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Combinatorial chemistry: A novel method in drug discovery and its application

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Combinatorial chemistry is a new technique developed in pharmaceutical industry, which involves synthesis of compounds in mass instead of a single compound, which are screened as a whole mixture for particular biological activity. Because of the rapid synthesis of compounds, this method saves the time and cost associated with the drug discovery. This brief review article includes combinatorial strategies, screening methods and encoding technologies and some of the applications in drug discovery.

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In this new era of medicinal chemistry the target is focused on the preparation of chemical libraries for the generation of new lead for drug discovery.

Chemical libraries[1] are intentionally created collections of differing molecules, which can be prepared either synthetically or biosynthetically and screened for biological activity in variety of formats. (e.g. libraries of soluble molecules; libraries of compounds tethered to resin beads, solid support or silica chips; recombinant peptide libraries on bacteriophage and other biological display vectors; etc).

Combinatorial chemistry is one of the new techniques developed in pharmaceutical industries to reduce the time and cost associated with producing effective and competitive new drugs. It is used to create a large population of structurally different molecules called chemical libraries in a short time that can be screened in one time against a variety of targets by high throughout screening or used for pharmacological assay.

The development of ugi-multicomponent reaction in 1962 and Merrifield solid phase synthesis[2] in 1963, offered the necessary tools to synthesize libraries of small organic compounds, but the first combinatorial synthesis did not result until 20 years. Later, this method made their first appearance in the development of peptide libraries by Houghton *et al*[3]. From 1990 onwards, there was an upsurge in combinatorial synthesis and small molecules were also synthesized as multicomponent mixtures. Since then combinatorial chemistry has expanded from peptides to organic, organometallic, inorganic and polymer chemistry.

Combinatorial chemistry: The process

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 entities[1].

In orthodox synthesis, there is a stepwise-directed synthesis of one specific product using basic fundamentals of organic chemistry

$$A + B \rightarrow C$$

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.

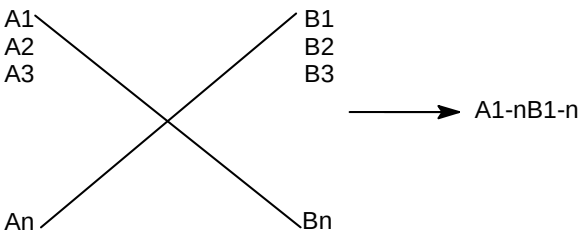


Figure 1 — Combinatorial synthesis

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 flask[4], 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 purified[5], instead each mixture is tested for biological activity as a whole. 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 generated[1].

(a) *Biological Library Approach*
- (i) Filamentous phage approach
- (ii) Plasmid approach
- (iii) Polysome approach

Table I—Milestones in combinatorial chemistry[6]

Year	Milestone
1984	Limited peptide library with the multi-pin technology
1985	Limited peptide library using tea-bag method
1986	Iterative approach on solid phase peptide library screening using the multi-pin synthesis
1986-90	Development of Polynucleotide library methods
1988	Introduction of the split synthesis method on synthesizing a limited library of solution peptides
1990	Light directed parallel peptide synthesis of a library of 1024 peptides on chip
1990	Successful application of the filamentous phage displayed peptide library method on a huge library of peptides
1991	Introduction of the one bead-one compound concept and successful application of this concept to a huge bead-bound peptide library
1991	Successful application of the iterative approach on a huge solution phase peptide library
1992	Synthesis of a limited benzodiazepine-based small molecule library
1992-93	Development of encoding methods for the one bead-one compound non-peptide library

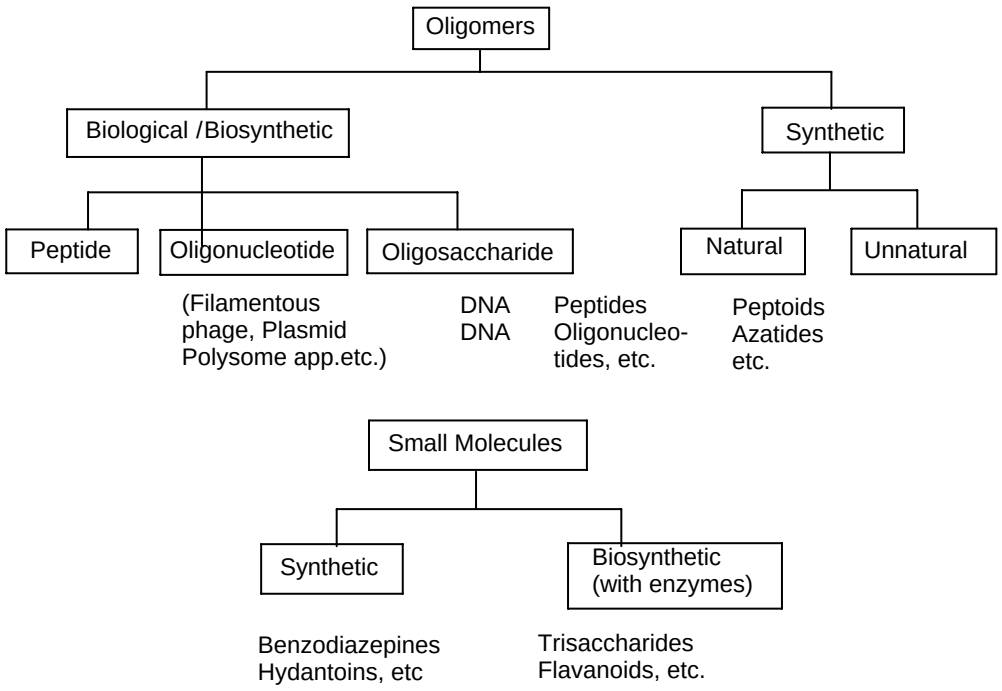


Figure 2 —Different types of combinational libraries

(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[1, 6]

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 antibodies[8] was isolated. Subsequently, related techniques on expressing peptides in libraries of plasmids and polysomes[9, 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 multipeptides. Brief overview of the main methods is provided below.

(a) Multi pin methodology[11]

In this method, the synthesis, of peptides takes place on polyethylene pins (4×40 mm) functionalized with acrylic acid arranged in 96 well formats[12]. 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**).

(b) Tea bag method

Houghten first developed this method of multiple peptide synthesis[13]. 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**).

(c) 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.

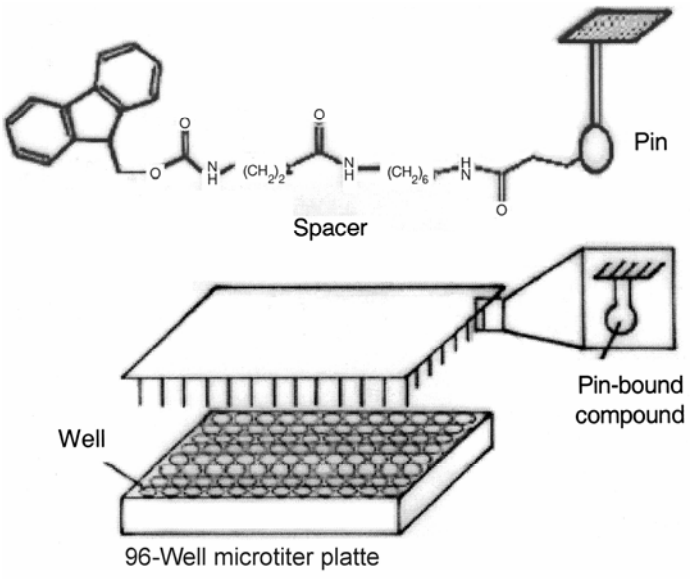


Figure 3 — Multi-pin methodology

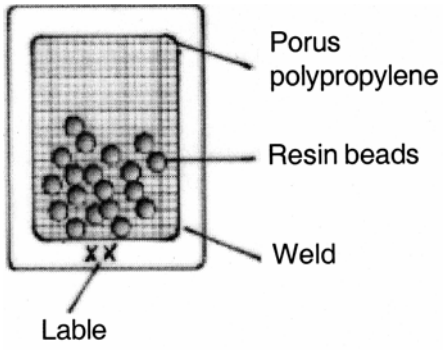


Figure 4 — Tea bag method

(d) Light directed spatially addressable parallel chemical synthesis[14]

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 nitroveratryloxycarbonyl (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

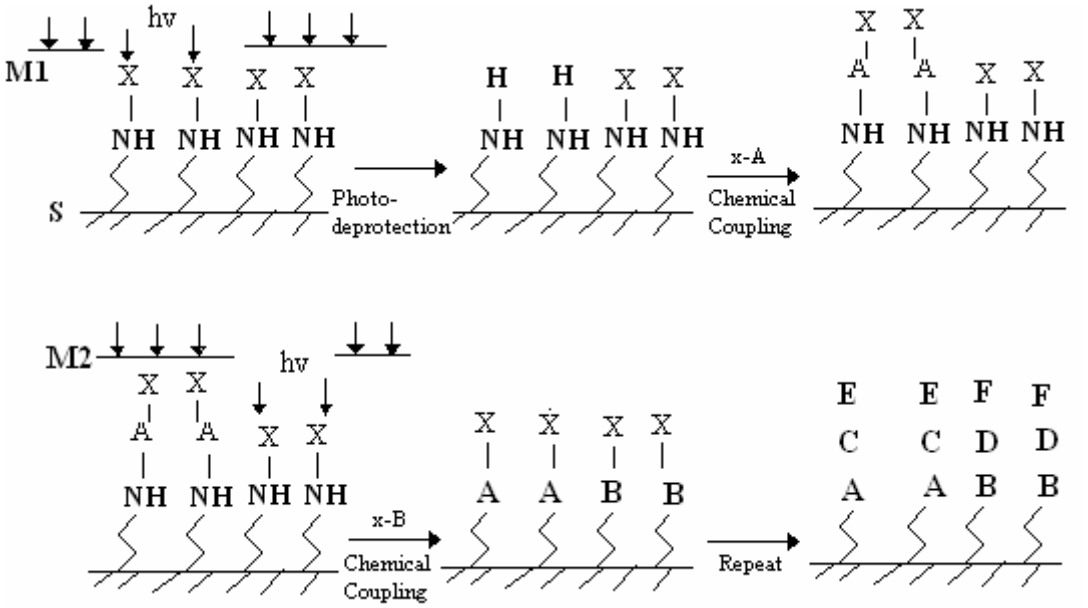


Figure 5 —Concept of Light directed spatially addressable parallel chemical synthesis

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.

(ii) Solid phase synthesis

In this method, the reaction is carried out on a solid support such as resin beads[4], a range of different starting materials can be bound to separate resin beads, which are mixed together, 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 by-products can be easily removed by washing with appropriate solvent. Large excess of reagent solvents can be used to drive the reactionto completion. Intermediates in a 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**).

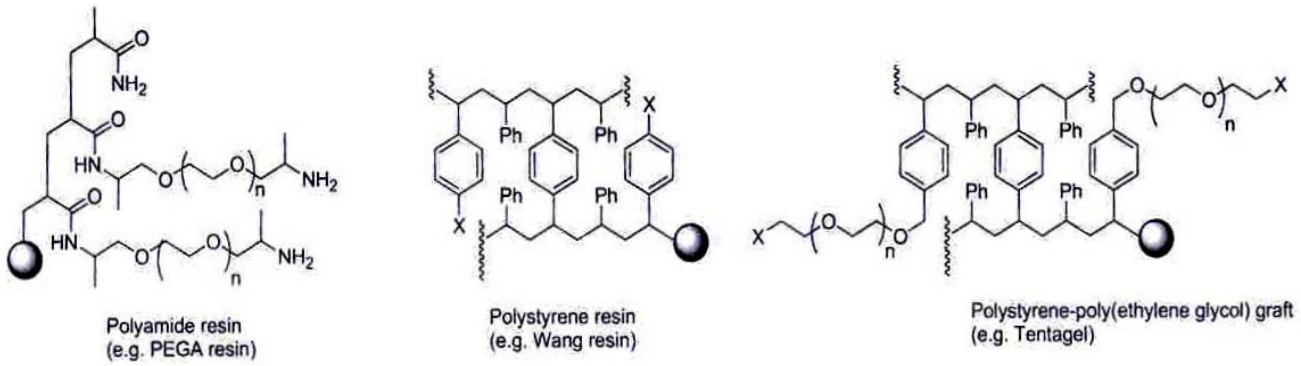


Figure 6 — Example of polymeric support[15, 16]

Figure 7 — example of linkers

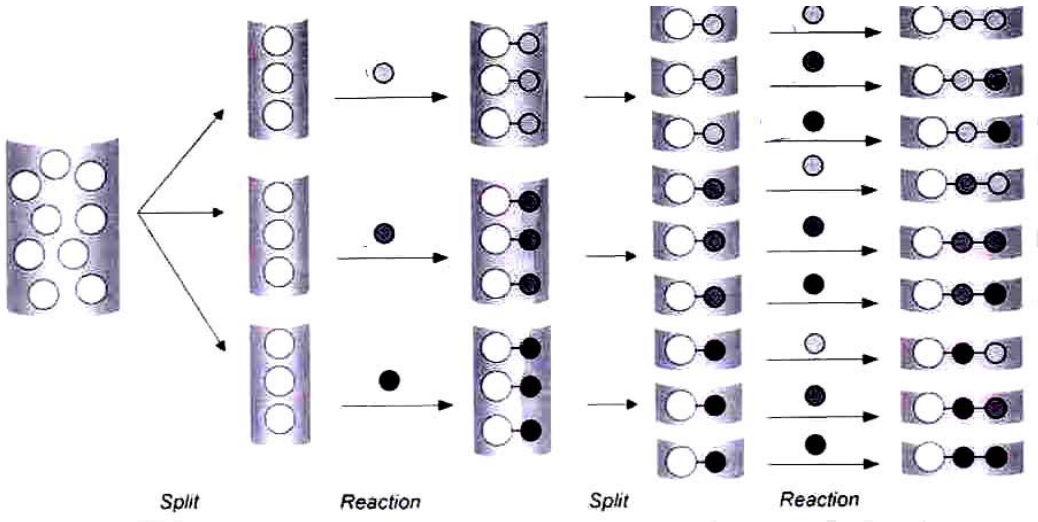


Figure 8 — Parallel synthesis

(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).

Two distinct mechanisms for elaborating molecular diversity may be defined by (i) parallel synthesis and (ii) split and mix synthesis.

(i) Parallel synthesis[4]. In this method, each starting material is reacted with each building block separately. After each reaction step, the product is split into n partitions before it is reacted with next building block (**Figure 8**).

Advantages

(a) No deconvolution is required.
(b) No risk of synergistic effects leading to false positive results during screening.

(ii) Split and mix synthesis[17]. The starting material is split in 'n' portions, reacted with n building blocks, and recombined in one flask for the second step, this procedure is repeated. This method is particularly employed for solid phase synthesis (**Figure 9**).

Advantages

(a) Large libraries are readily available.

Limitations

(a) Complex mixtures are formed.
(b) Deconvolution or tagging is required.
(c) Synergistic effect may be observed during screening, leading to false positive.

Criteria for selection of building block

The selection depends on the availability of large number of diverse, fairly complex, easily accessible starting material. Member of building block should reflect broad array of physiological properties, e.g. functionality, charge, confirmation, etc.

Screening

An essential element of the combinatorial discovery process is that one must be able to extract the information made available by library. This can be achieved by Library screening methods[11]. There are two types of screening.

(i) *Random screening*. In this, the task is to identify a lead compound in the absence of any structural information about active molecules.

(ii) *Directed screening*. In this, the objective is to evaluate closely related structure analogues of lead molecule, to establish SAR and optimize biological activity (**Table II**).

The importance of differentiating between random screenings versus directed screening is particularly related to the design of assay for library evaluation. In searching large, highly diverse libraries for novel lead compound, a premium is placed on the ability to detect rare ligands that may have modest affinity for the target receptor. The assay strategy may differ in screening analogue libraries, since here we try to develop quantitative SAR on a large number of compounds and to increase potency of a lead.

In broad terms, the assay procedures are grouped into three categories[11].

(i) Isolation of ligand by affinity purification.
(ii) Binding of receptor to immobilized ligand.
(iii) Testing the activity of libraries of soluble compounds.

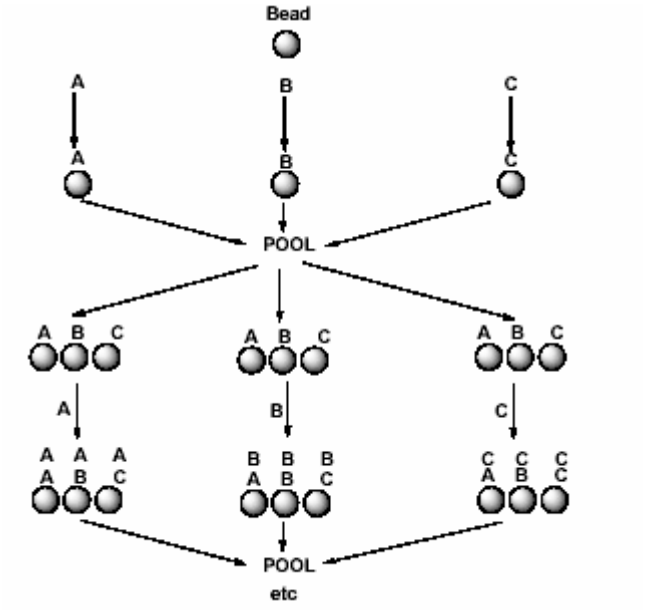


Figure 9 — Split and mix synthesis

Table II — Comparison of broad screening verses directed screening

Broad screening	Directed screening
Huge size library	Modest size library
Broadest structural diversity	Relatively narrow structural diversity.
Any building block	Specific retro combinatorial building block
No special initial structural goal	Specific structural goal
Undefined order of reaction	Specific order of combination
Flexible synthetic strategy	Well designed synthetic strategy

Each format presents different challenges with regard to the minimum affinity requirement for ligand detection, the demonstration of binding specificity and the ability to discriminate among compounds in the library on the basis of their affinities for the target.

(i) Isolation of ligands by affinity purification

Two distinct methods have been employed to achieve purification of peptide/nucleic acid complexes. The first involves incubation of a receptor in solution with the mixture of compounds. After allowing sufficient time for binding, the receptor is captured by immobilizing it on anti-receptor antibody. The second approach calls for pre-immobilization of the receptor on beads, or a chromatography support, followed by capture of the complex. In both cases, a solid support facilitates the separation and washing of receptor bound complex.

To enhance the probability of isolating peptide ligands with biological function, the receptor should be active when immobilized.

Immobilization of receptor protein on beads can be accomplished by covalent attachment. Biotinylation and immobilization on streptavidin or capture with high affinity non-blocking antibodies. But the first two processes may result in inactive protein and also some drug discovery targets may not be readily available as soluble receptors.

(ii) Binding of receptor to immobilized ligand

The various libraries of compounds bound to solid support (pins, beads, etc.) can be screened by detecting direct binding of a labeled receptor to an immobilized ligand; the identity of the ligand is determined directly by mass spectroscopy. There are numbers of important issues related to this assay, first the ability of a receptor to interact with a tethered ligand may be influenced by the site or nature of its covalent bonding with support and the chemical nature of the linkage between the ligand and support may affect receptor ligand interaction.

(iii) Testing the activity of libraries of soluble compounds

The classical method of screening for a desired biological activity is to test soluble compounds one at a time in a competition-binding assay, enzyme inhibition assay or in a cell-based bioassay.

This approach has been applied to library screening by releasing small compounds synthesized on solid support (e.g. pins, beads). The compounds on individual beads are released locally onto a lawn of confluent mammalian cells and cause activation of cells in the area surrounding the bead. The bead responsible for cell activation is isolated and detected by various methods. But**,** the activity of a given pool is based on the cumulating activity of all the compounds in the pool that is pools with the same activity may contain some high affinity compound and some low affinity compound, which will cause difficulty in separation.

Encoding

The combinatorial libraries are collection of unique molecules, which are the source of molecular diversity[18]. By the process of screening the number of this library that has "desirable properties" are sorted out. It is now very important to learn the identity of "winning" library member. The process of identification of active compound in a mixture is known as Encoding.

(a) Positional Encoding (iterative resynthesis and rescreening)

In this method the resynthesis and rescreening is carried out to know the identity of the active compound. After checking back we can isolate the active compound (**Figures 10a** and **10b**).

Limitations

(i) In large pools compound with modest activity cannot be detected.

(ii) Activity in any given pool is generally a result of the sum of more than one active compounds, the most active pool may therefore not contain the most potent compound.

(iii) Iterative resynthesis and biological testing can be time consuming and laborious.

(b) Chemical Encoding

This method is used for peptide libraries. The vast numbers of RNA sequence in a small volume of solution are generated. The solution was passed through an affinity column to which the ligands are bound. The RNA sequences that bound tightly to immobilize ligand eluted more slowly than others. The small amounts of sequences were eluted and amplified through polymerase chain reactions technology, these RNA sequences can be determined by using existing analytical techniques. By establishing the RNA sequence of 'Winner" its chemical identity becomes known.

(c) Electronic Encoding

This technique uses a micro electronic device called a Radio frequency (rf) memory tag[19]. The tag measuring 13×3 mm is encased in heavy walled glass and contains the following. A silicon chip, onto which laser-etched a unique binary code, a rectifying circuit, with which absorbed rf energy is converted to D.C. electrical energy, a transmitter/receiver circuit, an antenna, through which energy is received and rf signals are both received and sent.

Applications in Drug Discovery

The combinatorial chemistry first shows its presence in synthesis of peptide libraries. The peptide

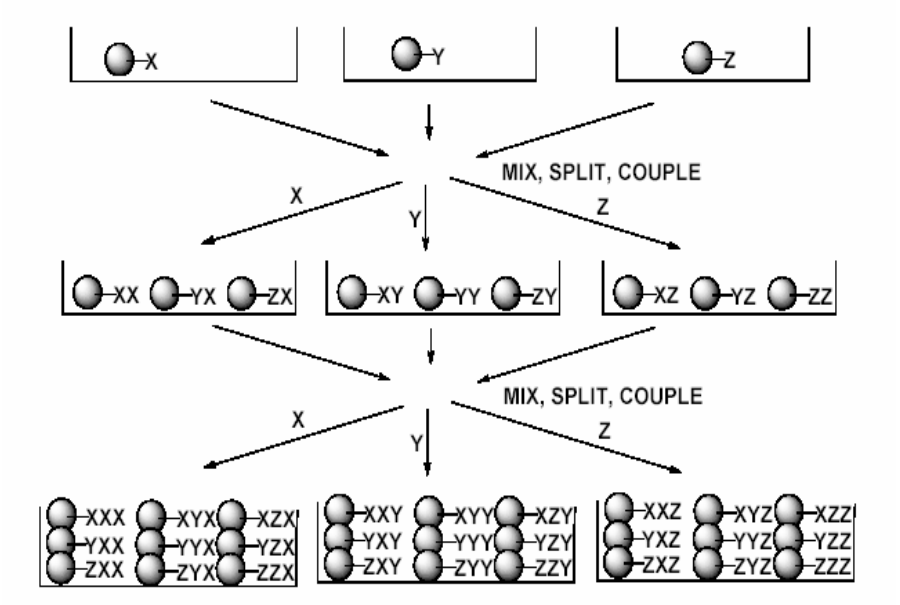


Figure 10a — Synthesis of compounds by split and mix synthesis

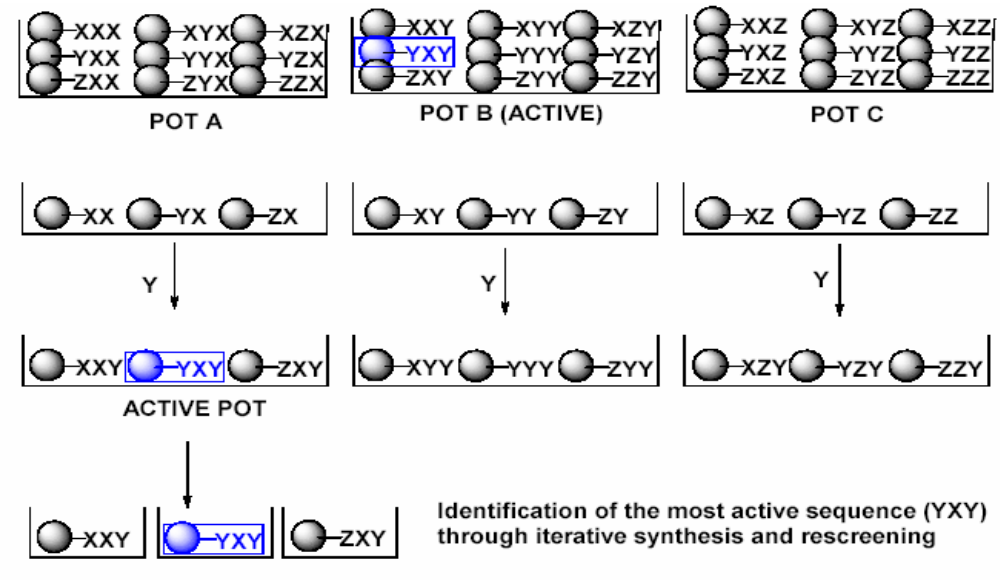


Figure 10b — Deconvolution of active compound

plays varying role in body. By the use of combinatorial chemistry we can generate vast peptide, which may be active. Biologically active peptide hormones play an important role in regulating a multitude of human physiological response and many low molecular weight bioactive peptides can act as a hormone receptor against or antagonists. In addition, peptide structure commonly is found in molecules designed to inhibit enzymes that catalyze proteolysis, phosphorylation and other past translational protein modification that may play important role in pathologies of various disease states.

A few examples of the application of combinatorial chemistry in lead optimization and drug discovery are given below.

(a) Synthesis of peptoids

Some of the polypeptides of polypeptide libraries were found to be potent inhibitors for enzyme like kinases and proteases useful in treatment of AIDS and cancer[20], but these peptides have a poor bio-availability and unfavourable pharamcokinetic properties. So, the focus has been shifted on developing synthetic peptido mimetic like peptoides,

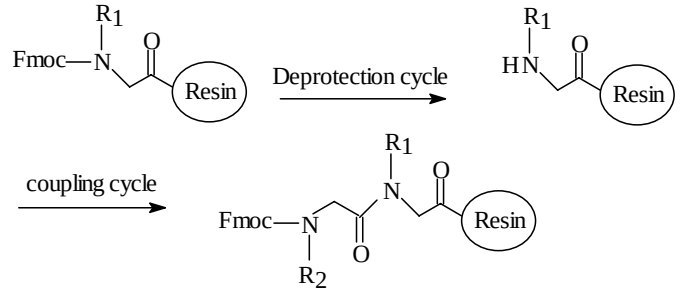
one of the synthetic diversities has been developed by Simon *et. al*[21].

This group has created a basic set of monomers N-substituted glycine units, each bearing a nitrogen substitute similar to natural α-amino acid side chain. The formal polymerization of these monomers resulted in a class of polymeric diversity which was termed as 'PEPTOIDS". Peptoides may be synthesized either by "Full monomer" oligomers synthesis "Sub monomer" oligomers synthesis **(Figure 11a** and **11b)**.

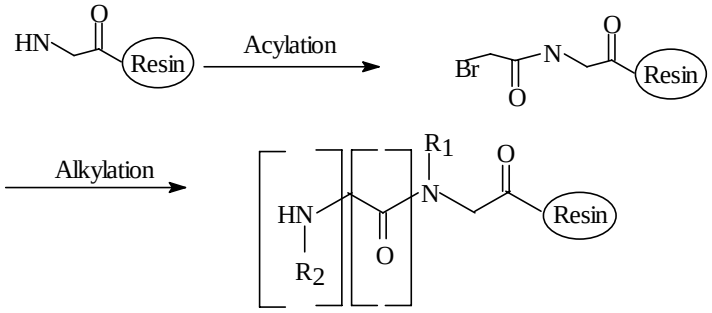
Various biological activities have been established for specific peptoid synthesized, including inhibition of α-amylase and the hepatitis-A viruses 3C protease, binding to that fat RNA of HIV, antagonism at α_1 adrenergic receptor.

(b) Combinatorial lead optimization of a Neuropeptide-FF antagonist[22] Neuropeptide-FF (Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH_2) has been identified as a high affinity ligand for a G-protein coupled receptor HLWAR 77. It is an anti-opioid and has been implicated in pain modulation, morphine tolerance, and morphine abstinence. Centrally administered Neuropeptide-FF also has been known to precipitate quasi-Morphine abstinence syndrome (QMAS) in opiate-naive animals. Therefore, antagonist of Neuropeptide-FF may allow easier management of withdrawal symptoms that adversely affect the treatment of opiate abuse.

Desaminotyrosyl-Phe-Leu-Phe-Gln-Pro-Gln-Arg-NH_2, the first antagonist of Neuropeptide-FF was discovered but this analogue does not show any CNS bioavailability after systemic administration and, thus could not be considered as a potential lead compound. Derivatization with 5-(dimethylamino)-1-naphthalenesulfonyl (dansyl) at the secondary amino group of the N-terminal proline residue of the tripeptide Pro-Gln-Arg-NH_2, obtained from the sequence of



(a) Full monomer oligomers synthesis



(b) Submonomer oligomer synthesis

Figure 11a — Synthesis of peptoides

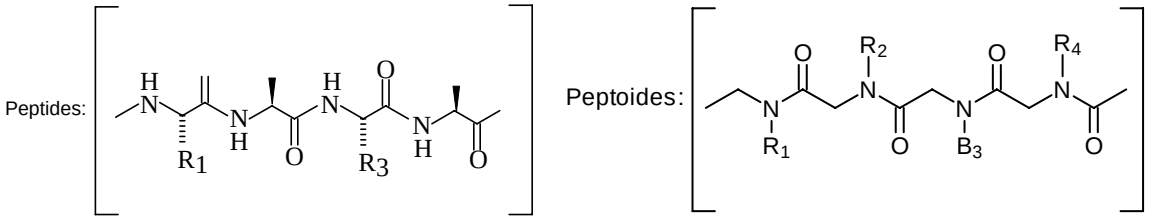
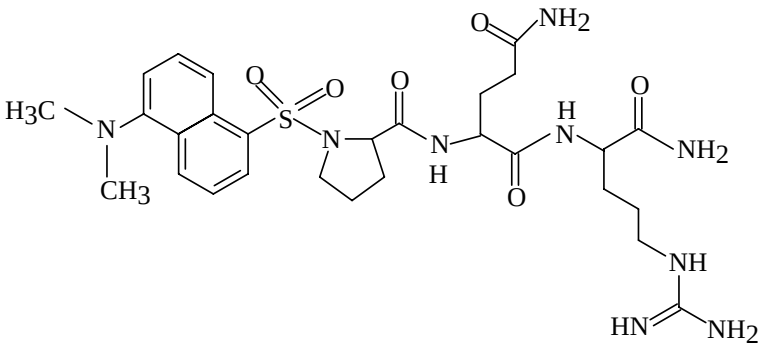
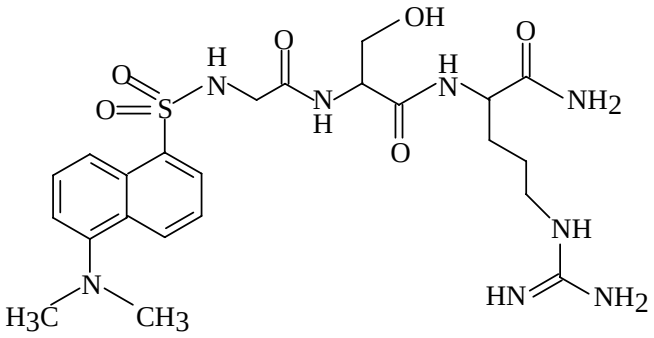


Figure 11b — Comparison of peptide and peptoid backbone



Dansyl-Pro-Gln-Arg$_{NH_2}$



Dansyl-Gly-ser-Arg$_{NH_2}$

Figure 12 — Structure of Neuropeptide-FF antagonists

Neuropeptide-FF, has afforded an antagonist with significant lipid solubility to cross the BBB.

For a combinatorial optimizationto improve potency, libraries focused on the possible replacement of the proline and glutamine residues of this lead compound were obtained by a solid phase split and mix method using coded amino acid as building blocks. After screening for competitive binding against a radioiodinated Neuropeptide-FF analogue, 5-(dimethylamino)-1-naphthylene sulfonyl-Gly-Ser-Arg-NH_2 (dansyl-GSR-NH_2) has emerged as one of the compounds having high affinity to the Neuropeptide-FF receptor and over with a moderate increase in CNS penetration power compared to lead compound **(Figure 12)**.

(c) Generation of a benzodiazepin library

The seminar work of Ellman[23] on solid phase synthesis of 1,4-benzodiazepin lays the ground work for creation of small molecules library and is considered as one of the most advancements in medicinal chemistry and represents the first example of the application of combinatorial organic synthesis to non polymeric organic compounds.

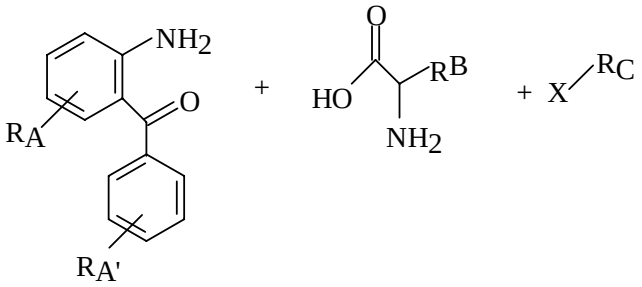


Figure 13 — Components of a benzodiazepine library

The benzodiazepines were synthesized on a solid support by the connection of three building blocks, and of different chemical families **(Figure 13)**.

Following the attachment of 2-aminobenzophenone hydroxy or carboxy derivative to the support using an acid cleavable linker [(N-hydroxymethyl) phenoxy-acetic acid], the N-protecting group is deblocked (piperdine/DMF), the weak nucleophilic amine is acylated with an α-Fmoc-protected amino acid fluoride, using 4-methyl-2, 6-di-*tert*-butylpyridine as an acid scavenger. Fmoc deprotection followed by treatment with 5% acetic acid in DMF, causes the general cyclizationto the intermediate lactam.

Capitalizing on the ability of lithiated 5-(phenyl methyl)-2-oxazolidinone to selectively deprotonate the anilide NH_2, alkylation was achieved with a variety of alkylating reagents. Aqueous acid cleaves the new benzodiazepine from the support in very high overall yields **(Figure 14)**.

(d) Combinatorial lead optimization of Histamine H_3 receptor antagonist[24]

The H_3 receptors are primarily located in the CNS in a presynaptic receptor that modulates the production and release of histamine. Blockade of this receptor leads to increased level of histamine and other neurotransmitters throughout the brain via effects on the pre and post synaptic H_3 receptors. The wide distribution of H_3 receptor in the mammalian CNS indicates a physiological role for this receptor. Therefore, its therapeutic potential as a novel drug development target has been proposed for indications associated with neurological disorders such as Alzheimer's disease, Parkinson's disease and epilepsy, as well as metabolic disorders such as obesity.

A series of biaryl derivatives has been investigated to develop selective H_3 blocker[25]. A small library of 49 biphenyl-O-propylamine amides (a) were synthesized as singletons in solutions; the resulting products were purified using high throughput HPLC-MS techniques and assayed in a binding experiment using cloned human H_3 and rat cortex H_3 receptors. A number of potent inhibitors were found, one most potent being (b), was demonstrated excellent selective towards H_3 receptors **(Figure 15)**.

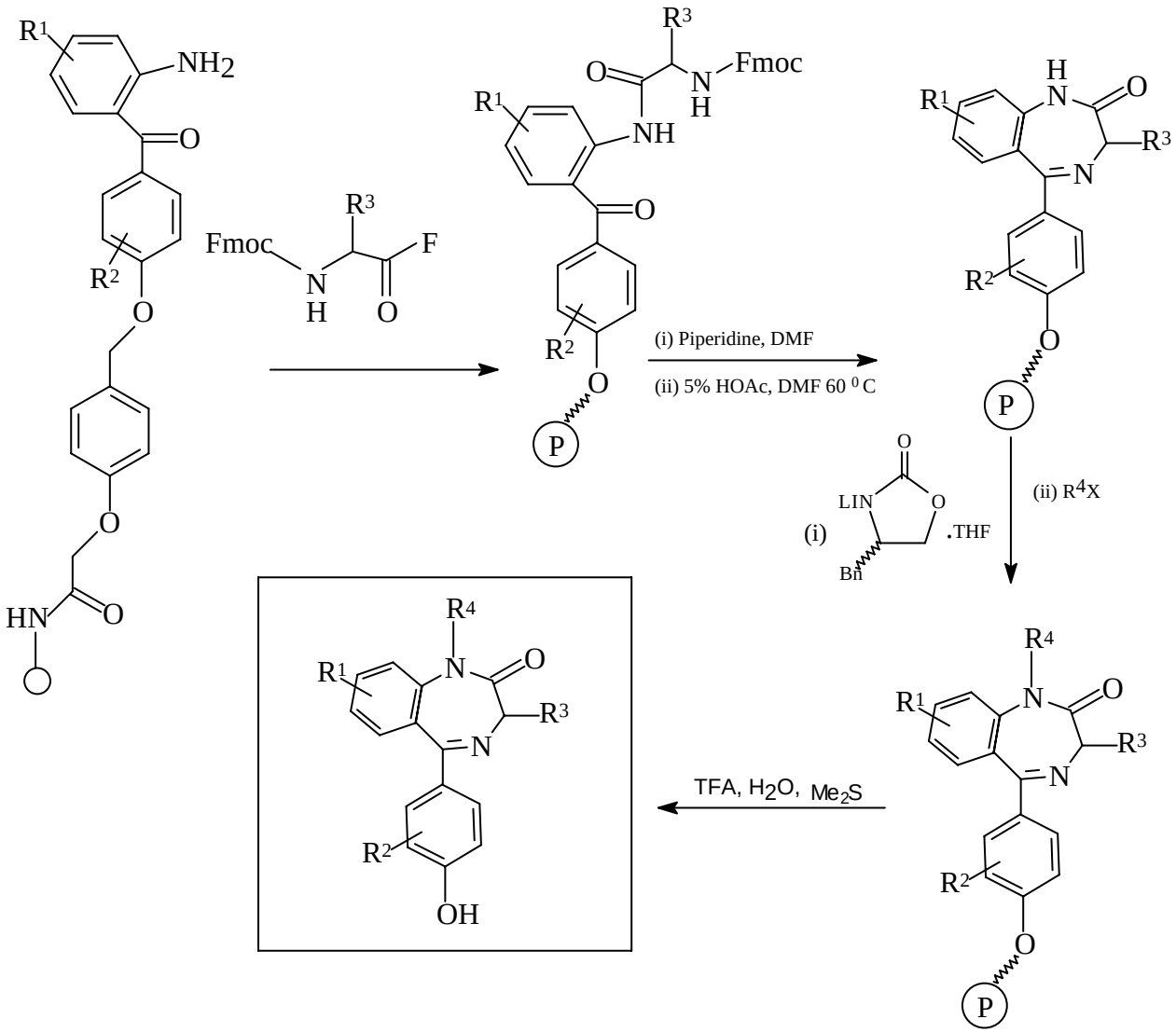
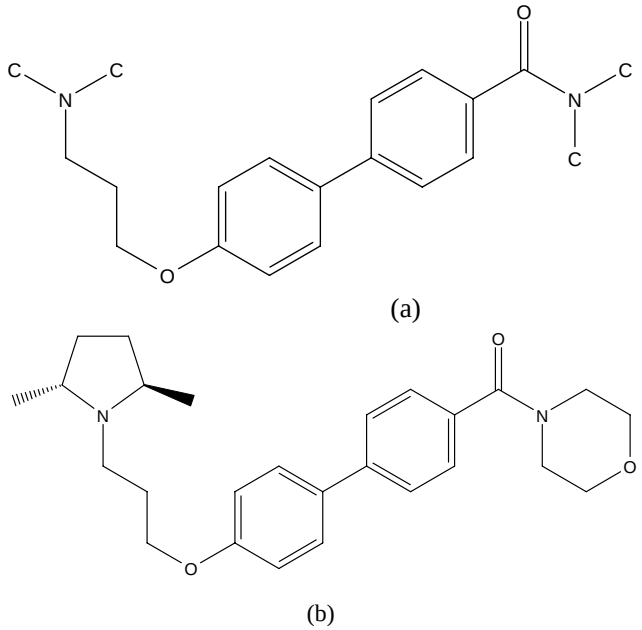


Figure 14 — synthesis of a benzodiazepine library



(a)

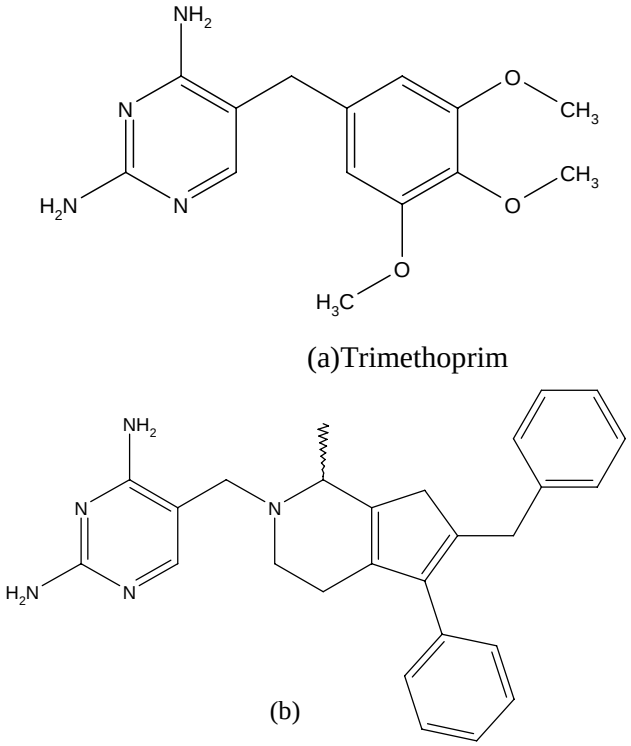
(b)

Figure 15 — H3 receptor antagonists

(e) Combinatorial lead optimization of dihydrofolate reductase inhibitor

The spread of antibiotic resistance has reached alarming proportions in some species, and one of the most worrying trends is the increasing incidence of methicillin resistant *S.aureus* in hospitals and multiresistant *S. pneumoniae* in the community. Therefore, there is an urgent need for effective antibacterial agents to treat infections caused by these organisms. The enzyme dihydrofolate reductase (DHFR) has been established in the clinic as a proven target for chemotherapy. The DHFR inhibitor Trimethoprim was introduced primary for the treatment of community-acquired infections and urinary track infections, with emphasis on gram-negative pathogens. The enzyme remains an under-exploited target in the antibacterial field and now optimization of inhibitors against gram-positive pathogens has been performed. Recent work has been conducted for improving the pharmacokinetic properties of DHFR inhibitors[25].

A library of 1392 compounds was synthesized in solution. The compounds were evaluated for inhibition of human DHFR and the bacterial enzymes from TMP-sensitive *S. aureus* and TMP-resistant *S. pneumoniae*. Several potent inhibitors were found, with one of the most potent compound (b) possessing IC_{50} value of 42 nM against *S. aureus* and 550 nM against *S. pneumoniae* **(Figure 16)**.



(a)Trimethoprim

(b)

Figure 16 — Dihydrofolate reductase inhibitor

Limitations of combinatorial chemistry

A major problem in the combinatorial organic synthesis is the difficulty of confirming the degree to Which the expected chemistry has proceeded on the entire population of the substrate molecule. In the characterization of combinatorial products the presence of “by-products” (unexpected products) combine with the difficulty of detecting these compounds, will cause problems, if one mistakenly concludes that screening “hit” is expected product.

Conclusion

Combinatorial chemistry field has advanced rapidly over past ten years. This method has been considered as a most important advancement in medicinal chemistry and is widely exploited by pharmaceutical industries in drug discovery. Whether the aim is a broad discovery search or optimization of a lead, combinatorial chemistry is a process for integration of synthesis and screening.

In this competitive market, pharmaceutical industry must require efficient research work to stand in market and combinatorial chemistry offers higher productivity at lower expenses. This technique has definitely decreased the cost associated with the drug

research and increased the chances of finding new lead molecule.

Within a short time, promising drug leads have already been developed using combinatorial library methods and several are currently in preclinical studies. Further, in conjunction with the advent of computational chemistry and molecular modeling techniques, combinatorial chemistry can now be applied to various new drug targets developed from our recent understanding of the molecular basis of disease.

By considering all these aspects it is understandable that this method will definitely become helpful to mankind in development of new drugs and lead molecule at lower expenses.

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