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DECLARATION

I hereby declare that the thesis entitled "Structural, Conformational and Biochemical Aspects of Thiosemicarbazones and Crystallization and Modeling Studies on Lectins" submitted to the University of Madras in February 2006 for the degree of Doctor of Philosophy is the original and independent work carried out by me during the period 2001-2006 in the Department of Crystallography and Biophysics, University of Madras under the supervision of Prof. M.N. Ponnumswamy, and the thesis has not formed previously the basis for the award of any Degree, Diploma, Associateship, Fellowship or any other similar titles.

N. Sampath
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References
This thesis entitled "Structural, Conformational and Biochemical Aspects of Thiosemicarbazones and Crystallization and Modeling Studies on Lectins" is a report on the research work carried out by the candidate in the Department of Crystallography and Biophysics, University of Madras, Guindy Campus, Chennai-600 025, under the guidance of Prof. M.N. Ponnuswamy, during the period 2001-2006.

The thesis consists of two parts. Part I deals with the structural and biochemical aspects of thiosemicarbazone derivatives and their biological activities. This part is further divided into two sections. Section A, consisting of three chapters, describes the synthesis, characterization and three-dimensional structure determination of various thiosemicarbazone derivatives. Section B, also consisting of three chapters, explains the biological activities of thiosemicarbazones against the hepatocellular carcinoma (HCC) and some other microorganisms. Modeling studies have also been carried out to curtail the action of ribonucleotide reductase (RNR) enzyme through specific inhibitors. Part II explains the procedures adopted to extract, purify and crystallize two lectin proteins. An appendix, consisting three crystal structures, is appended at the end of the thesis.

Part I: Crystal Structure of Thiosemicarbazones and their Biochemical Studies

Thiosemicarbazide is a metal scavenger and combines with aldehydes or ketones to generate the thiosemicarbazone derivatives, which possess a wide range of biological activities due its reduction capability. It is planar and adopts an extended (E) conformation due to extensive electron delocalization throughout the moiety. It is a tridentate ligand which shows two major changes during the biological reaction, namely (i) 180° rotation about the hydrazinic C-N bond and (ii) switch over the S atom to cis
position for imine nitrogen. These changes are ideal for tridentate ligands favouring metal chelation. All these factors reinforce each other and render the ligand an efficient scavenger of metal ions present in the biological molecule.

Section A: Structural Studies on Thiosemicarbazones

Chapter 1 deals with the crystal structure of piperidine thiosemicarbazone derivatives. These derivatives possess many pharmaceutical activities. The structural studies are carried out to understand the relationship between the conformation of the piperidine ring and thiosemicarbazone moiety. In this direction, four different derivatives, namely N-free piperidines and N-methyl piperidines are discussed in detail.

Chapter 2 describes the crystal structures of azabicyclic thiosemicarbazone derivatives. They possess medicinal and biological activities such as analgesic, anti-inflammatory, hypotensive etc. Two different azabicyclic thiosemicarbazone derivatives are discussed here. These compounds are derived from primary amines and aldehydes under suitable conditions.

Chapter 3 presents the details of the crystal structure of phenyl thiosemicarbazone derivatives. The pharmacological study of these compounds reveals the inhibitory activity against influenza, protozoa, smallpox and several kinds of tumors. All these activities of thiosemicarbazones are mainly due to the chelating properties of this moiety and its ability to bind with the transition metal ions by the sulfur and hydrazinic nitrogen atoms. In view of this, two phenyl thiosemicarbazones were designed and crystallographic studies were carried out.
Section B: Biochemical Studies of Thiosemicarbazones

Chapter 4 deals with the anticancer study against *Hepatocellular carcinoma (HCC)* using the derived piperidine thiosemicarbazone derivatives. HCC is a malignancy, responsible for more than one million deaths annually worldwide. HCC occurs for the patients with cirrhotic liver disease and it develops both epithelial and mesenchymal tissues. During cancer development, the clinical serum markers such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) levels are increased, thus confirming the liver damage due to cancer.

Herein, the synthesized piperidine thiosemicarbazones were treated against the HCC induced rats and a significant decrease of serum marker enzyme levels were noticed in the cancer developed liver after oral treatment. The structure activity relationship of the treated drugs is described elaborately.

Chapter 5 describes the antibacterial and antifungal studies of thiosemicarbazone derivatives against the microorganism such as the human bacterial and fungal pathogens. For antibacterial studies ten bacterial strains and for antifungal studies two *candida* human pathogens were taken into account. For this anti-microbial study, synthesized thiosemicarbazones were used as drugs for inhibition of microorganisms by agar well diffusion method. The inhibition zone indicates that the thiosemicarbazones are sensitive to the microorganisms. The results reveal the structure activity relationships of drugs that were analyzed.

Chapter 6 deals with the modeling studies to curtail the action of RNR using two different approaches. Ribonucleotide reductase (RNR) is an enzyme required for DNA synthesis and thus for cell division, which is a potential target for drugs designed to
inhibit the cell growth. The interest in RNR as a target for cancer therapy is increased due to a newly identified human RNR which is regulated by enzyme p53. The p53 protein actively suppresses the tumor formation but on mutation induces several forms of cancer.

The RNR inhibitor must be a potential radical scavenger to destroy the tyrosyl radical or iron metal scavenger. Flavin and Phenosafranine are the radical scavengers used in our study to reduce the radical activity. Chelating agents such as thiosemicarbazones, normally play a vital role to remove or prevent the incorporation of iron into the enzyme. The details pertaining to dock the radical scavengers and the mechanism adopted to chelate the iron by thiosemicarbazones are discussed here.

PART II: STRUCTURAL STUDIES ON LECTINS

Chapter 7 describes the extraction, purification and crystallization of lectin proteins from the insect grasshopper and mollusc in details. Lectins bind with carbohydrate chains on the cell wall and play an important biological role in many cellular processes. The purified proteins were crystallized using the protocol of McPherson (Crystallization of Biological Molecules, 1999, Spring Harper Laboratory Press, Cold Spring Harper, New York, 67). A survey in Protein Data Bank (PDB) on insect lectins reveals the non-availability of the three-dimensional crystal structures. An attempt was made to model the structure based on human galactin-3 (PDB: 1a3k). The docking study was done for this modeled structure with two sugars, namely N-acetylgalactosamine and lactose. The lectin-sugar complexes were analyzed and the activity was compared with the human galactin-3 structure.
Three biologically important crystal structures, namely two spiro derivatives and a carbazole, are added as an Appendix at the end of the thesis.

The figures and tables are numbered serially chapter wise. The figures are given at appropriate places in the text and the tables are given at the end of each chapter. The references cited in the text are given in alphabetical order at the end of the thesis. The structure factor tables for the small molecular structures are provided in the Compact Disc (CD) enclosed at the back cover of the thesis.

Based on the above work, the following research articles have been published/communicated:


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