CHAPTER 31

MEDICAL SCIENCES
BIOMEDICAL RESEARCH

Doctoral Theses

01. ABHISHEK KUMAR
Development and Characterization of Ca2+ and Zn2+ Enriched Alginate Composites for Post-traumatic Hemorrhage Control and Wound Management.
Supervisor: Prof. Mohd Saquib Ansari
Th 27267

Abstract

Post-Traumatic Hemorrhage, Which Can Result From Accidents Or Battlefield Injuries, Is A Significant Global Concern Due To The High Pre-Hospital Mortality Rate. Substantial Efforts Have Been Made To Develop Hemostatic Agents That Can Effectively Reduce Hemorrhage In The Immediate Aftermath Of A Traumatic Event. The Utilization Of Naturally Abundant And Highly Biocompatible Polymers Such As Alginate Can Significantly Influence The Safety, Effectiveness, And Cost-Efficiency Of Hemostatic Agents. The Present Study Aimed To Investigate The Potential Efficacy Of The Developed Ca2+ And Zn2+ Supplemented Sodium Alginate-Based Hemostatic Hydrogel (Sa-Cz) For The Management Of Low-Pressure Bleeding Wounds With The Added Advantage of Accelerated Wound Healing And Subsequently Developed Ca2+ And Zn2+ Supplemented Sodium Alginate-Based Dry Hemostatic Particles (Sa-Cz Dhp) To Manage Excessive Blood Loss Following Post-Traumatic Hemorrhage. Sa-Cz Hydrogel Showed Substantial In-Vitro Efficacy, As Observed By The Significant Reduction In Coagulation Time With Better Blood Coagulation Index (Bci) And No Evident Hemolysis In Human Blood. Sa-Cz Significantly Reduced Bleeding Time (≈60%) And Mean Blood Loss (≈65%) In The Tail Bleeding and Liver Incision in the Mice Hemorrhage Model ($P \leq 0.001$). Sa-Cz Also Showed Enhanced Cellular Migration (1.58-Fold) In-Vitro and Improved Wound Closure (≈70%) As Compared With Betadine (≈38%) At The 7th-Day Post-Wound Creation In-Vivo ($P < 0.005$). The Safe Sa-Cz Dhp Showed High Absorption And Accelerated Blood Clotting Kinetics With Reduced Coagulation Time (≈70%, $P < 0.0001$) In Whole Human Blood, Observed With Insignificant Hemolysis And Uninterrupted Rbc Morphology. Sa-Cz Dhp Significantly Reduced the Mean Blood Loss (≈90% In Sd Rats Tail Incision), and Bleeding Time (≈60% In Balb/C Mice Tail Incision) Was At Par With Commercially Available Celox™ Hemostatic Granules. In Conclusion, the Biocompatible Sa-Cz and Sa-Cz Dhp Exhibited Rapid and Effective Management Of Excessive Blood Loss. It Is Also Pertinent To Note That The Developed Formulations Could Be A Cost-Effective Alternative To Their Commercial Counterparts.

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02. AGRAWAL (Nishtha)
Modulating Host Signaling Cascade: Potential Antiviral Tool against Chikungunya Virus.
Supervisors: Prof. Sunit K. Singh and Prof. Gagan Dhawan
Th 26787

Abstract

Chikungunya virus is an arthropod-borne arthritogenic alphavirus that re-emerged in 2005 around the islands of Indian Ocean and poses a potential danger as there are no specific antiviral or vaccines available. Viruses invade the host cells and maneuver the cellular translation machinery to translate the viral proteins in substantial amounts, which disturbs ER homeostasis leading to induction of UPR, a host response pathway involved in viral pathogenesis. Here, we investigated the effect of chikungunya virus infection on UPR pathways in HEK293 cells. We confirmed the induction of IRE1 and ATF6 branch of UPR during chikungunya virus infection. The PERK branch of UPR was suppressed. We further examined the effect of inhibition of individual UPR pathways on chikungunya virus replication. We observed that the virus replication was significantly downregulated in presence of 3-ethoxy-5,6-dibromosalicylaldehyde (IRE1 inhibitor) and AEBSF (ATF6 inhibitor). Thus, this study provides a novel outlook in designing chikungunya virus antivirals. We observed the effect on autophagy when the HEK cells were infected with chikungunya virus and incubated in presence of UPR inhibitors. The two UPR inhibitors (AEBSF, 3-ethoxy-5,6-dibromosalicylaldehyde) resulted in significant downregulation of viral replication, highlighting the possible pro-viral role of autophagy induced by UPR. We also analysed the therapeutic role of MicroRNAs (MiR-141, MiR-155) during chikungunya virus replication

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03. BANSAL (Aniket Kumar)
Investigating the Effect of Osmolytes on Hela Cell Proliferation and Efficacy of Cisplatin.
Supervisor: Prof. Laishram Rajendrakumar Singh
Th 27122

Abstract

Osmolytes are heterogenous group of small organic molecules that accumulate in the body to circumvent extreme osmotic insults including temperature, pH, salinity, etc. Classically, osmolytes are broadly classified into three major groups i.e. polyols (mannitol, glycerol, sorbitol, inositol, pinitol, sugar and sugar derivatives), free amino acids (glycine, alanine, proline) and their derivatives (taurine, octopine, β-alanine), and methylamines including trimethylamine-N-oxide (TMAO), glycerophosphocholine (GPC), glycine betaine (betaine) and sarcosine. Osmolytes are known to be permeable through biological membranes and accumulate in cells up to millimolar concentrations, increase thermodynamic stability of proteins, modulate enzyme activity and prevent protein aggregation. Osmolytes are also known to protect cells by increasing stability and inhibiting aggregation of proteins. A recent study by Younus et al. on intrinsically disordered proteins (IDPs), class of proteins that lack ordered three-

dimensional structure, reveals that osmolytes modulate structural and functional integrity of different IDPs and therefore might have clinical implications for a large number of human diseases (e.g., amyloidosis, diabetes, and neurodegeneration, etc.). Since, most transcription factors contain intrinsically disordered domains and are involved in oncogenesis and metastasis, osmolytes could modulate structural and functional behavior of many transcription factors thereby impacting cancer disease pathology. These findings pave foundation for the possible involvement of osmolytes in cancer cell signaling. The thesis therefore, aimed at investigating the effect of osmolytes on the proliferation of cancer cell with particular emphasis on cervical cancer.

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04. BHATTACHARYA (Reshmee)
Structural and Functional Consequences of AGE-Induced Covalent Modification of Protein and Preventive Strategies: A Study Based on Diabetes Mellitus.
Supervisor: Prof. Laishram Rajendrakumar Singh
Th 27123

Abstract

Diabetes is a metabolic disease characterized by elevated levels of glucose in the blood. Chronic diabetes leads to impaired vision (retinopathy), renal failure (nephropathy), foot ulcers, charcot joints, surgical removal of limbs (peripheral neuropathy), autonomic neuropathy, gastrointestinal dysfunction and even cardiovascular symptoms. Diabetes is broadly classified into two categories. Type 1 diabetes, characterized by deficiency of insulin secretion by β-cells of pancreas. Individuals developing type 1 diabetes is exhibit autoimmune pathological conditions and genetic abnormalities in the pancreatic β-cells. Type 2 diabetes primarily develops due to inadequate insulin action or in combination with deficient compensatory mechanism of insulin secretory response. If the high blood glucose levels persist for a prolonged period of time, it predisposes to a condition called hyperglycemia. However, not all patients with hyperglycemia are diabetics. Eating more carbohydrates, contracting an infection, carrying a chronic complication like kidney/heart disease or trauma of any kind causes a bodily response characterized by enhance metabolism and hyperglycemia. Hyperglycemia is characterized by the accumulation of highly reactive reducing sugars such as glucose, fructose, ribose-5-phosphate etc. These reducing sugars have the ability to covalently modify important proteins and enzymes, specifically at lysine residues (a process termed as glycation), making them non-functional. Such modification (known as Maillard reaction) is initiated by a nucleophilic addition between a free amino group of protein and electrophilic carbonyl groups of glucose forming an unstable Schiff's base (Aldimine). Subsequently, the Schiff's base undergoes Amadori rearrangement to form a stable ketoamine called Amadori product. These Amadori products further undergo enolization reactions to form dicarbonyl compounds (glyoxal (GO), methylglyoxal (MGO), 1-deoxyglucosone, etc). Eventually, the advanced glycation end-products (AGEs) are formed by following two different pathways: the irreversible rearrangement of the Amadori products through both oxidative and non-oxidative pathways and through subsequent condensation reaction between the dicarbonyls and side chain of lysine, cysteine and arginine residues as described in scheme 1. In spite of the fact that sugars are the main precursors of AGE formation, these intermediate dicarbonyls are 2 much more

reactive than other reducing sugars (like ribosyl or D-glucose), and act as propagators of the non-enzymatic glycation and crosslinking of several proteins. Since aldehyde carbonyls are comparatively more electrophilic than ketoses, aldose sugars generally react more rapidly than ketose sugars. Glucose is the least reactive one among aldose sugars, but quantitatively it is the most abundant carbohydrate in humans making adducts with most of the proteins. In addition to reacting with proteins, AGEs per se are also known to interact with their receptors called RAGE. This AGE-RAGE interaction is known to activate robust inflammatory response. In fact, binding of AGE to RAGE results in dimerization of RAGE followed by recruitment of an adaptor molecule, mDia1 to the cytosolic domain. RAGE-mDia1 interaction then activates several key molecules (Ras/Raf, JAK/STAT, Cdc42/Rac, PI3K/AKT, etc.) ultimately activating inflammatory response. Additionally, AGE-RAGE axis is also involved in other biological processes including tissue remodeling, BBB injury, apoptosis, oxidative stress, etc. thereby leading to a spectrum of disease pathologies.

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05. JHA (Praksh)
Target Based Computational Design and Modeling of Selective Molecules As Potential Therapeutic Against Cancer, SARS-CoV-2 and Tuberculosis.
Supervisor: Prof. Madhu Chopra
Th 26790

Abstract

Lately, several microorganisms are showing low sensitivity/resistance to commonly used antibiotics. This has resulted in poor treatment and management of infectious diseases. Conventional methods of drug discovery are time-consuming and expensive. Structure- based drug discovery approach is a promising computational technique to identify and explore the potential of small molecules as inhibitors for the identified target protein from the pathogen of interest. Computational validation confirms the authenticity of identified novel molecules. This study focuses on the application of structure-based drug discovery to design inhibitors against microbes of global concern like Neisseria gonorrhoeae, SARS-CoV-2, and Trypanosoma cruzi. Addressing the global health challenge by using a structure-based drug discovery approach signifies its vital role in combating various diseases. The first objective of our study has two components, one focuses on structure-based drug discovery for Neisseria gonorrhoeae whereas the second sub-objective focuses on the identification of its potential vaccine candidates. The second objective is based on using a structure-based drug discovery pipeline to identify novel inhibitors for RBD-ACE2 interaction in the case of SARS-CoV2. The third objective is focused on molecular docking studies and interaction analysis of three novel compounds targeting Trypanothione reductase in Trypanosoma cruzi. The results of the first objective identified two novel molecules that were validated using MD simulation and MM-PBSA analysis. The second objective came up with a natural compound Curcumin as an inhibitor of RBD-ACE2 interaction in the case of SARS-CoV2 by hindering the interaction at the junction of the two. The third objective computationally deciphered the interaction site of three small molecules, identified in vitro as the inhibitors for Trypanothione reductase in Trypanosoma cruzi. Briefly, the study demonstrates the significance and potential of a structure-based drug discovery approach in

addressing global health challenges like AMR and the urgent need for the development of a gonococcal vaccine for Neisseria gonorrhoeae, finding more effective treatments for SARS CoV2 and developing some novel compounds for the treatment of Chagas disease caused by Trypanosoma cruzi.

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1. Introduction and review of literature 2. Aims and objectives 3. Targeting peptidyI arginine deiminase type-2 (PAD2) using structure based pharmacophore modeling, molecular docking,and molecular dynamics study for the development of anti-cancer therapeutics 4. Classification of $HDAC_6$ inhibitors by machine learning methods 5. Structure-guided pharmacophore based virtual screening, Docking and molecular dynamics to discover novel inhibitors against endoribonuclease (Nsp15) and RNA-dpeneder RNA polymerase (RDRP) of SARS-CoV-2 6. Ligand based pharmacophore modeling against patonthenate kinase (PanK) o tuberculosis and multi-targeting approach involving panK and PyrG. Summary and conclusion. Annexures.

06. KAUR (Sumeet)
To Understand Molecular Mechanism of HDAC6 Inhibition With Subtype Selective Inhibitor,'Ricolinostat' Alone and in Combination With Other Anticancer Agents In Cervical Cancer Cells.
Supervisor: Prof. Madhu Chopra
Th 26791

Abstract

Ricolinostat, HDAC6 specific inhibitor exhibits promising anticancer effects alone as well as in combination with various other chemotherapeutic drug in several cancer types. In this study, we evaluated the effect of ricolinostat in cervical cancer as a single agent as in combination with topotecan/etoposide as a single agent suppressed proligeration, and induced G2/M phase cell cycle arrest and apoptosis in cervical cancer cells. In addition to that, ricolinostat treatment alone resulted in increased ROS production,p21 expression and decreased Bel-xL expression. We also found that ricolinostat significantly enhanced the antiproliferative activity of both, topotecan and etoposide in cervical cancer cells. Ricolinostat/topoisomerase inhibitor combination induced G2/M phase cell cycle arrest in GI phase arrest in SiHa cells. Ricolinostat when used in combination with etoposide result in G2/M phase arrest in SiHa cells. Apoptosis induction was observed in both cervical cancer cells, after combined simultaneous treatment with ricolinostat and topoisomerase inhibitors. ROS production was also observed in HeLa and SiHa cells that were treated with ricolinostat and topoisomerase inhibitor combination, indicating that ROS production was a contributing factor in cell death mediated by ricolinostat and topoisomerase inhibitor combination.

Contents

1. Introduction and review of literature 2. Aim and objective 3. Materials and methods 4. To study the anticancer effect of ricolinostat on cervical cancer cells 5. Evaluation of the effect of combination therapy of ricolinostat and topoisomerase in cervical cancer cells 6. Evaluation of the effect of combination therapy of ricolinostat and MLN4924 in He La cancer cells. Summary. List of publications.

07. MANN (Kiran)
Identification of High Throughput Radiation Biomarkers Using Integrated Metabolomics Approach.
Supervisors: Prof. Radhika Bakhshi and Dr. Poonam Rana
Th 26786

Abstract

The present title entitled "Identification of High Throughput Radiation Biomarkers Using Integrated Metabolomics Approach**"** deals with the investigation of metabolic changes following different radiation doses with the investigation of metabolic changes following different radiation doses using ^{1}H NMR and LC-MS along with integrated analysis. In the case of a radiological scenario, mass screening of massive populations will be an essential part of the public health and medical response to a radiological incident to distinguish the exposed from the non-exposed. In the development of radiation countermeasures, biomarkers are the immediate tools that are required to determine the absorbed radiation dose during mass casualty triage and the proper medical treatment of years since metabolites are crucial participants in biological systems. To date, many substantial evidence supports radiation-induced metabolic changes in urine and plasma. The work under this thesis has focused on identifying a panel of metabolic biomarkers 24 hrs. post radiation exposure in a preclinical model that can provide information about the status of radiation injury.

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08. POONIA (Priya)
Virtual Screening & Molecular Modeling for Development of Subtype HDAC Inhibitors and Evaluation of Their Antiproliferative Activity in Cervical Cancer Cell.
Supervisor: Prof. Madhu Gupta
Th 26792

Abstract

In the present study, we blended multiple computer aided drug screening approaches systematically. We used "Molecular Modeling" and "Drug repurposing" approaches to obtain new potent HDAC6 inhibitors. To achieve this aim HDAC6 pharmacophore based virtual screening of two different databases: the ZINC database and the Drugbank was performed. To focus on the high selectivity of the inhibitors candidates, we applied stringent screening methods and resolute validation at each step: Pan HDAC inhibitors and HDAC6 inhibitors were kept as controls the study that helped not only helped in validation the outcomes of the study but also helped in setting the reference limits or cutoff parameters for screening of the hits in the studies.

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09. RATHI (Bhawna)
Investigation and Analysis of Novel Antibiofilm Compounds Inhibiting Curli Biogenesis in *E.coli*.
Supervisor: Prof. Sunit K.Singh
Th27252

Abstract

Bacterial biofilms are complex multicellular communities embedded in a dense extracellular matrix, which play a significant role in chronic human infectious diseases like cystic fibrosis, UTIs, and implant-associated infections. Curli fimbriae are crucial for bacterial adhesion and extracellular matrix development in biofilms. Targeting these curli could be viewed as an antibiofilm strategy. Keeping this in mind we have identified two novel compounds A01 and A02 targeting curli biogenesis in E.coli biofilms. The identified compounds were obtained by virtual screening from a natural products database having a high binding affinity to the curli regulator protein (csg D), which is the master regulator of curli biogenesis and MD simulations were conducted to determine the structural stability of these compounds with csg D protein. A druggable profile was assessed in silico for these compounds to evaluate their potential for drug-like properties. The compounds were then further tested for their antibiofilm activity and drug likeliness in vitro using various molecular biology techniques and these two promising candidates were also tested for their antibiofilm potential in UPEC biofilms. Our study demonstrated that two potential compounds are likely to be effective against curli-dependent E. coli biofilm infections. In addition, we have also explored the antibiofilm properties of caffeine against Uropathogenic E. coli which is mediated by curli biogenesis. Furthermore, we have evaluated the combination of caffeine and certain antibiotics for their efficacy against strong biofilm-forming UPEC isolates from UTI patients. Overall, our findings suggest that targeting curli biogenesis could be used to combat E. coli biofilm formation owing to rapidly emerging resistance to traditional antibiotics in clinical settings. This study has not only gained insights into a better understanding of biofilm formation butalso proposed novel antimicrobials that can effectively target E. coli biofilm formation.

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10. RAVIKANT

Structure based drug discovery to design inhibitors against microbes of global concern and reverse vaccinology based immunoinformatics approach to identity novel vaccine candidate in Neisseria Gonorrhoeae

Supervisors: Prof. Daman Saluja and Prof. Myron

Th 26788

Abstract

Lately, several microorganisms are showing low sensitivity/resistance to commonly used antibiotics. This has resulted in poor treatment and management of infectious diseases. Conventional methods of drug discovery are time-consuming and expensive. Structure- based drug discovery approach is a promising computational technique to identify and explore the potential of small molecules as inhibitors for the identified target protein from the pathogen of interest. Computational validation confirms the authenticity of identified novel molecules. This study focuses on the application of structure-based drug discovery to design inhibitors against microbes of global concern like Neisseria gonorrhoeae, SARS-CoV-2, and Trypanosoma cruzi. Addressing the global health challenge by using a structure-based drug discovery approach signifies its vital role in combating various diseases. The first objective of our study has two components, one focuses on structure-based drug discovery for Neisseria gonorrhoeae whereas the second sub-objective focuses on the identification of its potential vaccine candidates. The second objective is based on using a structure-based drug discovery pipeline to identify novel inhibitors for RBD-ACE2 interaction in the case of SARS-CoV2. The third objective is focused on molecular docking studies and interaction analysis of three novel compounds targeting Trypanothione reductase in Trypanosoma cruzi. The results of the first objective identified two novel molecules that were validated using MD simulation and MM-PBSA analysis. The second objective came up with a natural compound Curcumin as an inhibitor of RBD-ACE2 interaction in the case of SARS-CoV2 by hindering the interaction at the junction of the two. The third objective computationally deciphered the interaction site of three small molecules, identified in vitro as the inhibitors for Trypanothione reductase in Trypanosoma cruzi. Briefly, the study demonstrates the significance and potential of a structure-based drug discovery approach in addressing global health challenges like AMR and the urgent need for the development of a gonococcal vaccine for Neisseria gonorrhoeae, finding more effective treatments for SARS CoV2 and developing some novel compounds for the treatment of Chagas disease caused by Trypanosoma cruzi.

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11. SHAKUNTALA

Investigations on the Immunological Roles of Host Derived Heat Shock Proteins During Mycobacterial Infection.

Supervisor: Prof. Krishnamurthy Natarajan

Th27251

Abstract

Mycobacterium tuberculosis attenuates many defence responses from alveolar macrophages to create a niche at sites of infection in the human lung. Levels of Heat Shock Proteins have been reported to increase many folds in the serum of active TB patients than in latently infected individuals. Heat shock proteins constitutes a large group of proteins which maintain the protein homeostasis in the living cells. Heat shock proteins constitutes around 10% of total cellular protein content. Protein homeostasis is important to maintain integrity and survival of the cell. Apart from their chaperonin functions HSPs are also reported in various disease conditions like cancers, cardiovascular diseases, neurodegenerative disease, autoimmune diseases. Here we investigated the regulation of key defence responses by HSPs during mycobacterial infection. We show that infection of macrophages with M. bovis BCG induces higher expression of HSP-27 and HSP-70. Inhibiting HSP-27 and HSP-70 prior to mycobacterial infection leads to a significant decrease in mycobacterial growth inside macrophages. Further, inhibiting HSPs resulted in a significant increase in intracellular oxidative burst levels. This was accompanied by an increase in the levels of T-cell activation molecules CD40 and IL-12 receptor and a concomitant decrease in the levels of T-cell inhibitory molecules PD-L1 and IL-10 receptor. Furthermore, inhibiting HSPs significantly increased the expression of key proteins in the autophagy pathway along with increased activation of pro-inflammatory promoting transcription factors NF-kB and p-CREB. Interestingly, we also show that both HSP-27 and HSP-70 are associated with anti-apoptotic proteins Bcl-2 and Beclin-1. These results point towards a suppressive role for host HSP-27 and HSP-70 during mycobacterial infection.

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12. SOPHORONEA (Tuithung)

Study the role of $[Ca^{2+1}]I$,$[Ca^{2+}]m$ and TOR/AMPK pathway in neuroprotection mediated through adenosine A_{2A} receptor in the neurodegenerative model of Parkinson's disease.

Supervisor: Prof. Pratibha Mehta Luthra

Th 26789

Abstract

Parkinson's disease (PD) is a chromic neurodegenerative disease with no cure. The impairment of Ca^{2+} homeostasis has a role in the alteration of various physiological functions like oxidative stress, protein aggregation autophagy and apoptosis leading neurodegeneration. A_{2A} R antagonists have been suggested to have a role in Ca^{2+} signaling as well as reported to have a neuroprotective effect in the experimental models of PD. However the underlying mechanisms remain elusive. Therefore, we attempt to explore the mechanism of A_{2A}R in calcium signaling using HEK 293-A_{2A} R cells *in vitro* and *in vivo* 6-OHDA, model of PD. This study includes three objectives,

firstly the study was taken to elucidate the mechanism of $[Ca^{2+}]_i$ release via A_{2A} R using stably transfected HEK 293 expressing A_{2A} R cells. Second objective we demonstrated that 6-OHDA-induced toxicity in PMDN cells caused mitochondrial Ca^{2+} overload via inhibition of NCLX and activation of MCU leading to mitochondrial dysfunction. The third objective includes the study of both in vitro(6-OHDA –induced toxicity in PMDN cells) and in vivo (unilateral 6-OHDA lesioned rat model) where the result showed that 6-OHDA caused intracellular Ca^{2+} overload leading to dysregulated autophagic induction and apoptosis.

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