CHAPTER 5

BIOMEDICINE

Doctoral Theses

031. BRAHAM PARKASH
Modulation of Dimerization and Translational Frameshifting Processes of Human Immunodeficiency Virus Type-1 by Using Polyamide Nucleic Acid (PNA)
Supervisor : Dr. Vibha Tandon
Th 15230

Abstract

Synthesizes PNA monomers and then a series of PNAs directed against the crucial sequences in HIV genome responsible for dimerization and frameshifting processes of HIV-1. These PNAs were tested under various in-vitro and ex-vitro conditions. The data shows that the PNAs targeted against the DLS region of HIV-1 exhibit the maximum inhibition on the dimer formation under in-vitro dimerization condition. The results of the effect of PNAs on the LDI-BMH equilibrium show a shift towards the BMH conformer except PNA targeted to AUG region of gag gene. In in-vitro coupled dimerization and template-switching study, a decrease in template, switching efficiency during reverse transcription with HIV-1 RT between donor and acceptor viral RNA transcripts has been reported with PNAs targeted DLS region. PNAs complementarity to the stem-loop structure of frameshift signal in Gag and Pol overlapping region also show the significant effect on the expression ratio of Gag and Gag-Pol fusion precursor proteins. These results clearly demonstrate PNAs to be a powerful modulator and a potential antiviral drug candidate against HIV-1.

Contents

1. Introduction and review of literature. 2. Synthesis of Boc/acy1 PNA monomers and solid phage synthesis of PNA oligomer targeted against various domains in HIV-1 RNA genome. 3. Effect of PNA oligomers on the equilibrium between LDI-BMH conformations and related processes under in-vitro conditions. 4. Effect of PNA oligomers on the efficiency of translational frameshifting.

032. CHAUDHARY (Preeti)
Synthesis and Antimicrobial Activity of N-Alkyl and N-Aryl Piperazine Derivatives using Benzotriazole Methodology.
Supervisor : Dr. Akhilesh Kumar Verma
Th 15465

Abstract

Piperazines and substituted piperazines are important pharmacophores that can be found in many marketed drugs, such as the Merck HIC protease inhibitor Crixivan, and durgs under development. Piperazinyl-Linked Ciproflozacin dimers reported as potent antibacterial agents against resistant strains, a novel class of mixed D_2/D_4 receptor antagonist, dual calcium antagonist, antimalarial agents and potential antipsychotic agents. Recently piperazine derivatives containing tetrazole nucleus has been reported as an antifungal agent. Many synthetic procedures exist for the synthesis of various derivatives of N-alkyl and N-aryl piperazine in the literature. Reported methodologies for the synthesis of various derivatives of N-alkyl and N-aryl piperazine suffer from several practical disadvantages such as the use of costly catalyst, multistep synthesis long reaction times, elevated temperatures, difficult to recover the solvent and moderate yields. Due to medicinal importance of pipezine derivatives, the study aim to synthesize N-alkyl and N-aryl piperazine derivatives in mild reaction condition using cheap and easily available chemical.

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033. CHOITHANI (Jyoti)
Development of New Heterobifunctional Reagents for the Preparation of Biochips.
Supervisors : Dr. K. C. Gupta and Dr. Pradeep Kumar
Th 15231

Abstract

Two DNA microarray strategies dominate the field, namely, the deposition method and the On-Chip technology. The deposition method has become a method of choice, which offers excellent

flexibility in the sense that a wide variety of ligands can be immobilized. Covalent as well as non-covalent attachment of oligonucleotides to surfaces have been undertaken, amongst them, the earlier one has been found to be the method of choice. The methods, reported so far, engross the modifications of solid surfaces and oligonucleotides for immobilization. Some of the methods employ coupling reagents or activators. Thus the immobilization process requires several steps and expensive reagents to obtain surface bound oligonucleotides. To minimize the number of steps in the preparation of arrays, homo- and heterobifunctional reagents have been used to couple oligonucleotides directly to virgin/functionalized glass surfaces. The GOPTS method requires longer reaction time (~8h), while the MPTS results in immobilization of oligonucleotides via a rather labile disulfide linkage. Looking to the limitations of the existing heterobifunctional reagents with respect to reaction time and stability of the linkage, preparation of new heterobifunctional reagents and there use in construction of oligonucleotide microarrays have been undertaken.

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034. JAIN (Monika)

Investigation of Therapeutic Potential and Adjuvant Efficacy of Seabuckthorn Extract Against Dengue Virus Infection

Supervisors : Dr. Anju Katyal and Dr. Lilly Ganju

Th 15235

Abstract

Explored aqueous extract of Amla (Emblica officinalis), Bahera (Terminalia balerica), Shankhpushpi (Evolvus alsinoides) and alcoholic extract of Seabuckthorn (Hippophae rhamnoides) for their cytotoxicity. Further the therapeutic potential and adjuvant efficacy of Seabuckthorn leaf alcoholic extract (SBTLAE)

against Dengue virus infection was evaluated.

Contents

1. Introduction. 2. Aims and objectivs. 3. Therapeutic potential of Seabuckthorn during Dengue virus infection. 4. Adjuvant efficacy of Seabuckthorn against Dengue virus infection. 5. Conclusion and Bibliography.

035. KASHIF HANIF

Designing, Synthesis and Biological Studies of Peptidomimics as Novel ACE-Inhibitor

Supervisors : Dr. Santosh Pasha and Dr. M. A. Qadar Pasha

Th 15233

Abstract

Chimeric Peptide - 2 has been designed with the aim of obtaining an enzymatically more stable analog of Chimeric Peptide-1. Endogenous opioid peptides like endomorphins, met-enkephalin and NPFF/FMRF amide family of neuropeptides, besides playing a role in modulation of antinociception, also affect cariovascular system. Based on MERF, which consists of overlapping sequences of FMRFa and met-enkephalin, two chimeric peptides YGGFMKKKFMRFamide (YFa) and (D-Ala2) YAGFMKKKFMRF amide {(D0Ala2) YFa} were designed and synthesized. Effect of YFa and (D-Ala2) YFa on arterial blood pressure and heart rate was evaluated in anaesthetized rats. Both YFa and (D-Ala2) YFa showed a dose-dependent fall in mean arterial pressure in dose range of 13-78 micromol/kg. After naloxone treatment (5 mg/kg), vasodepressor effect of (D-Ala2) YFa and YFa was only partially blocked as compared to metenkephalin. Partial blockade of vasodepressive effect of YFa and (D-Ala2) YFa by naloxone may be attributed to interaction of these chimeric peptides with receptors other than naloxon-sensitive receptors such as anti-opioid receptors, adrenergic receptors and D-analogue receptors.

Contents

1. Hypertension and angiotensin converting enzyme inhibitor. 2. Designing, synthesis, characterization and in-vitro screening of petidomimics as novel ACE inhibitors. 3. Biological activity of peptidomimics in animal models of hypertension. 4. Insight : Antinociception, antiopioids and designing of chimeric peptides. 5. Hypotensive effect of chimeric peptides of met-enkephalin and FMRFa.

036. MAHAJAN (Shweta)
Novel Chemical Routes for the Construction of Oligonucleotide Microarrays (Bio-Chip) and Their Applications
Supervisors : Dr. K. C. Gupta and Dr. Pradeep Kumar
Th 15234

Abstract

Oligonucleotides microarrays have gained increasing use and acceptance in the study of genetic and cellular processes in the last few years. Today, the technology of arrayed nucleic acids as a multi-facette, interdisciplinary field revolving around the application of nucleic acids in many areas, from genetics and molelcular medicine to industrial production. The different and sometimes specialized uses put varied demands on the oligonucleotide arrays, requiring alternative approaches for preparing arrays that fulfill changing requirements. Deals with the approach for the cost-effective synthesis of oligonucleotides, labeling techniques as well as the methods for the estimation of functional group density on the polymer supports by employing universal reagents. In addition, efficient and reproducible strategies of arraying modified oligonucleotides on glass surface have also been highlighted.

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037. MIRZA IRFAN BEIG
Neural and Cardiovascular Responses in Conscious Animals during Epilepsy
Supervisors : Dr. Anju Katyal and Dr. M. Fahim
Th 15237

Abstract

Demonstrates that pretreatment with nifedipine alone or in combination with valproic acid provided significant protection

against PIZ induced seizures. Pretreatment with nifedipine in combination with valproic acid also reduced the required dosage of valproic acid for seizure suppression. Verapamil did not show significant protection against PTZ induced seizures and also could not potentiate the protective effect of valproic acid significantly. Reveales that seizures, particularly generalized tonic clonic were accompanied with lack of blood pressure regulatory mechanism and were accompanied by hypertension and bradyarrhythmia. Pretreatment of valproic acid was not able to block seizure induced hypertension and bradyarrhythmia. Pretreatment with nifedipine alone or in combination with valproic acid was able to provide significant protection against seizure induced hypertension and bradyarrhythmia. Pretreatment with verapamil alone or in combination with valproic acid was not able to block seizure induced hypertension and bradyarrhythmia. If seizures are not being controlled by valproic acid alone, it may be achieved by combining valproic acid with nifedipine instead of another AED. If nifedipine is given in combination with valproic acid, it will reduce the required dosage of valproic acid which inturn will reduce the toxicities associated with higher doses. As the combination is also carioprotective, it may prevent various complications like SUDEP, if it is because of cardiovascular complications. However, the dose of nifedipine should be selected carefully as it may cause complications due to hypertension and tachycardia.

Contents

038. NIMESH (Surendra)

Development of Nanoparticle Based Carrier Systems for the Delivery of Biomolecules

Supervisors : Prof. Ramesh Chandra and Dr. K. C. Gupta

Th 15236

Abstract

The continuous increasing wealth of knowledge about the role of genes involved in acquired or hereditary diseases renders the delivery of regulatory genes or nucleic acids into affected cells, a potentially promising strategy. Apart from viral vectors, non-viral gene delivery systems such as polycationic polymers, have recently received increasing interest, because of the safety concerns associated with insertional mutagenesis of retro-viral

vectors. Amongst polycationic polymers, PEI takes prominent position due to its potential for endosomal escape. To date, a large number of studies regarding incorporation of modifications into PEI to improve upton the gene transfer efficacy have been proposed. However, the problems of toxicity and poor gene delivery efficiency associated with PEI poses a major hurdle for in vivo studies. The work embodied in thesis started with the purpose of incorporating suitable modifications into PEI to improve in vitro cell viability and transfection efficiency.

Contents

1. Introduction. 2. Material and methods. 3. Polyethylenimine nanoparticles as efficient transfecting agents for mammalian cells. 5. Hexane-1, 6-bis (phosphat) cross-linked polyethylenimine nanoparticles for enhanced gene delivery and Summary.

039. SACHDEVA (Suraksha)
Cloning, Expression, Purification and Immunization Studies of MSP-1_{19} and MSP-1_{42} (Vaccine Candidate Antigens) of P. Falciparum and P. Vivax
Supervisors : Dr. Anju Katyal and Dr. Pawan Malhotra
Th 15464

Abstract

Compares the immunogenicities of both MSP-1_{42} and MSP-1_{19} of P. falciparum and P. vivax, the two most prevalent human malaria parasites, in recently developed human compatible adjuvants. E. coli expressed PfMSP-1_{42} formulated in Montanide ISA 720 and alum generated protective antibody responses which protected mice against a parasite challenge.

Contents

1. Introduction. 2. Review of literature. 3. Materials and methods. 4. Results. 5. MSP-1_{42} and MSP-1_{19} of P. vivax. 5. Discussion. Summary and Conclusion.

040. SARVESH KUMAR
Studies on Cell Adhesion Molecules : Modulation of Expression and Function by Novel Aromatic Ester from Piper Longum
Supervisor : Dr. Balaram Ghosh
Th 15228

Abstract

The migration of the leukocytes to the site of inflammation is regulated in part by the expression of cell adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecules (VCAM-1), and E-selectin. The expression of these molecules at the right levels and the right time is critically controlled. Disregulation of this controlled process is involved in inflammation and leads to development of various inflammatory disorders including asthma. Here, investigations were undertaken on using cell adhesion molecules as targets for identifying small molecules with potential to be used for the development of anti-inflammatory compounds. The work presented is on the identification and characterization of one such compound, ethyl 3', 4', 5' - trimethoxycinnamate from herbal plant piper longum, which modulates the expression and function of cell adhesion molecules viz. ICAM-1, VCAM-1 and E-selectin. Further, in search for finding a better molecule, the thio analogues of the identified compound were designed, synthesized and evaluated for their effect on modulation of expression and function of cell adhesion molecules viz. ICAM-1, VCAM-1 and E-selectin. The most potent molecule from the screening study was further tested for its efficacy in in-vivo animal model by using mouse model of asthma. Further part of the study is focused on elucidating the molecular mechanism of action of the most potent molecule i.e. ethyl 3', 4', 5' - trimethoxy thiocinnamate.

Contents

1. Review of literature. 2. Rationale and scope of the study. 3. Materials and methods. 4. Results and discussion. 5. Summary, Conclusions, Bibliography and Appendix.

041. SHARMA (MAMTA)
Molecular and Genetic Studies in Asthma
Supervisor : Dr. Balaram Ghosh
Th 15226

Abstract

The recent sequencing of the human genome combined with advances in bioinformatics and molecular genetics, and high speed internet communication provide an unprecedented and readily accessible set of discovery tools. Here, using a combinatorial approach involving bioinformatics and experimental methods,

attempted to identify new asthma associated genes. Reports the identification of a novel asthma gene : INPP4A. The protective role of INPP4A in asthma is evident from our observatins and the biochemical role of INPP4A. INPP4A enzyme dephosphorylates and inactivates PtdIns(3,4)P_2, an important messenger in the PI3K-Akt pathway, which is associated with proliferation of airways smooth muscle, platelet activation, mast cell degranulation and bronchial hyperresponsiveness in mouse model of asthma.

Contents

1. Review of literature. 2. Rationale and scope of the study. 3. Materials and methods. 4. Results and discussion, Summary, Conclusions and Bibliography.

042. SHARMA (Shilpy)
Molecular Studies on Respiratory Disorders
Supervisor : Dr. Balaram Ghosh
Th 15225

Abstract

Details on two major candidate genes, the beta subunit of the high affinity receptor for IgE (FcεRIβ) and transforming growth factor beta 1 (TGFβ1), including the identification of novel polymorphisms in the Indian population, their association with asthma and its associated quantitative trait-serum IgE levels and the functional implication of the associated polymorphisms and the haplotypes. A population based (case control) as well as a family based design for the identification of functional polymorphisms associated with atopic asthma was undertaken. The identification of novel risk and protective genotypes and haplotypes in these two asthma associated genes, FcεRiβ and TGFβ1, combined with their functional correlation could be helpful in predicting susceptibility towards developing respiratory disorders, including atopic asthma in the Indian population. Furthermore, these results could also help in predicting the response of drugs that target these pathways.

Contents

1. Review of literature. 2. Rationale and scope of the study. 3. Materials and methods. 4. Results and discussion. 5. Summary, Conclusions, Bibliography and Appendices.

043. SHARMA (Shipra)
Mitochondrial Polymorphisms in Neurological Disorders
Supervisors : Prof. Vani Brahmachari and Dr. Mitali Mukerji
Th 15229

Abstract

Proves the involvement of variations of mitochondria in cases of ataxia (both sporadic and hereditary) wherein screened for variations in the entire mitochondrial genome and then studied the consequence of these variations through computational approaches. These ataxia cases did not have expansion in the so far reported trinucleotide repeat associated loci. We also studied conserved regions of mitochondrial proteins (both nuclear and mitochondrial encoded) using in silico approaches and identified susceptible regions for deleterious mutations.

Contents

1. Review of literature. 2. Spectrum of mitochondrial polymorphisms in sporadic ataxia patients. 3. In silico analysis of conserved region of mitochondrial protein.

044. VIMAL KISHOR SINGH
Functional Evaluation of Hematopoietic Stem Cell Specific Antigen CD34 by Genetic Engineering and Gene Transfection Studies
Supervisors : Dr. P. M. Luthra and G. U. Gurudutta
Th 15232

Abstract

Reports the putative structure of Hu-CD34 that can help in identifying the functional domain. The whole Hu0CD34 molecule is likely to attain an extended far N-terminus region that possesses large number of N/O- linked carbohydrate attachment sites. This highly glycosylated region may not have most of the secondary structural elements as demonstrated. Whereas region with conserved cysteine residues was observed to likely attain globular structure that perhaps acts as a scaffold similar to immunoglobulins. There is no enzymatic or catalytic domain cytoplasmic region. However, study demonstrated its possible interaction to the SH3 domain through non-P-X-X-P motifs and that might facilitate its interaction(s) with various intracellular molecules bearing SH3 domain(s) leading to downstream signaling. In agreement to previous observations study confirmed the

functional significance of Hu-CD34 antigen as an adhesion molecule. The induction of Hu-CD34 induced is likely dependent upon phosphorylation of Ser^{306} residue.

Contents

1. Introduction and review of literature. 2. Structural analysis of CD34. 3. Three-Dimensional structure prediction of the interaction of CD34 with the SH3 Domain of Crk-L. 4. Genetic engineering of human CD34 gene. 5. Effect of inducible expression of human CD34 and mutants on adhesion, and proliferation. Epilogue. References.

045. VERMA (Yogesh Kumar)
Response Study of Multipotential Hematopoietic Stem Cells by Co-Expressing Survival and Proliferative Regulator Genes by Transfection
Supervisors : Prof. H. G. Raj and Dr. G. U. Gurudutta
Th 15227

Abstract

Generates 3D homology model of Bcl-2 and BH3 domain of Bax, and shown the existence of a sequentially and structurally conserved active site in Bcl-2 that plays an important role in pro-apoptotic proteins heterodimerization such as Bax etc. The docking of Bcl-2 and BH3 domain of Bax helped us to elucidate the mechanism for decreased anti-apoptotic activity of Bcl-2 mutants (G145/E, W188A). Elucidate the mechanism responsible for enhanced anti-apoptotic activity of survival enhancing mutant forms of Bcl-2 (D34A, S70E, V931 and ΔFLD) and observed that only in D34A and S70E mutants the structural integrity of active site is being maintained and enhanced negative charge in the active site which is translated into their higher heterodimerization potential with Bax and anti-apoptotic activity. Functionally these forms display higher affinity to heterodimerize with Bax at optimized cellular non-toxic expression induction dose to be one of the mechanism through which Bcl-2 exerts its anti-apoptotic effects. Mutants show higher radioresistance than Bcl-2 when induced under controlled induction and higher superoxide anion reduction potential by maintaining SOD2 activity after irradiation. In addition, these mutants display higher potential for the suppression of DNA strand breaks by probably initiating DNA repair pathway in growth arrested cells (in G2 phase), which likely prevents radiation injury.

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