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COMBINATORIAL CHEMISTRY AND ITS APPLICATION - A MODERN SYNTHETIC APPROACH

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ABSTRACT

Combinatorial chemistry is defined as the systemic and repetitive covalent connection of asset of different building blocks of varying array of diverse molecular entities. Combinatorial chemistry is a new methodology by which we can simultaneously synthesize a number of possible compounds that could produce simultaneously a very large number of compounds, called libraries. Combinatorial chemistry involves the rapid synthesis or the computer simulation of a large number of different but often structurally related molecules or materials. Combinatorial chemistry is especially common in CADD (Computer aided drug design) and can be done online with web based software, such as Molinspiration. In the past, chemists have traditionally made one compound at a time. For example compound A would have been reacted with compound B to give product AB, which would have been isolated after reaction work up and purification through crystallization, distillation, or chromatography. In contrast to this approach, combinatorial chemistry offers the potential to make every combination of compound A1 to Am with compound B1 to Bn. Although combinatorial chemistry has only really been taken up by industry since the 1990s, its roots can be seen as far back as the 1960s when a researcher at Rockefeller University, Bruce Merrifield, started investigating the solid-state synthesis of peptides.

KEYWORDS: Combinatorial chemistry, Libraries, Rapid synthesis, Computer simulation, Computer aided drug design.

INTRODUCTION

Combinatorial chemistry[1-3] is defined as the systemic and repetitive covalent connection of asset of different building blocks of varying array of diverse molecular entities. Combinatorial chemistry coupled to HTS and computational methods and has been integrated into lead discovery and optimization process throughput the pharma industry. Combinatorial chemistry involves the rapid synthesis or the computer simulation of a large number of different but often structurally related molecules or materials. In a combinatorial synthesis, the number of compounds made increases exponentially with the number of chemical steps. In a binary light-directed synthesis, 2n compounds can be made in n chemical steps. Combinatorial chemistry is especially common in CADD[4-6] (Computer aided drug design) and can be done online with web based software, such as Molinspiration.

The Combinatorial Chemistry is a scientific method in which a very large number of chemical entities are synthesized by condensing a small number of chemical compounds together in all combinations defined by a small set of chemical reactions.

Combinatorial technologies provided a possibility to produce new compounds in practically unlimited number. New strategies and technologies have also been developed that made possible to screen very large number of compounds and to identify useful components of mixtures containing millions of different substances. Instead of preparing and examining a single compound, families of new substances are synthesized and screened. In addition, combinatorial thinking and practice proved to be useful in areas outside the pharmaceutical research Such as search for more effective catalysts and materials research. Combinatorial chemistry became an accepted new branch within chemistry.

The aim of this project is to provide a basic introduction to the field of combinatorial chemistry describing the development of major techniques and some applications. Synthesis of molecules in a combinatorial fashion can quickly lead to large numbers of molecules. In order to handle the vast number of structural possibilities, researchers often create a 'virtual library', a computational enumeration of all possible structures of a given pharmacophore with all available reactants. Such a library can consist of thousands to millions of 'virtual' compounds. The researcher will select a subset of the 'virtual library' for actual synthesis, based upon various calculations and criteria.[7] Finding of novel drug is a complex process. Historically, the main source of biologically active compounds used in drug discovery programs has been natural products, isolated from plant, animal or fermentation sources. 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. By accelerating the process of chemical synthesis, this method is having a profound effect on all branches of chemistry, but especially on drug discovery.[8-10] Through the rapidly evolving technology of combichemistry, it is now possible to produce compound libraries to screen for novel bioactivities. This powerful new technology has begun to help pharmaceutical companies to find new drug candidates quickly, save significant money in preclinical development costs and ultimately change their fundamental approach to drug discovery.[11,12]

Historical Development

Combinatorial chemistry was first conceived about 15 years ago - although it wasn't called that until the early 1990s. Initially, the field focused primarily on the synthesis of peptide and oligonucleotide libraries. H. Mario Geysen, distinguished research scientist at Glaxo Wellcome Inc., Research Triangle Park, N.C., helped jump-start the field in 1984 when his group developed a technique for synthesizing peptides on pin-shaped solid supports. At the Coronado conference, Geysen reported on his group's recent development of an encoding strategy in which molecular tags are attached to beads or linker groups used in solid-phase synthesis. After the products have been assayed, the tags are cleaved and determined by mass spectrometry (MS) to identify potential lead compounds. Although combinatorial chemistry has only really been taken up by industry since the 1990s, its roots can be seen as far back as the 1960s when a researcher at Rockefeller University, Bruce Merrifield, started investigating the solid-state synthesis of peptides 19. In the past decade there has been a lot of research and development in combinatorial chemistry applied to the discovery of new compounds and materials. This work was pioneered by P.G. Schultz et al. in the mid-nineties (Science, 1995, 268: 1738-1740) in the context of luminescent materials obtained by code position of elements on a silicon substrate. Since then the work has been pioneered by several academic groups as well as industries with large R&D programs (Symyx Technologies, GE, etc).

Principle of Combinatorial Chemistry

The basic principle of these studies is to prepare very large number of compounds and then identify more components from these compounds.[13-17] It is a technique by which distinct molecule which is structurally large may be synthesized in a short time and submitted for pharmacological study. Researchers can synthesize many numbers of compounds in a short time by using simple methodology. Basic Concept of Combinatorial Chemistry The concept of combinatorial chemistry is very important in material science and drug discovery.

Basic idea of this study includes,

- Formation of number of compounds in one time.
- High throughput-screening which gives effective substance.

Design of Combinatorial Chemistry

One of the two general strategies may be followed while designing a combinatorial synthesis.

- A sequential attachment of building blocks.
- The non-sequential attachment of building blocks using B as a template.

In the first case, the building blocks are successively added to the preceding structure so that it can grow in only one direction.

Conventional Reaction: A + B----------> AB

Combinatorial Chemistry: $A_{1-n} + B_{1-n}$---------> $A_{1-n}B_{1-n}$

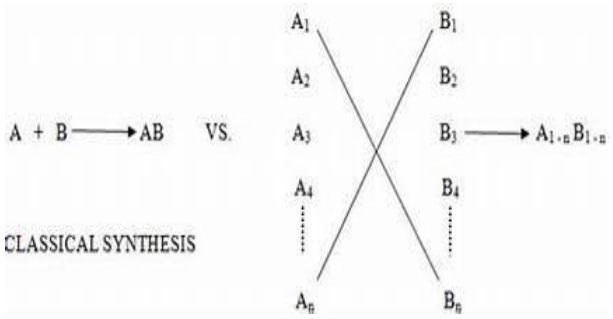

Fig. 1: Orthodox synthesis Vs Combinatorial synthesis.

Hence, In combinatorial chemistry, large numbers of compounds are made at the same time in small amounts, forming libraries which can be assayed for desired properties all at once. Finally the active compound is identified and made in quantity as a single compound.[18-21]

Combinatorial Chemistry Approach

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.[22-25]

Combinatorial chemistry encompasses many strategies and processes for the rapid synthesis of large, organized collections of compounds called libraries. The collection is then tested for the biological activity. Finally the active compound is identified and made in quantity as a single compound.

Thus the combinatorial chemistry approach has two phases:

1. Making a combinatorial library.
2. Finding the active compound. Screening mixtures for biological activity has been compared to finding a needle in a haystack.

In the past, chemists have traditionally made one compound at a time. For example compound 'A' would have been reacted with compound 'B' to give product 'AB', which would have been isolated after reaction work up and purification through crystallization, distillation or chromatography. In contrast to conventional approach, combinatorial chemistry offers the potential to make every combination of compound 'A1' to 'An' with compound 'B_1' to 'B_n'

The range of combinatorial techniques is highly diverse, and these products could be made individually in a parallel or in mixtures, using either solution or solid phase techniques. Whatever the technique used the common denominator is that productivity has been amplified beyond the levels that have been routine for the last hundred years. Combinatorial chemistry-a technology for creating molecules en masse and testing them rapidly for desirable properties-continues to branch out rapidly. Compared with conventional one-molecule-at-a-time discovery strategies, many researchers see combinatorial chemistry as a better way to discover new drugs, catalysts and materials. The development of new processes for the generation of collection of structurally related compounds (libraries) with the introduction of combinatorial approaches has revitalized random screening as a paradigm for drug discovery and has raised enormous excitement about the possibility of finding new and valuable drugs in short times and at reasonable costs.

Creating Chemical Libraries Compound library or chemical library is a collection of chemicals storage regularly used in industrial manufacturing and high-throughput screening. These chemical libraries are simple in terms of a series of excessively stored chemicals. Each stored chemical has associated information such as the chemical structure, physiochemical characteristics, purity, and quantity of the compounds.[26-29]

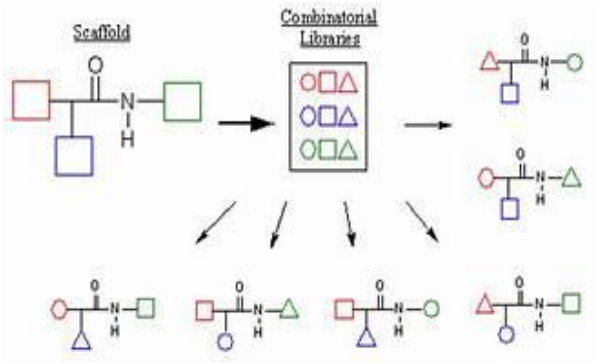

Fig. 2: Flow chart of combinatorial chemical Libraries.

Types of Combinatorial Libraries

Scaffold-based Libraries: Core-structure, which is common to all compounds of the library. Several single building blocks can consist of Scaffold.

Ex: Amino acid and Amino Benzophenone.

Backbone-based Libraries

Ex: Nucleic acid and Carbohydrate.

Two approaches to generate libraries are Random libraries and Focused libraries.

Methods In Combinatorial Chemistry[30,31]

a) Solid Phase Technique

Reactants are bound to a polymeric surface and modified whilst still attached. Final product is released at the end of the synthesis.

Requirements

- A resin bead or a functionalised surface to act as a solid support
- An anchor or linker.
- A bond linking the substrate to the linker.
- Be stable to the reaction conditions used in the synthesis
- A means of cleaving the product from the linker at the end.
- Protecting groups for functional groups not involved in the synthesis.

Solid phase tool

- Beads must be able to swell in the solvent used, and remain stable.
- Most reactions occur in the bead interior.

Anchor or linker

A molecular moiety which is covalently attached to the solid support, and which contains a reactive functional group

- Allows attachment of the first reactant
- The link must be stable to the reaction conditions in the synthesis but easily cleaved to release the final compound
- Different linkers are available depending on the functional group to be attached and the desired

functional group on the product
- Resins are named to define the linker

Eg: Merrifield, Wang, Rink
Solid phase synthesis: protecting groups
A few protecting groups used in solid phase synthesis.

For amines
Boc (t-butoxycarbonyl)
Fmoc (9-fluorenylmetoxy carbonyl)
Tmsec (2 [trimethylsilyl] ethoxycarbonyl)

For carboxylic acids
- Tertiary Butyl ester(t-butyl ester)
- Fm ester(9-fluronyl methyl ester)
- Tmse ester(2 [trimethylsilyl] ethyl)

Advantages
- Specific reactants can be bound to specific beads
- Beads can be mixed and reacted in the same reaction vessel
- Products formed are distinctive for each bead and physically distinct
- Excess reagents can be used to drive reactions to completion.
- Excess reagents and by products are easily removed
- Reaction intermediates are attached to bead and do not need to beisolated and purified
- Individual beads can be separated to isolate individual products
- Polymeric support can be regenerated and re-used after cleavingthe product
- Automation is possible.

b) Parallel Synthesis
Parallel Synthetic method
- To use a standard synthetic route to produce a range of analogues, with a different analogue in each reaction vessel, tube or well
- The identity of each structure is known
- Useful for producing a range of analogues for SAR or drug optimisation.

Houghton's Tea bag Method procedure
- Each tea bag contains beads and is labeled.
- Separate reactions are carried out on each tea bag
- Combine tea bags for common reactions or work up procedures
- A single product is synthesised within each teabag
- Different products are formed in different teabags
- Economy of effort - e.g. combining tea bags for workups
- Cheap and possible for any lab
- Manual procedure and is not suitable for producing large quantities of different products.

Automated parallel synthesis
- Automated synthesisers are available with 42, 96 or 144 reaction vessels or wells.
- Use beads or pins for solid phase support.
- Reactions and work ups are carried out automatically.
- Same synthetic route used for each vessel, but different reagents.
- Different product obtained per vessel.

c) Mixed Combinatorial Synthesis
- To use a standard synthetic route to produce a large variety of different analogues where each reaction vessel or tube contains a mixture of products.
- The identities of the structures in each vessel are not known with certainty.
- Useful for finding a lead compound.
- Capable of synthesising large numbers of compounds quickly each mixture is tested for activity as the mixture.
- Inactive mixtures are stored in combinatorial libraries.
- Active mixtures are studied further to identify active component.

The Mix and Split Method
Ex:
Synthesis of all possible dipeptides using 5 amino acids
Standard methods would involve 25 separate syntheses.

d) Solution phase synthesis
Solution phase assays, usually in the 96-well plate format, have been used in mass screening for most drug discovery programmes. There are many solution phase assays available. Eg. Competetive receptor binding assays with radiolabelled ligands, various enzymatic assays, cell based signal transduction assays, antibacterial assays, antiviral assays, anticancer assays. All these solution-phase assays, in principle can be adapted to combinatorial library. Because the number of compounds mixture of compounds generated by combinatorial methods are enormous, the current trend is to miniaturize and automate. These solutions- phase assays.

There are two general approaches to screen one bead one compound library with the solution phase.
1. The 96 well two stage release assays and
2. The insitu releasable solution phase assay with immobilized beads.

Combination of on Bead and Solution Phase Screening Assay
In some instances, it may be advantageous to combine solution phase assays with on bead assays to screen a specific target. Positive beads isolated by this approach are more likely to be true positives Eg. The compound beads are partitioned in to 1000 beads per well and a portion of the compound on each bead is released into the solution for biological testing.

The 1000 beads from a positive well can then be recycled and an on – bead binding assay performed to

identify single positive bead. Using this approach SALMON et al, successfully isolated ligands that bind to an anti – beta endorphin monoclonal antibody. Alternatively, an on bead binding assay can be performed. Positive beads can then be collected for a releasable functional solution – phase assay to identify true positive bead. Eg. The beads that bind to a protein kinase can first be identified and isolated by an enzyme – linked colorimetric assay. Compounds from each positive bead can then be released and tested for protein –kinase inhibitory activity.

e) Other Methods

They includes following methods.

i) The Multipin Method: In parallel procedures an array of different substances are simultaneously prepared. The first example of parallel synthesis was published by Geysen his colleagues. They synthesized a series of peptides epitopes in an apparatus developed for this purpose. The multipin apparatus had a block of wells serving as reaction vessels and cover plate with mounted polyethylene rods fitting into well. The first amino acid was attached to the end of polyethylene rods (pins) grafted with derivatized polyacrylic acid (marked by gray) The solutions of protected amino a coupling reagent were added to the wells (dark gray). The peptides formed on the pins immersed into solutions. The sequence of peptides depended on the order of amino acids of added to the wells. The peptides were screened after deprotection without leaving them from the pins.

ii) One bead one compound technique: With this strategy, a specific quantity of beads is allocated for each possible structure in the library; those beads contain only molecules of the given library member. The beads may be tagged in various ways to help identify the synthetic compound. The advantage of the one bead one compound strategy is the simplicity of analysis & screening. The disadvantage is keeping the beads separate & having to deal with a large number of synthesis in parallel. It is otherwise called as Split & Mix technique.

iii) Iterative deconvulution: This is the strategy first described 20 yrs ago when combinatorial chemistry was started. Each group has beads bearing a variety of compounds, but a given structure only appears in one of the groups. Suppose the active structure is ABC in the 3rd group. Since it is in the 3 rd group, we know a C in position 3 is needed for activity. We synthesize a smaller library of the structures, in 3 groups. (AAC+BAC+CAC, ABC+BBC+CBC, &ACC+BCC+CCC.) Now when we screen those mixtures, we find activity in the middle group of beads. This tells us that a B in position 2 is required for activity. The final step is to synthesize ABC,BBC, & CBC, keeping them separate, & screen each to find ABC as the active structure.

iv) Subtractive deconvulution: This is the strategy similar to iterative deconvulution but uses negative logic, namely, leave out a functional group, & if activity is absent, the functional group that is missing must be needed for activity. This is particularly useful for QSAR-type studies in which, say, a cl group is placed at several positions on a phenyl ring. The entire library is screened as a mixture to get the baseline activity level. If activity is detected, a set of sub libraries is prepared, with each missing one building block (subtraction of a functional groups from the active compounds) will be less active than the parent library. The Least active sub libraries identify the most important functional groups. A reduced library containing only these functional groups is then prepared, and the most active compounds are identified by either one compound synthesis or iterative deconvulution.

v) Bogus-coin detection: This begins with generating & screening the entire library as a single mixture. If activity is detected, the building blocks are divided into 3 groups (alpha, beta, gamma) & additional sub libraries are prepared. In these subsets, the number of building blocks from the alpha group is decreased, the number from the beta group is increased, & the number from the gamma group is unchanged. The resulting effect on activity (up.down, unchanged) suggests which group of building blocks was contributing most to activity. This approach is applied iteratively to zoom in one of the groups that are most active.

vi) Orthogonal pooling: The orthogonal pooling means perpendicular or uncorrelated. In this type of pooling, we distribute the functional groups to be considered into sets of libraries A,B,C etc.,which can contain mixtures of the same compounds. However, the functional groups are distributed such that any subset in A,B shares only one functional group, For example, if we have a very small library of structures aa,ab & ac. We might put aa & ab into group A, aa & ac into group B,ab & ac into group C. If ab is the active structure, screening A,B,C would show activity in A & C, but not in B, telling us that ab is the active one. 6. Positional scanning10: This is a noninterativedeconvulution screening strategy in which a subset library is created with a single building block fixed at one position & all building blocks in the other positions. In principle, by selecting the functional group from the most active subset at each position, the most active compound overall is discovered. This ignores interaction between building blocks, which may complicate the results.

The Application of Combinatorial Chemistry In Drug Discovery

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

- Synthesis of peptoids.
- Combinatorial lead optimization of a Neuropeptide-FF antagonist.
- Generation of a benzodiazepine library.
- Combinatorial lead optimization of Histamine H3 receptor antagonist.
- Combinatorial lead optimization of dihydro-folate reductase inhibitor.

Advantages

1. Applications of combinatorial chemistry are very wide Scientists use combinatorial chemistry to create large populations of molecules that can be screened efficiently.
2. By producing larger, more diverse compound libraries, companies increase the probability that they will find novel compounds of significant therapeutic and commercial value.
3. Provides a stimulus for robot-controlled and immobilization strategies that allow high-throughput and multiple parallel approaches to drug discovery.
4. Compounds that cannot be synthesized using traditional methods of medicinal chemistry can be synthesized using combinatorial techniques.
5. The cost of combinatorial chemistry library generation and analysis of said library is very high, but when considered on a per compound basis the price is significantly lower when compared to the cost of individual synthesis.
6. More opportunities to generate lead compounds.
7. Combinatorial chemistry speeds up drug discovery.

Future of Combinatorial Chemistry

The last ten years has seen an explosion in the exploration and adoption of combinatorial techniques. Indeed, it is difficult to identify any other topic in chemistry that has ever caught the imagination of chemists with such fervor. For pharmaceutical chemists at least the reason for this change is not hard to fathom. 20 years ago the market for pharmaceuticals was growing at around 10% per annum but more recently the rate of the market growth as decline. At the same time, cost constraints on pharmaceutical research have forced the investigation of methods that offer higher productivity at lower expenses. The belief that combinatorial chemistry will allow the productive and cost-efficient generation of both compounds and drug molecules has fuelled enormous investment in this area.

CONCLUSION

Combinatorial chemistry is a technology for creating molecules en masse and testing them rapidly for desirable properties-continues to branch out rapidly. One-molecule-at-a-time discovery strategies, many researchers see combinatorial chemistry as a better way to discover new drugs, catalysts, and materials. Compared with conventional one-molecule-at-a-time discovery strategies, many researchers see combinatorial chemistry as a better way to discover new drugs, catalysts, and materials. It is a method for reacting a small number of chemicals to produce simultaneously a very large number of compounds, called libraries, which are screened to identify useful products such as drug candidates and a method in which very large numbers of chemical entities are synthesized by condensing a small number of reagents together in all combinations defined by a small set of reactions.

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