SYNTHESIS AND BIOLOGICAL EVALUATION OF TSPO SELECTIVE LIGANDS

Abstract of

Thesis

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Abstract

The thesis entitled "***Synthesis and Biological Evaluation of TSPO Selective Ligands***" have 5 objectives:

1. Designing of new TSPO selective compounds computationally by using chemoinformatic tools such as molecular docking, ADME, and DFT analysis.

2. Synthesis and spectroscopic characterization of designed compounds and their Photophysical analysis to evaluate their binding interactions with serum albumin.

3. Polymeric and lipidic nanoformulation of synthesized compounds to examine their efficacy as a targeted drug delivery carrier.

4. In-vitro evaluation through cytotoxicity (SRB/MTT), drug release and hemolysis test.

5. Ex-vivo/in-vivo assessment in different animal species to identify the potential of synthesized molecules to become a ^{99m}Tc based specific TSPO SPECT marker.

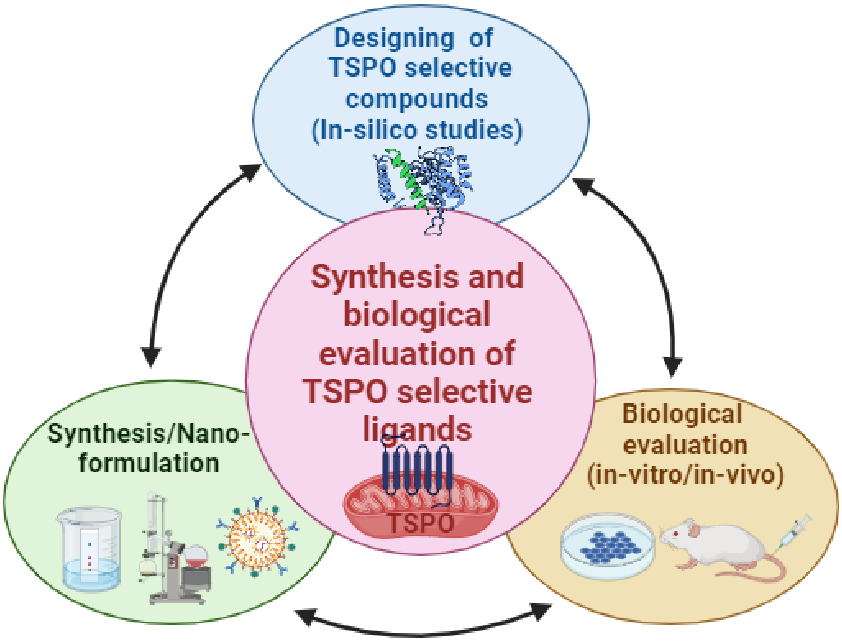

Figure 1: Overview of the thesis objectives

To accomplish these objectives, the thesis has been divided into 5 chapters.

Chapter 1 have been divided into three parts, the first part describes about different imaging modalities (such as PET, SPECT, CT, MRI and Optical imaging) and their comparative aspects, the current limitations associated with these modalities have also described. Subsequently, in addition to that more focus was given on Positron Emission Tomography (PET) and their application in CNS. Different CNS targets (like Purinergic receptor, TSPO, 5HT, Cyclooxygenase receptor and others) and their pre-clinical and clinical probes have also been described.

The thesis is based on TSPO selective ligands, so a detailed description and literature search have been given for TSPO and its PET/SPECT ligands which have been classified in three generations (First generation, Second generation and New generation ligands) with chronological alteration in their structure including the potentials and limitations associated with them. Along with wet synthesis/radiochemistry for PET/SPECT probes, the same pharmacophore has been also assessed for nanoformulation, therefore a detailed overview of two synthetic approaches that is polymeric and lipid based have been discussed.

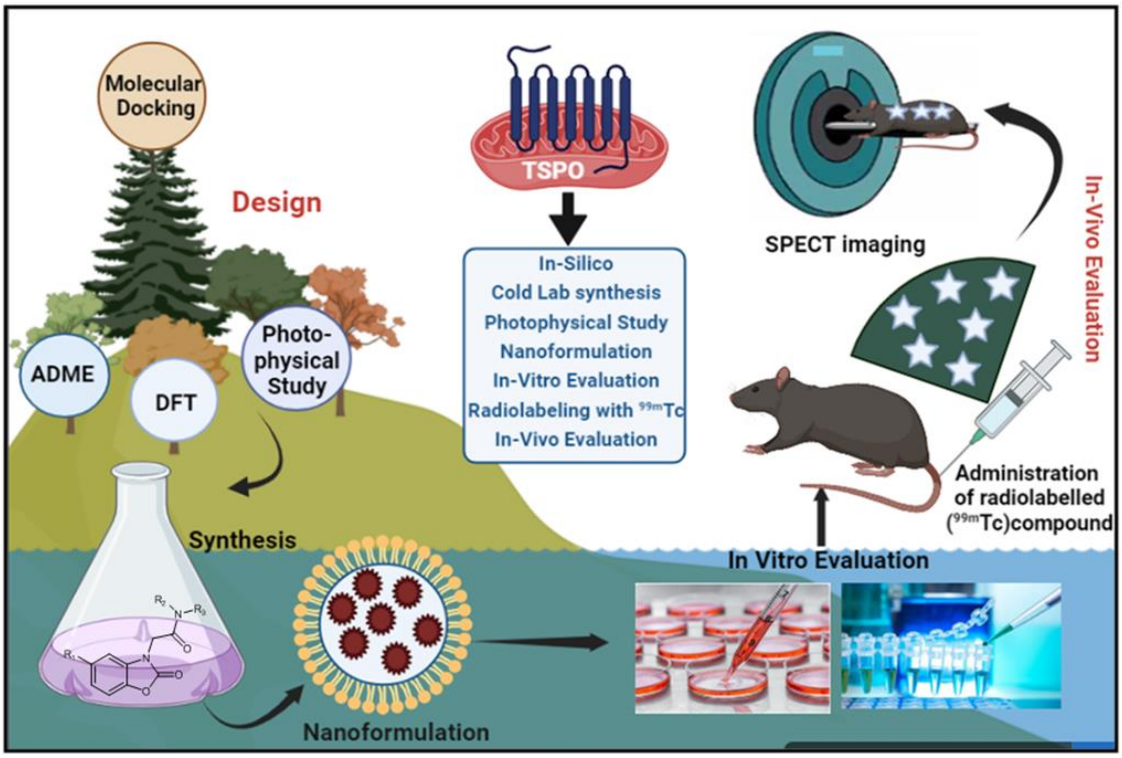

Figure 2: Representative image encompassing the different approaches discussed in Chapter 1

Total 8 probes (including 3 Radio probes and 5 Nano probes) have been designed and synthesized based on acetamidobenzoxazolone pharmacophore, which were developed by opening the diazepine cycle of Ro5-4864, a specific TSPO ligand from first generation.

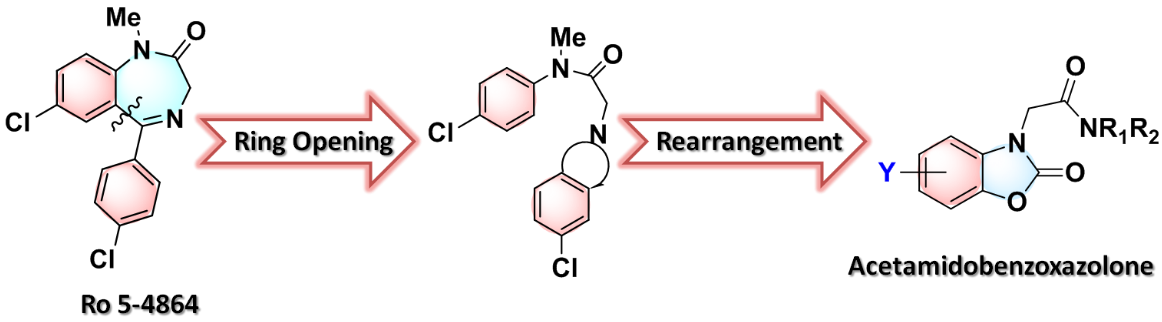

Figure 3: Development of acetamidobenzoxazolone pharmacophore by opening the diazepine cycle of Ro5-4864

So, maintaining all the necessary pharmacophoric properties in this framework we have explored 8 new TSPO selective compounds, namely **DOTA-MBP, PIC-MBP, EDTA-MBP, MCBP, T1**, **T2, QBZ and IQBZ**.

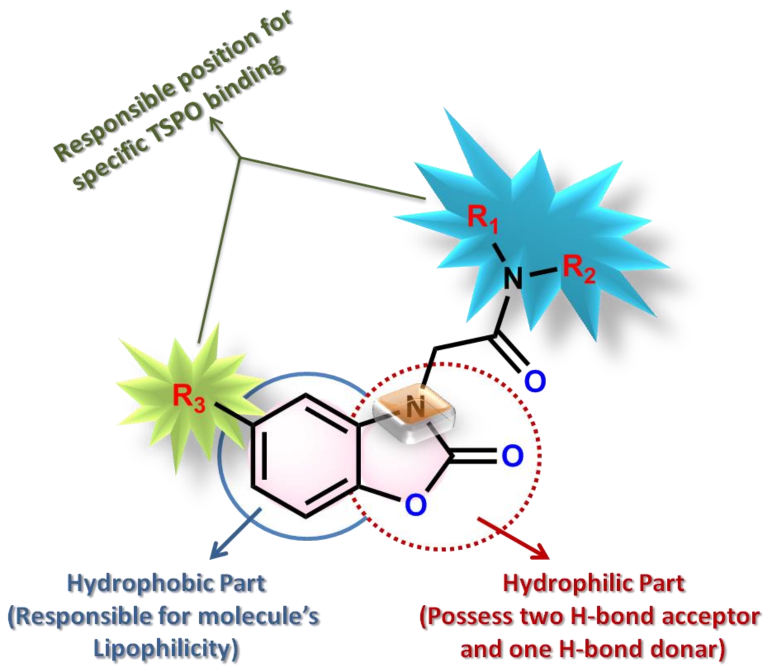

Figure 4: Pharmacophoric properties of acetamidobenzoxazolone

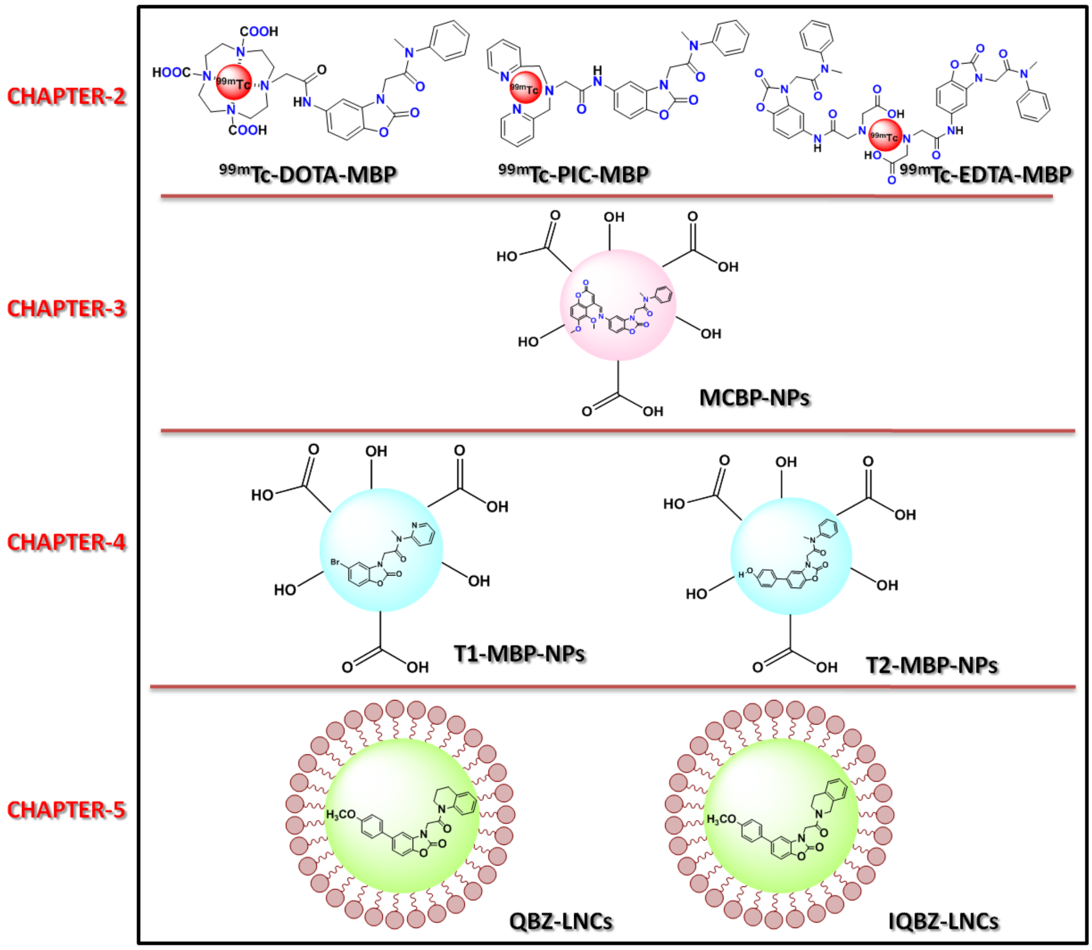

Figure 5: Representative structures of designed compounds in their radio/nano form.

In the second chapter, we have attempted to conjugate the TSPO compound 2-(5-amino-2-oxobenzo[*d*]oxazol-3(2*H*)-yl)-*N*-methyl-*N*-phenylacetamide (MBP) with cyclic and acyclic chelators DOTA, PIC and EDTA (abbreviated by DOTA-MBP, PIC-MBP and EDTA-MBP respectively). These chelator conjugated compounds have been synthesized with 60-80% yield and subsequently characterized by using different spectroscopic approaches like ^{1}H NMR, ^{13}C NMR, and IR. Prior to wet lab synthesis, all compounds have been validated computationally which revealed better binding affinity of PIC-MBP for TSPO than other two compounds DOTA-MBP and EDTA-MBP. This compound has been further evaluated photophysically to determine the binding constant, nature of interaction with serum albumin. Bovine Serum Albumin (BSA) was selected as the reference protein for studies due to its easy and widespread accessibility. The interaction between PIC-MBP and serum albumin was examined with reference to quenching, thermodynamics and the nature of their interaction.

After synthesis, chelating vehicles were subjected to complexation with ^{99m}Tc which give 98-99% radiolabelling efficiency. To ensure the prevention of transchelation during in-vivo imaging experiments, it is imperative that the chelator conjugated to the biological carrier possesses stronger and more stable complexation characteristics with the ligand. The in-vivo serum stability test confirmed the stable nature of ^{99m}Tc-DOTA-MBP, ^{99m}Tc-PIC-MBP and ^{99m}Tc-EDTA-MBP, without any transchelation to serum protein. Later ^{99m}Tc labelled compounds were analyzed for ex-vivo/in-vivo distribution in different organs of normal and lung-inflamed animal models.

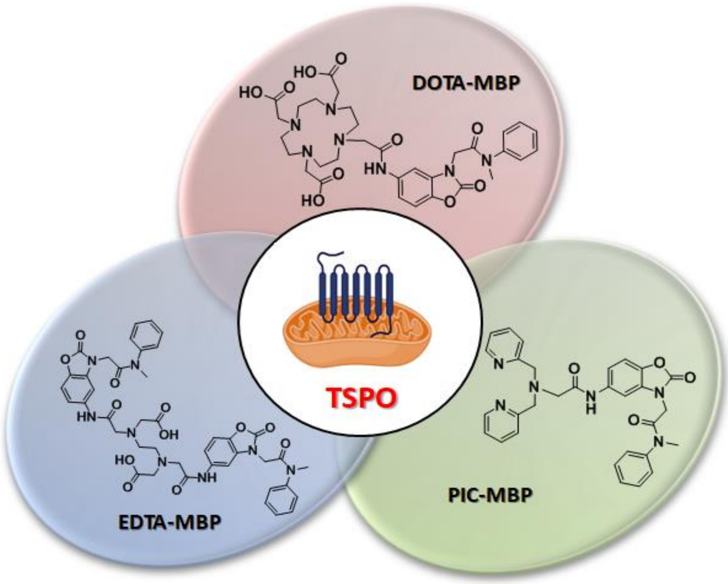

Figure 6: Structural representation of designed TSPO compounds in chapter 2

The third chapter encloses the computational validation of new TSPO selective compound named as MCBP ((E)-*N*-methyl-2-(2-oxo-5-(2-(2-oxo-2*H*-chromen-4-yl)vinyl)benzo[*d*]oxazol-3(2*H*)-yl)-*N*-phenylacetamide) through molecular docking and DFT analysis which revealed its effective binding affinity and mechanistic potential towards TSPO. We have tried to conjugate coumarin based moiety to MBP ligand. The synthesis was achieved in 5 chemical steps with 65 % yield. Subsequently, to analyze the pharmacokinetic and pharmacodynamic behavior of MCBP, as well as its interaction with the serum albumin (BSA), we conducted a comprehensive photophysical assessment using various spectroscopic techniques, including UV-absorption and fluorescence spectroscopy.

Here we have also tried to check MCBP's efficacy in its nano form. So wet lab synthesis followed by nanoformulation of MCBP with biocompatible PLGA polymer was performed. Encapsulation of MCBP in PLGA polymer was achieved to examine a targeted drug delivery and enhanced solubility of MCBP. The MCBP loaded nanoparticles were prepared with less than 100 nm particle size, exhibiting a low Polydispersity Index (PDI), and possessing a zeta potential of -29.2 mV. The morphology of these nanoparticles were observed through scanning electron microscopy (SEM) analysis.

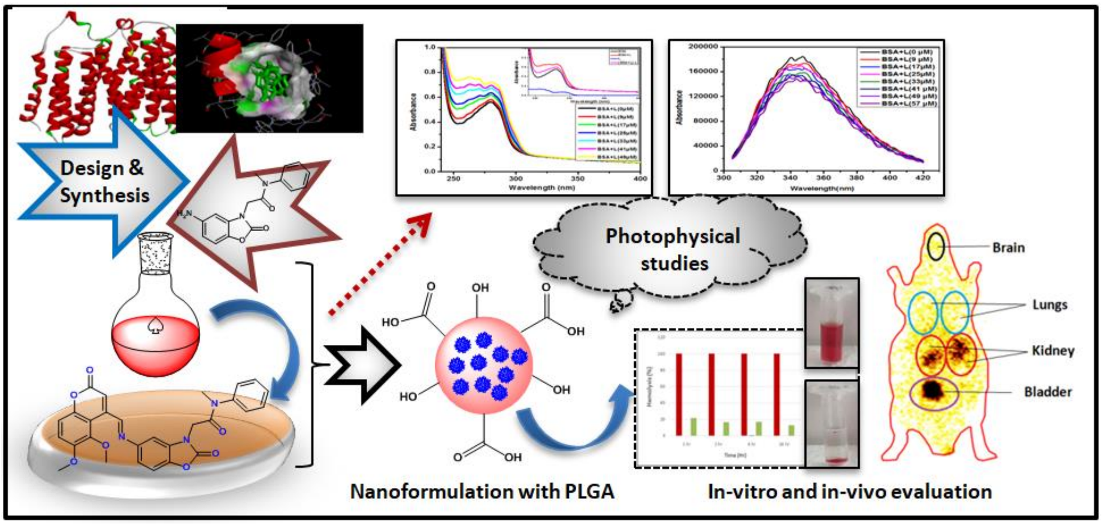

Figure 7: Descriptive depiction showcasing the various approaches implemented within chapter 3

Furthermore, in-vitro release studies of MCBP loaded nanoparticles were conducted under physiological conditions (pH 7.4) and the controlled release mechanism of MCBP signifies the potential of these nanoparticles for sustained drug delivery applications. Furthermore, we assessed the hemo-compatibility of MCBP through hemolysis study and its cytocompatibility via in-vitro cytotoxicity test. In-vivo SPECT imaging was performed to determine the extent of radioactivity uptake in the brain and peripheral organs, providing insights into the compound's distribution patterns.

In the fourth chapter, chemoinformatic tools (Molecular docking and ADME) have been used to design two skeleton named as 2-(5-bromo-2-oxobenzoxazol-3(2*H*)-yl)-*N*-methyl-*N*-(pyridin-2-yl)acetamide (T1) and 2-[5-(4-[^{11}C]methoxyphenyl)-2-oxo-1,3-benzoxazol-3(2*H*)-yl]-*N*-methyl-*N*-phenyl-acetamide (T2). Both these compounds were synthesized with overall 70-80 % yield. Further nanoformulation of T1/T2 were prepared with biodegradable and biocompatible polymer PLGA to develop nanoprobes for determination of 18 kDa Translocator protein.

Physico-chemical analysis was performed through photo-spectroscopic methods to determine the pharmacokinetics and pharmacodynamics of these two compounds. Serum albumin was taken as a model protein to determine quenching aspects of these compounds under ex-vivo conditions, their binding constant, impact on their 2D/3D structure and impact of these compounds on enzyme kinetics for esterase activity.

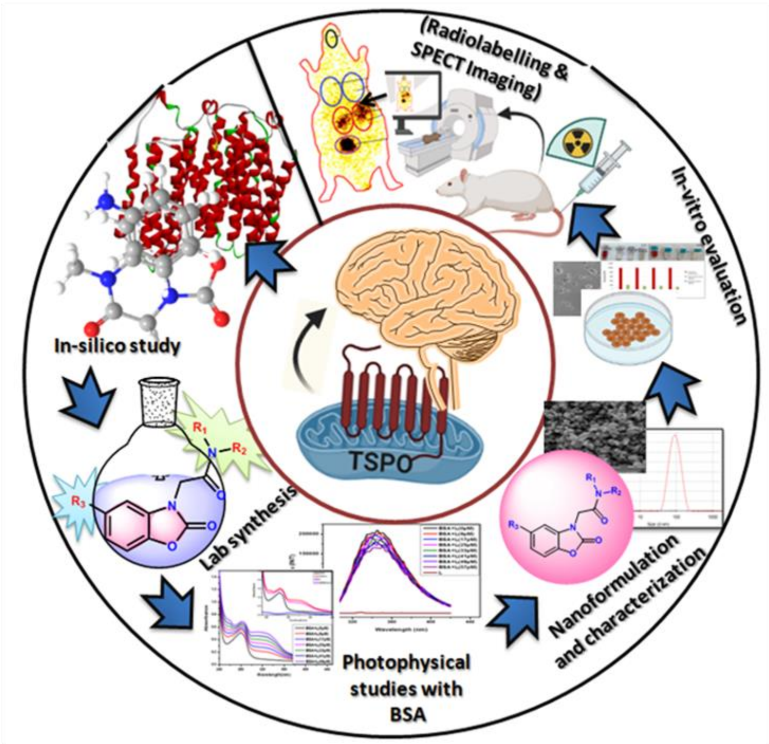

Figure 8: Comprehensive overview of the systematic processes utilized in Chapter 4

For appropriate pre-clinical analysis, animal study was performed in Balb/c mice to track the real time distribution, elimination and excretion. This has been accessed through SPECT imaging and the results shows that these nanoparticles were selective for TSPO specific organs.

The fifth chapter covers the brief assessment of another category of two newly screened TSPO compounds, in terms of their performance, namely 3-(2-(3,4-dihydroquinolin-1(2*H*)-yl)-2-oxoethyl)-5-(methyl(pyridin-3-yl)amino)benzo[*d*]oxazol-2(3*H*)-one (**QBZ**) and 3-(2-(3,4-dihydroisoquinolin-2(1*H*)-

yl)-2-oxoethyl)-5-(methyl(pyridin-3-yl)amino)benzo[*d*]oxazol-2(3*H*)-one (**IQBZ**). Both compounds were selected based on studies with chemoinformatic tools including molecular docking, ADME prediction and DFT analysis. We have tried to prepare QBZ and IQBZ encapsulated lipid nanocapsules for monitoring the alteration in TSPO density. The encapsulation of both compounds in lipid were achieved by phase inversion method with good yield. The loaded nanoparticles prepared with less than 100 nm particle side and highly stable surface charge.

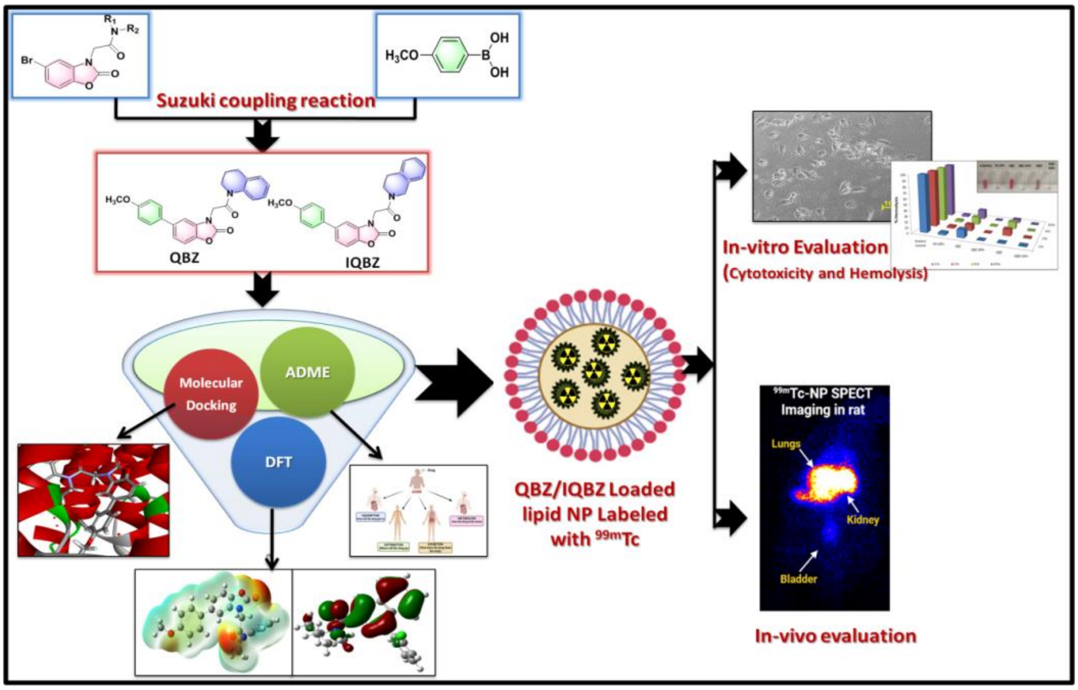

Figure 9: Illustrative view of various computation and biological tools (Chapter 5)

The hemocompatible and cytocompatible behavior were confirmed by hemolysis and SRB assay. A gamma nuclide technetium-99m (^{99m}Tc) based radiolabelling of both QBZ-LNPs and IQBZ-LNPs have also been described to attain further insights into the pharmacokinetics and pharmacodynamics. SPECT imaging described the capability of both lipid nano-vehicles as specific delivery carrier for TSPO specific peripheral organs.

Conclusion

A total of 8 compounds were designed and synthesized on the basis of previously reported acetamidobenzoxazolone pharmacophore specific for TSPO receptor. These compounds were categorized into 3 different series on the basis of their analysis. The

first series includes 3 compounds with chelating properties (DOTA-PIC, PIC-MBP, and EDTA-MBP). The second series consists of 3 compounds formulated as polymeric nanostructures (MCBP, T1, and T2). The third series comprises 2 compounds formulated as lipid nanostructures (QBZ and IQBZ). Prior to synthesis all compounds validated through computational approaches to assess their binding efficacy with specific protein TSPO and revealed their effective binding affinity towards TSPO within the range of -15 to -45 kJ/mol. Out of 3 synthesized chelating vehicles PIC-MBP has better binding profile and distribution pattern during in-vivo experiments compared to DOTA-MBP and ETDA-MBP. Next 3 synthesized compounds subjected to nanoformulation with polymeric systems and revealed their controlled and sustained drug release profile over 50 to 52 hrs. Hemocompatible and cytotoxic behavior were also found appropriate for clinical applications. Two compounds namely QBZ and IQBZ were nanoformulated using cationic DSPC lipids through a phase inversion approach, and their properties were compared. The results indicated that IQBZ exhibited a higher binding affinity, greater cytotoxicity, and improved distribution pattern in TSPO-specific organs.