Computer-aided Validation of Fluorobenzimidazole as Soluble Epoxide Hydrolase Inhibitor: An *In-Silico* Study

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ABSTRACT

Soluble epoxide hydrolase (sEH) is an enzyme and is encoded by the gene EPHX2 in human beings. Indeed, by inhibiting the biotransformation of these epoxide(s) *via* arresting the action of the mammalian sEH one can develop a promising antihypertensive drug(s). The present work was emphasized the possible binding mode of fifteen fluorobenzimidazole (4a-4o) derivatives as soluble epoxide hydrolase (sEH) inhibitors. The X-ray crystal structure of human sEH in complex with the inhibitor 6NJ [(3-(2-phenylethyl)-1*H*-indazole] has been utilized to select the specific active site of protein data bank (PDB) ID: 5AKY with resolution: 2.18 Å for the docking study. The energy conformer of *trans*-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (tAUCB) was docked using the prepared grid towards validation of the generated grid and docking protocol. The best results obtained by the ligands 4f, 4g and 4m with a dock score -9.94, -9.75, and -11.56, respectively. Among all the ligands, 4m exhibited considerable “drug-like” characteristics with the highest G score and possess acceptable *in-silico* pharmacokinetic by obeying Lipinski rule of five. In conclusion, compound 4m might be used as a lead molecule in the antihypertensive drug discovery.

Keywords: Docking, *In-Silico*, Soluble epoxide hydrolase, Fluorobenzimidazole

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INTRODUCTION

The science of medicinal chemistry is concerned with the discovery and creation of new therapeutic moiety, as well as their development into a dosage form. Drugs are compounds that are used to diagnose, treat, alleviate, or prevent disease¹. A lead chemical is a pharmacologically active molecule that serves as a starting point for drug development². Medicinal chemistry may entail the

isolation of chemicals from nature or the creation of novel molecules, as well as the study of the link between the structure of natural and synthesized compounds and their biological activities¹. The discovery, design, and development of a new medicine is a difficult and expensive process. There are several drugs under development but only a few pass the clinical trials. According to the current data,

only 11 out of 100 compounds become able to qualify the phase I clinical trials, and 1 out of 10 new chemical entities can qualify the phase III clinical trials. Hence the success rate of the drug discovery and development process of a new drug may be the key factor for the survival of Pharmaceutical and bulk drug industries. A biomolecular target (proteins such as enzymes, ion channels, receptors, or nucleic acids) is the central molecule in a metabolic or signaling pathway that leads to a certain medical condition or pathology. Drug design entails the creation of small molecules that are morphologically similar and configuration to the biomolecular target at which compounds bind, allowing them to attach to

the target's unique active site/allosteric site and operate as modulators or inhibitors³⁻⁵. The drug designing mainly focuses on various properties like target selectivity, minimum side effects, higher efficacy, easily synthesized, chemical stable, non-toxic properties⁶.

In summary, drug discovery and development are an approach that involves various steps like Chemical synthesis, QSAR, molecular modeling, toxicological studies, biotransformation, assay, and biopharmaceutical studies. The drug discovery pipeline may be summarized in the following flowchart (Fig 1)⁷.

Fig 1: Various stages of drug discovery

The epoxide hydrolase family includes soluble epoxide hydrolase (sEH), which is a bifunctional homodimeric enzyme that has both epoxide hydrolase and phosphatase activity at its C

terminus. It is located in the cytosol and peroxisomes and transforms epoxyeicosatrienoic acid to their respective diols. The gene EPHX2 in humans codes for sEH. In male mice, deletion of the

enzyme sEH, which converts EETs to DHETs, lowers blood pressure, as does treatment with a selective sEH inhibitor. Inhibition of EET conversion to DHET could be a new way to treat hypertension, according to this mechanism⁸.

The sEH metabolizes the biologically active epoxyalcohol metabolites of arachidonic acid. Hepoxilin A3 to Trioxilin A3 and Hepoxilin B3 to Trioxilin B3 Eicosatrienoic acid. sEH can be found in practically every mammalian organ, including the heart, liver, lungs, spleen, and brain. The kidney, which is an important component of the urinary and excretory systems, likewise releases sEH. As a result, inhibition of sEH has been demonstrated to be renoprotective in a variety of renal diseases⁹. Furthermore, several therapeutically utilized medications, such as sorafenib, were discovered to be powerful sEH inhibitors.

In the present work, few reported potential ligands having *in vivo* soluble epoxide hydrolase activity were chosen for docking studies. The present work was carried out to developed insight into the interaction pattern of the ligands within the active site of the protein 5AKY.

MATERIALS AND METHODS

Molecular Docking

Molecular docking studies were performed on the selected fluorobenzimidazole derivatives to get a detail knowledge about their binding preferences at the active site of the protein 5AKY. The molecules were selected based on range of activities from moderate to highest active molecules¹⁰.

The current investigation was conducted out on a PC running Windows 7 and a trial version of the Schrödinger molecular modelling software suite. On the Windows 7 operating system, docking was accomplished with the Glide coupled with Maestro (Schrödinger, LLC) interface. In order to anticipate *in silico* pharmacokinetic properties, the compounds were also submitted to Qikprop® analysis¹¹. Physically significant descriptors as well as pharmaceutically relevant qualities are predicted by the QikProp® algorithm. The ligands were neutralised before being employed by QikProp®, which is necessary because QikProp® is unable to neutralise a structure and hence no properties are generated in the regular mode. The chosen

compounds were preserved for docking investigations after the Qikprop® was completed¹¹.

Selection of Crystal Structure (PDB ID:5AKY)

The current docking investigation was carried out using the X-ray crystal structure of human sEH in association with the inhibitor 6NJ [(3-(2-phenylethyl)-1H-indazole] (PDB ID: 5AKY) (Resolution: 2.18). The protein preparation wizard was used to rectify mistakes in the protein. The binding site was created by putting the co-crystallized ligand (6NJ) in the middle of a rectangular box inside the enzyme (Fig 2). Using the software's Glide grid module, a 20 grid area was created for the co-crystallized ligand. To make low-energy conformers of all the ligands, the LigPrep module was employed. The enzyme was kept rigid throughout the docking investigations, whilst the ligands were kept flexible by creating ring conformations and penalising non-polar amide bond conformations. The default settings for all other Glide module settings were kept¹².

3-(2-phenylethyl)-1H-indazole (6NJ)

Fig 2: Structure of the co-crystallized ligand (native ligand) 6NJ[(3-(2-phenylethyl)-1H-indazole] of X-ray crystal structure of human soluble epoxide hydrolase

Preparation of Protein Structure and Grid Generation

A standard PDB shape document was created up entirely of heavy atoms, but it can also include water, cofactors, and metal ions, and it can be multimeric. Bond ordering, topologies, and formal atomic charges are usually not included in the structure. Because X-ray structural studies cannot usually discriminate between O and NH₂, terminal amide groups can also be misplaced. The protein

preparation wizard included with the Maestro Schrödinger package handles protein preparation.

Preparation of Ligands

4a-4o and trans-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (t-AUCB) (standard) were sketched on the Maestro build panel and created by Ligprep (Schrodinger, LLC, USA), which uses the OPLS 2005 molecular mechanics force field to construct a single low energy 3D structure with proper chiralities for each properly processed input structure. Ligprep may also generate a variety of structures from each input structure, including tautomers, stereochemistries, and ring conformations, as well as delete molecules based on molecular weight or the quantity and types of functional groups present¹³.

Molecular Docking

The default settings for all other parameters were used, and a grid of X = 19.28, Y = -23.49, Z = 1.16 three points on the x, y, and z directions was built. Glide was used to run docking simulations, and the stiff protein structure and flexible ligands were docked using the conventional docking approach. The ligands with the lowest energy were docked into the prepared grid using Glide XP (increased precision)¹¹.

In-silico Pharmacokinetic Prediction

This methodology has been extensively utilised as a filter for compounds with the potential to be developed further in drug development programmes. QikProp® was utilised to do in silico pharmacokinetic profiling. Both pharmaceutically relevant characteristics and physically meaningful descriptors are predicted. Prior to QikProp® analysis, ligands were neutralised. Because the QikProp® algorithm is unable to neutralise a structure, no properties will be generated in normal mode. QPlogP (Octanol/Water), QplogBB, Lipinski's rule of five, detection of reactive functional groups, percent human oral absorption, and CNS activity were some of the pharmaco-relevant features anticipated¹².

RESULTS AND DISCUSSIONS

Using the X-ray crystal structure of human sEH, docking simulation studies were done using the

Maestro Schrödinger programme to gain insight into probable protein-ligand interactions (5AKY). The trans-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (t-AUCB) lowest energy conformer was docked utilising the provided grid to validate the created grid and docking methodology (Fig 3). The protein-ligand interactions indicated that t-AUCB binds to the active region in a similar manner as previously reported.

Fig 3: t-AUCB interaction within the active site of sEH

As a result, the Glide-XP docking mode parameters were capable of providing repeatable in silico binding mode for sEH inhibitors. The LigPrep® module generated the low-energy conformers of the fluorobenzimidazole derivatives that were chosen (4a-4o). The binding pose of 4f, 4g, 4m, and tAUCB showed hydrogen bonding exclusively with TYR 466; HIS 420, PHE 497; TYR 466, PHE 497, HIP 524; ASP 335, TYR 383, TYR 466, LYS 495, PHE 497, Hip 524 respectively with a G score of -9.94; -9.75; -11.56 and -10.24 respectively (Fig 4 to Fig 7) (Table 1). Ligand 4f and 4m exclusively exhibited non-covalent hydrogen bonding (electrostatic) interaction with TYR 466 amino acid residue. Based on the G score (docking score), the more negative the value better is the affinity for the enzyme active site. Taking into consideration the G-score values of 4f, 4g and 4m, it was found that interaction with TYR 466 indicate better binding profile. Although, 4g binds with PHE 497, an amino acid residue that is part of active site but the G-score is comparably poorer than showed by 4f and 4m. The hydrogen

bond interaction demonstrates that the imidazole's nitrogen works as a hydrogen bond acceptor for the hydroxyl group of the tyrosine residue.

Table 1: G-Score and Hydrogen bond interaction of docked ligands

Sl. No.	Ligands	Glide Score	Hydrogen Bonding
1	4a	-8.59	VAL 416, SER 418
2	4b	-9.63	TYR 466
3	4c	-9.08	-
4	4d	-8.80	TYR 466
5	4e	-8.83	-
6	4f	-9.94	TYR 466
7	4g	-9.75	HIS 420, PHE 497
8	4h	-8.49	ASP 335, TYR 383
9	4i	-8.19	LEU 417
10	4j	-9.23	TYR 466
11	4k	-9.22	TYR 466
12	4l	-9.50	ASP 335, TYR 383
13	4m	-11.56	TYR 466, PHE 497, HIP 524
14	4n	-8.71	SER 418, TYR 466
15	4o	-9.45	TYR 383
16	t-AUCB	-10.24	ASP 335, TYR 383, TYR 466, LYS 495, PHE 497, HIP 524

The ligand 4m received the highest G score of -11.56 compare to all the other ligands including tAUCB because of better hydrogen bonding

interaction. As G score is a sum of various XP terms eg Lipophilic term derived from total ligand-protein vDW energy, hydrophobic enclosure reward, H Bond, π stacking etc.), this particular ligand received more H bond score of -1.65. However, 4f and 4g received an H Bond score of -0.18 and -0.81 respectively. Further, even though the standard tAUCB got an H bond score of -2.54, but it received a polar atom burial and desolvation penalty of 1.51 (the polar ligand atom orient itself to nonpolar-hydrophobic protein environment).

The QikProp® module of Maestro Schrödinger was used to predict “drug-likeness” for 15 different ligands (4a-4o) (Table 2 and Table 3). Computational methods to forecasting critical aspects of medications, including as absorption, distribution, metabolism, excretion, and toxicity, increase the likelihood of developing medications with qualities that translate to a safe and orally effective medicine.

Amongst the predicted parameters, the ligands are devoid of any reactive functional groups except 4m, which might probably undergo metabolism to acetal or analog due to the presence of dioxolane moiety as predicted by Qikprop. They have moderate to high percentage oral absorption. The predicted octanol/water partition coefficients (QP log O/W) are in the range of 3-6. Most of the ligand possesses high lipophilicity and the ligand has the tendency to cross blood brain barrier as predicted by QplogBB values. This suggests the ligands might possibly penetrate the CNS and cause neurotoxicity. Further, the outcome of Lipinski's rule of five (mol_MW < 500, QPlog Po/w < 5, donorHB < 5, accptHB < 10) showed that majority of the ligand violate (4b-4g; 4l, 4n and 4o) because the QP log O/W>5. Among all the ligands, 4m emerged to exhibit better drug like molecule compare to others.

CONCLUSION

The present work emphasise on the possible binding mode of fifteen fluorobenzimidazole derivatives as sEH inhibitors. The docking pose of all the ligands exhibited that they bind in a similar pattern within the active site of sEH. The best results obtained by the ligands 4f, 4g and 4m with a dock score of -9.94, -9.75, and -11.56 respectively. Based on the Glide visualizer, it can be inferred that hydrogen bond interaction reveals that the nitrogen of imidazole served as hydrogen bond acceptor that interacts

with the hydroxyl group of tyrosine residue (TYR 466). The ligand 4m received the highest G score of -11.56 compare to all the other ligands including tAUCB because of better hydrogen bonding interaction. The *in silico* pharmacokinetic prediction

studies of the ligands under consideration showed that most of the ligands violate Lipinski rule of five due to high octanol/water partition coefficient. Among all the ligands, 4m possess considerable “drug-like” characteristics with the highest G score.

Fig 4: By using the Glide XP visualizer of the Schrodinger Maestro module, above mentioned figure indicated the 3D view of the docking study of the minimum energy structure of the complex of ligand 4f docked within the active site of sEH (PDB ID: 5AKY). Ligand 4f hydrogen bonded with TYR 466 and shown as yellow dash.

Fig 5: By using the Glide XP visualizer of the Schrodinger Maestro module, above mentioned figure indicated the 3D view of the docking study of the minimum energy structure of the complex of ligand 4g docked within the active site of sEH (PDB ID: 5AKY). Ligand 4g hydrogen bonded with HIS 420 and PHE 497 shown as yellow dash

Fig 6: By using the Glide XP visualizer of the Schrodinger Maestro module, above mentioned figure indicated the 3D view of the docking study of the minimum energy structure of the complex of ligand 4m docked within the active site of sEH (PDB ID: 5AKY). Ligand 4m hydrogen bonded with TYR 466, PHE 497, HIP 524 shown as yellow dash

Fig 7: By using the Glide XP visualizer of the Schrodinger Maestro module, above mentioned figure indicated the 3D view of the docking study of the minimum energy structure of tAUCB (Standard) docked within the active site of sEH (PDB ID: 5AKY). tAUCB hydrogen bonded with ASP 335, TYR 383, TYR 466, LYS 495, PHE 497, HIP 524 shown as yellow dash

Table 2: Pharmacokinetic Prediction of selected compounds by QikProp®

Sl. No.	Ligands	Reactive functional Groups	QPlogBB	QPlogPO/W	% Human Oral Absorption	Lipinski rule of five
1	4a	0	-0.758	4.04	86.9	0
2	4b	0	-1.224	6	85.4	1
3	4c	0	-1.348	6	89.3	1

4	4d	0	-1.191	5	81.7	1
5	4e	0	-1.292	6	87.1	1
6	4f	0	-1.007	5	82.8	1
7	4g	0	-1.052	5	81.6	1
8	4h	0	-1.058	4	88.9	0
9	4i	0	-1.062	4	90.7	0
10	4j	0	-1.361	3	80.3	0
11	4k	0	-1.584	3	72.7	0
12	4l	0	-1.405	6	89.1	1
13	4m	1	-1.112	4	90.2	0
14	4n	0	-1.645	5	76.1	1
15	4o	0	-1.157	5	79.9	1

Table 3: QikProp® properties and descriptors

Sl. No.	Descriptor	Description	Recommended Range
1	#rtvFG	The presence of reactive functional groups might cause false positives in HTS assays as well as breakdown, reactivity, or toxicity issues in vivo. .	0-2
2	QPlogBB	Coefficient of brain/blood partition predicted. Note that QikProp predictions are for medications that are taken orally, therefore dopamine and serotonin, for example, are CNS negative since they are too polar to pass through the blood-brain barrier.	-3.0 – 1.2
3	QPlogPo/w	Predicted octanol/water partition coefficient	-2.0 TO 6.5
4	Percent Human Oral Absorption	On a scale of 0 to 100 percent, predicted human oral absorption. A quantitative multiple linear regression model is used to make the prediction. Because both measure the same property, this metric usually correlates well with Human Oral Absorption.	>80% is high <25% is poor
5	Rule of Five	The number of times Lipinski's rule of five has been broken. Mol MW 500, QPlogPo/w 5,	Maximum is 4

donorHB 5, accptHB 10 are the regulations.
Druglike compounds are those that meet these
criteria. (The number "five" relates to the
limitations, which are all multiples of five.)

CONFLICT OF INTEREST

There are no conflicts of interests.

AUTHORS' CONTRIBUTIONS

BB and ND conducted experimental work, while RB, KK and AMAI drafted the manuscript. All authors read and approved it for publication.

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