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Review Article

Role of Combinatorial Chemistry in the Field of New Drug Design

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Combinatorial chemistry is a new technique developed in pharmaceutical industry, which involves synthesis of compounds in mass instead of single compound, which are screened as whole mixture for particular biological activities. Combinatorial Chemistry is set to become a core technology for pharmaceutical and chemical companies. In combination with technologies such as High Throughput Screening (HTS), robotics, advanced software and genetics, it has the ability to shorten the time to market for new drugs and make drug discovery a less costly process. Combinatorial chemistry is now also moving into new fields of application like agrochemicals and advanced materials. Through the rapidly evolving technology of combi-chemistry, it is now possible to produce chemical libraries to screen for novel bioactivities. The focus of drug discovery is to replace the sequential approach with the most effective parallel approach. Combinatorial chemistry is especially common in CADD (Computer aided drug design) and can be done online with web based software, such as molinspiration. This is very helpful in the new drug discovery, as large number of structurally distinct molecules may be synthesized in a time and submitted for pharmacological assay. It is faster lead generation with low risk of failure. This save significant money in preclinical development costs and ultimately change their fundamental approach to drug discovery.

Keywords: combinatorial, robotics, genetics, agrochemicals, CADD, molinspiration

1. INTRODUCTION

Combinatorial chemistry is the synthesis of all possible combinations of chemical building blocks. When it first originated only a few years ago, the ability of this technology to generate millions of novel compounds seemed highly desirable.

Combinatorial chemistry is the technique which shortens the time and synthesized at lower cost with producing strong response and competitive new drugs in pharmaceutical industry by researchers. The systematic and repetitive, covalent connection of a set of different "building blocks" of varying structures to each other in order to yield a large array of diverse molecular entities is called as combinatorial chemistry. It provides a means for rapid synthesis of new compounds, and high-throughput screening technology provides a mechanism to test them for beneficial properties.

The first method which was applied to oligonucleotides and peptides in combinatorial chemistry libraries which include proteins, synthetic oligomers, small molecules, and oligosaccharides. The required type of library is based upon the method of library preparation. There are three main steps involved in the combinatorial library:

a) Preparation of the library
b) Screening of the library compounds
c) Chemical structure determination of active compounds.

The design of combinatorial library synthesis is such that a range of analogues can be produced using similar reaction conditions, either in same reaction vessel, or separately in parallel synthesis using semi-automated synthesis. The biological activity of the

entire collection is then tested. The active compound is then identified in the final step and made in quantity as a single compound. The combinatorial chemistry approach has two discrete phases:

a) Preparing a library.
b) Searching the active compound. Screening mixtures for biological activity which can be compared to finding a needle in a haystack.

The combinatorial chemistry is the latest methodology to modern drug development, like as Polymerase chain reaction was to molecular biology. The compounds that looked successful in vitro unfortunately didn't always remain so in vivo, often due to bioavailability or toxicity problems. Chemists used to synthesize one-by-one of variant parent molecule and tested each compound-- a time-consuming and expensive process yielding very few, if any, leading to the development of new compounds with biochemical properties similar to known therapeutics. Chemists looked to independent research to overcome this technological hurdle and modern combinatorial chemistry was born. [1-3]

History:

The combinatorial chemistry was first developed before 15 years but it was granted from 1990s. H.Mario Gevsen, the research scientist at Glaxo Welcome Inc., Research Triangle Park, N.C., & A technique for synthesizing peptides on pin-shaped solid supports was established by his group in 1984. This was the first jump in this field. After assaying the product, the tags have been cleaved & determined using Mass Spectrometry to identify potential lead compounds. But before that, Bruce Merrifield, a researcher at Rockefeller University, had already started investigating the solid-state synthesis of peptides in 1960 although the industries adopted it only after 1990s. Over the years, a lot of research & development has been carried out on combinatorial chemistry. These developments are used to explore new compounds & materials. At starting, P.G. Schultz et al. engineered this work in the mid nineties in the context of luminescent materials obtained by co-deposition of a silicon substrate. The key objective of combinatorial chemistry is rapid production of novel molecules i.e. the ability to purify intermediates and final products easily by using various separation techniques. To achieve this, Solid Phase Synthesis in particular has been the main influence on library methodology. [4]

Objectives:

- Synthesis of libraries based on lead molecules.
- Synthesis of libraries based on heterocyclic structures.
- Synthesis of libraries based on drug like structures.
- Synthesis of libraries based on natural product like structures.
- Synthesis of libraries based on novel structures.
- Peptide library synthesis

Principles of Combinatorial Chemistry [5]

1 .Basic ideas & concepts

- Preparation of a large number of different compounds at the same time
- High throughput-screening provides the most promising substances

Conventional Reaction: $A + B \longrightarrow A\text{-}B$

Combinatorial Chemistry: $A(1\text{-}n) + B(1\text{-}n) \longrightarrow A(1\text{-}n) - B(1\text{-}n)$

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2. Synthetic methods & technique

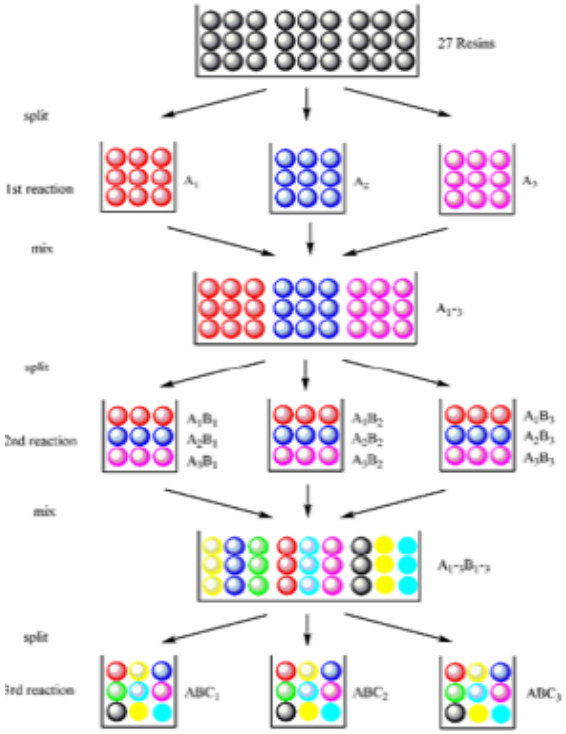

Fig. 1: Split pool synthesis

Split-Pool-Synthesis

The split and mix synthesis technique is method in combinatorial chemistry which was developed by Furka and has been used increasingly. For example, Split and mix synthesis is used by houghten for the creation of large libraries of peptides on a macro scale in a 'tea bag' approach in which the starting material is first split to 'n' portions, reacted with 'n' building blocks, and then re-combined in one flask and then repeated same procedure for the second step.

- Splitting of the resin, coupling with building block A1-A3
- Pooling, washing, deprotection
- Splitting, coupling with B1-B3
- Pooling, washing, deprotection
- Splitting, coupling with C1-C3

Parallel Synthesis: Each building block is separately reacting with each starting material. After each reaction step the product is split into 'n' portions previously it is reacted with 'n' new building blocks. Orthodox synthesis involves a multistep sequence. From initial product through to the last product is purified and fully characterized before screening. The biological activity considered for previous compound and guides the next analogue, prepared, and then screened. To optimize both activity and selectivity the process is repeated. [5, 6]

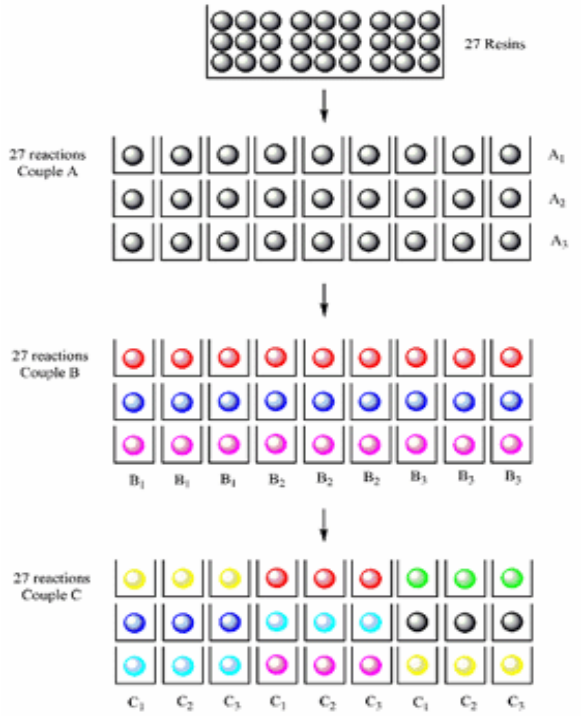

Fig.2: Parallel synthesis

- Coupling with building block A1-A3(1/3 of the resin beads for each building block), then
- washing, deprotection
- Coupling with building block B1-B3(1/3 of the resin beads for each building block), then washing, deprotection
- Coupling with building block C1-C3(1/3 of the resin beads for each building block), then

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International Journal of Pharmaceutical Erudition

washing, deprotection

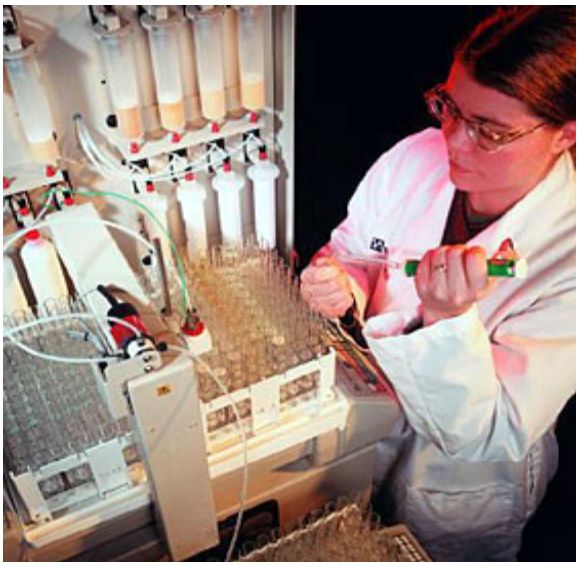

Fig.: 3 Combinatorial chemist performs parallel fractionation at Albany Molecular Research

Methods Used In Combinatorial Synthesis

There are two approaches by which the combinatorial libraries can be generated. [7]

1) **Biological library approach**
   a) Filamentous phase approach
   b) Plasmid approach
   c) Polysome approach
2) **Spatially addressable parallel solid phase library approaches**
   a) Multi-pin methodology
   b) Tea bag methodology
   c) SPOTS membrane method
   d) Light directed peptide synthesis on resin support

1) **Biological approach to generate molecular diversity**

The use of biological system for 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 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.

2) Spatially addressable parallel solid phase library approach

They are desire to develop and explore SAR around peptide lead compound has placed tremendous demands on the productivity of peptide chemistry. Variety of methods have been developed that permit simultaneous synthesis of multi peptides. They are as follows;

a) Multi pin methodology [8,9]

In this method, the synthesis, of peptides take place on polyethylene pins functionalized with acrylic acid arranged amino acids in 96 well formats. [9] The wells contain activated amino acids monomers. Peptide synthesis is carried out at the end of a spacer. Screening is done by means of enzyme linked immunosorbent assay (ELISA) to determine the binding capability of covalently bound peptide to antibodies.

b) Tea bag method

Houghten first developed this method of multiple peptide synthesis. The peptide synthesis occurs on resin that is sealed inside polypropylene bags. Amino acid are coupled o 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.

c) SPOTS membrane method

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

d) Light directed spatially addressable parallel chemical synthesis[10]

A scheme of combinatorial synthesis in which the identity of 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 chemical reagent to specific location on solid support.

Diversity-Oriented Synthesis

Researchers today are showing "how one might consider combining natural product synthesis with combichem," Hodges said. "In the past, combichem has largely focused on simple tried-and-true synthetic sequences, whereas the natural product route tends to be infinitely more complicated and creative. Combichem is now growing into those more complex realms"--an approach called diversity-oriented synthesis. "Diversity-oriented synthesis is taking on a new level," Combs added. "People are advancing it to the point where they're going after much more complex syntheses than in the initial years of combinatorial chemistry. They're tackling compounds with multiple stereocenters and very complex natural-product-like libraries, and that just wasn't happening three years ago."

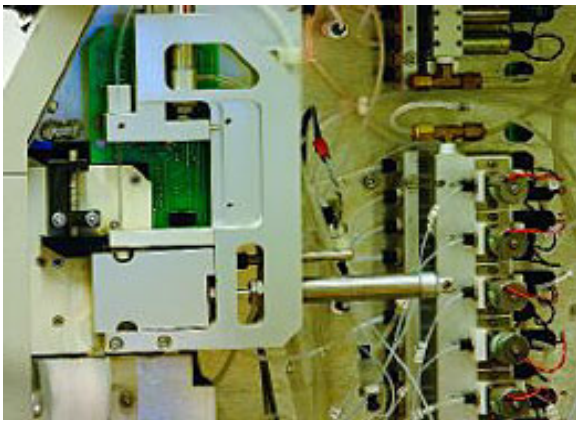

Fig. 4 :AUTO CARBS Seeberger's automated oligosaccharide synthesizer

Microwave-Assisted Organig Synthesis (MAOS)

Microwave- assisted organic synthesis (MAOS), fueled by the development of precision controlled, single-mode microwave reactors with robotic autosamplers for serial synthesis, has had a profound impact on organic and parallel synthesis. Reaction times are reduced by orders of magnitude; a diminution in side product formation is typically observed, and MAOS reactions are readily scalable. Moreover, MAOS reactions tend to be general in scope and lend themselves to the synthesis of libraries to rapidly develop SAR. These advantages, easily appreciated when considering established routes with successful reactions, are even more valuable when working out robust conditions for novel reactions and allowing one to approach reaction development from a combinatorial chemistry perspective. Exploratory reactions can be conducted in minutes to hours instead of days and speculative, higher-risk ideas can be pursued with minimal time investment, yet with complete testing of a hypothesis. Indeed, MAOS allows any chemistry to be pursued in

parallel (via 60-position autosamplers) and allows chemistries historically avoided for library synthesis (mulitcomponent reactions, organometallics, transition-metal -catalyzed couplings, etc.) to be completed successfully in minutes[11, 12 13].Other MAOS instruments allow for parallel microwave heating employing rotor systems[14] or special materials that allow multiple MAOS reactions to occur simultaneously.[15] which work by heating reaction vessels first instead of the reaction materials themselves as in the serial autosamplers. Beyond the speed advantage two additional merits of MAOS and modern reactors should be highlighted: precision and reaction scope. As has been noted in this text and elsewhere, the benefits of MAOS have been studied in multimode "kitchen microwaves" for decades; what prevented acceptance in the wider community was in multimode "kitchen microwaves" for decades; what prevented acceptance in the wider community was irreproducibility because of a lack of pressure and temperature control. In addition, kitchen microwaves use multimode resonators, which lead to a heterogeneous field and local "hot" spots, but despite this disadvantage, early work demonstrated the utility of MAOS. Modern systems provide a homogeneous field, precise control of temperature and pressure, and little resemblance to kitchen microwaves. Importantly, the mechanism of heating in a microwave reactor is quite different from classical thermal convection heating with a heating mantle or oil bath. MAOS relies on dipolar oscillations and ionic conduction, that is, molecular friction, to generate heat and afford uniform heating of the sample. In contrast, conventional thermal heating relies on heat transfer from the walls of a reaction vessel and affords non uniform heating of the sample .This uniform heating and rapid time to set temperature delivers reproducible results with fewer side products, and as a result, higher chemical yields.

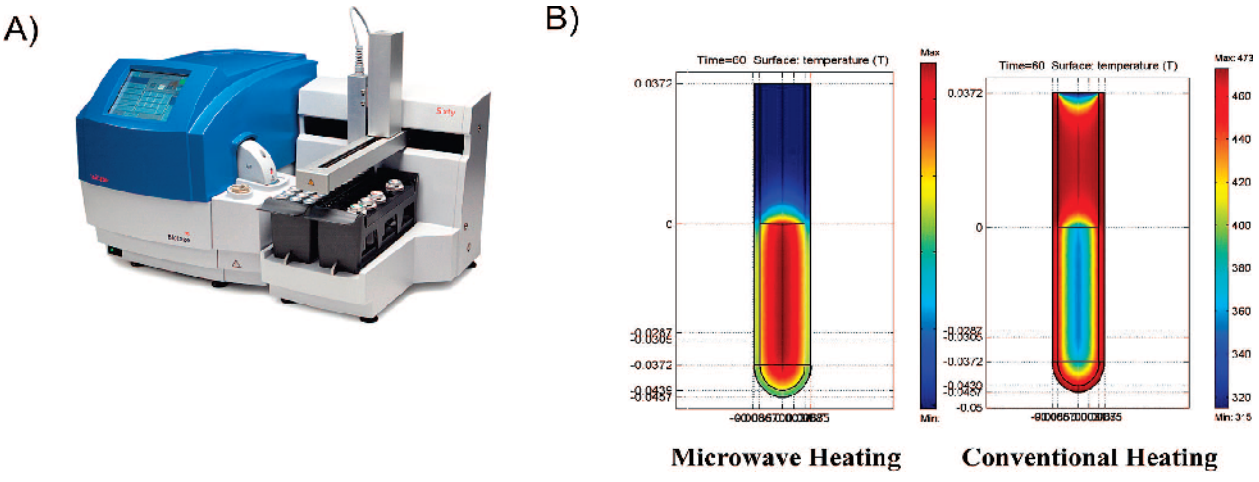

Fig.: 5(A) a single-mode microwave reactor for organic synthesis and (B) comparison of surfacetemperature between microwave and conventional heating.

There are three main steps involved in the combinatorial library:

- Preparation of the library
- Screening of the library compounds
- Chemical structure determination of active compounds

MODERN POSTPURIFICATION SAMPLE HANDLING AND COMPOUND CHARACTERIZATION

Modern parallel synthesis, that is, high-throughput medicinal chemistry, laboratories have industrial revolution (i.e., the automotive industry) and developed highly efficient assembly lines for postpurification sample handling and compound characterization. Automated weighing systems with bar-code readers scan and record weights on unique bar-coded vials into which pure compounds from the preparative LCMS systems are transferred for concentration in a sample evaporator. After the drying step, the bar-coded vials with pure, solid sample are transferred to a liquid handling robot. This instrument scans each bar code, weighs the vial, and determines the net weight of the pure product. This data file is merged with a registration file containing the molecular weight of the compound, and the system software then calculates the volume of DMSO required to dilute the samples to a preset concentration for screening. The system then dilutes the samples, transfers the DMSO stock solution to a 96-well plate, and generates an electronic plate map file for submission to the primary screen, in vitro drug metabolism assays, pharmacokinetic cassettes, and for flow-cell NMR (vide infra). With this highly automated workflow, a single scientist can oversee the postpurificat sample handling of thousands of samples per week.

TYPES AND FEATURES OF COMBINATORIAL LIBRARIES

1) Random libraries

   a) Drug like
   b) Diverse scaffolds

2) Targeted libraries

   a) Target-directed
   b) Diverse substitution

3) Focused libraries

   a) Similar to lead
   b) Complete

Development Of Combinatorial Chemistry: [16]

- From peptides to organic molecules
- From large to small libraries
- From mixtures to single compounds
- From combinatorial synthesis to automated parallel syntheses of molecules with drug-like character

Approaches to Combinatorial Chemistry: [17]

By means of traditional drug design, combinatorial chemistry depends on organic synthesis methodologies. Both are relies on each other but difference is only the scope. In organic synthesis methodologies, a single compound is synthesized while in the combinatorial chemistry; large libraries of compounds are synthesized automatically. But because large libraries do not produce active compounds independently, it is necessary for scientists to find out the way of producing the active components within these large populations. Thus, combinatorial organic synthesis (COS) is not random process but it is a systematic and repetitive process.

- **Dynamic Combinatorial Chemistry**

Under thermodynamic control, combinatorial chemistry is known as dynamic combinatorial chemistry. In a dynamic combinatorial library, all constituents are in equilibrium. The thermodynamic

stability of each of the library members determines composition of the library under the particular conditions of the experiment. It is suitable for screening affinity and may be having great interest in drug research.

From the available building blocks, dynamic combinatorial chemistry consists of using the target as a template which is build with best complement(s) [18-32] .In the field of supramolecular chemistry, where DCC is rooted by where molecular diversity generated by the use of self-assembling systems through the reversible association of a few components.

- **Concepts of Combinatorial Chemistry and Combinatorial Technologies** [32]

Automated screening follows combinatorial chemistry and combinatorial technology which is joined to computer to assisted combinatorial chemistry with automated parallel synthesis of chemical libraries. To obtain lead optimization, conventional and combinatorial strategy is used. In conventional method, hundreds of molecules can be prepared in a month where in combinatorial method, thousands of molecules can be prepared in a month. Convention method having slower lead generation with high risk of failure where combinatorial methods having faster leads generation with low risk of failure.

- **Combinatorial Chemistry Project Management**

To regulate the path of information for mixture and discrete or separate compound libraries, a combinatorial chemistry project management tool must be allow the researcher to associate data with:

a) The library itself (represented as a generic structure or parent library)
b) Mixtures of compounds within the library (represented as sub generics, or child libraries);
c) Individual compounds in the library.

By the support of Project Library the combinatorial researchers organize libraries into databases, where all information about parent and child libraries and discrete compounds can be stored and evaluated and associations between them is automatically managed. The load on tables of administrative, biological, physical, and encoding data to the appropriate parent library, child library, or specific structure is made easy by quick-loading features which are searchable by structure or associated data.

- **High Throughput Screening**

HTS is the most recent, fastest-growing area in synthetic and biochemistry. It enables screening of several thousand's of molecules in a small period of time to put forward a possible drug candidate by using sophisticated equipments. HTS methods are now a day's used to characterize the metabolic and pharmacokinetic data of new drugs. Traditional screening methods like NMR, IR, Mass, chromatography, elemental analysis etc are still being used, but the focus is mainly on high-throughput analytical techniques like gel-phase, high-performance liquid chromatography (HPLC) etc. Ultra high throughput screening and high content screening methods are newer approaches used in along with HTS. Pharmaceutical companies focus less on HTS as the construction of workstation needed for doing HTS requires huge investment. It provides high quality quantitative data which in turn provides time

and cost effective service. Certain compounds put forward by HTS have failed to come out as potent drugs, due to certain problems which were detected later.

- **Intelligent Enumeration**

The process of automatic generation of either sub generic or individual compounds from a generic structure is termed Enumeration. Structural representations of child libraries, or discrete compounds within the library by the researchers were done by enumeration of a parent library. Project library produces the appropriate structures on demand as well as automatically maintains the relationship between parent, child, and specific structures. (Encoding, component names, and parent library information are included in the data which are inherent by the child library or particular structure.

- **Communication Management**

In combinatorial chemistry, researchers must be capable to enter data by themselves and access it by themselves which is known as tracking data. They have ability to generate reports when necessary. Thus, they make the data readily available to all other members of the research team. The researchers can enter data into Project Library by using the guided, graphical user interface and generate standard reports quickly and easily export data into word processor programs for custom reports. They can also do data analysis by making spreadsheet included with structures and data for SAR work or export the data into other software programs for analysis. Project Library runs on Microsoft Windows and Apple Macintosh computer systems.

- **Cost Management** [33]

Combinatorial synthesis helps in developing more no. of drugs in single process that's why it helps in cost reduction. It is a highly sophisticated technological method. Combinatorial synthesis and screening of active compound invest huge amount of capital in machines. Laboratories cannot have enough money for the management of scarce data. Based on ASCII robot file which generates information, project library permits researchers to make the record of specific structures from library. By using the ASCII file from the Project Library robots can be programmed to synthesize compounds elucidated from virtual libraries. The work flow of the combinatorial chemistry is going smoothly in Project Library.

Combinatorial Synthesis in Solution:

Instead of using solid-phase techniques, libraries have been made successfully and screened in solution for the synthesis of combinatorial compounds. Only some groups gave a preference for solution libraries because of workable solid-phase coupling [34]. The rigid core molecule combines supporting multiple reactive sites with a mixture of building blocks which produce a random mixture of poly-functionalized structures. This is the idea behind the method of generating libraries of small organic molecules. In a single combinatorial step, library generation method can generate molecular diversity very powerfully. In this method excess quantities of the reactive reagent is used that pushes to complete the reactions and the solvent-solvent extraction can be isolated. In this method there is no need for further purification so they give the first priority to these

samples which are known as 'reaction products'. Solution phase chemistry is a time consuming method.[35, 36]

Combinatorial Synthesis on Solid-Phase: [35, 36]

Synthesis of peptides using chloromethylated-polystyrene containing immobilized in Solid-phase synthesis excess amount of reagents and monomers are used which has ability to synthesize compounds on an static polymeric resin bead, then move forward a reaction to complete and simple filtration will remove all the unwanted material and wash it is in the mind of most library synthesis. There are two interconnected requirements for using the solid supports for chemical as well as biological synthesis:

- A cross-linked, polymeric, insoluble material that is not moving to the situation of synthesis.
- A chemical protection strategy to allow selective orthogonal safety and deprotection of reactive groups in the monomers

There are certain advantage of the solid phase synthesis on a polymeric support greatly, simplifies the problem of product isolation from reaction mixture, moreover we 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:

a) Polymeric solid support
a) A linker
b) Protecting groups

a) Polymeric solid support

The choice of solid support depends on the type of chemistry of reaction. In addition, resion used must be stable under all those reaction conditions.

b) Linker

The linker is the molecule that sits between our compounds and the solid support. Linker 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.

c) Protecting group

Protecting groups are important for blocking and regenerating certain functional groups in a (Tertiarybutyloxy reaction sequence. Some examples of the protecting groups are as follows; FMOC (Fluromethoxy carbonyl benzyl ester) and BOC carboyl).

Combinatorial Chemistry in Drug Resarch

1) Drug research is an evolutionary process

Nature developed higher organisms from more primitive forms. Over the decades, lead structure search and optimization followed the same principles.

2) Combinatorial chemistry speeds up drug discovery

Automated parallel syntheses reduce the time needed for each evolutionary cycle.

3) Drug-like character of libraries

Biological properties are more important than synthetic accessibility.

4) Similarity and diversity

Similarity can be better defined than diversity, the "lack of similarity".

5) Size and diversity of libraries

Huge libraries are most often a waste of time and resources, because of the time spent for chemistry

optimization and limited diversity.

6) Combinatorial chemistry and rational drug design

Structure-based and computer-assisted design and virtual screening of protein ligands supplement combinatorial chemistry.

7) Combinatorial design of drugs

The necessary tools are already available but scoring function have to be improved.

8) Success criteria in drug research

Decisive for industrial success is not "me too" but "me better", "me faster", "me first" or "me only".

Good Combinatorial Chemistry Practice

- Drug Design is an evolutionary procedure Combinatorial chemistry speeds up drug discovery
- Lead discovery libraries shall have a high degree of chemical diversity
- Lead optimisation libraries shall have a high degree of similarity, to cover the chemical space around a lead structure
- Several small libraries generate a higher diversity than one large library
- Drug-like character is more important than synthetic accessibility

Future of Combinatorial Chemistry:

Now a day the market growth of pharmaceuticals has decreased. Because of limited investment on pharmaceutical research, the researchers were under high pressure to search new methods that gives higher productivity at lower expenses. Combinatorial chemistry can allow the productive and cost-efficient generation of both compounds and drug molecules so as to promote the investment in this area. Solid phase synthesis is highly suitable for the synthesis of biopolymers such as DNA, RNA and peptides. To achieve maximum effect of combinatorial chemistry, it is necessary to develop a large range of bond-forming reaction on solid phase because the history of drug discovery suggests that no single class of compound will provide all the drugs of the future. However, more work will be remaining to do in this field. Thus combinatorial chemistry has been taken as a tool kit for development of newer compounds by the medicinal chemists.

Advantages:

- In short time, large libraries of molecules can be created.
- For generation and analysis of said library, price of combinatorial chemistry library is extremely high, but price is significantly lower per compound compared to the cost of individual synthesis.

Disadvantages:

- Time limitation for using solid phase synthesis to the chemistry.
- The available types of reaction can affect the resin and for the attachment of the reagent to the substrate and bead, care must be taken so that it can be unaffected.
- Planning is necessary for each reaction step.

CONCLUSION

Combinatorial chemistry had played an important role in the drug discovery by identification of a new leads. It helps in the preparation of library, screening of library compound along with structure determination. By the application of the various synthesis methods,

many active compounds have been selected and have progressed into clinical trials. Various approaches of combinatorial chemistry such as high throughput screening, communication management, cost management, etc are beneficial for pharmaceutical industries along with other laboratories. Combinatorial chemistry is much more helpful and beneficial for new drug design.. As combinatorial chemistry give higher productivity at lower expenses, it do have good future in the pharmaceutical industries and other industries.

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