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UNRAVELING THE POTENTIAL, A COMPREHENSIVE ANALYSIS OF COMBINATORIAL CHEMISTRY

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ABSTRACT: In the pharmaceutical industry, combinatorial chemistry has emerged as a novel approach, facilitating the synthesis of multiple compounds simultaneously rather than focusing on individual compound. The quick production of the chemical means that this technology reduces the time and expense involved in drug research. In this review article, the topic of combinatorial chemistry is discussed in general terms, along with the concepts of combinatorial libraries, methodologies and applications. This technique significantly lowering the cost involved in new drug research and increases the chances of finding new lead molecules. Most commonly used combinatorial techniques are solid phase synthesis, Parallel synthesis, mixed combinatorial synthesis and solution phase synthesis. Combinatorial chemistry is useful for the efficient detection of big molecules as well as the production of tiny molecules and peptides. The aim of this project is to give a fundamental overview of combinatorial chemistry, outlining the evolution of key methods and a few applications.

INTRODUCTION: Combinatorial chemistry is a new technique developed in pharmaceutical industry which involves synthesis of compound in mass instead of a single compound, which are screened as a whole mixture for particular biological activity. Because of rapid synthesis of compound, this method saves the time and cost associated with drug discovery. It is used to create large population of structurally different molecules called chemical libraries in a short time that can be screened in one time against variety of targets by high throughput screening or used for pharmacological assay [1, 2].

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Definition: Combinatorial chemistry is defined as the systemic and repetitive covalent connection of a set of different "building blocks" of varying structures of each other to yield a large array of diverse molecular entities [3].

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. 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 [4].

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

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

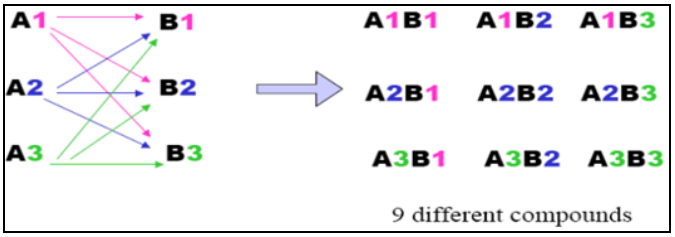

FIG. 1: COMBINATORIAL REACTION

Conventional synthesis is not the same as combinatorial chemistry. This entails the simultaneous reaction of one group of chemicals with another group of compounds to generate a combinatorial library, which is a collection of products. Formation of large number of compounds which are structurally different is very important to increase the chances of finding 'HIT' and to increase the diversity of compounds and number produced in each reaction

Advantages and Dis-Advantages of Combinatorial Chemistry:

Advantages:

Fast: Millions of compounds can be produced using a combinatorial approach in the same amount of time as one product produced using a conventional synthesis process.

Economical: A mixture that yields a negative result spares the work of synthesizing, purifying, and identifying each constituent. Simple it is not too difficult to isolate, purify, and identify the active chemical from a combinatorial library.

Drug Discovery: The chemical pool is produced by mixed combinatorial synthesis. The number of molecules treated to a random screening procedure determines the probability of detecting a particular molecule.

Drug Optimization: Parallel synthesis produces analogues with slight differences which is required for lead optimization [5].

Disadvantages:

1. Efficiency is highly affected by compound's size, solubility and function group.
2. Synthesized Compounds present in Achiral of Racemic form [5].
3. Difficult to characterize the identification of unexpected or unwanted product combination there is difficult to analyze will cause problems [4].

Combinatorial Approach:

Combinatorial approach has two phases:

- ❖ Creating chemical libraries.
- ❖ Identification of active ingredients.

Creating Chemical Libraries: A compound library, also known as a chemical library, is a collection of stored chemicals that are frequently used in high-throughput screening and industrial manufacture. In essence, these chemical libraries are just collections of substances that have been kept in excess. Information about each chemical that is stored includes its physiochemical properties, purity, amount, and chemical structure [6].

Types of Combinatorial Library:

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

Example: Amino acid and Amino Benzophenone.

Backbone Based Libraries:

Example: Nucleic acid and Carbohydrate. Two approaches to generate libraries are Random libraries and Focused libraries.

Techniques Used in Combinatorial Chemistry:

Solid Phase Synthesis: In the pharmaceutical industry as well as other discovery-related fields, combinatorial chemistry is crucial to the lead finding and hit optimization processes [7]. Solid phase techniques have only recently been used more widely in organic synthesis due to the influence of combinatorial chemistry. Originally, they were developed for oligonucleotide and then peptide synthesis [8].

Solid phase combinatorial chemistry involves attaching the starting molecule to an insoluble resin bead, adding excess reagent to the solution, and then isolating the products using straightforward filtration that captures the beads and removes the excess reagent [9].

Requirements of Solid Phase Synthesis: Polymeric Solid supports, a linker, protecting group

Method: This approach uses solid support, like resin beads, to carry out the reaction. Starting materials can be bound to separate resin beads. Then it is mixed with another reagent to get the product which is bound to solid support. Since, the products are bound to solid support, access reagents or by-products can be easily removed by reagent solvents can be used to drive the reaction complete [10].

Solid Support used in Solid Phase Synthesis:

Polystyrene Resins: In this, Polystyrene is cross linked with divinyl benzene (about 1% cross linking). Polystyrene resin is suitable for non-polar solvents.

Tenta Gel Resins: It consists of about 80% polyethylene glycol (PEG) grafted to cross-linked polystyrene 1. It combines the benefit of the soluble polyethylene glycol support with the insolubility & handling characteristics of the polystyrene bead. PEG containing resins are suitable for use in polar solvents.

Poly Acrylamide Resins: Like super blue these resin swell better in polar solvent, since they contain amide bonds, more closely resemble biological materials.

Glass and Ceramic Beads: These types of solid supports are used when high temperature and high pressure reaction are carried out [11].

Anchors/Linkers: A molecular moiety which is covalently attached to the solid support, and which contains a reactive functional group. The linker is a bifunctional molecule.

The link must be stable to the reaction conditions in the synthesis but easily cleaved to release the final compound. The linker is bound the resin is called anchor.

Protecting Groups: A protecting group is reversibly attached to the functional group to convert it to a less reactive form. When the protection is no longer needed, the protecting group is cleaved and the original functionality is restored. Protecting group to be stable under the expected reaction conditions and to be cleavable - if possible-at mild reaction conditions.

Some of the protecting groups most widely used in peptide synthesis are:

1. Benzyl carbonyl (Z) group
2. t-butoxy carbonyl (Boc) group
3. 9-fluorenyl methoxy carbonyl (Fmoc) group

Advantages of Solid Phase Synthesis:

- In the same reaction vessel, beads can be combined and reacted.
- Beads can be linked to specific reactants.
- Reactions can be accelerated to completion by using excess reagents.
- It is easy to eliminate excess reagents and byproducts.
- Reaction intermediates do not require isolation or purification because they are affixed to the bead.
- After the product is cleaved, the polymeric support can be reused.
- Automation is possible.

Parallel Synthesis: In parallel synthesis, a reaction is conducted in a sequence of wells, with a single product present in each well. It can be done without solid support or in a solution.

In this approach, each starting material is reacted with each building block separately (i.e. in separate vessel). After each reaction step the product is split into ‘n’ portions before it is reacted with n new building blocks.

In following figure spheres represents resin beads, A, B & C represent the sets of building block and borders represents the reaction vessels.

In the case, when three building blocks are utilized, in each coupling step after three stages, a total number of 27 different compounds, one on each resin bead, are formed using 9 individual reactions.

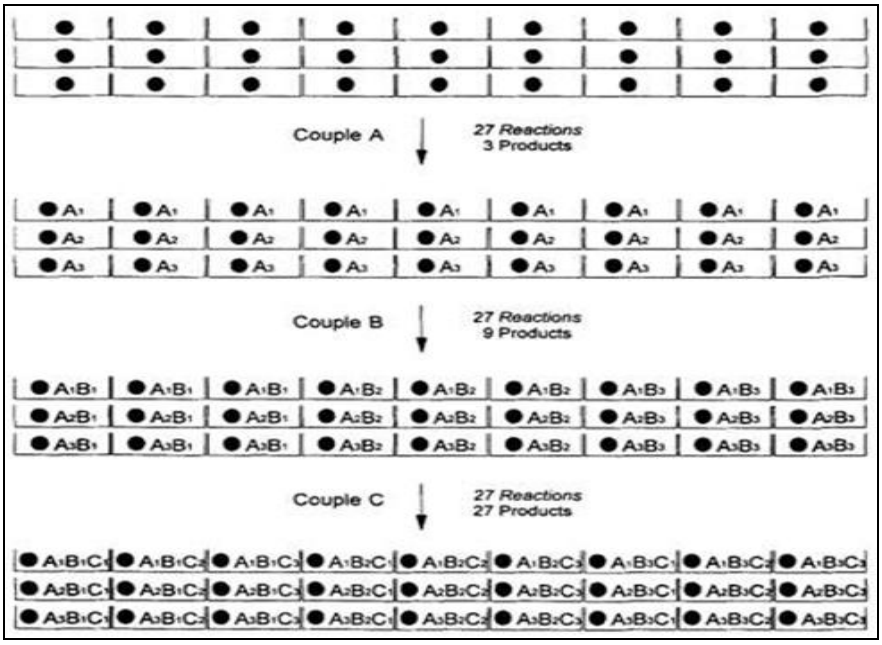

FIG. 2: SCHEMATIC REPRESENTATION OF PARALLEL SYNTHESIS

Methods for Parallel Synthesis:

- Houghton's Tea bag method
- Automated parallel synthesis method
- Multipin synthesis

Houghton's Tea bag Method: Hough ten created this technique for multiple peptide synthesis in 1985. The solid support beads are encased in permeable polypropylene (plastic) bags (5 x 22 mm mesh packets), and they are subsequently put into a reaction vessel with an amino acid solution and coupling reagent for coupling. All procedures are carried out on sturdy supports that are encased in bags, including the removal of protective groups, couplings, washings, and even cleavages. Each bag has a single peptide at the end of synthesis [12].

Using this procedure, each tea bag is named and has resin beads inside. Every tea bag undergoes a distinct response before being combined with other tea bags for a common reaction. Within each teabag, a single product is synthesized; in other teabags, distinct products are generated.

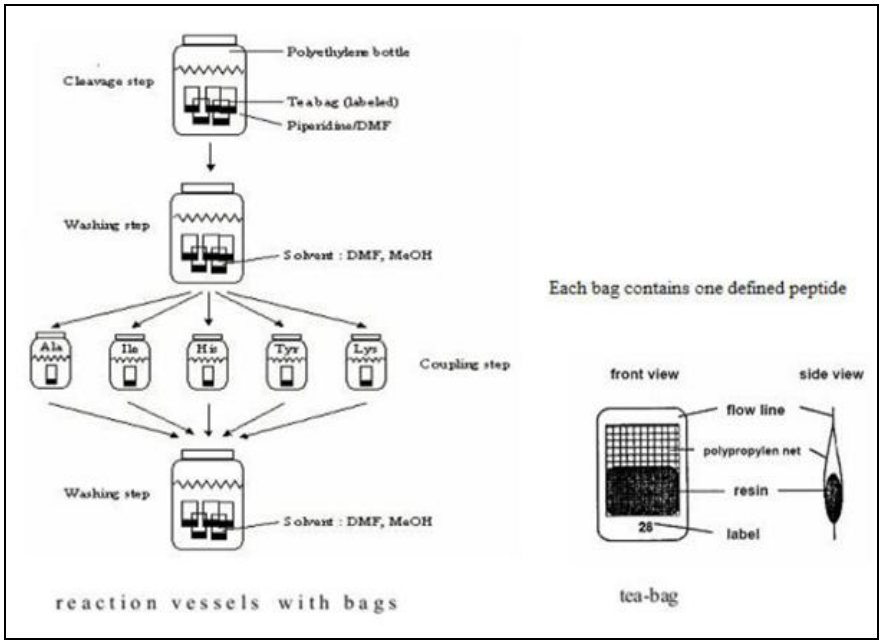

FIG. 3: SCHEMATIC REPRESENTATION OF HOUGHTON'S TEA BAG METHOD

Automated Parallel Synthesis: Automated parallel synthesis in combinatorial chemistry represents a revolutionary approach to the rapid generation of diverse molecular libraries. Automated synthesizers are available with 42, 96 or 144 reaction vessels or wells. Resin beads or pins are producing solid phase support to the drug synthesis. Chemical reactions and work ups are carried out automatically in reaction vessels. Same synthetic route used for each vessel, but different reagents are used then produce the different products per each vessel. Automatic parallel synthesizers are developed for both peptide and organic synthesis. The peptide synthesizers generally do not need heating or cooling. The organic synthesizers are more complex than the peptide synthesizers [13].

Method: The reagent and amino acid solutions are kept in septum-sealed containers. Bottles are used to store the solvents. Through the use of a needle-like probe that can pass through the septum, the solvents and solutions are automatically transported into reaction vessels. The probe is fitted to an arm that can be moved in x, y and also in z direction.

The Apex 396 comes with two different types of reactors in addition to one or two arms. The generated peptides have the ability to automatically separate from the resin. There is a range of 0.005 to 1 mmol for the amount of synthesized peptides.

Multipin Synthesis: In 1984, Geysen and associates described a method for creating a sequence of peptide epitopes using the Multipin apparatus. A block of wells (96 wells formatted in a 12×8 way) serves as reaction vessels in the Multipin apparatus, together with a cover plate that has affixed polyethylene rods or pins (4×40 mm) that fit into the well.

The reaction vessel is made out of a brush-like array of pins, with a bead and an appropriate linker at the end, where the synthesis is done. It is placed onto the plates that contain the solvents and reagents that are kept in the reaction vessel. Throughout the course of the reaction, solvents and reactants are constantly altered [15].

Mixed Combinatorial Synthesis: This method, in which each reaction vessel or tube contains a mixture of products, uses a typical synthetic route to make a broad variety of distinct analogues. It is uncertain with certainty which structures in each vessel are responsible for the presence of a lead compound. It can quickly synthesize a vast number of chemicals. Combinatorial libraries are used to hold inactive mixes, while active mixtures are further examined to determine the active component.

Split and Mix Synthesis: Ingredients are assembled using this process on the surface of the microparticles or beads. Beads from previous processes are divided into new construction blocks and many groups are added in each step. As a result, new groups are formed and the various bead groups are joined and divided once more. The process never ends; building blocks are added one after the other until the desired library is put together [16].

Advantages:

1. Method of having choice for large libraries.
2. Less reaction vessels required.

Disadvantages:

1. Less amounts of the synthesized compounds available.
2. Three-fold amount of resin beads necessary.

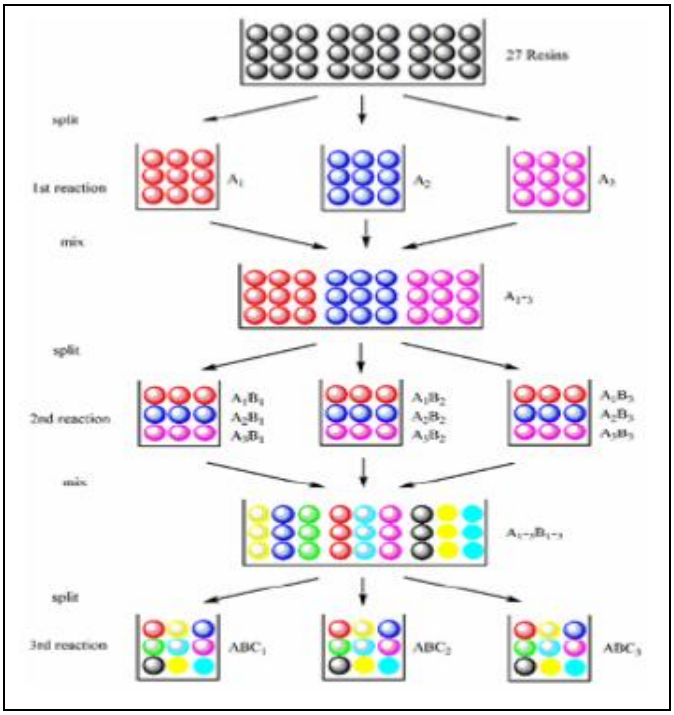

FIG. 4: SCHEMATIC REPRESENTATION OF SPLIT AND MIX SYNTHESIS

Solution Phase: The solution phase synthesis involves conducting chemical reaction simultaneously, preferably in well-ordered sets (arrays) of reaction vessels in solution. Soluble polymers are used in solution phase synthesis to support the product.

PEG is a frequently employed vehicle in solution phase synthesis; at ambient temperature, it can be either liquid or solid, and it has variable solubility in both organic and aqueous solvents. By converting one OH group of PEG to methyl ether (MeO-PEG-OH) it is possible to attached a carboxylic acid to the free OH and use in solution phase combinatorial synthesis. The main difficulty in this synthesis is isolating the product.

CONCLUSION: The current review came to the conclusion that combinatorial chemistry is crucial to the synthesis of many compounds. In the pharmaceutical industry, this method was frequently employed to quickly identify and characterize the structure of novel molecules. Particularly for medicinal chemists working on lead optimization, combinatorial chemistry remains a valuable tool.

The distinct qualities provided by new synthetic technologies can be extremely advantageous to combinatorial chemistry and parallel synthesis. These include the potential for quick reaction condition tuning and high-speed parallel processing of chemical transformations in the context of library production. Between the phase of solution and solid synthesis solid-phase organic synthesis, is the most significant technique.

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