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PhD Engineering Sciences *(Thesis Submitted)*
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PhD Enrollment Date	12.01.2015
Thesis Submission Date	29.08.2019
PhD Viva Voce Date	28.01.2020 (Scheduled)

PHD THESIS TITLE

Binding Mechanism of Anticancer Flavonoids and their Derivatives with Nucleic Acid

Mechanistic understanding of cytotoxicity exerted by chemodrugs is crucial to elucidate their mode of action and to improve their efficacy. The structural stability and conformational features of nucleic acid (DNA and RNA) holds significance due to its central role in life cycle of a cell. In this context, elucidation of physical and chemical basis of binding phenomena such as base sequence selectivity, correlation of structure-activity relationship, linkage between geometry and the thermodynamic properties becomes important.

RESEARCH EXPERIENCE
More than 06 Years

SPECIALIZATION
Biochemistry & Biophysics

RESEARCH SKILLS

- Spectroscopy
  Infrared Spectroscopy, Circular Dichroism Spectroscopy, Surface Enhanced Raman Spectroscopy, UV-visible Spectroscopy
- Microscopy
  Atomic Force Microscopy, Near-field Scanning Optical Microscopy
- Isothermal Titration Calorimetry
- Molecular Docking *(in-silico)* Simulations

WORK EXPERIENCE

Project-Junior Research Fellow | 2013-2015 (1.5 Years)
Title: Infrared Spectroscopic Study for Tumor diagnosis, Phase-II

- *Handled end to end project on breast cancer; collaborative project between CSIR-NPL, DST and Max Super Specialty Hospital, Delhi.*
- *Interacted with Research Scientist, Doctors, Cancer Patients, Hospital's Scientific & Ethical committee for their valuable inputs.*
- *Created dashboards to reflect health and progress of project.*
- *Developed and validated less invasive breast cancer diagnostic tool and wrote research publications.*

Project | Jan-June 2013 (06 Months), CSIR-NPL, New Delhi
Title: Noble Metal Nanoparticles: Synthesis, Characterization and Application in Surface Enhanced Raman Spectroscopy

- *Worked on biological applications of several gold & silver nanoshapes.*
- *Optimized size-controlled synthesis of metal nanostructures.*
- *Characterized via X-ray diffraction, electron microscopy (SEM & TEM).*

Project | June-July 2010 (06 Weeks), ILBS, New Delhi
Title: Hepatitis B Virus and Its Genotypes

- *Isolated genomic DNA and Hepatitis B viral DNA from Patients.*
- *Identified genotype variants from the studied population.*
- *Gained experience on RT-PCR, western blotting methods.*

EDUCATION

Class X
CBSE Board (2003-04, Marks-403/500, 80.6%)

Class XII (Biology, Physics, Chemistry)
CBSE Board (2005-06, Marks-357/500, 71.4%)

BSc in Biology
Kurukshetra University, Kurukshetra (2006-09, Marks-1059/1450, 73.3%)

MSc in Biotechnology
Kurukshetra University, Kurukshetra (2009-11, Marks-1398/2000, 69.9%)

MTech in Nanotechnology (First Rank)
NIT, Kurukshetra (2011-13, CGPA-9.94/10)

PG Diploma in Intellectual Property Rights Law
NLSIU, Bangalore (Distance Course, 2016-17, CGPA-4.4/7.0)

OTHER SKILLS

- Laboratory Skills ★★★★★
- Result Analysis ★★★★★
- Time Management ★★★★
- Public speaking ★★★★

FELLOWSHIPS, AWARDS & RECOGNITION

- 07 (Seven) Best Poster/Paper Presentation Award
  National & International Conferences/Symposia
- INSPIRE-Junior Research Fellowship, Year 2015
  Department of Science and Technology, Govt. of India
- Gold Medal, MTech, Year 2013
  National Institute of Technology, Kurukshetra
- Qualified GATE in Biotechnology, Year 2011 & 2012
- Awarded Roll of Honor by Chief Minister of Haryana, Year 2009

REFERENCES

- Dr. Ranjana Mehrotra, Former Chief Scientist
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List of Publications/Conference Proceedings

✓ **Publications in Peer-Reviewed SCI Journals**	**08**
✓ **Publications in Peer-Reviewed Open Access Journals**	**01**
✓ **Conference Proceedings (Abstract)**	**02**
✓ **Under Review**	**02**
✓ **Total Citations**	**46**
✓ **'h' Index**	**04**
✓ **Source**	**Google Scholar**

1. Ranjana Mehrotra, and **Bhumika Ray** (2019) Substantial role of infrared spectroscopy in lung cancer detection. Clinics in Oncology 4,1577 ***(Editorial, Open Access, Peer-Reviewed), IF-1.920***
2. **Bhumika Ray#,** Bhumika Gupta# and Ranjana Mehrotra (2019) Binding of platinum derivative, oxaliplatin to deoxyribonucleic acid: structural insight into antitumor action. Journal of Biomolecular Structure and Dynamics 37 (14), 3838-3847 ***(Research Article), IF-3.310***
3. Ranjana Mehrotra, Gunjan Tyagi, Sonika Charak, **Bhumika Ray**, Geeta Kadayaprath, Harit Chaturvedi, Urmi Mukherji and Andleeb Abrari (2018) Biospectroscopic analysis of human breast cancer tissue: probing infrared signatures to comprehend biochemical alterations. Journal of Biomolecular Structure and Dynamics 36 (3), 761-766 ***(Letter To The Editor), IF-3.310***
4. **Bhumika Ray**, Shweta Agarwal, Heena Kadyan, Kaweri Gambhir, Parag Sharma and Ranjana Mehrotra (2017) Deciphering molecular aspects of interaction between anticancer drug mitoxantrone and tRNA. Journal of Biomolecular Structure and Dynamics 35 (10), 2090-2102 ***(Research Article), IF-3.310***
5. Kaweri Gambhir, **Bhumika Ray**, Ranjana Mehrotra and Parag Sharma (2017) Morphology dependent two photon absorption in plasmonic structures and plasmonic-organic hybrids. Optics and Laser Technology 90, 201-210 ***(Research Article), IF-3.319***
6. Ranjana Mehrotra, **Bhumika Ray** and Harit Chaturvedi (2016) Biochemical characterization of lung cancer tissues using Fourier transform infrared spectroscopy. Journal of Cancer and Clinical Oncology, 2 (1), 1-8 ***(Research Article, Open Access, Peer-Reviewed)***
7. **Bhumika Ray**, Shweta Agarwal, Neelam Lohani, Moganty Raja Rajeswari and Ranjana Mehrotra (2016) Structural, conformational and thermodynamic aspects of groove directed-intercalation of flavopiridol into DNA. Journal of Biomolecular Structure and Dynamics 34 (11), 2518-2535 ***(Research Article), IF-3.310***
8. Shweta Agarwal, **Bhumika Ray** and Ranjana Mehrotra (2015) Surface enhanced Raman spectroscopy as an advanced tool for investigating chloroethyl nitrosourea derivatives complexation with DNA. International Journal of Biological Macromolecules 81, 891-897 ***(Research Article), IF-4.784***
9. Ranjana Mehrotra, Deepak Kumar Jangir, Shweta Agarwal, **Bhumika Ray**, Parul Singh and Avinash Kumar Srivastava (2013) Interaction studies of anticancer drug lomustine with calf thymus DNA using surface enhanced Raman spectroscopy. Mapan 28 (4), 273-277 ***(Research Article), IF-1.250***

Abstract Conference Proceedings

10. **Bhumika Ray** and Ranjana Mehrotra (2018), Preferential Binding of Flavonoids with Bovine Serum Albumin: In-silico and Spectroscopic Insight into Cytotoxic Competence. *BPS 2018* ***(Abstract Issue)****, Volume 114, Issue 3, Supplement 1, p55a, Biophysical Journal*. https://doi.org/10.1016/j.bpj.2017.11.356
11. Kaweri Gambhir, **Bhumika Ray**, Parag Sharma and Ranjana Mehrotra (2016) Morphology dependent enhancement of linear and third order optical nonlinearities of plasmonic-organic hybrids. *13th International Conference on Fiber Optics and Photonics* ***(Abstract Issue)****, OSA Technical Digest (Online), Optical Society of America, W3A.26.* https://doi.org/10.1364/PHOTONICS.2016.W3A.26

Under Review

12. **Bhumika Ray** and Ranjana Mehrotra, Probing Molecular Binding Characteristics of tRNA with Flavopiridol and Comparison with Flavopiridol-DNA Adducts. International Journal of Biological Macromolecules
13. **Bhumika Ray** and Ranjana Mehrotra, Nucleic Acid Binding Mechanism of Flavone Derivative, Riviciclib: Structural Analysis to Unveil Anticancer Potential. Journal of Physical Chemistry B

List of Papers Presented in National/ International Conferences (Oral/Poster)

Best Paper Presentation Awards 07 (Seven)

- ✓ **Bhumika Ray**, Satyendra Mourya, Anuradha and Gaurav Singh, Optical and electrical study of Au nano-thin films using spectroscopic ellipsometry, International Conference on Nanomaterials and Nanotechnology, University of Delhi, Delhi, December 18-21, 2011 ***(Oral Presentation)***

- ✓ **Bhumika Ray** and B.K. Kaushik, Synthesis and characterization of PVP encapsulated CdS nanoparticles, National Conference on NexGen Biotechnology: Amalgamating Science & Technology, UIET, Kurukshetra University, Kurukshetra, November 23-24, 2012 ***(Best Poster Award)***

- ✓ **Bhumika Ray** and B. K. Kaushik, Structural and optical properties of chemically synthesized polymer capped cadmium sulphide (CdS) nanoparticles, National Conference on Nanoscience and Instrumentation Technology (NCNIT-2013), National Institute of Technology (NIT), Kurukshetra, March 28-29, 2013

- ✓ **भूमिका रॉय** और रंजना मेहरोत्रा, नोबल धातु नैनोकणों का संश्लेषण, विश्लेषण और एस. ई. आर. एस. में आवेदन, बायो-मेडिकल विज्ञान एवं प्रोधौगिक़ी पर राष्ट्रीय सम्मेलन, सी.एस.आई.आर.- राष्ट्रीय भौतिक प्रयोगशाला, नई दिल्ली, नवम्बर 21-22, 2013 ***(Best Poster Award)***

- ✓ **Bhumika Ray**, Shweta Agarwal and Ranjana Mehrotra, A surface enhanced Raman spectroscopic investigation on flavopiridol-calf thymus DNA complexation, International Conference on Translational Medicine (T-NANO 2014), Ahmedabad University, Ahmedabad, December 15-17, 2014 ***(Best Poster Award)***

- ✓ **Bhumika Ray**, Shweta Agarwal and Ranjana Mehrotra, Groove-directed intercalation of calf thymus DNA by flavone derived antitumor drug: A spectroscopic approach, International Congress on Friedreich's Ataxia and DNA Structure in Health & Disease, All India Institute of Medical Sciences (AIIMS), New Delhi, April 11-13, 2015

- ✓ **Bhumika Ray**, Ranjana Mehrotra, Flavopiridol binding with tRNA: Spectroscopic analysis, Global Cancer Summit-2015: International Collaborative Conference, Indian Institute of Science (IISc), Bengaluru, November 18-20, 2015 ***(Best Poster Award)***

- ✓ **Bhumika Ray** and Ranjana Mehrotra, Delineating tRNA binding aspects of antitumor flavoalkaloid: A spectroscopic study, *RSC Symposium* on Advanced Nanomaterials for Energy, CSIR-National Physical Laboratory, New Delhi, October 07, 2016 ***(Best Poster Award)***

- ✓ **Bhumika Ray** and Ranjana Mehrotra, Molecular characteristics of ribonucleic acid binding with an anthracycline derivative: A spectroscopic and melting study, International Conference on Vibrational Spectroscopy 2016 (ICOPVS 2016), Lucknow University, Lucknow, November 05-08, 2016

- ✓ Kaweri Gambhir, **Bhumika Ray**, Parag Sharma and Ranjana Mehrotra, Morphology dependent enhancement of linear and third order optical nonlinearities of plasmonic-organic hybrids, 13th International Conference on Fiber Optics and Photonics, IIT Kanpur, December 04-08, 2016

  Conference Proceeding Published (Abstract Issue) - 13th International Conference on Fiber Optics and Photonics, OSA Technical Digest (Online), Optical Society of America, W3A.26

- ✓ **Bhumika Ray** and Ranjana Mehrotra, Nucleic acid binding with an anticancer anthracycline derivative-Spectroscopic and melting analysis, 2nd India International Science Festival (IISF 2016), CSIR-National Physical Laboratory, New Delhi, December 07-11, 2016

- ✓ **Bhumika Ray** and Ranjana Mehrotra, Delineating interaction mechanism of an anthracycline derivative with deoxyribonucleic acid: A spectroscopic and melting study, 5th National Conference on "Advances in Metrology" (AdMet-2017), The Northcap University, Gurugram, March 23-25, 2017 ***(Best Poster Award)***

- ✓ **Bhumika Ray** and Ranjana Mehrotra, Preferential binding of flavonoids with bovine serum albumin: in-silico and spectroscopic insight into cytotoxic competence, 62nd Annual Meeting of Biophysical Society (BPS 2018), San Francisco, California, USA, February 17-21, 2018

  Conference Proceeding Published (Abstract Issue) - Volume 114, Issue 3, Supplement 1, p55a, Biophysical Journal

- ✓ **Bhumika Ray** and Ranjana Mehrotra, Binding of alkaloid, nobiletin to double stranded DNA-Spectroscopic insights into nature of interaction and anticancer potential, XLII Annual Meeting of the Optical Society of India, OSI-International Symposium on Optics (OSI-ISO 2018), Indian Institute of Technology Kanpur (IITK), Kanpur, September 19-22, 2018

- ✓ **Bhumika Ray**, Parag Sharma and Ranjana Mehrotra, Studying circadian and vision performance of artificial lighting systems, Photonics 2018-The International Conference on Fiber Optics and Photonics, Indian Institute of Technology Delhi (IITD), December 12-15, 2018

- ✓ **Bhumika Ray** and Ranjana Mehrotra, Nucleic acid binding mechanism of flavonoid derived, riviciclib: Comparative spectroscopic analysis to unveil cytotoxic activity, 10th International Conference on "Advances in Metrology" (AdMet-2019), CSIR-National Physical Laboratory, New Delhi, February 20-22, 2019 ***(Best Poster Award)***

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Binding of platinum derivative, oxaliplatin to deoxyribonucleic acid: structural insight into antitumor action

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Communicated by Ramaswamy H. Sarma

ABSTRACT

Platinum-derived chemodrugs constitute an active class in cancer therapeutics. Besides being potent against various solid tumors, oxaliplatin has been recognized as the first platinum compound to be approved for the treatment of colorectal cancer. Structurally, oxaliplatin consists of a platinum metal complexed to oxalate and diaminocyclohexane (DACH) and exert its anticancer action by inhibiting DNA replication and transcription. The present study highlights the binding properties of oxaliplatin with calf thymus DNA using spectroscopic methods to comprehend its binding mechanism at molecular level to overcome associated cellular resistance and side effects. Attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopic outcomes confirm that oxaliplatin is a covalent binding agent and also provide sequence specificity in DNA molecule. Infrared spectral results further indicate that oxaliplatin alkylates purine nitrogenous bases majorly guanine residues (G) in the major groove via formation of either interstrand or intrastrand guanine–guanine *d*(GpG) and guanine–adenine *d*(GpA) (N7 position) crosslinks accompanied with a slight external binding to sugar–phosphate backbone. Again, circular dichroism (CD) spectroscopic results suggest subtle conformational changes in DNA molecule due to its complexation with oxaliplatin and duplex attains an intermediate conformational state, having characteristics of both B- and C-forms. Further, a moderate binding strength of $4.12 \pm 0.2 \times 10^4$ M^{-1} for the interaction has been estimated via ultraviolet–visible spectroscopy. The inferences obtained from these investigations are encouraging and can form the basis for further exploration in the field of rational drug development based on platinum compounds possessing preferential binding for nucleic acid with improved competence.

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KEYWORDS
Oxaliplatin; DNA–drug interaction; FTIR spectroscopy; UV–visible spectroscopy; circular dichroism

1. Introduction

Platinum drugs hold an integral position in conventional anticancer chemotherapy (Kelland, 2007; Wang & Lippard, 2005). Primarily synthesized platinum drugs were cisplatin (first generation) (Dasari & Tchounwou, 2014) and carboplatin (second generation) (Kelland, 2007). These were highly nephrotoxic (Pabla & Dong, 2008) and limited by intrinsic and/or acquired resistance (Galluzzi et al., 2012; McWhinney, Goldberg, & McLeod, 2009; Stewart, 2007), which led to the development of other platinum derivatives that can bypass their inherent resistance (Graham, Muhsin, & Kirkpatrick, 2004; Johnstone, Suntharalingam, & Lippard, 2016). Of these newly synthesized analogs, oxaliplatin (third generation, Figure 1) has demonstrated favorable cytotoxicity and also lack cross-resistance with cisplatin and carboplatin (Alcindor & Beauger, 2011; Graham et al., 2004). Further, it is less mutagenic and exhibits a broad spectrum of *in vitro* and *in vivo* antiproliferative activity that differs from parent compounds, so it has been approved for first-line therapy of various solid malignancies (Yu, Xiao, Yang, & Cao, 2015) including colorectal cancer (unresponsive to other drugs) (Gaur et al., 2014; Grothey & Goldberg, 2004; Iveson et al., 2018; Satake et al., 2018) and advanced ovarian cancer (Nessa, Beale, Chan, Yu, & Huq, 2011). It can be safely administered in an outpatient setting with no specific requirement of hydration treatment. Preclinical studies unveil that the combination of oxaliplatin with other agents such as topoisomerase-I inhibitors, thymidylate synthase inhibitors, gemcitabine, taxanes, and few more (Deng et al., 2017; Gaur et al., 2014; Nessa et al., 2011) augment tumor suppression and has also shown synergism in many *in vivo* tumor models (Deng et al., 2017; Nessa et al., 2011; Wang et al., 2017).

Structurally, oxaliplatin comprises platinum metal complexed with oxalate as hydrolyzable ligand and diaminocyclohexane (DACH) complex as carrier ligand. It is slightly soluble in water and methanol, while almost insoluble in ethanol and acetone (Graham et al., 2004). Several cytotoxic mechanisms are known for the members of platinum-derived anticancer molecules (Wexselblatt, Yavin, & Gibson, 2012); however, their complete action activity is still under clinical investigation. It is suggested that these drugs inhibit cancer cell proliferation and growth by targeting different biomolecules and their assisted pathways within cancerous cell (Wang & Lippard, 2005; Wexselblatt et al., 2012); therefore, they have attracted cancer biologists and other related research groups. Among these biomolecules, the structure

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LETTER TO THE EDITOR

Biospectroscopic analysis of human breast cancer tissue: probing infrared signatures to comprehend biochemical alterations

Ranjana Mehrotra[a]*, Gunjan Tyagi[a], Sonika Charak[a], Bhumika Ray[a], Geeta Kadayaprath[b], Harit Chaturvedi[b], Urmi Mukherjee[c] and Andleeb Abrari[c]

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Communicated by Ramaswamy H. Sarma.

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1. Introduction

Breast cancer (BC) is one of the most studied and leading form of malignancy in human females. Currently, studies conducted in the field of breast cancer focuses on its early detection using noninvasive or minimally invasive techniques in lieu of traditional excisional biopsy, as cancer treatment is often simpler and effective, when diagnosed at an early stage. Mammography is the first step, usually performed in diagnosing breast cancer, but at times mammogram may not be able to provide a clear picture. In addition, biopsy is performed to confirm the presence or absence of tumor, which is associated with false-positive results. Consequently, the limitations of current screening methods have shifted the focal area of oncological research in applying biospectroscopy techniques for diagnostics (Gajjar et al., 2014).

Infrared (IR) and Raman spectroscopy are versatile vibrational spectroscopy methods that have been used to discriminate normal and cancer tissue and/or cell of different kinds, including endometrial cancer, cervical cancer, lung cancer, precancerous lesion, and brain tumors (Gajjar et al., 2013). Coupled with some algorithms (Gajjar et al., 2013), these spectroscopic outcomes can deliver an objective, high throughput and low-cost solution to breast cancer diagnosis. Infrared spectroscopy (IR) has expanded its application in the field of human biology, since it was revealed that biological molecules present in a living tissue possess vibrational features that can be studied to derive their molecular information. Thus, the biochemical modification in a normal tissue/cell can be analyzed and compared to its malignant state (Gajjar et al., 2014). Further, several reports have highlighted its advancement in both near- and mid-infrared regions, making it an efficient and convenient method for clinical purposes. From last few years, FTIR spectrophotometer has been exploited to study the molecular and structural characteristics of proteins, carbohydrates, lipids, and nucleic acids. Initially, Chirgadze and Nevskaya in 1976 studied the infrared spectral features of amide I and amide II (Chirgadze & Nevskaya, 1976). Further, in an investigation, Liquier and his colleagues (Liquier, Taboury, Taillandier, & Brahms, 1977) demonstrated that FTIR spectroscopy could be utilized to identify the different conformations of DNA (Liquier et al., 1977). In the year 2000, using infrared spectroscopic vibrations, Bouchard and his co-researchers, revealed the structure of insulin and described the formation of amyloid fibrils via insulin, which involves substantial unfolding of the native protein (Bouchard, Zurdo, Nettleton, Dobson, & Robinson, 2000). Since then, many more complex studies have been conducted on proteins and nucleic acids (DNA/RNA) structures, their conformations and interactions with small ligands. The biochemical changes in a cell/tissue generally lead to nuclear, cytoplasmic and morphological variations and hence, FTIR spectroscopy could detect these alterations during the developmental stages of cancer before morphological and cytological changes are evident under light microscope. Many studies have shown that spectroscopic techniques (with different sampling modes) can differentiate the biochemistry of normal and neoplastic cells. It has been employed to investigate the carcinoma of the breast, esophagus, colon, stomach, and prostate significantly (Pu, Wang, Tang, & Alfano, 2010; Wang et al., 2003).

In the present study, we report the infrared signatures of breast normal and cancer tissues that have been acquired using FTIR spectrophotometer to observe the

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Deciphering molecular aspects of interaction between anticancer drug mitoxantrone and tRNA

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Mitoxantrone (1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione) is a synthetically designed antineoplastic agent and structurally similar to classical anthracyclines. It is widely used as a potent chemotherapeutic component against various kinds of cancer and possesses lesser cardio-toxic effects with respect to naturally occurring anthracyclines. In the present study, we have investigated the binding features of mitoxantrone–tRNA complexation at physiological pH using attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy, circular dichroism (CD) spectroscopy, isothermal titration calorimetry, and UV–visible absorption spectroscopic techniques. FTIR analysis reveals that mitoxantrone interacts mainly with heterocyclic base residues of tRNA along with slight external binding with phosphate–sugar backbone. In particular, mitoxantrone binds at uracil (C=O) and adenine (C=N) sites of biomolecule (tRNA). CD spectroscopic results suggest that there is no major conformational transition in native A-form of tRNA upon mitoxantrone–tRNA adductation except an intensification in the secondary structure of tRNA is evident. The association constant calculated for mitoxantrone–tRNA association is found to be 1.27×10^5 M^{-1} indicating moderate to strong binding affinity of drug with tRNA. Thermodynamically, mitoxantrone–tRNA interaction is an enthalpy-driven exothermic reaction. Investigation into drug–tRNA interaction can play an essential role in the rational development of RNA targeting chemotherapeutic agents, which also delineate the structural–functional relationship between drug and its target at molecular level.

Keywords: Mitoxantrone; tRNA–drug interaction; FTIR spectroscopy; CD spectroscopy; ITC

Highlights

- Interaction of mitoxantrone with tRNA was investigated using spectroscopy approach.
- FTIR analysis suggests mitoxantrone binds with tRNA nitrogenous bases.
- CD spectroscopic results confirm no transition in native A-conformation of tRNA.
- Mitoxantrone–tRNA binding is an exothermic reaction favored by negative enthalpy.

1. Introduction

Investigation into interaction between nucleic acid and small molecules has been a dynamic area of research, which has drawn much attention of researchers in the past decades (Jaumot & Gargallo, 2012; Rehman, Sarwar, Husain, Ishqi, & Tabish, 2015). The rationale behind these studies is the thrust to delineate action mechanism of drug at the molecular level. Such extensive exploration has led to the discovery of large number of compounds ranging from natural products to purely structure-based designed drugs that interact with DNA in a variety of ways and modulate its various biological activities and cellular functioning (Basu & Suresh Kumar, 2016; Da Costa & Dieckmann, 2013; Ray, Agarwal, Lohani, Rajeswari, & Mehrotra, 2016). These small molecules act as key compounds in the recognition of protein–DNA complexes and as an impending raw material for the rational development of new chemotherapeutics with more efficacy and specificity (Agarwal, Chadha, & Mehrotra, 2014; Giri & Kumar, 2009; Jeon, Jin, Kim, & Lee, 2015). In contrast to DNA, much attention has been given to the studies on the recognition of RNA binding small molecules (Hermann & Westhof, 1998; Hermann & Westhof, 2000). Since, the information on the participation of RNA molecules in regulating a vast range of biological activities and cellular functioning has emerged the curiosity in investigating RNA as targeted molecule for various chemotherapeutic compounds (Wilson & Li, 2000). However, success in this area is limited by intricate structural assortment and inadequately available high-resolution structural information of RNA molecules in comparison to DNA.

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Structural, conformational and thermodynamic aspects of *groove-directed-intercalation* of flavopiridol into DNA

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[b]*Quantum Phenomena and Applications, CSIR-National Physical Laboratory, Dr. K. S. Krishnan Marg, New Delhi 110012, India;*
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Certain plant-derived alkaloids and flavonoids have shown propitious cytotoxic acitvity against different types of cancer, having deoxyribose nucleic acid (DNA) as their main cellular target. Flavopiridol, a semi-synthetic derivative of rohitukine (a natural compound isolated from *Dysoxylum binectariferum* plant), has attained much attention owing to its anticancer potential against various haematological malignancies and solid tumours. This work focuses on investigating interaction between flavopiridol and DNA at molecular level in order to decipher its underlying mechanism of action, which is not well understood. To define direct influence of flavopiridol on the structural, conformational and thermodynamic aspects of DNA, various spectroscopic and calorimetric techniques have been used. ATR-FTIR and SERS spectral outcomes indicate a novel insight into *groove-directed-intercalation* of flavopiridol into DNA via direct binding with nitrogenous bases guanine (C6=O6) and thymine (C2=O2) in DNA groove together with slight external binding to its sugar–phosphate backbone. Circular dichroism spectral analysis of flavopiridol–DNA complexes suggests perturbation in native B-conformation of DNA and its transition into C-form, which may be localized up to a few base pairs of DNA. UV–visible spectroscopic results illustrate dual binding mode of flavopiridol when interacts with DNA having association constant, $K_a = 1.18 \times 10^4\ M^{-1}$. This suggests moderate type of interaction between flavopiridol and DNA. Further, UV melting analysis also supports spectroscopic outcomes. Thermodynamically, flavopiridol–DNA complexation is an enthalpy-driven exothermic process. These conclusions drawn from this study could be helpful in unveiling mechanism of cytoxicity induced by flavopiridol that can be further applied in the development of flavonoid-based new chemotherapeutics with more specificity and better efficacy.

Keywords: flavopiridol; drug-DNA interaction; vibrational spectroscopy; CD spectroscopy; groove-directed-intercalation

1. Introduction

Flavopiridol,{2-(2-Chlorophenyl)-5,7 dihydroxy-8-[(3R,4S)-3-hydroxy-1 methyl piperidinyl]-4H-chromen-4-one} (Figure 1), is a semi-synthetic flavoalkaloid. It is derived from a natural chromone alkaloid rohitukine, which has been isolated from the stem bark of an Indian medicinal plant, *Dysoxylum binectariferum* (Naik et al., 1988; Sedlacek et al., 1996). From the last two decades, flavopiridol has achieved a lot of consideration due to its antiproliferative activity against a wide range of haematological malignancies and solid tumours *in vitro* and *in vivo*. Administration of flavopiridol alone and in combination with radiation, and other anticancer agents have been reported by Wang et al. (Jain, Bharate, & Vishwakarma, 2012; Sedlacek, 2001; Wang & Ren, 2010). Recent studies unveil that combination therapy of flavopiridol with other chemotherapeutic agents is highly dose- and schedule-dependent. Single administration of flavopiridol has been given a status of orphan drug in Europe for the treatment of chronic lymhocytic leukaemia (Jain et al., 2012).

Flavopiridol possess strong cytotoxic activity against different cyclin-dependent kinases (CDKs), which are typical cell cycle regulators. It represses cell cycle progression at G1/S and G2/M phases of cell cycle and stimulates cell death (Carlson, Dubay, Sausville, Brizuela, & Worland, 1996). Several reports on structural and kinetic investigations have shown that flavopiridol competitively inhibits binding of adenosine triphosphate on the nucleotide-binding site of CDKs (Kaur et al., 1992; Losiewicz, Carlson, Kaur, Sausville, & Worland, 1994; Sedlacek, 2001). It suppresses positive transcription elongation factor b (P-TEFb) and interferes into transcriptional process. The inactivation of P-TEFb results in decreased phosphorylation and transcriptional activity of enzyme RNA polymerase II (Blagosklonny, 2004). Further, it has been reported that flavopiridol induces mitochondrial injury, which finally leads to

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SERS as an advanced tool for investigating chloroethyl nitrosourea derivatives complexation with DNA

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ABSTRACT

We report surface-enhanced Raman spectroscopic (SERS) studies on free calf thymus DNA and its complexes with anti-tumor chloroethyl nitrosourea derivatives; semustine and nimustine. Since, first incident of SERS in 1974, it has rapidly established into an analytical tool, which can be used for the trace detection and characterization of analytes. Here, we depict yet another application of SERS in the field of drug–DNA interaction and thereby, its promising role in rational designing of new chemotherapeutic agents. Vibrational spectral analysis has been performed in an attempt to delineate the anti-cancer action mechanism of above mentioned nitrosourea derivatives. Strong SERS bands associated with the complexation of DNA with semustine and nimustine have been observed, which reveal binding of nitrosourea derivatives with heterocyclic nitrogenous base pair of DNA duplex. Formation of dG–dC interstrand cross-link in DNA double helices is also suggested by the SERS spectral outcomes of CENUs–DNA adduct. Results, demonstrated here, reflect recent progress in the newly developing field of drug–DNA interaction analysis via SERS.

1. Introduction

Surface-enhanced Raman spectroscopy (SERS) is an advanced area with a wide range of analytical applications [1–5]. Few techniques are known to be present for the selective and direct investigation of molecular interactions within intricate system (biological system) [6,7]. When compared, Raman spectroscopy has an excellent capability of providing molecular fingerprints of analytes [8,9]. However, the potential of Raman spectroscopy, to recognize and provide structural information of molecules through narrow spectral features, has been limited by the requirement of highly concentrated sample and poor Raman signal [10,11]. These limitations were overcome by the discovery of surface-enhanced Raman scattering effect. With the SERS based technique, i.e. surface-enhanced Raman spectroscopy, valuable knowledge about the primary/secondary level structure present in the molecule and chemical surrounding of its specific moieties can be achieved [12,13]. In addition, SERS spectroscopy is highly selective and responsive analytical technique, which can be further employed in the area of bio-imaging and probing the living tissues and cells [5]. Principally, SERS depends on the collective oscillations of conduction band electrons (surface plasmons), which are excited by illumination over the metallic nanostructure surface at resonant frequency. Upon the adsorption of an analyte onto gaps and junctions of clustered metallic nanostructures, there is an augmentation in Raman signal by many orders due to the electromagnetic effect and/or charge transfer between the adsorbed molecule and metallic conduction band [14]. This renders SERS as an outstanding tool for ultrasensitive detection of nature and structural deviations associated with the complexation of biomolecule with ligand at molecular level [15,16]. Keeping this in view, here, we report the surface-enhanced Raman spectroscopic studies on free calf thymus DNA and its complexes with anti-tumor chloroethyl nitrosourea derivatives; semustine (SMT) and nimustine (NMT).

Chloroethyl nitrosourea derivatives represent a major class of alkylating agents, which have been regarded as potent anti-tumor drugs. They manifest cytotoxic activity against a variety of cancers such as metastatic brain tumor [17], Lewis lung carcinoma [18] and leukemia [19]. One of the Chloroethyl nitrosourea derivatives i.e. semustine (Fig. 1a) is a 4-methyl derivative of lomustine (a nitrosourea derivative), mainly used in the chemotherapy of primary and metastatic brain tumors [17] and L1210 leukemia [19]. Like other members of the nitrosourea family, semustine is also

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