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COMBINATORIAL CHEMISTRY: A NEW APPROACH OF DRUG DISCOVERY

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ABSTRACT

Combinatorial chemistry is providing significant impetus to new innovations in synthetic chemistry. Combinatorial chemistry has rapidly become the rising star among research methods, allowing scientists to efficiently test the feasibility of a multitude of new compounds. The pursuit of new drugs is but one challenging field in which these combinatorial methods are particularly advantageous, helping researchers meet the modern-day demands of a highly competitive environment. This article emphasises that modern combinatorial synthesis is possible not only in the solid phase, but also in solutions. Moreover, it discusses computer-assisted methods as well as the apparatus and instrumentation required for the combinatorial method. Combinatorial chemistry is to be able to synthesize, purify, chemically analyze & biologically testing all structures in library, here it describes the use in drug design & discovery to find new lead structures in shorter time.

KEYWORDS: Combinatorial chemistry, Drug discovery, Combinatorial library, Resins, Combinatorial synthesis.

INTRODUCTION

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. Synthesis of molecules in a combinatorial fashion can quickly lead to large numbers of molecules. For example, a molecule with three points of diversity (R_1, R_2, and R_3) can generate possible structures, are the numbers of different substituent utilized.

The basic principle of combinatorial chemistry is to prepare libraries of a very large number of compounds then identify the useful components of the libraries.

Combinatorial chemistry is a technique by which large numbers of structurally distinct molecules may be synthesised in a time and submitted for pharmacological assay. The key of combinatorial chemistry is that a large range of analogues is synthesised using the same reaction conditions, the same reaction vessels. In this way, the chemist can synthesise many hundreds or thousands of compounds in one time instead of preparing only a few by simple methodology.

Introduction to drug discovery

Drug discovery and development is an expensive process due to the high costs of R&D and human clinical tests. The typical development time is 10-15 years. R&D of a new drug involves the identification of a target (e.g. protein) and the discovery of some suitable drug candidates that can block or activate the target. Clinical testing is the most extensive and expensive phase in drug development and is done in order to obtain the necessary governmental approvals. In the US drugs must be approved by the Food and Drug Administration (FDA).

R&D – Finding the Drug

One of the most successful ways to find promising drug candidates is to investigate how the target protein interacts with randomly chosen compounds, which are usually a part of compound libraries. This testing is often done in so called high-throughput screening (HTS) facilities. Compound libraries are commercially available in sizes of up to several millions of compounds. The most promising compounds obtained from the screening are called hits – these are the compounds that show binding activity towards the target. Some of these hits are then promoted to lead compounds – candidate structures which are further refined and modified in order to achieve more favourable interactions and less side-effect.

Drug Discovery Methods

The following are methods for finding a drug candidate, along with their pros and cons:

1. Virtual screening (VS) based on the computationally inferred or simulated real screening;

The main advantages of this method compared to laboratory experiments are:

-low costs, no compounds have to be purchased externally or synthesized by a chemist;

-it is possible to investigate compounds that have not been synthesized yet;

-conducting HTS experiments is expensive and VS can be used to reduce the initial number of compounds before using HTS methods;
-huge amount of chemicals to search from.

The disadvantage of virtual screening is that it cannot substitute the real screening. 2. The real screening, such as high-throughput screening (HTS), can experimentally test the activity of hundreds of thousands of compounds against the target a day. This method provides real results that are used for drug discovery. However, it is highly expensive.

Quantitative Structure-Activity Relationships (QSAR)

As mentioned in the previous paragraph it is necessary to know the geometrical structure of both the ligand and the target protein in order to use molecular docking methods. QSAR (Quantitative Structure-Activity Relationships) is an example of a method which can be applied regardless of whether the structure is known or not.

QSAR formalizes what is experimentally known about how a given protein interacts with some tested compounds. As an example, it may be known from previous experiments that the protein under investigation shows signs of activity against one group of compounds, but not against another group.

In terms of the lock and key metaphor, we do not know what the lock looks like, but we do know which keys work, and which do not. In order to build a QSAR model for deciding why some compounds show sign of activity and others do not, a set of descriptors are chosen. Typical descriptors are parameters such as molecular weight, molecular volume, and electrical and thermodynamically properties. QSAR models are used for virtual screening of compounds to investigate their appropriate drug candidates descriptors for the target.

Synthesis of Combinatorial Library

Combinatorial synthesis on solid phase can generate very large numbers of products, using a method described as mix and split synthesis. This technique was pioneered by Furka and has been enthusiastically exploited by many others since its first disclosure. For example, Houghton has used mix and split on a macro scale in a "tea bag" approach for the generation of large libraries of peptides.

The method works as follows: a sample of resin support material is divided into a number of equal portions (x) and each of these are individually reacted with a single different reagent.

After completion of the reactions, and subsequent washing to remove excess reagents, the individual portions are recombined, the whole is thoroughly mixed, and may then be divided again into portions. Reaction with a further set of activated reagents gives the complete set of possible dimeric: units as mixtures and this whole process may then be repeated as necessary (for a total of n times). The number of compounds obtained arises from the geometric increase in potential products; in this case x to the power of n.

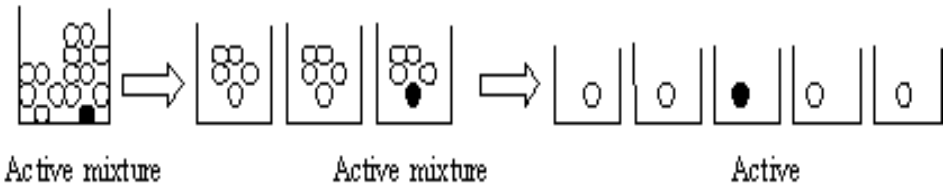
A simple example of a 3 x 3 x 3 library gives all 27 possible combinations of trimeric products. X, Y and Z could be amino acids, in which case the final products would be tripeptides, but more generally they could be any type of monomeric unit or chemical precursor. It can be seen that the mix and split procedure finally gives three mixtures each consisting of nine compounds each, and there are several ways of progressing these compounds to biological screening. Although the compounds can be tested whilst still attached to the bead, a favoured method is to test the compounds as a mixture following cleavage from the solid phase. Activity in any given mixture reveals the partial structure of active compounds within the library, as the residue coupled last (usually the N-terminal residue) is unique to each mixture. Identification of the most active compound relies on deconvoluting the active mixtures in the library through further synthesis and screening.

In the example where the active structure is YXY, the mixture with Y at the terminal position will appear as the most active. Having retained samples of the intermediate dimmers on resin addition of Y to each of the three mixtures will give all nine compounds with Y at the terminal position, and the second position defined by the mixture. The most active mixture here defines the middle position of the most active trimmer to be residue X. Finally, the three individual compounds can be independently resynthesised and tested to reveal both the most potent compound and also some structure activity relationship data.

By using a colourimetric assay, beads bearing peptide sequences that bound tightly to the protein streptavidin or to an antibody raised against β-endorphin were revealed by visual inspection. Bead picking using micromanipulation isolated the beads, and the active peptide structures were determined by microsequencing.

A modification of this method has allowed screening of such libraries in solution. Linkers have been devised that allow several copies of the library compounds to be released sequentially. Using this method it is possible to identify an active mixture using a solution

assay, and then return to the beads that produced these compounds, and redistribute them into smaller mixtures for retest. By repeatedly reducing the mixture size, ultimately to single compounds, the bead containing the most potent sequence may be identified and the peptide product sequenced.



METHODS OF COMBINATORIAL CHEMISTRY

There are two major approaches in combinatorial chemistry. The "pool and split method" involves attaching the starting compounds to polymer beads. The beads are then split into 50 groups and reacted with the second set of reagents. After this reaction, all the beads are pooled, mixed together, and split into 50 groups again. If mixing is efficient, each group should contain approximately equal numbers of beads representing each of the 1000 first-generation products.

The groups of beads are then reacted with the next set of reagents. If the beads are tagged in some way after each reaction (for example, with a different fluorescent label to identify each reagent) the combination of tags will characterize each of the 50 000 second-generation products exactly. The finished beads can be screened for their ability to bind a particular target protein in the body. The compound with the best performance can be identified and tested further. Additional rounds of pooling and splitting allow libraries with millions of compounds to be generated and subjected to high-throughput screening (HTS).

The second method is called "parallel synthesis". All the different chemical structure combinations are prepared separately, in parallel, using thousands of reaction vessels and a robot programmed to add the appropriate reagents to each one. This method is unsuitable for the creation of very diverse libraries but is very useful for the development of smaller and more specialized libraries based on a particular skeleton (starting compounds).

1. Combinatorial Synthesis in Solution

Despite the focus on the use of solid-phase techniques for the synthesis of combinatorial libraries, there have been few examples where libraries have successfully been made and

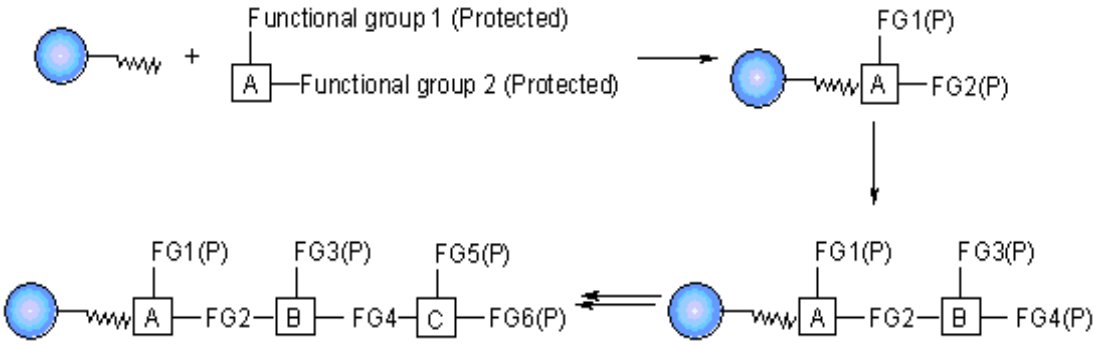
screened in solution. The benefit of preparing libraries on resin beads has been explained as offering advantages in handling, especially where a need to separate excess reagents from the reaction products is attached to the resin. In most of case a simple filtration effects a rapid purification and the product are ready to further synthetic transformation. But it should be remember that using solid phase chemistry brings several disadvantages as well. Clearly the range of chemistry available on solid phase is limited and it is difficult to monitor the progress of reaction when the substrate and product are attached to the solid phase.

Indeed some groups have expressed a preference for solution libraries because there is no prior requirement to develop workable solid phase coupling and linking techniques. The difficulty in purifying large number of compounds without sophisticated automated processes.

2. Combinatorial Synthesis on Solid-Phase

Since Merrifield pioneered solid phase synthesis back in 1963, work, which earns him a Nobel Prize, the subject, has changed radically. Merrifield's Solid Phase synthesis concept, first developed for biopolymer, has spread in every field where organic synthesis is involved. Many laboratories and companies focused on the development of technologies and chemistry suitable to SPS. This resulted in the spectacular outburst of combinatorial chemistry, which profoundly changed the approach for new drugs, new catalyst or new natural discovery.

The use of solid support for organic synthesis relies on three interconnected requirements:



1) A cross linked, insoluble polymeric material that is inert to the condition of synthesis;
2) Some means of linking the substrate to this solid phase that permits selective cleavage of some or all of the product from the solid support during synthesis for analysis of the extent of reaction(s);
3) A chemical protection strategy to allow selective protection and deprotection of reactive groups.

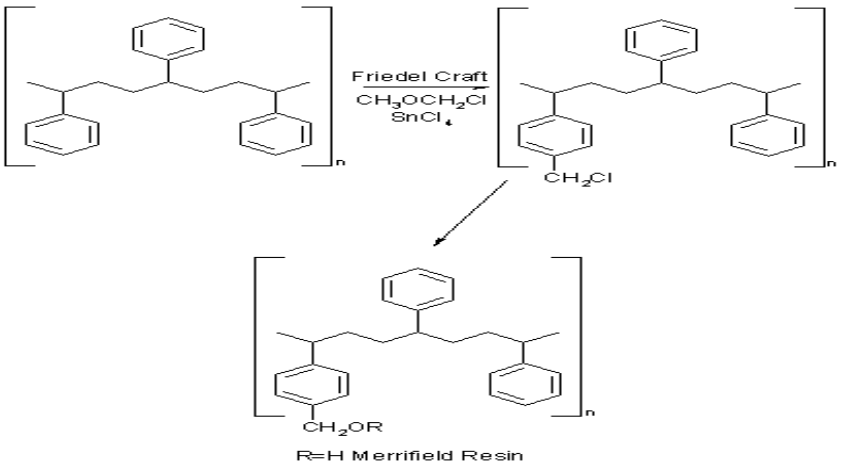
Merrifield developed a series of chemical reactions that can be used to synthesise proteins. The direction of synthesis is opposite to that used in the cell. The intended carboxy terminal amino acid is anchored to a solid support. Then, the next amino acid is coupled to the first one. In order to prevent further chain growth at this point, the amino acid, which is added, has its amino group blocked. After the coupling step, the block is removed from the primary amino group and the coupling reaction is repeated with the next amino acid. The process continues until the peptide or protein is completed. Then, the molecule is cleaved from the solid support and any groups protecting amino acid side chains are removed. Finally, the peptide or protein is purified to remove partial products and products containing errors.

Resins for Solid Phase Synthesis

In solid phase support synthesis, the solid support is generally based on a polystyrene resin. The most commonly used resin supports for SPS include spherical beads of lightly cross linked gel type polystyrene (1–2% divinylbenzene) and poly (styrene-oxyethylene) graft copolymers which are functionalised to allow attachment of linkers and substrate molecules. Each of these materials has advantages and disadvantages depending on the particular application.

Cross linked Polystyrene

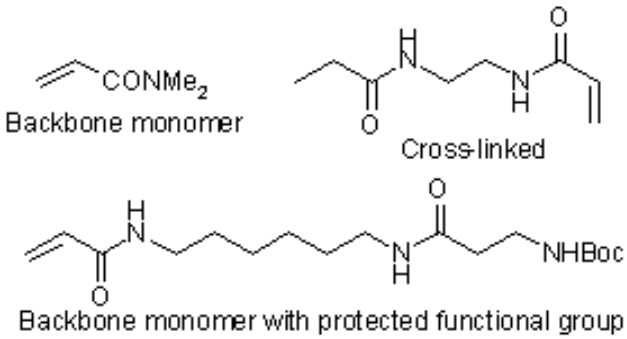
Lightly cross-linked gel type polystyrene (GPS) (Figure) has been most widely used due to its common availability and inexpensive cost. GPS beads which are functionalised with chloromethyl-, amino methyl-, and a variety of linkers are commercially available from a variety of sources. This swelling causes a phase change of the bead from a solid to a solvent-swollen gel, and therefore, the reactive sites are accessed by diffusion of reactants through a solvent-swollen gel network. In solvents, which swell the polymer well, the gel network consists of mostly solvent with only a small fraction of the total mass being polymer backbone. This allows relatively rapid diffusional access of reagents to reactive sites within the swollen bead. In solvents, which do not swell the polymer, the cross-linked network does not expand and the diffusion of reagents into the interior of the bead is impeded.



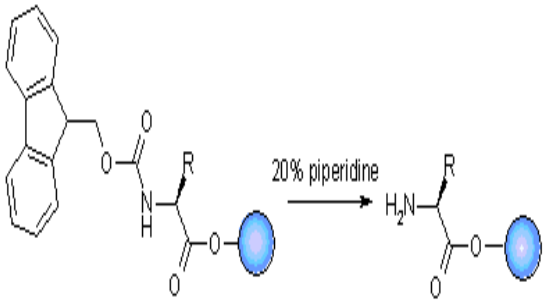
GPS has good swelling characteristics in solvents of low to medium polarity ranging from aliphatic hydrocarbons to dichloromethane. Polar, protic solvents, such as alcohols and water, do not swell GPS resins, and accessibility to all reaction sites may be compromised. Hence GPS supports are most suitable for chemistry performed in solvents of low to medium polarity.

Polyamide Resins

Sheppard designed polyacrylamide polymers for peptide synthesis as it was expected that these polymers would more closely mimic the properties of the peptide chains themselves and have greatly improved salvation properties in polar, aprotic solvents (e.g. DMF, or N-methyl pyrrolidinone).

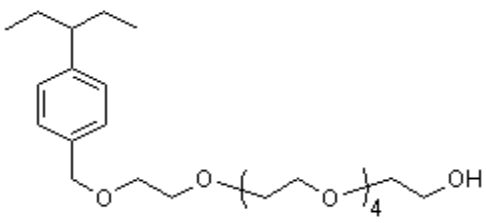


Sheppard also proposed the use of a new protection and linking strategy. The Merrifield approach depended on a benzyl ester linkage and Boc protection. But a more mild protection/deprotection were sought. The protecting group finally chosen was the fluorenylmethoxycarbonyl (Fmoc) which can be removed by base (usually piperidine).



TentaGel Resins

Poly (styrene-oxyethylene) graft copolymers, first reported by Bayer and Rapp, are another class of widely used supports for organic synthesis. As with the polyacrylamide resins, in order to produce a polar reaction milieu that is closer to the solvents generally used by solution synthetic chemists, grafted polymer beads have been prepared. The most pre-eminent of these is TentaGel resin which consists of polyethylene glycol attached to cross-linked polystyrene through an ether link, and combines the benefits of the soluble polyethylene glycol support with the insolubility and handling characteristics of the polystyrene bead. The resin was originally prepared by the polymerisation of ethylene oxide on cross-linked polystyrene already derives with tetraethylene glycol to give polyethylene glycol chains. Poly (styrene-oxyethylene) graft copolymers beads display relatively uniform swelling in a variety of solvents from medium to high polarity ranging from toluene to water. The polymers are produced by grafting ethylene oxide from the polystyrene backbone creating long flexible chains that terminate with a reactive site spatially separated from the more rigid polystyrene backbone.



Some disadvantages of Poly (styrene-oxyethylene) graft copolymers supports are:
- Relatively low functional group loading compared with GPS; the potential for the PEG chains to complex Lewis acids;
-The potential instability of PEG;
- The presence of linear PEG impurities found in the small molecule products after cleavage from the resin;

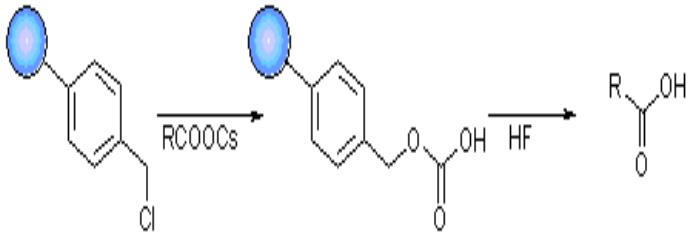
- The tendency for resins to become sticky and difficult to handle as the synthesis progress.

Linkers

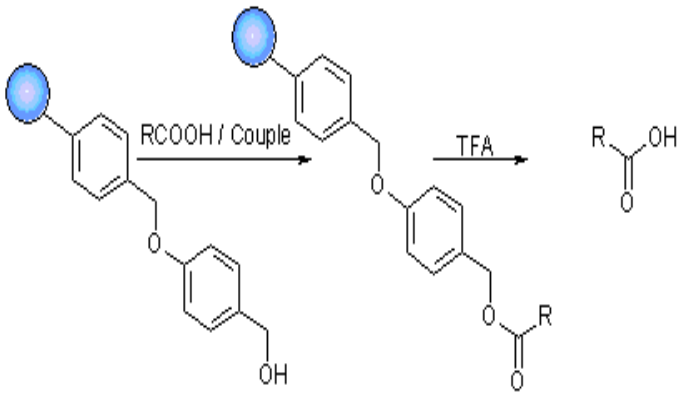
The group that joins the substrate to the resin bead is an essential part of solid phase synthesis. The linker is a specialised protecting group, in that much of the time, the linker will tie up a functional group, only for it to reappear at the end of the synthesis. The linker must not be affected by the chemistry used to modify or extend the attached compound. And finally the cleavage step should proceed readily and in a good yield. The best linker must allow attachment and cleavage in quantitative yield.

Carboxylic acid linkers

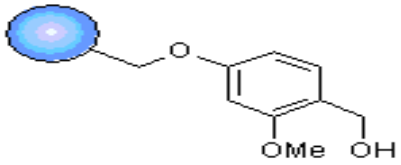
The first linking group used for peptide synthesis bears the name of the father of solid phase synthesis. Merrifield resin is cross-linked polystyrene functionalised with a chloromethyl group. The carbonyl group is attached by the nucleophilic displacement of the chloride with a cesium carboxylate salt in DMF. Cleavage to regenerate the carboxylic acid is usually achieved by hydrogen fluoride.



The second class of linker used for carboxylic acid is the Wang linker. This linker is generally attached to cross-linked polystyrene, TentaGel and polyacrylamide to form Wang resin. It was designed for the synthesis of peptide carboxylic acids using the Fmoc-protection strategy, and due to the activated benzyl alcohol design, the carboxylic acid product can be cleaved with TFA.



A more acid-labile form of the Wang resin has been developed. The SASRIN resin has the same structure as the Wang linker but with the addition of a methoxy group to stabilise the carbonium ion formed during acid catalysed cleavage.



Approaches to Combinatorial Chemistry

As with traditional drug design, combinatorial chemistry relies on organic synthesis methodologies. The difference is the scope--instead of synthesizing a single compound, combinatorial chemistry exploits automation and miniaturization to synthesize large libraries of compounds. But because large libraries do not produce active compounds independently, scientists also need a straightforward way to find the active components within these enormous populations.

Thus, combinatorial organic synthesis (*COS*) is not random, but systematic and repetitive, using sets of chemical "building blocks" to form a diverse set of molecular entities. Scientists have developed several different COS strategies, each with the same basic philosophy--stop shooting in the dark and instead, find ways to determine active compounds within populations, either spatially, through chemical encoding, or by systematic, successive synthesis and biological evaluation.

There are three common approaches to COS. During arrayed, spatially addressable synthesis, building blocks are reacted systematically in individual reaction wells or positions to form separated "discrete molecules." Active compounds are identified by their location on the grid.

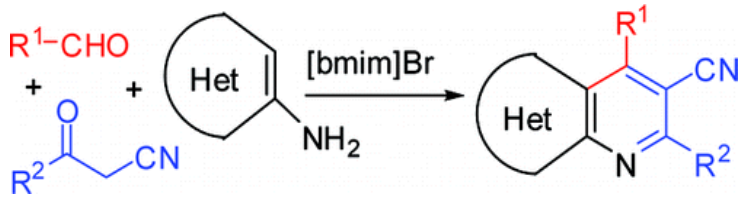
This method has been applied in scale (as in the Parke-Davis Pharmaceutical DIVERSOMER technique), as well as in miniature (as in the Affymax VLSIPS technique). The second technique, known as encoded mixture synthesis, uses nucleotide, peptide, or other types of more inert chemical tags to identify each compound.

During deconvolution, the third approach, a series of compound mixtures is synthesized combinatorially, each time fixing some specific structural feature. Each mixture is assayed as a mixture and the most active combination is pursued. Further rounds systematically fix other structural features until a manageable number of discrete structures can be synthesized and screened.

APPLICATION OF COMBINATORIAL CHEMISTRY

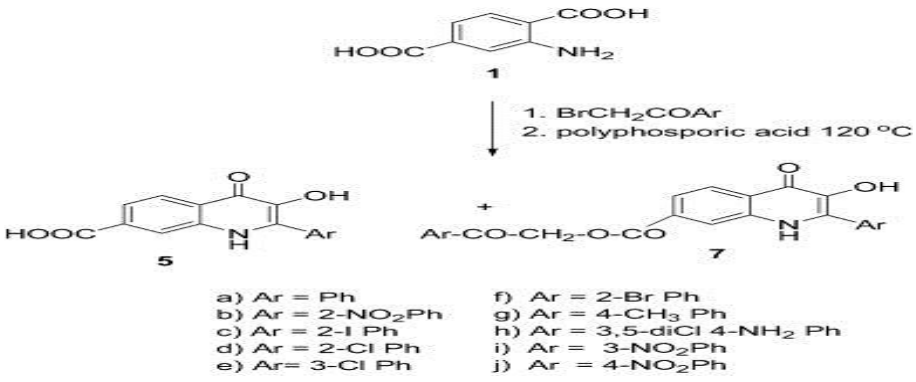
Applications of combinatorial chemistry are very wide.

1) Efficient One-Pot Three-Component Synthesis of Fused Pyridine Derivatives in Ionic Liquid.



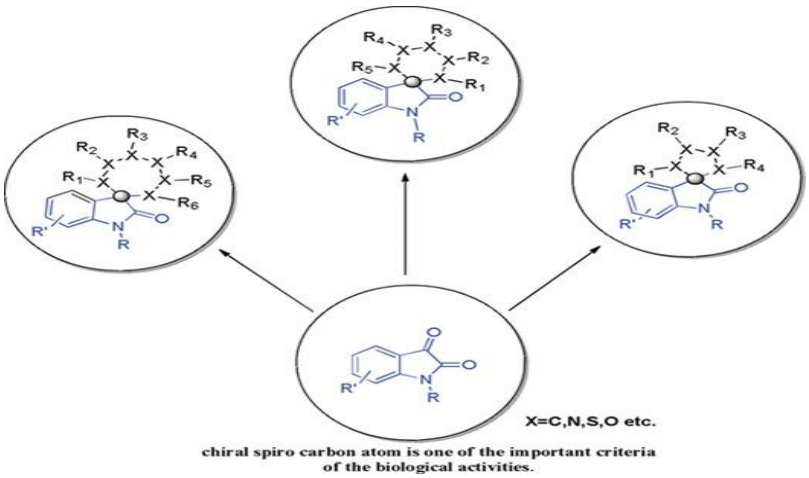
An efficient one-pot synthesis of fused pyridine derivatives (including pyrazolo [3,4-*b*]pyridine and pyrido[2,3-*d*]pyrimidine) by three-component reaction of aldehyde, acyl acetonitrile, and electron-rich amino heterocycles (including aminopyrazole and aminouracils) in ionic liquid is reported. This new protocol has the advantages of environmental friendliness, higher yields, shorter reaction times, and convenient operation.

2) Synthesis and cytotoxic activity of substituted 2-phenyl-3-hydroxy-4(1*H*)-quinolinones-7-carboxylic acids.



The preparation of 3-hydroxy-2-phenyl-4(1*H*)-quinolinones substituted in position 7 with a carboxyl group is described. The synthesis is based on the reaction of 2-aminoterephthalic acid with substituted α-bromoacetophenones and subsequent cyclization of the resulting bisphenacylesters in polyphosphoric acid.

3) Review of Synthesis of Spiro Heterocyclic Compounds from Isatin and 1,3-dipolar cycloaddition reactions.



The most attractive application of isatin in organic synthesis is undoubtedly in the highly reactive C-3 carbonyl group, which is a prochiral center as well. The construction of a spiroheterocyclic framework has always been a challenging endeavor for synthetic organic chemists as it frequently requires synthetic design based on specific strategies. Synthesis of various spiroheterocyclic compounds through multicomponent reactions and 1,3-dipolar cycloaddition reactions.

RESULT

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. Thus the combinatorial chemistry approach has two phases:

1. making a library.

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

Combinatorial synthesis is a process which rapidly produces many different compounds by using a few starting reagents and varying the reactions used. The objective is to build a large library of compounds from a starting "scaffold" to interact with specific biological targets. From this library, the most potent hits can then be isolated for further testing and development, eventually leading to a finished product to begin clinical trials.

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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