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METHYLATION OF THIONICOTINAMIDE FOR IMPROVED BLOOD BRAIN BARRIER PERMEABILITY IN THE TREATMENT OF CNS CANCERS

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Abstract

The inhibition of NAD+ kinase (NADK) in cancer cells may represent a novel treatment strategy. Cytosolic NADK is an enzyme responsible for generating NADP, which is then rapidly converted to NADPH by reductases. Together, NAD and NADP are involved in a variety of cellular pathways, including metabolism, energy production, protein modification, and ROS detoxification. Treatment of cancer cells with thionicotinamide lowered NADPH pools, compromised biosynthetic capabilities, and inhibited cell growth. The 2D structure of thionicotinamide was obtained from the PubChem database and sketched alongside its methylated analogue using the ChemAxon software. The in-silico pharmacokinetic parameters of the compounds were predicted using the SwissADME online server. This was also used to construct the boiled-egg plot which confirmed the blood brain barrier permeability of the two compounds. Results from the in-silico pharmacokinetics parameters obtained from the SwissADME prediction showed that the methylated analogue of thionicotinamide might be a better therapeutic option for the treatment of CNS cancers as it showed an improved blood brain barrier permeability propertiy.

Keyword: *NAD+ kinase, Thionicotinamide, Inhibition, Blood Brain Barrier*

Introduction

NAD+ kinase is an enzyme that converts nicotinamide adenine dinucleotide (NAD+) into NADP+ through phosphorylating the NAD+ coenzyme [1]. NADP+ is an essential coenzyme that is reduced to NADPH primarily by the pentose phosphate pathway to provide reducing power in biosynthetic processes such as fatty acid biosynthesis and nucleotide synthesis [2]. The structure of the NADK from the archaean Archaeoglobus fulgidus has been determined [3]. In humans, the genes NADK and MNADK encode NAD+ kinases localized in cytosol and mitochondria [4], respectively. Similarly, yeast have both cytosolic and mitochondrial isoforms, and the yeast mitochondrial isoform accepts both NAD+ and NADH as substrates for phosphorylation [5].

NADK phosphorylates NAD+ at the 2' position of the ribose ring that carries the adenine moiety. It is highly selective for its substrates, NAD and ATP, and does not tolerate modifications either to the phosphoryl acceptor, NAD, or the pyridine moiety of the phosphoryl donor, ATP [6]. NADK also uses metal ions to coordinate the ATP in the active site. In vitro studies with various divalent metal ions have shown that zinc and manganese are preferred over magnesium, while copper and nickel are not accepted by the enzyme at all. A proposed mechanism involves the 2' alcohol oxygen acting as a nucleophile to attack the gamma-phosphoryl of ATP, releasing ADP [7].

A central nervous system (CNS) tumor begins when healthy cells in the brain or the spinal cord change and grow out of control, forming a mass. A tumor can be either cancerous or benign. A cancerous tumor is malignant, meaning it can grow and spread to other parts of the body. A benign tumor means the tumor can grow but will not spread [8]. A CNS tumor is especially problematic because a person's thought processes and movements may be affected. This type of tumor may be challenging to treat because the tissues around the tumor are often vital to the body's functioning. The treatment of CNS tumors in infants and young children may be especially challenging because a child's brain is still developing [9].

There are different types of CNS tumors. Some are cancerous and very likely to grow and spread. These are often called very aggressive or high grade [10]. There are also less aggressive types, often called low grade. And some types are noncancerous and not likely to grow and spread. In addition, there are variations within each type that affect how quickly the tumor will grow. Many of these differences depend on genetic changes found within the tumor [11].

Due to the essential role of NADPH in lipid and DNA biosynthesis and the hyperproliferative nature of most cancers, NADK is an attractive target for cancer therapy [12]. Furthermore, NADPH is required for the antioxidant activities of thioredoxin reductase and glutaredoxin. Thionicotinamide and other nicotinamide analogs are potential inhibitors of NADK [13], and studies show that treatment of colon cancer cells with thionicotinamide suppresses the cytosolic NADPH pool to increase oxidative stress and synergizes with chemotherapy [14].

This experiment is aimed at the modification of the thionicotinamide 2D structure to achieve an improved blood brain barrier permeability for CNS cancer treatment.

Materials and Methods

Ligand preparation

The 2D structure of thionicotinamide and its CH_3 analogue were designed using the MarvinSketch software [15]. All designed structures were downloaded and saved as mrv files.

File conversion

Saved mrv files from the ligand preparation process were converted into SMILES strings (Simplified Molecular Input-Line-Entry System) using the Open Babel Open Source Chemistry Toolbox. Open Babel, a chemical toolbox is designed to speak many of the languages of chemical data [16].

Ligand minimization

Thionicotinamide and its CH3 analogue were minimized using the UCSF Chimera software [17]. UCSF Chimera is an extensible program for analyzing and interactively visualizing molecular structures and related data which include supramolecular assemblies, density maps, alignment of sequences, results from molecular docking trajectories and conformational ensembles [18].

Visualization of atoms

Atoms making up the thionicotinamide and its CH3 analogue were visualized using the Pymol molecular visualizer [19]. PyMOL is an open-source tool for model visualization and it is made available for utilization in structural biology [20]. Text

Discussion

The polar surface area (PSA), also known as the topological polar surface area (TPSA) of a molecule is defined as the sum of all polar atoms (oxygen and nitrogen), with the inclusion of the hydrogen atom attachments. The polar surface area is a metric that is often used in medicinal chemistry to optimize the cell permeation ability of drugs. Molecules with a PSA value higher than 140 angstroms squared are known to be poor in cell membrane penetration [21]. For molecules to penetrate the blood–brain barrier (BBB) (in order to act on the central nervous system receptors), the value assigned to the polar surface area must be less than 90 angstroms squared [22]. The CH3 analogue of thionicotinamide has been shown to possess the blood-brain barrier permeation attributes as its TPSA value appeared lower than 90 angstroms .

The partition coefficient between n-octanol and water (log Po/w) serves as the classical method for the description of lipophilicity. The diversity of the models backing the predictors will increase the accuracy in the prediction using the consensus log Po/w [23]. The lipinski's rule [24] was used as the drug likeness descriptor for the purpose of this study and the optimal lipophilicity range (Log Po/w) allowed should not exceed 5. The observation from the concensus lipophilicity column of figure 3 and 4 shows that thionicotinamide and its CH3 analogue are within the optimal lipophilicity range and as such can be regarded as drug like compounds.

Activities regarding drug development can be facilitated and made easier in cases where molecules are soluble. This brings about ease in drug handling and its formulation [25]. Moreover, for discovery projects that target the oral form of administration, one of the major absorption property influencers the solubility of the compound [26]. Also, drugs that are designed for parenteral administration requires a high solubility attribute to aid the delivery of an appreciable amount of the active ingredient in smaller volumes of pharmaceutical dosage [30]. A compound can be considered as soluble if the Log S value is less than 6 [25]. Thionicotinamide and its CH3 analogue, according to the column projecting the solubility result in figure 3 and 4 are all water soluble, implying that they might be easily absorbed.

The nature of the gastrointestinal mucosal membrane surface area plays an important role in the process of drug absorption and it has a varying and differential effect from the stomach to the rectum. The physiochemical properties of the luminal content are also implicated to have an influence in drug absorption process [26]. The absorption process itself is continually described in terms of hypothesis of simple partition of pH, where absorption is controlled by the equilibrium position between the ionized and non-ionized forms of the drug at varying physiological pH values encountered in the gastrointestinal tract [27]. Thionicotinamide and its CH3 analogue possess a high gastrointestinal absorption rate, indicating their increased drug bioavailability.

The P-glycoprotein (P-gp) is involved physiologically in the reduction of the harmful effects of toxic compounds, xenobiotics and drugs which the body is exposed to by constantly pumping them out of cells. The need for the role played by the P-glycoprotein has led to the recognition of the

modulation it confers on many important and clinical therapeutic agents and this pharmacokinetic importance has led to the incorporation of its screening in any process involving drug discovery [28]. Drug pharmacokinetic parameters can also be affected through various drug induced induction or inhibition directed at modulating drug transporters and this can lead to a significant drug-drug interaction [29]. Both thionicotinamide and its CH3 analogue were no substrates to the Pglycoprotein hence their oral bioavailability remains intact.

The boiled-egg is a robust and intuitive graph prediction of passive intestinal absorption and brain penetration, as a function of lipophilicity and apparent polarity (described by WLOGP and TPSA, respectively). If plotted molecule falls inside the white ellipse, the probability of a good intestinal absorption is high. If plotted molecule falls inside the yellow ellipse (i.e. the yolk), the probability of a good BBB crossing is high. The white and yolk of the BOILED-Egg are not mutually exclusive and molecules predicted as not absorbed by the GI and BBB non-permeant are located in the grey area or even further outside the range of the plot [30]. The presence of the CH3 analogue of thionicotinamide (molecule 2) in the egg yolk of the boiled egg plot is and indication of the compound's ability to cross the blood brain barrier

Conclusion

The structural modification effected on thionicotinamide through the substitution of the terminal sulphur (S) atom for a methyl group has proven to be effective in the alteration of the blood brain barrier permeation properties of the drug while it retains other important pharmacokinetics parameters.

The molecular docking protocol to determine the efficacy of this new analogue of thionicotinamide in CNS cancer treatment is therefore recommended and we further recommend more modifications on this drug for better anticancer activity.

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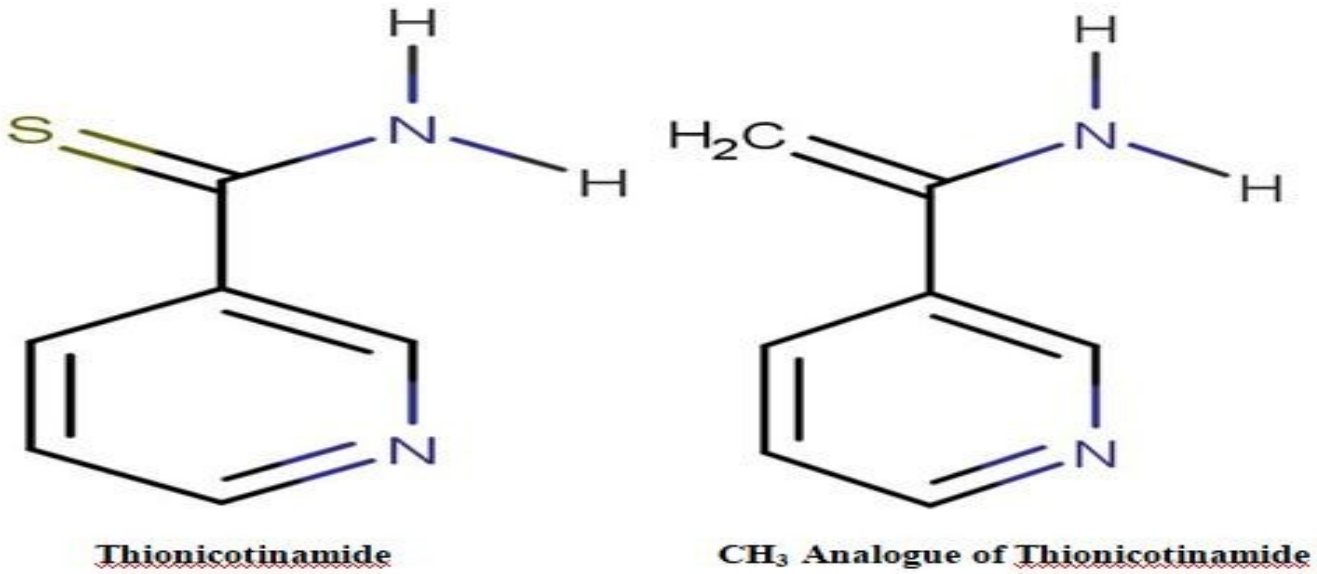

Figure 1: 2D Structure of thionicotinamide and its analogue

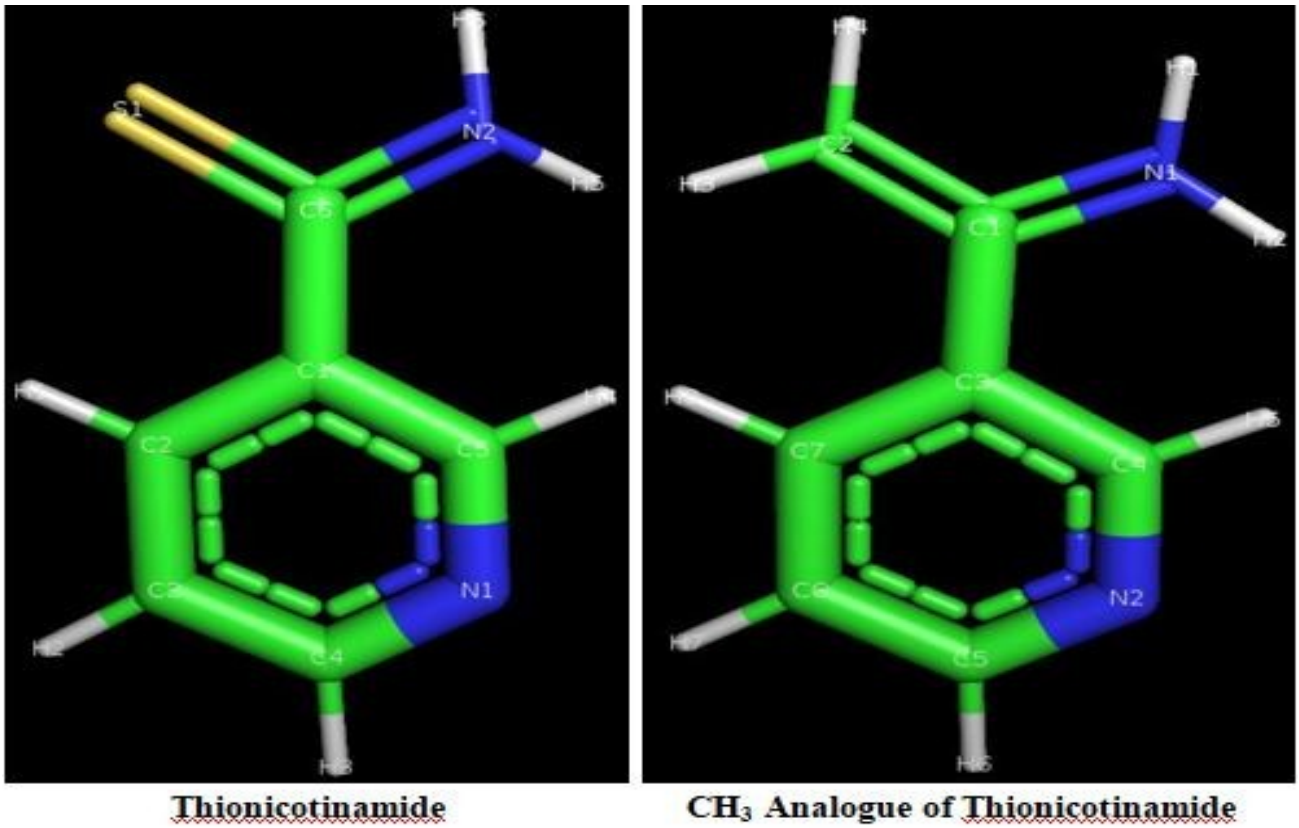

Figure 2: The 3D structure of thionicotinamide and its analogue

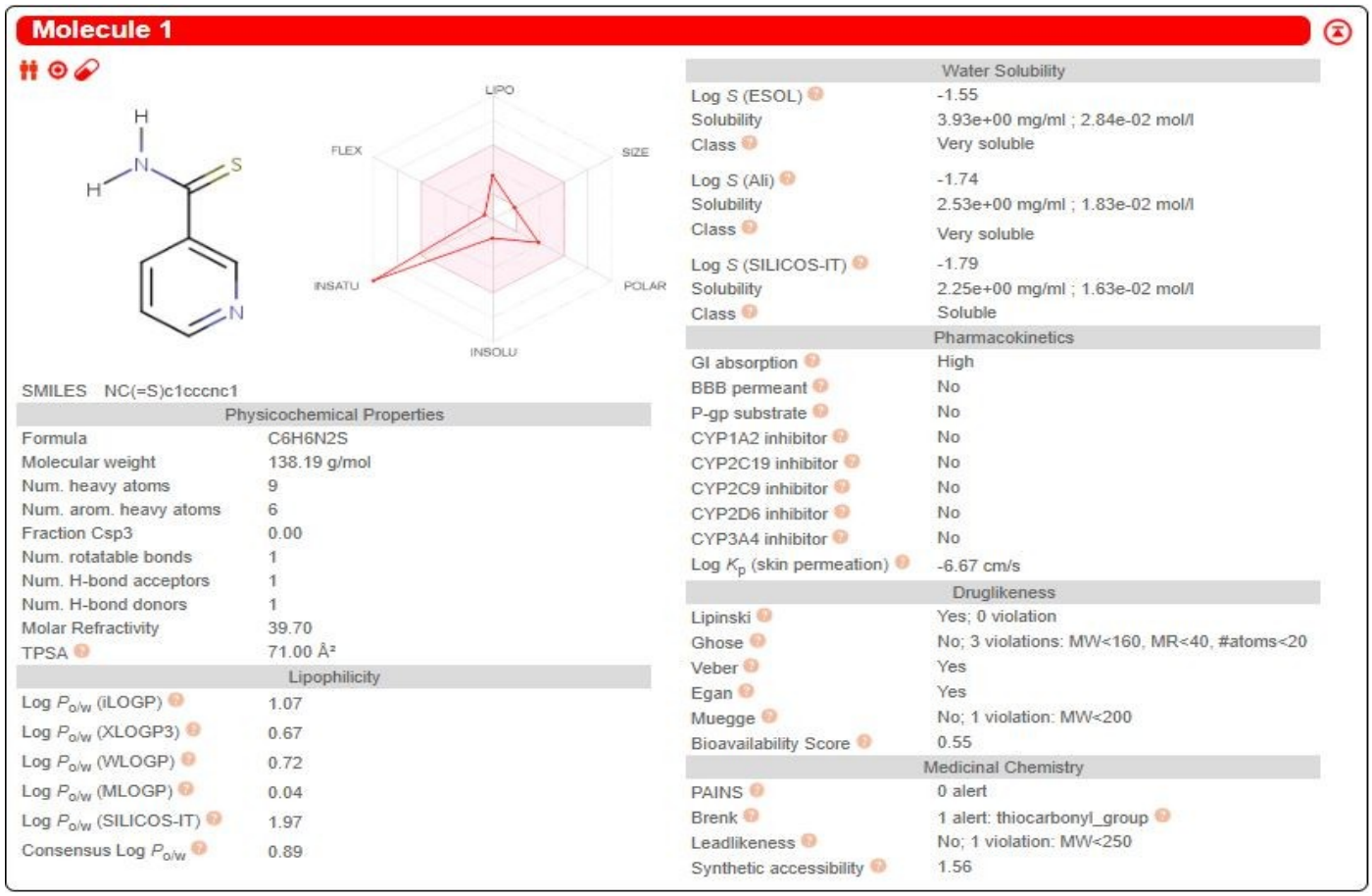

Figure 3: *In-Silico* pharmacokinetics of thionicotinamide

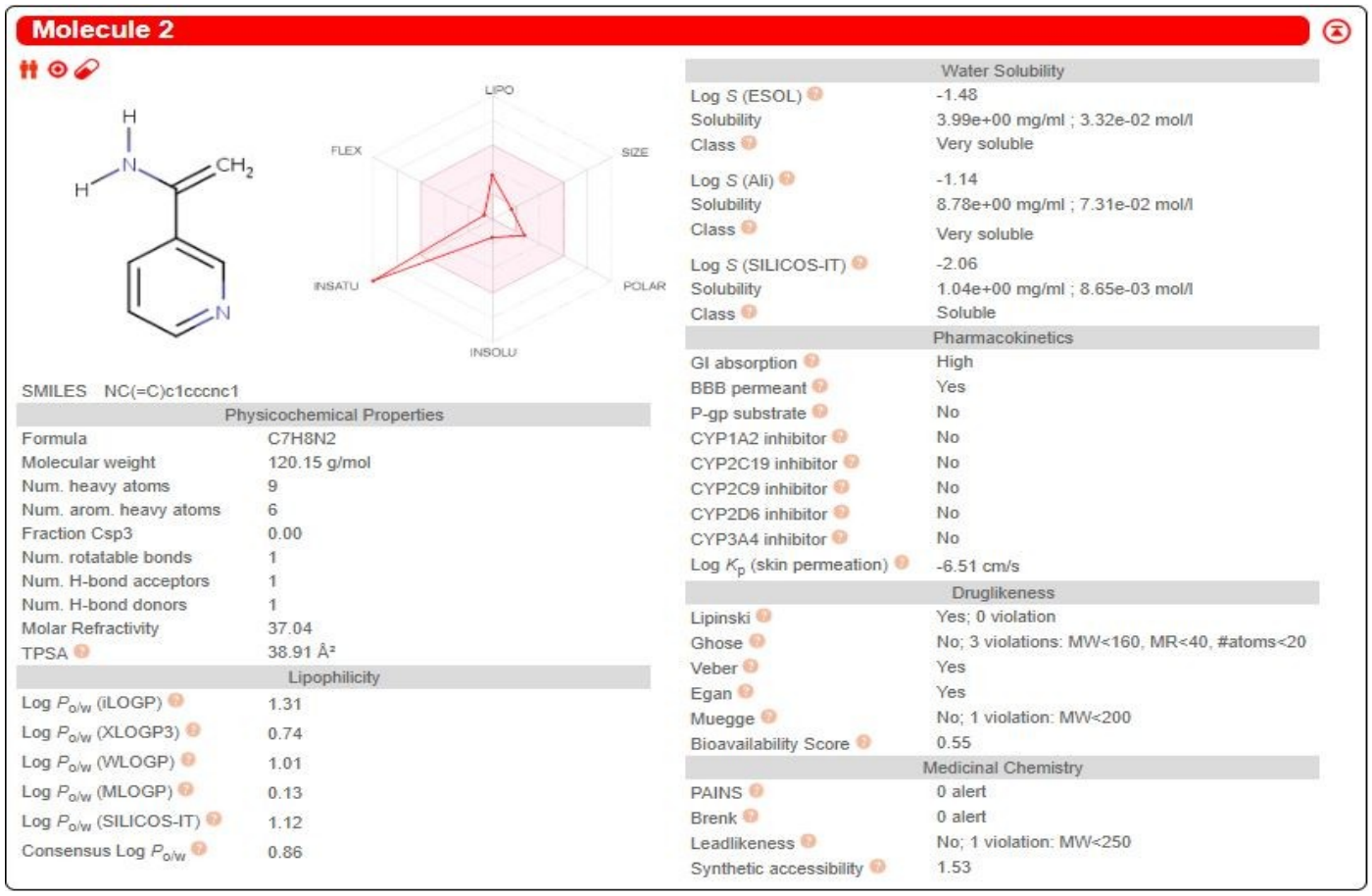

Figure 4: *In-Silico* pharmacokinetics of the CH_3 analogue of thionicotinamide

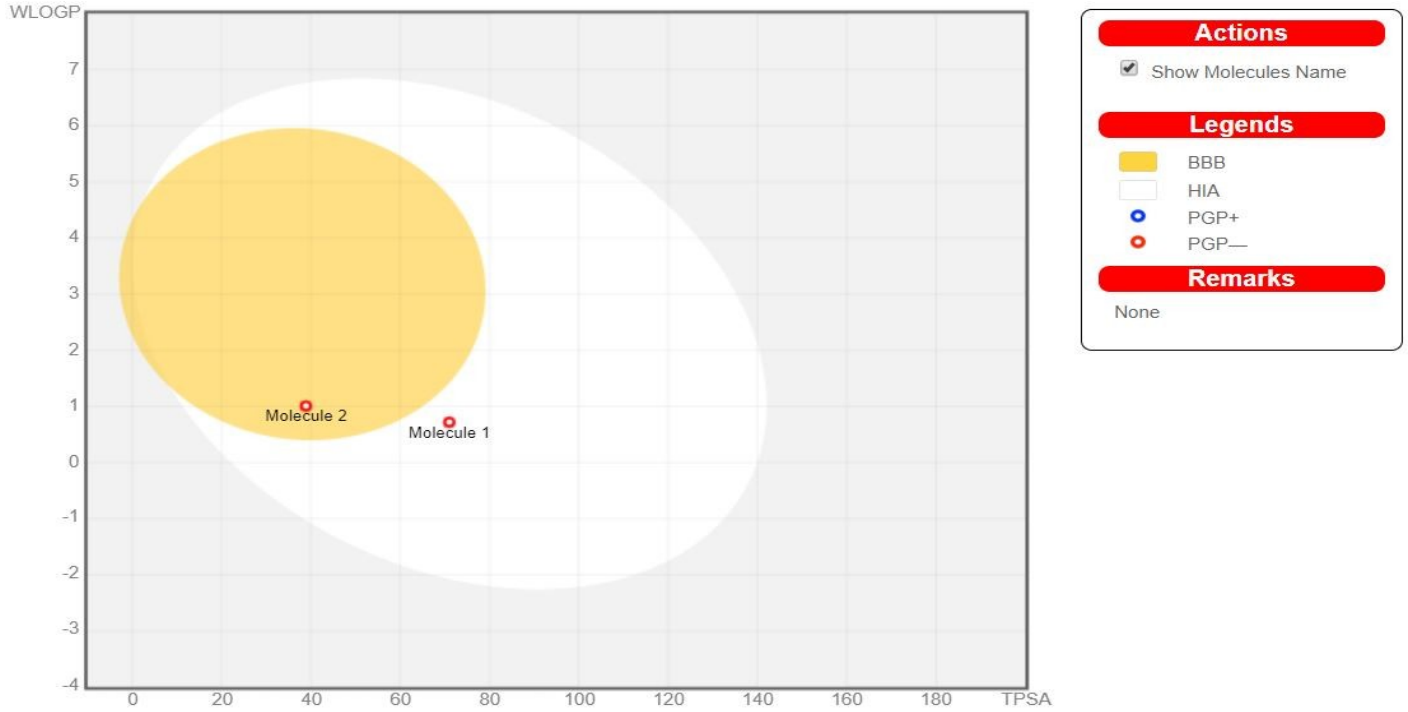

Figure 5: Boiled-Egg Plot