CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

https://zenodo.org/records/11076546

https://www.iajps.com/volumes/volume11-april-2024/16-issue-04-april-24/

Available online at: http://www.iajps.com *Review Article*

A REVIEW ARTICLE ON ETODOLAC: ANTI-INFLAMMATORY

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Article Received: March 2024 **Accepted:** March 2024 **Published:** April 2024

Abstract:

A robust, accurate and sensitive automated procedure for the determination of etodolac, an anti- inflammatory drug, in pharmaceutical formulas by sequential injection analysis, is reported. The same system was also applied to evaluate the antioxidant activity of the drug expressed as Trolox equivalent antioxidant capacity (TEAC). The methodology is based on measuring at 734 nm the decay of absorbance of a solution with the radical 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) after its reduction by etodolac. Optimum ABTS•+ -etodolac reaction was achieved with 0.329 mL of sample and 0.205 mL of ABTS•+ solution. Etodolac was determined at concentrations up to 4.5 × 10−5 mol L−1.A solution detection limit of 6.6 × 10−6 mol L−1 was obtained under the optimised experimental conditions. A relative standard deviation (n = 10) lower than 4.7% with a sample throughput of more than 21 samples/h was obtained. No interference from excipients was observed. The developed methodology was applied in the analysis of pharmaceutical preparations and the obtained results were in good agreement with those furnished by the reference procedure with relative deviations lower than 1.4%.

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QR CODE

Please cite this article in press K. Bhargavi et al., A review article on etodolac: Anti-inflammatory., Indo Am. J. P. Sci, 2024; 11 (04).

INTRODUCTION:

Etodolac is a nonsteroidal anti-inflammatory drug (NSAID) that is available by prescription only and is used long term for therapy of chronic arthritis and short term for acute pain. Etodolac has been linked to rare instances of clinically apparent drug induced liver disease.

BACKGROUND:

Etodolac belongs to the acetic acid derivative class of NSAIDs similar to diclofenac, sulindac, ketorolac and indomethacin. Like other NSAIDs, etodolac is a potent cyclooxygenase (Cox-1 and -2) inhibitor which blocks the formation of prostaglandins that are important in pain and inflammatory pathways. It has analgesic as well as antipyretic and antiinflammatory activity. Etodolac was approved in the United States in 1991 and is available by prescription only. Currently more than 3 million prescriptions are filled yearly. Current indications include treatment of osteoarthritis and rheumatoid arthritis and for short term treatment of acute pain. Etodolac is available as capsules or tablets in doses of 200, 300, 400 and 500 mg generically and under the trade name Lodine. Extended-release formulations of 400, 500 and 600 mg are also available for once or twice daily dosing. The recommended dose is 400 to 1200 mg in 2 to 4 divided doses daily, based upon response and tolerance. Like other NSAIDs, etodolac is generally well tolerated, but side effects can include headache, dizziness, somnolence, dyspepsia, nausea, abdominal discomfort, heartburn, diarrhea, peripheral edema, pruritus and hypersensitivity reaction.Etodolac/cyclodextrin formulation: physicochemical characterisation and invivo pharmacological studies :

CHARACTERISATION AND INVESTIGATIONAL DRUG MATERIAL AND METHODS

Chemicals

Etodolac (molecular weight, 287.35g/mol, 95.42%)was gifted by M/S Fleming Laboratory Limited, Dist.Medak, Andhra Pradesh, India. Crude Gum Katira was obtained from Seoni District of Madhya Pradesh.Eudragit®RS75 and Eudragit®RL75 polymer granules were obtained as gift sample from Evonik Rohm, Pharma Polymers, Kirsechenallee, Darmastand, Germany.Span 80 (Loba Chemie Pvt. Ltd, Mumbai, India), TriSodium Orthophosphate (Loba Chemie Pvt. Ltd. India),Hydrochloric Acid 35% (Merck Life Science Pvt. Ltd.India), Tween 80 (Merck Specialties Pvt. Ltd, India),Dichloromethane (Merck Specialities Pvt. Ltd, India), Potassium dihydrogen phosphate (Merck Specialties Pvt.Ltd, India), and all others analytical grade chemicals were purchased and used as received.

Animal

Male albino rats (150-200 g) and rabbits (1.5-2 kg) were taken as experimental animal. The animals were acclimatized to laboratory conditions for 3days before the commencement of the experiment and kept under standard condition of temperature (25 ºC), relative humidity (70±10%) and a 12 hours light/12 hours dark cycle environment. During the study period, guideline of Committee for the Purpose of Control and Supervision of Experiment on Animal (CPCSEA), Institutional Animal Ethics Committee (IAEC) were properly followed for the maintenance of animal and the experiment protocol was approved by Animal Ethics Committee of Jadavpur University, Ref. No. AEC/PHARM/1601/02/2016 dated 22/04/2016.

Preparation of etodolac loaded gum Katira Microspheres

Etodolac loaded gum Katira microspheres were prepared by double-emulsion solvent evaporation technique. Gum Katira (50 mg) was mixed with 4ml of phosphate buffer (pH6.8) to form a homogeneous mixture in a magnetic stirrer for 1hr at a constant temperature of 40-450C. Etodolac was added to the homogeneous mixture and stirring was continued for another 1hr. The prepared etodolac and gum Katira mixture was then dispersed in a solution of Eudragit®RS100 and Eudragit®RL100 (7:1), Dichloromethane, Acryflow (Lubricating agent) and Span 80 (30 µL) through a 20-gauge syringe. The above mixture was homogenized well for 5-10 minutes using magnetic stirrer (1000rpm) to form W1/O emulsion. A separate acidic aqueous solution (100 mL) containing Tween 80 (50 µL) and a slight amount of polyvinyl alcohol was subjected to mechanical stirring (900 rpm) and to it the previously prepared W1/O emulsion was added drop wise using a 16-gauge syringe to form W1/O/W2 emulsion with a continuous stirring for 2-2.5 hours. The resultant microspheres formed were washed with distilled water followed by air drying for 24 h and final storage in desiccators for pharmacological investigation (Ruhidas et al. 2016)

FORMULATION OF INVESTIGATION DRUG PURPOSE AND METHODS

The critical formulation parameters (CFP) were selected as ETD amount, stabilizer type and ratio as well as critical process parameters (CPP) which were bead size, milling time and milling speed. The two-factorial-23 and The Box-Benkhen Designs

were generated to evaluate CFP and CPP, respectively. Particle size (PS), polydispersity index (PDI) and zeta potential (ZP) were analyzed as dependent variables. Characterization, physical

Pharm kinetics Aspects and Drug Disposition

Etodolac is a chiral nonsteroidal anti-inflammatory drug (NSAID) that is marked as the racemate. Currently, the drug is available in several countries for the treatment of arthritis and the alleviation of pain. Etodolac possesses several unique disposition features mainly due to its stereoselective pharmacokinetics. In plasma, the concentrations of the 'inactive' R-enantiomer are about 10-fold higher than those of the active S-enantiomer, an observation that is novel among the chiral NSAIDs. In common with other NSAIDs, the drug is highly plasma protein bound, and undergoes virtually complete biotransformation to oxidised metabolites and acyl-glucuronides. Etodolac is well absorbed, with maximal plasma concentrations attained within 1 to 2 hours in healthy volunteers. The area under the plasma concentration-time curve of racemic etodolac increases linearly with doses used clinically. The elimination half-life of etodolac is between 6 and 8 hours in plasma, and is similar for both enantiomers. The volume of distribution (Vd) of racemic etodolac is higher than that of most other NSAIDs mainly because of the extensive distribution of the S-enantiomer. The very large Vd of the S-enantiomer, compared with its antipode is, at least in part, due to its less extensive plasma protein binding. In addition to the unchanged drug, substantial concentrations of the acyl-glucuronides of etodolac are found in both plasma and the synovial fluid of patients with arthritis. A limited amount of conjugated etodolac is found in the bile of patients following cholecystectomy. Hepatic cirrhosis has no effect on the pharmacokinetics of racemic etodolac, although the effect of hepatic dysfunction on the pharmacokinetics of the individual enantiomers has yet to be determined. In elderly non-arthritic individuals with excellent kidney function, aging does not affect the pharmacokinetics of etodolac. The pharmacokinetics of the drug in patients with renal failure have not been published, and may be important because the acyl-glucuronides are renally cleared. The area under the plasma concentration-time curve of racemic etodolac increases linearly with doses used clinically. The elimination half-life of etodolac is between 6 and 8 hours in plasma, and is similar for both enantiomers. The volume of distribution (Vd) of racemic etodolac is higher than that of most other NSAIDs mainly because of the extensive distribution of the S-enantiomer. The very large Vd of the S-enantiomer, compared with its antipode is, at least in part, due to its less extensive plasma protein binding. In addition to the unchanged drug, substantial concentrations of the acyl-glucuronides of etodolac are found in both plasma and the synovial fluid of patients with arthritis.

APPROACHES IN DRUG DISCOVERY

Indian System of Traditional Medicines like Yajurveda, Siddha and Unani ETIOLOGY OF RA

The exact cause of RA is not known. In the previous study, it was identified that transcription of pro-inflammatory cytokines was induced by certain environmental factors such as smoking, gingivitis, and intestinal bacterial flora. Various disease-susceptible genes have also been identified previously, namely, human leukocyte antigens, cytotoxic T-lymphocyte antigen-4, peptidyl arginine deiminase 4, signal transducer and activator activator of transcription 4, tumor necrosis factor (TNF) alphainduced protein 3, and C-C motif chemokine ligand 21. It is suggested that induction of autoimmunity, breaking of immune tolerance to antigens, and epigenetic modifications occur due to citrullination of extracellular matrix molecules and reactions between environmental and genetic factors. The synovial tissues of patients with RA have autoreactive T-cells and B cells. When a person's self-tolerance breaks down, activation of T-cells takes places which in turn stimulate B cells to produce autoantibodies. Allergic reaction takes place when deposition of immune complex in tissues and activation of complement occur due to autoantibodies and antigen reactions. In the inflamed tissue, the generation of lymphoid follicle-like and germinal central-like structures occurs due to the accumulation of memory T-cells and B cells. In these areas, the expression of pro- inflammatory cytokines and costimulatory is high-Inflammatory cytokines like interleukin-1 (IL- 1), IL-6, and TNF-α are produced in large amount in inflamed tissues by synovial cells and lymphocytes, in turn they are responsible for the inflammation of joints.Furthermore, cartilage is degraded by the enzyme matrix metalloproteinases produced by cytokine-stimulated synovial cells. In addition, receptor activator of nuclear factor kappa B ligand is also expressed by synoviocytes and lymphocytes to bring the maturation and activation of osteoclasts. The bones are destroyed and absorbed by multinucleated osteoclasts, which causes joint destruction at the contact point.[3,7,8]

SIGN AND SYMPTOMS

In a few patients, the disease starts silently and shows symptoms such as fatigue, loss of appetite, malaise, and later synovitis. The early symptoms of RA continue from weeks to months. In the primary stage, RA consists of symmetric polyarthritis, which includes the small joints of the ankle, foot, and wrist. 10% of patients have polyarthritis, fever, and lymphadenopathy. Some patients often have delicate, warm, enlarged, and swollen joints. Joint stiffness also occurs in the morning and after inactivity. As the disease progressed, the symptoms spread to the elbows, hips, and shoulders. Pleuritis, pericarditis, interstitial lung disease, rheumatoid nodules, and foot ulcers are also affiliated in the patients of RA.[9,10]

TREATMENT OF RA

The main objective of the treatment is to minimize inflammation and pain, increase joint function, and avoid destruction and deformation of joints. The treatment strategies for RA include pharmaceutical products, various exercises, lifestyle modification, and, if required, surgery. At every stage of treatment, an assessment of response will be done.

First-line management

NSAIDS and corticosteroids; the overall objective of first-line management is to reduce pain and minimize inflammation. Fast-acting medication includes NSAIDS such as aspirin, naproxen, ibuprofen, and etodolac. Some newer analgesics include etorocoxib aspirin, naproxen, ibuprofen, and etodolac. Some newer analgesics include etorocoxib and celecoxib. Corticosteroids are more specific anti-inflammatory drugs, but the side effects are more severe as compared to NSAIDS. For this reason, they are administered in low doses and for a short duration. Intra-articular injections are also administered in some patients.

Second-line management

Disease-modifying antirheumatic drugs (DMARDS).The goal of second-line management is to support remission by preventing the progression of joint destruction and deformation. In this stage, the drugs are slow acting, and the response develops in weeks to months. The DMARDS also reduce the risk of lymphoma with RA.The initial drug in second-line management is methotrexate. It is an immunosuppressant drug. Regular monitoring of blood parameters can be done due to the higher side effects of methotrexate.

The antimalarial drug hydroxychloroquine can be used long-term in RA. Sulfasalazine can also be used in combination with anti-inflammatory drugs. Targeted Synthetic DMARDS: Barcitinib and Tofacitinib. Biological DMARDS: TNF-alpha inhibitors (Adalimubab, Certolizumab pegol, Etanercept, Golimubab); anti-B-cell (Rituximab); anti-T-cell costimulation (Abatecept); anti IL-6 inhibitors (Sarilumab, Tocilizumab). IL-1 inhibitors (anakinra, canakinumab, and rilonacept). Granulocyte macrophage colony-stimulating factor inhibitor (mavrilimumab, otilimab). Listed above are the current pharmacological approaches to treat rheumatoid arthritis.

Surgery

Surgery is mainly used in the end stage of RA. When all the non-surgical approaches have failed, surgery is the last option to reduce the pain in deformed joints. The main objective of surgery is to alleviate pain and restore the functionality of joints. Surgical procedures are mainly customized according to the needs of the patient. Some important surgical procedures are tenosynovectomy, arthroscopy, osteotomy, arthroplasty, and joint replacement.

SIDE EFFECTS OF TREATMENT OF RA

The drugs used in the management of RA have various side-effects, and this consideration limits the use of drugs at different stages of the disease. Some of the side effects are depicted in Table 1.

APPROACH OF HERBAL TREATMENT IN ARTHRITIS

India is known for the presence of a vast variety of herbal medicines. Ayurveda, Unani, and Siddha system in India are well-known systems that were used conventionally. In Ayurveda, a healthy body means a metabolic balance in a body. As long as people's metabolism is balanced, they will live a healthy life. At present, 60% of the world's population uses alternative systems of medicine. Both developing and developed countries are using these alternative medicines despite the availability of allopathic medicines. Alternative medicines are composed of herbals, minerals, and organic matter in the traditional system of medicines. The source of herbal drugs is naturally occurring medicinal plants. The Indian subcontinent has a plethora of medicinal plant varieties that are used conventionally. More than 20 thousand medicinal plants are recorded in India; however, more than 25 thousand herbal formulations are used as traditional and folk medicines.

The history of the Ayurvedic system of medicine in India is about 5000 years old. It includes herbal

medicines and diets for the prevention and treatment of disease. In developed countries, plants and their secondary metabolites, as well as phytocontituents, have a long history in traditional systems of medicine. In the middle of the twentieth century, the use of herbal medicines increased very rapidly. The full details, in terms of pharmacology and indication, of selected medicinal plants as herbal drugs are available in various monographs in developed countries.

At present, worldwide, people are using herbal medicines for primary health care. Herbal medicines and their active constituents are proficiently used to treat chronic disorders. The affordability of herbal drugs compared to typical pharmaceuticals is the key advantage.

NEED OF ALTERNATIVE AND COMPLIMENTARY MEDICINES IN THE TREATMENT OF RA

At present, the drugs used in the treatment of RA are not effectively capable of restoring the structure and function of damaged cartilage. Except for analgesics, the use of synthetic pharmaceuticals in the management of RA warranted the need for new, safe, and effective therapy for arthritis patients. This problem can be solved with the potential of herbal medicines. The popularity of herbal medicines and nutraceuticals now may be due to several factors, like the fact that present allopathic therapy may not be capable and people don't like it due to various side effects; the patient needs more relief from symptoms and disability; they wish to minimize the stress generated due to chronic illness; and patients also believe that herbal medicines are safe and effective as the history of use of herbal medicines is very old. These reasons augment the patient driven exploration of alternative medicines in the treatment of RA.

TRADITIONAL CHINESE MEDICINE HISTORICAL DEVELOPMENTS

The anti-inflammatory analgesic drugs have their origins in the use of extracts of salicylate containing plants, especially the bark of the willow tree (Salix alba andother members of the Salix species), in the treatment of fever, pain and inflammatory conditions (Rainsford, 2004a). These treatments date from early Chinese, Indian, African and American eras and were initially described in some detail by Romanand Greek medical authorities. During the 17th–19th centuries, the popularity of these plant extracts became evident following the publication by the Reverend Edward Stone in the 17th century of probably what were the first clinical trials of willow bark extract for the treatment of agues or fever. Isolation of the principally active salicylate components followed in the early 19th century and with advancesin chemistry in Europe and developments in the German chemical industry inthe mid-late 19th century, there followed the synthesis or salicylic and acetylsalicylic acids, the latter being highly successfully commercialized by Bayer AG asAspirin™ over 100 years ago. The historical aspects of the origins and development of aspirin and other salicylates are told in detail elsewhere (Rainsford, 2004a).During the period of the exploitation of the by-products of the coal tar industry in Germany in the 19th century came also the development of antipyretic/analgesic agents, antipyrine, aminopyrine, phenacetin and later following recognition of paracetamol (acetaminophen) as the active metabolite of phenacetin, this was eventually commercially developed for use as an analgesic/antipyretic agent in the 1950's(Prescott,
2001).

DISCOVERY OF NSAIDs

The development of the first of the category of what are now known as the nonsteroidal anti-inflammatory drugs (NSAIDs) of which aspirin has now become recognized as the progenitor, was phenylbutazone in 1946 (by JR Geigy, Basel, Switzerland) and later indomethacin in the 1960's (by Merck & Co, Rahway, NJ,USA) (Otterness, 1995). Phenylbutazone was initially employed as a combination with antipyrine in the belief it would enhance the actions of the latter. However, it emerged to have greater anti-inflammatory/analgesic activity than antipyrine and was for the best part of 30 years successfully used for the treatment of arthritic and other painful inflammatory conditions until its popularity progressively waned after associations with life-threatening agranulocytosis and bone marrow suppression (still essentially not conclusively proven today), upper gastrointestinal ulcers and bleeding and subsequent popularity of more advanced NSAIDs. Ibuprofen was developed by Boots (UK) in the 1950–1960's and after establishing its favorable safety profile at dose ranges for analgesic and anti-pyretic efficacy (up to 1200mg daily) it was the first NSAID (other than aspirin) tobe approved for non-prescription (over-the-counter or OTC sale) use in the UK(in 1963), then the USA (in1964) and later in many other countries worldwide(Rainsford, 1999). Just after ibuprofen was developed, a large number of

pharmaceutical companies undertook the discovery and development of NSAIDs with arange of chemical and biological properties (Evans & Williamson, 1987; Otterness,1995; Rainsford, 1999, 2004a; 2005a). The general chemical categorization ofthese drug classes are shown in Figure 1. Most of these drugs developed in the1960's were discovered in the pre-prostaglandin era (i.e. before Vane and his colleagues had discovered the inhibitory actions of aspirin and related drugs on the production of prostaglandins). Their anti-inflammatory, analgesic and antipyretic properties were discovered using animal models with some supportive properties being established in some biochemical systems which were known also to be important in inflammation (e.g. mitochondrial oxidative, intermediary and connective tissue collagen and proteoglycan metabolism; stability of albumin; and later oxyradicals).

Combinatorial Chemistry

Combinatorial chemistry can be used to generate new lead for a specific target, as well as powerful alternative method to optimize the initial lead. Combinatorial chemistry, may be defined as the systematic and repetitive, covalent connection of a set of different "building blocks" of varying structures to each other to yield a large array of diverse molecular entities1. In orthodox synthesis, there is a stepwise-directed synthesis of one specific product using basic fundamentals of organic chemistry A + B → C In combinatorial chemistry, we synthesize directly large number of compounds through preparing many single compounds in parallel or many compounds simultaneously in mixtures (Figure 1).This process is: (i) Faster, more efficient, cheaper and can give rise to millions of compounds in the same time, as it takes to make one compound, (ii) If we want to find a lead compound quickly and efficiently combinatorial chemistry provides a means of producing this quantity of compounds.

To increase the chances of finding a "hit", to increase the number and diversity of compounds produced; combinatorial synthesis is carried out in such a way that, mixtures of compounds are produced in each reaction flask4, allowing a single chemist to produce thousands of novel structures.

In combinatorial chemistry, the emphasis is on producing mixtures of thousand compounds. The structure of compound in a mixture is not known with certainty and the components are not separated or purified5, instead each mixture is tested for biological activity. If there is no activity than there is no need to study that mixture any more. If activity is observed then the challenge is now to identify which component(s) of mixture is active.

Milestone in combinatorial chemistry: see Table I and Figure 2.Methods in combinatorial synthesis There are two approaches by which the combinatorial libraries can be generated1. (a) Biological Library Approach

(i) Filamentous phage approach
(ii) Plasmid approach
(iii) Polysome approach

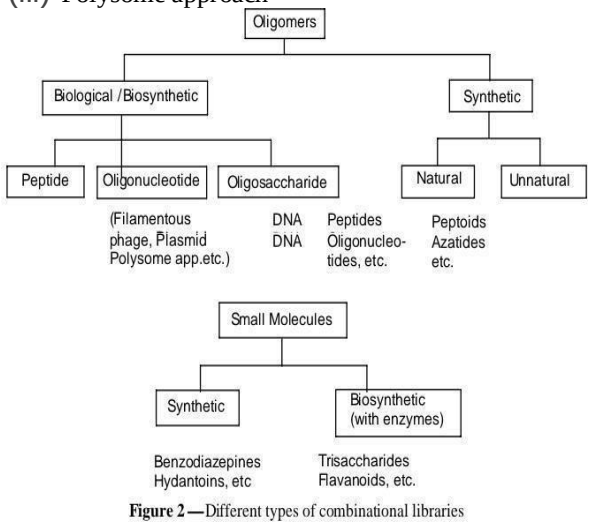

Figure 2—Different types of combinational libraries

(b) Spatially addressable parallel solid phase library approach

(i) Multi-pin methodology
(ii) Tea bag methodology
(iii) SPOTS membrane method
(iv) Light directed peptide synthesis on resin support

Except for biological library approach, which is limited to peptide libraries with eukaryotic amino acids, other synthetic approach is applicable to peptide, nonpeptide oligomers or small molecule libraries.

(a) Biological approach to generate molecular diversity

The use of biological system for the generation of peptide diversity mimics the evolutionary creation of protein diversity. Artificial evolution can be greatly enhanced by the introduction of diversity in to the system at a much higher rate than that occurs naturally. The source of the diversity in the combinatorial chemical synthesis is the structure of oligonucleotides. Oligonucleotide synthesis is a well characterized chemistry that allows tight control of the composition of mixture created. The degenerated sequence produced are then cloned and expressed as peptides.

Peptides displayed on phage particles

This method involves displaying the peptide on the much less complex surface of the bacteriophage particles. In 1988, Parmley and Smith proposed the use of filamentous phage to display random oligopeptides on the amino terminal of the viral P III coat protein. This was accomplished by the insertion of a strech of random deoxynucleotide into P III gene of filamentous phage with the help of ECoRI restriction endo nuclease. The normal function of P III is to mediate adsorptionto host cell as a prelude to the entry of phage into a bacterial cell. The guest peptide could be detected on the surface of mature phage with anti-ECoRI antibodies. This method was primarily considered as a tool for cDNA expression library cloning. However, the library of short, randomly created peptides could serve as an "epitope library" for mapping the binding specificities of antibody.

In 1990, three groups reported successful application of this approach in generating millions of random peptide libraries from which specific ligand against monoclonal antibodies8 was isolated. Subsequently, related techniques on expressing peptides in libraries of plasmids and polysomes9, 10 were developed.

The biological approach enables one to take the advantage of known protein folds (e.g. immunoglobulin fold) by grafting random oligopeptides on such tertiary folds. However, there are also some limitations like, (i) the biological approach in general is limited to the 20 eukaryotic amino acid. (ii) Incorporation of unnatural amino acid or other organic moieties into this library is not feasible.

(ii) Spatially addressable parallel solid phase library approach

The desire to develop and explore SAR around peptide lead compound has placed tremendous demands on the productivity of peptide chemistry. Over the last 15-20 years variety of methods have been developed that permit simultaneous synthesis of multi peptides. Brief overview of the main methods is provided below.

Multi pin methodology

In this method, the synthesis, of peptides takes place on polyethylene pins (4×40 mm) functionalized with acrylic acid arranged in 96 well formats12. The wells contain activated amino acid monomers. Peptide synthesis is carried out at the end of a spacer (e.g. NB - Fmoc - β - alanyl - 1, 6-diaminohexane). Screening is done by means of enzyme linked immunosorbent assay (ELISA) to determine the binding capability of covalently bound peptide to antibodies (Figure 3).

(a) Tea bag method

Houghten first developed this method of multiple peptide synthesis13. The peptide synthesis occurs on resin that is sealed inside polypropylene bags. Amino acids are coupled to the resin by placing the bag in solution of the appropriate individual activated monomers. All common steps such as resin washing and amino group deprotection are performed simultaneously. At the end of synthesis, each bag contains a single peptide (Figure 4).

(b) SPOTS membrane method

Frank (1992) has followed Geysens strategy except that a cellulose membrane or paper was used instead of the polyethylene pins as the solid support for peptides synthesis.

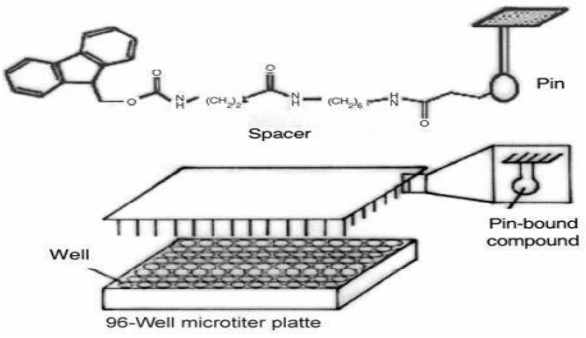

Figure 3 — Multi-pin methodology

Figure 1: MULTI PIN METHODOLOGY

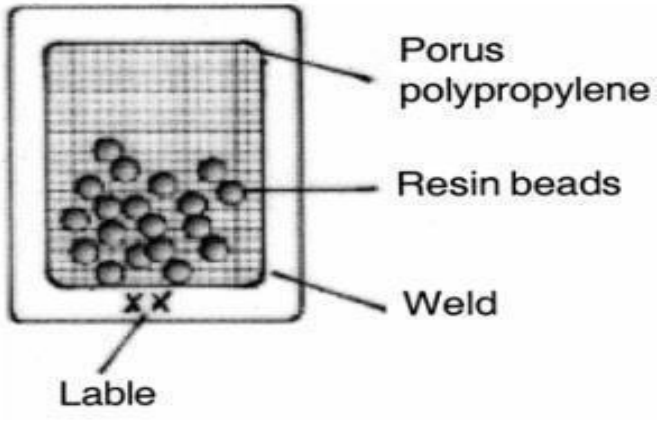

Figure 4—Tea bag method

Figure 2:TEA BAG METHOD

d) Light directed spatially addressable parallel chemical synthesis

A scheme of combinatorial synthesis in which the identity of a compound is given by its location on a synthesis substrate is termed as spatially addressable synthesis. Here the combinatorial process is carried out by controlling the addition of a chemical reagent to specific location on a solid support. This technique combines two technologies: (i) Solid phase peptides synthesis chemistry and (ii) Photolithography. The key point of this technology is shown in Figure 5.

A synthesis substrate is prepared for amino acid coupling through the covalent attachment of photo labile nitro veratryl oxy carbonyl (NVOC) protected amino linker, light is used to selectively activate a specified region of the synthesis support for coupling. Removal of the photo labile protecting groups by light (deprotection) results in activation of selected areas. After activation the first set of amino acids, each bearing a photo labile protecting group on the amino terminus is exposed to the entire surface. Amino acid coupling only occurs in region that was addressed by light in the preceding step. The solution of amino acid is removed and the substrate is again illuminated through a second mask, activating a different region for reaction with a second protected building lock. The pattern of masks and sequence of reactance define the products and their location. Since this process utilizes photolithographic technique, the number of compounds that can be synthesized is limited only by number of synthesis sites that can be addressed with appropriate resolution. The position of the compound is precisely known hence, its interaction with other molecules can be directly assessed.

Combinatorial chemistry can be applied to: (i) Solution phase synthesis (ii) Solid phase synthesis. In solution phase synthesis, the library members are typically synthesized as individual compound, so called parallel synthesis. On solid support, the split and mix technique as well as parallel synthesis can be applied. **(i) Solution phase**

synthesis

The solution phase synthesis involves conducting chemical reaction simultaneously, preferably in well-ordered sets (arrays) of reaction vessels in solution for example, the preparation of small array of amides, which consists of placing different acid chlorides and amines in each of matrix reaction vessel (along with tertiary amine to neutralize liberated hydrochloric acid), incubating and performing liquid-liquid extraction. Evaporation of the solvent gives crude amides, which can be tested directly in biological assay. The main disadvantage of this method is when number of reagents are taken together in solution, it can result in several side reactions and may lead to polymerization giving a tarry mass. Therefore, to avoid this, the new approach is developed in which all chemical structure combinations are prepared separately, in parallel on a given building block using an automated robotic apparatus. Hundreds and thousands of vials are used to perform the reactions and laboratory robots are programmed to deliver specific reagents to each vial.

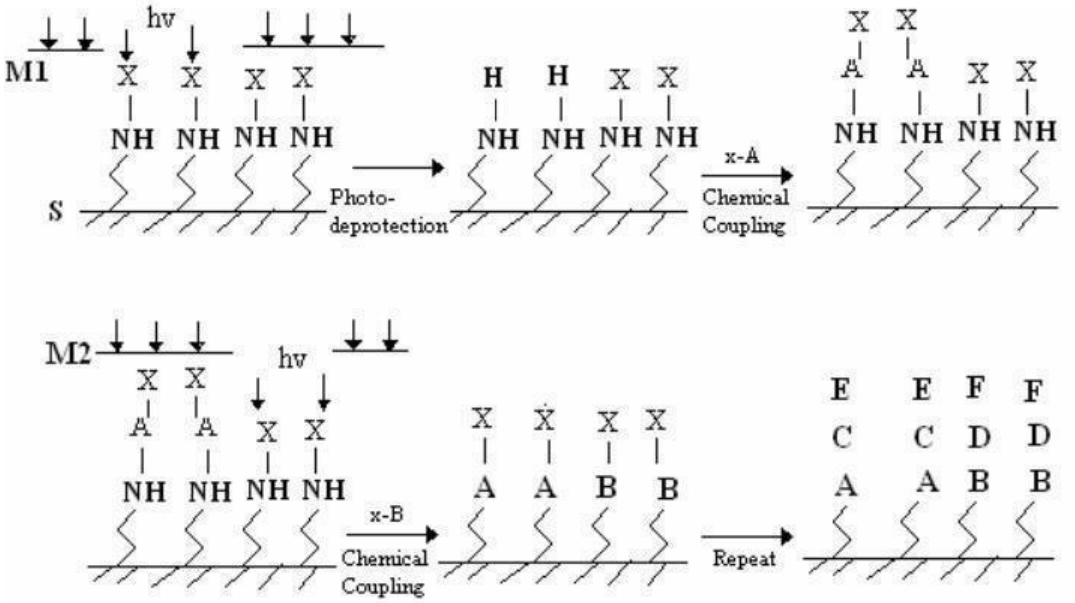

Figure 5 — Concept of Light directed spatially addressable parallel chemical synthesis

(ii) Solid phase synthesis

In this method, the reaction is carried out on a solid support such as resin beads4, a range of different starting materials can be bound to separate resin beads, which are mixed, such that all the starting material can be treated with another reagent in a single experiment. Since the products are bound to solid support, excess reagents or byproducts can be easily removed by washing with appropriate solvent. Large excess of reagent solvents can be used to drive the reaction to completion. Intermediates in reaction sequences are bound to the bead and need not be purified. Individual beads can be separated at the end of the experiment to get individual products; the polymeric support can be regenerated and reused if appropriate cleavage conditions and suitable anchor/linker groups are chosen. There are certain advantages of the solid phase synthesis over the solution phase synthesis, which includes synthesis on a polymeric support greatly, simplifies the problem of product isolation from reaction mixture, moreover we can take the advantage of the support-tethered diversity in the design of convenient receptor binding assay for library evaluation. The use of solid support for organic synthesis relies on three interconnected requirements:

(i) Polymeric solid support
(ii) A linker
(iii) Protecting groups

(i) Polymeric solid support

The choice of solid support depends on the type of chemistry of reaction. In addition, resin used must be stable under all those reaction conditions (Figure 6).

(ii) Linker

The linker is the molecule that sits between our compound and the solid support. The linker's role is to keep our compound attached to the solid support during synthesis and allows us to cleave off the final product in a high yield under conditions that do not destroy the product (Figure 7).

(iii) Protecting group

Protecting groups are important for blocking and regenerating certain functional groups in a reaction sequence. Some examples of the protecting groups are as follows. Fmoc (Fluoromethoxy carbonyl benzyl ester) and Boc (Tertiarybutyloxy carbonyl).

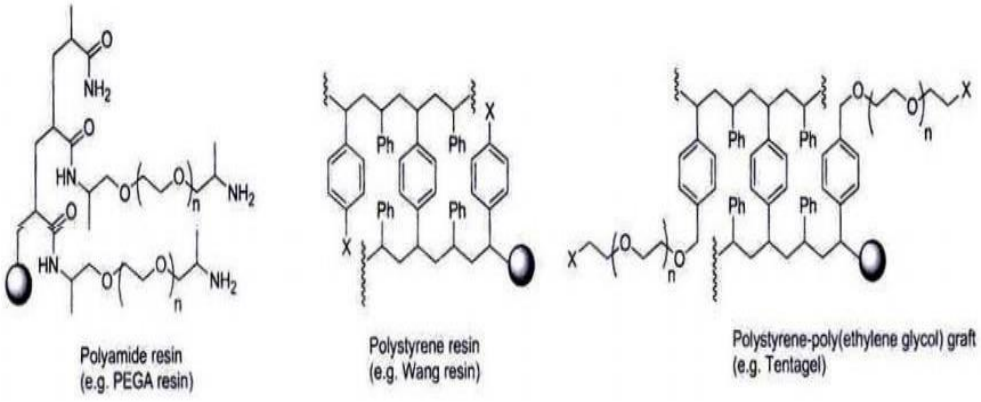

Figure 6—Example of polymeric support[15, 16]

Figure 7— example of linkers

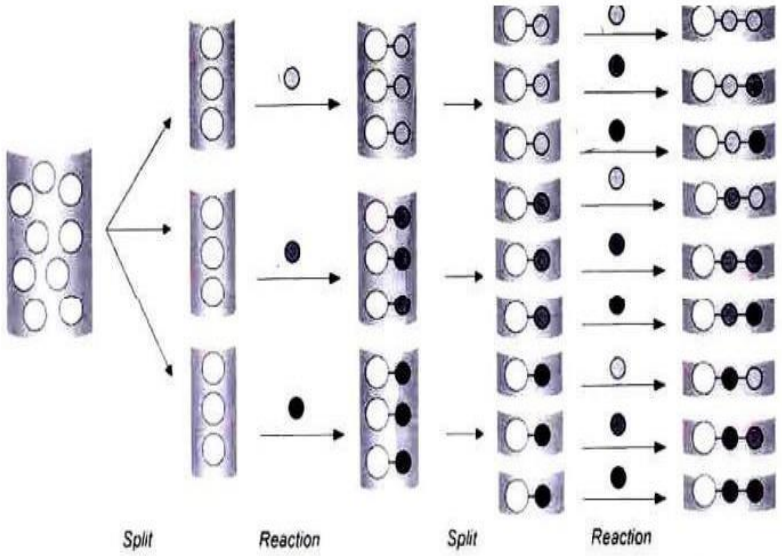

Figure 8—Parallel synthesis

The committee for the purpose of control and supervision of experiments on animals (CPCSEA) Guidelines for the care and use of laboratory: Animals:

Good Laboratory Practices (GLP) for animal facilities is intended to assure quality maintenance and safety of animals used in laboratory studies while conducting biomedical and behavioral research and testing of products.

GOAL:

The goal of these Guidelines is to promote the humane care of animals used in biomedical and behavioral research and testing with the basic objective of providing specifications that will enhance animal wellbeing, quality in the pursuit of advancement of biological knowledge that is relevant to humans and animals.

VETERINARY CARE:

Adequate veterinary care must be provided and is the responsibility of a veterinarian or a person who has training or experience in laboratory animal sciences and medicine. Daily observation of animals can be accomplished by someone other than a veterinarian; however, a mechanism of direct and frequent communication should be adopted so that timely and accurate information on problems in animal health, behavior, and well-being is conveyed to the attending veterinarian. The veterinarian can also contribute to the establishment of appropriate policies and procedures for ancillary aspects of veterinary care, such as reviewing protocols and proposals, animal husbandry and animal welfare; monitoring occupational health hazards containment, and zoonosis control programs; and supervising animal nutrition and sanitation. Institutional requirements will determine the need for full-time or part-time or consultative veterinary services

QUARANTINE, STABILIZATION AND SEPARATION:

Quarantine is the separation of newly received animals from those already in the facility until the health and possibly the microbial status of the newly received animals have been determined. An effective quarantine minimizes the chance for introduction of pathogens into an established colony. A minimum duration of quarantine for small lab animals is one week and larger animals is 6 weeks (cat, dog and monkey) Effective quarantine procedures should be used for nonhuman primates to help limit exposure of humans to zoonotic infections. Regardless of the duration of quarantine, newly received animals should be given a period for physiologic, psychologic and nutritional stabilization before their use. The length of time stabilization will depend on the type and duration of animal transportation, the species involved and the intended use of the animals.

Physical separation of animals by species is recommended to prevent interspecies disease.

OF DISEASE SURVEILLANCE, DIAGNOSIS, TREATMENT AND CONTROL:

All animals should be observed for signs of illness, injury, or abnormal behavior by animal house staff. As a rule, this should occur daily, but more-frequent observations might be warranted, such as during postoperative recovery or when animals are ill or have a physical deficit. It is imperative that appropriate methods be in place for disease surveillance and diagnosis (Annexure 1 and 2). Unexpected deaths and signs of illness, distress, or other deviations from normal health condition in animals should be reported promptly to ensure appropriate and timely delivery of veterinary medical care. Animals that show signs of a contagious disease should be isolated from healthy animals in the colony. If an entire room of animals is known or believed to be exposed to an infectious agent (e.g., Mycobacterium tuberculosis in non-human primates), the group should be kept intact and isolated during the process of diagnosis, treatment, and control. Diagnostic clinical laboratory may be made available

ANIMAL EXPERIMENTATION INVOLVING HAZARDOUS AGENTS:

Institutions should have policies governing experimentation with hazardous agents. Institutional Biosafety Committee whose members are knowledgeable about hazardous agents are in place in most of the higher-level education, research institutes and in many pharmaceutical industries for safety issues. This committee shall also examine the proposal on animal experiments involving hazardous agents in addition to its existing functions (Annexure – 8). Since the use of animals in such studies requires special consideration, the procedures and the facilities to be used must be reviewed by both the Institutional Biosafety Committee and Institutional Animal Ethics Committee (IAEC).

DURATIONS OF EXPERIMENTS:

No animal should be used for experimentation for more than 3 years unless adequate justification is provided.

PHYSICAL RESTRAINT OF ANIMALS FOR

EXAMINATION:

Brief physical restraint of animals for examination, collection of samples, and a variety of other clinical and experimental manipulations can be accomplished manually or with devices be suitable in size and design for the animal being held and operated properly to minimize stress and avoid injury to the animal. Prolonged restraint of any animal, including the chairing of non-human primates, should be avoided unless essential to research objectives. Less restrictive systems, such as the tether system or the pole and collar system, should be used when compatible with research objectives. The following are important guidelines for the use of restraint equipment's: Restraint devices cannot be used simply as a convenience in handling or managing animals. The period of restraint should be the minimum required to accomplish the research objectives. Animals to be placed in restraint devices should be given training to adapt to the equipment. Provision should be made for observation of the animal at appropriate intervals. Veterinary care should be provided if lesions or illness associated with restraint are observed. The presence of lesions, illness, or severe behavioral change should be dealt with by the temporary or permanent removal of the animal from restraint.

FUNCTIONAL AREAS OF ANIMAL HOUSE:

The size and nature of a facility will determine whether areas for separate service functions are possible or necessary. Sufficient animal area is required to: ÿ ensure separation of species or isolation of individual projects when necessary ÿ receive, quarantine, and isolate animals and ÿ provide for animal housing. In facilities that are small, maintain few animals or maintain animals under special conditions (e.g., facilities exclusively used for housing germfree colonies or animals in runs and pens) some functional areas listed below could be unnecessary or included.

PHYSICAL FACILITIES OF ANIMAL HOUSE:

(a) Building materials: should be selected to facilitate efficient and hygienic operation of animal facilities. Durable, moisture-proof, fire-resistant, seamless materials are most desirable for interior surfaces including vermin and pest resistance.

(b) Corridor(s):

Should be wide enough to facilitate the movement of personnel as well as equipment's and should be kept clean.

(c) Utilities

such as water lines, drain pipes and electrical connections should preferably be accessible through service panels or shafts in corridors outside the animal rooms.

(d) Animal room doors

Doors should be rust, vermin and dust proof. They should fit properly within their frames and provided with an observation window. Door closures may also be provided. Rodent barriers can be provided in the doors of the small animal facilities.

(e) Exterior windows

Windows are not recommended for small animal facilities. However, where power failures are frequent and backup power is not available, they may be necessary to provide alternate source of light and ventilation. In primate rooms, windows can be provided.

(f) Floors

Floors should be smooth, moisture proof, nonabsorbent, skid-proof, resistant to wear, acid, solvents, adverse effects of detergents and disinfectants. They should be capable of supporting racks, equipment, and stored items without becoming gouged, cracked, or pitted, with minimum number of joints. A continuous moisture-proof membrane might be needed. If sills are installed at the entrance to a room, they should be designed to allow for convenient passage of equipment. (g)Drains

Floor drains are not essential in all rooms used exclusively for housing rodents. Floor in such rooms can be maintained satisfactorily by wet vacuuming or mopping with appropriate disinfectants or cleaning compounds. Where floor drains are used, the floors should be sloped and drain taps kept filled with water or corrosion free mesh. To prevent high humidity, drainage must be adequate to allow rapid removal of water and drying of surfaces.

(h)Walls and ceilings

Walls should be free of cracks, unsealed utility penetrations, or imperfect junctions with doors, ceilings, floors and corners. Surface materials should be capable of withstanding scrubbing with detergents and disinfectants and the impact of water under high pressure

(i) Storage areas:

Separate storage areas should be designed for feed, bedding, cages and materials not in use. Refrigerated storage, separated from other cold storage, is essential for storage of dead animals and animal tissue waste. Facilities for sanitizing equipment and supplies An area for sanitizing

cages and ancillary equipment is essential with adequate water supply.

(j) Experimental area:

All experimental procedures in small animals should be carried out in a separate area away from the place where animals are housed. For larger animal functional areas for aseptic surgery should include a separate surgical support area, a preparation area, the operating room or rooms, and an area for intensive care and supportive treatment of animals.

ENVIRONMENT:

(a) Temperature and humidity control:

Air conditioning is an effective means of regulating these environmental parameters for laboratory animals. Temperature and humidity control prevents variations due to changing climatic conditions or differences in the number and kind of room occupants. Ideally, capability should be provided to allow variations within the range of approximately 18 to 29°C (64.4 to 84.2øF), which includes the temperature ranges usually recommended for common laboratory animals. The relative humidity should be controllable within the range of 30% to 70% throughout the year. For larger animals a comfortable zone (18 to 37°C) should be maintained during extreme summer by appropriate methods for cooling.

(b) Ventilation: In renovating existing or in building new animal facilities, consideration should be given to the ventilation of the animals' primary enclosures. Heating, ventilating, and airconditioning systems should be designed so that operation can be continued with a standby system.

The animal facility and human occupancy areas should be ventilated separately.

(c) Power and lighting:

The electrical system should be safe and provide appropriate lighting and a sufficient number of power outlets. It is suggested that a lighting system be installed that provides adequate illumination while people are working in the animal rooms and a lowered intensity of light for the animals.

Fluorescent lights are efficient and available in a variety of acceptable fixtures. A time-controlled lighting system should be used to ensure a regular diurnal lighting cycle wherever required.

Emergency power should be available in the event of power failure.

(d) Noise control:

The facility should be provided with noise free environment. Noise control is an important consideration in designing an animal facility. Concrete walls are more effective than metal or plaster walls in containing noise because their density reduces sound transmission.

ANIMAL HUSBANDRY:

(A) Caging or housing system:

The caging or housing system is one of the most important elements in the physical and social environment of research animals. It should be designed carefully to facilitate animal well-being, meet research requirements, and minimize experimental variables. The housing system should: ÿ provide space that is adequate, permit freedom of movement and normal postural adjustments, and have a resting place appropriate to the species; (Annexure – 3) ÿ provide a comfortable environment ÿ provide an escape proof enclosure that confines animal safety ÿ provide easy access to food and water; ÿ provide adequate ventilation ÿ meet the biological needs of the animals, e.g., maintenance of body temperature, urination, defecation and reproduction the animals dry and clean, consistent with species requirements ÿ facilitate research while maintaining good health of the animals. They should be constructed of sturdy, durable materials and designed to minimize cross-infection between adjoining units. Polypropylene, polycarbonate and stainless-steel cages should be used to house small lab animals, Monkeys should be housed in cages made of steel or painted mild steel and for other animals such as sheep, horses, the details can be seen in Annexure – 3. To simplify servicing and sanitation, cages should have smooth, impervious surfaces that neither attract nor retain dirt and a minimum number of ledges, angles, and corners in which dirt or water can accumulate. The design should allow inspection of cage occupants without disturbing them. Feeding and watering devices should be easily accessible for filling, changing, cleaning and servicing. Cages, runs and pens must be kept in good condition to prevent injuries to animals, promote physical comfort, and facilitate sanitation and servicing. Particular attention must be given to eliminate sharp edges and broken wires, keeping cage floors in good condition.

(B) sheltered or outdoor housing:

When animals are maintained in outdoor runs, pens, or other large enclosures, there must be protection from extremes in temperature or other harsh weather conditions and adequate protective and escape mechanism for submissive animals, as in case of monkeys by way of an indoor portion of a run,

should be provided. Shelter should be accessible to all animals, have sufficient ventilation, and be designed to prevent buildup of waste materials and excessive moisture. Houses, dens, boxes, shelves, perches, and other furnishings should be constructed in a manner and made of materials that allow cleaning or replacement in accordance with generally accepted husbandry practices when the furnishings are soiled or worn-out. Ground-level surfaces of outdoor housing facilities can be covered with absorbent bedding, sand, gravel, grass, or similar material that can be removed or replaced when needed to ensure appropriate sanitation. Buildup of animal waste and stagnant water should be avoided for example, by using contoured or drained surface. Other surfaces should be able to withstand the elements and be easily maintained.

(C) Social environment:

The social environment includes all interactions among individuals of a group or among those able to communicate. The effects of social environment on caged animals vary with the species and experience of the animals. In selecting a suitable social environment, attention should be given to whether the animals are naturally territorial or communal and whether they will be housed singly or in groups. When appropriate, group housing should be considered for communal animals. In grouping animals, it is important to take into account population density and ability to disperse; initial familiarity among animals; and age, sex, and social rank. Population density can affect reproduction, metabolism, immune responses, and behavior. Group composition should be held as stable as possible, particularly for canine, non-human primates, and other highly social mammals, because mixing of groups or introducing new members can alter behavioral and physiological functions. Non-human primates should have a run for free ranging activities.

Food:

Animals should be fed palatable, non-contaminated, and nutritionally adequate food daily unless the experimental protocol requires otherwise. Feeders should allow easy access to food, while avoiding contamination by urine and feces. Food should be available in a mounts sufficient to ensure normal growth in immature animals and maintenance of normal body weight, reproduction, and lactation in adults. Food should contain adequate nutrition, including formulation and preparation; freedom from chemical and microbial contaminants; bio-availability of nutrients should be at par with the nutritional requirement of the animal. Laboratory animal diets should not be manufactured or stored in facilities used for farm feeds or any products containing additives such as rodenticides, insecticides, hormones, antibiotics, fumigants, or other potential toxicants. Areas in which diets are processed or stored should be kept clean and enclosed to prevent entry of insects or other animals. Precautions should be taken if perishable items such as meats, fruits, and vegetables are fed, because these are potential sources of biological and chemical contamination and can also lead to variation in the amount of nutrients consumed. Diet should be free from heavy metals (e.g., lead, arsenic, cadmium, nickel, mercury), naturally occurring toxins and other contaminants. Exposure to extremes in relative humidity, unsanitary conditions, light, oxygen, and insects hasten the deterioration of food. Meats, fruits, vegetables, and other perishable items should be refrigerated if required to be stored. Unused, open food should be stored in vermin – proof condition to minimize contamination and to avoid potential spread of disease agents. Food hoppers should not be transferred from room to room unless cleaned and sanitized. The animal feed should contain moisture, crude farceuse protein, essential vitamins, minerals crude fat and carbohydrate for providing appropriate nutrition.

WATER:

Ordinarily animals should have continuous access to fresh, potable, uncontaminated drinking water, according to their requirements. Periodic monitoring of microbial contamination in water is necessary. Watering devices, such as drinking tubes and automatic waterers if used should be examined routinely to ensure their proper operation. Sometimes it is necessary to train animals to use automatic watering devices. It is better to replace water bottles than to refill them, however, if bottles are refilled, care should be taken that each bottle is replaced on the cage which it was removed.

BEDDING:

Bedding should be absorbent, free of toxic chemicals or other substances that could injure animals or personnel, and of a type not readily eaten by animals. Bedding should be used in amounts sufficient to keep animals dry between cage changes without coming into contact with watering tubes. Bedding should be removed and replaced with fresh materials as often as necessary to keep the animals clean and dry. The frequency is a matter of professional judgement of the animal care

personnel in consultation with the investigation depending on the number of animals and size of cages. However, it is ideal to change the bedding twice a week. The desirable criteria for rodent contact bedding is ammonia binding, sterilizable, deleterious products not formed as a result of sterilization, easily stored, nondesiccating to the animal, uncontaminated, unlikely to be chewed or mouthed, non-toxic, no malodorous, nestable, disposable by incineration, readily available, remains chemically stable during use, manifests batch uniformity, optimizes normal animal behavior, nondeleterious to cage-washers, non-injurious and nonhazardous to personnel, no nutritious and nonpalatable. Nesting materials for newly delivered pups wherever can be provided (e.g., paper, tissue paper and cotton).

SANITATION AND CLEANLINESS:

Sanitation is essential in an animal facility. Animal rooms, corridors, storage spaces, and other areas should be cleaned with appropriate detergents and disinfectants as often as necessary to keep them free of dirt, debris, and harmful contamination. Cleaning utensils, such as mops, pails, and brooms, should not be transported between animal rooms. Where animal waste is removed by hosting or flushing, this should be done at least twice a day. Animals should be kept dry during such procedures. For larger animals, such as dogs, cats, and non-human primates, soiled litter material should be removed twice daily. Cages should be sanitized before animals are placed in them. Animal cages, racks, and accessory equipment's, such as feeders and watering devices, should be washed and sanitized frequently to keep them clean and contamination free. Ordinarily this can be achieved by washing solid bottom rodent cages and accessories once or twice a week and cages, racks at least monthly. Wire – bottom rodent cages for all other animals should be washed at least every 2 weeks. It is good practice to always have extra cages available so that a systematic cage-washing schedule can be maintained. Cages can be disinfected by rinsing at a temperature of 82.2oC (180oF) or higher for a period long enough to ensure the destruction of vegetative pathogenic organisms. Disinfection can also be accomplished with appropriate chemicals; equipment's should be rinsed free of chemicals prior to use. Periodic microbiologic monitoring is useful to determine the efficacy of disinfection or sterilization procedures. Rabbits and some rodents, such as guinea pigs and hamsters, produce urine with high concentration of proteins and minerals. Minerals and organic compounds in the urine from these animals often adhere to cage surfaces and necessitate treatment with acid solutions before washing. Water bottles, sipper tubes, stoppers, and other watering equipment should be washed and then sanitized by rinsing with water of at least 82.2oC (180oF) or appropriate chemicals agents. Hyper chlorite) to destroy pathogenic organisms, if bottles are washed by hand, powered rotating brushes at the washing sink are useful, and provision should be made for dipping or soaking the water bottles in detergents and disinfectant solutions. A two compartment sink or tub is adequate for this purpose. Some means for sterilizing equipment's and supplies, such as an autoclave or gas sterilizer, is essential when pathogenic organisms are present. Routine sterilization of cages, food and bedding is not considered essential if care is taken to use clean materials from reliable sources. Where hazardous biological, chemical, or physical agents are used, a system of equipment monitoring might be appropriate. Deodorizers or chemical agents other than germicidal should not be used to mask animal odors. Such products are not a substitute for good sanitation.

WASTE DISPOSAL:

Wastes should be removed regularly and frequently. All waste should be collected and disposed of in a safe and sanitary manner. The most preferred method of waste disposal is incineration. Incinerators should be in compliance with all central, state, and local regulations. Waste cans containing animal tissues, carcasses, and hazardous wastes should be lined with leakproof, disposable liners. If wastes must be stored before removal, the waste storage area should be separated from other storage facilities and free of flies, cockroaches, rodents, and other vermin. Cold storage might be necessary to prevent decomposition of biological wastes. Hazardous wastes should be rendered safe by sterilization, contamination, or other appropriate means before they are removed from an animal facility for disposal.

RECORD KEEPING:

Training record of staff involved in animal activities ÿ Water analysis report The animal house should maintain the following records: ÿ Animal house plans, which includes typical floor plan, all fixtures etc. ÿ Animal house staff record-both technical and non -technical ÿ Health record of staff/ animals ÿ All standard operating procedures (SOPs) relevant to the animals ÿ Breeding, stock, purchase and sales records ÿ Minutes of institute Animals Ethics Committee Meetings ÿ Records of experiments conducted with the number of animals used (copy of Form D) ÿ Death Record ÿ Clinical

record of sick animals.

PERSONNEL AND TRAINING:

The selection of animal facility staff, particularly the staff working in animal rooms or involved in transportation, is a critical component in the management of an animal facility. The staff must be provided with all required protective clothing (masks, aprons, gloves and gumboots and other footwear) while working in animal rooms. Facilities should be provided for change over with lockers, wash basin, toilets and bathrooms to maintain personal hygiene. It is also important a regular medical check-up is arranged for the workers to ensure that they have not picked up any zoonotic infection and also that they are not acting as a source of transmission of infection to the animals. The animal house in-charge should ensure that persons working in animal house do not eat, drink, smoke in animal room and have all required vaccination, particularly against tetanus and other zoonotic diseases. Initial in-house training of staff at all levels is essential. A few weeks must be spent on the training of the newly recruited staff, teaching them the animal handling techniques, cleaning of cages and importance of hygiene, disinfection and sterilization. They should also be made familiar with the activities of normal healthy and sick animals so that they are able to spot the sick animal during their daily routine checkup of cages (Annexure – 7).

ANAESTHESIA AND EUTHANASIA:

The scientists should ensure that the procedures, which are considered painful, are conducted under appropriate anesthesia as recommended for each species of animals. It must also be ensured that the anesthesia is given for the full duration of experiment and at no stage the animal is conscious to perceive pain during the experiment. If at any stage during the experiment the investigator feels that he has to abandon the experiment or he has inflicted irreparable injury, the animal should be sacrificed. Neuromuscular blocking agents must not be used without adequate general anesthesia (Annexure – 5). In the event of a decision to sacrifice an animal on termination of an experiment or otherwise, an approved method of euthanasia should be adopted (Annexure – 6) and the investigator must ensure that the animal is clinically dead before it is sent for disposal.

The data about large animals, which have been euthanized, should be maintained.

Anesthesia:

Unless contrary to the achievement of the results of study, sedatives, analgesics and anesthetics should be used to control pain or distress under experiment. Anesthetic agents generally affect cardiovascular, respiratory and thermo-regulatory mechanism in addition to central nervous system. Before using actual anesthetics, the animal is prepared for anesthesia by overnight fasting and using pre-anesthetics, which block parasympathetic stimulation of cardio-pulmonary system and reduce salivary secretion. Atropine is the most used anticholinergic agent. Local or general anesthesia may be used, depending on the type of surgical procedure. Local anesthetics are used to block the nerve supply to a limited area and are used only for minor and rapid procedures. This should be carried out under expert supervision for regional infiltration of surgical site, nerve blocks and for epidural and spinal anesthesia. Several general aesthetic agents are used in the form of inhalants. General anesthetics are also used in the form of intravenous or intramuscular injections such as barbiturates. Species characteristics and variation must be kept in mind while using an aesthetic. Side effects such as excessive salivation, convulsions, excitement and disorientation should be suitably prevented and controlled. The animal should remain under veterinary care till it completely recovers from anesthesia and postoperative stress. Euthanasia: is resorted to events where an animal is required to be sacrificed on termination of an experiment or otherwise for ethical reasons. The procedure should be carried out quickly and painlessly in an atmosphere free from fear or anxiety. For accepting a euthanasia method as humane it should have an initial depressive action on the central nervous system for immediate insensitivity to pain. The choice of a method will depend on the nature of study, the species of animal to be killed (Annexure – 6). The method should in all cases meet the following requirements: (a) Death, without causing anxiety, pain or distress with minimum time lag phase. (b) Minimum physiological and psychological disturbances. (c) Compatibility with the purpose of study and minimum emotional effect on the operator. (d) Location should be separate from animal rooms and free from environmental contaminants. Tranquilizers have to be administered to larger species such as monkeys, dogs and cats before a euthanasia procedure.

PRECLINICAL STUDY

In this study we report that R-etodolac (SDX-101), at clinically relevant concentrations, induces potent cytotoxicity in drug-sensitive multiple myeloma (MM) cell lines, as well as in dexamethasone

(MM.1R)-, doxorubicin (Dox40/RPMI8226)-, and bortezomib (DHL4)resistant cell lines. Immunoblot analysis demonstrates that R-etodolac induces apoptosis characterized by caspase-8, -9, and -3 and PARP (poly-ADP [adenosine diphosphate]ribose polymerase) cleavage and down-regulation of cyclin D1 expression. Subcytotoxic doses of R-etodolac up-regulate myeloid cell leukemia-1 proapoptotic variant (Mcl-1S), while enhancing dexamethasone (Dex)induced caspase activation and apoptosis. The combination of R-etodolac with Dex results in a highly synergistic cytotoxic effect. Retodolac also induces apoptosis against primary cells isolated from patients with MM refractory to chemotherapy. Although interleukin 6 (IL-6) and insulin-like growth factor-1 (IGF-1) abrogate Dex-induced MM cell cytotoxicity, neither IL-6 nor IGF-1 protects against R-etodolac-induced cytotoxicity in MM cells. R-etodolac also inhibits viability of MM cells adherent to bone marrow stromal cells (BMSCs), thereby overcoming a mechanism of drug resistance commonly observed with other conventional chemotherapeutic agents. Our data, therefore, indicate that R-etodolac circumvents drug resistance in MM cells at clinically relevant concentrations, targets Mcl-1, and can be synergistically combined with Dex. (Blood. 2005;106:706-712)

Multiple myeloma (MM) remains an incurable plasma cell malignancy, despite treatment with alkylating agents, anthracyclines, corticosteroids,1,2 as well as high-dose chemotherapy combined with stem cell transplantation,3, 4, 5 because of, at least in part, both intrinsic and acquired drug resistance.6, 7, 8 Although initial treatment with conventional drugs such as dexamethasone (Dex) effectively induces death in MM cells, prolonged drug exposure results in the development of drug resistance. Furthermore, the bone marrow (BM) microenvironment confers drug resistance in MM cells via at least 2 different mechanisms: (1) cell adhesion-mediated drug resistance (CAM-DR) through adhesion of MM cells to fibronectin found on BM stromal cells (BMSCs) and (2) activation of phosphatidylinositol 3-kinase (PI3-K)/a PI3-K target (Akt) and/or Janus kinase 2 (JAK2)/signal transducers and activators of transcription 3 (STAT3) signaling because of high levels of cytokines found in the BM milieu (such as interleukin-6 [IL-6] and insulin like growth factor-1 [IGF-1]).9, 10, 11, 12 Rationally designed treatments targeting the BM microenvironment, as well as the MM cell, can overcome drug resistance in both preclinical and early clinical studies.13, 14, 15, 16

Etodolac (1,8-diethyl-1,3,4,9-tetrahydropyrano-[3,4-b] indole-1-acetic acid) is a nonsteroidal anti-inflammatory drug (NSAID) that inhibits both cyclooxygenase 1 (COX1) and -2, and is approved for treatment of degenerative joint disease and rheumatoid arthritis.17,18 Etodolac exists as a racemic mixture of the R- and S-enantiomers, and, unlike all other chiral NSAIDs, the 2 enantiomers of etodolac are not metabolically interconvertible. The R-etodolac isoform (SDX-101) lacks significant COX inhibitory activity and, thereby, has a more favorable safety profile than the racemic etodolac because of lack of COX-dependent side effects, such as gastrointestinal toxicity.

R-etodolac was recently demonstrated to inhibit transcription of a β-catenin-dependent Tcell- and lymphoid-enhancing transcription factor (TCF/LEF) receptor gene in HEK293 cells and to diminish the in vitro survival of chronic lymphocytic leukemia (CLL) cells.19 R-etodolac also has in vivo prostate cancer antitumor activity via its direct effect on the peroxisome proliferator activated receptor-γ (PPAR-γ) and retinoid X receptor-α (RXR-α) pathways.20,21

R-etodolac (SDX-101) is currently being tested (Salmedix, San Diego, CA) in phase 2 clinical trials for treatment of refractory CLL. Reduction in average lymphocyte counts have been observed in patients with CLL administered 1000 to 2400 mg R-etodolac twice daily, at steady-state blood levels of approximately 300 to 600 μM.

REFERENCES:

1. Abadie E, Ethgen D, Avouac B, Bouvenot G, Branco J, Bruyere O, Calvo G, Devogelaer JP, Dreiser RL, Herrero-Beaumont G, Kahan A, Kreutz G, Laslop A, Lemmel EM, Nuki G, Van De Putte L, Vanhaelst L, Reginster JY; Group for the Respect of Excellence and Ethics in Science. Recommen.for the use of new methods to assess the efficacy of disease-modifying drugs in thetreatment of osteoarthritis. Osteoarthritis Cartilage. 2004;12:263–268.
2. Adams SS, Cobb R. Non-steroidal anti-inflammatory drugs. In: *Progress in Medicinal Chemistry*, 5, GP Ellis, GB West, eds. London: Butterworth, 1967, 59–133.
3. Allgayer H. Review article: mechanisms of action of mesalazine in preventing colorectal carcinoma in inflammatory bowel disease. Aliment Pharmacol Ther. 2003;18 Suppl 2:10–14.
4. Ambrosini MV, Mariucci G, Rambotti MG, Tantucci M, Covarelli C, De Angelis L, Del

Soldato P. Ultrastructural investigations on protective effects of NCX 4016 (nitro aspirin) on macrovascular endothelium in diabetic Wistar rats. J Submucosa Cytol Pathol. 2005;37:205–213.

5. Bengmark S. Curcumin, an atoxic antioxidant and natural NFkappaB, cyclooxygenase-2, lipO xygenase, and inducible nitric oxide synthase inhibitor: a shield against acute and chronic diseases. JPEN J Parenter Enteral Nutr. 2006;30:45–51.
6. Bennett A, Charlier EM, McDonald AM, Simpson JS, Stamford IF. Bone destruction by breast tumours. Prostaglandins. 1976;11:461–463.
7. Bennett A. Prostaglandins as factors in diseases of the alimentary tract. Adv Prostaglandin Thromboxane Res. 1976;2:547–555.
8. Claria J, Romano M. Pharmacological intervention of cyclooxygenase-2 and 5-lipoxygenase pathways. Impact on inflammation and cancer. Curr Pharm Des. 2005;11:3431–3447
9. Corazzi T, Leone M, Roberti R, Del Soldato P, Gresele P. Effect of nitric oxide-donating agents on human monocyte cyclooxygenase-2. Biochem Biophys Res Commun. 2003;311:897–903.
10. Corazzi T, Leone M, Maucci R, Corazzi L, Gresele P. Direct and irreversible inhibition of cyclooxygenase-1 by nitroaspirin (NCX 4016). J Pharmacol Exp Ther. 2005;315:1331–1337.
11. Crum NF, Lederman ER, Wallace MR. Infections associated with tumor necrosis factor-alpha antago nists. Medicine (Baltimore). 2005;84:291–302.