

Synthesis of novel bioactive azocoumarin derivatives: Cytotoxicity, DNA binding, BSA binding study, and their *in silico* analysis

Thesis

Submitted to Vidyasagar University

For award of the degree of

Doctor of Philosophy (Science)

By

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2025

Dedication

*I dedicate this thesis to my
parents and all my family
members*

MIDNAPORE CITY COLLEGE

Certificate from the Supervisor

It is hereby certified that the thesis entitled “Synthesis of novel bioactive azocoumarin derivatives: Cytotoxicity, DNA binding, BSA binding study, and their *in silico* analysis” is being submitted by Putul Karan, Reg. No.: 972 / Ph. D (Sc.), for the award of the degree of Doctor of Philosophy in Science to the Vidyasagar University, Midnapore, West Bengal, India. This thesis is a record of trustworthy research work that she absolutely carried out under my direct supervision. The matter incorporated in this Ph.D. thesis has not been submitted for the award of any other degree/diploma anywhere before. In my opinion, the thesis has fulfilled all the requirements according to the regulations and has reached the standard necessary for submission.


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Abstract:

The design and synthesis of novel azo-coumarin derivatives represent a promising area of research in medicinal chemistry due to their potential pharmacological activities. In this study, a series of novel azo-coumarin derivatives such as 6-[3-pyridyl]azocoumarin, 6-[Phenylazo]coumarin, 6-[2-Chlorophenylazo]coumarin, 6-[3-Chlorophenylazo]coumarin, and 6-[4-Chlorophenylazo]coumarin were synthesized using diazo-coupling reaction. The synthesized compounds were fully characterized using FT-IR, NMR, and mass spectrometry, and biological studies were carried out. The structures of the synthesized compounds were optimized using DFT calculation and the frontier molecular orbital calculations reveal that synthesized compounds were more biologically and chemically active than coumarin. The cytotoxicity of these derivatives was evaluated against human cancer cell lines LN-229 and the IC₅₀ values were evaluated and it was found that 6-[4-Chlorophenylazo]coumarin was most effective. The interaction of these derivatives with CT-DNA was investigated using UV-visible and fluorescence spectroscopy. All the synthesized compounds bound at the minor groove of CT-DNA and the binding constant values showed the order of the binding affinities was 6-[4-Chlorophenylazo]coumarin > 6-[2-pyridyl]azocoumarin > 6-[3-Chlorophenylazo]coumarin > 6-[Phenylazo]coumarin > 6-[3-pyridyl]azocoumarin > coumarin and all the compounds bound with CT-DNA through minor groove. The BSA binding study of the synthesized compounds was also carried out using UV-visible and fluorescence spectroscopy which showed the same binding order of the compounds observed with the DNA binding study. *In silico* analysis supported all the experimental outcomes regarding CT-DNA and BSA binding.

Keywords: Azo-coumarins, DFT calculations, Cytotoxicity, BSA binding, DNA binding, Molecular docking.

Declaration by the Candidate

I, Putul Karan, Reg. No.: 972 / Ph. D (Sc.), hereby declare that this dissertation entitled "**Synthesis of novel bioactive azocoumarin derivatives: Cytotoxicity, DNA binding, BSA binding study, and their *in silico* analysis**" has been completed by me under the supervision of Dr. Avishek Ghosh, in the Department of Pure and Applied Science (Chemistry), Midnapore City College, Midnapore-721129, West Bengal, India. I also approve that this work is original and has not been submitted partially or entirely for any degree/diploma or academic award anywhere before. I have maintained the guidelines provided by the University in preparing the thesis. Whenever, I have used any objects (data, figures, theoretical analysis, and texts) from other sources, I have given their credit by citing in the text and also referred in this thesis. Any contribution made to this research by others, with whom I have worked at Midnapore City College or elsewhere, is explicitly acknowledged in the dissertation.

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