An Introduction to in silico toxicokinetics prediction and ADME profiling of medicinal plants

Abstract

From time immemorial, natural products were the source for food and medicine. The unique properties of plants helped mankind to survive through centuries. The unique chemical structure resulted in peculiar pharmacodynamic and pharmacokinetic properties and thus made them suitable drug candidates. Last century witnessed emergence of a wide array of new epidemic diseases. To discover drugs to combat these situations are really a challenging task for the scientists. This can be simplified by mining the data regarding ADME and toxicokinetic profile of plants. This will help the research community to narrow down the search strategies for new drug development and streamline the advancements in drug research. This article gives an insight about the preliminary techniques adopted for in silico drug designing, toxicokinetics and ADME profiling of a newly discovered plant or research on an existing plant/plant part for a new disease.

Introduction

Natural products are the major sources for food and medicine for the humans from time immemorial. The unique chemical structure and pharmacodynamic and pharmacokinetic properties made them suitable drug candidates. It offers therapeutic alternatives showing wide range of biological activities.[1] As per the WHO statistics, about 40% of approved drugs are derived from natural product resources. In the past few years there is a huge increase in the publicly accessible natural product database.[2] Over 120 different natural product databases and collections are published since 2010. All these database targets to the therapeutic indication of products. The data mining proves that only a few data is available on the toxicokinetic and ADME profile. [3]

Absorption, distribution, metabolism, and excretion properties plays a significant role in drug development. About 45% of drug candidates fails due to poor ADME results. The preclinical ADME studies reduced the number but the drug toxicity remains as a major hurdle. [4] The non optimal ADME and toxicity ends upon later stage failures in drug development resulting in a huge wastage of time, energy, and money.

The in-silico models contribute largely towards the drug optimization by improving the ADME prediction. [5]

In silico toxicology methods includes computational approaches which visualize, simulate, analyse, or predict toxicity of drug molecules. It analyses the chemical and biological properties of drug molecules based upon the actual or virtual chemical structure of the concerned molecule. It is a predictive technique that helps in retrieving relevant data and/or make predictions regarding the effects of chemicals. It is intertwined with physics, chemistry, biology, mathematics, computer science, and informatics with toxicology, which uses information from computational tools to analyse beneficial or adverse effects envisaging toxicity endpoints of compounds. [6]

Methods used in in silico drug design

Drug discovery and development is a complicated, time-consuming process and it depends on many factors such as pharmacokinetic data, effectiveness, safety profile and marketable reasons. The in-silico drug design in a newly emerging field in which different sides of basic research and practice are combined to obtain a precise result. The important methods in in-silico drug design are described below.[7]

1. Homology modelling:

   It is also recognised as comparative modelling of protein and it allows to generate an unknown atomic resolution model of the "target" protein from its amino acid sequence and an experimental three-dimensional structure of a related homologous protein.[7] It involves the recognition of one or more identifies protein structures probably to show resemblance with the structure of the query sequence to residues in the template sequence. It is reported that the protein structures are more conserved than protein sequence among homologous.[8] Since the protein structures are more conserved than DNA sequences, detectable levels of sequence similarity usually involve substantial structural similarity. Bioinformatics software tools are used to generate the 3D structure of the target based on the known 3D structures of templates.[9] The Modeller is a popular tool in homology modelling, and SWISS-model repository is a database of protein structures created with homology modelling. [10,11]

2. Molecular docking:

It is a unique technique which envisages the favoured orientation of one molecule to a second, when bound to each other to form a stable complex.[12] Molecular docking denotes ligand binding to its receptor or target protein. Molecular docking is used to recognize and optimize drug candidates by examining and modelling molecular interactions between ligand and target macromolecules. Molecular docking is used to generate multiple ligand conformations and orientations and the most appropriate ones are selected.[13, 14] There are several molecular docking tools available that includes ArgusDock, DOCK, FRED, eHITS, AutoDock and FTDock. Molecular modelling involves scoring methods that are used to rank the affinity of ligands to bind to the active site of a receptor. In virtual highthroughput screening, compounds are docked into the active site and then scored to determine which one is more likely to bind tightly to the target macromolecule.[15]

3. Virtual high throughput screening:

   It is a computational technique where large libraries of compounds are evaluated for their potential to bind specific sites on target molecules such as proteins, and well-matched compounds tested. The research in the drug discovery process involves virtual screening (VS) which is a computational method used for the rapid exploration of large libraries of chemical structures in order to identify those structures that are most likely to bind to a drug target, usually a protein receptor or enzyme.[16] Virtual screening plays a vital role in the drug discovery process. The term "virtual screening" is relatively new as compared to the more general and older concept of database searching. Walters, et al. defines, virtual screening as "automatically evaluating very large libraries of compounds" using a computer program.[17] It is clear from above definition that VS has been a numbers game at large scale and it is focusing to find out answers of questions like how can we screen down the huge chemical space of over 1060 possible compounds to a practicable number that can be synthesized, purchased, and tested. It is less expensive than High-Throughput Screening, Faster than conventional screening, scanning many potential drugs like molecules in very little time. HTS itself is a trial-and-error approach but can be better complemented by virtual screening.[18]

4. Quantitative structure activity relationship (QSAR):

Quantitative structure-activity relationships (QSAR) methods are used to show a relationship of structural and/or property descriptors of compounds with their biological activities. These descriptors explaining the properties like steric, topologic, electronic, and hydrophobic of numerous molecules, have been determined through empirical methods, and only more recently by computational methods.[19]

5. Hologram quantitative structure activity relationship (HQSAR):

In Hologram QSAR, a distinctive QSAR procedure, there is no need for precise 3D information about the ligands. In this method, the molecule breaks to a molecular fingerprint encoding the frequency of occurrence of various kinds of molecular fragments. Simply, the minimum and maximum length of the fragments depends on the size of the fragment to be included in the hologram fingerprint. Molecular holograms are caused by a generation of linear and branched fragments, ranging in size from 4 to 7atoms.[20]

6. Comparative molecular field analysis (CoMFA):

Comparative molecular field analysis (CoMFA) is a constructive novel technique to explain structure activity relationship. It is a well-known 3D QSAR method and work on CoMFA began in the 70's. It delivers values of ClogP which means the solvent repellent constraints the ligands and explains the steric and electrostatic values of the ligands. [21,22]

7. Comparative molecular similarity indices analysis (Co-MSIA):

Comparative Molecular Similarity Indices Analysis (CoMSIA) is recognized as one of the new 3DQSAR approaches. It is generally used in the drug discovery process to locate the common characteristics, essential for the proper biological receptor binding. This method deals with the steric and electrostatic characteristics, hydrogen bond acceptors, hydrogen bond donor and hydrophobic fields.[23]

8. 3D pharmacophore mapping:

The 3D pharmacophore search is an imperative, vigorous and simple method to quickly recognize lead compounds alongside a preferred target. Conventionally, a pharmacophore is defined as the specific 3D arrangement of functional groups within a molecular framework that are indispensable to attach to an active site of an enzyme or bind to a macromolecule. It is essentially the

first step to describe a pharmocophore in order to understand the interaction of a ligand with a receptor. Once a pharmacophore is recognized, the medicinal chemist utilizes the 3D database search tools to retrieve novel compounds that are suitable for the pharmacophore model. The modern drug design process has been used to make it one of the most successful computational tools because the search algorithms have made advancements over the years to efficiently identify and optimize lead focus combinatorial libraries and help in virtual high-throughput screening. [24]

9. Microarray analysis:

    Microarray analysis is a DNA technology which plays a very significant role in the advancement of biotechnology. These are basically properly arranged sets of known sequence DNA molecules. Mostly rectangular, which can be consisted of hundreds of thousands sets. Each single feature drives on the array at the accurately demarcated position on the substrate. The identity of the DNA molecule associated to each feature does not change. Scientists use this information to know the results of their experiments. The microarray study helps scientists to perceive numerous genes in a small sample immediately and also to carry out the analysis of the expression of these genes. That safety is given to facilitate biotechnology and pharmaceutical companies to identify target molecules. Microarray analysis can assist medical companies to participate in the selection of the most suitable candidates in clinical trials of new drugs. This development has a potential as a future technology to help medical experts in the selection of the most effective drugs, or to help those with less side effects for individual patients. It has wide applications in many fields, such as transgenic animal studies, cancer tissue microarrays and other diseases, normal tissues, and cells during development. This approach can be used to develop new and potent drugs.[25]

10. Conformational analysis:

    Conformational analysis deals with deformable molecules and their minimum energy configurations through various calculation methods and interaction networks involves comparing a molecular receptor site of another molecule and calculating the most energetically satisfactory3-D conformation.[26]

11. Monte Carlo simulation:

The principles of statistical mechanics are involved in Monte Carlo simulation which produces adequate different conformations of a system by computer simulation to permit the preferred thermodynamic, structural, and numerical properties to be calculated as a weighted average of these properties over these conformations. A valuable presentation has joined Monte Carlo sampling with flexible temperatures (simulated annealing) to enhance the fixing of ligands into active sites.[27]

12. Molecular dynamic (MD) simulation:

    Molecular dynamics is an effective procedure and depends on the molecular motion simulation by solving Newton's equations of motion for each atom and increasing the speed and position of each atom by a small increase of the time duration. MD simulations characterize alternative methods to sample configuration space, based on the above-mentioned rule. That is shared with temperatures using "reasonable" (a few hundreds or thousands of degrees), this means that only the local area around the sampled point, and only relatively small barriers (a few tens of kJ / mol) are overcome. Generation may be different (local), minimum may be accomplished by selecting configuration appropriate times during the simulation and thus minimize these structures. MD methods utilize the inherent dynamics of the system to search deformation modes of low energy and can be used for sampling of the conformational space of a large confined system.[28]

Methods for in silico predictive toxicology

Several approaches are developed for the prediction of in-silico toxicology. It is broadly classified into four major categories.

1. Structure activity modelling: quantitative structure activity relationships (QSAR), expert systems, grouping and read-across techniques
2. Chemo-informatics: generating molecular descriptors using computational tools including quantum chemical methods and molecular dynamics simulations for toxicity prediction
3. Databases and gathering biological data that contain relations between chemicals and toxicity endpoints, databases for storing data about chemicals, toxicity and chemical properties

4. Data mining and analysis: calculating molecular descriptors, generating a prediction model, evaluating the accuracy and interpreting the model, statistical methods and prebuilt models in web servers or standalone applications for predicting toxicity.

There are various procedures to unravel general or specific compound toxicity/safety and each method has respective strengths, limitations, the scope of application and interpretation. The underlying principle is to find the suitable and the most effective method to address the particular issue. However, all the four categories mentioned in this section for in silico predictive tools are highly interrelated. [29]

Tools used for predicting toxicity endpoints of drugs

In-silico predictive toxicology is used in combination with in vitro and in vivo experimental data obtained at the molecular, cellular, organ, organism, and population levels, provide the possibility of improved safety at molecular and functional changes occurring across multiple levels of biotic organization to characterize and evaluate interactions between potential hazards with the components of biological system. Predictive toxicology extrapolates quantifiable chemical toxicity end points and this application is a new paradigm for risk assessment.[30]

Table:1. In silico tools used for predicting toxicity endpoints of drugs

In silico methods	Description	Software/Database
Quantitative structure activity relationship models	Use molecular descriptors to predict chemical toxicity	OECD QSAR
		Top-Kat
		Derek Nexus
		VEGA
		METEOR
		vLife-QSARpro
Structural alerts and rule-based models	Chemical structures that indicate or	OECD QSAR
		Tox-tree
		OCES
		Derek Nexuc

	associate to toxicity	HazardExpert
		Meteor
		CASE
		PASS
		Cat-SAR
Read-across	Predicting unknown toxicity of a chemical using similar chemicals with known toxicity from the same chemical category	OECD QSAR
		Toxmatch
		Tox Tree
		AMBIT
		Ambit Discovery
		AIM
		DSSTox
		ChemlDplus
Dose–response and time–response models	Relation between doses (or time) and the incidence of a defined biological effect.	CEBS
		PubChem
		ToxRefDB
Pharmacokinetic (PK) and Pharmacodynamics (PD) models	PK models calculate concentration at a given time. PD models calculate effect at a given concentration	WinNonlin
		Kinetica
		ADAPT

Advantages

In following situations these methods serve as an important tool for the hazard assessment of existing or newly introduced drug molecules.[31]

1. Emergency situations where rapid assessment required to understand the knowledge on potential toxicity.
2. Cases with limited availability of test material.

3. Challenges for laboratory-based assays.
4. Non feasibility of synthesis of complex test drug
5. Situation demands a less time consuming and less expensive experimental test.
6. Supports the principle of 3R (replacement, refinement, and reduction)

Applications of in-silico toxicology: [31]

It includes,

1. Alternative to test data: These assays are alternative assays for animal testing assays. It is an alternative method to quickly fill the data gap in the toxicity profile.
2. As a part of weight of evidence in regulatory submissions: In current situation, regulatory frameworks accept only certain specific laboratory tests as endpoints. In such cases in silico assessment will complement the toxicology data.
3. Mixtures assessment: The toxicology profile of mixtures is dangerous and much hazardous to assess in an in vivo testing condition. Synergistic effects of such compounds can be better evaluated with in silico approaches.
4. Assessment of impurities and degradation products: The chemicals or drug molecules may contain small level of impurities produced during the manufacturing process. The ICH M7 guidelines provides specific recommendations for assessing these impurities by using two complementary computational toxicology methodologies i.e. statistical based and expert rule-based models to predict the level of toxicity.
5. Residues of plant protection products: This serves an effective alternative approach to evaluate the residue of plant protection products for dietary risk assessment of various plant products.
6. Assessment of extractables and leachable: Medical devices, food contact substances, consumer product packaging materials etc may cause a risk for human health due to the release of potential toxic substances. An in-silico toxicology assessment will provide sufficient data for the risk assessment in these situations.
7. Worker's safety and occupational health: Chemicals using in many industries may possess unexplored mutagenicity, carcinogenicity, reproductive and development toxicity and acute toxicity. These potential toxic effects can be

studied using in silico methods. In silico approaches utilises specific end points to predict major toxicological effects like respiratory sensitization.

8. Metabolite analysis: The metabolites can present with increased or decreased risk of local or systemic toxicity effects when compared to parent chemical. If a reactive, toxic metabolite is formed by an organism, their identification, separation, and possible synthesis are challenging. In silico methods provide a practical alternative approach to understand the safety profile of potentially large number of chemicals as well as un explored metabolites.
9. Ecotoxicology: Various chemicals molecules are discharged into the environment which may cause potential harm. These parent compounds can be transformed into various additional chemicals by hydrolysis, redox-reactions or photolysis. Prediction of physico-chemical parameters supports the assessment of potential environmental exposure to the chemical.
10. Green chemistry and safer alternatives: It identify drug molecules with a safer profile than existing one's with toxicity potential. These assays will provide an insight about structural features which are responsible for toxicity.
11. Selection of product development candidates: This provide a helpful approach to shortlist the drug candidates as it is inexpensive, rapid to perform, and high throughput. It helps in optimisation by suggesting the toxicophores.
12. Emergency response situations: When there is an unexpected human exposure to some new chemical or drug molecules, this will help to evaluate the toxicity effects quickly. In the absence of previously generated data, this alone serves as a quick practical option to tackle the hazardous situation.
13. Prioritizing chemical testing: this can help to prioritise the in vitro and in vivo toxicology testing based upon the chemical exposure and toxicity prediction based on potential toxicological liabilities.
14. Rationalization of in vitro and in vivo study results: The results from different in silico models can be used in conjunction with biological data to infer the mechanism of action or mode of toxicity. This information will facilitate to tailor made the in vivo assays.

ADME prediction

ADME is used to describe the pharmacokinetic properties such as, absorption, distribution, metabolism, and excretion of drugs. It is a useful tool to predict the pharmacological properties of drug candidates, especially during the pre-clinical stage of drug development. Usage of in-silico models in ADME prediction contributes to drug optimization and prevents late-stage drug candidate failures.[32]

Absorption:

Absorption is predicted from water solubility, lipophilicity, and percentage of intestinal human absorption (HIA) properties. Water solubility is predicted using the Silicos IT LogSw descriptor of *Swiss ADME*. In the *Swiss ADME* LogSw scale, compounds with values less than (more negative than) −6 are poorly soluble. Lipophilicity was assessed using the logarithm of the *n*-octanol/water partition coefficient, which is predicted using the Consensus $LogP_{o/w}$ descriptor of *Swiss ADME*. $LogP_{o/w}$ is closely related to transport processes, including membrane permeability, and distribution to different tissues and organs.[33] The general criteria to have good oral bioavailability i.e. good permeability and solubility is to have a moderate logP ($0 < \log P < 3$).[34]

Distribution:

Distribution is predicted using the glycoprotein P (P-gp) substrate, blood–brain barrier (BBB) permeability and fraction unbound descriptors. P-gp is an ATP-dependent drug-extracting pump, and it is found in various human tissues. All of the new synthesized molecules were predicted to be substrates of P-gp. The BBB is a complex structure that separates the central nervous system (CNS) from the peripheral tissue. In order to maintain homeostasis in the CNS, the BBB controls the transfer of material, nutrients and cells from the blood to the brain and from the brain to the blood. It also participates in the clearance of cellular metabolites and toxins from the brain to the blood.[35]

Metabolism:

Metabolism is estimated using *Swiss ADME* according to inhibition of the main cytochromes (CYP) of the P450 superfamily, namely CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4. CYP enzyme inhibition, a principal mechanism for metabolism-based drug–drug interactions, usually involves competition with another

drug for the same enzyme binding site. Enzyme inhibition impairs the biotransformation or clearance of all clinically used drugs including several anticancer agents, resulting in higher plasma levels of drugs that influence the therapeutic outcome. If the drug is a prodrug, then the effect is decreased. Thus, inhibition of CYPs may lead to toxicity or lack of efficacy of a drug.[36]

Excretion:

Excretion occurs primarily as a combination of hepatic and renal clearance, is related to bioavailability, and is important for determining dosing rates to achieve steady-state concentrations. Excretion values were predicted, using the total clearance (CLtot) descriptor of *pkCSM-pharmacokinetics*, to range from 0.02 to 0.138 ml/min/kg.[37]

In silico tools for ADME prediction

The major softwares and models used for in silico ADME prediction are tabulated below.[38]

Table:2. Absorption, distribution, metabolism, and excretion models

Software/model	**Features**
Cerius2-ADME	Provides computational ADME/Tox prediction tools with the ability to predict problematic new chemical entities at an early stage.
Physico-chemical laboratory	Predicts pKa, LogP, LogD, solubility, boiling point, and vapour pressure.
Artificial Intelligence software	Uses the chemical descriptors and numerical modelling techniques. Series of predictive algorithms which are used to assess the likely ADMET properties of new chemical structures
Predictive ADMET	HIA model significantly improves the prediction of problematic compounds with respect to their passive absorption profiles. This model has addressed the issue of

	over-prediction of absorption, which has been a major problem with previous HIA models.
iDEA™ predictive ADME stimulations system	The absorption module predicts permeability and FDp over time. The metabolism module predicts bioavailability, linked to the absorption module for inputs.
ADME/Tox screens ADME boxes	Classifies compounds as permeable (% HIA >10–15) or nonpermeable (% HIA < 10-15). Three modes of permeation are considered: paracellular, non-restricted transcellular, restricted transcellular.
PreADME	Web-based application for predicting ADME data and building drug-like chemical library using neural network with back propagation method.
QikProp	QikProp results have been fitted to datasets of drug-like molecules, based on 2D and 3D descriptors reflecting Monte Carlo simulation studies as well as experiment
GastroPlus QMPRPlus	Biopharmaceutical property estimation, solubility, permeability, absorption, and distribution. Simulations and prediction of gastrointestinal dissolution, transit, and pharmacodynamics.
VolSurf	Predicts ADME properties using pre-calculated models; computes unique, ADME-relevant descriptors and creates QSAR models of bioactivity or property

Conclusion

In the present era, application of machine learning in prediction of toxicity profile of new drugs can be evaluated and documented. An increase in the access to data and computing power have contributed to the use of in silico methods for prediction of toxicity. Easily accessible open-source tools and heterogenous high dimensional data sets are equally available for pre-processing and predictive modelling of data. The success rates of these efforts improve over time. Most machine tools are often referred as “black boxes”, as the rational interpretations behind the underlying mechanisms are difficult. Even those models with high accuracy do not readily

discloses the biological mechanisms behind predictions. The quality of data used for train a model is more vital than the choice of algorithm used.

Even though the in-silico data is not sufficiently accurate to replace the in vitro and in vivo assays, they will provide a baseline idea about the toxicology profile of concerned drug entity. These in silico models do not consider dose or exposure into account for the toxicity study. So, they are unable to produce the absolute level of toxicity, but furnishes supplementary information for the overall risk assessment process. Therefore, data on internal exposure must be taken into consideration. The results obtained from these assays contributes to the screening process of new drugs.

The in silico/in vitro/in vivo/PK profiling architecture of a new drug molecule is the vital ingredient in the drug discovery and development process. This can prioritise the candidates in drug development process. This proper establishment of in silico-in vitro correlation (ISIVC) and in vitro-in vivo correlation (IVIVC) will eventually increases the productivity of pharmaceutical industry.

A wide variety of tools exists to assess the general toxicity and organ specific toxicity effects. Standardisation of in silico tool use and interpretation of results would greatly reduce the burden on both industry and regulatory authorities and will provide confidence for the use of these approaches. Integration and implementation of in silico methodologies for toxicity evaluation are rapidly evolving. In silico predictive toxicology enables regulation or safety formulation to impact the discovery of drugs with a superior safety profile. Development of more physiologically relevant predictive toxicity model systems, advanced algorithms and analysis methods will successfully replace in vitro and in vivo toxicity tests.

References:

1. Yongye A. B.; Waddell J.; Medina-Franco J. L. Molecular scaffold analysis of natural products databases in the public domain. Chem. Biol. Drug Des. 2012, 80, 717–724. 10.1111/cbdd.12011.
2. Pilón-Jiménez B. A.; Saldívar-González F. I.; Díaz-Eufracio B. I.; Medina-Franco J. L. BIOFACQUIM: A Mexican compound database of natural products. Biomolecules 2019, 9, 31 10.3390/biom9010031.
3. Sánchez-Cruz N.; Pilón-Jiménez B. A.; Medina-Franco J. L. Functional group and diversity analysis of BIOFACQUIM: A Mexican natural product database. F1000Research 2019, 8, 2071 10.12688/f1000research.21540.1
4. Gupta P. K, Disposition. In Illustrated Toxicology; Elsevier, 2018; pp 67–106. ISBN 9780128132135.
5. Bocci G.; Carosati E.; Vayer P.; Arrault A.; Lozano S.; Cruciani G. ADME-Space: a new tool for medicinal chemists to explore ADME properties. Sci. Rep. 2017, 7, 6359 10.1038/s41598-017-06692-0.
6. Durán-Iturbide NA, Díaz-Eufracio BI, Medina-Franco JL. *In Silico* ADME/Tox Profiling of Natural Products: A Focus on BIOFACQUIM. ACS Omega. 2020 Jun 25;5(26):16076-16084. doi: 10.1021/acsomega.0c01581. PMID: 32656429; PMCID: PMC7346235.
7. Bernard D, Coop A, Mac Kerell AD Jr. Computer-Aided Drug Design: Structure-Activity Relationships of Delta Opioid Ligands. Drug Des Rev. 2005,2: 277-291.
8. Kaczanowski S, Zielenkiewicz P. Why similar protein sequences Encode similar three M dimensional structures. Theoretical Chemistry Accounts. 2010,125:543–50.
9. Park DS, Kim JM, Lee YB, Ahn CH. QSID Tool: anew three-dimensional QSAR environmental tool. Journal of Computer-Aided Drug Design. 2008, 22(12): 873-883.
10. Richard C. Bioinformatics in Computer-Aided Drug Design. 2005.
11. Lengaure T, Rarery M. computational methods for molecular docking. curropninstructbiolo. 6:402- 406.
12. Soloman AK. Molecular modelling and drug design.2nd edition (Chennai), MB publisher, 2008.
13. Lesk AJM. Introduction to bioinformatics. Oxford university press inc. New York. 2002.

14. Perdo HL. A systematic review on CADD: Docking and Scoring. JMPI. 2010, 47-51.
15. http: en.wikipedia.org/wiki/Virtual _high_throughput_screening., last accessed on 21/05/2023.
16. Walters WP, Stahl MT, Murcko MA. Virtual screening – an overview. Drug.Discov. Today. 1998, 3 (4): 160– 178.
17. Bohacek RS, McMartin C, Guida WC. The art and practice of structurebased drug design: a molecular modeling perspective. Med. Res. Rev. 1996,16: 3–50.
18. Kovesdi I, Rodriguez MFD, Orfi L, Naray-Szabo G, Varro A, Papp JG, Matyus P. Application of neural networks in structure-activity relationships. Med Res Rev. 1999, 19:249.
19. Suh M, Park S, Jee H. Comparison of QSAR Methods (CoMFA, CoMSIA, HQSAR) of Anticancer 1-N-Substituted Imidazoquinoline-4,9-dione Derivatives. Bull. Korean Chem. Soc. 2002, 23: 417-422.
20. Cramer RD, Patterson DE, Bunce JD. Comparative Molecular Field Analysis (CoMFA). 1. Effect of Shape on Binding of Steroids to Carrier Proteins. J. Am. Chem. Soc. 1988, 110: 5959-5967.
21. Wold S, Ruhe A, Wold H, Dunn WJ. The collinearity problem in linear regression. The partial least squares approach to generalized inverse. SIAM J. Sci.Stat. Comput. 1984, 5: 735-743.
22. Malinowski ER, Howery DG. Factor Analysis in Chemistry; Wiley: New York. 1988.
23. . Kurogi Y, Guner OF. Pharmacophore modeling and three-dimensional database searching for drug design using catalyst. Curr.Med.Chem. 2001, 8:1035-1055.
24. Meyer EF, Swanson SM, Williams JA. Molecular modeling and drug design. PharmacolTher. 2000, 85:113-121.
25. Go N, Scherga HA. Ring closure and local conformational deformations of chain molecules. Macromolecules. 1970, 3(2).178-187.
26. Allen MP, Tildesley DJ. Computer Simulation of Liquids, Oxford Science Publications, Oxford, UK, 1989, 385.
27. Tuckerman ME, Martyna GJ. Understanding Modern Molecular Dynamics: Techniques and Applications. J. Phys. Chem. B. 2000, 104: 159-178.

28. Wadood A, Ahmed N, Shah L, Ahmad A, Hassan H, Shams S. In-silico drug design: An approach which revolutionarised the drug discovery process. OA Drug Design & Delivery 2013 Sep 01;1(1):3.
29. Raies AB, Bajic VB. In silico toxicology: computational methods for the prediction of chemical toxicity. Wiley Interdiscip Rev Comput Mol Sci 2016;6(2):147–7
30. Parthasarathi Ramakrishnan. Dhawan Alok, Chapter 5 - In Silico Approaches for Predictive Toxicology, Editor(s): Alok Dhawan, Seok Kwon, In Vitro Toxicology, Academic Press, 2018, Pages 91-109, ISBN 9780128046678, https://doi.org/10.1016/B978-0-12-804667-8.00005-5.
31. Myatt GJ et al, In silico toxicology protocols. Regul Toxicol Pharmacol. 2018 Jul; 96:1-17. doi: 10.1016/j.yrtph.2018.04.014. Epub 2018 Apr 17. PMID: 29678766; PMCID: PMC6026539.
32. Durán-Iturbide N.A., Díaz-Eufracio B.I., Medina-Franco J.L. ACS Omega. 2020; 26:16076.
33. H. Van de Waterbeemd, in:, Comprehensive Medicinal Chemistry II, Elsevier, 2007, pp. 669–697.
34. Zerroug A., Belaidi S., BenBrahim I., Sinha L., Chtita S. *J. King Saud Univ. – Sci.* 2018;4:595.
35. Małkiewicz M.A., Szarmach A., Sabisz A., Cubała W.J., Szurowska E., Winklewski P.J. *J. Neuroinflammation.* 2019;16:15.
36. Manikandan P., Nagini S. *Curr. Drug Targets.* 2018;19:38.
37. Pires D.E.V., Blundell T.L., Ascher D.B. *J. Med. Chem.* 2015;58:4066.
38. Paranjpe, P.V., Grass, G.M. & Sinko, P.J. In silico tools for drug absorption prediction. Am J Drug Deliv 1, 133–148 (2003). https://doi.org/10.2165/00137696-200301020-00005.