider exploration for biomedical applications.

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Table 1 Physicochemical Properties of Ellagic Acid

Property	Value
Molecular weight	302.19 g/mol
LogP	1.31
LogD	0.53
LogSw	-2.83
Number of stereocenters	0
Stereochemical complexity	0.000
Fsp3	0.000
Topological polar surface area	141.34 Å ²
Number of hydrogen bond donors	4
Number of hydrogen bond acceptors	8
Number of smallest set of smallest rings (SSSR)	1
Size of the biggest system ring	16
Number of rotatable bonds	0
Number of rigid bonds	21
Number of charged groups	0
Total charge of the compound	0
Number of carbon atoms	14
Number of heteroatoms	8
Number of heavy atoms	22
Ratio between the number of non-carbon atoms and the number of carbon atoms	0.57

Table 2 Druggability Properties of Ellagic Acid

Property	Value
Lipinski's rule of 5 violations	0
Veber rule	Good
Egan rule	Good
Oral PhysChem score (Traffic Lights)	2
GSK's 4/400 score	Good
Pfizer's 3/75 score	Good
Quantitative estimate of drug-likeness score	0.245
Solubility	17843.03
Solubility Forecast Index	Good Solubility
Log $P_{o/w}$ (iLOGP)	0.79
Log $P_{o/w}$ (XLOGP3)	1.10
Log $P_{o/w}$ (WLOGP)	1.31
Log $P_{o/w}$ (MLOGP)	0.14
Log $P_{o/w}$ (SILICOS-IT)	1.67
Consensus Log $P_{o/w}$	1.00
Log S (ESOL)	-2.94
Solubility	3.43e-01 mg/ml ; 1.14e-03 mol/l
Class	Soluble
Log S (Ali)	-3.66
Solubility	6.60e-02 mg/ml ; 2.18e-04 mol/l
Class	Soluble
Log S (SILICOS-IT)	-3.35
Solubility	1.36e-01 mg/ml ; 4.49e-04 mol/l
Class	Soluble
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	No; 1 violation: TPSA>140
Egan	No; 1 violation: TPSA>131.6
Muegge	Yes
Bioavailability Score	0.55

Table 3 ADMET Properties of Ellagic Acid

Property	Value	Probability
Human Intestinal Absorption	HIA+	0.720
Blood Brain Barrier	BBB+	0.564
Caco-2 permeable	Caco2-	0.831
P-glycoprotein substrate	Substrate	0.538
P-glycoprotein inhibitor I	Non-inhibitor	0.938
P-glycoprotein inhibitor II	Non-inhibitor	0.964
CYP450 2C9 substrate	Non-substrate	0.834
CYP450 2D6 substrate	Non-substrate	0.910
CYP450 3A4 substrate	Non-substrate	0.721
CYP450 1A2 inhibitor	Non-inhibitor	0.591
CYP450 2C9 inhibitor	Non-inhibitor	0.559
CYP450 2D6 inhibitor	Non-inhibitor	0.958
CYP450 2C19 inhibitor	Non-inhibitor	0.802
CYP450 3A4 inhibitor	Non-inhibitor	0.908
CYP450 inhibitory promiscuity	Low CYP Inhibitory Promiscuity	0.957
Ames test	Non AMES toxic	0.913
Carcinogenicity	Non-carcinogens	0.958
Biodegradation	Not ready biodegradable	0.805
Rat acute toxicity	2.621 LD50, mol/kg	NA
hERG inhibition (predictor I)	Weak inhibitor	0.972
hERG inhibition (predictor II)	Non-inhibitor	0.915

Table 4a Performance of vNN models in 10-fold cross validation using a restricted or 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

^aNumber of compounds in the dataset; ^bTanimoto-distance threshold value; ^cSmoothing factor; ^dPearson's correlation coefficient; ^eRegression model.

Table 4b Summary of vNN model for cross validation of ADMET data for EA

Query	Liver Toxicity		Metabolism						Membrane Transporters			Others			
	DILI	Cyto-toxicity	HLM	Cyp inhibitors for					BBB	P-gp Inhibitor	P-gp Substrate	hERG Blocker	MMP	AMES	MRTD (mg/day)
				1A2	3A4	2D6	2C9	2C19							
	Yes	⊗	⊗	No	No	No	No	No	⊗	No	No	Yes	No	No	20

Table 5 Predicted Human Target Proteins (STITCH database) towards EA

ENSP00000314099	CA5B	812
ENSP00000376889	NME2	820
ENSP00000364898	SYK	816
ENSP00000358107	CA14	812
ENSP00000217244	CSNK2A1	958
ENSP00000356958	NR1I3	700
ENSP00000345659	CA7	812
ENSP00000256119	CA1	816
ENSP00000366662	CA6	812
ENSP00000225831	CCL2	700
ENSP00000408695	PRKCA	800
ENSP00000377192	G6PD	700
ENSP00000384408	PARG	700
ENSP00000311032	CASP3	738
ENSP00000219070	MMP2	725
ENSP00000265896	SQLE	827
ENSP00000376886	NME1-NME2	820
ENSP00000305355	PRKCB	800
ENSP00000283916	TMPRSS11D	786
ENSP00000367608	CA9	812
ENSP00000270776	PGD	700
ENSP00000178638	CA12	812
ENSP00000297494	NOS3	828
ENSP00000253496	F12	966
ENSP00000231449	IL4	834
ENSP00000263321	TYR	847
ENSP00000285379	CA2	817
ENSP00000300900	CA4	812
ENSP00000309591	PRKACA	800
ENSP00000285381	CA3	812
ENSP00000309649	CA5A	812
ENSP00000221515	RETN	800

ADMET features were predicted using admetSAR (23092397) open-source tool. The physicochemical properties were computed using FAF-Drugs4 (28961788) and RDKit open-source cheminformatics platform. The druggability scoring schemes were computed using FAF-Drugs4 (28961788) and FAF-QED (28961788) open-source cheminformatics platform. The human target proteins were predicted using STITCH (26590256), a database of Chemical-Protein Interaction Networks

