Significance of Combinatorial chemistry in Drug Discovery

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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 redefining the way pharmaceuticals and other high performance chemicals and materials are discovered and developed. Using a novel technique called combinatorial chemistry; we can create several potential molecules that could concurrently produce a huge number of libraries of compounds. In CADD, combinatorial chemistry is particularly prevalent (Computer aided drug design), which can be carried out online using web-based technologies such Mol inspiration. Traditionally, chemists have created one compound at a time. It afforded faster, less expensive and more comprehensive exploitation of new drug targets. In fact, these techniques are now routinely used in tandem in any given discovery project.

I INTRODUCTION

Combinatorial chemistry is systematic and recurring covalent bonding of various chemical entities'. The lead discovery and optimization process in the pharmaceutical business has been merged with combinatorial chemistry coupled with HTS and computational approaches. A huge number of various but frequently structurally related compounds or materials are rapidly synthesized or computer simulated in combinatorial chemistry. In a combinatorial synthesis, the number of compounds produced rises exponentially as more chemical reactions are performed. 2n molecules can be created in n chemical steps during a binary light-directed synthesis. Combinatorial

chemistry is frequently used in CADD (computer assisted drug design) and can be performed online using web-based applications like Molinspiration. The basic combinatorial chemistry paradigm was first articulated by Eddington in 1927, “If an army of monkeys were strumming on typewriters, they might write all the books in the British Museum.” Combinatorial chemistry is a method for systematically combining immense numbers of small molecule chemical building blocks together to make all possible combinations according to a specified chemical reaction sequence. This provides for the rapid production of ***libraries*** of hundreds, or thousands, of diverse molecules that would have previously taken chemists years to complete, synthesizing them one-at-time. The more compounds in a library, the greater the chance that one will be found to match the active site on a biological target. Ultimately, this should lead to reduced time for drug discovery and more cost effective use of pharmaceutical discovery resources.

Synthesis of molecules in a combinatorial fashion can quickly lead to large numbers of molecules. Researchers frequently build a "virtual library," a computer tool, to handle the enormous number of structural options. Listing all potential pharmacophore structures with all available data reactants. These "virtual" compounds can number in the thousands to the millions in such a library. Based on a variety of factors, the researcher will choose a portion of the "virtual library" for actual synthesis. One of the key new fields in chemistry is combinatorial chemistry created by experts in the pharmaceutical sector to speed up the process. The expenses related to creating innovative drugs that are both effective and affordable. By quickening chemical synthesis, this approach is having a significant impact on all fields particularly on the discovery of new drugs. 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.

Historical Development

Key milestones of Drug Discovery

Year	Class	Contributors	Development.
1963	P	Merrifield	Solid-phase peptide synthesis
1970	SM	Leznoff	Early nonpeptide solid-phase synthesis
1984	P	Geysen	Multipins for parallel synthesis
1985	P	Houghten	Teabags
1988	P	Furka	Mix-and-split synthesis
1991	P	Fodor	Light-directed spatially addressable parallel synthesis
	P	Houghten	Screening of mixtures
	P	Lam	On-bead screening: one bead, one peptide
1992	P	Houghten	Positional scanning
	SM	Ellman	Solid-phase synthesis of Benzodiazepines
1993	SM	De Witt	Diversomers; parallel solid-phase synthesis on resin
1994	SM	Smith	Indexed libraries
1995	P	Deprez	Orthogonal libraries
1996	P	Ni	Secondary amine tags for encoding
	SM	Curran	Fluorous tags for reagents and Substrates
	SM	Cheng, Boger	Mixtures by solution chemistry
1997	SM	Lipinski	Design: developability
1999	SM	Ley	Multistep solution synthesis with supported reagents

1. PRINCIPLE OF COMBINATORIAL CHEMISTRY

The fundamental idea behind these investigations is to produce a huge number of compounds and then extract additional components from them. It is a method by which separate molecules with high structural dimensions can

be created in a short time and submitted for pharmacological study. Researchers can combine numerous numbers of compounds quickly utilize a straight forward process. 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.

2. COMBINATORIAL CHEMISTRY APPROACH

Combinatorial chemistry is the systematic and repetitive connection of a set of different "building blocks" of varying structures to each other to create a large array of diverse molecular entities. Combinatorial chemistry covers many strategies for the rapid synthesis of large, organized collections of compounds called libraries. The collection is screened for the biological activity. Finally, the active compound is identified and mass-produced as a single compound. Therefore, the combinatorial chemistry approach has two stages:

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 Anwith compound B1 to Bn

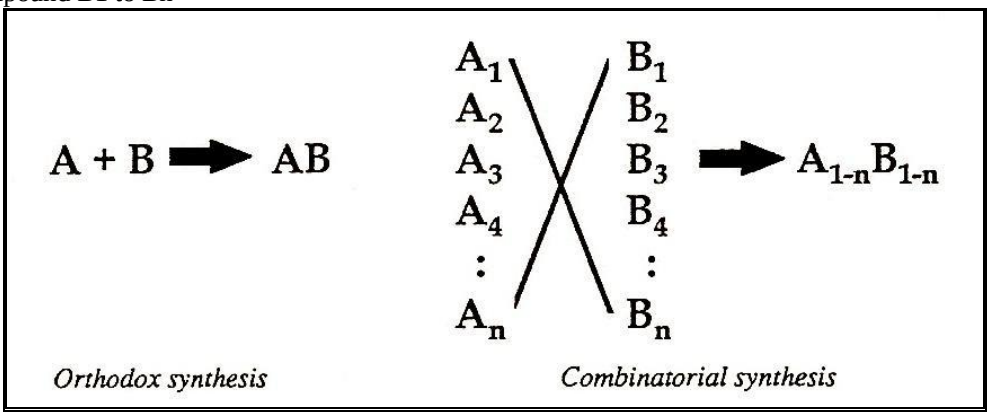

Fig. 1: Orthodox synthesis Vs Combinatorial synthesis.

The range of combined technologies is very diverse and these products can be manufactured individually in parallel or in mixtures using solution or solid phase technologies. Whatever the technique used, it was found that productivity levels have been greatly increased beyond what has been typical in the last hundred years. Combinatorial chemistry is a technology that is used to create molecules and test them quickly for desirable properties. This field of research is expanding rapidly, and new applications are being developed all the time. Many researchers see combinatorial chemistry as a better way to discover new drugs, catalysts and materials than using one-molecule-at-a-time discovery strategies. The development of new processes for the generation 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 timeframes.

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.

3. 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.

6. COMBINATORIAL CHEMISTRY-METHODS

6.1 Solid Phase Technique

Reactants are attached to a polymeric surface and modified at the same time attached. Final product is released at the end of the synthesis.

6.1.1 Requirements

• A resin bead acts 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.

6.1.2 Solid phase tool

• Beads must be able to remain stable & swell in the solvent used.
• Most reactions occur in the bead interior.

6.1.3 Anchor or linker

A molecular moiety which is covalently attached to the solid support, and which contains a reactive functional group
• Enable attachment of the first reactant
• The link must be easily cleaved to release the final compound and remain
Stable to the reaction conditions in the synthesis
• 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, and Rink
Solid phase synthesis: protecting groups
A few protecting groups used in solid phase synthesis.

Amines

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

Carboxylic acids

• Tertiary Butyl ester(t-butyl ester)
• Fmester(9-fluronyl methyl ester)
• Tmseester(2 [trimethylsilyl] ethyl)

6.1.4 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
• More amount of reagents can be used to drive reactions to completion.
• More amount of reagents and by products are easily removed
• Reaction intermediates do not need to be isolated and purified and are attached to bead.
• Individual beads can be separated to isolate individual products
• Polymeric support can be regenerated and re-used after cleaving the product
• Automation is possible.

6.2 Parallel Synthesis

6.2.1 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 drug optimization and SAR

6.2.2 Houghton's Tea bag Method procedure

• Each tea bag contains beads and is labelled.
• Separate reactions are carried out on each tea bag
• Combine tea bags for work up procedures and for common reactions
• Within each teabag a single product is prepared.
• In different teabags various products are formed
• Economy of effort - e.g. combining tea bags for workups
• Cheap and accessible for many labs

• Manual procedure and is not suitable for producing large quantities of different products.

6.2.3 Automated parallel synthesis

• Automated preparations are available with 42, 96 or 144 reaction vessels or wells.
• For solid phase support use beads or pins
• Automatically reactions and work ups are carried out
• Same synthetic route used for each vessel, but different reagents.
• Every vessel different products are obtained

6.3 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 synthesizing large numbers of compounds quickly each mixture is tested for activity as the mixture.
• In combinatorial libraries inactive mixtures are stored
• Active mixtures are studied further to identify active component.

6.3.1 The Mix and Split Method

Ex: Synthesis of all possible dipeptides using 5 amino acids
Standard methods would involve 25 separate syntheses.

6.4 Solution phase synthesis

Solution phase assays, usually in the 96-well plate format, have been used in mass screening for most drug discovery programs. There are various solution phase assays available. 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 large, 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.

6.4.1 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. By considering 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.

6.5 Other Methods

They includes following methods.

6.5.1 The Multipin Method: In parallel procedures an array of different substances are simultaneously prepared. Geysen and his colleagues published the first example of parallel synthesis . 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.

6.5.2 One bead one compound technique: 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.

6.5.3 Iterative deconvolution: It is first described 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.

6.5.4 Subtractive deconvolution: This is the strategy similar to iterative deconvolution but uses negative logic, namely, leave out a functional group, & if activity is absent, the functional group that is missing must beneeded 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 deconvolution.

6.5.5 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, and gamma) then 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 suggests which group of building blocks was contributing most to activity.

6.5.6 Orthogonal pooling: It 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.

7. IMPORTANCE OF COMBINATORIAL CHEMISTRY IN DRUG DISCOVERY

The drug discovery process is highly complex, it may be considered to consist of four key stages, the first being the discovery and definition of a *biological target*. In the majority of cases this is a protein with involvement in a disease process. A consequence of the success of the Human Genome Project in providing a blueprint of the human genome, in combination with the complementary approach of proteomics, is the unprecedented rate of discovery and understanding of new targets. This is set to have a profound effect on drug discovery in the new millennium. Once a relevant target has been identified, assays are developed to identify molecules that bind to the target and modify its behavior in the desired manner.

The process now enters the *lead generation stage*. Here, the goal is to identify a lead compound whose properties (principally, but not exclusively its potency against the target) make it a suitable candidate for more in-depth exploration. In most cases this involves a high-throughput screening exercise, where large numbers of compounds are screened against the target in the assay developed previously. When the greater the number of appropriate compounds available for screening then the greater the chance of success. The production of lead discovery libraries was an early driver for combinatorial chemistry and continues to be an important application. Libraries for this purpose are typically of significant size, from a few thousand to tens or hundreds of thousands of compounds and may exist in a number of formats that match the screening protocols in use. Libraries on solid supports require alternative screening strategies, which may be particularly suited to certain specific target classes but are unlikely to be as generally applicable as solution assays.

When knowledge of the three-dimensional structure of a protein is known, either throughx-ray diffraction studies or through homology modeling, de novo design of a library ofcompounds is a possibility. Computer modeling of the interactions between candidate small molecules and the known active site of the protein can provide a startingpoint for library design. Such an approach will often take place in parallel with highthroughputscreening exercises.

Once a suitable lead is identified, the compound enters the third stage of the process,

optimization. The goal here is to modify the lead structure such that a number of criteria for progression into the final, *development, stage* are satisfied. Important criteria, the actual values of which will be set on a case-by-case basis, include high potency against the target as well as selectivity against related targets. The development candidate will also exhibit appropriate absorption, distribution, metabolism, and excretion (ADME) and toxicity properties and will have a positive profile against P450 enzymes. Screening for these properties at this stage, whether by in vitro, in vivo, or computational protocols is becoming an essential filter prior to entering the costly development phases.

Combinatorial chemistry at this point is to provide sets of compounds with which to derive an understanding of the contribution of structural elements of the molecule toward its binding to the protein target, so that structure–activity relationships (SAR) can be developed. Lead optimization libraries typically consist of hundreds or a few thousand compounds at most. As the iterative cycles of library design, synthesis, and screening progress and more is known

about SAR, the library size might fall to a handful of compounds focused on fine-tuning some aspect of the overall profile. The optimized product will enter the final, although most lengthy and costly stage, that of development into a marketable drug, including optimization of synthetic routes for production-scale synthesis, stringent trials in patients to prove clinical efficacy and safety, regulatory approval, and marketing; It is only once sales have begun that the massive research and development costs can start to be recovered, and this is possible onlyuntil the patent life of the compound expires. Any time savings in the overall process thereforerepresent opportunities for increased revenue, and combinatorial chemistry clearly has an important role to play in this respect.

7.1 Advantages

1. Combinatorial chemistry to create large populations of molecules that can be screened efficiently.
2. Companies increase the probability that they will find novel compounds of significant therapeutic and commercial value.
3. A stimulus for robot-controlled and immobilization strategies that allow high-throughput and multiple parallel approaches to drug discovery.
4. Using combinatorial techniques Compounds that cannot be synthesized using traditional methods of medicinal chemistry can be synthesized.
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.

8. CHALLENGES IN COMBINATORIAL CHEMISTRY

- Planning and matching a target with the diversity-producing chemical scheme-including the choice to synthesize individually or as a mixture.
- Assuring that the desired range of compounds is produced.
- Finding which compound is active within mixtures that test “active”-deconvolution of the mixture.
- Engineering a robust scheme for anchoring the synthesized compound in thecase of solid-phase synthesis.
- Automation of certain synthesis and screening schemes.
- Managing the data pertaining to the synthesis and assays.

The total program, including the choice of compounds, chemical reactions, deconvolution scheme, assay method, compound identification, data handling, automation, and quality control needs to work together harmoniously to fulfill the original purpose, namely-to improve the degree of novelty and productivity in drug discovery or lead optimization.

9. FUTURE OF COMBINATORIAL CHEMISTRY

In the past decade has seen an explosion in the exploration and adoption of combinatorial techniques. 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. Twenty years ago the market for pharmaceuticals was growing at around ten percent 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 fueled enormous investment in this area.

10. CONCLUSION

- Combinatorial chemistry is a technology for creating molecules masse and testing them rapidly for desirable properties-continues to branch out rapidly.
- Many researchers see combinatorial chemistry as a better way to discover new drugs, catalysts, and materials.
- Many researchers conclude that combinatorial chemistry as a better way to discover new drugs, catalysts, and materials.
- 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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