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Research Article

In silico comparative study of drug designing and development of L-Citrulline and its analogues

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Abstract: L-citrulline is one of the important agents in the treatment of heart diseases, obesity, type 2 diabetes, trauma, severe stress, carcinogenesis, tumor growth, wound healing, immune system and to reduce blood pressure; and particularly erectile dysfunction. However, expansion of the clinical utility of l-citrulline has been limited as it is not very organ specific. Computational design of analogues of l-citrulline has been reported in this paper. The structure and relative energies of the target molecules are predicted using Hartree- Fock method. The methods of theoretical chemistry have been used to elucidate the molecular properties. The analysis of molecular descriptors defined by Lipinski has shown that the candidate analogues obey 'rule of five'. The solubility of drugs in water have been determined as it is of useful importance in the process of drug discovery and development from molecular design to pharmaceutical formulation and biopharmacy. All toxicities associated with candidate drugs have been calculated. P-glycoprotein (P-gp) is a cell membrane-associated protein that transports a variety of drug substrates. P-gp expressed in normal tissues as a determinant of drug pharmacokinetics and pharmacodynamics been examined. Drug plasma-protein binding and volume of distribution are one of the many factors which influences bioavailability of a drug , hence its value have also been calculated. To avoid rejection of drugs, it is becoming more important to determine pKa, absorption, polar surface area and other physiochemical properties associated with a drug, before synthetic work is undertaken.

Introduction

L-Citrulline, a colorless, water soluble, is a non-essential amino acid first identified from the juice of watermelon, *Citrullus vulgaris* Schrad, and later obtains from tryptic digestion of casein[1]. Watermelon is the richest known source of L-Citrulline, and it is thought that this amino acid plays an important role in drought tolerance [2].

When consumed, citrulline is converted to arginine through certain enzymes. Arginine is an amino acid that has a significant role in nutrition due to its multiple physiological and pharmacological activities. While it is

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classified as a nonessential amino acid in unstressed animals and humans, it becomes an indispensable in times of heart diseases, obesity, type 2 diabetes, trauma, severe stress, carcinogenesis, tumor growth, wound healing, immune system and reduce blood pressure. Arginine is one of the most versatile amino acids, functioning as precursor for nitric oxide, urea, ornithine, proline, polyamines, agmatine, creatine and several other body proteins [3].

A drawback to administering L-arginine orally is that a large portion of the L-arginine passes through the gastrointestinal tract and the hepatic portal system where it is catabolized by arginase I to ornithine and urea [4]. Moreover, chronic L-arginine treatment may have adverse effects on cardiovascular function [5]. Therefore, oral L-citrulline supplementation is an important substitute for L-arginine supply under pathologic conditions that increase arginase activity and or limit L-arginine availability. By converting into L-arginine, L- citrulline plays an important role in supplying L-arginine to NOS. Unlike L-arginine, it bypasses hepatic metabolism and it is not a substrate of arginase. Also, there is no evidence of transporter dysfunction for L-citrulline under pathological conditions, such as oxidative stress, that can occur for L-arginine transport. It also has been demonstrated that L-citrulline cannot sustain NO production by iNOS, but that it can support NO production with eNOS activation [6]. L-citrulline supplementation to patients with sickle cell disease, in which elevated arginase activity is manifest raised plasma L-arginine levels and reduced their symptoms [7]. Arginine boosts nitric oxide, which is a cellular signaling molecule that has multiple biological functions and which relaxes blood vessels, the same basic effect that sildenafil (Viagra), has, to treat erectile dysfunction and maybe even prevent it. Chief among the beneficial effects of NO in the body is its role in improving blood flow. Other biological activities include muscle relaxation, modulation of immune responses, reduced inflammation, increased kidney function, enhanced sexual performance (notably with respect to penile erections) and stimulated hormone secretion. NO also have favorable effects on nervous system and brain function. In short, NO is a remarkably versatile, multifunctional biological molecule [8]. Citrulline may not be as organ specific as Viagra, but it's a great way to relax blood vessels without any drug side-effects.

The analogues of an existing drug molecule shares chemical and therapeutic similarities with the original compound. The chemical design of analogues makes use of simple and traditional procedures of medicinal chemistry [9].

Various analogues of citrulline has been reported and compared with citrulline and with each other to test that which analogue is more organs specific and involve vascular smooth muscle relaxation and increased blood flow in the penile tissues. Our objectives of analogue design are the identification and development of a possibly improved version of a prototype L-Citrulline drug, which will become suitable drug. Such compounds are often “direct analogues”, and therefore are chemically and pharmacologically similar to the prototype drug.

For comparing various analogues we have used the results of large-scale theoretical calculations for the study of the lipophilicity, solubility, absorption, polar surface area, solubility, bioavailability, and partition coefficient, volume of distribution, gastro intestinal absorption, clearance and toxicity. Drug plasma-protein binding which

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is one of the many factors which influences bioavailability of a drug has been calculated. P-glycoprotein which plays a role in the protection of the organism against potentially toxic substances has also been calculated. We have also evaluated of the "druglikeness" and predicted the probable activity profile by an approach which consists of comparing a newly prepared molecule to a training set of about 35,000 active compounds for which the main and the side pharmacological effects, the mechanism of action, the mutagenicity, the carcinogenicity, the teratogenicity, and the embryotoxicity are (at least partly) known. The program then predicts the potential biological activity of the new molecule. These predictions, if confirmed experimentally, may provide new leads from drugs that are already on the market.

As experimentally determined values are not directly useful in the design process, hence we need the properties before the compounds are made, thus potential sue of this drug has been proposed.

Conversion of L-citrulline to L-arginine

Conversion of L-citrulline to L-arginine occurs not only in the cells of the kidney proximal tubules, but also in the cells of many tissues [10] . Cells involved in the production of NO as well as cells that produce ornithine and urea by the catabolism of L-arginine also produce L-citrulline, which can then be recycled to arginine. The process of L-citrulline catabolism to L-arginine is a two-step enzymatic process involving the rate limiting enzyme arginosuccinate synthase (ASS) and arginosuccinate lyase (ASL) . In the presence of aspartate and ATP, L-citrulline is converted to arginosuccinate by ASS. Arginosuccinate is cleaved by ASL to form fumarate and L-arginine [11].

Computational Methods

Hartree- Fock calculations were performed using Spartan' 06 program [12] at the B3LYP [13] levels of theory with 6-31G** basis set [14] . The compounds were built with a standard bond length and angles using the PC SPARATN Pro Ver 1.08 molecular modeling program. The molecular mechanic methods minimized the energy and then by the Hartree-Fock method at 6-31G** level**.** Molecular modeling and determination of molecular properties of drug structures as accomplished by Chem-Sketch [15] , Molinspiration [16] and MolSoft [17]. Solubility, Log Kow, and dermal permeation coefficient were determined by EpiSuite software (AllidSystems, Sylmar, A). Drug likeness was determined by methods of Actelion and MolSoft. Values of pKa were determined by using SPARC On Line Calculator for properties (Version August 2003, University of Georgia, Athens, GA, www.uga.edu). Prediction of the probable activity profile was done by PASS (Prediction of Activity Spectra for Substances) developed by Poroikov and
his team [18] .

Molecular Modeling

To obtain the most stable conformation, we used a combination of molecular mechanics and quantum chemical semi empirical calculations. Structure was built in HyperChem [19] Release 7 for Windows (Hypercube Inc. Gainesville, Florida) using a molecular mechanics procedure under MM+ [20]. The geometry was optimized to a rms (root mean square) gradient of 0.001 in vacuo (Polak-Ribière method). Then a molecular dynamics programme was run for 1 ps, with 0.001 ps steps, relaxation time 0.1 ps, to a simulation temperature of 300 K. This was followed by MM+ geometry optimization to a rms gradient of 0.2. The

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molecular dynamics run was repeated and a further MM+ protocol was carried out to a gradient of rms 0.004 on the selected drug. Angles and bond-lengths were measured on the models. Dipole moments were determined using the semi-empirical PM3 programme [21,22] in singly-excited configuration interaction. (RHF [Restricted Hartree-Fock], charge 0, spin multiplicity 1, lowest state, orbital criterion, five occupied and five unoccupied orbital.)

Results and Discussions

Lipinski's rule of five: As per Lipinski's rule of five [23], an orally active drug has (i) not more than 5 hydrogen bond donors (OH and NH groups), (ii) not more than 10 hydrogen bond acceptors, (iii) a molecular weight under 500, (iv) a partition coefficient log P under 5. A close study of our molecules fulfills nearly all requirements; hence they can be used as oral drug.

H- Bond donors and acceptors: A poor permeation or absorption is more likely when there are more than 5 H-bond donors, 10 H-bond acceptors. Hydrogen-bonding capacity has been also identified as an important parameter for describing drug permeability [24]. Its abnormal increase may result in considerably lowered absorption. . Value of HBD is 6 for A1, A2 and A4, which is slightly greater than 5, but it will not have much effect on oral usage. For A3 it is 5, which is well within the range. Value of HBA is also within upper limit for all target molecules and shown in table 1.

Lipophilicity and Partition Coefficient Log P: An important consideration, although somewhat underrated, for the predictive design of drugs is their lipophilicity and it has been interpreted as a measure of the permeation of drugs across cell membranes and their subsequent migration into the nucleus [25].

Partition or distribution coefficients are critical elements in efforts designed to describe the uptake, distribution, biotransformation, and excretion of organic chemicals in biological systems [26]. High log P values imply high solubility and good penetration of lipid membranes, but by implication, low solubility in aqueous phases, and, hence the inability for the molecule to be transported through the body. Molecules with high log P also tend to be substrates of the metabolizing cytochrome P450 enzymes in the liver, in which case, first pass effects can remove much of the administered drug candidate before it can reach its target area [27]. Log P value predicted for target drugs are shown in table 2.

***Polar Surface Area (PSA)*:** Molecular polar surface area (PSA) is a very useful parameter for prediction of drug transport properties and has been shown to correlate very well with the human intestinal absorption, Caco-2 monolayers permeability, and blood-brain barrier penetration [28]. Calculated surface characteristics of molecules have been correlated with several physicochemical properties of drug molecules including lipophilicity, the energy of hydration and the hydrogen bond formation capacity [29]. An increase in the value of PSA in the optimum model corresponded to an initial positive effect on bioavailability but then caused predicted bioavailability to drop substantially [30]. PSA of investigated molecules are within range and are shown in table 1.

Traffic lights and determination of oral absorption: Based on the Ro5 and additional properties, an elaborate and promising set of ‘Traffic Lights’ (TLs) which addresses oral

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absorption has recently been proposed by Lobell et al. for the in silico prioritization of hits[31]. Five properties have been found to be of primary importance in determining oral absorption, namely

- molecular size (as assessed by MW but including a correction for halogens), with optimal values ≤ 400;
- lipophilicity as calculated by the ClogP algorithm, with optimal values ≤ 3;
- solubility at pH 6.5 (i.e. resulting from the balanced contribution of neutral and ionised species), with optimal values ≥ 50 mg L^{-1};
- polarity (as assessed by the polar surface area (PSA)), with optimal values ≤ 120 $A°^{2}$;
- the number of rotatable bonds, with optimal values ≤ 7.

Each compound is assigned an in silico oral PhysChem score by summing up the values taken by its five TLs. The values of the PhysChem score can range from 0 to 10, and 'the lower the score, the more favourable the in silico evaluation of a compound's physicochemical properties in serving as a lead for the discovery of an orally administered drug'. Values are shown in table 3. PhysChem score is 0 for A1 and A, for A3 it is 1 and for A2 it is 2.

Blood Brain Barrier: The blood-brain barrier (BBB) is of pivotal importance to maintain homeostasis of the central nervous system, CNS, as it closely regulates the composition of the interstitial fluid in the brain [27]. The experimental determination of log*BB* is a time-consuming, expensive, and difficult technique, requiring animal experiments and the synthesis of the test compounds, usually in radio labeled form [31,32]. It is of considerable value to predict log*BB* values of compounds from their physicochemical parameters or, ideally, from their molecular structures.

So, the value ascribed to this ability is calculated as demonstrated by Clark [33]

$$\text{LogBB} = -0.0148(\text{PSA}) + 0.152\ \text{logP} + 0.139 \quad \ldots\ldots(1)$$

Clearly this gives the experimentalist a calculable value of Log BB to aim for in the experimental design of CNS active drugs. Published values of logBB range from approximately – 2.00 to + 1.00. Within this range, compounds with logBB > 0.3 cross the BBB readily, while those with logBB < -1.0 are only poorly distributed to the brain [34]. Calculated values for our molecules are shown in table 2. LogBB values for A1, A2 ad A4 is less than – 1.0, hence these are poorly distributed to brain. Values for A3 is within 0.5 and -1.0, hence it is moderately distributed to brain.

Drug Dissolution log S: The solubility of drugs in water is of central importance in the process of drug discovery and development from molecular design to pharmaceutical formulation and biopharmacy because oral absorption is dependent on the compound dissolving in the aqueous constants of gastrointestinal tract (dissolution) and then traversing the actual barrier of the gastrointestinal tract to reach the blood [35]. Dissolution depends on the surface area of the dissolving solid and solubility of the drug at the surface of the dissolving solid. Yalkowsky [36] has noted that log S correlate well with log P with an additional term involving the melting point (mp) for crystalline solute, it is given as:

$$\log S = 0.8 - \log P - 0.01(mp-25) \quad \ldots\ldots..(2)$$

Virtually all drugs have aqueous solubility [37] log S> -6.

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Solubility of candidate drugs at different constituents of body (at different pH) is shown in table 4. Probability solubility has been also shown in this table.

Drug Absorption, Permeability and Transport: It is difficult to predict drug absorption after oral dosage due to complex drug-specific parameters and physiological processes, including: drug release from the dosage form and dissolution, aqueous solubility, Gastro intestinal (GI) motility and contents, pH, GI blood flow, membrane transfer or permeability and active transport systems, and pre-systemic and first pass metabolism [38,39]. Drugs are categorized based on permeability, aqueous solubility and elimination mechanisms to improve the ability to anticipate transporter effects, and food and drug–drug interactions [40].

Watari et al [41] evaluated the pharmacokinetics of barbiturates in rabbits and found a linear relationship between the logarithm of ka (drug absorption) and log P, as in equation 4,

$$\text{Log } K_a : 0.193 \log P + 0.0148 \ldots\ldots\ldots\ldots (3)$$

Values for target drug molecules calculated by above equation are shown in table 2. Maximum passive absorption for A1, A2 is 15% and contribution from paracellular route is 100%. . Maximum passive absorption for A3 is 34% and contribution form trancellular route is 3% and from paracellular route is 97%. Similarly maximum passive absorption for A4 is 14% and 100% contribution is from paracellular route. Hence, passive absorption is not very good for these candidate drugs, except for A3 which is much better than others.

The prediction of the fraction of a drug absorbed in humans (denoted as FA) has been aided by the efforts of Abraham and co-workers who carefully complied and analyzed a set of FA data for 241 drugs [42]. Using Abraham's solute descriptor, FA is given by

$$FA = 90 + 2.11E + 1.70\ S - 20.7A - 22.3\ B + 15.0\ V \ldots (4)$$

where E is the excess molar refraction, S the dipolarity, A the hydrogen bond acidity of the compound, B is the hydrogen bond bascity compound and V its characteristics McGowan volume. This model suggests, in keeping with other work, that increasing the hydrogen bonding capacity is deleterious to facile intestinal absorption [43]. If FA is greater than 90%, compound is considered to be well absorbed. Values for A1, A2, A3 and A4 are 51.012, 46.652, 64.399 and 47.918 respectively. A3 will be better absorbed as compared to others.

Similarly, percentage human intestinal absorption (HIA), calculated by Abraham et al [42], is given by

$$\% \text{ HIA} = 100/ [\ 1 + 10^{-(1.02 + 0.062E + 0.098S - 0.60\ A - 0.68B + 0.45V)}\ ] \ldots\ldots (5)$$

For A1, A2, A3 and A4 % HIA comes out to be 46.895, 39.674, 68.48 and 99.14 respectively.

Candidate molecules are checked for their absorption properties using Caco-2 (a human intestinal epithermal cell line derived from a colorectal carcinoma) for their susceptibility to metabolic degradation using liver microsomes or hepatocytes [44].

Waterbeemd[45] developed the QSPR (quantitative structure-property relationship) model to describe the Caco-2 permeabilities. This equation is as:

$$\log P_{app} = 0.008 \text{ X MW} - 0.043 \text{ X PSA} - 5.165 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

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Here, log P_{app} is the logarithm of the apparent permeability (cm s^{-1}) through the monolayer and the PSA indicates the polar surface area of the compound. The equation indicates that permeability increases with increasing MW and decreasing . values of log P_{app} comes out to be
- 7.862, - 7.862, - 7.542 and – 8.632 respectively.

Winiwarter [46] investigated a different measure of intestinal absorption in humans, effective permeability (P_{eff}), which is given as:

$$\text{Log } P_{eff} = = 2.546 - 0.011 \text{ X PSA} - 0.278 \text{ X HBD} \quad \text{.....(7)}$$

where PSA is polar surface area and HBD is number of hydrogen bond donors. According to this equation, decreasing the number of hydrogen bonding functional groups in a molecule favors passive absorptions. Calculated values for Log P_{eff} are – 0.1704 forA1 and A2, 0.835 for A3 and – 0.403 for A4.

As, candidate drugs are not polar (hydrophilic) hence they can penetrate through tight junctions. They can unzip the junction locally as they can transmigrate, with minimal leakage, or to adhere in the region of the junction then migrate through the cell. Possible active transport for A1, A2 is amino acids. A3 and A4 are not transported at all.

Aqueous Solubility Log W: The solubility of drugs in water is of central importance in the process of drug discovery and development from molecular design to pharmaceutical formulation and biopharmacy. An insufficient aqueous solubility is likely to hamper bioavailability of the drugs. In recent years, high throughput screening (HTS), where collections of thousands of compounds are screened with the intention of finding relevant biological activity has proven valuable in finding new lead drugs [47]. Gao *et al* [48] estimated aqueous solubility of drug -like molecules with QSPR approach and found that it is between -5.16 to 0.92. For Citrulline and its analogues value of log w are shown in table 2.

P-Glycoprotein: P-Glycoprotein is a membrane-associated protein that has affinity for a variety of large, structurally unrelated, neutral or cationic amphipathic compounds. By pumping substrate drugs out of the cell, this protein decreases the intracellular drug accumulation, resulting in a diminished therapeutic efficacy[44]. The results of several studies also suggested that P-glycoprotein plays a role in the protection of the organism against potentially toxic substances, e.g., by limiting the absorption of orally ingested compounds, by mediating the elimination of substrates from the body, and by protecting crucial organs such as the brain and the testis against toxic substances in the circulation [45,46].

Citrulline and its analogues are neither substrates nor inhibitors.

Plasma Protein Binding: Drug binding to plasma proteins is an essential step in both drug discovery and in clinical phases of drug development. Binding of drugs to plasma proteins is important in understanding the pharmacokinetics and pharmacodynamic relationship of a drug [47,48]. Therefore, plasma protein binding (PPB) is normally recognized as an important factor in assessing drug disposition, efficacy, and safety [50]. In the early drug development stage, the knowledge of drug protein binding property is essential in extrapolating preclinical animal data to predict the drug's efficacy and toxicity in human subjects.

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Also, plasma protein binding propensity of a drug affects the amount of drug available to diffuse into target tissues, for example brain, the calculation of in vivo hepatic clearance, and the interpretation of the drug's bioavailability [51].

Although the main drug-binding proteins are albumin and alpha 1-acid glycoprotein, plasma contains many other proteins; consequently, there is a high probability that many small molecules will exhibit some levels of binding. To determine the extent of PPB, the molecule should be tested directly in a protein-binding assay using plasma or serum. This is a critical step in characterizing the distribution of a small molecule with respect to the plasma compartment [52,53]. But, it is a hectic and time consuming process, this value can be determined computationally and probability that it is near to experimental value is very high.

The strength of an interaction between plasma proteins and a drug is usually expressed as a *%PPB* value. % PPB for A1 is 1.19% and HSA affinity constant [54] ($LogK_A^{HSA}$) is 1.81.In this molecule acid and base are separated by less than 4 atoms, so it is zwitterionic and it is likely to bind to the majority of plasma proteins.

For A2, % PPB is 1.15% and $LogK_A^{HSA}$ is 1.64, distance between acid and base group is 4 to 7, so it is zwitterionic and special structural effects on protein binding may be observed.

For A3 %PPB is 44.76% and $LogK_A^{HSA}$ is 1.54. As base pKa is < 8.5, this candidate drug will predominately bind to alpha-1-acid glycoprotein and albumin.

For A4 %PPB is 1.25% and $LogK_A^{HSA}$ is 2.22, acid and base are separated by less than 4 atoms, so it is zwitterionic and it is likely to bind to the majority of plasma proteins.

Volume of Distribution: Volume of distribution (V_d) is also an important parameter characterizing drug disposition. V_d is a measure of relative partitioning of drugs between plasma (the central compartment) and the tissues. All tissues are considered as a single homogenous compartment. *Vd* is necessary for simulating plasma concentration of a drug (C_p). V_d is a composite parameter and it depends on many chemical and biological factors. *Vd* is a function of the sum of binding interactions with various tissue components *vs* binding to plasma proteins [55]. Compounds that can distribute within the body water typically show V_d representative of the body water volume, 0.8 l/kg. For predicting V_d, we has used the software developed by ap-algorithms. For A1 and A2, V_d is 0.38 L/Kg, so these are zwitterionic with a strong acidic group. For A3, V_d is 0.57 L/Kg, it is moderately hydrophilic basic drug. For A4, V_d is 1.57 L/Kg, so it is zwitterionic drug with a moderately acidic group.

Clearence: Clearance is defined as the volume of blood cleared of drug per unit of time and directly influences plasma concentration–time profiles. It is difficult to correlate clearance with physicochemical and molecular descriptors owing to the complexity of the biological system, the influence of transporters, and the vast range of sites and mechanisms of drug biotransformation and elimination [56]. Mayer *et al* [57] shows relation between renal clearance values and log D as in equation 5,

$$\text{Log } CL_R = - 0.22 \text{ LogD} - 0.84 \quad \text{…….(8)}$$

Thus, there is a simple linear relationship between log D of barbiturates and the

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logarithm of intrinsic clearance. For target drug molecules, these values are shown in table 5.

Toxicity : Toxicity determination is very important because numerous examples exist of drugs that have had to be withdrawn, because of unacceptable toxicity, in clinical trials and even after reaching the market-place. Traditionally, toxicity studies have been experimental in nature and in most cases have involved animal studies. Such studies can be time-consuming and expensive. As a result, computationally predicting the toxicity of a given molecule has been intensively studied, as a means to avoid animal testing [58,59].

We have used the Ames test, which is used worldwide as an initial screen to determine genotoxic properties of NCE's (new chemical entities) for the pharma- and chemical industry. It is the short term bacteria reverse mutation test that is performed on various *S. typhimurium* and *E. coli* bacteria strains. As a standardized screen it is one of the most popular tests for assessing genotoxic properties of compounds. We have predicted the AMES genotoxicity from structure using the software developed by pharma-algorithms. Probabilities of positive AMES test for the candidate drug molecules are shown in table 2. Except for A2, all have probabilities less than 0.8, it means only A2 is genotoxic but it has medium mutagenicity, no tumorigenicity, nono irritant and no reproductive effect. A1 and A3 are mild genotoxic and have high risk mutagenicity, medium risk of tumorigencity and no risk of irritant and reproductive effect and there is 99.3 percent chances that A4 is safe as far as genotoxicity is concern but it also have high risk of mutagenicity, medium risk of tumorigenicity, no irritant nd no reproductive effect

Predictions are displayed, in figure, in terms of color coded atomic/fragmental contributions "color coded potentials"). This allows identifying and visualizing specific structural toxicophores: genotoxicity potential in the Ames test (green part is not involved in genotoxic activity, red part is associated with genotoxic properties).

Hanse and Clayton [60] modeled the acute toxicity of barbiturates to the mouse using only the octanol-water partition coefficient (P), a measure of hydrophobicity:

$$\log 1/LD50 = 1.02\log P - -0.27(\log P)2 + 1.86 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(9)$$

n = 13 r2 = 0.852 s = 0.113

where LD50 = dose to kill 50% of mice, n = number of compounds used in developing the QSAR (the training set), r = correlation coefficient, and s = standard error of the estimate.

Calculated value of LD50 and pLD50 (predicted LD) for mouse and rat are shown in table 8 and 9 respectively. All the values for the drug taken through various routes are well in the range. Our drug can be used as oral as the value of LD50 is in between 500 – 2500, so it is slightly toxic. According to Gosselin *et al* [61], if drug is slightly toxic, then Probable Oral Lethal Dose for Human can be 5-15 g/kg

There are certain well known protein targets that can lead to toxicity, such as the human Ether-a-go-go Related Gene (hERG) [62]. For such scenarios, one can apply a number of methods to decide whether a compound will be toxic by virtue of interacting with hERG, for example.

Toxicity is also expressed through biological activity data (pIC50) defined as molar concentration of those chemicals necessary

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to displace 50% of radiolabeled tetrachlorodibenzo-p-dioxin (TCDD) from the aryl hydrocarbon (Ah) receptor.

Guha *et al* [58] used 775 pIC50 values since they could not evaluate descriptors for some of the molecules. They then selected a cutoff of 5.5, such that molecules with a pIC50 greater than this value were classified as toxic and the remainder as non-toxic. We used a model of hERG force field, developed by quantum pharmaceuticals, for predicting a molecule structure in its inhibition constant for hERG channes. This model is very useful for molecular acido toxicity prediction. Calculated values for A1, A2, A3 and A4 comes out to be 0.7, 1.1, 2.7 and 0.8 respectively which are less than 5.5, hence targeted compounds are non-toxic.

Prediction of toxic properties of small drug like molecules is a big challenge both from theoretical and practical points of view. Quantitatively people use different measures of toxicity such as Maximum Recommended Daily Dose (MRDD) or Lethal Dose (LD50) [63]. calculated MRDD for A1, A2, A3 and A4 are 3.3, 1.8, 2.9 and3.0 respectively.

Organ specific health effects: We have predicted organ specific health effects using the software ToxBoxes V1.1. This software uses health effects predictive algorithms based on long term toxicity studies with adverse effects reported on particular organs or organ systems. Data has been incorporated from chronic, subchronic, acute and carcinogenicity studies encompassing various species and routes of administration.

The structural features contributing to the adverse health effect are identified and highlighted using color mapping as shown in figure 6. Red sections are associated with the toxic action of the compound on a particular organ, while green sections of the molecule are not related to the health effect under investigation.

Bioavailability: The bioavailability of a drug is the rate at which the drug becomes available to the body and the extent to which the dose is ultimately absorbed after administration. The extent of bioavailability directly influences plasma concentrations, as well as the therapeutic and toxic effects resulting from oral drug administration. Drugs with poor bioavailability are inefficient because a major portion of a dose never reaches the plasma to exert a pharmacological effect. Low bioavailability is also associated with large inter-subject variability in plasma concentrations and effects. Incomplete oral bioavailability has various causes. These include poor dissolution or low aqueous solubility, degradation of the drug in gastric or intestinal fluids, poor intestinal membrane permeation, and pre systemic intestinal or hepatic metabolism [64]. The best equation for prediction of bioavailability values for drugs, derived by them, is :

Bioavailability (%) = −45.20 + 5.08 (electron affinity) + 4.09 (aromatic ring count) –

15.83 (HOMO)− 3.34 (log *P*) − 0.09 (molar volume) − 0.72 (volumetric HLB) − 4.75 × 10−7 (water solubility) + 1.18 (Hansen's hydrogen-bonding solubility parameter).

Predicted bioavailability of the drugs in the test set was used to evaluate overall predictive performance best optimum model. Calculated bioavailability for target drug molecules are above 68%, 70%, 65% and 63% for A1, A2, A3 and A4 repectiviely. For A1 and A2 probability of %F(Oral) > 30% is 0.757 and %F(Oral) > 70% is 0.097, for A3, probability of %F(Oral) > 30% is 0.515 and %F(Oral) >

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70% is 0.029 and for A4 probability of %F(Oral) > 30% is 0.583 and %F(Oral) > 70% is 0.359.

Druglikeness*:* It is a complex balance of various molecular properties and structure features which determine whether particular molecule is similar to the known drugs. It, generally means 'molecules which contain functional groups and/or have physical properties consistent with most of known drugs'. These properties, mainly hydrophobicity, electronic distribution, hydrogen bonding characteristics, molecule size and flexibility and presence of various pharmacophoric features influence the behavior of molecule in a living organism, including bioavailability, transport properties, affinity to proteins, reactivity, toxicity, metabolic stability and many others. The presence of structural fragements typically found in drugs. Molecules with score between 2 and 7 are classified as drugs; otherwise they are classified as non-drugs. Our candidate drugs have C=O functional groups whose score is 3.4; hence they can be used as drug. As this drug contains a single pharmacophoric group, it attacks central nervous system. Drug is a single pharmacophoric group, and contains amine functional group; hence it can be classified as drug.

A more recent example of the functional approach to identify drug like molecules is the work of Muegge *et al* [65]. They assigned each molecule a score based on the presence of structural fragments typically found in drugs. Compounds containing some specific single pharmacophoric group can also classify as drug. One of the groups is amine. Candidate drug contains amine group, accordingly they can be classified as drug. As our drug doesn't have nitro group, so there is less probability that they will be rejected because Nitro groups, tend to begin aromatic rings, may increase a molecule's tendency to generate false positives under assay conditions [44].

Expert system for calculation of druglikeness score towards GPCR ligands, ion channel modulators, kinase inhibitors and nuclear receptor ligands based on Molinspiration technology [63] were done. For A1 and A2 our score comes out to be 0.98, for A3 it is - 1.77 and for A4 it is 0.55. So, druglikeness score is good for A1 and A2

Drug Score: The drug score combines druglikeness, LogP, logS, molecular weight and toxicity risks in one handy value than may be used to judge the compound's overall potential to qualify for a drug. This value is calculated by multiplying contributions of the individual properties with the first equation:

$$dS = \left(\frac{1}{2} + \frac{1}{2} S_i\right) . \pi\, t_i$$

$$S = \frac{1}{1 + e^{ap+b}}$$

dS is the drug score. S_i are the contributions calculated directly from of cLogP, logS, molweight and druglikeness (pi) via the second equation which describes a spline curve. Parameters a and b are (1, -5), (1, 5), (0.012, -6) and (1, 0) for cLogP, logS, molweight and druglikeness, respectively. t_i are the contributions taken from the 4 toxicity risk types. The t_i values are 1.0, 0.8 and 0.6 for no risk, medium risk and high risk, respectively.

Drug score for A1, A2, A3 and A4 are 0.24, 0.39, 0.25 and 0.33 respectively. For A2 drug score is high as compared to other three.

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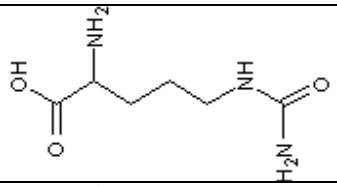
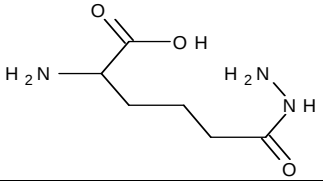
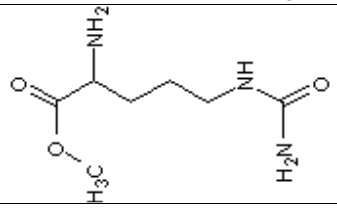
Conclusion

Molecular diversity is approached with a versatile range of tools and techniques. Drug designing, a specialized stream uses diversity analysis as a vital component. Two kinds of analysis, namely, computational sensitivity analysis and structure analysis are used to compare the candidate drug and its analogues. Computer modeling has some extra benefits. One of the prime advantages is speed and accuracy that increases the efficiency of time and on comparative improvisations and developments over the previous ones in a simulated environment. Theoretical study is used to determine stable conformation, pKa, lipophilicity, solubility, absorption, BBB, HBD, HBA, PSA drug dissolution, drug permeability, electrostatic potential map, P glycoprotein, plasma protein binding, volume of distribution, gastro intestinal absorption, drug clearance, toxicity, bioavailability and drug likeness of candidate drug and its analogues, for no experimental physicochemical data exits. Value of HBA and HBD is well within the range for A3. log p is lowest for A3, hence it will be well transported through the body. As PSA for A3 is lowest among other investigated molecules, hence predicted bioavailability will not drop substantially for this analogue. Except A3, other investigated molecules are poorly distributed to brain. Solubility of A3 is also appreciable. Passive absorption for A3 is comparatively better than others. It is also confirmed by the prediction of the fraction of drug absorbed in human (FA). Aqueous solubility is within the permitted limit. More important, acute toxicity is lowest for A3. Hence, it can be said that A3 have much better drug like properties.

Figure 1: Structure of Citrulline and its analogs

S. N.	Citrulline and its analogs	Name and Smiles	Structure
1	Citrulline **"A1"**	2-amino-5-(carbamoylamino)pentanoic acid NC(=O)NCCCC(N)C(O)=O	
2	Amide changed to Retroamide **"A2"**	2-amino-6-hydrazino-6-oxohexanoic acid NC(CCCC(=O)NN)C(O)=O	
3	Hydroxy changed to Methoxy **"A3"**	methyl 2-amino-5-(carbamoylamino)pentanoate NC(=O)NCCCC(N)C(=O)OC	

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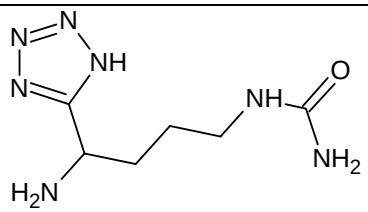
4	Carboxylate changed to Tetrazole **"A4"**	1-[4-amino-4-(2*H*-tetrazol-5-yl)butyl]urea NC(CCCNC(N)=O)c1nnnn1	

Table 1: Molecular properties of Citrulline and its analogs

S.N	Property	Value			
		A1	**A2**	**A3**	**A4**
1.	Molecular Formula	$C_6H_{13}N_3O_3$	$C_6H_{13}N_3O_3$	$C_7H_{15}N_3O_3$	$C_6H_{13}N_7O$
2.	Molecular Weight in amu	175.18572	175.18572	189.2123	199.21372
3.	Molecular Volume in cm^3	135.8 ± 3.0	135.8 ± 3.0	161.2 ± 3.0	143.8 ± 3.0
4.	Composition	C(41.14%) H(7.48%) N(23.99%) O(27.40%)	C(41.14%) H(7.48%) N(23.99%) O(27.40%)	C(44.43%) H(7.99%) N(22.21%) O(25.37%)	C(36.17%) H(6.58%) N(49.22%) O(8.03%)
5.	Number of HBA	4	4	4	5
6.	Number of HBD	6 (> 5)	6 (> 5)	5	6 (> 5)
7.	Polar Surface Area in A^2	95.31	95.31	88.41	117.70
8.	Number of Stero centres	1	1	1	1
9.	Energy in au	-1625950.71	-1625805.91	-1625805.85	-1625805.79
10.	Energy(aq) in au	-1626016.69	-1625866.41	-1625866.55	-1625866.43
11.	Dipole Moment in debye	8.07429374	8.40105588	8.50106718	8.62871401
12.	Parachor in cm^3	381.1 ± 4.0	381.1 ± 4.0	423.8 ± 4.0	435.0 ± 4.0
13.	No. of rotatable bonds	5	5	6	5
14.	pKa (Base)	9.90	9.90	7.40	8.80
15.	pKa (Acid)	1.90	2.30	No pKa	5.30

Table 2: Physiochemical properties of Citrulline and its analogs

S.N.	Property	Value			
		A1	**A2**	**A3**	**A4**
1.	Molar Refractive Index (in cm^3)	42.06 ± 0.3	1.531 ± 0.02	46.90 ± 0.3	49.10 ± 0.3
2.	Partition Coefficient log P	-3.82	-4.33	-1.74	-3.43
3.	Solubility (in mg L^{-1})	84.4	74.8	34.5	4.4
4.	Aqueous Solubility log S_W	-0.32	-0.37	-0.74	-1.66

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5.	Blood Brain Barrier Log BB	-1.744	-1.744	-0.439	-1.750
6.	Absorption Log k_a	-0.5860	-0.5860	-0.3934	-0.17301
7.	Volume of distribution V_d (in L/Kg)	0.38	0.38	0.57	1.57
8.	Probability of positive Ames test	0.358	0.931	0.231	0.007

Table 3: Score of Traffic Light (TL) and oral absorption

Drug and Analogs → / Traffic Lights ↓	**A1**	**A2**	**A3**	**A4**
MW ≤ 400 (TL = 0) 400-500 (TL = 1) ≥ 500 (TL = 2)	0	0	0	0
LogP ≤ 3 (TL = 0) 3-5 (TL = 1) ≥ 5 (TL = 2)	0	0	0	0
Solubility Mg L^{-1} ≥ 50 (TL = 0) 10–50 (TL = 1) ≤ 5 (TL = 2)	0	0	1	2
PSA (A^2) ≤ 120 (TL = 0) 120-140 (TL = 1) ≥ 140 (TL = 2)	0	0	0	0
Rot Bonds ≤ 7 (TL = 0) 8-10 (TL = 1) ≥ 11 (TL = 2)	0	0	0	0
Total	0	0	1	2

Table 4: Solubility in buffer (log S)at different constituents of body

S.N.	Part of body	pH	Log S			
			A1	**A2**	**A3**	**A4**
1.	Stomach	1.7	- 0.13	0.02	0.71	0.03
2.	Duodenum	4.6	- 0.43	- 0.47	0.67	- 0.91
3.	Jejunum and Ileum	6.5	- 0.43	- 0.47	- 0.05	- 1.63
4.	Blood	7.4	- 0.43	- 0.47	- 0.53	- 1.64
5.	Colon	8.0	- 0.43	- 0.47	- 0.70	- 1.59
Probability that compound solubility is		10.0 mg/ml	0.832	0.667	0.688	0.232
		> 1.0 mg/ml	0.982	0.977	0.961	0.797
		> 0.1 mg/ml	0.999	1.00	0.998	0.999

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Table 5: Log D and clearance log CL_R different constituents of body

S.N.	Part of body	pH	A1		A2		A3		A4	
			logD	log CL_R	logD	log CL_R	logD	log CL_R	logD	log CL_R
1.	Stomach	1.7	-5.02	0.2644	-4.08	0.0576	-4.84	0.2248	-6.53	0.5966
2.	Duodenum	4.6	-4.33	0.1126	-3.82	0.004	-4.36	0..1192	-6.34	0.5548
3.	Jejunum and Ileum	6.5	-4.33	0.1126	-3.82	0.004	-2.69	- 0.2482	-5.91	0.4602
4.	Blood	7.4	-4.33	0.1126	-3.82	0.004	-2.04	- 0.3912	-5.91	0.4602
5.	Colon	8.0	-4.33	0.1126	-3.82	0.004	-1.84	- 0.4352	-5.96	0.4712

Table 6: Abraham's Descriptors for Human Intestinal Absorption of Citrulline and its analogs

	Solute Descriptors	A1	A2	A3	A4
1.	Excess molar refraction E	0.90	0.94	0.79	1.53
2.	Dipolarity S	1.90	2.02	1.86	2.48
3.	Hydrogen bond acidity A	1.37	1.25	0.80	1.36
4.	Hydrogen bond basicity B	1.61	1.93	1.62	1.95
5.	McGowan Volume V	1.343	1.343	1.484	1.474

Table 7: Permeability scale of Citrulline and its analogs

	Permeability Scale	A1	A2	A3	A4
1.	Human Jejunum Scale (in cm s^{-1}) pH = 6.5	0.14×10^{-4}	0.14×10^{-4}	0.23×10^{-4}	0.14×10^{-4}
2.	Caco-2 Scale (in cm s^{-1}) pH = 7.4	0.31×10^{-6}	0.31×10^{-6}	0.52×10^{-6}	0.26×10^{-6}

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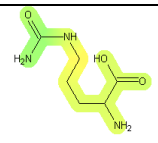
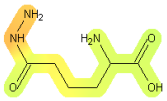
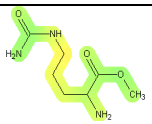
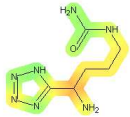
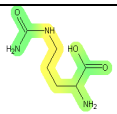
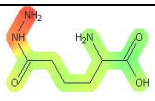
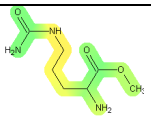
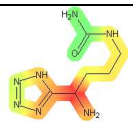
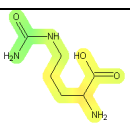
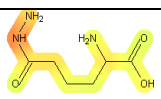
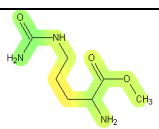
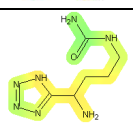
Table 8: Acute toxicity LD50 Citrulline and its analogs (for mouse)

	A1				**A2**				**A3**				**A4**			
	LD50	pLD50	Lower limit	Upper limit	LD50	pLD50	Lower limit	Upper limit	LD50	pLD50	Lower limit	Upper limit	LD50	pLD50	Lower limit	Upper limit
Intraperitoneal	1400 mg/kg	0.89	-1.74	-0.22	1400 mg/kg	0.92	1.74	0.25	270 mg/kg	-0.15	-0.87	0.47	1500 mg/kg	-0.87	-1.88	-0.24
Oral	2000 mg/kg	1.07	-2.48	-0.75	2200 mg/kg	-1.10	-2.60	-0.68	840 mg/kg	-0.65	-2.12	-0.45	1500 mg/kg	-0.89	-2.48	-0.52
Intravenous	270 mg/kg	-0.18	-1.03	1.10	400 mg/kg	-0.36	-1.06	0.80	110 mg/kg	0.23	-0.44	1.12	340 mg/kg	-0.23	-1.04	1.16
Subcutaneous	1100 mg/kg	-0.79	-2.09	0.27	1300 mg/kg	-0.87	-2.33	0.05	250 mg/kg	-0.13	-1.20	0.86	1700 mg/kg	-0.94	-2.65	-0.07

Table 9: Acute toxicity LD50 Citrulline and its analogs (for rat)

	A1				**A2**				**A3**				**A4**			
	LD50	pLD50	Lower limit	Upper limit	LD50	pLD50	Lower limit	Upper limit	LD50	pLD50	Lower limit	Upper limit	LD50	pLD50	Lower limit	Upper limit
Intraperitoneal	1700 mg/kg	0.98	2.06	0.03	1500 mg/kg	-0.93	-2.15	0.15	290 mg/kg	-0.18	-1.22	0.57	1700 mg/kg	-0.93	-2.31	0.29
Oral	5500 mg/kg	1.50	3.20	0.91	4200 mg/kg	-1.38	-3.42	-0.55	1000 mg/kg	-0.73	-2.39	-0.08	1100 mg/kg	-0.74	-2.66	0.17

Table 10: Probability health effect due to toxicity on various parts of body and color mapping highlighting structural features contributing to a adverse health effect

S.N.	Part of Body	Probability and Color Mapping			
		A1	**A2**	**A3**	**A4**
1	Blood	0.07 	0.46 	0.67 	0.94 
2.	Cardiovascular System	0.13 	0.17 	0.97 	0.55 
3.	Gastrointestinal System	0.35 	0.31 	0.32 	0.88 

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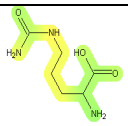
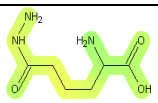
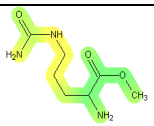
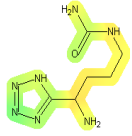
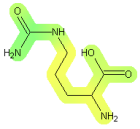
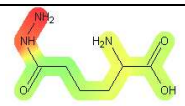
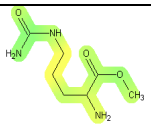
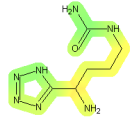
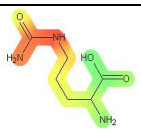
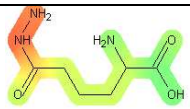
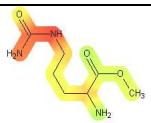
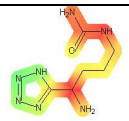
4.	Kidney	0.08 	0.09 	 0.14	 0.32
5.	Liver	0.16 	0.16 	 0.22	 0.54
6.	Lungs	0.01 	0.01 	 0.42	 0.13

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