Quick Peek

Editorial
Students Activity
Students Achievement
Photo Gallery
Scientific write-ups
Students’ corner and poetry
Editorial Board

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Editorial

The academic year 2014-15 witnessed outstanding contributions from the students for the past two issues for the newsletter, which showcased artistic creativity and writing genius. This venture has certainly provided a platform to the students to explore their creativity to the hilt.

Dissertation topics have been included, herein for creating awareness of the wide diversity of projects undertaken by the Department, in addition to poetic contributions and write-ups.

The International Yoga Day celebration at the Department was a huge success, and the photographs vouch for this. A glimpse in the form of memories is offered for the various programs conducted by the Department.

Hopefully, this issue will be appreciated. Thank you Readers for your love, support and feedback.

Keep Reading!!!

Dr. Archana Moon, e-BioResource Editor
STUDENTS ACTIVITIES DURING JANUARY 2015 TO JULY 2015

10th January  Prof. M. C. Nath memorial Lecture Series.
20th-21st January  Sports Day.
23rd January  Annual Cultural Day “Paraquats”
25th-27th February  Workshop Cum Training on Animal & alternatives in Toxicology testing.
8th April  Valedictorial Ceremony of M.Sc II batch 2015 “Hasta-la-Vista”
21st June  International Yoga Day
30th June  Guest Lecture by Dr. Pravat Mandal, TATA innovation Fellow, John Hopkins University, USA & Professor, National Brain Research Centre, India.

Subject: “Brain Biochemistry through brain imaging- a multimodel research initiative for direct clinical application”

10th July  Guest Lecture by Dr. Atul Kulkarni, Professor, Translational Research Group, Rutgers Cancer Institute of New Jersey (CINJ).

Subject: “Cancer Genomics from Bench to Bed”

STUDENTS ACHIEVEMENTS

100% inhouse Dissertation
100% result of M.Sc Final Year students
Placements-
   Mr. Gunjan Ganvir placed at WCL.
Inhouse Dissertation Topics

- Phytochemical analysis of *Ailanthus excelsa* leaves in different Solvents - Amita A. Bhandarkar
- An age Comparative study of serum bone biomarkers of Albino Wistar rats - Anuja C. Rai
- Antiemetic Potential of Gomutra Ark of different breeds of Cow in diabetic rat model - Chaitali M. Bobade
- A Comparative analysis of L-Nicotine from various Tobacco Samples - Drishti C. Singh
- Isolation & Purification of Seed Protein of *Zizyphus jujuba* - Gunjan B. Ganvir
- Studies on Antioxidant enzyme of *Ailanthus excelsa* leaves extract - Joyti H. Singh Kachawah
- Isolation and Characterization of cancer Stem cells from breast cancer cell line - Amrita A. Tripathi
- Biochemical Studies on Rhizosphere of some *Ziziphus* species - Madhuri C. Pandey
- Antibacterial activity of *Zizyphus* sp. in some Diarrehea causing microorganisms - Neha V. Lakde
- Molecular analysis of dihydrofolate reductase from multidrug resistant strain - Nisha U. Singh
Bone biochemical histology and morphometric studies in ovariectomised female rats - Pallavi V. Masram

Studies on enzyme activity of Zizyphus Seeds - Pallavi V. Nagpure

Glutathione dependent antioxidant enzymes and A8344 G mt DNA mutation in maternally inherited type 2 diabetes - Pragnya Venkatrama

Ant diabetic potential of cow urine extracts of a single strain in diabetic rat model - Puja S. Kushwaha

Phytochemical analysis & determination of quercetine from methanic extract of Alianthus excelsa leaves - Raksha U. Bhoyar

Phytochemical profiling of Brassica - Ravina N. Guriya

Studies amylase inhibitory activity in Zizyphus species - Sapna K. Lonare

Study antioxidant enzymes and mitochondrial DNA (UuR) mutation in mitochondrial encephalomyopathy, lactic acids and stroke like Episodes (MELAS) - Shipra S. Bodele

Targeting cancer stem Cells for cancer chemoprevention by natural dietary constituents - Shivani C. Sahay

In Vivo toxicity profile of Brassica oleracea L.var.Capitata - Surbhi R. Shivhare
Dr. Pravat Mandal delivered a lecture on “Neurobiological approach”

Hon’able Vice Chancellor Dr. Vilas Sabkal was guest of Honour.

Dr. Atul Kulkarni gave a talk on “Cancer genomics from bench to bed”

Dr. Shekhar Mande, Director, NCCS Pune addressed the assembly.
Sports Day and Cultural Day were celebrated by Students, Teachers, Research Scholars and Non-Teaching Staff with great enthusiasm.
International 1st Yoga day was celebrated by the Department
Molecular Docking: An Approach to Computer Aided Drug Designing

The age of botanicals (Somberg 1996) when human made use of herbs for food, religious significance and medications was known as the pharmaceutical years. But, the process of discovery of new drug molecules has changed dramatically in the last century since; drugs were discovered accidentally including quinine, digitalis, cocaine, antipyrine and aspirin with one of the most accidental discovery of penicillin in 1928 by Alexander Fleming. Moreover, the acceptance of “microbial theory of disease” explained the infectious diseases caused by microorganisms put a key development in medicinal science and drug discovery.

Earlier, the process of drug discovery was random with limited scientific knowledge and limited technology. For pharmaceutical industries, the number of years required to bring a drug from discovery to market was approximately 12-14 years and cost 1.2$-1.4$ billion dollars. But, today’s drug discovery is based on the integration of knowledge from various disciplines including molecular biology,
biochemistry, physiology, kinetics, etc. The process of drug discovery has been revolutionised with advent of genomics, proteomics, bioinformatics, high throughout screening (HTS), virtual screening, de novo design, in vitro, in silico ADMET screening and structure based drug design.

Molecular docking is an important tool used in computer aided drug designing. Docking is a method used to predict preferable binding analysis of ligand with receptor using computational methods. Compared to traditional experimental techniques, virtual screening (VS) for hit identification and lead optimisation permits all aspects of drug discovery. The VS is classified into ligand based and structure based drug discovery. When the structure of active ligand is known but little structural information is available for target, then ligand based methodology such as pharmacophore modelling and quantitative structure activity relationship (QSAR) can be used.

In structure based drug designing, molecular docking is the common method used. To perform structure based molecular docking, two things are needed. First is the structure of protein. The structure of protein can be determined by X-ray crystallography, NMR (Nuclear Magnetic Resonance) spectroscopy and/or electron microscopy. The protein data bank (PDB) is a database that gives information about the deposited protein structures, the method used to elucidate the structure, the organism from which protein is purified, the
apostructure or crystal bound information, etc. The second requirement is the structural information of ligand. This information can be accessed from PubChem online portals, from literature search, etc.

The ligand-receptor binding mechanism is same as the “lock and key theory” proposed by Fisher. The earlier docking methods were based on this old theory, where both the ligand and the receptor were treated as rigid bodies. But, then this theory was replaced by the ‘Induced fit theory’ of Koshland. This theory stated that the active site of protein is continually reshaped and hence ligand and receptor should be treated as flexible entities.

The molecular docking is performed with induced fit theory. At the end of docking, the results are evaluated on the basis of the docking score and minimum binding energies. Also the interactions between ligand and receptor were analysed that could be H-bond, hydrophobic interactions, π-π stacking, π-cation, salt bridge interactions, Vander Waals forces, etc.
Dihydropteroate Synthase

Dihydropteroate synthase (DHPS) EC: 2.5.1.15 is a pterin binding enzyme. It is an enzyme which produces dihydropteroate in bacteria, but not expressed in most eukaryotes including humans. It is a functional homodimer which catalyses the condensation of p-aminobenzoic acid (PABA) in the de novo biosynthesis of folate, which is an essential cofactor in both nucleic acid and protein biosynthesis. The enzyme dihydropteroate synthase (DHPS) is encoded by the folP gene and catalyzes the formation of 7,8-dihydropteroate from p-aminobenzoic acid (pABA) and 6-hydroxymethyl-7,8-dihydropterin-pyrophosphate (DHPH). This reaction is a key step in the folate biosynthetic pathway of bacteria and primitive eukaryotes. Higher eukaryotes utilize dietary folate and therefore lack DHPS expression which makes it an attractive focus for antimicrobial drug discovery.

In addition to DHPS, many other enzymes participate in folate pathway, including: 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase (HPPK), dihydrofolate synthetase (DHFS) and dihydrofolate reductase (DHFR). Drugs that inhibit DHFR and DHPS are used in the treatment of infections by the
apicomplexan parasites *Plasmodium sp.* and *Toxoplasma gondii*. In these species DHPS is a part of a bifunctional enzyme fused to HPPK.

DHPS is a single domain protein that forms an eight-stranded TIM alpha/beta barrel, where the 7,8-dihydropterin pyrophosphate substrate binds in a deep cleft in the barrel. Bacterial DHPS (gene sul or folP) is a protein of about 275 to 315 amino acid residues that is either chromosomally encoded or found on various antibiotic resistance plasmids. In the lower eukaryote *Pneumocystis carinii*, DHPS is the C-terminal domain of a multifunctional folate synthesis enzyme (gene fas).

Other proteins contain a DHPS-like domain, including members of the methyltetrahydrofolate (corrinoid iron-sulphur protein methyltransferase (MeTr)) family. MeTr catalyses a key step in the Wood-Ljungdahl pathway of carbon dioxide fixation. Other members of this family that contain a DHPS-like domain include methionine synthase and methanogenic enzymes that activate the methyl group of methyltetrahydrodromethano (or -sarcino) pterin.

DHPS is inhibited by sulphonamides which are substrate analogues of pABA. Thus, in bacteria, antibacterial sulphonamides act as competitive inhibitors of the enzyme dihydropteroate synthase, DHPS. The effect of dihydropteroate synthetase inhibition is comparable to that of dihydrofolate reductase inhibition by trimethoprim, another bacteriostatic agent. Together these two drugs - trimethoprim-sulfamethoxazole [TMP-SMX] - are commonly used to treat recurrent urinary tract, *Shigella*, *Salmonella*, and *Pneumocystis jivoreci* infections.

Though higher eukaryotes including humans lack DHPS expression, DHPS is the target for the highly effective sulfa drugs that have been used for over 70 years. However, the emergence of sulfa drug resistance has seriously compromised their utility and has spawned a number of efforts to develop new classes of DHPS inhibitors. The sulphonamide drugs were among the first effective synthetic antimicrobial agents to be developed and are still widely used in clinical and veterinary practice. Widespread resistance to sulphonamides has now developed in many organisms, a phenomenon which frequently arises from specific mutations within the *dhps* gene.
A macrophage-stimulating compound from a screen of microbial natural products

To harness the capacity of our innate immune system to overcome infections, scientists here have developed a screening protocol for screening a large in-house collection of extracts from Actinobacteria for molecules with the ability to activate the NF-κB pathway and increase macrophage mediated killing of bacteria. Scientists here report the identification of a small molecule streptazolin as a novel macrophage stimulating molecule. The macrophage cell line used is THP-1 Blue (Invivogen) expressing a Secreted Embryonic Alkaline Phosphatase (SEAP)-Quanti-Blue reporter gene under the control of master transcriptional regulator NF-κB. Heat Killed Listeria monocytogenes (HKLM) was used as the positive control. The macrophages that showed a reporter activity equal to or higher than that induced by HKLM were used in a follow-up study (killing assay) wherein the bacterial uptake, binding and killing by macrophages was allowed and all the extracellular bacteria were killed using antibiotics like gentamicin, penicillin; intracellular bacteria were thus enumerated. Since a few extracts, as a result of screening, also showed an unspecified antibiotic activity against Streptococcus mutans, that the extracts used for follow-up study did not contain any spurious antibiotic activity was also ensured. A strain WAC1325 was found to show a very high NF-κB reporter expression and was thus used for activity guided purification of streptazolin using reverse phase HPLC, 1D and 2D NMR studies, HR-MS. Purified streptazolin was assessed for antibiotic activity for concentrations upto 512µg/ml with negative results. That the macrophage stimulatory activity of streptazolin was due to the presence of a characteristic diene moiety within the
its structure was supposed by the scientists on the basis of comparative studies with the reduced product of streptazolin that is tetrahydrostreptazolin (lacking diene moiety) for a concentration range of 30µg/ml - 240µg/ml with null activity of tetrahydrostreptazolin whatsoever while streptazolin was found to be most active at a concentration of 60µg/ml.

Priming studies were performed to assess the cytokine stimulating potential of streptazolin on THP1-Blue cells by Enzyme Linked Immunosorbent Assay (ELISA). The results can be stated as - 1)Streptazolin alone cannot induce the secretion of Tumour Necrosis Factor-α (TNF-α), 2)it can boost its secretion in presence of lipopolysaccharide (LPS), 3)PAM3CSK4 - a synthetic agonist of Toll Like Receptor (TLR)-2 alone can boost TNF-α secretion but 4)it gives a only a little additive induction of TNF-α in combination with PAM3CSK4  further 5)streptazolin can alone bring about Interleukin-8 (IL-8) induction, 6)an additive response to IL-8 secretion in conjunction with LPS, 7)an almost a similar response of PAM3CSK4 as compared to that in TNF-α case, these findings were supported by graphical and statistical analysis and suggest that streptazolin inhibits the activation of NF-κB neither through TLR-2 nor through TLR-4 (through which LPS brings about macrophage stimulation). Also adding an equal volume of streptazolin and HKLM resulted in a remarkable additive expression of NF-κB which suggests that they act through different pathways. All the above results imply that streptazolin may have a role in priming macrophages in response to bacterial agonists.

Kinase inhibitor study was performed which involved the treatment of the four mitogen activated promoter kinases (MAPKs) i.e phosphatidylinositide 3 kinase (PI3K), c-Jun NH₂ terminal kinase 1 (JNK), extracellular signal regulated kinases 1 & 2 (ERK 1&2) and p38, to determine the mechanism of streptazolin-induced activation of NF-κB. It was found that the addition of PI3K inhibitor LY294002 significantly reduced the NF-κB expression as compared to streptazolin alone, suggesting that streptazolin acts through PI3K signalling.
Streptazolin is known to induce NF-κB on a time-scale comparable to that of HKLM-positive controls (supported by graphical analysis), suggesting that this molecule acts directly on macrophages to trigger NF-κB expression. On the basis of assessmentment of number of bacteria associated with macrophages (both bound and internalised) at early time-points of infection and found that streptazolin appeared to increase the ability of macrophages to bind bacteria at early time points, which may contribute to increased bacterial killing in the presence of streptazolin.

Rising rates of antibiotic resistance in bacterial pathogens is a medical crisis of global concern, in the face of which exploring different immunostimulatory strategies seems to be the right move and represents a promising alternative for antibiotics.
Students’ Corner

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आई......
एक सावली
क्षणक्षणाला सोबत असणारी
मनामनाच्या कोप-यात वसणारी.
लहानपणी बोट धरून,
चालायला शिकविणारी...
आणि !
छोट्या-छोट्या आनंदात
सोबत असणारी.
चुकलेल्या वठणावर
रस्ता दाखविणारी
संस्काराच्या बझावर खंबीर करणारी.
प्रत्येक दुःख स्त्त: झेलून
उमलण्याचा मोक्षा श्वास
हद्यात भरणारी
आई ती आईच...

काळोख
डोळायात ....
अगदी खोलवर बसलेला काळोख
मनात कधी घर करून जातो
कठतच नाही.
एक उन्माद असतो,
काळोखात;
मनात दडलेल्या कवडश्या सारखा
कधी हेलावणारा
तर कधी
तरंगावर स्वार होणा-या
हेलकाळ्यांसारखा.

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