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Review Article

A REVIEW ON CURRENT INDUSTRIAL TRENDS FOR SYNTHESIS OF MEDICINAL COMPOUNDS

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ABSTRACT

In the pharmaceutical industry, computational methods have gained a major role in the discovery and development of new drugs. Yet, synthesis of the pharmaceutical compounds is among the most crucial steps of drug design along with the life science discoveries complementing it. The review focuses on all the recent developments in the application of computational methods as well as organic synthetic methodology in the field of pharmaceutical research. The most modern synthetic developments of pharmacologically interesting compounds (carbohydrates and nucleotides) as well as important synthetic methods such as combinatorial chemistry, solid-phase reactions, bio-assisted organic synthesis and asymmetric synthesis as applicable to drug discovery and development are critically discussed.

Keywords: Lead Discovery, Computational chemistry, Asymmetric synthesis, Biocatalysis, Green synthesis.

Drugs play important and a vital role in everybody's life in today's world. This is what drives to set up new R&Ds in pharmaceutical industry to study and develop knowledge databases on diseases, mechanisms treatment and medicines. Undoubtedly, pharmaceutical research is an interdisciplinary area and needs successful integration of people from various branches of sciences viz. Organic chemistry, analytical chemistry, biology, biotechnology and Informatics but the whole cumbersome process initiates with the efforts of synthetic chemistry and now, also with the simultaneous contribution from Informatics scientists.

In today's scenario of advanced scientific and technical knowledge and professionals of exceeding qualifications and of course the advancement in the field of instrumentation evident from the wealth of literature, including publications and patents, available and added each day, it is not a question today, how to synthesize new molecules but the challenges presented to the chemical research division of the drug discovery team are:

(a) How to synthesize molecules with increased possibility of showing therapeutic activity so as to minimize the cost of unproductive research (Lead Discovery) and

(b) Successful large-scale manufacture of Investigational New Drug (IND) candidates.

There had been and are currently ongoing, efforts which have revolutionized the field of synthesis, particularly of drugs to effectively circumvent these roadblocks. A review of such options available to the drug discovery unit for successful development of new drug substances is provided, divided into two sections concerned with two major roadblocks i.e. the Lead Discovery and the Scale-up synthetic methodologies.

1. LEAD DISCOVERY

R&D productivity has become a major concern lately as the business analysts and observers question the future viability of the pharmaceutical industry looking at the current trends. The average cost of bringing a new molecular entity into the market has now risen to 1.8 billion, the major pressure being created by the various key factors like expiration of key patents, cost-constrained healthcare and increasing demands of regulatory authorities [1]. In the light of the above and to prevent our healthcare from such economic losses, it has been proposed to adopt such methods which improve the efficiency and effectiveness of current research efforts. It is important to sustain in the world of growing competition to be updated with new methodologies as well as capture the best practice that suits one. The various upcoming options which are in trend followed by leading healthcare providers to increase their profit scale include:

1.1 Computational Drug Design:

Against the traditional trial and error based synthetic approach comprising synthesis of drug candidates and analyzing the biological activity blindly, a more rational, million-saving and high-throughput approach has developed with the advent of the computers. In the pharmaceutical industry today, high performing computational methods has become an intricate step in the process of early discovery and development of new drug candidates [2].

The concept of drug design can be defined as designing an ideal drug for a particular desired action in terms of selectivity and efficacy based on the molecular understanding of interactions between the drug and its target and then going for actual synthesis. With the help of computer technology and various modeling softwares and detailed information on molecular basis of the disease, informatics scientists can model a receptor or protein or any target supposed to be the site of action of the drug. After designing the biological target various site parameters and the proximity of the active site gives a wealth of information as to what molecules can fit in there and hence block the faulty mechanism of disease progression. Designing a molecule according to this information will give a more rational drug candidate and also help fighting the problem of toxicity as such a candidate will be selective. This is the basis of ***structure based drug design*** [3].

A process of designing analogues computationally by changing groups and atoms in a molecule followed by screening molecules against the modeled receptors is called ***virtual screening*****.**

There are two approaches for adopting a structure-based drug design in cases where protein structure details of the binding site and its active ligand is available: one is based on focusing on the active site structure and the other is to place the basis of research on the active ligand that he/she has at her convenience to understand the pharmacology and come up with a successful drug candidate.

In ***ligand-based drug design***, one studies the two-dimensional and three-dimensional structure of the available drug molecules and designs the new molecule such that the basic pharmacophore is maintained and further optimizes the other parts of the molecule meeting all other aspects to develop a drug like easier development process, solubility characteristics, compatible with formulation development process and ingredients etc. Designing of Celecoxib and other selective Cox-2 receptor inhibitors can be illustrated as an example of Ligand based Drug Design.

The impetus for the discovery of Cox-2 inhibitors was the gastrointestinal side effects of Non-steroidal Anti-inflammatory Drugs (NSAIDs). NSAIDs, discovered since 1971, were known to have pharmacological effects in pain and fever, inflammation and undesired effects like GIT ulcers. The separation of the beneficial effects from the undesirable effects waited till the discovery of another isoform of Cox

in 1991, i.e. Cox-2 which was found to be located in leucocytes, synoviocytes and in CNS locations whereas Cox-1 distributed primarily in GIT [4]. So, it became obvious to search for selective Cox-2 receptor inhibitors. The first breakthrough reports came for the compounds DuP697 and NS398 which were anti-inflammatory but not ulcerogenic and were modified to clinically active forms, marketed as Celebrex and Vioxx [5]. The two drugs were among the most frequently prescribed new drugs in the United States. Vioxx, however, was later withdrawn voluntarily in 2004, due to an increased risk of myocardial infarction and stroke [6].

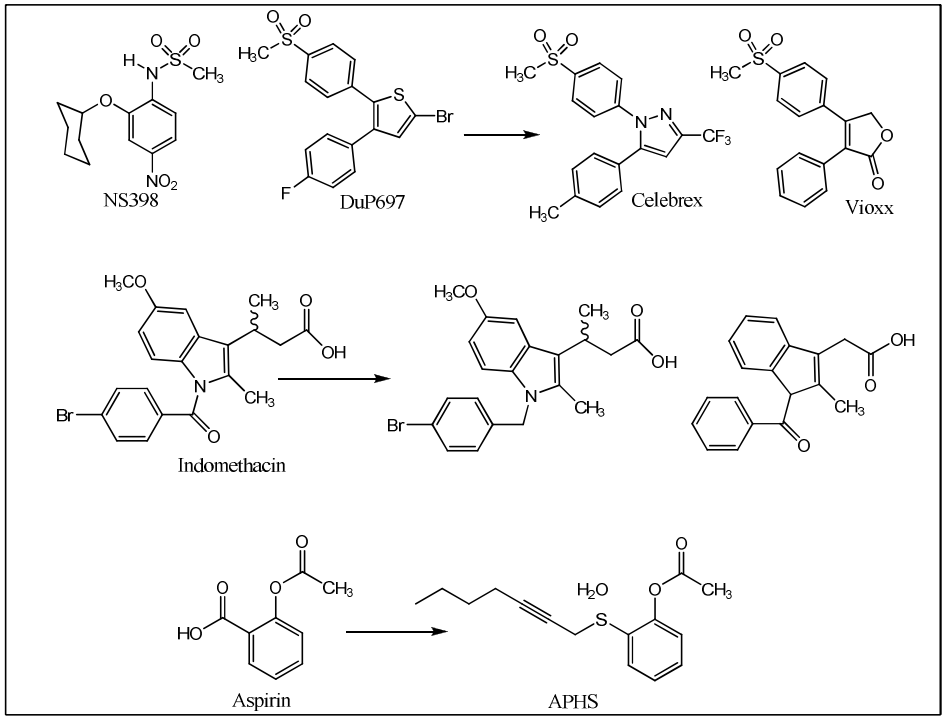


Fig. 1: Origin of different classes of Cox-2 Inhibitors [5]

Ligand based design is especially an advantage in research areas where the target or receptor has not yet been identified. Although various tools for this application have been developed, they all suffer from their own limitations particularly referring to a thorough understanding of the principle of method chosen and thus accuracy is largely user dependent. The recent pharmacophore approach methods have also been developed to take into account conformational flexibility of the molecules while binding to the receptor [7].

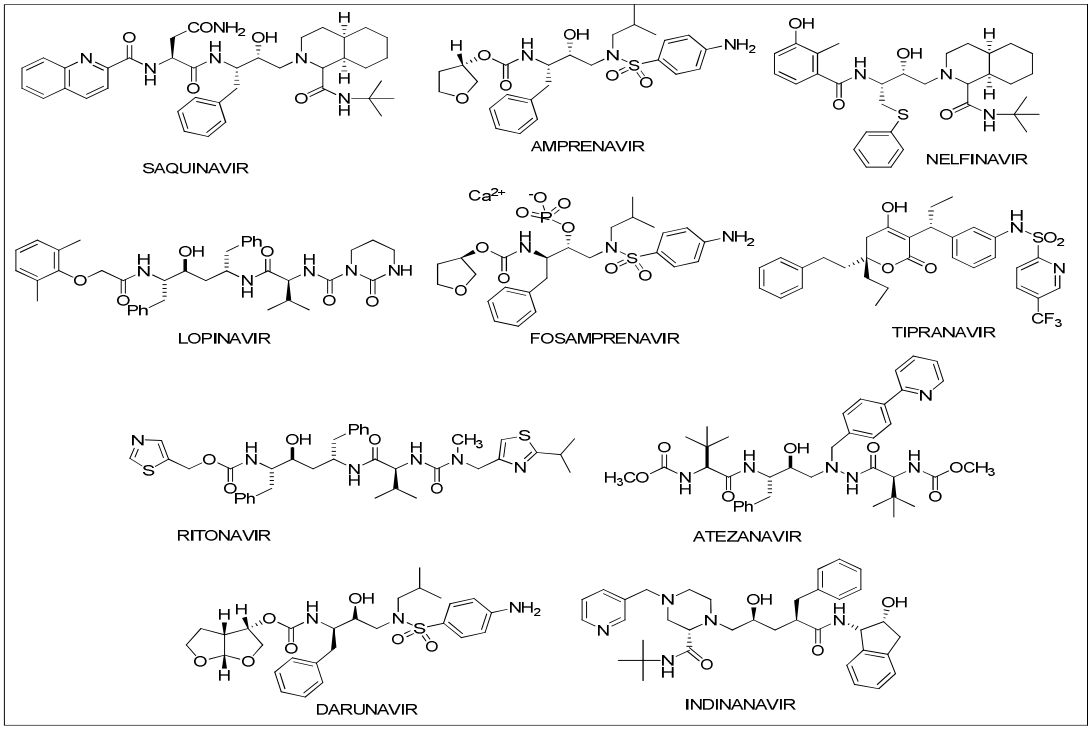


Fig. 2: The FDA-approved protease inhibitors [9].

In ***Receptor-based drug design***, the steps include a complete study and understanding of the receptor, designing a virtual model where all the distances and angles i.e. all three dimensional parameters imitate the real receptors followed by construction of template molecules which could fit in the simulated receptors. HIV protease inhibitor designing was an application of this concept.

HIV belongs to a class of viruses called retrovirus and presents three main enzymes reverse transcriptase (RT), integrase and HIV protease. As the virus enters a cell the RT generates the DNA from the viral RNA, integrase integrates this DNA into the cell nucleus and HIV protease is needed to cleave viral polyprotein precursor into individual mature proteins synthesized from the infected nuclear material of the cell. The structure and the mechanism of action of HIV protease has been studied extensively till now and more than 140 structures are available for this enzyme. The knowledge accumulated through the last two decades about the structure and mechanism of action of this enzyme has paved way for designing inhibitors for the enzyme [8,9].

A more developed and more rational version of structure based drug design is the ***Fragment based Drug Design***. Fragments are the small, low molecular weight molecules that would usually form a part of the drug. In contrast to the high throughput screening (HTS) approach involving sequentially the steps of designing, synthesizing and screening of synthesized compounds against drug targets, fragment based lead discovery involves generation of a library of fragments, identification of fragments suitable for developing biologically active candidates followed by combination or optimization of these fragments to generate lead compounds without the need of knowing any of the structural or mechanistic details. Hence fragment based approach is based on a logical approach of designing ligands emphasizing on efficiency and selectivity and applicable to large group of targets as the need for knowledge of the structure of targets, mechanisms or the lead compounds is negated [10].

Discovery of Protein kinase inhibitors and metalloproteinase inhibitors were areas where fragment based approach was exploited. The development of small molecule inhibitors for the non-receptor type tyrosine kinase C-src, involved the steps of preparing a set of potential binding fragments containing a common chemical linkage group, screening of the prepared library of fragments against tyrosine kinase enzyme, preparing again a combinatorial library of molecules by linking two of the screened positive fragments linked by the common chemical linkage groups and final screening of the combinatorial library to identify the tightest-binding ligands [11]. Similarly the concept was used to discover potent ligands for the enzyme matrix metalloproteinase stromelysin (MMP-3). Here again, two of the ligands which showed the binding to the enzyme were linked to give inhibitors for the enzyme. The scientists used the NMR technology to observe the binding between the enzyme and fragments [12].

In a recent study, fragment-based ligand approaches was exploited in the area of Alzheimer Disease and several guidelines were provided for the design of multi-target inhibitors of protein linked to cause and progression of Alzheimer Disease [13].

1.2 Natural Product based Drug Discovery:

Natural products provide a rich source of biologically active molecules and many of the approved drugs are derived from natural sources in spite of the fact that only 10% of the total biodiversity have been explored for biological activity till date.

The bioactive constituents distributed distinctly in various parts of the nature may serve us as potential therapeutic candidates while others may be poisonous. These active constituents are actually secondary metabolites and biosynthesized naturally. The primary metabolites viz. Carbohydrates, proteins, fats are present ubiquitously in all but the constitution of secondary metabolites varies every time as a result of adaptation of the organisms to the natural environment depending on source species, area, climate, season etc. It can be observed that nature synthesizes a diversity rich infinite collection of constituents starting from the same building blocks [14,15].

Natural product based drug discovery may not be considered the most fashionable trend in the field of pharmaceuticals by the researchers but the reality is that still a large number of drugs are derived from natural molecules. An example to model the statement is the present chemotherapy of Malaria. There is no compound other than natural products that have established their role in the treatment of Malaria or which is a part of lead optimization or preclinical development phase in Drug Discovery of Anti-malarials. The major advantage of this strategy is diversity and complexity which is difficult to achieve in synthesized library of new molecules [16].

The conventional process of natural product based drug development include the steps of drug discovery (serendipitous or rational or traditional), processing of extracts followed by identification and isolation of the active constituent, total synthesis of identified compounds and/or its analogs, and optimization of the molecule with parallel bioassays as to overcome physicochemical/ biological barriers. The rich natural resources have led to the discovery of various compounds. But there are disadvantages with the conventional approach:

(1) Non-availability of reliable resources

(2) Limited supply

(3) Complexity of isolation, very small quantities of bioactive constituents.

(4) Formidable analog synthesis

Apart from the above disadvantages, it is also evident that natural compounds mostly present us with hits rather than potential leads as from their activity/ toxicity profile. And there exists always a need to optimize these hits to successfully convert to a new medicinal entity. Here a combination of strategies like *in silico* techniques, High Throughput Screening, Fragment Based Drug Design, ligand or receptor-based techniques and others discussed may be helpful.

Therefore, the ideal approach would be to explore biodiversity presented by the nature to get a diverse library of hits and then engineer these hits with the help of novel strategies to serve us with molecules of medicinal importance [17].

Bio-Organic Synthesis: Apart from the secondary metabolites which provide direct contribution to the list of hits or leads, primary metabolites also exhibits potential importance in drug discovery. These biomolecules and their derivatives have their importance in transforming lead molecules into active therapeutic candidates by improving activity/ toxicity profile and as inspiration for mimetic design. Four major types of biomolecules are nucleic acids, carbohydrates, peptides and lipids and the others are the various possible hybrids of these four. The success of the strategy can be seen from the wealth of literature present in this area.

Since lipids are molecules with a large molecular formula and high molecular weight, they are mostly used in drug delivery formulations and also in gene delivery [18]. While other biomolecules like peptides, sugars and nucleic acids are extensively explored for options in drug design. These provide an advantage of better absorption, easy excretability, lesser problems of toxicity over the completely synthetic or the natural products obtained from non-human sources. Carbohydrates for example are very complex and diverse structures and play important biological role in human body. And the synthesis and analysis of oligosaccharides and glycoconjugates like glycosaminoglycans, and development of carbohydrate-based vaccines has recently been the major exploration area in the field of drug research [19]. Similarly nucleic acids and peptides, being present as essential components of structure of cells in human body, are important targets for therapeutics. Drugs like Acyclovir and Cytarabine are the examples of successful breakthroughs in the area [20-22].

Peptide research is still in its infancy but an important area in medicinal chemistry research. Synthetic amylin was the first peptide drug approved by FDA to lower blood sugar in Type-1 Diabetes. Since then the interest developed and today the science is used in research of almost all therapies. An example is the tetrapeptide Carfilzomib recently approved by FDA on 20 July 2012 for use in relapsed and refractory multiple myeloma which is derived from naturally-occurring epoxomicin [23].

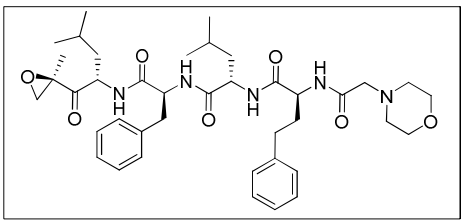


Fig. 3: Carfilzomib [23]

Combinatorial Synthesis: The aim of this approach is to increase the number of compounds in the library and then screen and select those with most promising biological profile. The methodology includes selecting a core motif and appending various building blocks to design and synthesize a number of compounds. However, as this approach works around the same central core structure, the library misses on the diversity front. As chirality and rigidity are the two most important features distinguishing approved drugs and natural products from compounds in combinatorial chemistry libraries, these are the two issues emphasized in so-called diversity oriented libraries [24].

	X_1	X_2	X_n
Y_1	X_1-Y_1	X_2-Y_1	X_n-Y_1
Y_2	X_1-Y_2	X_2-Y_2	X_n-Y_2
Y_m	X_1-Y_m	X_2-Y_m	X_n-Y_m

Fig. 4: Principles of combinatorial chemistry [4]

As against the rational structure based drug design, the focus here is not on a particular biological target or a particular therapeutic area but generating a rich and diverse pool and the idea that the eventual target for a compound in phenotypic screening can be selected from any one of the cell's or organism's entire collection of proteins [25].

Diversity oriented synthesis is principally inspired from the natural product drug discovery and adds the element of diversity to combinatorial library synthesis to overcome its drawbacks. In combinatorial chemistry, the structural framework was same and only the building blocks were varied but in Diversity oriented synthesis, the building blocks, the structural framework and the stereochemistry all are varied [26].

An example of application of this methodology is illustrated by the study conducted for the discovery of a novel antimalarial compound comprising phenotypic screening of lead compound library generated using DOS approach against *Plasmodium falciparum* asexual blood-stage parasites to generate initial hits. Structure-activity relationships guided further synthesis of compounds with improved potency and water solubility, yielding a potent inhibitor of parasite asexual blood-stage growth [27].

Another recent example employing a DOS library of 55 compounds quickly generated from 1,3-cyclohexanediones, dimethylformamide dimethylacetal, and various *N*-substituted cyanacetamides led to synthesis of 2,5-dioxo-5,6,7,8-tetrahydro-2*H*-chromene-3-carboxamide derivatives *via* hydrolysis of 4-cyanobuta-1,3-dienolate salts. These chromenes contain a 2-pyrone scaffold which is present in many well-known natural and synthetic biologically active compounds [28].

While DOS stresses on diversity, **Chemogenomics** works on the factor of therapeutic efficacy and helps in providing a smaller and better libraries. In chemogenomics, the ultimate goal is somewhat broad, including discovery of novel drugs and identification of new drug targets. It is the systemic screening of chemical libraries of small molecules against individual drug target families e.g. G-protein coupled receptors, nuclear receptors, proteases, kinases etc. By identifying screening hits that modulate the activity of the less well characterized members of the target family, the function of these novel targets can be elucidated. Furthermore the hits for these targets can be used as a starting point for drug discovery [29].

Library construction is somewhat typical in this approach as it includes known ligands of at least one and preferably several members of the target family. Since a portion of ligands that were designed and synthesized to bind to one family member will also bind to additional family members, the compounds contained in a targeted chemical library should collectively bind to a high percentage of the target family [30].

1.3 Recent applications in Drug Discovery of Protein Kinase Inhibitors:

Protein Kinases are at present, one of the most pursued targets for anticancer drug discovery owing to their role in cell growth, proliferation and survival. These studies have led to the approval of not less than 22 anticancer agents by US Food and Drug Administration till date and many more are under the stages of clinical evaluation. These kinases are either self mutated and directly responsible for the survival and/or proliferation of cancer cell or may be required for different stages of tumor formation and maintenance as an adjunct factor. The mutationally affected enzymes are among the most clinically exploited ones as the response translated by inhibiting these is the most dramatic [31]. The compounds of the present class of protein kinase inhibitors useful as anticancer agents are divided into the subgroups according to the sites of their action *in vivo*. These inhibitors either act on the active site of protein kinases in the active or inactive conformation, competing with the ATP site or these may be allosteric inhibitors, that is, bind to the site adjacent to ATP site and inhibit the binding of ATP [32]. The direct inhibitors includes the FDA approved Imatinib and Nilotinib (inhibitor of ABL1, KIT and platelet-derived growth factor) [33] and Sorafenib (KIT, PGDFR and Raf inhibitor) [34]. Examples of the allosteric inhibitors include the CI-1040 (MEK1 and MEK2 inhibitor) [35] and BMS-345541 (inhibitor of nuclear factor κB kinase) [36]. All these inhibitors have been discovered using high through-put screening approach and identification by chemical assays. Another class of inhibitors identified is the covalent inhibitors which bind to the ATP-receptor irreversibly. These have been designed rationally which bind to the cysteine residue of the EGFR, VEGFR2 and few others.

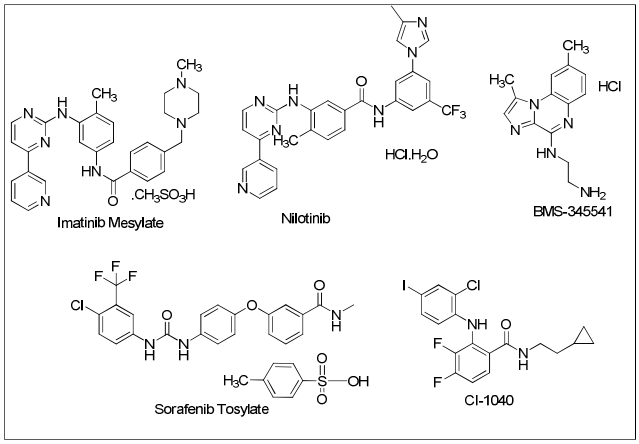


Fig. 5: Structures of some validated protein kinase inhibitors [32-36]

As there is still a need to develop strategies for discovery of new anticancer drugs, and HTS being proven to be an outdated approach, the new discovery scientists are focusing on analogue synthesis, structure informed design and fragment-based design strategies. Though these methods helps in identifying diverse set of molecules, Homology modeling is a technique where the identified scaffold can be co-crystallized with the kinase of interest and preliminary information about efficacy and selectivity can be obtained.

Thus, discovery of new effective and selective molecules against kinases can be followed using the present available techniques. But the challenges that are present today are the unpredictable toxicity patterns, and development of resistance for which what is required is a more detailed study about functioning of the protein kinases in the human system and an insight into their mechanistic pathways.

2. DEVELOPING THE LARGE SCALE SYNTHETIC ROUTE FOR INVESTIGATIONAL NEW DRUG CANDIDATES

The second section of this article deals with the large-scale synthesis of molecules which are screened positive for therapeutic efficacy and can now be labeled as Investigational New Drug. After the leads are discovered and preclinical trials are conducted, there occurs a need of transferring of technology from lab-scale synthetic experiments to plant-scale manufacture to meet further regulatory and market requirements.

An ideal synthetic route is one which has (a) Maximum yield of desired product (b) Minimum waste or byproducts (c) Minimum use of toxic and hazardous chemicals (d) Minimum steps and simple route (e) Regio/enantio/stereoselective (f) Easy isolation of desired.

In total, the route should be simple, economical, green and safe to the environment and to the scientists and also easily scalable with minimum plant requirements. Green Chemistry are based on the similar principles be it employed in lab-scale or manufacturing scale. In an effort aimed at efficient large scale manufacturing of lead molecules, the recent trends in the synthetic chemistry are presented.

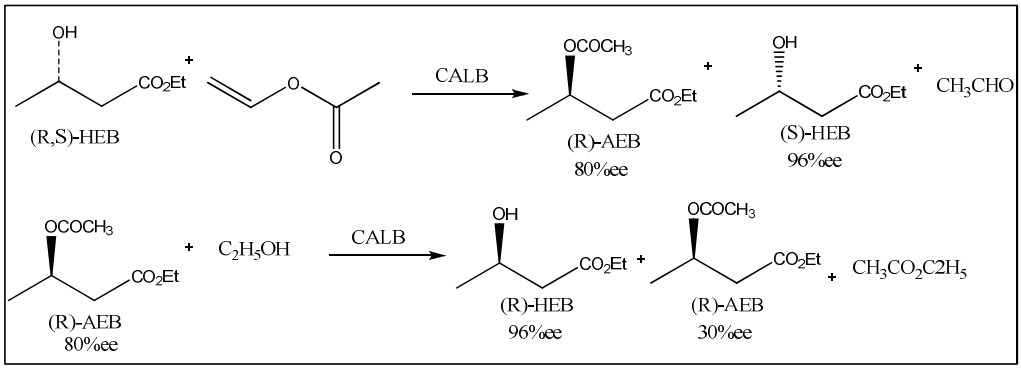
2.1 Bioassisted organic synthesis or Biocatalysis:

Biocatalysis is the use of biological catalysts to perform chemical modifications. These biological catalysts may be isolated enzymes or enzymes present in whole cells. Use of such alternatives helps in synthesizing molecules of diverse and complex structures which are difficult to synthesize by using chemical methods. In addition to the advantage of complexity, biocatalysis provides advantages of selectivity (chemoselectivity, regioselectivity and enantioselectivity), environmental acceptability, mild condition for reactions and easy scale-up due to bioreactors and fermenters. However, the challenges presented to the organic chemist include restriction of solvents due to solubility limitations and strict substrate specificity. The solubility limitations can be overcome by mixture of solvent, ionic liquids etc. and the substrate specificity can be broadened by using wild or bioengineered enzymes [37].

The use of biomaterial to obtain enantiopure compounds can be divided into three different methods:

2.1.1 Enzymatic Resolution of racemic mixture: Use of chiral objects (enzymes) to convert one of the enantiomers from the racemic mixture into product at a greater reaction rate than the other enantiomer. The biocatalysed reactions can give a maximum theoretical yield of 50% and enantiomeric excess of 100% but practically the theoretical yield can also be improved if the undesirable enantiomer is racemized back and recycled.

Ethyl-3-hydroxybutyrate (HEB) is an important precursor for the synthesis of many drugs and fine chemicals. For example the *(R)*-enantiomer is an intermediate for an anti-glaucoma drug and *(S)*-HEB is used for synthesizing pheromones and carbapenem antibiotics. An efficient, scalable two-step process for large-scale production of *(S)* and *(R)*-HEB is given employing a lipase from *Candida antarctica* B (CALB) [38].



Scheme 1: A two step enzymatic resolution process for production of *(S)*- and *(R)*-HEB [38]

Another example is the synthesis of D-amino-acids, an important intermediate in the production of antibiotics, agrochemicals and novel pharmaceuticals. Synthesis of D-amino-acids using chemical methods have been a challenge whereas synthesis of D-/L- racemic mixture is easy to synthesize which if combined with biocatalytic technologies can give enantiopure D-amino acids. For instance, some D-amino acids can be cost-effectively produced from the corresponding D,L-hydantoins with the D-hydantoinase carbamoylase system [39] or from D,L-amino acid amides using an L-amidase, followed by chemical amide hydrolysis [40]. D-Amino acids are also industrially accessible by enzymatic hydrolysis of the N-acylated racemates with an L-amino acid acylase [41].

2.1.2 Biocatalysed Asymmetric Synthesis: It is the synthesis of chiral molecules from non-chiral units using chiral biocatalysts in such a way that the different possible isomers are formed in different quantities.

A special example in this category is the biocatalysed Baeyer Villiger oxidation reaction using monooxygenases named BVMO i.e. Baeyer-Villiger Monooxygenases. The Baeyer-Villiger Oxidation of ketones to form esters and lactones is an important reaction in the pharmaceuticals and agrochemicals [42].

A rich amount of references showing applications of enzymatic catalysis in the synthesis of pharmaceutical drugs have been published including many books. One such review which presents the synthesis of chiral intermediates of drugs covers widespread therapeutic areas like antianxiety, antidiabetic, antihypertensive, anti-infective, anti-alzheimer therapy and thus shows the importance and popularity of the approach [43].

2.1.3 Stereoinversion: In stereoinversion, one of the stereoisomer from the racemic mixture is converted to the other giving a product rich in a specific stereoisomer. For example, an unnatural amino acid can be synthesized from natural amino acids using this method. The approach combines the biocatayzed oxidation (deracemization) with the chemocatalyzed reduction in a single pot. Examples of application of this approach can be in the stereoinversion of alcohols such as beta-hydroxyesters, aryl ethanols and terminal 1,2-diols, substrates with two stereocentres such as cyclohexan-1,2-diol, cis and trans indan-1,2-diol and pentan-2,4-diol [44] or in stereoinversion of amino acids.

The enantioselective biocatalyst converts undesired enantiomer of amino acid to its keto acid or imine intermediate, which is then converted by the metal catalyst (for example ammonia-borane

reducing couple) to the original enantiomer and the desired enantiomer. Repeating this cycle resulted in a stereospecifically pure product [45].

Enzymes like epimerases and racemases are some classes of enzyme which act specifically on chiral carbon. Racemases catalyze the stereochemical inversion around the asymmetric carbon atom in a substrate having only one center of asymmetry. Epimerases catalyze the stereochemical inversion of the configuration about an asymmetric carbon atom in a substrate having more than one center of asymmetry [46]. Application of these has been seen in the carbohydrate chemistry.

Other than asymmetric synthesis and resolution of racemic mixture, application of enzymes such as for determining enantiomeric excess in stereochemical reactions has also provided additional resources to synthetic chemists. Enzymatic method for determining enantiomeric excess (EMDee) can be used for the high throughput screening of asymmetric catalysts. In this method a specific enzyme is used to selectively process one enantiomer of a product from a catalytic reaction and determine the enantiomeric excess based on its affinity to the product. For example, acylases can be used to determine the products like amides, esterases and lipases for ester products and alcohol dehydrogenases for alcohol products [47].

Table 1: Examples of Chiral Drugs from Various Therapeutic Classes

Therapeutic Class	Examples
Antibiotic	Ofloxacin, Moxalactam
Antihypertensive	Enalapril, Captopril, Diltiazem
Antihypercholesteremic	Atorvastatin, Lovastatin
Antiarrhythmics	Propafenone, Tocainide
Antineoplastics	Cyclophosphamide, Iphosphamide
Antimalarials	Chloroquine, Halofantrine, Mefloquine
Muscle relaxants	Methocarbamol, Baclofen
Antiinflammatory	Ibuprofen, Naproxen, Ketorolac
Anticoagulants	Warfarin, Acenocoumarol
Anesthetics	Prilocaine, Ketamine, Pentobarbital
Antiemetics	Ondansetron
Antihistamine	Terfenadine, Loratadine

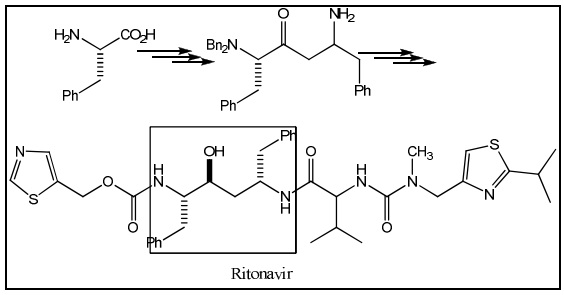
2.1 Asymmetric Synthesis:

Although chirality and isomers have been known to exist since long, its importance in pharmaceuticals has been appreciated only recently. Difference in pharmacological activity of enantiomers of chiral drug molecules is often displayed as biological receptors are all chiral molecules (proteins of L-amino acids and D-configured carbohydrates, etc.) Thanks to the awareness created in the industry, FDA now recommends activity assessment of each enantiomer for racemic drugs in body [48].

Table 1 gives examples of chiral drugs marketed as enantiopure compounds.

Asymmetric Synthesis broadly covers methods to prepare enantiopure products. There are four approaches for synthesis of enantiomer of the target molecule. (a) Chiral pool synthesis (b) using chiral auxilliaries (c) asymmetric catalysis (d) resolution of racemic mixture.

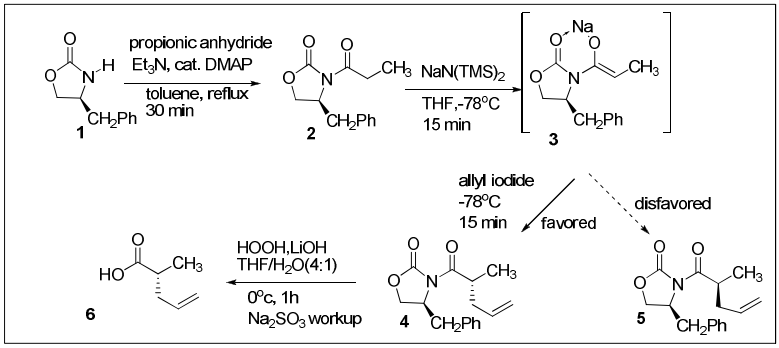
Chiral Pool Synthesis is the easiest approach of all where an enantiopure starting material is manipulated through a synthetic scheme designed to convert it into an enantiopure product. An example is the synthesis of HIV protease inhibitor Ritonavir developed by the Abbott group where scientists used readily available *(S)*-phenylalanine as starting material [49].



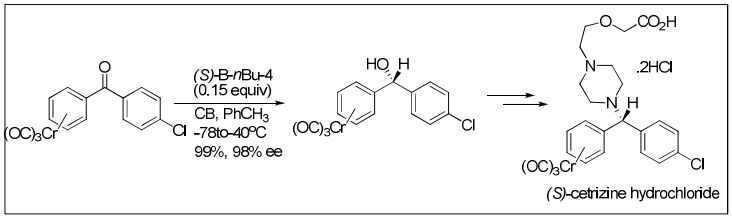
Scheme 2: Synthesis of Ritonavir from *(S)*-phenylalanine [49]

Chiral Auxilliaries is an approach which involves use of a chiral auxiliary, which forms an adduct to the starting material and physically blocks one of the possible trajectories favoring the synthesis of one of the isomer. The chiral auxiliary is then removed in later steps. Application of oxazolidinones as chiral auxiliaries in chiral allylation is shown as an illustration of the concept. The approach has been applied in diastereoselective alkylations, aldol additions, α-aminations, Michael additions, and Diels–Alder cycloadditions, among others to generate a number of synthetically useful enantiopure intermediates of industrial importance where chiral oxazolidinones were used as chiral auxiliaries [50-51].

The third approach is using **enantioselective catalysts and reagents** to induce chirality in achiral starting materials. An example is the synthesis of Cetrizine hydrochloride where the Corey-Bakshi-Shibata reduction conditions were employed [52].



Scheme 3: Use of oxazolidinones as chiral auxiliary for chiral allylation [50-51]



Scheme 4: Enantioselective synthesis of *(S)*-cetrizine hydrochloride [52]

And the reaction has since been exploited by organic chemists in a number of natural product syntheses lactones, terpenoids, alkaloids, steroids, and biotins, and has been utilized on large scale in industry [53]. A few examples of industrial application of Sharpless Asymmetric Epoxidation in the preparation of pharmaceuticals are given below [54].

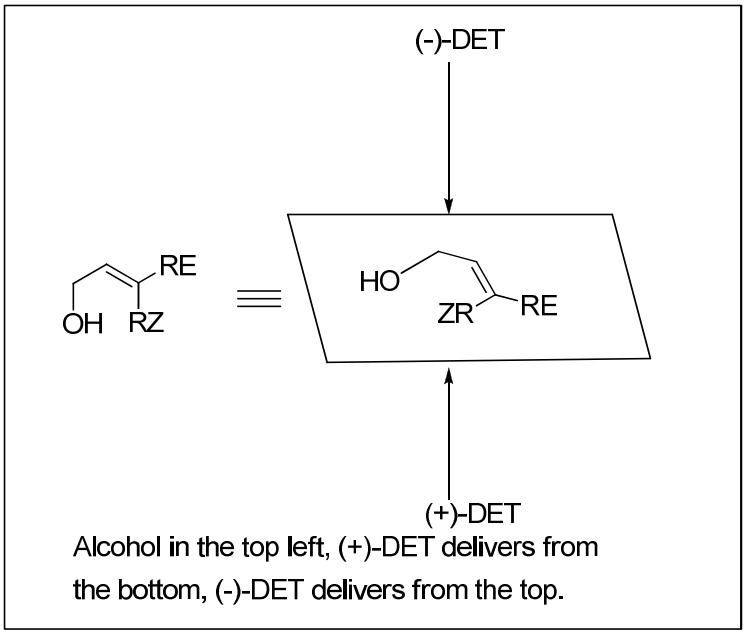


Fig. 6: Illustration of Sharpless Epoxidation reaction to prepare 2,3-epoxyalcohols from primary and secondary allylic alcohols

(Source: http://en.wikipedia.org/wiki/Sharpless_epoxidation)

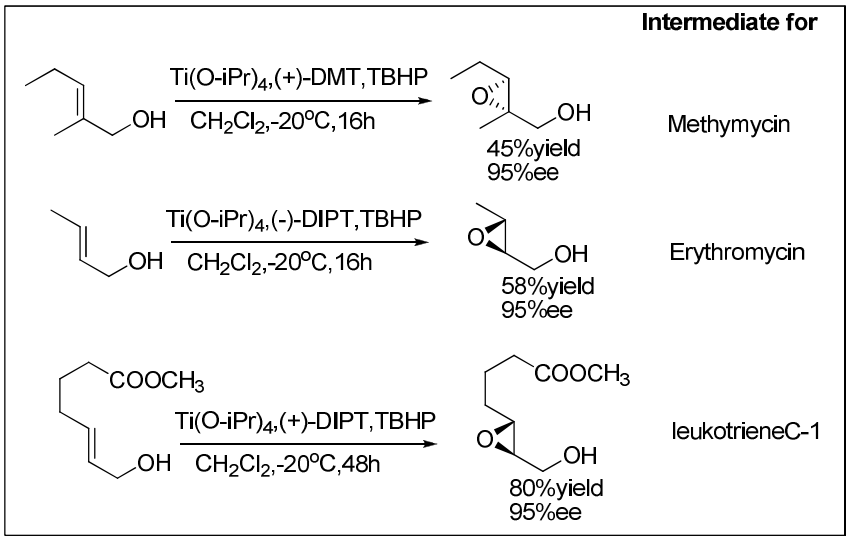
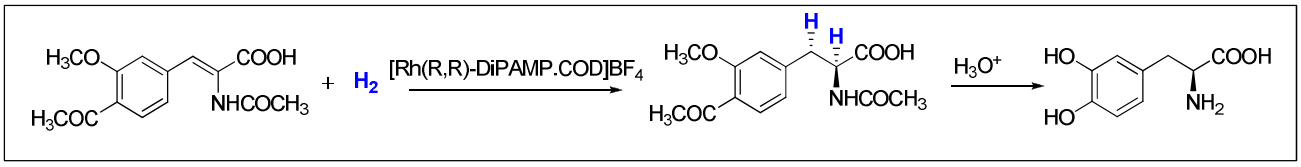


Fig. 7: Application of Sharpless Epoxidation reaction for synthesis of pharmaceuticals [54]



Scheme 5: Hydrogenation step in the industrial production of L-DOPA [56]

The Sharpless Epoxidation has also been used for the total synthesis of various carbohydrates, terpenes, leukotrienes, pheromones, and antibiotics [55].

The methodology used by Knowles while working for the Monsanto Company in an enantioselective hydrogenation step in the industrial production of L-DOPA also works on the principle of enantioselective catalysis [56].

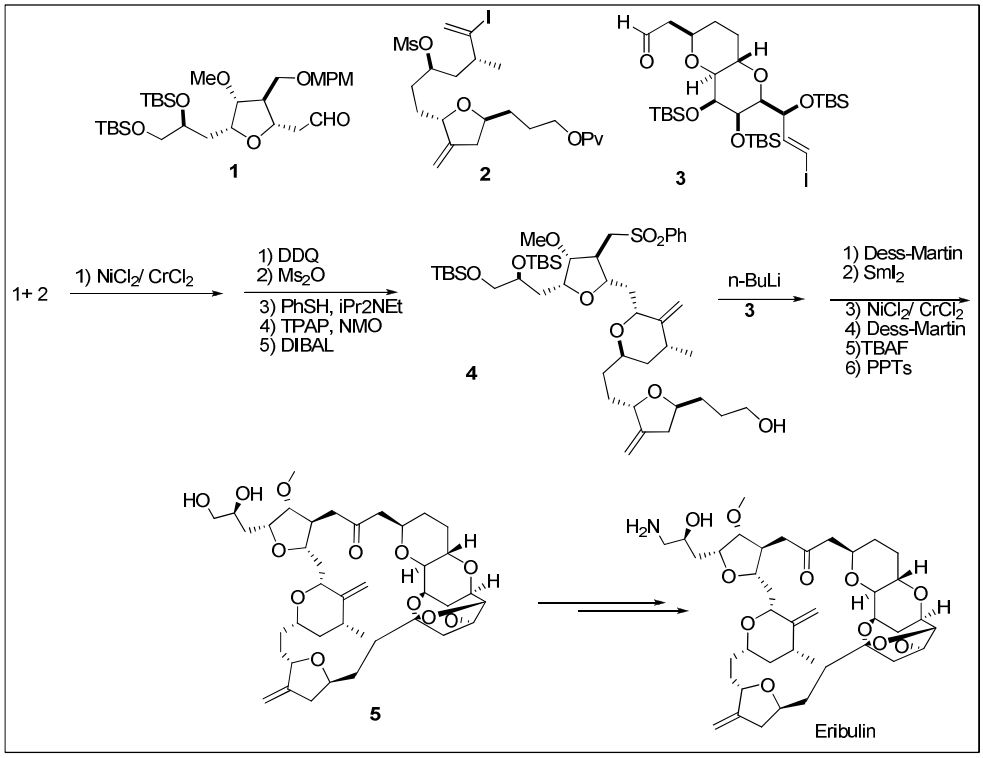
2.2.1 Application of asymmetric synthesis in preparation of Eribulin:

With the advent of such advanced synthetic techniques, the challenging synthesis of molecules with complex stereochemistry is also been brought inside the scope of synthetic skills of the chemical research scientists today.

For example, the newly approved anticancer drug Eribulin mesylate, approved by FDA in the year 2010 is marketed by Eisai Company under the name HALAVEN for use in treatment of patients with metastatic breast cancer who have received at least two prior chemotherapy regimens for late-stage disease, including both anthracycline- and taxane-based chemotherapies [57].

The molecule is derived from a natural compound, Halichondrin B [58], isolated from a marine sponge, *Halichondria okadai* in 1986. The total synthesis of the macrocyclic, Halichondrin B was carried out at Harvard in 1992 [59]. The scientists by synthesizing halichondrins and all its structural variants studied the structure activity relationship and optimized the molecule to make possible the drug discovery and development of Eribulin [60-61].

The challenging synthesis of such a complex molecule was made possible by the today's new and advanced synthetic techniques and the transition metal catalysis which enabled outstanding control on the diastereoselctivity of macrocyclic analogs [62]. The macrocyclics were prepared utilizing the principle of convergent organic synthesis involving coupling of the three building blocks **1**, **2** and **3** and employing protective group strategy. Fragment 2 (C14-26) [59] and 3 (C-1-13) [63] were synthesized using methods published before. Fragment **1** was synthesized and the three were coupled together to form the final compound **5** which is later converted to eribulin. The scheme includes fragment **1** coupling with **2** under Nozaki-Hiyama-Kishi conditions [64-65] followed by cyclization and removal of protecting group MPM and introduction of sulfone moeity to form **4**. Coupling of fragment **4** was afforded with fragment **3** followed by modification of ketone group at C1 to obtain an intermediate which underwent an intramolecular macrocyclic cyclization at C13-14 under Nozaki-Hiyama-Kishi conditions. Finally treatment with tetrabutylammonium flouride and pyridinium *p*-toluenesulfonate generated the compound **5** in good yields. Modification of side chain at C32 afforded Eribulin.



Scheme 6: Stereoselective synthesis of Eribulin [59,62-65]

Chiral Resolution is the simplest method among all other approaches of asymmetric synthesis, without requiring any advanced catalyst or reagent systems and involves separation of enantiomers from the racemic mixture. However this method has the disadvantage that the maximum yield of the desired enantiomer is only 50%.

The various methods include: Resolution by crystallization, using chiral resolving agent like tartaric acid and brucine to form diastereomers where one of those is favored thus allowing separation. Another method is by using chiral stationary phases to separate enantiomers by chromatography. This strategy is very commonly employed in small scale synthesis but others are important for large scale production of medicinal [66].

2.2 Green Chemistry: The 12 principles [67] of green chemistry are:

1. Minimum waste generation
2. Atom Economy of schemes
3. Designing less hazardous chemical syntheses
4. Designing safer chemicals with minimum toxicity
5. Use of safer solvents and auxiliaries
6. Design for Energy Efficiency (preferable reaction conditions-ambient temperature and pressure conditions)
7. Use of Renewable Feedstocks.
8. Reduce Derivatization steps
9. Use of selective catalysis
10. Design of products which degrade after their use
11. Real-time analysis for Pollution Prevention
12. Inherently Safer Chemistry for Accident Prevention

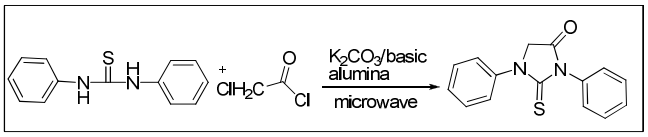
A few examples of recent trends presented here demonstrate the growing awareness towards the application of green chemistry in synthetic medicinal chemistry.

2.3.1 Solid Phase Synthesis: Solid phase synthesis is a methodology whereby one of the reactant undergoing a chemical transformation is attached to an insoluble solid support and after the completion of the transformation; it is cleaved to release the product. This methodology was first developed in 1963 for the synthesis of a tetrapeptide [68] which was later extrapolated for the synthesis of polynucleotides [69] and oligosaccharides [70].

Recently, application of combinatorial chemistry strategies in solid phase synthesis has allowed the extension of strategy for synthesis of multiple non-oligomeric organic molecules at same time. An advantage with this chemistry is that a number of reactions can be performed in a mixture simultaneously by linking the reactant to a resin and easy purification or isolation of products by simple filtration technique [71].

Solid phase Freidel Crafts reaction was employed in the synthesis of 2,3-benzodiazepines with good yields and purities. A specific example is the solid-phase synthesis of 1-aryl-3,5-dihydro-4*H*-2,3-benzodiazepin-4-ones, which are potentially useful for the treatment of epilepsy comprising friedel-crafts acylation of resin-bound 3,4-dimethoxyphenylacetate using various acyl chlorides yielding resin bound ketones as products which are ultimately converted into the corresponding 2,3-benzodiazepines [72].

Also the technique has been combined with microwave conditions to synthesize molecules having pharmaceutical applications [73].



Scheme 7: Solventless synthesis of Thiohydantoin [73]

A successful application of solid phase technique in microbial transformation of Cortexolone, an anti-dementia drug, is another example broadening the scope of synthesis from synthetic laboratory to biochemicals. [74]

2.3.2 Microwave chemistry: Microwave assisted heating offers the advantage of controlled conditions with short reaction times, and rapid optimization of reaction conditions to make it feasible for small scale synthesis and contribute to in the lead optimizations phase of drug development.

In contrast with conventional heating methods, microwave energy heats up the reaction media directly as the reaction vessel is transparent to microwave radiations allowing molecules of the reactants to couple with microwaves directly. This causes instantaneous localized superheating of molecules which respond to either dipole rotation or ionic conductivity due to interaction with magnetic and electric fields of microwaves.

The microwave-assisted reactions can be divided into two categories: Microwave reactions with solvents and solvent-free microwave reactions.

The 'green' Microwave assisted reaction can be made more greener by using water as solvents. Water has been proved to be an excellent solvent in microwave reactions [75].

The review presented by Besson *et al.* deals with the synthesis of novel heterocyclic structures using microwave radiations. In addition to the successful application of microwave in heterocycle synthesis, the review also suggests use of solid supports and appropriate reaction conditions which are advantageous over conventional methods [76].

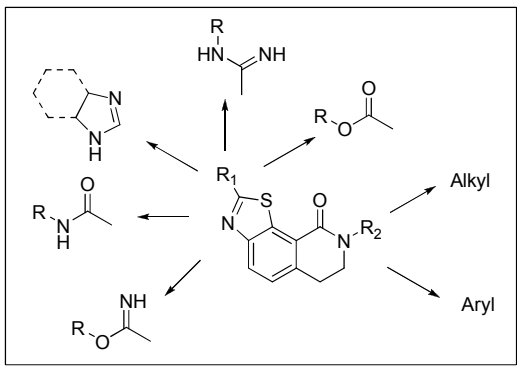


Fig. 8: Studied modulations of the ring in various positions under microwave-accelerated conditions. [76]

A synthesis of neuroleptic molecules Trazodone hydrochloride, Aripiprazole and their key process intermediates using conventional and microwave assisted reaction enhancement technique (MORE) is presented by Nandini R. Pai *et al.* highlighting the differences and advantages of microwave synthesis [77]. Use of microwave conditions has also been exploited for green synthesis of 1,5-Benzothiazepines, an important chromophore with broad range of therapeutic activities like anticonvulsant, CNS depressant, Ca^{++} channel blockers, anticancer, anti fungal, anti-HIV and antimicrobial activities. [78]

Another review by Anshul Chawla *et al.* details the the efficient "green" synthesis of benzimidazole derivatives by microwave induced reactions [79].

2.3.3 Nanocatalysis: Catalysis is one of the twelve principles of Green Chemistry as they are superior to the use of stoichiometric reagents. A green catalyst should possess higher activity, selectivity, efficient recovery from reaction mixture, recyclability and cost-effectiveness. Nanocatalysts, having particle size in the nano-range not only has higher surface area but has more important advantages. These are also known as quasi-homogenous systems as they have advantages of both homogenous and heterogenous catalyst systems. They have high activity and selectivity like homogenous catalysts and are easy to separate and are recyclable like heterogeneous catalysts [80].

The properties of these catalysts can be tuned by altering their size, shape and composition. For example, hexagonal Pt(111) surfaces are approximately 3-7 times more active than cubic Pt(100) surfaces for aromatization reactions [81].

Nanokinetix's new technology nanoparticle are designed such so that every nanoparticle will behave exactly the same. Also they contain a tethering agent which allows the individual particles to remain as discrete units in the reaction medium and also increases the catalyst bulk so they can be easily filtered after the reaction is over [82].

2.3.4 Solventless Synthesis Approach: Although the use of solvent is linked deeply and inseparably in a chemical reaction, use of extensive solvents is one of the major reasons for low sustainability of the reactions. The various methods developed by the synthetic chemists to develop sustainable chemistry in practice include developing solventless reactions, using water as solvent, or using green substitutes like ionic liquids and supercritical fluids as the reaction media.

Mechanochemistry (Using Ball mill for reactions)- Although solvent free reactions may go efficiently in homogenous conditions (solid-gas, solid-liquid and liquid-liquid), heterogeneous situations are challenging to develop efficient chemical reactions. As in the second example, in order to carry out reactions in solid-solid state in the absence of solvents, a medium is required which provides the reactants the required spatial and energy environment so that the reactant molecules can have effective collisions and form the products. For this, supporting techniques like solid phase reactions employing solid supports for reactants, thermal processes or irradiation with UV, microwave or ultrasound or mechanical energy by using ball mills or their combination are being exploited to replace the role of solvent [83]. Ball mills are ideal tools among the tools for non-classical ways of energy entry (microwave, photochemistry, ultrasound, and electrochemistry) combining a high mixing efficiency combined with high energy densities due to frictional forces [84]. In planetary ball mills, the grist is crushed by high-powered blows from the grinding balls. This mechanical energy is used to provide threshold energy for chemical reactions [85].

In an example of application of ball mill, the two components of an aldol reaction are combined together with the asymmetric catalyst S-proline in a ball mill in a mechanosynthesis. The reaction product has 97% enantiomeric excess. In a recent similar example α,α-Dipeptide *(*methyl ester of (S)-proline-(S)-phenylalanine), catalyzed the stereoselective formation of the expected aldol products in ball mill in solvent free conditions, with higher diastereo- and enantioselectivity relative to similar reactions in solution, up to 91:9 anti:syn diastereomeric ratio and up to 95% enantiomeric excess [86].

Using Ionic liquids- Another approach in this area which is gaining wide acceptance is using ionic liquids as they have potential applications as pharmaceutical solvents/cosolvents. Other than

solvents, ionic liquids have also been exploited as catalysts in the synthesis of API.

Application of ionic liquids has been extended to many important reactions used commonly in the synthesis of pharmaceuticals like Knoevenagel condensations and Robinson annulations [87a], Friedlander synthesis, Heck reactions, Diels-Alder reaction, Nucleophillic Displacement reactions (SN^2 reactions), and Friedel-Crafts reactions [87b] and heterocycle synthesis [87c] Another specific example is the synthesis of modafinil, wherein enantioselective oxidations leading to chiral sulfoxides was utilized in the route to synthesis [88].

2.3.5 Recycling Technology: Artimesinin is a known antimalarial and an anticancer drug, the synthetic method for the production of which is very complicated. Artemisinic acid is a substance produced as a by-product from the isolation of artemisinin from sweet wormwood, which is produced in volumes ten times greater than the active ingredient itself. Moreover, artemisinic acid can easily be produced in genetically modified yeast as it has a much simpler structure. Chemical researchers have developed a process to convert the artemisinic acid into artemisinin in a single step and a simple apparatus for this process, which enables the production of large volumes of the substance under very controlled conditions.

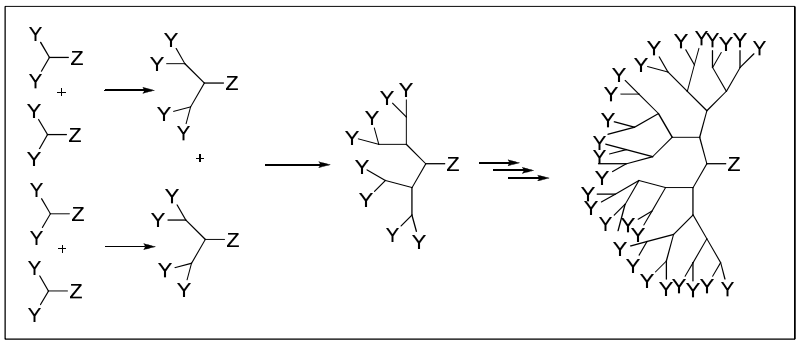
The antimalarial activity and other effect of the molecule to treat other infections and even breast cancer is due to an endoperoxide group which is a very reactive chemical group formed by two neighboring oxygen atoms. Photochemistry is used to incorporate this structural element into the artemisinic acid by converting oxygen into a form that can react with molecules to form peroxides [89].

The example illustrating the recycling technology concentrates on the economy of the reaction sequence to develop the pharmaceuticals by utilizing the waste products into useful entities.

2.3.6 Multicomponent/Convergent synthesis: is an approach which aims at improving yields. In linear synthesis, the overall yield of the product is reduced at every step. Hence this method involves synthesis of building blocks and coupling of all the fragments thus minimizing the sequential isolation and purification processes and improving the yields. Most advantageously, the methodology can be extended to solid phase synthesis, microwave synthesis or other green synthetic techniques [90].

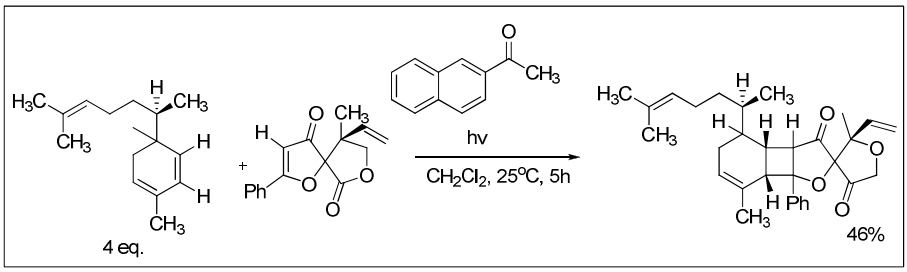
The situation can be exemplified by an illustration of synthetic scheme for the synthesis of:

Dendrimers: These are typically branched molecules with 3D symmetric morphology. Applications of dendrimers typically involve conjugating other chemical species to the dendrimer surface that can function as detecting agents (such as a dye molecule), affinity ligands, targeting components, radioligands, imaging agents, or pharmaceutically active compounds [91].



Scheme 8: Convergent synthesis of a dendrimer

(Source: http://en.wikipedia.org/wiki/Dendrimer#cite_note-holister-19)



Scheme 9: Use of Microwave in total synthesis of natural product *Biyouyanagin A* [93]

Proteins (Polypeptides) are again polymers of amino acids. Many amino acids/peptides are polymerized together in a convergent synthesis to yield a polypeptide or a protein [92].

An example of its use in total synthesis is the final step (photochemical [2+2]cycloaddition) towards the compound *Biyouyanagin A,* an inhibitor of HIV replication. The convergent synthesis involves two cascade sequences and a remarkably selective [2+2] cycloaddition reaction to forge the cyclobutane ring of the target molecule in the ultimate step [93].

CONCLUSION

Organic synthesis is the branch of science which focuses on generating compounds in laboratory and this science is extended for biologically active small molecules in pharmaceutical industry. A practical compilation of the chemical sciences with the life sciences discoveries like that of the target molecule and the disease mechanisms is the basis of rational drug discovery of small molecule therapeutics. Apart from the application of principles of rational drug discovery and other approaches like natural products based or diversity based drug discovery, modern concepts like green chemistry and applications like microwave chemistry help in developing an economic and more potential process to generate new therapeutic scaffolds. It is also evident from the literature that more often a combination of one or more of these strategies can help abridging the 'valley of death'. The examples, presented here, although not exhaustive, may help the pharmaceutical society in presenting an outlook to appreciate the new trends and techniques.

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