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In-vitro ADME studies of TUG-891, a GPR-120 inhibitor using Swiss ADME predictor

Mishra Shweta*, Dahima Rashmi

School of Pharmacy, Devi Ahilya Vishwavidyalaya, Takshashila Campus, Khandwa Road, Indore-452001, M.P., India

ABSTRACT

Predicting the absorption, distribution, metabolism and elimination (ADME) profile of drug candidates before their synthesis, in the early stage of drug discovery, could help in selecting candidates with the less critical ADME profile. *In vivo* ADME assessment is found to be costly, time consuming and involve the lives of animals, so the *in vitro* ADME analysis is better, cheaper and provides accurate results quickly. TUG-891 is a GPR-120 inhibitor under clinical trials. The aim of the present study is to predict the *in vitro* ADME studies of TUG-891, to know the expected outcome of the clinical trials and finding the correlation between the *in vivo* and *in vitro* results along with the improvisation in the structure of the TUG-891, so that the biological activity remains unaffected, but reduces the unwanted ADME effects. The 2D and 3D structures of TUG-891 were drawn on chemdraw 3D-Ultra version 8.0 by minimizing the energy using MM2 and MOPAC setting the minimum RMS gradient to 0.01. The structure was imported, the structure smiley was entered and the Swiss ADME drug design study was run. The bioavailability radar showed that the colored zone is the suitable physicochemical space for oral bioavailability where the following properties were taken into consideration as flexibility, lipophilicity, saturation, size, polarity and solubility. The pharmacokinetic properties were studied using the boiled egg model allows for intuitive evaluation of passive gastrointestinal absorption and brain penetration in function of the position of the molecules in the WLOGP-versus-TPSA referential. The white region is for high probability of passive absorption by the gastrointestinal tract and the yellow region that is yolk, is for high probability of brain penetration. Yolk and white areas are not mutually exclusive. Through the study conducted it could be concluded that the aqueous solubility of the compound should be increased along with the fraction of sp_3 hybridized carbon atoms. The molecule should not be the inhibitor of metabolizing enzymes and so further modifications need to be done on the lead structure.

Keywords: ADME, GPR-120 inhibitor, *in vitro* prediction, TUG-891

*Address for Correspondence:

Shweta Mishra, Doctoral Research Fellow, School of Pharmacy, Devi Ahilya Vishwavidyalaya, Takshashila Campus, Khandwa Road, Indore-452001, M.P., India.

INTRODUCTION

Drug development involves the assessment of efficacy and toxicity of the new drug candidates and includes generating a hypothesis of the target receptor for a particular disorder and screening the *in vitro* and/or *in vivo* biological activities of the new drug candidates. A critical piece in drug discovery and development is conducting DMPK (drug metabolism and pharmacokinetics) studies, often referred to as ADMET (absorption, distribution, metabolism, elimination and toxicity) studies. The initiation of early absorption, distribution, metabolism and excretion (ADME) screening has dramatically decreased the proportion of compounds failing in clinical trials. The main aim of preclinical ADME is to eliminate weak drug candidates in the early stages of drug development, which allow resources to be focused on potential drug candidates.[1] Regulatory authorities have relied on *in vivo* testing to predict the behavior of new molecules in the human body since the 1950s.

Bioavailability, tissue distribution, pharmacokinetics, metabolism and toxicity are typically assessed in one rodent and one non-rodent species (dog or non-human primate) prior to administering a drug to a human to evaluate pharmacokinetics and exposure in a clinical trial, phase 1. The standard required methodology for bio-distribution assessment uses radioactively labeled compounds. This is time- and resource-intensive both in terms of synthesizing sufficient amounts of radioactively labeled compound and of performing the animal experiments.[2] Therefore, these assays are implemented rather late in the preclinical development process when more resources are released to study the few molecules that have advanced to that stage. With advances in cell and molecular biology, high-throughput screening and miniaturization technologies in the 1990s, as well as stem cell-derived models at the beginning of this century, early *in vitro* ADME studies have been developed to predict *in vivo* animal and human results at a level of speed and cost effectiveness appropriate for the early discovery stage.[3] The

preclinical studies for drug screening involve the use of animals which is very time consuming and expensive and at times leads to suffering of the used organism. This has forced the researchers to find ways to not only decrease the time involved in drug screening procedures but also decrease the number of animals used and also increase the humane care of animals. To fulfill this goal a number of new *in vitro* techniques have been devised which are called 'Alternatives' or 'Substitutes' for use of animals in research involving drugs. The advantages of these alternatives include the decrease in the number of animals used, ability to obtain the results quickly, reduction in the costs and flexibility to control the variables of the experiment.[2] The progress of ADME profiling has decreased the proportion of drug candidates failing in clinical trials for ADME reasons and providing important early input into safety and toxicity prediction of drug candidates.

Swiss ADME web tool is freely available software used to predict the physicochemical properties, absorption, distribution, metabolism, elimination and pharmacokinetic properties of molecules, which are key endeavors to further, go for the clinical trials. It takes into account six physicochemical properties, which are most important like flexibility, lipophilicity, saturation, size, polarity and solubility.[4]

Drug likeliness parameters can be studied by using few rules which were set by some pharmaceutical companies setting the relationship between the pharmacokinetic properties and biological activities which should be followed for having *in vivo* activity. Lipinski rule of five was given by Pfizer stating that there should be less than 500 dalton molecular weight, the hydrogen bond acceptor should be not more than 10, the hydrogen bond donor should be more than 5, log P value should be not more than 5 and the biological transporters should not be a part of drug. Ghose rule was given by Amgen which stated that log P value should be from -0.4 to +5.6, molar refractivity should be between 40-130, the molecular weight should be between 180-480 and the number of atoms should be 20-70, including hydrogen bond donor and hydrogen bond acceptor. The Egan rule was given by Pharmacia Company for the prediction of human intestinal absorption stating the log P value should not be more than 5.88 and topological polar surface area should not be more than 131.6. Some drugs like steroids the molecular weight should be more than 500 D, 10 or fewer rotatable bonds should be there, polar surface area should not be more than 140 A° are orally active stated by Verber, given by GSK pharmaceuticals. Muegge rule was given by Bayer Pharmaceuticals, stated the parameters as, molecular weight 200-600 D, log P between -2 to +5, topological surface area not more than 150, number of rings not more than 7, number of carbon atoms not less than 4, number of heteroatoms more than 1, number of rotatable bonds not more than 15, hydrogen bond donor atoms not more than 5 and hydrogen bond acceptor atoms should be more than 10. Abbott bioavailability F should not be more than 10%.[5]

For having a good biological activity, a drug should have sufficient lipophilic character so that it can cross the cell membrane. The lipophilicity parameter can be calculated by using several methods. ilogP stands for in-house physics based method relying on free energies of solvation in n-octanol and water calculated by generalized born and solvent accessible surface area model. The XlogP3 is an atomistic method including corrective factors and knowledge based library calculated by XlogP program. WlogP is an atomistic method based on fragmental system. MlogP is topological method relying on a linear relationship with 13 molecular descriptors. SILICOS-IT logP is a hybrid method relying on fragment and topological descriptors. Consensus logP is arithmetic mean of all five predictions of lipophilic character.[5]

A drug should have good aqueous solubility for oral bioavailability and absorption. The water solubility is predicted by three methods namely ESOL, (ALI) logS and (SILICOS-IT) logS. ESOL stands for estimating aqueous solubility directly from molecular structure directly from molecular structure followed by molecular weight, proportion of heavy atoms in aromatic system and number of rotatable bonds. The model performed consistently well across 3 validation sets predicting solubility within a factor of 5-8. Log S (Ali) determines the *in silico* prediction of aqueous solubility incorporating the effect of topological polar surface area. Log (SILICOS-IT) determines the negative logarithm of water solubility of a compound by fragmental method. The log S scale value ranges between -10 (insoluble), -6 (poorly soluble), -4 (soluble), -2 (very soluble) and 0 (highly soluble).[4,5]

Pharmacokinetic parameters include gastrointestinal absorption, blood-brain barrier permeation, P-gp substrate, CYP 1A2 inhibitor, CYP2C19 inhibitor, CYP2C9 inhibitor, CYP2D6 inhibitor, CYP3A4 inhibitor and Log K_p (skin permeation). One model is a multiple linear regression, which aims at predicting the skin permeability coefficient (Kp). It is adapted from Potts and Guy, who found Kp linearly correlated with molecular size and lipophilicity (R_2=0.67). The more negative the log Kp (with Kp in cm/s), the less skin permeant is the molecule. The predictions for passive human gastrointestinal absorption and blood-brain barrier permeation both consist in the readout of the BOILED-Egg model 17. Brain or Intestinal Estimated Permeation method (BOILED egg) is proposed as an accurate predictive model that works by computing the lipophilicity and polarity of small molecules. Permeability glycoprotein (P-gp) substrate is key to appraise active efflux through biological membrane. It protects CNS from xenobiotics and is overexpressed in tumor cells. 50-90% molecules are substrates of 5 major isoforms CYP 1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4. Inhibition of these isoenzyme is certainly one major cause of pharmacokinetics related drug-drug interactions leading to toxic ADME due to accumulation of drug/ metabolites.[6]

Pan assay interference compounds (PAINS) are chemical compounds that often give false positive results in high throughput screens. It tends to react non-specifically with numerous biological targets rather than specifically affecting one desired target. Common PAINS include curcumin, phenl sulfonamides, isothiazolones, quinanones, catechols etc. Synthetic accessibility score ranges from 1 (very easy) to 10 (very difficult).

TUG-891 is a GPR-120 inhibitor which is under clinical trials. The aim of the present study is to predict the *in vitro* ADME studies of TUG-891, to know the expected outcome of the clinical trials and finding the correlation between the *in vivo* and *in vitro* results along with the improvisation in the structure of the TUG-891, so that the biological activity remains unaffected, but reduces the unwanted ADME effects.

MATERIALS AND METHOD

Swiss ADME is a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Compared to the state-of-the art of free web-based tools for ADME and pharmacokinetics e.g. pk-CSM[4] and admetSAR[5] and apart from unique access to proficient methods e.g. iLOGP[7] or the BOILED-Egg,[8] strong points of Swiss ADME are non-exhaustively: different input methods, computation for multiple molecules and the possibility to

display, save and share results per individual molecule or through global intuitive and interactive graphs. Finally, Swiss ADME is integrated in the Swiss Drug Design workspace.[9]

The 2D structure of TUG-891 was drawn on chemdraw, 2D and 3D structures were drawn on chemdraw 3D-Ultra version 8.0 by minimizing the energy using MM2 and MOPAC setting the minimum RMS gradient to 0.01. The structure was imported and the structure smiley was entered. The Swiss ADME drug design study was run and the reading were noted down.

RESULT AND DISCUSSION

The bioavailability radar (Figure 1) showed that the colored zone is the suitable physicochemical space for oral bioavailability where the following properties were taken into consideration as flexibility, lipophilicity, saturation, size, polarity and solubility. The lipophilicity of the compound log P can range from -0.7 to +5.0. The molecular weight can range from 150 g/mol to 500 g/mol. The topological polar surface area ranges from 20-130 $A^{\circ 2}$. The insolubility studied using log S (ESOL) ranges between 0 to 6. The number of rotatable bonds should be between 0-9 and the unsaturation fraction ranges from 0.25 to 1.0 indicating the fraction of carbon atoms in the sp_3 hybridization should not be less than 0.25.

The physicochemical properties states that the molecular formula of the compound is $C_{23}H_{21}FO_3$. The molecular weight was 364.41 g/mol. The number of heavy atoms is 27 and number of aromatic heavy atoms was 18. The fraction of carbon atoms in the sp3 hybridization was 0.17. The numbers of rotatable bonds were 7, the numbers of hydrogen bond acceptors were 4 and the number of hydrogen bond donor was 1. The molar refractivity was 104.13 and the topological polar surface area was found to be 46.53 $A^{\circ 2}$.

The log $P_{o/w}$ (ilog P) is 3.00, the log $P_{o/w}$ (Xlog P3) is 5.24, the log $P_{o/w}$ (Wlog P) is 5.67, the log $P_{o/w}$ (MlogP) is 4.78, the log

$P_{o/w}$ (SILICOS-IT) is 6.12 and the consensus log $P_{o/w}$ is 4.96 respectively. From the log P values overall it can be concluded that the compound is having good lipophilic character.

The water solubility of the compound was studied using log S (ESOL) value as -5.43 depicting the compound belongs to moderately water soluble class.
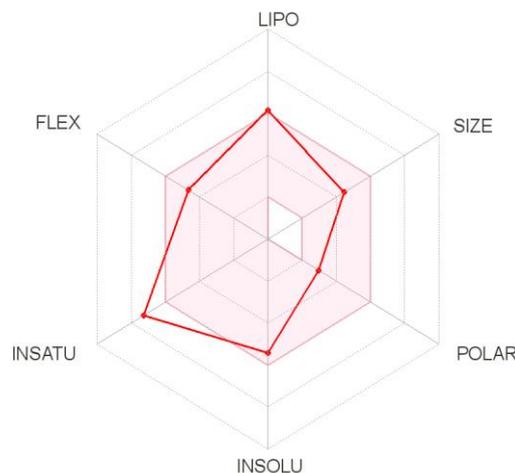

Figure 1: The bioavailability radar of TUG-891 using Swiss ADME predictor.

The pharmacokinetic properties were studied using the boiled egg model (7) allows for intuitive evaluation of passive gastrointestinal absorption (HIA) and brain penetration (BBB) in function of the position of the molecules in the WLOGP-versus-TPSA referential (Figure 2). The white region is for high probability of passive absorption by the gastrointestinal tract, and the yellow region (yolk) is for high probability of brain penetration. Yolk and white areas are not mutually exclusive.
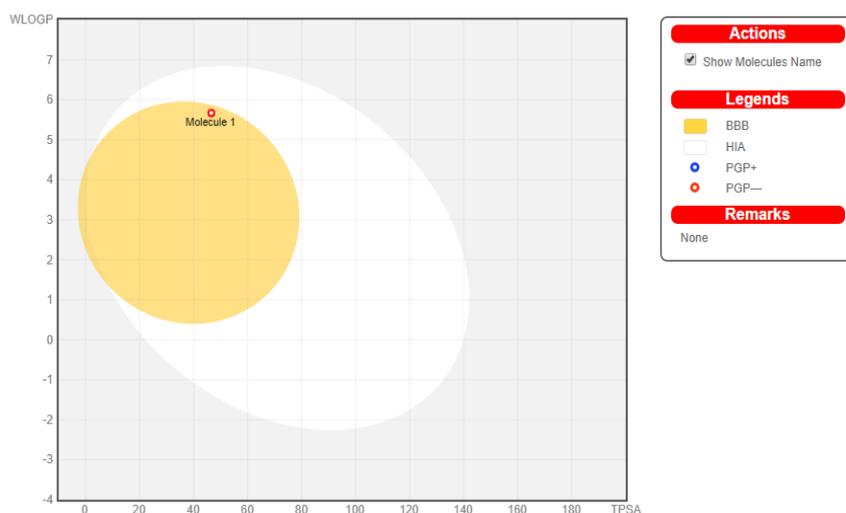

Figure 2: Molecule falling in egg's yolk is predicting the molecule is able to penetrate through the blood brain barrier.

The molecule TUG 891 is showing high gastrointestinal absorption and easily permeate blood brain barrier. It is not a P-gp substrate means there would not be an issue in the excretion of drug. P-glycoprotein plays a significant role in drug absorption and disposition. Because of its localization,

P-glycoprotein appears to have a greater impact on limiting cellular uptake of drugs from blood circulation into brain and from intestinal lumen into epithelial cells than on enhancing the excretion of drugs out of hepatocytes and renal tubules into the adjacent luminal space. It is an inhibitor of

isoenzyme CYP2C19 and CYP2D6 which means there might be the chances of accumulation or drug-drug interaction resulting in toxicity.

Drug likeness parameter is high as it is following Lipinski, verber, egan rule with the bioavailability score of 0.56. Swiss ADME Synthetic Accessibility (SA) Score is based primarily on the assumption that the frequency of molecular fragments in 'really' obtainable molecules correlates with the ease of synthesis. The fragmental contribution to SA should be favorable for frequent chemical moieties and unfavorable for rare moieties. The synthetic accessibility score was found to be 2.78 which means it would not be tough to synthesize the molecule. There is no alert for PAINS indicating the compound is quite specific in nature.

CONCLUSION

The traditional clinical trial approaches needs a lot of time and money investment after which it might conclude that the molecule fails, so in order to reduce or modify the lead structure it is important to go for the *in vitro* studies rather than blindly following monopoly and rushing for the animal studies. Swiss ADME web tool enables the computation of key physicochemical, pharmacokinetic, drug-like and related parameters for one or multiple molecules. Through the study conducted it could be concluded that the aqueous solubility of the compound should be increased along with the fraction of sp_3 hybridized carbon atoms. The molecule should not be the inhibitor of metabolizing enzymes and so further modifications need to be done on the lead structure.

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