CHAPTER 4

BIOMEDICINE

Doctoral Theses

024. BAWEJA (Renu B)
Studies on Sporulation in Bacillus Anthracis.
Supervisors : Dr. W. N. Gade and Dr. Yogendra Singh
Th 14655

Abstract

Concludes that conditions during sporulation play significant role in Bacillus anthracis sporulation. Various factors namely temperature, pH and nutrient deprivation were found to bring about changes in sporulation efficiencies of B. anthracis cultures. B. anthracis spores formed under various stress conditions have varied suceptibility towards physical and chemical denaturants used for spore inactivation. Spores formed at 45 degree Celcius were observed to be more resistant while those formed in nutrient deficient medium (PBS) were more sensitive as compared to spores formed at 37 degree celcius in mG sporulation medium (control). Spores of B. anthracis formed under various stress conditions vary in their age of germination and dipicolinic acid content (DPA). Amount of DPA was observed to be two fold higher in spores formed at 45 degree celcius as compared to spores formed at 37 degree celcius. Atomic force microscopy revealed significant variation in size of spores of B. anthracis formed at different temperatures during sporulation. Spores formed at 45 degree celcius were smaller whereas spores formed at 25 degree celcius were elongated as compared to spores formed at 37 degree celcius. Analysis of proteins of spores formed in PBS and in mG sporulation medium revealed the downregulation of metabolic proteins and upregulation of stress proteins specifically those involved in oxidative stress.

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025. BHUPENDER SINGH

Synthesis of Bifunctional Chelating Agents to Label Monoclonal Antibodies for Radioimmunodiagnosis of Cancer.

Supervisors : Dr. Vibha Tandon and Dr. Anil K. Mishra

Th 14659

Abstract

Develops new synthetic strategies for construction of aromatic ring based new bifunctional chelating agents to study and evaluate the complexation with different radiometals particularly with technetium-99m. Stability and pharmacokinetics of new bifunctional chelating agents complex with different metals especialy technetium was followed by bioconjugation of new BFCs with biotin and proteins (mAb) for the bioevaluation of new conjugate for targeted scintigraphy. As a evaluation part of this study, bioconjugate radiolabeled with radioactive metal (targeted bullet) were screened by in vivo biodistribution studies and scintigraphic application for tumor imaging in animal models. Further, as a part of development of newer radiopharmaceuticals, in second project new organic molecules were synthesized, radiolabeled with ^{99m}Tc and further evaluated for infection imaging (in vivo diagnosis of bacterial infections).

Contents

026. GOYAL (Ankur)

Experimental Strategies for Studying Hepatitis B and C Virus Infection and its Treatment.

Supervisors : Dr. Naveen Arora and Dr. Shiv K. Sarin

Th 14653

Abstract

Genotype 3 was found to be the most prevalent genotype in patients with chronic hepatitis C in North and Central India. Detailed mutational analyses of a large number of aa sequences

showed, that a higher number of non-conservative aa variations within the PKR binding domain and an extended V3 region of NS5A is correlated with virologic response to Pegylated interferon alpha+Ribavirin based treatment in HCV 3a infected patients. Upon phylogenetic analysis of 61 NS5A sequences, no near clustering of HCV 3a isolates derived from patients who did or did not respond to interferon based treatment was detectable. Interestingly, HCV 3a isolates from the same geographical origin clustered together. The study demonstrates exemplarily that a large sample size is crucial for genotype to phenotype analyses of interferon alpha-based treatment resistance in chronic hepatitis C. VDR Taq 1 tt was more common in HCV patients as compared to healthy controls. In multivariate analysis, VDR Apa 1 aa correlated with histological HCV disease severity (p=0.01). CCR4 Delta32 mutation does not have a role in disease susceptibility, severity or response to therapy in patients with chronic hepatitis C infection. TNF-Beta A/A allele correlated significantly with hepatic inflammation (p=0.01). Number of CpG repeats did not have any influence on anti-HBs titer. However shorter mer gave a fold better immune response. Chemical conjugation of HBsAg with CpG ODN significantly enhances its immunogenicity towards Th1 response with higher levels of IFN-gama.

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027. MOHD. SAIF ZAMAN

Understanding the Mechanism of Sporulation in Bacillus Anthracis.

Supervisor : Dr. Yogendra Singh

Th 14660

Abstract

Describes the Bacillus anthracis spore and the changes it undergoes in the process of its transformation into a vegetative cell and investigates the role of the metabolic enzyme nucleoside diphosphate kinase in the sporulation process of Bacillus

anthracis. Concludes that Germination in Bacillus anthracis occurs in the same way as reported in other Bacillus species. Ndk mutant spores failed to outgrow either in liquid media, solid media or in presence of macrophages, suggesting that Ndk is apparently controlling the morphogenesis and physiological properties of the spores. Ndk is expressed during the initial stages of germination, which is in keeping with it's metabolic role. The enzyme was found to be active over a range of pH and high ionic strength, which correlates well with it's in vivo involvement in germination.

Contents

1. Introduction. 2. Review of Literature. 3. Materials and Methods. 4. Summary, Conclusions and Bibliography.

028. MYTHILY (G)
Mapping of Nucleosomes on Eukaryotic Genome Sequences.
Supervisor : Prof. Samir K. Brahmachari
Th 14657

Abstract

The study, carried out a comparative in silico analysis of chromatin characteristics in terms of the scaffold/matrix attachment regions, nucleosome formation potential and the occurrence of repetitive sequences, in the upstream regulatory regions of different sets of eukaryotic genes with contrasting spatial patternsof expression and found that chromatin landscape in 5' regulatory regions of genes, with contrasting spatial patterns of expression differ significantly. Observations suggest that nucleosome exclusion is a common phenomenon in the 5' regulatory regions of human genes. Further analysis of nucleosome exclusion sequences in 5' regulatory regions showed that these are enriched in gene dense chromosomes and many of them belonged to the category of TATA-less promoters, indicating a role for nucleosome exclusion in TATA-less promoter activation. The study of genomic nucleosome positioning signals enables to understand the sequence preferences exhibited by the histone octamer in eukaryotes. Describes the isolation, cloning and analyses of human dinucleosome DNA library from placental nuclei. Analysis shows that >75% of the dinucleosome DNA arise from repetitive elements. Among these elements, Alu were the predominant class followed by Satellite sequences. Alu Y subfamily was found to be significantly over-represented and Alu J subfamily was

significantly under-represented in the dinucleosome clones. Also found that binding sites for transcription factors were significantly under-represented in the dinucleosome clones. The analysis has also revealed several short sequence motifs with a role in nucleosome positioning.

Contents

1. Review of literature. 2. In Silico analysis of the chromatin architecture of genes with spatially distinct patterns of expression. 3. Sequences influencing nucleosome positioning in the 5' regulatory regions of human genes. 4. Results, discussion and Bibliography.

029. PANWAR (Puja)
Syntheses and Evaluation of Specific Radiopharmaceuticals for Target Specific Scintigraphy and Cancer Therapy.
Supervisors : Prof. Ramesh Chandra and Dr. Anil Kumar Mishra
Th 14658

Abstract

Attempts to highlight the syntheses of metallopharmaceuticals and evaluated biologically for the diagnostic and therapeutic application. Also synthesized another class of acyclic bifunctional chelating agents based on Schiff based ligands. Chiral salen ligands have several attractive features that constitute the basis for their utility in diagnostic imaging. The two potentially tetradentate (N_2O_2) and pentadentate (N_3O_3) bifunctional Schiff-base ligands : N, N'-bis(2-hydroxybenzyl)-1-(p-aminobenzyl) ethylenediamine, Bhabed (2g') and N, N'-bis(2-hydroxybenzyl)-2-(p-aminobenzyl)-3-monooxo-1,4,7-triazaheptane, Bhabmt (2k') were successfully labeled with ^{99m}Tc. These C-substituted ligand were found to be stable under physiological conditions. Presents an exhaustive account of work starting from the synthesis of a new conjugate in good yield and its biological evaluation as a drug candidate in cell cultures and animal models. The serum stability of ^{99m}Tc labeled proteins through several bifunctional chelates has been investigated. In DOTA-Ph-Al the metabolizable linker greatly reduces the in vivo loss of metal ion and also increases the clearance of the radioactivity from background. In addition, the non-specific labeling could be easily avoided by using a buffer with weak metal-binding properties in an appropriate concentration, or by adding of EDTA (or DTPA etc.) to the reaction mixture to scavenge unchelated metal ions. Hence, the effects of instability due to the cleavage of covalent bonds

and non-specific binding are negligible. The greater serum stability of ^{99m}Tc labeled protein conjugated with the DOTA and cyclam analogues has been explained by steric hinderance toward dissociation resulting from the presence of the macrocyclic ring and the denticity of the donor atoms. The mono-N-substitution of DOTA with amino acid significantly presents it utility as a BFC with a free amine, which can be conjugated to wide variety of protein molecules. The metal chelate can significantly impact the tumor uptake and biodistribution of radiopharmaceuticals based on small biomolecules, due to the fact that in many cases the metal chelate contributes greatly to the overall size and molecular weight of the radiopharmaceutical. Therefore, the design and selection of BFC is very important for the development of a clinically useful therapeutic agent.

Contents

030. RUPESH KUMAR

Copper Nanoparticle Catalyzed C-N Bond Formation : Michael Reaction and Amination of Aryl Halides.

Supervisors : Prof. Ramesh Chandra and Prof. Vani Brahmachari
Th 14656

Abstract

Copper nanoparticles have been prepared by the reduction of Cu^{2+} ions to Cu (0) in a reverse micellar system. The nanoparticles prepared were round in shape, with an average size of 14-17nm as confirmed by the TEM photograph and QELS data. The UV spectrophotometery was used for characterizing metallic nature of the nanoparticles. Prepared copper nanoparticles of 14-17 nm size were successfully used as a c atalyst in the C-N bond formation (aza-Michael reaction and amination of aromatic halides. Demonstrates that Cu-nanoparticles (14-17 nm) can catalyze C-N bond formation (aza-Michael reaction) with various N-alkyl-and N-aryl-piperazines in mild reaction condition in high yiels. Copper-nanoparticles were successfully applied for the amination of aromatic halides with various amines. 10-15mol% copper nanoparticles of 14-17 nm efficiently catalyzed the : (i) cross

coupling reactionof aryl iodides with N-alkyl- and N-aryl-piperazines using KO-tBu base in dioxane at 110 degree celcius in good to excellent yield; (ii) cross coupling reaction of 4-bromoiodobenzene with amines using 10-15 mo1% copper nanoparticles of 14-17 nm showing the chemoselective amination reaction; (iii) cross coupling reaction of N-Methylpiperazine with 2,4-difluoronitrobenzene using 15mo1% Cu (14-17 nm) as catalyst, 1.5 equivalent of KO-tBu as base in dioxane resulted in chemoselectively amination reaction. N-Methylpiperazine replaced the fluoro at ortho position to the nitro group of 2,4-difluoronitrobenzene, whereas no reaction occurs at para fluoro. Cu-nanoparticles efficiently catalyse the C-N bond formation in mild reaction condition and the catalyst can be recovered and have much promise for further applications.

Contents

031. SARKAR (Mita)

Studies on Oxidative Stress and Antioxidants in Thyroid Dysfunction.

Supervisors : Dr. Madhu Chopra and Dr. Rajeev Varshney

Th 14654

Abstract

Demonstrates oxidative stress and oxidative stress induced damage in hyperthyroid patients based on the following findings : (i) Increased ROS generation observed in hyperthyroid patients and assayed directly in the erythrocytes and peripheral blood mononuclear cells. (ii) Perturbations in the cellular physiology including changes in the mitochondria and cellular growth and proliferation. (iii) Oxidative damage to the macromolecules leading to apoptosis in hypermetabolic condition. (iv) Disturbances in the antioxidant defense system. Shows that flow cytometric measurement of ROS generation could be useful in the early detection of the onset of damage, complementing the biochemical evaluations and early diagnosis of the disease. The study was carried out in the Indian perspective with the understanding that, besides providing an appropriate background for future application based studies; together with the information obtained from systemic studies

in established cell lines, it also provide an insight and elucidate the role of oxidative damage in the pathophysiology of thyroid dysfunction thereby enabling proper managementof the disease.

Contents

1. Introduction. 2. Scientific Background. 3. Material, Subjects and Methods. 4. Results and Discussion. 5. General Discussion. 6. Summary, Conclusion and Bibliography.

032. SAROJ KUMARI

Synthesis and Evaluation of Cholecystokinin Receptor Specific Antagonists Taking Lead From Naturally Occurring Ligands.

Supervisors : Dr. Madhu Chopra and Dr. Anil Kumar Mishra
Th 14652

Abstract

Cholecystokinin (CCK) is an important gastrointestinal hormone, and it is one of the most abundant neurotransmitter peptides expressed in brain. CCK receptors have been classified into two subtypes, CCK-A and CCK-B on the basis of their affinities for structurally and functionally related family of peptides with identical COOH - terminal pentapeptide sequences but with differences in sulfation at the sixth (gastrin) and seventh (CCK) tyrosyl residues, and also with differences in response to specific antagonists. Two series of compounds were synthesized, one series with hydrazino linker and other with thiourea linker. The pharmacological assessment of the synthesized ligands was performed using gastric acid secretion assay in isolated perfused stomach of mouse. Results indicate that all synthesized ligands are acting as insumountable antagonists because no maximum response of CCK-8 (0.21pH) was observed in presence of ligand. At increasing concentration of synthesized ligand the gastric acid secretion is occurring from the stomach comes down. It means that inhibition of gastric acid secretion from the stomach is due to CCK-8 application. One of the compound (3Z)-1H-indole-2,3-dione 3-[(3-(4-methylphenyl)-4-oxo-3,4-dihydroquinazolin-2-yl]hydrazone(5b) was radiolabeled with ^{99m} Tc and was studied. The results of pretreatment studies of ^{99m} Tc labeled 5b shown that blocking with 1 mg/ml of CCK-8, 10 minutes before the injection of the radiolabeled-ligand reduced the accumulation in stomach to about 30% of control where as the activity in the intestine was reduced to 50% at 1

hr p.i. There was increased accumulation of activity in liver, but reduction in case of rest of the organ studies. Two fluorescein derivatives were synthesized. In one the fluorescein moiety was directly attached to the compound {N(4-ethoxyphenyl)-2-[3,4-dihydro-3-(3-carboxyphenyl)-4-oxo-2-quinazonyl)]} hydrazine carboxamide (6a). The other in which one linker was provided in between fluorescein moiety and compound {N(4-ethoxyphenyl)-2-[3,4-dihydro-3-(3-carboxyphenyl)-4-oxo-2-quinazonyl]} hydrazine carboxamide (6a). Purification of ligand was done by using HPLC. The synthesized compounds were characterized using spectroscopic techniques such as IR and NMR.

Contents

1. Introduction and Scientific Background. 2. Review of Literature and Objectives of Present Work. 3. Synthesis of the Designed quinazolinone derivatives, an active pharmacophore in Asperlicin and their characterization with the help of organic spectroscopy. 4. Estimation of antagonistic activity of the newly synthesized cholecystokinin - B/Gastrin receptor antagonists using an in vitro acid secretion assay. 5. Synthesis of the DTPA derivative of the gastrin, purification, characterization and biological evaluation. 6. Determination of affinity of ^{99m} Tc-radiolabeled quinazolinone derivative and its biodistribution in mice and scintigraphic studies in rabbits. 7. Synthesis of fluorescein derivative of one of the most active quinazolinone compound 6a and determination of ligand receptor interaction with the help of fluorescence spectroscopy. Bibliography.

033. SHARMA (Kirti)
Role of PknH/EmbR Signaling System of Mycobacterium Tuberculosis in Regulation of Arabinan Metabolism.
Supervisors : Dr. Yogendra Singh and Dr. S. Ramachandran
Th 14661

Abstract

Delineate the role of protein serine/threonine kinase H and the cognate transcriptional regulator, EmbR of M. tuberculosis in its survival/pathogenesis. M. tuberculosis possesses a functional, transmembrane protein kinase H (PknH) which specifically autophosphorylates and phosphorylates the Ser/Thr residues of histone. Lysine at 45 position is essential for catalytidc activity of PknH. Acidic and heat stress significantly decreased the level of PknH transcript suggesting that the

kinase may be involved in respective stress mediated signaling in M. tuberculosis. PknH was restricted to the slow growing mycobacterial species and was absent in fast growing saprophytic species suggesting a possible role of PknH in the processes unique to the members of slow growing mycobacterium. EmbR interacts with RNA polymerase in a dose-dependent manner and exhibits phosphorylation dependent ATPase activity tht is suggested to provide energy for catalyzing the isomerization of the closed complex between EmbR and RNAP to a transcriptionally competent open complex. EmbR binds to upstream region of embCAB genes in M. tuberculosis and phosphorylation of EmbR by PknH enhances its DNA binding activity towards promoter regions of embCAB genes. Expression of PknH in M. smegmatis significantly increased the phosphorylation of endogenous EmbR as well as levels of embC, embA and embB gene transcripts thus demonstrating the positive regulatory effect of EmbR on transcription of embCAB operon after its phosphorylation by PknH in vivo.PknH mediated increase in transcription of embCAB genes alters LAM/LM ratio and resistance to Ethambutol, a fronline antituberculosis drug. In vitro kinase assays and GST-pull down assays revealed that EmbR acts as a substrate of atleast three mycobacterial STPKs viz. PknH, PknA and PknB. Mstp was found to act as a negative regulator of STPK-EmbR signaling system, as it converted phosphorylated forms of PknA, PknB , PknH and EmbR into dephosphorylated (inactive) state. Mstp mediated dephosphorylation brings back the level of DNA binding and ATPase activity of EmbR equivalent to tht of unphosphorylated protein. Exposure to intracellular environment increased the level of pknH in processes that promote intracellular survival of the pathogen.

Contents

034. TIWARI (Rakesh Kumar)

Synthesis of Substituted 1,2,3,4-Tetrahydropyrazino (1,2-a) Indoles and 1,2,3,4,-Tetrahydroisoquinolines via Intramolecular Cyclization Using Benzotriazole Methodology.

Supervisors : Prof. Ramesh Chandra and Prof. Vani Brahmachari

Th 14651

Abstract

The antifungal activity evaluation studies on 1-substituted-10-methyl-1,2,3,4-tetrahydropyrazino (1,2-a) indole 6a-1 and 1,2-disubstituted-10-methyl-1,2,3,4-tetrahydropyrazino (1,2-a) indole 9a-f has revealed that 1-(4-chlorophenyl)-10-methyl-1,2,3,4-tetrahydropydropyrazino (1,2-a) indole 6e is a potent antifungal agent. All synthesized compounds of this series have shown mild to moderate activity. The antibacterial activity shown by pyrazino (1,2-a) indole derivaties revelead that the compound 6g, 6b and 6d has shown potent antibacterial activity against most of the strain used in the study. Also the toxicity data on the haemolytic cell showed that these derivatives are less toxic than the standard drug Gentamycin. So the pyrazino (1,2-a) indoles derivatives could be considered for the safer drug candidate for the development of suitable antimicrobial drug. The result of antibacterial activity of all synthesized isoquinoline derivatives had shown significant activity towards most of the strains used in the study. The antibacterial activity shown by THIQ derivatives a listed by there MIC values showed that the compound (12a-c) with free NH group in the isoquinoline moiety showed 3.5 to 20 ug/ml against all the standard strains of the bacteria used in the study where as for compound 13a-c, showed moderate MIC values revealing that moiety with free NH group had significant antibacterial activity. The results of anti Aspergillus activity evaluation revealed that one of the 1,2,3,4-tetrahydroisoquioline, that is, 14b is a potent inhibitor of the growth of A. fumigatus, A. Flavus and A. Niger. The compound 14b exhibited activity in the range of 93.7-187.5 ug/disc in disc diffusion and 125-250 ug/ml in microbroth dilution assays. The MIC_{90} value of 14b by percentage spore germination inhibition assay was found to be in the range of 93.5 to 125 ug/ml, whereas other derivatives showed mild to moderate activity towards strains used in the study. The results of anti Candida activity showed that all the synthesized compounds had variable activity against the pathogenic strains of C. Albicans. The most active compound 15a was a potential inhibitor of the growth of C. Albicans with MIC_{90} value of 62.5 ug/ml by microbroth dilution assay. The moderate activity was

also found for compounds 12a and 14b, which exhibited activity at 125 ug/ml in microbroth dilution assay.

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