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A Brief Introduction of Combinatorial Chemistry

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ABSTRACT

Combinatorial chemistry is one of the important new methodologies developed by researchers in the pharmaceutical industry to reduce the time and costs associated with producing effective and competitive new drugs. Through the rapidly evolving technology of combi-chemistry, it is now possible to produce chemical libraries to screen for novel bioactivities. In the age of high-throughput screening and combinatorial chemistry, the focus of drug discovery is to replace the sequential approach with the most effective parallel approach. By the completion of the human gene-map, understanding and healing a disease require the integration of genomics, proteomics, and, very recently, metabolomics with early utilization of diverse small-molecule libraries to create a more powerful "total" drug discovery approach.

Key words: combinatorial, gene-map, genomics

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INTRODUCTION

Combinatorial chemistry is difficult to introduce exactly in some measure. Synthesis of large numbers of compounds simultaneously by using a variety of starting materials is allowed by combinatorial chemistry. 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. Combinatorial chemistry took fourteen years to get the drug from market for a company in average time and cost of drug is about $359 million.

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:

- Preparation of the library
- Screening of the library compounds
- 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:

- Preparing a library.
- 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 from the mid-'80s on the solid-phase

synthesis of Oligonucleotides and peptides to overcome this technological hurdle and modern combinatorial chemistry was born. [1-3]

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.

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]

DEVELOPMENT OF COMBINATORIAL CHEMISTRY:

According to historical view, the research effort made in classical combinatorial chemistry can be described into three phases:

In the beginning of 1990s, the improvements made in high-throughput screening (HTS) technologies were the preliminary efforts which were the trigger off in the combinatorial chemistry arena. This creates a demand for accessing a large number of compounds for biological screening. The chemists were under constant pressure to produce an enormous numbers of compounds for screening purposes. For a practical point of view, in modern organic

synthesis generally some structural complexity was found in which the molecules in the first phase were simple peptides.

After 1990's, the chemists knew that some status was missing in compounds produced in a combinatorial chemistry in the second phase. Thus priority was given to quality instead of quantity.

Then third phase started in growing. This third phase was going in progress prepared with biochemical community. Due to the shifting of the scientific community into the post-genomic chemical biology age, the growing demand of newly discovered proteins and their interactions with other macromolecules can easily understood. [5]

APPROACHES TO COMBINATORIAL CHEMISTRY:

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. COS is repeatedly using sets of chemical "building block" to form a various sets of molecular entities. Several different COS strategies have been developed by scientists with same basic philosophy---stop shooting in the dark and instead, find out the various ways to determine active compounds within populations through chemical encoding, or by systematic, successive synthesis and biological evaluation (deconvolution).

There are three common approaches to COS. In the first approach, spatially addressable synthesis, the building blocks are systematically reacted in individual reaction to produce separated "discrete molecules." Active compounds are found out by their location on the grid. This method can be applied by two ways---First in scale as in the Parke-Davis Pharmaceuticals DIVERSOMER technique & second in miniature as in the Affymax VLSIPS techniques. In the second approach, the technique is known as encoded mixture synthesis. In this method nucleotide, peptide, or other types of more inert chemical tags are used to identify each compound.

In the third approach, the method is known as deconvolution. A series of compound is synthesized combinatorially, each time fixing some specific structural feature. Each mixture is assayed separately and from that most active combination is pursued. Again rounds systematically until a manageable number of separate structures can be synthesized and screened by fixing other structural features. Scientists working with peptides to find out the most active peptide sequence from number of possibilities could say that combinatorial chemistry is a technologically advanced way of searching a needle in a haystack.

The conclusion is that this is the idea which removes the estimation and instead of this it is used to create and test logically and systematically as many compounds or mixtures as possible to obtain a viable set of active leads. [6]

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 library members are interconverted into one another via reversible process in that covalent or non-covalent interactions are involved [7-25]. 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) [16]. 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. [17-20]

CONCEPTS OF COMBINATORIAL CHEMISTRY AND COMBINATORIAL TECHNOLOGIES

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. [21]

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.

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:

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

Anyone can easily build generic structures by using Project Library's tools and also assign names and encode information to the components. You can search for specific structures within the library by using sub structural, encoding, or other data constraints.

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.

This ability is known as “intelligent enumeration”. This intelligent enumeration of Project Library supports the three methods which are generally used today for the identification of the active components structure within a combinatorial synthesis:

- Arranged, addressable synthesis (molecular weight, components, or location identifies active compound);
- Nucleotides, peptides, or other chemical tag are used by encoded mixture synthesis.
- Deconvolution (To identify active compound by iterative synthesis of mixtures and subsequent screening)

Special navigation tools of project library permit the investigator to progress between enumerated parent libraries, child libraries, and particular compounds with easiness.

REAGENTS AND BUILDING BLOCKS

In combinatorial chemistry, the ability to manage the building blocks that formulate the libraries is as important as the ability to manage libraries themselves. So, in Project Library the researchers assign names and codes to specific building blocks included into a library, and to store the blocks and other associated data for future use.

Researchers used project library’s special processing tools to manipulate the reagents lists and quickly turn them into building blocks. The ability to search for structures of building block which makes it possible to put on new approach into biological effectiveness of individual building blocks by tracking their achievement across libraries.

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

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.

MDL has generated new software by using programs of project library. There are two phases to generate project library which was chosen by MDL. Using extensive industry research and software pilot program, MDL gained knowledge about which type of information was needed by combinatorial chemistry programs and which type of contribution is needed by this growing field. During the industry research phase, MDL technically assessed the requirements of the industry and the science involved in combinatorial chemistry by visiting 50 companies in the pharmaceutical, biotechnology, and agrochemical industries. MDL took the interview of the researchers involved in combinatorial chemistry programs. After discussion they found out possible software solution on paper and then MDL developed prototype software to promote information exchange and improve software requirements.

In the second phase, MDL put the prototype software in research groups. Combinatorial chemists were trained to use prototype software in their daily routine. Then feedback of pilot researchers was used to shape Project Library. Thus MDL proved that the software contains a guided user interface, supports multiple research methods faultlessly, and is complimented by other MDL software and scientific applications. [22]

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.

APPLICATION OF COMPOUND LIBRARIES TO DRUG DISCOVERY:

By using combinatorial chemistry, millions of products can be produced faster, cheaper and in more efficient manner. Chemical libraries were created through many combinations using chemical, biological, or biosynthetic procedures, the collection of building-block and assembling these blocks.

The way of discovery is a pragmatic process in compound libraries. The number and varieties of desirable structures can be recommended by libraries. However rational design has an important role in combinatorial chemistry.

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 [23]. 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. [24, 25]

COMBINATORIAL SYNTHESIS ON SOLID-PHASE:

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: [26]

- 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

SYNTHESIS OF A COMBINATORIAL LIBRARY:

The library members are typically synthesized as individual compounds in solution like parallel synthesis. Not only the split and mix technique but also parallel methodology has been used on solid supports.

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A. 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.

In contrast parallel analogue synthesis, reaction between substrate S with multiple reactants, R1, R2, R3 … Rn, to produce a compound library of n individual products SR1, SR2, SR3 … SRn. In this, purification is not necessary for screening the library. And minimal characterization of the individual compounds is screened by using a rapid throughput screening technique.

Characteristics:

- No risk of synergistic effects primarily to false positives throughout screening.
- No need of deconvolution.
- Increase in organization of complex task.

B. SPLIT AND MIX SYNTHESIS:

The split and mix synthesis technique is another 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.

Characteristics:

- Deconvolution or tagging is required.
- It forms complex mixtures.
- Synergistic effects may be observed during screening, leading to false positives.
- Simple complex task organization.
- Large libraries are readily obtainable.

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.

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