

**A BINDING FREE ENERGY BASED COMPUTATIONAL PATHWAY FROM
CHEMICAL TEMPLATES TO LEAD-LIKE MOLECULES:
A CASE STUDY OF CYCLOOXYGENASE -2 INHIBITORS**

by

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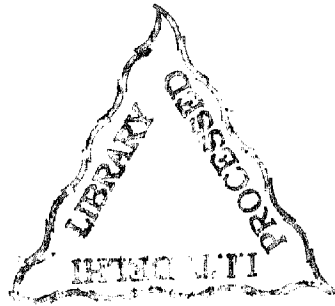
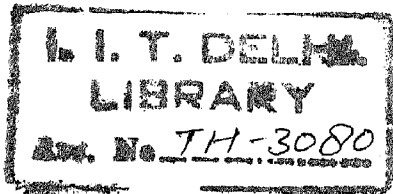
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CERTIFICATE

This is to certify that the thesis entitled "*A Binding Free Energy Based Computational Pathway from Chemical Templates to Lead-like Molecules: A Case Study of Cyclooxygenase-2 Inhibitors*" being submitted by **Ms. N. LATHA** to the Indian Institute of Technology, Delhi for the award of the degree of **Doctor of Philosophy** in Chemistry is a record of bonafide research work carried out by her. Ms. N. Latha has worked under my guidance and supervision, and has fulfilled the requirements for the submission of this thesis which, to my knowledge, has reached the requisite standard.

The results contained in this dissertation have not been submitted in part or full to other University or Institute for the award of any degree or diploma.



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The dawn of the 21st century heralds a new era in the drug discovery process. Access to the complete human genome sequence as well as to the complete sequences of pathogenic organisms (<http://www.ncbi.nlm.nih.gov/Genbank/>) provides us with information that can result in an avalanche of therapeutic targets. As more protein target structures become available with advances in crystallography, nuclear magnetic resonance and bioinformatics, the need grows for a set of computational tools that can identify and analyze active sites and suggest compounds that can bind to these sites specifically. Automation of lead compound design *in silico* given the structure of the protein target and a definition of its active site vies for the top of the wish list in any drug discovery programme. Several conceptual and methodological challenges remain before this goal could be accomplished. Some of the concerns are the novelty of the candidates generated, their geometries, the partial atomic charges, a proper account of the flexibility of the candidate molecule and the target, a consideration of solvent and salt effects in binding, a reliable methodology for developing quantitative estimates of binding affinities and finally the druggability of the candidates generated. This thesis is aimed at charting a computational pathway for lead design at the current state of the art tackling some of the above issues. The pathway developed is tested on a series of known drugs for cyclooxygenase-2 as the target enzyme and this forms the focus of the thesis.

The thesis is divided into seven chapters. The motivation behind this study and a background to computer-aided drug design, the role of biomolecular drug targets and some recent computational methods forms the main focus of **Chapter-1**. A comprehensive

computational protocol of starting from chemical templates to the design of lead-like molecule is presented in **Chapter 2**. This chapter discusses the strategy adopted for the design of template library and candidate molecules there from, development of their geometries and force field parameters, docking the candidates in the active site of the target protein and preparing the protein-ligand complexes for subsequent energy based processing. Binding free energy estimates as applied to protein-ligand complexes obtained from docking and energy minimization can at best be qualitative from a theoretical perspective. These are critically assessed in **Chapter 3**. The success of the method in sorting drugs from non-drugs for a given target is demonstrated using cyclooxygenase as a proof of concept. A case study of COX-2 inhibitors to test the methodology proposed is presented in **Chapter 4**. Flexibility of the target and that of ligand, roles of solvent and salt effects are of serious concern in any viable computational procedure for ranking candidates. Additionally, Boltzmann averaging tends to reduce the magnitudes of the binding free energies obtained from energy-minimized structures (single point calculations). These concerns are addressed by separate molecular dynamics simulations on the systems corresponding to initial and final states of the binding process. **Chapter 5** illustrates the application of molecular dynamics simulations in conjunction with the theory presented for a semi-quantitative estimate of binding free energies. An important question relating to generality of the method and transferability of the protocol is undertaken by testing binding of flurbiprofen, an NSAID specific to COX-1. A detailed structural and energetic analysis is undertaken in **Chapter 6** to account for specificity of flurbiprofen to cyclooxygenase-1. This is presented in this chapter together with some computational issues related to specificity. Finally a critical assessment of the steps in the proposed computational pathway and further improvements envisioned are summarized in **Chapter 7**.

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