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"Drug Design strategy and chemical process maximization in the light of Molecular Hybridization Concept."

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A Draft Thesis is submitted for the partial fulfilment of PhD in Chemistry Thesis/Degree proceeding.

DECLARATION

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b. The work has not been submitted to any other Institute for any degree or diploma.

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(Subhasis Basu)

ACKNOWLEDGEMENT

This preface is to extend an appreciation to all those individuals who with their generous co-operation guided us in every aspect to make this design and drawing successful.

We are deeply indebted to our guide ***Prof./ Dr. …………………………………………….. *** *for his excellent guidance, encouragement and support throughout our project work. There profound theoretical knowledge as well as his practical experience that he readily shared with us have helped us to overcome many difficulties.*

We also thank our library and Laboratory staffs of Visva Bharati University for supplying necessary books, and Laboratory equipment and set up time to time as per my requirement.

Date: SUBHASIS BASU

- Dedicated to my parents

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Contents**:**

Abstract

Molecular hybridization of nucleic acids is the process in which two single-stranded nucleic acid molecules with complementary base sequences form a double-stranded nucleic acid molecule. Nucleic acid hybridization technology is a fundamental tool in molecular biology, and has been applied in various fields such as detection of gene expression, screening specific clone from cDNA or genomic library, determining the location of a gene in chromosome and diagnosis of diseases.

Cage like property for eg graphene or glue like bio material from spider of even polymer can bind rapid growth of cell for its binding nature at micro level or can trap bacterial or viral growth by trapping within Net like structure. The idea of Quantum Dots may be implied to identify such net as drug to separate from diseases infected Zone. Some time electrification or by application of Radioactivity through this net may reduce harmful diseases spreading.

Molecular hybridization: a useful tool in the design of new generation drug prototypes using the sequencing of Nucleic acids for a particular patient. Molecular hybridization is a new concept in drug design and development based on the combination of pharmacophore moieties of different bioactive substances to produce a new hybrid compound with improved affinity and efficacy, when compared to the parent drugs. Additionally, this strategy can result in compounds presenting modified selectivity profile, different and/or dual modes of action and reduced undesired side effects. So, in this paper, we described several examples of different strategies for drug design, discovery and pharmacy modulation focused on new innovative hybrid compounds presenting analgesic, anti-inflammatory, platelet anti-aggregating, anti-infectious, anticancer, cardio- and neuroactive properties.

By this molecular hybridization concept in the field of genetic engineering and as well as molecular biology, formation of a partially or wholly complementary nucleic acid duplex as association of single strands, usually between DNA and RNA strands or previously unassociated DNA strands, but also between RNA strands; used to detect and isolate specific sequences, measure homology, or define other characteristics of one or both strands. If we can detect the particular cause of critical disease like Parkinson, Cancer or AIDS for a particular patient. We can synthesis the new drug design for that patient to treat them for complete cure in this molecular hybridisation concept. So this is a new generation concept for developing drug as well as the cause of the particular diseases for a particular patient. Not only that by fusing more than one drugs structure components by Molecular hybridisation concept we can make a single drug for a patient with multiple affected diseases using computer simulation more modern method in the field of medicinal chemistry. The idea will show us to find the way of sustainable health and immortality in future, based on Drug design Expert System.

Keywords: Molecular hybridization, Drug design, Hybrid compounds, Pharmacophoric group combination, DNA and RNA Sequencing, Drug design Expert System.

A. INTRODUCTION:

Over the last decade the registration of pharmaceuticals for the treatment of new pathologies or that represent therapeutic innovations or known illnesses, mostly infectious and with high social –economic impact , such as neurogenerative diseases and cancer, has suffered a continuous decrease, contrasting with the growing of technological and scientific advances pursuing the improvement of the quality of life. Reappearing diseases such as tuberculosis, hanseniasis, smallpox, schistosomiasis, infectious diseases associated to resistant microorganisms, such as malaria, still incurable new virus and tropical diseases, besides cancer, neurodegenerative and autoimmune diseases, still represent a big challenge for the pharmaceutical sector and demand a continuous effort to the development of new therapeutic tools: more efficient, selective and economically accessible

In this paradoxical context, the Pharmaceutical Industry has invested heavily in the development of new techniques of diagnosis, investigation and creation of chemical libraries with high molecular diversity, based on combinatorial chemistry, computer-aided drug design (CADD), simulation and prediction of physicochemical and structural properties associated to drug-receptor interactions (QSAR), automatized processes of pharmacological screening (High Throughput Screening – HTS), new methods of *in vitro* and *in vivo* pharmacological evaluation, based on the advances of the molecular biology, genomics and biotechnological approaches [1-3].

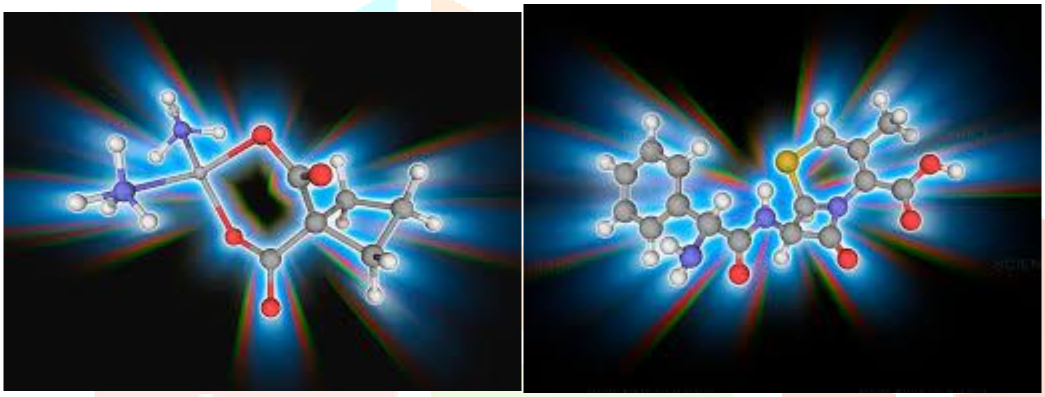
Recently, the natural products chemistry has been returning to a prominent position in the prospection of bioactive compounds, justifying the new investments in research from the pharmaceutical sector pursuing new pharmaceuticals, especially the ones whose origin is vegetal, marine or from microorganisms. The enormous chemical diversity of the secondary metabolites still challenges and inspires the synthetic and medicinal chemistry for their molecular complexity and diversity, working as role templates for the discovery of new drugs and the planning of new synthetic and semisynthetic derivatives [3-8].

On the other hand, the result of all effort was not yet able of promoting the quick access to massive quantities of new bioactive chemical entries (BioNCEs). Furnishing the expectation of high efficiency and productivity, which has not happened yet, since the number of new registered pharmaceuticals has been decreasing significantly year after year [4-5].The rational planning of new synthetic prototypes has been using a series of methods of structural modification that aim, *a priori*, at the generation of new compounds presenting optimized pharmacodynamics and pharmacokinetic properties, exploring bioactive substances' fragments (Fragment-Based Drug Design) [9], active metabolites of drugs [10], bioisosterism [11], selective optimization of side effects of drugs [12] and drug lamentation [13]. No doubt the whole idea is based on Drug design expert system, as described in the next topic.

B. Drug Design Expert System:

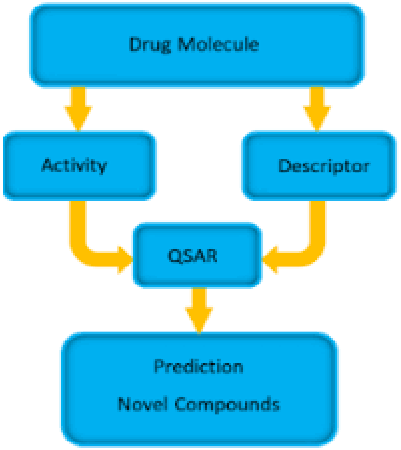
Drug Design Expert System or protocol is mainly based on the following two flowchart systems as follows.

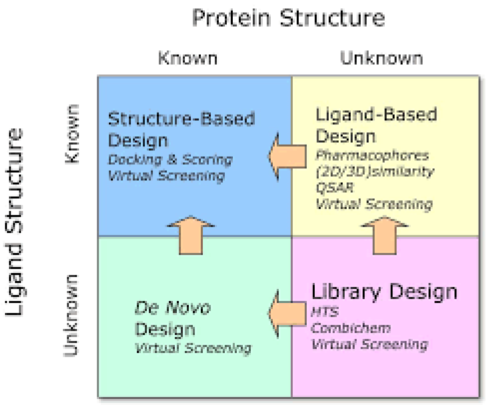
Cage like property for eg graphene or glue like bio material from spider of even polymer can bind rapid growth of cell for its binding nature at micro level or can trap bacterial or viral growth by trapping within Net like structure. The idea of Quantum Dots may be implied to identify such net as drug to separate from diseases infected Zone. Some time electrification or by application of Radioactivity through this net may reduce harmful diseases spreading.

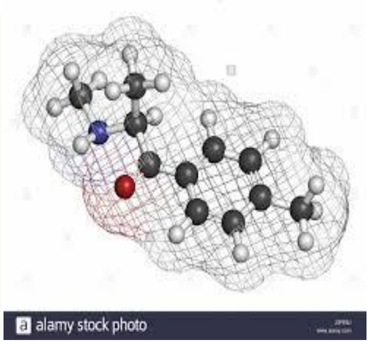


Cage like trapping and also electrification or Application of Radio activity in the cage..

Drug design strategy.







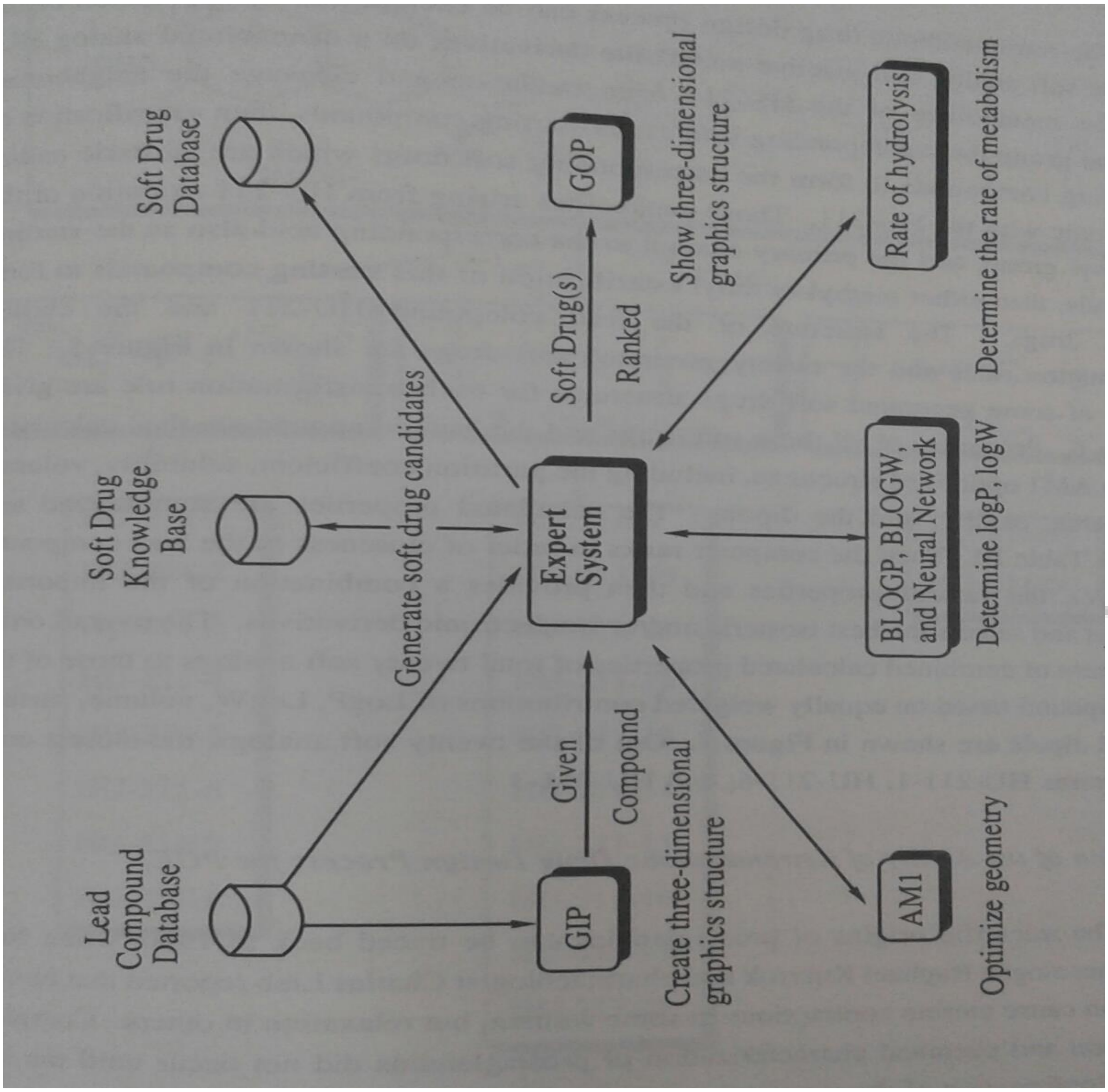
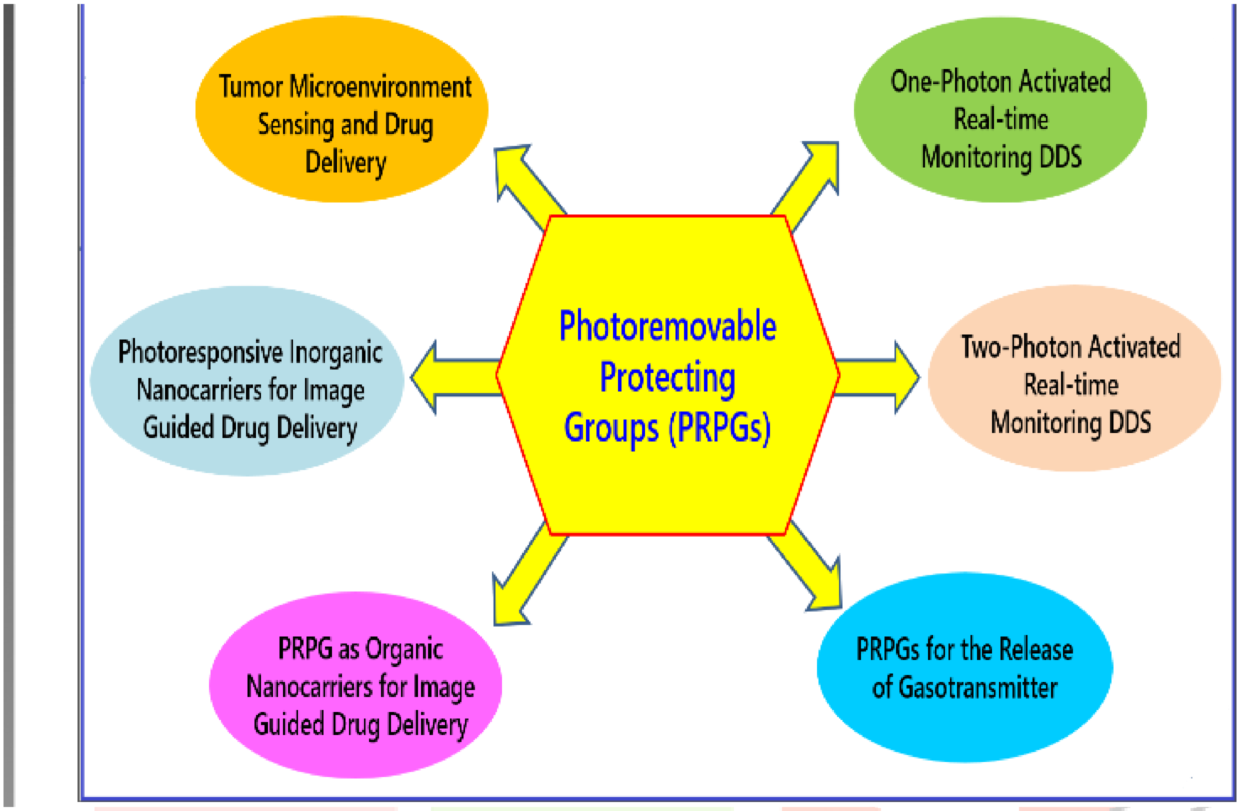


Fig. (B2). Architecture of Soft design System.

Design and Development of Fluorescent Photoremovable Protecting Groups for Controlled Release of Bioactive Molecules

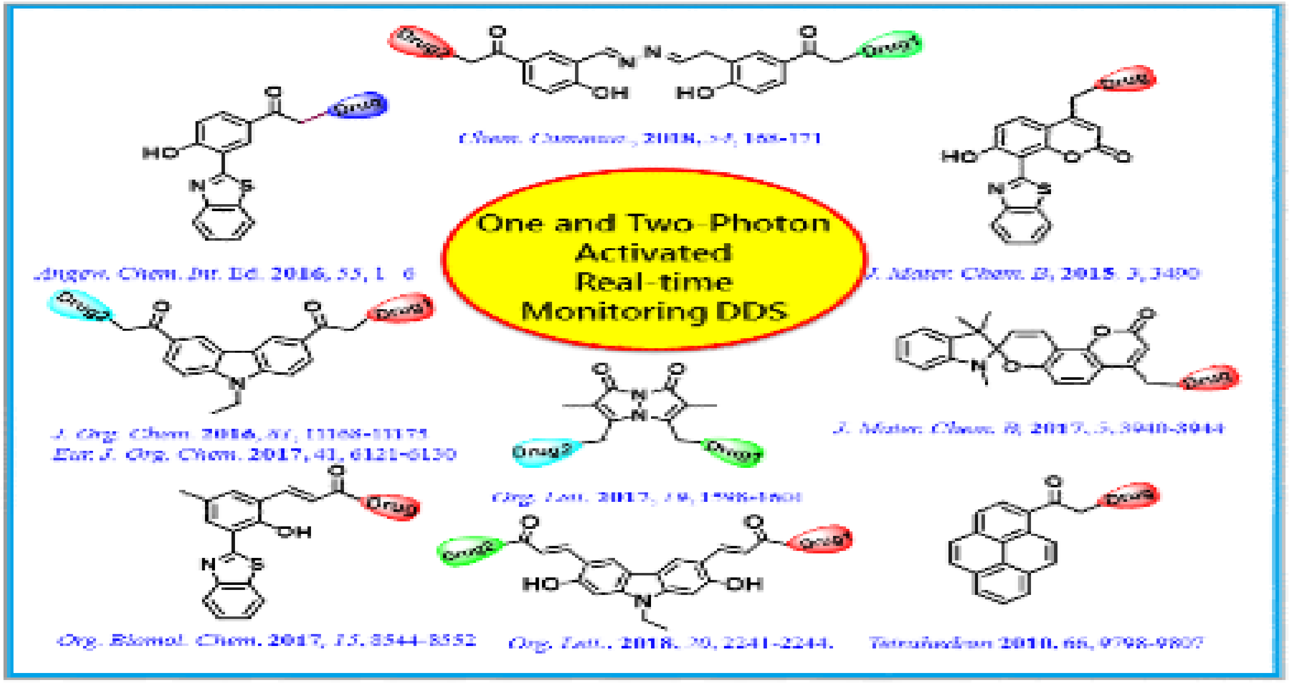
Photoremovable protecting groups (PRPGs) are of great interest science they allow precise spatial and temporal control over the release of the protected functionalities. So, design and synthesis of various photoactivable protecting groups which can cage and release bioactive molecules such as bioagents (neurotransmitters and cell-signaling molecules), various functional groups (alcohols, amines, thiols) acids, bases, Ca2+ ions, oxidants, insecticides, pheromones, enzymes, fragrances, etc., at a specific site, upon exposure to UV or visible light independent of the reaction media, are of great interest of our research. Photocaging refers to temporary inactivation of a biologically active molecule using a photoremovable protecting group (PRPG).



One and Two-Photon Activated Photoremovable Protecting Groups for Real-Time-Monitoring of Dual Drug Delivery

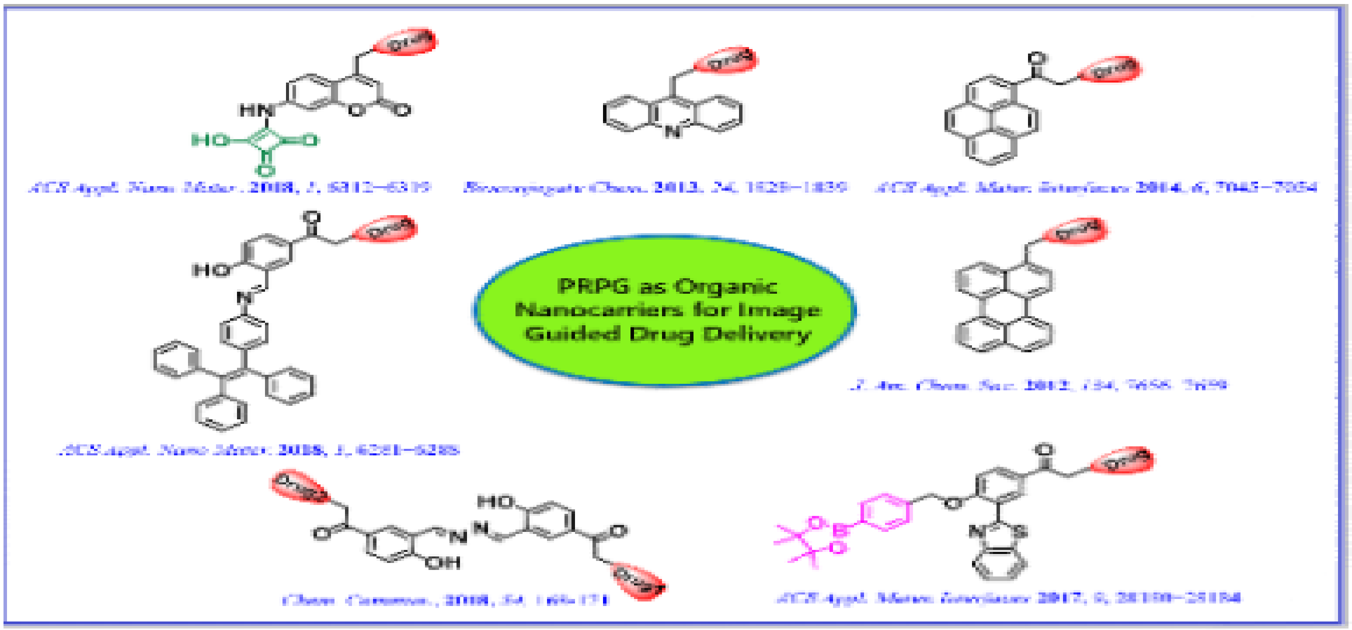
Our group has designed and synthesized various photoactivable protecting groups which can cage and release bioactive molecules. Further by tuning the substituents, we made it possible to control the wavelength and rate of release. We further utilized these photoremovable protecting groups for the controlled release of drugs and real time monitoring of the drug released. In this process, since the drug molecule is covalently attached with a photoactive molecule, it loses its activity temporarily, thereby ruling out the possibility of premature release or leaching of the drug. Once the system reaches the target site, the drug can be released from the photoactive molecule through photolysis, thus restoring its activity. Some of the newly developed protecting groups by our group are perylen-3-ylmethyl, 1-acetylpyrene, 1-hydroxyacetylpyrene, acridine-9-ylmethyl, acetyl carbazole. These newly developed protecting groups are highly fluorescent and can absorb in the visible region. We have been able to utilize these protecting groups in releasing single and dual anticancer drugs. We have recently developed p-hydroxyphenacyl-benzothiazole-chlorambucil conjugate as a real time monitoring drug delivery system assisted by excited state intramolecular proton transfer. Here, we have modified a well-known phototrigger, the p-hydroxyphenacyl (pHP) group by making it fluorescent and increasing its excitation wavelength above 400 nm so that it can be efficiently utilized as drug delivery agent in the area of theranostics.

Two-photon activated photoremovable protecting groups (PRPGs) have become a useful tool for the delivery of bioactive molecules. Because they provide advantages like (i) precise delivery of active molecules both spatially and temporally in a non-invasive way (ii) deeper penetration into biological samples and (iii) reduces the illumination duration, thus mitigating the harmful effects associated with the light on biological tissues. Our group has recently designed and synthesized various two-photon based protecting groups which can cage and release bioactive molecules, with substitutions like alcohols, acids, amines etc., at a specific site. We have further utilized these photoremovable protecting groups for the controlled release of drugs and real time monitoring of the drug release. Some of the newly developed two-photon activated protecting groups by our group are carbazole fused o-hydroxycinnamate and ESIPT based p-hydroxy phenacyl. These newly developed two-photon activated protecting groups are highly fluorescent and able to release dual anticancer drugs with real-time monitoring ability in the NIR region.



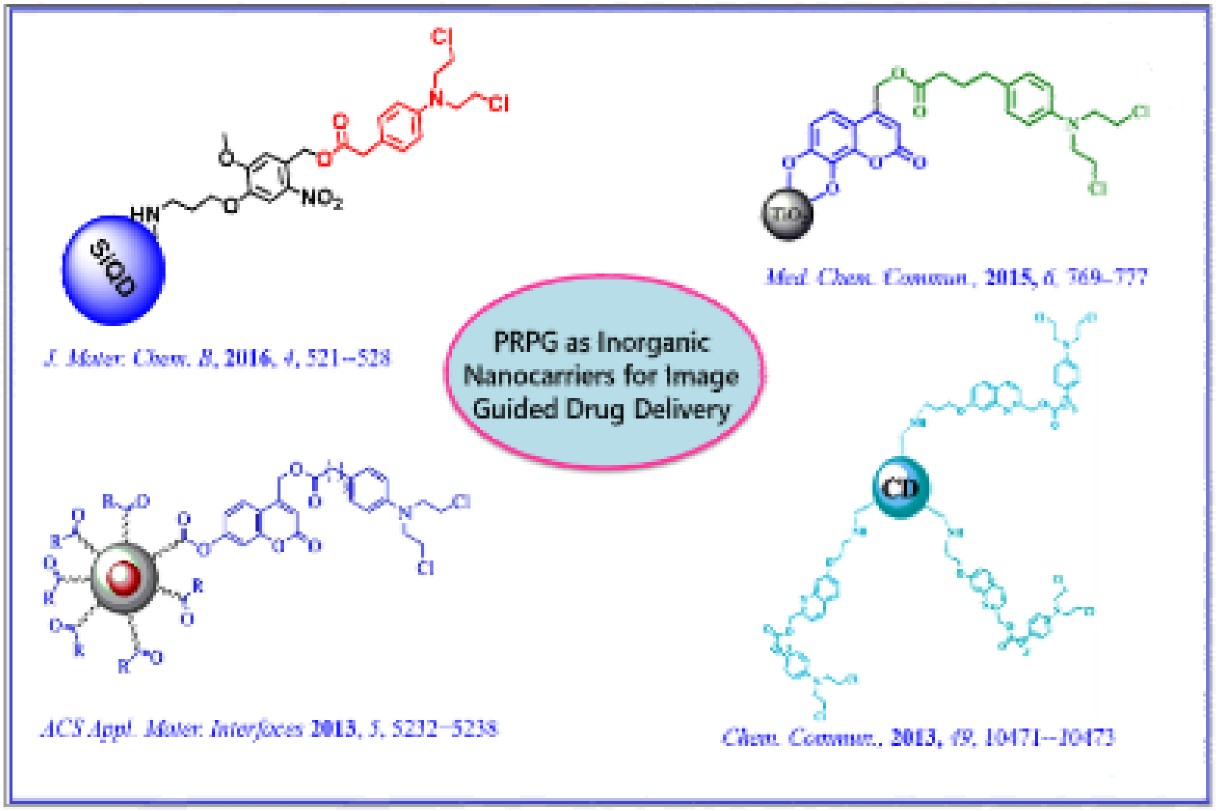
Photoremovable Protecting Groups as Organic Nanocarriers for Image Guided Drug Delivery

Our group has developed a nanoparticle-sized photoremovable protecting group for the first time, where PRPGs by itself can act as a nanocarrier for the drug delivery, thereby eliminating the key steps involved in synthesizing dual component photoresponsive nanocarriers. We have developed drug conjugated Perylene-3-ylmethanol, Acridin-9-methanol, 1-Acetylpyrene fluorescent photocages as organic nanoparticles. Perylene-3-ylmethanol organic nanoparticles showed real-time monitoring of anticancer drug release. In vitro biological studies of all these nanoparticles revealed that these newly synthesized organic nanoparticles exhibit good biocompatibility and cellular uptake as well as efficient photoregulated anticancer drug release ability. Such fluorescent organic nanoparticles which were developed by our group may open up new perspectives for designing a new class of promising photoresponsive nanocarriers for drug delivery..



Photoresponsive Inorganic Nanocarriers for Image Guided Drug Delivery

Generally, photoresponsive nanoparticles for drug delivery are constructed using two main ingredients: biocompatible nanoparticles and photoremovable protecting groups, which are nothing but small organic molecules which are able to release active molecule in presence of light. To date, the nominee has developed various photoresponsive nanoparticles such as coumarin tethered Fe/Si magnetic nanoparticles, quinoline decorated mesoporous silica nanoparticles, quinoline bounded carbon dots and silicon quantum dots for image guided drug delivery in vitro. With these newly developed functionalized organic and inorganic nanoparticles our group has shown efficient cell targeted drug delivery, nuclear targeted drug delivery via charge reversal and real time monitoring of the drug release via in vitro experiments.



Tumor Microenvironment Sensing and Drug Delivery

The tumor microenvironment is the cellular environment in which the tumor exists, including surrounding blood vessels, immune cells, fibroblasts, bone marrow-derived inflammatory cells, lymphocytes, signaling molecules, and the extracellular matrix. The tumor microenvironment contributes to tumour heterogeneity. Our group is using the tumour heterogeneity as a new and competent tool for cell-specific targeted drug delivery and cancer treatment.

pH Sensing and Drug Delivery:

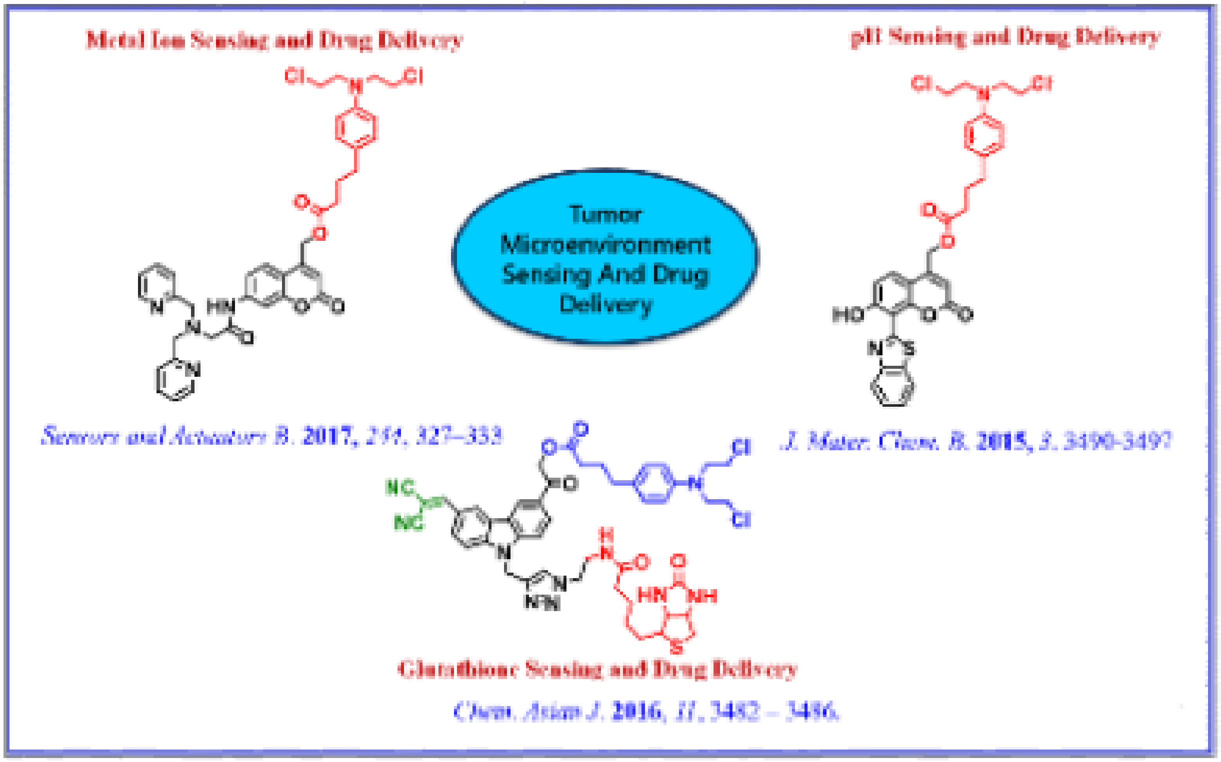
Cancer thrives in an acidic environment and doesn't survive in a normal, more alkaline environment. Cancer cells make our body even more acidic as they produce lactic acid. Our group has utilized this tumor microenvironment to develop pH sensing and drug delivery systems. We developed an ESIPT based drug delivery system for pH sensitive fluorescence properties and photocontrolled release of the anticancer drug chlorambucil.

Metal Ion sensing and Drug Delivery:

According to past research, healthy human prostate accumulates the highest level of zinc of any soft tissue in the body. But on the contrary, with the development of malignancy, there is a dramatic reduction in the zinc content of prostate tissue, associated with the inability of cancer cells to accumulate zinc. Our group has developed a system that first guided us to locate the diseased area by using cellular zinc concentration as a biomarker followed by the on-demand release of anticancer drug chlorambucil by employing the external stimulus, light.

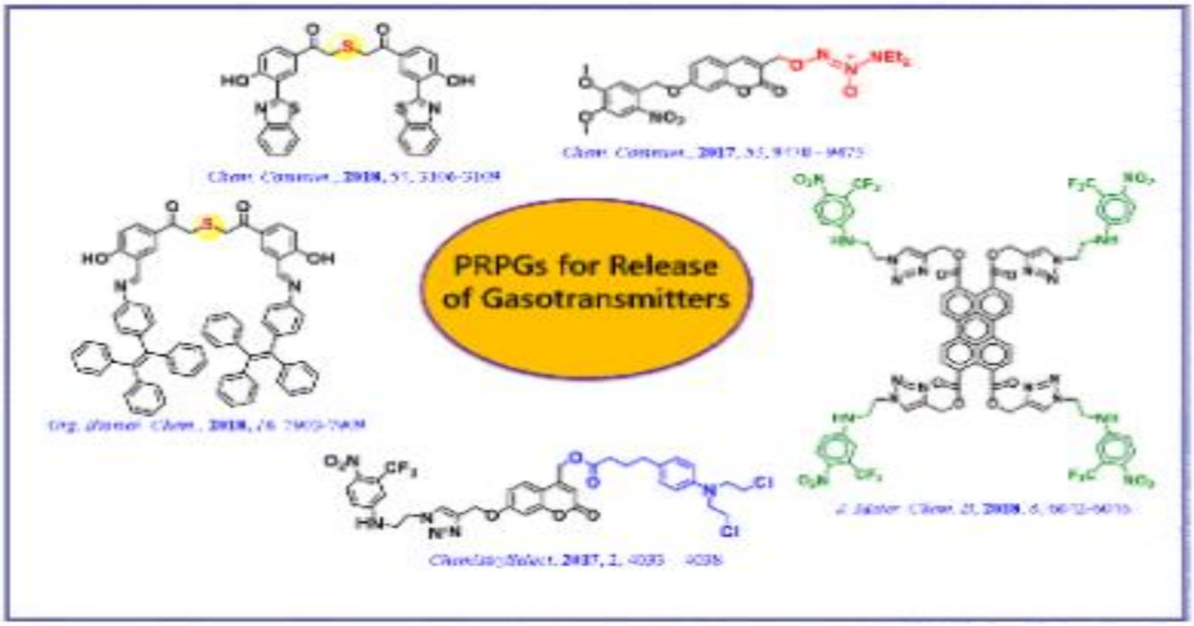
Glutathione Sensing and Drug Delivery:

Biological thiols, including glutathione(GSH), cysteine (Cys), and homocysteine (Hcy), play crucial roles in maintaining the appropriate redox status of biological systems. GSH is the most abundant cellular thiol, which is important for maintaining a cellular defense against free radicals and reactive oxygen species (ROS), and it also maintains exogenous antioxidants in their reduced forms. Our group has designed a redox-responsive drug delivery system (DDS) to exploit the higher level of thiols in tumor sites to trigger the release of drug from the DDS.



Photoremovable Protecting Groups for the Release of Gasotransmitter

Gasotransmitters are endogenously generated gaseous signaling molecules by specific enzymes and can elicit various biological functions at physiologically allowed concentrations by targeting specific cellular and molecular targets. Gasotransmitters are highly diffusible and their half-life period varies from a few seconds to minutes. Therefore, a variety of Gasotransmitter Donors (GDs) have been developed to enable the chemical storage and delivery of these gaseous signaling molecules to benefit a wider range of biological applications. Light triggered the release of gasotransmitter in a spatially and temporally controlled fashion has gained much interest. Our group has recently designed and synthesized many photoresponsive gasotrasmitter (NO, H2S, CO) releasing molecules based on perylene, tetraphenylethylene, p-hyroxyphenacyl, flutamide, and diazeniumdiolate.



C. Molecular Hybridization of Nucleic acids

- Molecular hybridization of nucleic acids is the process in which two single-stranded nucleic acid molecules with complementary base sequences form a double-stranded nucleic acid molecule. Nucleic acid hybridization technology is a fundamental tool in molecular biology, and has been applied in various fields such as detection of gene expression, screening specific clone from cDNA or genomic library, determining the location of a gene in chromosome and diagnosis of diseases.

1. Principles Of Nucleic Acid Hybridization

The technique of nucleic acid hybridization is established and developed on the basis of the denaturation and renaturation of nucleic acids. Hydrogen bonds in double- stranded nucleic acids can be disrupted by some physicochemical elements, and two strands of nucleic acids are separated into single strand.

2. Hybridization DNA from source “Y” TACTCGACAGGCTAG CTGATGGTCATGAGCTGTCCGATCGATCAT DNA from source “X” TACTCGACAGGCTAG Hybridization

3. If different single-stranded DNA molecules, or DNA and RNA molecules, or RNA molecules are mixed together in a solution, and the renaturation is allowed to occur under proper conditions, single- stranded DNA or RNA will bind with each other to form a local or whole molecule of double-stranded structure as long as the single-stranded molecules are complementary, no matter what kind of sources they come from.

4. Nucleic acid hybridization as a technique involves using a labelled nucleic acid probe, which is a known DNA or RNA fragment, to bind with the target nucleic acids, which is usually a poorly understood, heterogeneous population of nucleic acids. A probe labelled with detectable tracer is the prerequisite for determining a specific DNA sequence or gene in a sample or genomic DNA by nucleic acid hybridization.

5. The target nucleic acids to be analysed are usually denatured, and then mixed with the labelled probe in the hybridization system. The probe will bind to the segment of nucleic acid with complementary sequence under proper conditions. The hybridization can be identified by the detection of the tracer labelling the probe. Thus the existence or the expression of specific gene can be determined.

6. Preparation And Labelling Of Nucleic Acid

7. Preparation of probes • Probes may be single-stranded or double- stranded molecules, but the working probe must be single-stranded molecules. The probes used in hybridization of nucleic acids include oligonucleotide(15-50 nucleotides), genomic DNA fragment, cDNA fragment and RNA.

8. Oligonucleotide probes are short single- stranded DNA fragments designed with a specific sequence complementary to the given region of the target DNA. They are usually synthesized in vitro.

9. Genomic DNA probes can be prepared from the cloned DNA fragment in plasmid. • cDNA probes can be prepared from the cloned cDNA in plasmid, or amplified directly from mRNA by RT-PCR. • RNA probes are usually transcribed in vitro from a cloned cDNA in a proper vector. The size of genomic DNA probes, cDNA probes and RNA probes may be 0.1 kb to 1 kb.

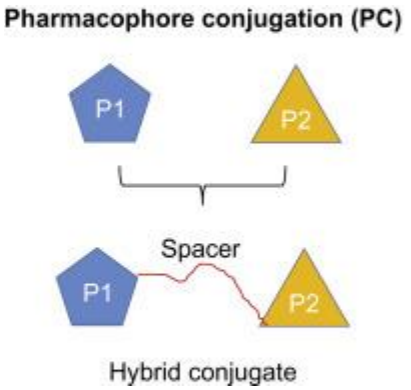
10. Labelling of probes • Probe is usually labelled with a detectable tracer, which is either isotopic or non-isotopic. The purified oligonucleotide is labelled in vitro by using a suitable enzyme to add the labelled nucleotide to the end of the oligonucleotide.

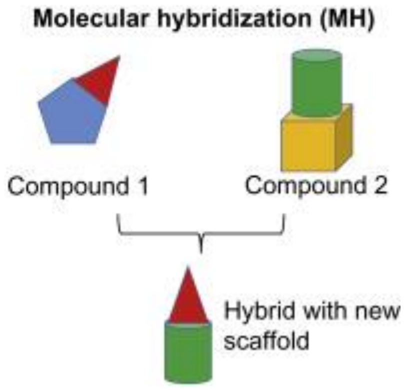
11. For the preparation of the labeled RNA probes, RNA probes are usually synthesized by RNA polymerase in the presence of ATP, GTP, CTP and the labelled UTP, with specific fragment of a gene or cDNA in a proper vector as template. RNA probes can then be generated and be labeled at the same time.

12. Genomic DNA probes and cDNA probes are usually labelled in the process of DNA synthesis in vitro. In the reaction of DNA synthesis with a DNA probe as template, if a labelled-dNTP, which can be incorporated into newly-synthesized DNA chain, is added as a substrate, the labelled DNA probe will be formed.

13. There are different, sensitive detecting methods for each of the labels used in nucleic acid hybridization. After hybridization, the location and the quantity of the hybrid molecules can be determined. The labels in common use include radioactive (32 P and 35 S) and nonradioactive (digoxigenin, biotin, fluorescein) substances which are used to label dNTP.

14. Hybridization Of Nucleic Acids





15. Southern blot hybridization • Southern blot hybridization is an assay for sample DNA by DNA-DNA hybridization which detects target DNA fragments that have been size-fractionated by gel electrophoresis (Figure 4-1). In Southern blot hybridization, the target DNA is digested with restriction endonucleases, size-fractionated by agarose gel electrophoresis, denatured and transferred to a nitrocellulose or nylon membrane for hybridization.

16. DNA fragments are negatively charged because of the phosphate groups so to migrate towards the positive electrode, and sieved through the porous gel during the electrophoresis. Shorter DNA fragments move faster than longer ones. For fragments between 0.1 and 20kb in length, the migration speed depends on the length of fragment. Thus, fragments in this size range are fractionated by size in a conventional agarose gel electrophoresis system.

17. Following electrophoresis, the sample DNA fragments are denatured in strong alkali, such as NaOH. Then, the denatured DNA fragments are transferred to a nitrocellulose or nylon membrane and become immobilized on the membrane. Subsequently, the immobilized single- stranded target DNA sequences are allowed to interact with labelled single- stranded probe DNA.

18. The probe will bind only to complementary DNA sequences in the target DNA to form a target-probe heteroduplex. As the positions of the immobilized single- stranded target DNA fragments on membrane are

faithful records of the sieve separation achieved by agarose electrophoresis, they can be related back to the original gel to estimate their size.

19. Figure 4-1 Southern blot hybridization detects target DNA fragments that have been size-fractionated by gel electrophoresis

20. Southern blot hybridization technique is widely applied in researches since its invention. It could be applied for analysis of gene expression, screening of recombinant plasmids, analysis of gene mutation, and identification of the existence of a given DNA such as DNA from pathogenic microorganism. It could also be used to detect deletion of gene by restrictions mapping.

21. Hybridization • The bases in DNA will only pair in very specific ways: G with C and A with T • In short DNA sequences, imprecise base pairing will not be tolerated • Long sequences can tolerate some mispairing only if hydrogen bonding of the majority of bases in a sequence exceeds the energy required to overcome mispaired bases • The source of any single strand of DNA is irrelevant, merely the sequence is important, thus complimentary DNA from different sources can form a double helix • This phenomenon of base pairing of single stranded DNA strands to form a double helix is called hybridization as it may be used to make hybrid DNA composed of strands from different sources

23. Because DNA sequences will seek out and hybridize with other sequences with which they base pair in a specific way much information can be gained about unknown DNA using single stranded DNA of known sequence • Short sequences of single stranded DNA can be used as “probes” to detect the presence of their complementary sequence in any number of applications including: – Southern blots – Northern blots (in which RNA is probed) – In situ hybridization – Dot blots . . . • In addition, the renaturation, or hybridization, of DNA in solution can tell much about the nature of organism’s genomes

D.MOLECULAR HYBRIDIZATION AS A TOOL IN THE PLANNING OF NEW LIGANDS AND PROTOTYPES

The molecular hybridization (MH) is a strategy of rational design of new ligands or prototypes based on the recognition of pharmacophore sub-unities in the molecular structure of two or more known bioactive derivatives which, through the adequate fusion of these sub-unities, lead to the design of new hybrid architectures that maintain pre-selected characteristics of the original templates.

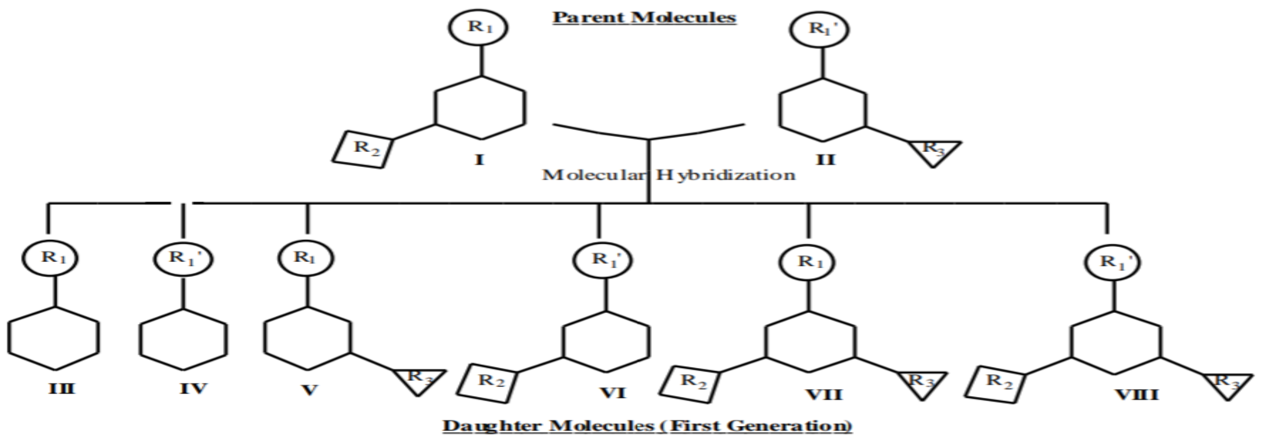


Fig. (1). Proposal of chemical evolution by combination of different structural sub-unities of parent molecules

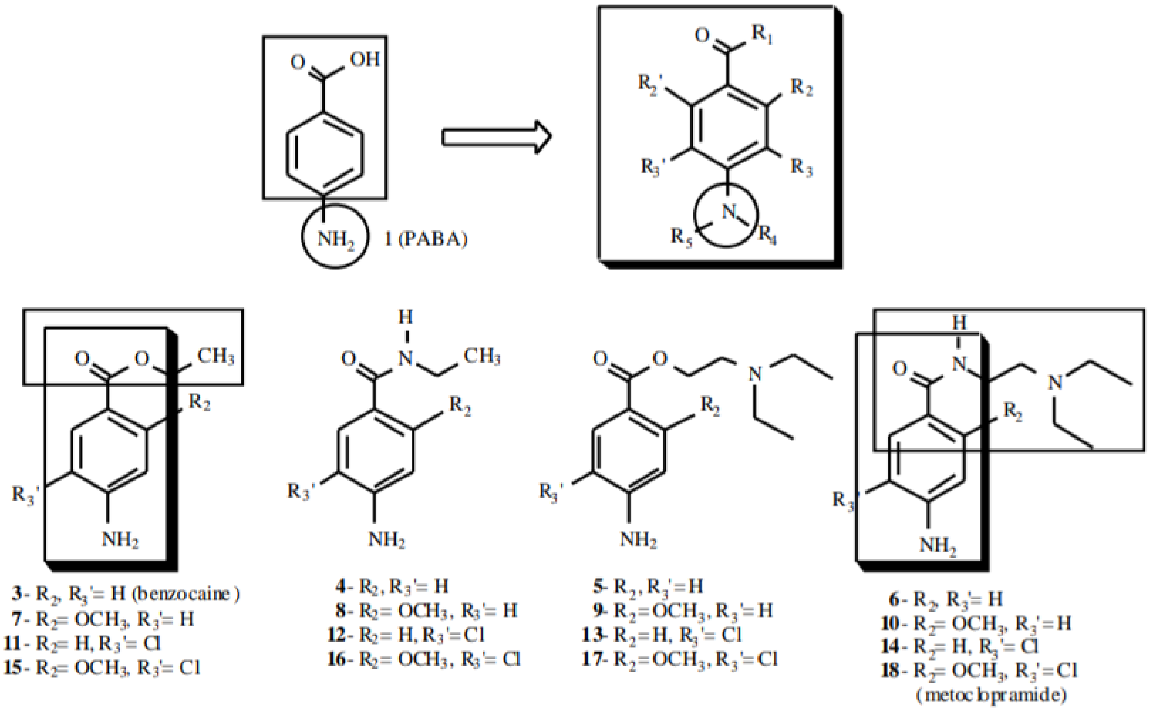


Fig. (2). Chemical library built with the PABA basic structure (**1**).

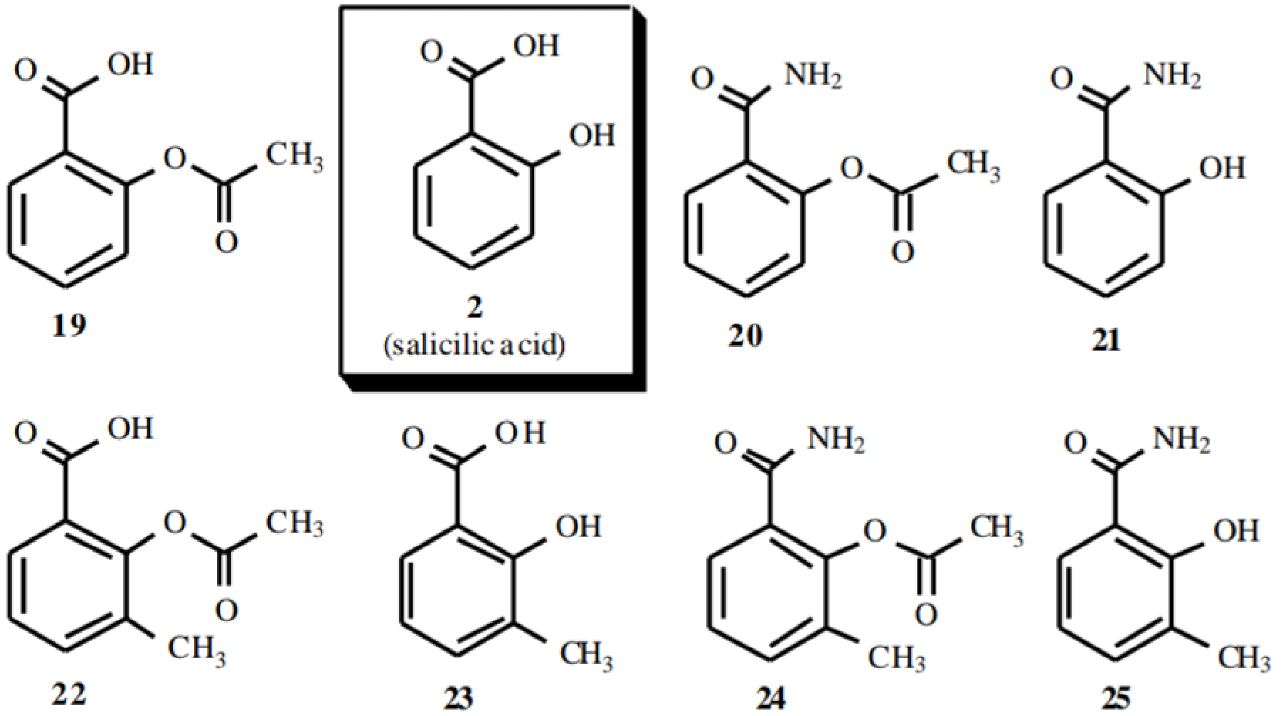


Fig. (3). Chemical library built with the basic structure of the salicylic acid (**2**).

E . CARDIOACTIVE AGENTS

Selective -adrenergic receptor antagonists are the most clinically used therapeutic class for the treatment of hypertension and angina pectoris [15]. Most of the available drugs of this class contain an 3-aryloxy-2-propanolamine structural sub-unity with an isopropyl or *t*-butyl substituent attached to the N atom, as represented by classical α-blocker agent propranolol (**26**) (Fig. (**4**)) [16]. Alternatively, these cardiovascular dysfunctions could be treated by the use of verapamil-analogue calcium antagonists as zatebradine (**27**), a benzazepinone derivative with vasodilator and bradycardic properties [17]. Collaborators [18] exploited the MH as strategy in the design of new cardio active hybrid molecules (28-33), in which aryl alkyl sub-unity of lateral chain of 27 was replaced by the2-hydroxy-3-aryloxypropylaminegroup(A), a particular structural sub unity of β-BLOCKERS(Figure-4). The pharmacological results showed that all the synthesized hybrid compounds 28-33 represented a bradycardic profile, as well as the prototype compound(27), besides vasorelaxant property of (-)-(*S*)-**30** (IC_{50} = 20 µM), probably due to its calcium antagonist effect evidenced by the inhibition of the aortic smooth muscle contraction induced by 80 mM of potassium chloride. However, despite the inclusion of 3-aryloxy-2-propanolamine (A) did not change the magnitude of the cardiovascular effects, all the hybrids **28-33** were better than the prototype **27** , considering the negative inotropic activity. It was shown that the association of the pharmacophore moiety of the lead-compound propranolol (**26**) to the zatebradine (**27**) core produced new derivatives with high affinity and selectivity for $_1$-adrenergic receptors. The compound (+)-(*R*)-**30** was the most potent of this series, revealing distinct activity profiles for both enantiomers, *R* enantiomer demonstrates affinity for $_1$-adrenergic receptors (pIC_{50} = 7.39), while *S* enantiomer showed specificity for relaxing smooth muscle vases, though in smaller extension [18].

[19]. These results indicated an evolution on the therapeutic application of this new class of hybrid compounds in comparison to the sartans, since the original antihypertensive properties were assured, added to the benefits from the NO production in the cardiovascular system.

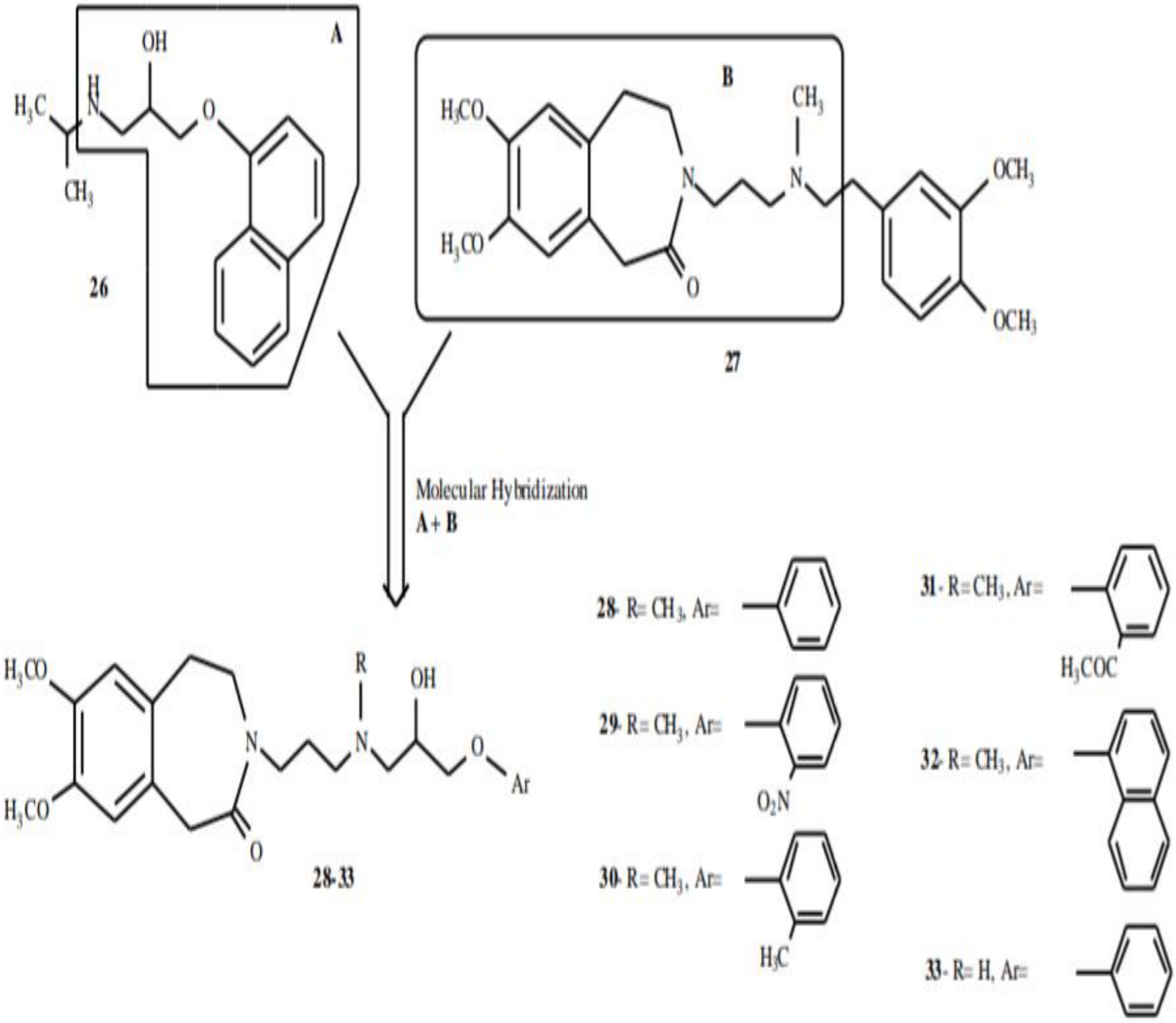


Fig. (4). Hybrid cardio active compounds planned out of propranolol (**26**) and zatebradine (**27**).

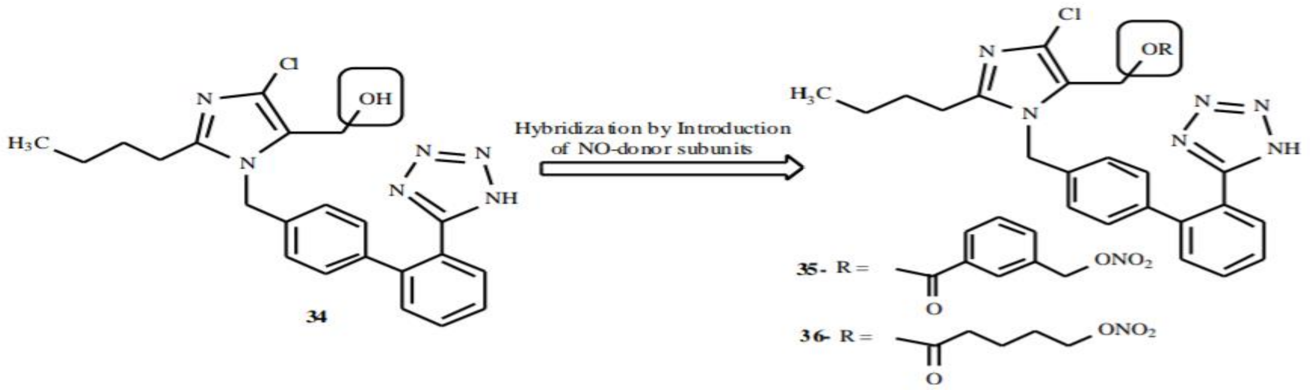


Fig. (5). Design of new hybrids **35** and **36** planned from the association of NO donor moieties to the antihypertensive drug losarta

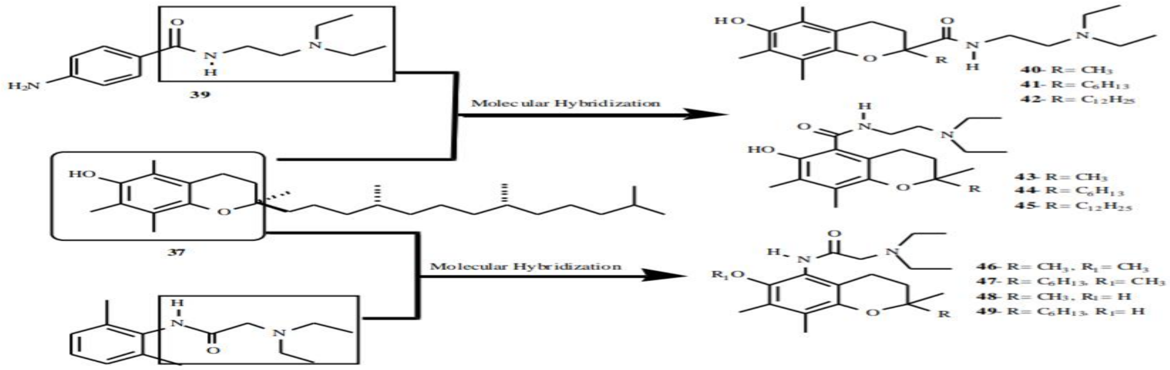


Fig. (6). Design of antiarrhythmic/antioxidant hybrids (**40-49**) based on the structure of α-tocopherol (**37**), lidocaine (**38**) and procainamide (**39**).

”Synthesis and development of stimuli-responsive nanomedicines for heart disease therapy”

Bioinspired approaches to create multi-functional nanoparticles capable of changing their surface or core properties in response to local or up-regulated disease markers for more effective and smart stimuli-responsive drug delivery. In this manner, potent biological therapeutics (such as enzymes) can be delivered in a spatiotemporally controlled manner, with the aim of reducing enzyme degradation, systemic toxicity and collateral damage to the host. Inparticular we are interested in the synthesis of nanogels with diverse properties that can be designed to facilitate ‘smart’ drug delivery in response to up-regulated disease biomarkers.

Nanogels are nanometer-sized nanoparticles that have the ability to retain high volumes of water or biological fluids, and maintain their structure. This excellent and unique property makes them an ideal nanoplatform for the delivery of biological drugs such as enzymes. Nanogels have superior properties as they offer: 1) encapsulation stability for biologically sensitive payloads, 2) they have low immunogenicity and toxicity, and can be designed to be fully biodegradable, 3) multiple biological payloads can be delivered in a single nanogel, facilitating combination therapies, 4) their synthesis can be aqueous based and easily scaled, and 5) they are soft nanoparticles that can easily squeeze through restricted sites under haemodynamic sheer flow.

Nanogels are prepared via the heterogeneous polymerisation of monomers or precursors by either chemical or physical cross-linking. Furthermore, nanogels can be created to be responsive to a wide variety of environmental stimuli such as enzymes, temperature, pH and ionic strength. They are extremely versatile since these functionalities can be bestowed within their monomer design. This strategy of triggered disintegration makes cross-linked polymeric nanogels a promising system for the controlled delivery of biologics.

Project aim: According to the WHO, each year, 17.9 million people die from cardiovascular diseases (CVDs), mostly due to heart attacks and stroke – with CVDs accounting for 31% of all deaths worldwide, making this disease the number 1 cause of death globally. This project aims to develop novel nanogel therapies for catalytic pharmacological thrombolysis in the event of acute myocardial infarctions (heart attacks), whereby blood clots that block arteries can rapidly be broken down due the action of locally delivered enzymes. This new strategy aims to develop an intelligent drug delivery system that can precisely target the thrombus (blood clot that forms in vessels and remains there). The project will involve the synthesis of nanogels for the delivery of fibrinolytic enzymes. In the first instance monomer libraries (that will allow stimuli-responsive release of enzymes) will be synthesized and characterized, and applied to nanogel synthesis using polymerisation in confined droplets. Depending on their level of skills and interest, the student may also carry out experiments to test the biological efficacy of the nanogels.

F. ANTI-TUMORAL AGENTS

Anew research [26] proposed the synthesis of hybrids of the geldanamycine (GDM, **51**) with estradiol (**52**, Fig. (**7**)), aiming at obtaining molecules capable of causing the specific degradation of proteins, such as ligands of estradiol receptors (ER) and of the transaminase of the HER2 membrane, which are highly expressed in several types of breast cancer. The appropriate intervention on these proteins could lead to the delay of the cellular growing and/or apoptosis [27].

The ansamicines inhibit this process and induce the degradation of Hsp90 substrates [26]. The fusion between the structures of the estradiol (**52**) and GDM (**51**) should lead to new hybrid ligands that maintain the activities of both original ligands. In order to do that, a way of gathering these two structural templates in the hybrid targets **53-56** (Fig. (**7**)) was investigated, exploring the C-16 position of the estradiol, whose relative stereochemistry should be avoid steric effects over the pharmacophore hydroxyl group at C-17. In relation to the GDM (**51**), prior studies revealed that the methoxy attached carbon of benzoquinone system could act as Michael acceptor when facing amines or other bio nucleophilic species, supporting the structural design that considered the connection of a spacer unit presenting a terminal primary amino group, stereo selectively placed at C-16 of the estradiol (**52**), with this electrophilic site of GDM (**51**) (Fig. (**7**)) [26].

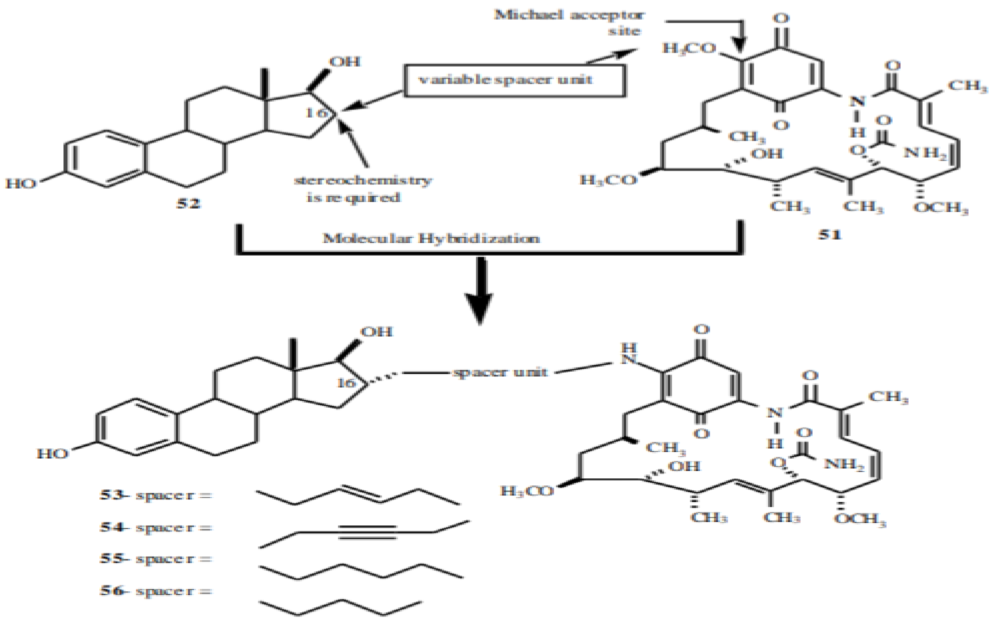


Fig. (7). Design of hybrids of the gendanamycin (**51**) and estradiol (**52**)

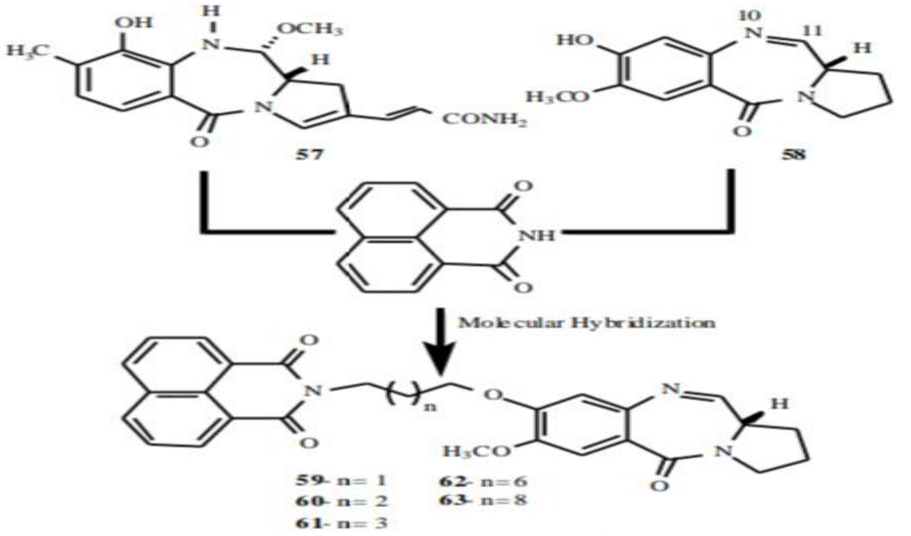
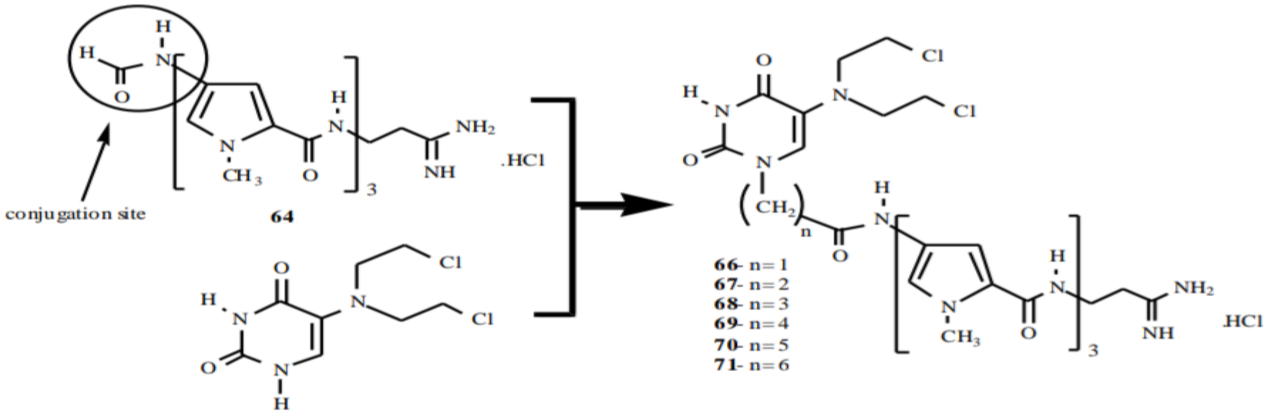


Fig. (8). Series of naphtalimide-PBD hybrids **59-63** planned as anti-tumoral agents.

The PBDs' mechanism of action is based on the nucleophilic attack of the guanine amino group to the iminic C-11 of **58** the system (or N-10-C-11 carbinolamine equivalent of **57,** Fig. (**8**)), creating adducts covalently bound to DNA sequences, being able to act not only as anti-tumoral agents but also as genes regulators and probes of the DNA structure. By exploring the naphtalimide derivative, which is a DNA intercalant agent and a potent anti-tumoral, Kamal and collaborators [28] synthesized the series of naphtalimide-PDB hybrids **59-63** (Fig. (**8**)) as drug candidates that combined the properties of DNA intercalating agent and ligand, and could present an improved anti-tumoral activity.(n = 2) over colon and renal tumour lines (log LC_{50} = -4.34 and -4.57, respectively) and **61** (n = 3) over colon and melanoma tumour cells (log LC_{50} = -4.41 and -4.43, respectively), indicating that the combination of intercalating and covalent bonding properties to the DNA in a single chemical entity represents a new approach in the development of new anti-tumoral agents [28]



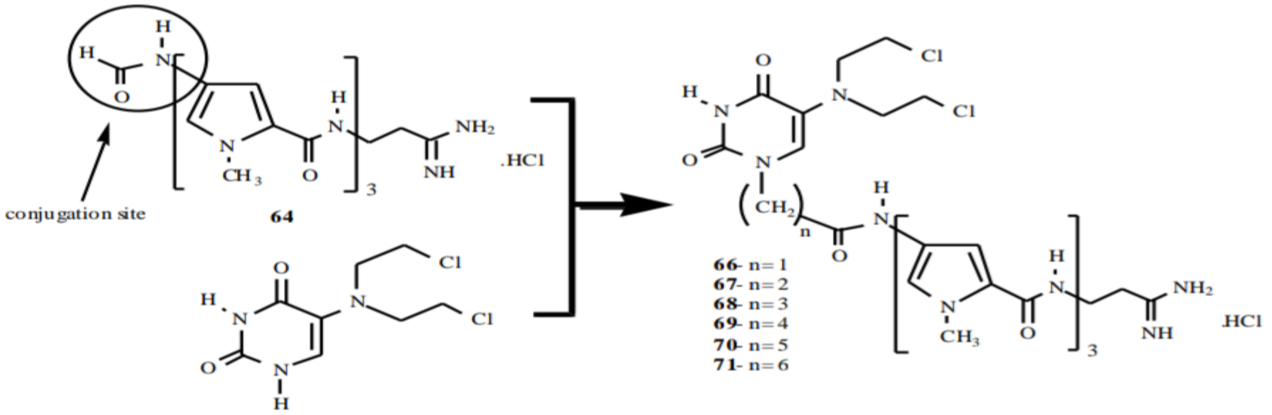


Fig. (9). Hybrids of the anti-tumoral distamicine-A (**64**) and uramustine (**65**).

Researchers [29] explored the structures of distamicine-A (**64**) and uramustine (**65**, Fig. (**9**)), a uracil mustard with strong DNA alkylating activity, to synthesize hybrid anti-tumoral agents. The result of the conjugation of the pharmacophore sub-unities of **64** and **65** , connected through polyethylene chains with diverse sizes, were six new hybrid compounds (**66-71**) with superior antitumor activity than distamicine-A (IC_{50} > 100 µM) and uramustine (IC_{50} = 5.1 µM) tested isolate against the human leukaemia K562 line. The compounds **66** (IC_{50} = 4.06 µM), **67** (IC_{50} = 2.54 µM) and **68** **(**IC_{50} = 7.26 µM), presenting short spacer unities (1 to 3 carbons), showed similar moderate anti-tumoral profile, which could be improved at least twenty times by the increase of the spacer chain (**69**, IC_{50} = 0.11 µM, n= 4; **70**, IC_{50} = 0.14 µM, n= 5; **71,** IC_{50} = 0.07 µM, n= 6, Fig. (**9**)). It was observed that the most potent cytotoxic hybrid derivative 71 presented a cytotoxic activity >1000 times superior to the distamicine-A (**64**) in the same experimental conditions [29].

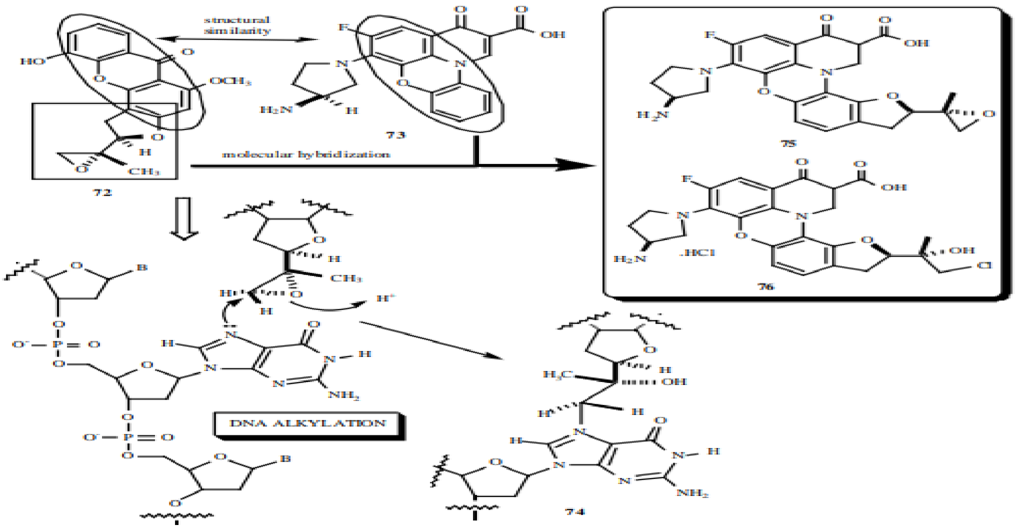
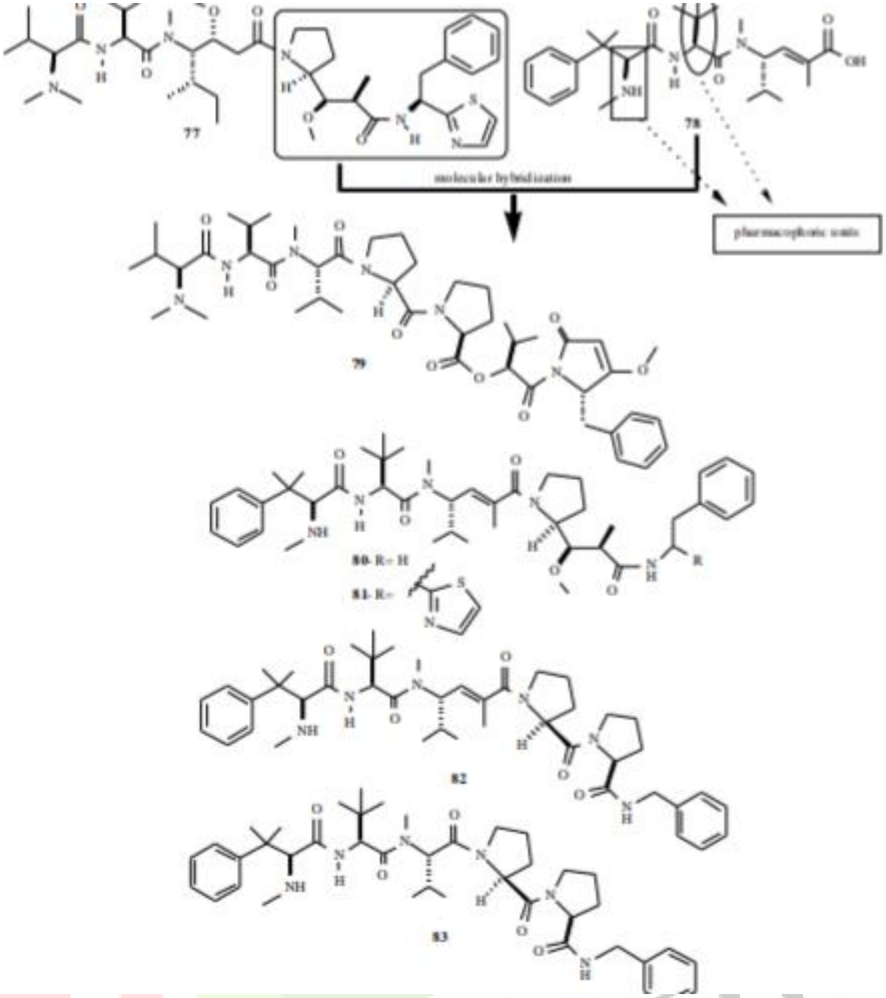


Fig. (10). Psorospermine (**72**), A-62176 (**73**), DNA alkylation adduct (**74**) formed through the nucleophilic attack of a guanine sub-unity to the oxirane ring of **72** and the molecular hybrids **75** and **76**

Other approaches on the planning of anti-tumoral agents have been based on the election of the topoisomerase II as target for DNA intercalating cytotoxic agents. This enzyme catalyses changes in the DNA topology, promoting important functions

In the DNA metabolism and in the structure of the chromosomes. Psorospermine (**72**), a natural antibiotic isolated from the shells of *Psorospermum febrifuge* and A-62176 (**73**, Fig. (**10**)),



(11). Molecular hybridization of the antitumor agents dolastatin (**77**) and taltobulin (**78**) as a tool for the design of the new analogues **79-83**.

Another example of rational planning of anti-tumoral drug candidates by molecular hybridization was related by scientists [31], who elected agents that act in the same biochemical pathway that taxanes and vinca alkaloids, useful drugs to the treatment of several cancer types. The mechanism of action of these two drugs involves the interference in the microtubules dynamics, which are proteins constituted by and b-tubulin heterodimers, preventing the chromosomal separation in the mitotic process and causing apoptosis. The dolastatin 10 (**77**) and taltobulin (HTI-286, **78**) (Fig. (**11**)) are peptide inhibitors of the tubulin polymerization, currently under clinical phase evaluation, which were elected as prototypes for the design of a new structural architecture, combining the common scaffold of analogs of **78** with structural carboxydipeptidyl sub-unity of dolastatin 10 (**77**). Previous structure-activity relationship (SAR) studies of taltobulin analogs had established some critical pharmacophorical sub-unities, such as the basic methylamine group and the demand of a bulky substituent group bound to the b-carbon of the amino acidic sub-unity of **77** (Fig. (**11**)), which were maintained in the design of the taltobulin/dolastatin hybrids **79-83** [31].

The hybrids **79-82** showed activities in resistant tumoral cellular lines, comparable to the taltobulin (**78**, IC$_{50}$ = 0.96 nM, KB-3-1; 2.3 nM, KB-8-5 and 77 nM, KB-V1), highlighting the results in cells KB-3-1 and KB-8-5 (**79**, IC$_{50}$ = 0.84 and 2.2 nM; **80,** IC$_{50}$ = 0.75 and 4.1 nM and **81,** IC$_{50}$ = 0.90 and 5.1 nM, respectively). However, all were less active than **77** (IC$_{50}$ = 0.073nM, KB-3-1; 0.34 nM, KB-8-5 and 43 nM, KB-V1). Besides that, **80** and **81** showed better resistance to the P-glycoprotein than the dolastatin 10 (**77**), as evidenced by the fraction KB-V1/KB-3-1 2 to 3 times smaller, but still greater than **77** (KB-V1/KB-3-1= 80). These two tumoral lines express a P-glycoprotein carrier and the susceptibility to the connection of this protein is related to the increase of the resistance to the treatment by multi-target drugs. On the other hand, the inferior vinylogue derivative **83** showed to be 3-4 times more potent than **78, 79** and **82** against KB-3-1 cells (IC$_{50}$ = 0.25 nM). Nevertheless, likewise the other hybrids and the dilatations, the compound **83** presented a pronounced susceptibility for the binding to the P-glycoprotein (KB-V1/KB-3-1 > 12000) in comparison with **78.** Besides the cytotoxic activity in KB cells, all the new hybrid analogs inhibited the tubulin polymerization by 56-69% (at 0.3 µM concentration), presenting superior antitumor profile than that observed for the lead-compounds **77** (78%) and78 (88%) [31].

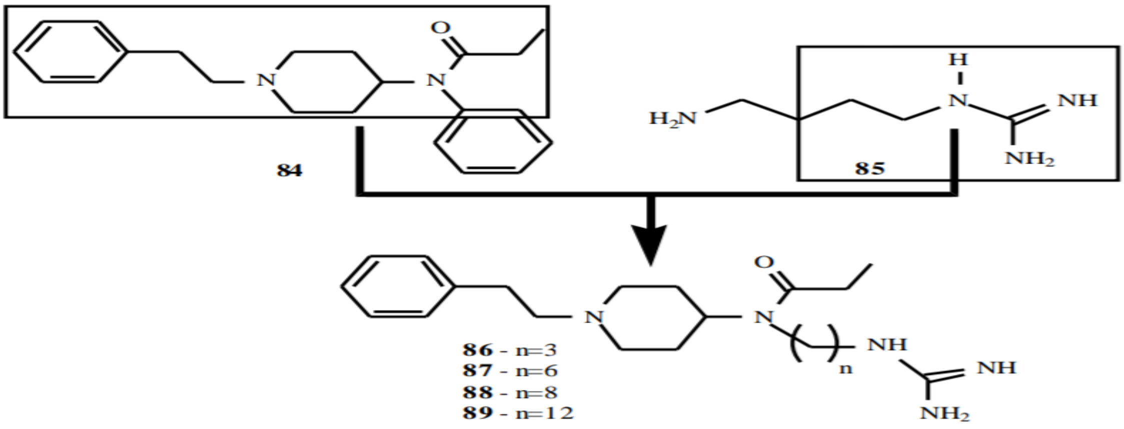


Fig. (12). Fentanyl-guanidine hybrids **86-89** planned as I$_2$-IBS / opioid ligands.

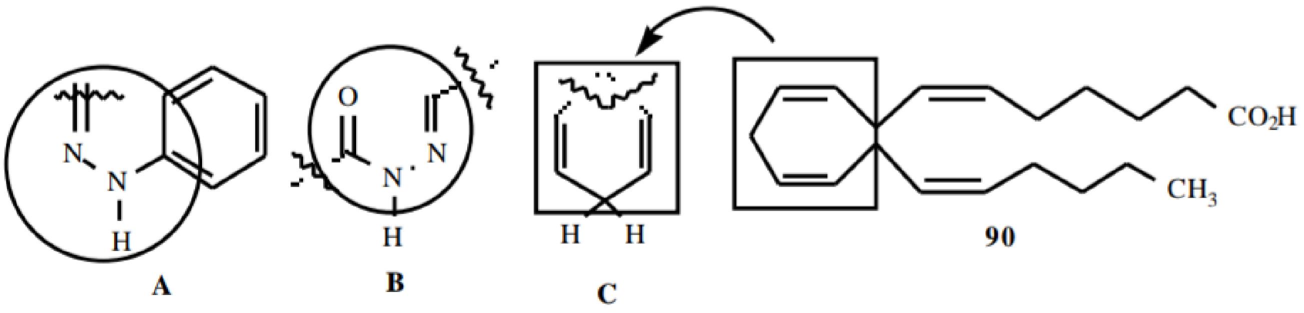


Fig. (13). Aryl- (**A**) and acyl-hydrazine (**B**) sub-unities mimicking the bis-allyl fragment (**C**) present in the arachidonic acid (**9**)

G.Discovery and development of HIV-protease inhibitors

Many major physiological processes depend on regulation of proteolytic enzyme activity and there can be dramatic consequences when equilibrium between an enzyme and its substrates is disturbed. In this prospective, the discovery of small-molecule ligands, like protease inhibitors, that can modulate catalytic activities has an enormous therapeutic effect.[1] Hence, inhibition of the HIV protease is one of the most important approaches for the therapeutic intervention in HIV infection[2] and their development is regarded as major success of structure-based drug design.[3] They are highly effective against HIV[4] and have, since the 1990s, been a key component of anti-retroviral therapies for HIV/AIDS.[5]

Contents

History

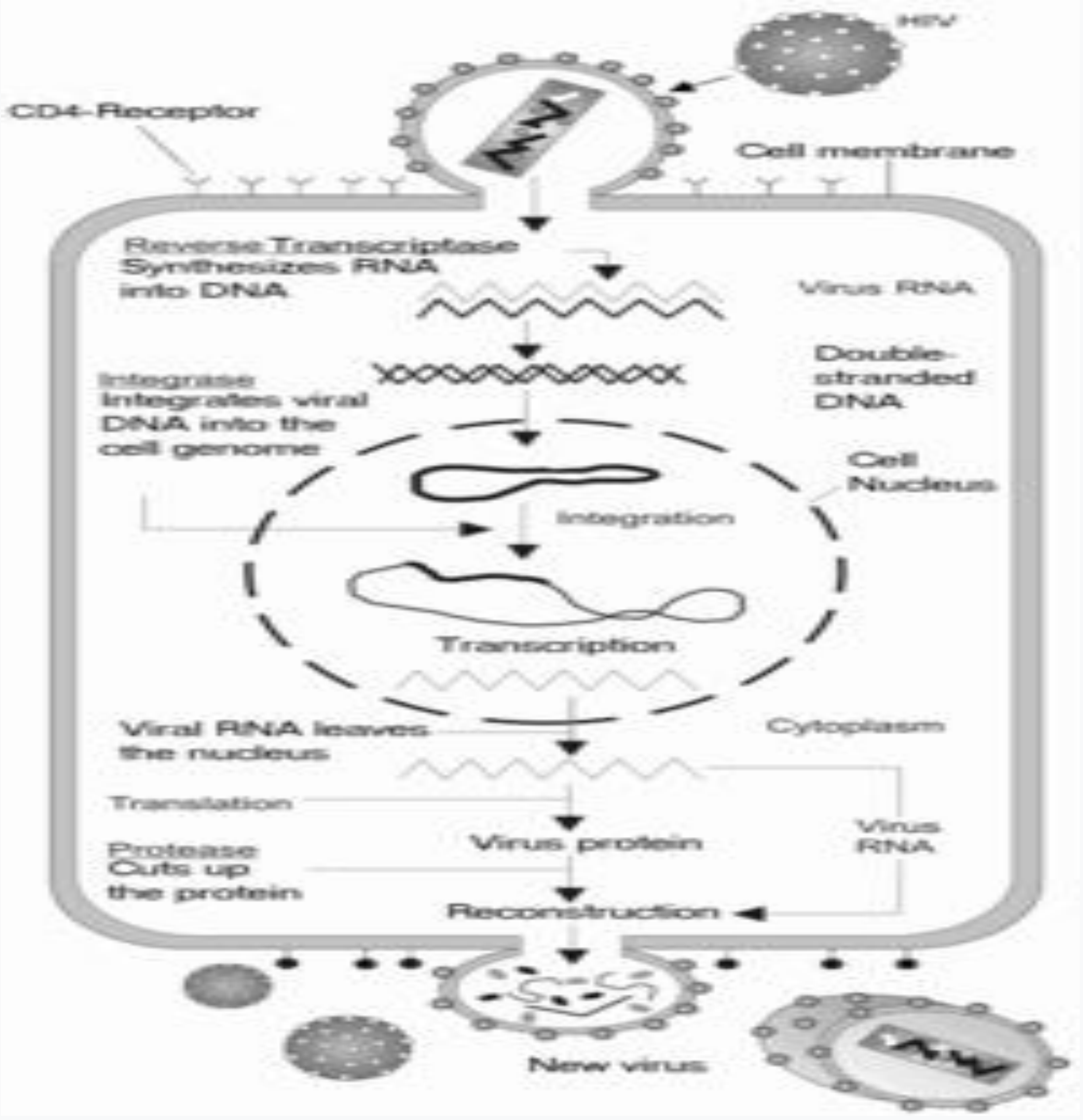
Human immunodeficiency virus (HIV) is a lentivirus that has two major species, HIV-1 which causes the majority of the epidemic, and HIV-2, a close relative whose distribution is concentrated in western Africa.[6] HIV infection was first described in 1981 in San Francisco and New York City.[7] In 1985, HIV was identified as the causative agent of acquired immune deficiency syndrome (AIDS) and its complete genome was immediately available. This knowledge paved the way for the development of selective inhibitors.[6]

HIV-2 carries a slightly lower risk of transmission than HIV-1 and infection tends to progress more slowly to AIDS.[7] In common usage HIV usually implies HIV-1.[8]

HIV-1 protease is one of the best known aspartic proteases, and an attractive target for the treatment of AIDS.[9]

After the discovery of HIV protease it only took 10 years for its first inhibitor to reach the market.[10] The first reports of highly selective antagonists against the HIV protease were revealed in 1987. Phase I trials of saquinavir began in 1989 and it was the first HIV protease inhibitor to be approved for prescription use in 1995. Four months later, two other protease inhibitors, ritonavir and indinavir, were approved.[6] In 2009, ten protease inhibitors have reached the market for treatment against HIV but one protease inhibitor, amprenavir, was withdrawn from the market in 2004.[6][11]

Life cycle of HIV



The HIV replication cycle

HIV belongs to the class of viruses called retroviruses, which carry genetic information in the form of RNA. HIV infects T cells that carry the CD4 antigen on their surface. When HIV infects its target cell it requires fusion of the viral and cellular membranes.[12] The first step is the interaction between envelope proteins of the virus (gp120, gp41) and specific host-cell surface receptors (e.g. CD4 receptor) on the target cell. Then the virus binds to the chemokine coreceptors CXCR4 or CCR5, resulting in conformational changes in the envelope proteins. This fusion creates a pore through which the viral capsid enters the cell.[13] Following entry into the cell the RNA of the virus is reverse-transcribed to DNA by the first virally encoded enzyme, the reverse transcriptase. The viral DNA enters the nucleus where it is integrated into the genetic material of the cell by the integrase, a second virally encoded enzyme. Activation of the host cell leads to the transcription of the viral DNA into mRNA. The mRNA is then translated into viral proteins and the third virally encoded enzyme, namely HIV protease, is required to cleave a viral polyprotein precursor into individual mature proteins. The viral RNA and viral proteins assemble at the surface of the cell into new virions. The virions bud from the cell and are released to infect other cells. All infected cells are eventually killed because of this extensive cell damage, from the destruction of the host's genetic system to the budding and release of virions.[12]

Mechanism of action

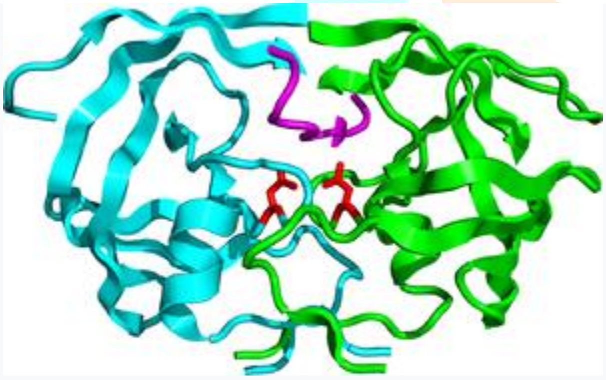
There are several steps in the HIV life cycle that may be interfered with, thus stopping the replication of the virus. A very critical step is the proteolytic cleavage of the polypeptide precursors into mature enzymes and structural proteins catalyzed by HIV protease.[12] HIV protease inhibitors are peptide-like chemicals that competitively inhibit the action of the virus aspartyl protease. These drugs prevent proteolytic cleavage of HIV Gag and Pol polyproteins that include essential structural and enzymatic components of the virus. This prevents the conversion of HIV particles into their mature infectious form.[6]

Protease inhibitors can alter adipocyte metabolism causing lipodystrophy, a common side effect associated with the use of most HIV protease inhibitors. Many mechanisms have been proposed, for example inhibition of adipocyte differentiation, triglyceride accumulation and increased lipolysis. Theories considering the effect of protease inhibitors on insulin-stimulated glucose uptake have also been linked to the lipodystrophic syndrome. It is possible that protease inhibitors can cause a decrease in insulin-stimulated tyrosine phosphorylation of IRS-1, representing inhibition of early steps in insulin signaling. Decreased adiponectin secretion and induced expression of interleukin-6 associated with HIV protease inhibitors may also contribute to inhibition of insulin-stimulated glucose uptake.[14]

Design

Protease inhibitors were designed to mimic the transition state of the protease's actual substrates. A peptide linkage consisting of –NH-CO- is replaced by an hydroxyethylen group (-CH_2-CH(OH)-) which the protease is unable to cleave. HIV protease inhibitors fit the active site of the HIV aspartic protease and were rationally designed utilizing knowledge of the aspartyl protease's mode of action. The most promising transition state mimic was hydroxyethylamine which led to the discovery of the first protease inhibitor, saquinavir. Following that discovery, other HIV protease inhibitors were designed using the same principle.[15]

Binding site**[edit]**



A schematic structure of a HIV-1 protease. The monomers are shown in green and cyan, the Asp-25 and Asp-25´ residues are shown in red, and Ile50 and Ile50´ residues linked to a water molecule are shown in purple.

The HIV protease is a C2-symmetric homodimeric enzyme consisting of two 99 amino acid monomers. Each monomer contributes an aspartic acid residue that is essential for catalysis,[6] Asp-25 and Asp-25´. The HIV protease has the sequence Asp-Thr-Gly, which is conserved among other mammalian aspartic protease enzymes. An extended beta-sheet region on the monomers, known as the flap, constitutes in part the substrate binding site with the two aspartyl residues lying on the bottom of a hydrophobic cavity.[12][16][17] Each flexible flap contains three characteristic regions: side chains that extend outward (Met46, Phe53), hydrophobic chains extending inward (Ile47, Ile54), and a glycine rich region (Gly48, 49, 51, 52). Ile50 remains at the tip of the turn and when the enzyme is unliganded a water molecule makes hydrogen bonds to the backbone of Ile50 on each monomer.[17]

HIV proteases catalyze the hydrolysis of peptide bonds with high sequence selectivity and catalytic proficiency. The mechanism of the HIV protease shares many features with the rest of the aspartic protease family although the full detailed mechanism of this enzyme is not fully understood.[12] The water molecule seems to play a role in the opening and closing of the flaps as well as increasing the affinity between enzyme and substrate. The aspartyl residues are involved in the hydrolysis of the peptide bonds.[17] The preferred cleavage site for this enzyme is the N-terminal side of proline residues, especially between phenylalanine and proline or tyrosine and proline.[6][16]

Development

The first HIV protease inhibitor, saquinavir, is a peptidomimetic hydroxyethylamine[6] and was marketed in 1995.[18] It is a transition state analogue of a native substrate of the protease.[6] The observation that HIV-1 protease cleaves the sequences containing the dipeptides Tyr-Pro or Phe-Pro was the basic design criterion.[19] Addition of the decahydroisoquinoline (DIQ) group was one of the most significant modifications that led to the discovery of saquinavir. This substituent improves aqueous solubility and potency by limiting the conformational freedom of the inhibitor.[20] Saquinavir is effective against both HIV-1 and HIV-2[5] and is usually well tolerated but high serum concentration is not achieved.[11]

Ritonavir, a peptidomimetic HIV protease inhibitor, was marketed in 1996.[18] It was designed to fit the C2-symmetry in the binding site of the protease.[6] The developers of ritonavir, Abbott Laboratories, started with compounds that were active against the virus but had poor bioavailability. Some improvements were made, for example the terminal phenyl residues were removed and pyridyl groups put instead to add water solubility. The final product of these improvements was ritonavir.[19] Significant gastrointestinal side effects and a large pill burden are ritonavir's main drawbacks and is therefore not used as a single treatment.[11] However, it is a strong inhibitor of the cytochrome P450 enzyme mediated metabolism[19] and it is only used in a combination therapy with other protease inhibitors for pharmacokinetic boosting.[11]

Indinavir, which is a peptidomimetic hydroxyethylene HIV protease inhibitor, reached the market in 1996.[6][18] The design of indinavir was guided by molecular modeling and the X-ray crystal structure of the inhibited enzyme complex. The terminal phenyl constituents contribute hydrophobic binding to increase potency.[19] It is an analogue of the phenylalanine-proline cleavage site of the HIV Gag-polyprotein.[6]

Nelfinavir was the first protease inhibitor that was not peptidomimetic. In the design process of nelfinavir, an orally bioavailable and nonpeptidic inhibitor, iterative protein cocrystal structure analysis of peptidic inhibitors was used and parts of the inhibitors were replaced by nonpeptidic substituents.[19] Nelfinavir contains a novel 2-methyl-3-hydroxybenzamide group, whereas its carboxyl terminal contains the same DIQ group as saquinavir.[19] Nelfinavir was marketed in 1997[18] and was the first protease inhibitor to be indicated for pediatric AIDS.[19]

Amprenavir reached the market in 1999.[18] It is an *N*,*N*-disubstituded amino-sulfonamide nonpeptide HIV protease inhibitor[6] and shares some common features with previous protease inhibitors. It has a core similar to that of saquinavir but with different functional groups on both ends. On one end it has a tetrahydrofuran carbamate group and on the other end is an isobutylphenyl sulfonamide with an added amide. This structure results in fewer chiral centers, that makes it easier to synthesize and gives it enhanced aqueus solubility. That in turn gives better oral bioavailability.[19] However, amprenavir was withdrawn from the market in 2004 since fosamprenavir, its prodrug, proved superior in many aspects.[6]

Lopinavir was marketed in 2000[18] and was originally designed to diminish the interactions of the inhibitor with Val82 of the HIV-1 protease, a residue that is often mutated in the drug resistant strains of the virus.[19] It is a peptidomimetic HIV protease inhibitor[6] and its core is identical to that of ritonavir. Instead of the 5-thiazolyl end group in ritonavir, lopinavir has a phenoxyacetyl group and the 2-isopropylthiazolyl group in ritonavir was replaced by a modified valine in which the amino terminal had a six-membered cyclic urea attached.[19]

Fosamprenavir was marketed in 2003[18] and is a phosphoester prodrug that is rapidly and extensively metabolized to amprenavir.[21] The solubility and bioavailability is better than of amprenavir[6] which results in reduced daily pill burden.[22]

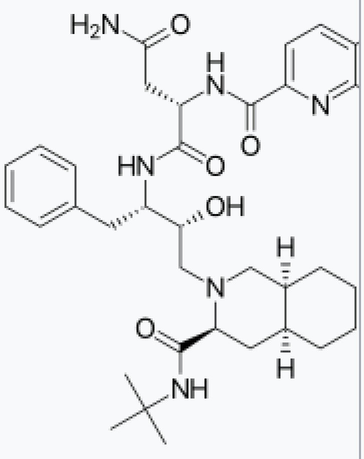
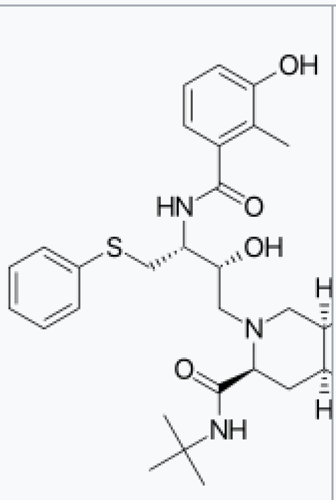
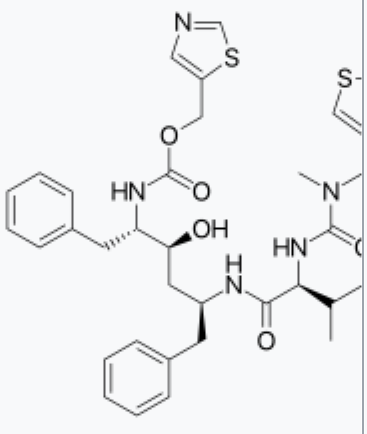
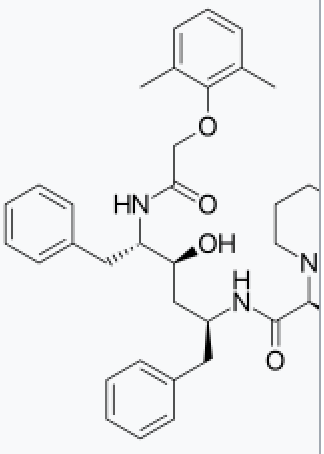
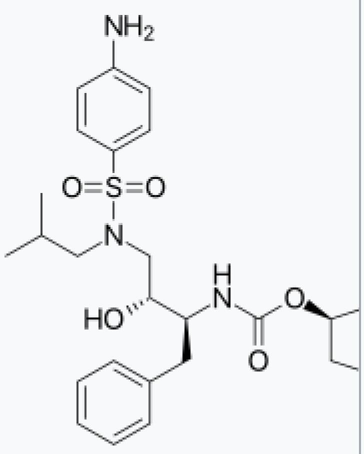
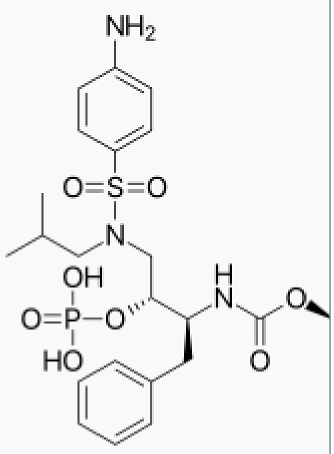
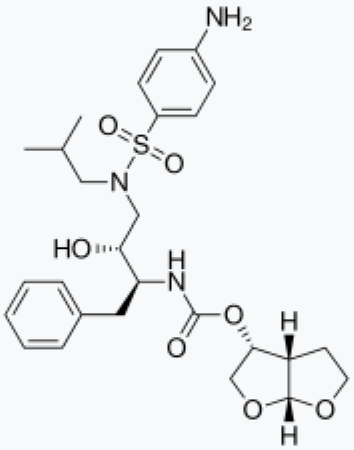
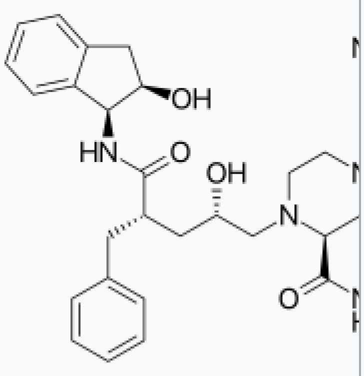
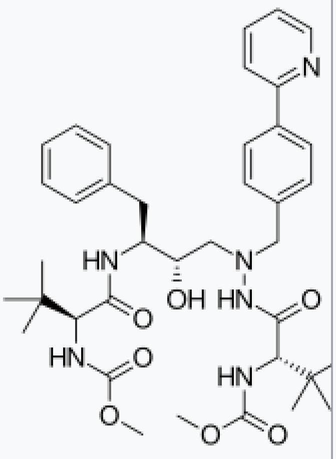
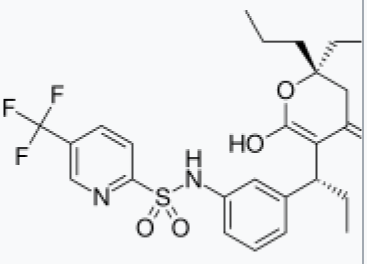
Atazanavir was marketed in 2003[18] and is an azapeptide protease inhibitor[18] designed to fit the C2-symmetry of the enzyme binding site.[11] Atazanavir showed better resistant profiles than previous HIV protease inhibitors.[4] It is unique among the other protease inhibitors as it can only be absorbed in an acidic environment.[11]

Tipranavir is a nonpeptidic HIV-1 protease inhibitor[11] and reached the market in 2005.[18] Unlike other HIV protease inhibitors on the market, tipranavir was developed from a nonpeptidic coumarin template and its antiprotease activity was discovered by high-throughput screening.[23] This sulfonamide containing 5,6-dihydro-4-hydroxy-2-pyrone had emerged from screenings of 3-substituted coumarins and dihydropyrones.[24] It possesses broad antiviral activity against multiple protease inhibitor resistant HIV-1.[25]

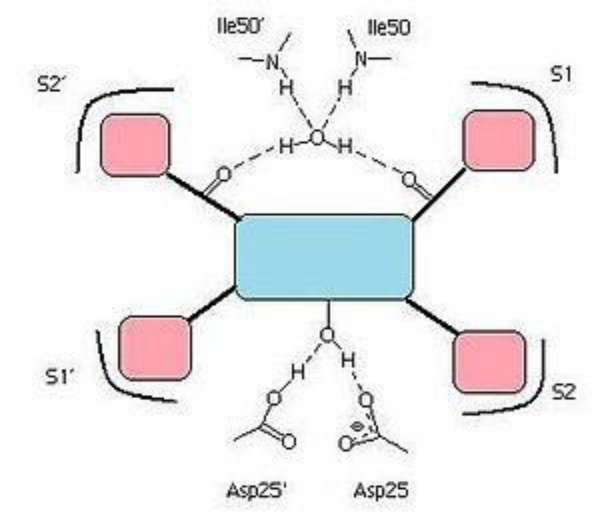
Darunavir reached the market in 2006[18] and is a nonpeptidic analogue of amprenavir, with a critical change at the terminal tetrahydrofuran (THF) group. Instead of a single THF group, darunavir contains two THF groups fused in the compound, to form a bis-THF moiety which makes it more effective than amprenavir. With this structural change, the stereochemistry around the bis-THF moiety confers orientational changes, that allows for continued binding with the protease which has developed a resistance for amprenavir.[26]

All the FDA approved protease inhibitors are listed below.

HIV protease inhibitors the FDA has approved

			
Saquinavir	Nelfinavir	Ritonavir	Lopinavir
			
Amprenavir	Fosamprenavir	Darunavir	
			
Indinavir	Atazanavir	Tipranavir	

Structure-activity relationship



A simplified image of a protease inhibitor binding to the active site of the HIV-1 protease. The central core motif is shown in blue with the hydroxyl group forming hydrogen bonds with Asp-25 and Asp-25´. Hydrogen bonds also connect carbonyl groups on the inhibitor to the water molecule linked to Ile50 and Ile50´. Hydrophobic groups are shown in pink and their complementing pockets referred to as S1, S1´, S2 and S2´.

All the HIV protease inhibitors on the market contain a central core motif consisting of a hydroxyethylen scaffold, with the only exception being the central core of tipranavir, which is based on a coumarin scaffold.[15] A very important group on the HIV protease inhibitors is a hydroxyl group on the core motif which forms a hydrogen bond with the carboxylic acid on the Asp-25 and Asp-25´ residues in the binding site.[16][27] Hydrogen bonds between the water molecule, which is linked to Ile50 and Ile50', and carbonyl groups of the peptidomimetic inhibitors seem to connect them with the flap regions.[19] On the other hand, on the nonpeptidic inhibitors, there is a proton acceptor which replaces the tetracoordinated water molecule and interacts directly with the two Ile50 residues on the flap of the enzyme.[28] Specific pockets in the binding site of the HIV protease, often referred to as S1, S1',S2 and S2', recognize hydrophobic amino acids on natural substrates. The potency of inhibitors bearing hydrophobic groups complementing these areas is therefore increased.[29] Some residues in the enzyme binding site are capable of forming hydrogen bonds with hydrophilic groups on the inhibitor, for example with the THF moieties on amprenavir and darunavir. Since darunavir has a bis-THF moiety, instead of a single THF moiety like on amprenavir, it can form more hydrogen bonds and increase binding energy.[26]

Resistance

Mutations that code for alterations of the conformational shape facilitate resistance of HIV to protease inhibitors.[26] The locations of these mutations are primarily in the active site of the HIV protease enzyme as well as outside of the active site, including those at protease cleavage sites in the Gag-Pol polyprotein precursors. The cleavage sites have highly diverse sequences, so the protease recognizes its substrates not based on sequence but rather the conserved 3D shape the substrates share when bound at the active site. This conserved shape has been named the *substrate envelope*.[30] The active site mutations have been shown to directly change the interactions of the inhibitors, and mostly occur at positions where inhibitors contact protease residues beyond the substrate envelope.[31] The non-active site mutations are considered to affect by other mechanisms, like influencing dimer stability and conformational flexibility.[32][33]

Over 100 single gene point mutations have been described, of which at least 26 are specific to protease inhibitors. Of these, there are about 15 primary or major mutations that are significant enough to change drug activity.[26] Many mutated residues have been found in HIV-1 protease which cause drug resistance, for example Leu33 changes to Ile, Val, or Phe; Val82 to Ala, Phe, Leu, or Thr; Ile84 to Val; and Leu90 to Met.[34] Different mutations affects different protease inhibitors. For instance, mutations at Leu90 evidently affect saquinavir and nelfinavir while indinavir activity is affected by mutations at Met46, Val82, and Ile84, and fosamprenavir is affected when Ile50 changes to Val and at Ile84. A combination of mutations can render high-level drug resistance but single mutations normally do not equate with drug resistance to protease inhibitors.[26] The mutations can be divided into primary mutations and secondary mutations. Primary mutations often have only a small effect on resistance. The chemical structures of most protease inhibitors are quite similar, so it is not surprising that some primary mutations lead simultaneously to resistance to multiple protease inhibitors. Cross-resistance is one of the major problems of protease inhibitor treatment.[35] Additional mutations emerging in the protease during continuous protease inhibitor therapy are commonly referred to as secondary mutations. This can lead to high-level protease inhibitor resistance.[35]

The Stanford HIV RT and Protease Sequence Database (also called the “HIV Drug Resistance Database”) was formed in 1998 with HIV reverse transcriptase and protease sequences from persons with well-characterized antiretroviral treatment histories, and is publicly available to query resistance mutations and genotype-treatment, genotype-phenotype, and genotype-outcome correlations: http://hivdb.stanford.edu

Although the substrate envelope provides the general strategy of designing inhibitors that mimic the substrate and stay within the envelope to avoid resistance conferred by most active site mutations,[36][37] there is no general strategy to tackle the problem of drug resistance, especially due to those away from the active site. Researches directed towards development of new therapies to cure AIDS are focused on avoiding cross-resistance to drugs that are already on the market.[12]

Current status

In January 2018 darunavir was still the most recent HIV protease inhibitor to reach the market.[38]

In 2006, GlaxoSmithKline discontinued the phase II clinical development of brecanavir, an investigational protease inhibitor for the treatment of HIV, due to insurmountable issues regarding formulation.[39]

In the summer of 2009, GlaxoSmithKline and Concert Pharmaceuticals announced their collaboration to develop and commercialise deuterium-containing medicines. One of them is CTP-518, a protease inhibitor for the treatment of HIV, expected to enter phase I clinical trials in the second half of 2009. CTP-518 is a novel HIV protease inhibitor developed by replacing certain key hydrogen atoms of atazanavir with deuterium. Pre-clinical studies have demonstrated that this modification fully retains the antiviral potency but can evidently slow hepatic metabolism and thereby increase the half life and plasma trough levels. CTP-518, therefore, has the potential to be the first HIV protease inhibitor to eliminate the need to co-dose with a boosting agent, such as ritonavir.[40]

H.Retrometabolic drug design

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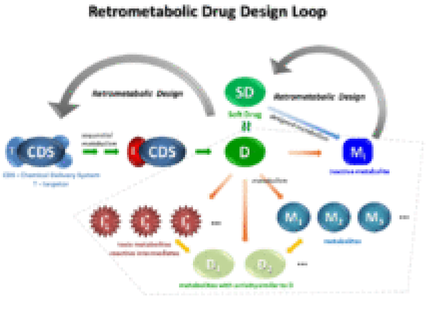
In the field of drug discovery, **retrometabolic drug design** is a strategy for the design of safer drugs either using predictable metabolism to an inactive moiety or using targeted drug delivery approaches. The phrase retrometabolic drug design was coined by Nicholas Bodor.[1] The method is analogous to retrosynthetic analysis where the synthesis of a target molecule is planned backwards. In retrometabolic drug design, metabolic reaction information of drugs is used to design parent drugs whose metabolism and distribution can be controlled to target and eliminate the drug to increase efficacy and minimize undesirable side effects. The new drugs thus designed achieve selective organ and/or therapeutic site drug targeting and produce safe therapeutic agents and safe environmental chemicals. These approaches represent systematic methodologies that thoroughly integrate structure-activity (SAR) and structure-metabolism (SMR) relationships and are aimed at designing safe, locally active compounds with improved therapeutic index (ratio of benefit vs. side effect).[2][3][4][5][6]



Retrometabolic drug design loop that includes chemical delivery systems (CDS) design and soft drug (SD) design. Possible metabolic pathways for drugs (D) in general are indicated within the dashed box.

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Classification

The concept of retrometabolic drug design encompasses two distinct approaches. One approach is the design of soft drugs (SDs),[4][7][8][9][10][11][12][13][14][15] new, active therapeutic agents, often isosteric or isolelectronic analogs of a lead compound, with a chemical structure specifically designed to allow predictable metabolism into inactive metabolites after exerting their desired therapeutic effect(s). The other approach is the design of chemical delivery systems (CDSs).[4][16][17][18][19][20][21][22][23] CDSs are biologically inert molecules intended to enhance drug delivery to a particular organ or site and requiring several conversion steps before releasing the active drug.

Although both retrometabolic design approaches involve chemical modifications of the molecular structure and both require enzymatic reactions to fulfill drug targeting, the principles of SD and CDS design are distinctly different. While CDSs are inactive as administered and sequential enzymatic reactions provide the differential distribution and ultimately release the active drug, SDs are active as administered and are designed to be easily metabolized into inactive species. Assuming an ideal situation, with a CDS the drug is present at the site and nowhere else in the body because enzymatic processes destroy the drug at those sites. Whereas, CDSs are designed to achieve drug targeting at a selected organ or site, SDs are designed to afford a differential distribution that can be regarded as reverse targeting.

Soft drugs**[edit]**

Since its introduction by Nicholas Bodor in the late 1970s, the soft drug concept generated considerable research both in academic and in industrial settings. Bodor defined soft drugs as biologically active, therapeutically useful chemical compounds characterized by a predictable and controllable in vivo metabolism to non-toxic moieties after they achieve their therapeutic role.[24] There are several rationally designed soft drugs that have either already reached the market, such as

- esmolol (Breviblock)
- landiolol (Onoact)
- remifentanil (Ultiva)
- loteprednol etabonate (Lotemax, Alrex, Zylet)
- clevidipine (Cleviprex)

or are in late-stage development (remimazolam, budiodarone, celivarone, AZD3043, tecafarin).[25] There are also compounds that can be considered as soft chemicals (e.g., malathion) or soft drugs (e.g., articaine, methylphenidate) even though they were not developed as such.[25]

Chemical delivery systems

Since their introduction in the early 1980s, CDSs have also generated considerable research work, especially for brain and eye targeting of various therapeutic agents, including those that cannot cross the blood-brain barrier or the blood-retinal barrier on their own. Within this approach, three major general CDS classes have been identified:

- *Enzymatic physicochemical-based (e.g., brain-targeting) CDSs:* exploit site-specific traffic properties by sequential metabolic conversions that result in considerably altered properties
- *Site-specific enzyme-activated (e.g., eye-targeting) CDSs:* exploit specific enzymes found primarily, exclusively, or at higher activity at the site of action
- *Receptor-based transient anchor-type (e.g., lung-targeting) CDSs:* provide enhanced selectivity and activity through transient, reversible binding at the receptor

This concept has been extended to many drugs and peptides, its importance illustrated by the fact that its first applications and uses were published in *Science*[26][27][28] in 1975, 1981 and 1983. Its extension to the targeted brain-delivery of neuropeptides was included by the *Harvard Health Letter*[29] as one of the top 10 medical advances of 1992. Several compounds have reached advanced clinical development phase, such as

- E_2-CDS (Estredox) for the brain-targeted delivery of estradiol[30] and
- betaxoxime for the eye-targeted delivery of betaxolol[31]

In the first example above, brain-targeted CDSs employ a sequential metabolic conversion of a redox-based targetor moiety, which is closely related to the ubiquitous NAD(P)H $\rightleftharpoons$ NAD(P)$^+$ coenzyme system, to exploit the unique properties of the blood-brain barrier (BBB). After enzymatic oxidation of the NADH type drug conjugate to its corresponding NAD$^+$- drug, the still inactive precursor, "locks-in" behind the BBB to provide targeted and sustained CNS-delivery of the compound of interest.

The second example involves eye-specific delivery of betaxoxime, the oxime derivative of betaxolol. The administered, inactive β-amino-ketoxime is converted to the corresponding ketone via oxime hydrolase, an enzyme recently identified with preferential activity in the eye, and then stereospecifically reduced to its alcohol form. IOP-lowering activity is demonstrated without producing the active β-blockers systemically, making them void of any cardiovascular activity, a major drawback of classical antiglaucoma agents. Because of the advantages provided by this unique eye-targeting profile, oxime-based eye-targeting CDSs could replace the β-blockers currently used for ophthalmic applications.

History and significance

These retrometabolic design strategies were introduced by Nicholas Bodor, one of the first and most prominent advocates for the early integration of metabolism, pharmacokinetic and general physicochemical considerations in the drug design process.[32][33][34] These drug design concepts recognize the importance of design-controlled metabolism and directly focus not on the increase of activity alone but on the increase of the activity/toxicity ratio (therapeutic index) in order to deliver the maximum benefit while also reducing or eliminating unwanted side effects. The importance of this field is reviewed in a book dedicated to the subject (Bodor, N.; Buchwald, P.; *Retrometabolic Drug Design and Targeting*, 1st ed., Wiley & Sons, 2012), as well as by a full chapter of *Burger's Medicinal Chemistry and Drug Design*, 7th ed. (2010) with close to 150 chemical structures and more than 450 references.[35] At the time of its introduction, the idea of designed-in metabolism represented a significant novelty and was against mainstream thinking then in place that instead focused on minimizing or entirely eliminating drug metabolism. Bodor's work on these design concepts developed during the late 1970s and early 1980s, and came to prominence during the mid-1990s. Loteprednol etabonate, a soft corticosteroid designed and patented[36][37] by Bodor received final Food and Drug Administration (FDA) approval in 1998 as the active ingredient of two ophthalmic preparations (Lotemax and Alrex), currently the only corticosteroid approved by the FDA for use in all inflammatory and allergy-related ophthalmic disorders. Its safety for long-term use[38] further supports the soft drug concept, and in 2004, loteprednol etabonate[39][40][41] was also approved as part of a combination product (Zylet). A second generation of soft corticosteroids such as etiprednol dicloacetate[42] is in development for a full spectrum of other possible applications such as nasal spray for rhinitis or inhalation products for asthma.

The soft drug concept ignited research work in both academic (e.g., Aston University, Göteborg University, Okayama University, Uppsala University, University of Iceland, University of Florida, Université Louis Pasteur, Yale University) and industrial (e.g., AstraZeneca, DuPont, GlaxoSmithKline, IVAX, Janssen Pharmaceutica, Nippon Organon, Novartis, ONO Pharmaceutical, Schering AG) settings. Besides corticosteroids, various other therapeutic areas have been pursued such as soft beta-blockers, soft opioid analgetics, soft estrogens, soft beta-agonists, soft anticholinergics, soft antimicrobials, soft antiarrhythmic agents, soft angiotensin converting enzyme (ACE) inhibitors, soft dihydrofolate reductase (DHFR) inhibitors, soft cancineurin inhibitors (soft immunosuppressants), soft matrix metalloproteinase (MMP) inhibitors, soft cytokine inhibitors, soft cannabinoids, soft Ca^{2+} channel blockers (see[35] for a recent review).

Following the introduction of the CDS concepts, work along those lines started in numerous pharmaceutical centers around the world, and brain-targeting CDSs were explored for many therapeutic agents such as steroids (testosterone, progestins, estradiol, dexamethasone), anti-infective agents (penicillins, sulfonamides), antivirals (acyclovir, trifluorothymidine, ribavirin), antiretrovirals (AZT, ganciclovir), anticancer agents (Lomustine, chlorambucil), neurotransmitters (dopamine, GABA), nerve growth factor (NGF) inducers, anticonvulsants (Phenytoin, valproate, stiripentol), Ca^{2+} antagonists (felodipine), MAO inhibitors, NSAIDs and neuropeptides (tryptophan, Leu-enkephalin analogs, TRH analogs, kyotorphin analogs). A number of new chemical entities (NCE) were developed based on these principles, such as E_2-CDS (Estredox[30] or betaxoxime[31] are in advanced clinical development phases.

A review of ongoing research using the general retrometabolic design approaches is conducted biennially at the *Retrometabolism Based Drug Design and Targeting Conference*, an international series of symposia developed and organized by Nicholas Bodor. Proceedings of each conference held have been published in the international pharmaceutical journal *Pharmazie*. Past conferences, and their published proceedings are:

I. Antiviral drug

"Antiviral" redirects here. For other uses, see Antiviral (disambiguation). For the computer software, see Antivirus.

Antiviral drugs are a class of medication used for treating viral infections.[1] Most antivirals target specific viruses, while a broad-spectrum antiviral is effective against a wide range of viruses.[2] Unlike most antibiotics, antiviral drugs do not destroy their target pathogen; instead they inhibit its development.

Antiviral drugs are one class of antimicrobials, a larger group which also includes antibiotic (also termed antibacterial), antifungal and antiparasitic drugs,[3] or antiviral drugs based on monoclonal antibodies.[4] Most antivirals are considered relatively harmless to the host, and therefore can be used to treat infections. They should be distinguished from viricides, which are not medication but deactivate or destroy virus particles, either inside or outside the body. Natural viricides are produced by some plants such as eucalyptus and Australian tea trees.[5]

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Medical uses

Most of the antiviral drugs now available are designed to help deal with HIV, herpes viruses, Covid 19, the hepatitis B and C viruses, and influenza A and B viruses. Researchers are working to extend the range of antivirals to other families of pathogens.

Designing safe and effective antiviral drugs is difficult because viruses use the host's cells to replicate. This makes it difficult to find targets for the drug that would interfere with the virus without also harming the host organism's cells. Moreover, the major difficulty in developing vaccines and anti-viral drugs is due to viral variation.

The emergence of antivirals is the product of a greatly expanded knowledge of the genetic and molecular function of organisms, allowing biomedical researchers to understand the structure and function of viruses, major advances in the techniques for finding new drugs, and the pressure placed on the medical profession to deal with the human immunodeficiency virus (HIV), the cause of acquired immunodeficiency syndrome (AIDS).

The first experimental antivirals were developed in the 1960s, mostly to deal with herpes viruses, and were found using traditional trial-and-error drug discovery methods. Researchers grew cultures of cells and infected them with the target virus. They then introduced into the cultures chemicals which they thought might inhibit viral activity and observed whether the level of virus in the cultures rose or fell. Chemicals that seemed to have an effect were selected for closer study.

This was a very time-consuming, hit-or-miss procedure, and in the absence of a good knowledge of how the target virus worked, it was not efficient in discovering effective antivirals which had few side effects. Only in the 1980s, when the full genetic sequences of viruses began to be unraveled, did researchers begin to learn how viruses worked in detail, and exactly what chemicals were needed to thwart their reproductive cycle.

Antiviral Drug Design

Anti-viral targeting

The general idea behind modern antiviral drug design is to identify viral proteins, or parts of proteins, that can be disabled. These "targets" should generally be as unlike any proteins or parts of proteins in humans as possible, to reduce the likelihood of side effects. The targets should also be common across many strains of a virus, or even among different species of virus in the same family, so a single drug will have broad effectiveness. For example, a researcher might target a critical enzyme synthesized by the virus, but not by the patient, that is common across strains, and see what can be done to interfere with its operation.

Once targets are identified, candidate drugs can be selected, either from drugs already known to have appropriate effects or by actually designing the candidate at the molecular level with a computer-aided design program.

The target proteins can be manufactured in the lab for testing with candidate treatments by inserting the gene that synthesizes the target protein into bacteria or other kinds of cells. The cells are then cultured for mass production of the protein, which can then be exposed to various treatment candidates and evaluated with "rapid screening" technologies.

Approaches by Virus life cycle stage

Viruses consist of a genome and sometimes a few enzymes stored in a capsule made of protein (called a capsid), and sometimes covered with a lipid layer (sometimes called an 'envelope'). Viruses cannot reproduce on their own and instead propagate by subjugating a host cell to produce copies of themselves, thus producing the next generation.

Researchers working on such "rational drug design" strategies for developing antivirals have tried to attack viruses at every stage of their life cycles. Some species of mushrooms have been found to contain multiple antiviral chemicals with similar synergistic effects.[6] Compounds isolated from fruiting bodies and filtrates of various mushrooms have broad-spectrum antiviral activities, but successful production and availability of such compounds as frontline antiviral is a long way away.[7] Viral life cycles vary in their precise details depending on the type of virus, but they all share a general pattern:

1. Attachment to a host cell.
2. Release of viral genes and possibly enzymes into the host cell.
3. Replication of viral components using host-cell machinery.
4. Assembly of viral components into complete viral particles.
5. Release of viral particles to infect new host cells.

Before cell entry

One anti-viral strategy is to interfere with the ability of a virus to infiltrate a target cell. The virus must go through a sequence of steps to do this, beginning with binding to a specific "receptor" molecule on the surface of the host cell and ending with the virus "uncoating" inside the cell and releasing its contents. Viruses that have a lipid envelope must also fuse their envelope with the target cell, or with a vesicle that transports them into the cell before they can uncoat.

This stage of viral replication can be inhibited in two ways:

1. Using agents which mimic the virus-associated protein (VAP) and bind to the cellular receptors. This may include VAP anti-idiotypic antibodies, natural ligands of the receptor and anti-receptor antibodies.[*clarification needed*]
2. Using agents which mimic the cellular receptor and bind to the VAP. This includes anti-VAP antibodies, receptor anti-idiotypic antibodies, extraneous receptor and synthetic receptor mimics.

This strategy of designing drugs can be very expensive, and since the process of generating anti-idiotypic antibodies is partly trial and error, it can be a relatively slow process until an adequate molecule is produced.

Entry inhibitor

A very early stage of viral infection is viral entry, when the virus attaches to and enters the host cell. A number of "entry-inhibiting" or "entry-blocking" drugs are being developed to fight HIV. HIV most heavily targets the immune system's white blood cells known as "helper T cells", and identifies these target cells through T-cell surface receptors designated "CD4" and "CCR5". Attempts to interfere with the binding of HIV with the CD4 receptor have failed to stop HIV from infecting helper T cells, but research continues on trying to interfere with the binding of HIV to the CCR5 receptor in hopes that it will be more effective.

HIV infects a cell through fusion with the cell membrane, which requires two different cellular molecular participants, CD4 and a chemokine receptor (differing depending on the cell type). Approaches to blocking this virus/cell fusion have shown some promise in preventing entry of the virus into a cell. At least one of these entry inhibitors—a biomimetic peptide called Enfuvirtide, or the brand name Fuzeon—has received FDA approval and has been in use for some time. Potentially, one of the benefits from the use of an effective entry-blocking or entry-inhibiting agent is that it potentially may not only prevent the spread of the virus within an infected individual but also the spread from an infected to an uninfected individual.

One possible advantage of the therapeutic approach of blocking viral entry (as opposed to the currently dominant approach of viral enzyme inhibition) is that it may prove more difficult for the virus to develop resistance to this therapy than for the virus to mutate or evolve its enzymatic protocols.

Uncoating inhibitor

Inhibitors of uncoating have also been investigated.[8][9]

Amantadine and rimantadine have been introduced to combat influenza. These agents act on penetration and uncoating.[10]

Pleconaril works against rhinoviruses, which cause the common cold, by blocking a pocket on the surface of the virus that controls the uncoating process. This pocket is similar in most strains of rhinoviruses and enteroviruses, which can cause diarrhea, meningitis, conjunctivitis, and encephalitis.

Some scientists are making the case that a vaccine against rhinoviruses, the predominant cause of the common cold, is achievable. Vaccines that combine dozens of varieties of rhinovirus at once are effective in stimulating antiviral antibodies in mice and monkeys, researchers have reported in Nature Communications in 2016.

Rhinoviruses are the most common cause of the common cold; other viruses such as respiratory syncytial virus, parainfluenza virus and adenoviruses can cause them too. Rhinoviruses also exacerbate asthma attacks. Although rhinoviruses come in many varieties, they do not drift to the same degree that influenza viruses do. A mixture of 50 inactivated rhinovirus types should be able to stimulate neutralizing antibodies against all of them to some degree.

During viral synthesis

A second approach is to target the processes that synthesize virus components after a virus invades a cell.

Reverse transcription

One way of doing this is to develop nucleotide or nucleoside analogues that look like the building blocks of RNA or DNA, but deactivate the enzymes that synthesize the RNA or DNA once the analogue is incorporated. This approach is more commonly associated with the inhibition of reverse transcriptase (RNA to DNA) than with "normal" transcriptase (DNA to RNA).

The first successful antiviral, aciclovir, is a nucleoside analogue, and is effective against herpesvirus infections. The first antiviral drug to be approved for treating HIV, zidovudine (AZT), is also a nucleoside analogue.

An improved knowledge of the action of reverse transcriptase has led to better nucleoside analogues to treat HIV infections. One of these drugs, lamivudine, has been approved to treat hepatitis B, which uses reverse transcriptase as part of its replication process. Researchers have gone further and developed inhibitors that do not look like nucleosides, but can still block reverse transcriptase.

Another target being considered for HIV antivirals include RNase H—which is a component of reverse transcriptase that splits the synthesized DNA from the original viral RNA.

Integrase

Another target is integrase, which integrate the synthesized DNA into the host cell genome.

Transcription

Once a virus genome becomes operational in a host cell, it then generates messenger RNA (mRNA) molecules that direct the synthesis of viral proteins. Production of mRNA is initiated by proteins known as transcription factors. Several antivirals are now being designed to block attachment of transcription factors to viral DNA.

Translation/antisense

Genomics has not only helped find targets for many antivirals, it has provided the basis for an entirely new type of drug, based on "antisense" molecules. These are segments of DNA or RNA that are designed as complementary molecule to critical sections of viral genomes, and the binding of these antisense segments to these target sections blocks the operation of those genomes. A phosphorothioate antisense drug named fomivirsen has been introduced, used to treat opportunistic eye infections in AIDS patients caused by cytomegalovirus, and other antisense antivirals are in development. An antisense structural type that has proven especially valuable in research is morpholino antisense.

Morpholino oligos have been used to experimentally suppress many viral types:

- caliciviruses[11]
- flaviviruses (including WNV)[12]
- dengue[13]
- HCV[14]
- coronaviruses[15]

Translation/ribozymes

Yet another antiviral technique inspired by genomics is a set of drugs based on ribozymes, which are enzymes that will cut apart viral RNA or DNA at selected sites. In their natural course, ribozymes are used as part of the viral manufacturing sequence, but these synthetic ribozymes are designed to cut RNA and DNA at sites that will disable them.

A ribozyme antiviral to deal with hepatitis C has been suggested,[16] and ribozyme antivirals are being developed to deal with HIV.[17] An interesting variation of this idea is the use of genetically modified cells that can produce custom-tailored ribozymes. This is part of a broader effort to create genetically modified cells that can be injected into a host to attack pathogens by generating specialized proteins that block viral replication at various phases of the viral life cycle.

Protein processing and targeting

Interference with post translational modifications or with targeting of viral proteins in the cell is also possible.[18]

Protease inhibitors

Some viruses include an enzyme known as a protease that cuts viral protein chains apart so they can be assembled into their final configuration. HIV includes a protease, and so considerable research has been performed to find "protease inhibitors" to attack HIV at that phase of its life cycle.[19] Protease inhibitors became available in the 1990s and have proven effective, though they can have unusual side effects, for example causing fat to build up in unusual places.[20] Improved protease inhibitors are now in development.

Protease inhibitors have also been seen in nature. A protease inhibitor was isolated from the Shiitake mushroom (*Lentinus edodes*).[21] The presence of this may explain the Shiitake mushroom's noted antiviral activity *in vitro*.[22]

Long dsRNA helix targeting

Most viruses produce long dsRNA helices during transcription and replication. In contrast, uninfected mammalian cells generally produce dsRNA helices of fewer than 24 base pairs during transcription. **DRACO** (**double-stranded RNA activated caspase oligomerizer**) is a group of experimental antiviral drugs initially developed at the Massachusetts Institute of Technology. In cell culture, DRACO was reported to have broad-spectrum efficacy against many infectious viruses, including dengue flavivirus, Amapari and Tacaribe arenavirus, Guama bunyavirus, H1N1 influenza and rhinovirus, and was additionally found effective against influenza *in vivo* in weanling mice. It was reported to induce rapid apoptosis selectively in virus-infected mammalian cells, while leaving uninfected cells unharmed.[23] **DRACO** effects cell death via one of the last steps in the apoptosis pathway in which complexes containing intracellular apoptosis signalling molecules simultaneously bind multiple procaspases. The procaspases transactivate via cleavage, activate additional caspases in the cascade, and cleave a variety of cellular proteins, thereby killing the cell.

Assembly

Rifampicin acts at the assembly phase.[24]

Release phase

The final stage in the life cycle of a virus is the release of completed viruses from the host cell, and this step has also been targeted by antiviral drug developers. Two drugs named zanamivir (Relenza) and oseltamivir (Tamiflu) that have been recently introduced to treat influenza prevent the release of viral particles by blocking a molecule named neuraminidase that is found on the surface of flu viruses, and also seems to be constant across a wide range of flu strains.

Immune system stimulation

Rather than attacking viruses directly, a second category of tactics for fighting viruses involves encouraging the body's immune system to attack them. Some antivirals of this sort do not focus on a specific pathogen, instead stimulating the immune system to attack a range of pathogens.

One of the best-known of this class of drugs are interferons, which inhibit viral synthesis in infected cells.[25] One form of human interferon named "interferon alpha" is well-established as part of the standard treatment for hepatitis B and C,[26] and other interferons are also being investigated as treatments for various diseases.

A more specific approach is to synthesize antibodies, protein molecules that can bind to a pathogen and mark it for attack by other elements of the immune system. Once researchers identify a particular target on the pathogen, they can synthesize quantities of identical "monoclonal" antibodies to link up that target. A monoclonal drug is now being sold to help fight respiratory syncytial virus in babies,[27] and antibodies purified from infected individuals are also used as a treatment for hepatitis B.[28]

Antiviral drug resistance

Antiviral resistance can be defined by a decreased susceptibility to a drug caused by changes in viral genotypes. In cases of antiviral resistance, drugs have either diminished or no effectiveness against their target virus.[29] The issue inevitably remains a major obstacle to antiviral therapy as it has developed to almost all specific and effective antimicrobials, including antiviral agents.[30]

The Centers for Disease Control and Prevention (CDC) inclusively recommends anyone six months and older to get a yearly vaccination to protect them from influenza A viruses (H1N1) and (H3N2) and up to two influenza B viruses (depending on the vaccination).[29] Comprehensive protection starts by ensuring vaccinations are current and complete. However, vaccines are preventative and are not generally used once a patient has been infected with a virus. Additionally, the availability of these vaccines can be limited based on financial or locational reasons which can prevent the effectiveness of herd immunity, making effective antivirals a necessity.[29]

The three FDA-approved neuraminidase antiviral flu drugs available in the United States, recommended by the CDC, include: oseltamivir (Tamiflu), zanamivir (Relenza), and peramivir (Rapivab).[29] Influenza antiviral resistance often results from changes occurring in neuraminidase and hemagglutinin proteins on the viral surface. Currently, neuraminidase inhibitors (NAIs) are the most frequently prescribed antivirals because they are effective against both influenza A and B. However, antiviral resistance is known to develop if mutations to the neuraminidase proteins prevent NAI binding.[31] This was seen in the H257Y mutation, which was responsible for oseltamivir resistance to H1N1 strains in 2009.[29] The inability of NA inhibitors to bind to the virus allowed this strain of virus with the resistance mutation to spread due to natural selection. Furthermore, a study published in 2009 in Nature Biotechnology emphasized the urgent need for augmentation of oseltamivir (Tamiflu) stockpiles with additional antiviral drugs including zanamivir (Relenza). This finding was based on a performance evaluation of these drugs supposing the 2009 H1N1 'Swine Flu' neuraminidase (NA) were to acquire the Tamiflu-resistance (His274Tyr) mutation which is currently widespread in seasonal H1N1 strains.[32]

Origin of antiviral resistance

The genetic makeup of viruses is constantly changing, which can cause a virus to become resistant to currently available treatments.[33] Viruses can become resistant through spontaneous or intermittent mechanisms throughout the course of an antiviral treatment.[29] Immunocompromised patients, more often than immunocompetent patients, hospitalized with pneumonia are at the highest risk of developing oseltamivir resistance during treatment.[29] Subsequent to exposure to someone else with the flu, those who received oseltamivir for "post-exposure prophylaxis" are also at higher risk of resistance.[34]

The mechanisms for antiviral resistance development depend on the type of virus in question. RNA viruses such as hepatitis C and influenza A have high error rates during genome replication because RNA polymerases lack proofreading activity.[35] RNA viruses also have small genome sizes that are typically less than 30 kb, which allow them to sustain a high frequency of mutations.[36] DNA viruses, such as HPV and herpesvirus, hijack host cell replication machinery, which gives them proofreading capabilities during replication. DNA viruses are therefore less error prone, are generally less diverse, and are more slowly evolving than RNA viruses.[35] In both cases, the likelihood of mutations is exacerbated by the speed with which viruses reproduce, which provides more opportunities for mutations to occur in successive replications. Billions of viruses are produced every day during the course of an infection, with each replication giving another chance for mutations that encode for resistance to occur.[37]

Multiple strains of one virus can be present in the body at one time, and some of these strains may contain mutations that cause antiviral resistance.[30] This effect, called the quasispecies model, results in immense variation in any given sample of virus, and gives the opportunity for natural selection to favor viral strains with the highest fitness every time the virus is spread to a new host.[38] Also, recombination, the joining of two different viral variants, and reassortment, the swapping of viral gene segments among viruses in the same cell, play a role in resistance, especially in influenza.[36]

Antiviral resistance has been reported in antivirals for herpes, HIV, hepatitis B and C, and influenza, but antiviral resistance is a possibility for all viruses.[30] Mechanisms of antiviral resistance vary between virus types.

Detection of antiviral resistance

National and international surveillance is performed by the CDC to determine effectiveness of the current FDA-approved antiviral flu drugs.[29] Public health officials use this information to make current recommendations about the use of flu antiviral medications. WHO further recommends in-depth epidemiological investigations to control potential transmission of the resistant virus and prevent future progression.[39] As novel treatments and detection techniques to antiviral resistance are enhanced so can the establishment of strategies to combat the inevitable emergence of antiviral resistance.[40]

Treatment options for antiviral resistant pathogens

If a virus is not fully wiped out during a regimen of antivirals, treatment creates a bottleneck in the viral population that selects for resistance, and there is a chance that a resistant strain may repopulate the host.[41] Viral treatment mechanisms must therefore account for the selection of resistant viruses.

The most commonly used method for treating resistant viruses is combination therapy, which uses multiple antivirals in one treatment regimen. This is thought to decrease the likelihood that one mutation could cause antiviral resistance, as the antivirals in the cocktail target different stages of the viral life cycle.[42] This is frequently used in retroviruses like HIV, but a number of studies have demonstrated its effectiveness against influenza A, as well.[43] Viruses can also be screened for resistance to drugs before treatment is started. This minimizes exposure to unnecessary antivirals and ensures that an effective medication is being used. This may improve patient outcomes and could help detect new resistance mutations during routine scanning for known mutants.[41] However, this has not been consistently implemented in treatment facilities at this time.

Vaccinations

Main article: Vaccination

While most antivirals treat viral infection, vaccines are a preemptive first line of defense against pathogens. Vaccination involves the introduction (i.e. via injection) of a small amount of typically inactivated or attenuated antigenic material to stimulate an individual's immune system. The immune system responds by developing white blood cells to specifically combat the introduced pathogen, resulting in adaptive immunity.[44] Vaccination in a population results in herd immunity and greatly improved population health, with significant reductions in viral infection and disease.[45]

Vaccination policy

Main article: Vaccination policy § United States

Vaccination policy in the United States consists of public and private vaccination requirements. For instance, public schools require students to receive vaccinations (termed "vaccination schedule") for viruses and bacteria such as diphtheria, pertussis, and tetanus (DTaP), measles, mumps, rubella (MMR), varicella (chickenpox), hepatitis B, rotavirus, polio, and more. Private institutions might require annual influenza vaccination. The Center for Disease Control and Prevention has estimated that routine immunization of newborns prevents about 42,000 deaths and 20 million cases of disease each year, saving about $13.6 billion.[46]

Vaccination controversy

Main article: Vaccination schedule § United States

Despite their successes, in the United States there exists plenty of stigma surrounding vaccines that cause people to be incompletely vaccinated. These "gaps" in vaccination result in unnecessary infection, death, and costs.[47] There are two major reasons for incomplete vaccination:

1. Vaccines, like other medical treatments, have a risk of causing complications in some individuals (allergic reactions). Vaccines do not cause autism, as stated by national health agencies, such as the US Centers for Disease Control and Prevention,[48] the US Institute of Medicine,[49] and the UK National Health Service[50]
2. Low rates of vaccine-preventable disease, as a result of herd immunity, also make vaccines seem unnecessary and leave many unvaccinated.[51][52]

Although the American Academy of Pediatrics endorses universal immunization,[53] they note that physicians should respect parents' refusal to vaccinate their children after sufficient advising and provided the child does not face a significant risk of infection. Parents can also cite religious reasons to avoid public school vaccination mandates, but this reduces herd immunity and increases risk of viral infection.[45]

Limitations of vaccines

Vaccines boosts the body's immune system to better attack viruses in the "complete particle" stage, outside of the organism's cells. They traditionally consist of an attenuated (a live weakened) or inactivated (killed) version of the virus. These vaccines can, in very rare cases, harm the host by inadvertently infecting the host with a full-blown viral occupancy[*citation needed*]. Recently "subunit" vaccines have been devised that consist strictly of protein targets from the pathogen. They stimulate the immune system without doing serious harm to the host[*citation needed*]. In either case, when the real pathogen attacks the subject, the immune system responds to it quickly and blocks it.

Vaccines are very effective on stable viruses but are of limited use in treating a patient who has already been infected. They are also difficult to successfully deploy against rapidly mutating viruses, such as influenza (the vaccine for which is updated every year) and HIV. Antiviral drugs are particularly useful in these cases.

Antiretroviral therapy as HIV prevention

Following the HPTN 052 study and PARTNER study, there is significant evidence to demonstrate that antiretroviral drugs inhibit transmission when the HIV virus in the person living with HIV has been undetectable for 6 months or longer.

Public policy

Use and distribution

Guidelines regarding viral diagnoses and treatments change frequently and limit quality care.[54] Even when physicians diagnose older patients with influenza, use of antiviral treatment can be low.[55] Provider knowledge of antiviral therapies can improve patient care, especially in geriatric medicine. Furthermore, in local health departments (LHDs) with access to antivirals, guidelines may be unclear, causing delays in treatment.[56] With time-sensitive therapies, delays could lead to lack of treatment. Overall, national guidelines, regarding infection control and management, standardize care and improve healthcare worker and patient safety. Guidelines, such as those provided by the Centers for Disease Control and Prevention (CDC) during the 2009 flu pandemic caused by the H1N1 virus, recommend, among other things, antiviral treatment regimens, clinical assessment algorithms for coordination of care, and antiviral chemoprophylaxis guidelines for exposed persons. [57] Roles of pharmacists and pharmacies have also expanded to meet the needs of public during public health emergencies.[58]

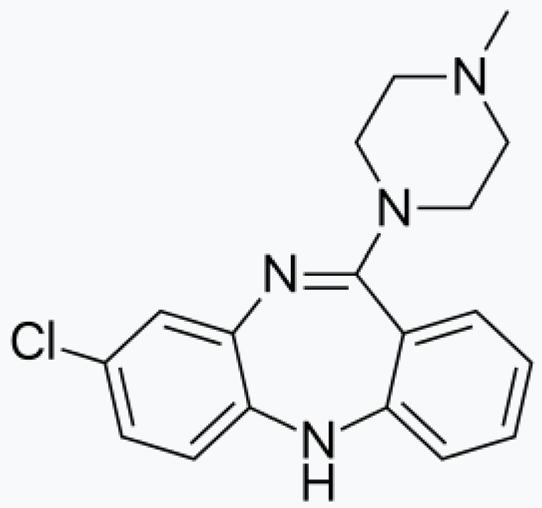
Stockpiling

Main article: Strategic National Stockpile

Public Health Emergency Preparedness initiatives are managed by the CDC via the Office of Public Health Preparedness and Response.[59] Funds aim to support communities in preparing for public health emergencies, including pandemic influenza. Also managed by the CDC, the Strategic National Stockpile (SNS) consists of bulk quantities of medicines and supplies for use during such emergencies.[60] Antiviral stockpiles prepare for shortages of antiviral medications in cases of public health emergencies. During the H1N1 pandemic in 2009–2010, guidelines for SNS use by local health departments was unclear, revealing gaps in antiviral planning.[56] For example, local health departments that received antivirals from the SNS did not have transparent guidance on the use of the treatments. The gap made it difficult to create plans and policies for their use and future availabilities, causing delays in treatment.

J. Atypical antipsychotic

Atypical antipsychotic
Drug class



Skeletal formula of clozapine, the first atypical antipsychotic

Synonyms	Second generation antipsychotic, serotonin–dopamine antagonist

The **atypical antipsychotics** (**AAP),** also known as **second generation antipsychotics** (**SGAs**) and **serotonin–dopamine antagonists** (**SDAs**),[1][2] are a group of antipsychotic drugs (antipsychotic drugs in general are also known as major tranquilizers and neuroleptics, although the latter is usually reserved for the *typical antipsychotics*) largely introduced after the 1970s and used to treat psychiatric conditions. Some atypical antipsychotics have received regulatory approval (e.g. by the FDA of the US, the TGA of Australia, the MHRA of the UK) for schizophrenia, bipolar disorder, autism, and as an adjunct in major depressive disorder.

Both generations of medication tend to block receptors in the brain's dopamine pathways. Atypicals are less likely than haloperidol — the most widely used typical antipsychotic — to cause extrapyramidal motor control disabilities in patients such as unsteady Parkinson's disease-type movements, body rigidity, and involuntary tremors. However, only a few of the atypicals have been demonstrated to be superior to lesser-used, low-potency first-generation antipsychotics in this regard.[3][4][5]

As experience with these agents has grown, several studies have questioned the utility of broadly characterizing antipsychotic drugs as "atypical/second generation" as opposed to "first generation," noting that each agent has its own efficacy and side-effect profile. It has been argued that a more nuanced view in which the needs of individual patients are matched to the properties of individual drugs is more appropriate.[4][3] Although atypical antipsychotics are thought to be safer than typical antipsychotics, they still have severe side effects, including tardive dyskinesia (a serious movement disorder), neuroleptic malignant syndrome, and increased risk of stroke, sudden cardiac death, blood clots, and diabetes. Significant weight gain may occur. Critics have argued that "the time has come to abandon the terms first-generation and second-generation antipsychotics, as they do not merit this distinction."[6]

Contents

Medical uses

Atypical antipsychotics are typically used to treat schizophrenia or bipolar disorder.[7] They are also frequently used to treat agitation associated with dementia, anxiety disorder, autism spectrum disorder, and obsessive-compulsive disorder (an off-label use).[8] In dementia, they should only be considered after other treatments have failed and if the patient is a risk to themselves and/or others.[9]

Schizophrenia

The first-line psychiatric treatment for schizophrenia is antipsychotic medication,[10] which can reduce the positive symptoms of schizophrenia in about 8–15 days. Antipsychotics only appear to improve secondary negative symptoms of schizophrenia in the short term and may worsen negative symptoms overall.[11] Overall there is no good evidence that atypical antipsychotics have any therapeutic benefit for treating the negative symptoms of schizophrenia.[12]

There is very little evidence on which to base a risk and benefit assessment of using antipsychotics for long-term treatment.[13]

The choice of which antipsychotic to use for a specific patient is based on benefits, risks, and costs.[14] It is debatable whether, as a class, typical or atypical antipsychotics are better.[15] Both have equal drop-out and symptom relapse rates when typicals are used at low to moderate dosages.[16] There is a good response in 40–50% of patients, a partial response in 30–40%, and treatment resistance (failure of symptoms to respond satisfactorily after six weeks to two of three different antipsychotics) in the remaining 20%.[17] Clozapine is considered a first choice treatment for treatment resistant schizophrenia, especially in the short term; in the longer-terms the risks of adverse effects complicate the choice.[18]

Efficacy in the treatment of schizophrenia

The utility of broadly grouping the antipsychotics into first generation and atypical categories has been challenged. It has been argued that a more nuanced view, matching the properties of individual drugs to the needs of specific patients is preferable.[3] While the atypical (second-generation) antipsychotics were marketed as offering greater efficacy in reducing psychotic symptoms while reducing side effects (and extrapyramidal symptoms in particular) than typical medications, the results showing these effects often lacked robustness, and the assumption was increasingly challenged even as atypical prescriptions were soaring.[19][20] In 2005 the US government body NIMH published the results of a major independent (not funded by the pharmaceutical companies) multi-site, double-blind study (the CATIE project).[21] This study compared several atypical antipsychotics to an older, mid-potency typical antipsychotic, perphenazine, among 1,493 persons with schizophrenia. The study found that only olanzapine outperformed perphenazine in discontinuation rate (the rate at which people stopped taking it due to its effects). The authors noted an apparent superior efficacy of olanzapine to the other drugs in terms of reduction in psychopathology and rate of hospitalizations, but olanzapine was associated with relatively severe metabolic effects such as a major weight gain problem (averaging 9.4 lbs over 18 months) and increases in glucose, cholesterol, and triglycerides. No other atypical studied (risperidone, quetiapine, and ziprasidone) did better than the typical perphenazine on the measures used, nor did they produce fewer adverse effects than the typical antipsychotic perphenazine (a result supported by a meta-analysis[3] by Leucht *et al*. published in *The Lancet*), although more patients discontinued perphenazine owing to extrapyramidal effects compared to the atypical agents (8% vs. 2% to 4%, P=0.002). A phase 2 part of this CATIE study roughly replicated these findings.[22] Compliance has not been shown to be different between the two types.[23] Overall evaluations of the CATIE and other studies have led many researchers to question the first-line prescribing of atypicals over typicals, or even to question the distinction between the two classes.[24][25][26]

It has been suggested that there is no validity to the term "second-generation antipsychotic drugs" and that the drugs that currently occupy this category are not identical to each other in mechanism, efficacy, and side-effect profiles. [27]

Bipolar disorder

In bipolar disorder, SGAs are most commonly used to rapidly control acute mania and mixed episodes, often in conjunction with mood stabilizers (which tend to have a delayed onset of action in such cases) such as lithium and valproate. In milder cases of mania or mixed episodes, mood stabilizer monotherapy may be attempted first.[28] SGAs are also used to treat other aspects of the disorder (such as acute bipolar depression or as a prophylactic treatment) as adjuncts or as a monotherapy, depending on the drug. Both quetiapine and olanzapine have demonstrated significant efficacy in all three treatment phases of bipolar disorder. Lurasidone (trade name Latuda) has demonstrated some efficacy in the acute depressive phase of bipolar disorder.[28][29][30]

Major depressive disorder

In *non-psychotic* major depressive disorder (MDD), some SGAs have demonstrated significant efficacy as adjunctive agents; and, such agents include:[31][32][33][34]

- Aripiprazole
- Brexpiprazole
- Olanzapine
- Quetiapine
- Ziprasidone[35]

whereas only quetiapine has demonstrated efficacy as a monotherapy in non-psychotic MDD.[36] Olanzapine/fluoxetine is an efficacious treatment in both *psychotic* and *non-psychotic* MDD.[37][38]

Aripiprazole, brexpiprazole, olanzapine, and quetiapine have been approved as adjunct treatment for MDD by the FDA in the United States.[39][40] Quetiapine and lurasidone have been approved, as monotherapies, for bipolar depression, but as of present, lurasidone has not been approved for MDD.[39]

Autism

Both risperidone and aripiprazole have received FDA labelling for autism.[37]

Dementia and Alzheimer's disease

Between May 2007 and April 2008, Dementia and Alzheimer's together accounted for 28% of atypical antipsychotic use in patients aged 65 or older.[41] The U.S. Food and Drug Administration requires that all atypical antipsychotics carry a black box warning that the medication has been associated with an increased risk of mortality in elderly patients.[41] In 2005, the FDA issued an advisory warning of an increased risk of death when atypical antipsychotics are used in dementia.[42] In the subsequent 5 years, the use of atypical antipsychotics to treat dementia decreased by nearly 50%.[42]

Adverse effects

The side effects reportedly associated with the various atypical antipsychotics vary and are medication-specific. Generally speaking, atypical antipsychotics are widely believed to have a lower likelihood for the development of tardive dyskinesia than the typical antipsychotics. However, tardive dyskinesia typically develops after long-term (possibly decades) use of antipsychotics. It is not clear if atypical antipsychotics, having been in use for a relatively short time, produce a lower incidence of tardive dyskinesia.[28][48]

Some of the other side effects that have been suggested is that atypical antipsychotics increase the risk of cardiovascular disease.[49] The research that Kabinoff et al. found that the increase in cardiovascular disease is seen regardless of the treatment they receive, instead it is caused by many different factors such as lifestyle or diet.[49]

Sexual side effects have also been reported when taking atypical antipsychotics.[50] In males antipsychotics reduce sexual interest, impair sexual performance with the main difficulties being failure to ejaculate.[51] In females there may be abnormal menstrual cycles and infertility.[52] In both males and females the breasts may become enlarged and a fluid will sometimes ooze from the nipples.[51] Sexual adverse effects caused by some anti-psychotics are a result of an increase of prolactin. Sulpiride and Amisulpiride, as well as Risperdone and paliperidone (to a lesser extent) cause a high increase of prolactin.

In April 2005, the US Food and Drug Administration (FDA) issued an advisory and subsequent black box warning regarding the risks of atypical anti psychotic use among elderly patients with dementia. The FDA advisory was associated with decreases in the use of atypical antipsychotics, especially among elderly patients with dementia.[53] Subsequent research reports confirmed the mortality risks associated with the use of both conventional and atypical antipsychotics to treat patients with dementia. Consequently, in 2008 the FDA issued although a black box warning for classical neuroleptics. Data on treatment efficacies are strongest for atypical antipsychotics. Adverse effects in patients with dementia include an increased risk of mortality and cerebrovascular events, as well as metabolic effects, extrapyramidal symptoms, falls, cognitive worsening, cardiac arrhythmia, and pneumonia.[54] Conventional antipsychotics may pose an even greater safety risk. No clear efficacy evidence exists to support the use of alternative psychotropic classes (e.g. antidepressants, anticonvulsants).[55]

Atypical antipsychotics may also cause anhedonia.[56]

Drug-induced OCD

Main article: Obsessive–compulsive disorder § Drug-induced OCD

Many different types of medication can create/induce pure OCD in patients that have never had symptoms before. A new chapter about OCD in the DSM-5 (2013) now specifically includes drug-induced OCD.

Atypical antipsychotics (second generation antipsychotics), such as olanzapine (Zyprexa), have been proven to induce de-novo OCD in patients.[57][58][59][60]

Tardive dyskinesia

All of the atypical antipsychotics warn about the possibility of tardive dyskinesia in their package inserts and in the PDR. It is not possible to truly know the risks of tardive dyskinesia when taking atypicals, because tardive dyskinesia can take many decades to develop and the atypical antipsychotics are not old enough to have been tested over a long enough period of time to determine all of the long-term risks. One hypothesis as to why atypicals have a lower risk of tardive dyskinesia is because they are much less fat-soluble than the typical antipsychotics and because they are readily released from D2 receptor and brain tissue.[61] The typical antipsychotics remain attached to the D2 receptors and accumulate in the brain tissue which may lead to TD.[61]

Both typical and atypical antipsychotics can cause tardive dyskinesia.[62] According to one study, rates are lower with the atypicals at 3.9% per year as opposed to the typicals at 5.5% per year.[62]

Metabolism

Recently, metabolic concerns have been of grave concern to clinicians, patients and the FDA. In 2003, the Food and Drug Administration (FDA) required all manufacturers of atypical antipsychotics to change their labeling to include a warning about the risks of hyperglycemia and diabetes with atypical antipsychotics. It must also be pointed out that although all atypicals must carry the warning on their labeling, some evidence shows that atypicals are not equal in their effects on weight and insulin sensitivity.[63] The general consensus is that clozapine and olanzapine are associated with the greatest effects on weight gain and decreased insulin sensitivity, followed by risperidone and quetiapine.[63] Ziprasidone and aripiprazole are thought to have the smallest effects on weight and insulin resistance, but clinical experience with these newer agents is not as developed as that with the older agents.[63] The mechanism of these adverse effects is not completely understood but it is believed to result from a complex interaction between a number of pharmacologic actions of these drugs. Their effects on weight are believed to mostly derive from their actions on the H_1 and 5-HT_{2C} receptors, while their effects on insulin sensitivity are believed to be the result of a combination of their effects on body weight (as increased body mass is known to be a risk factor for insulin resistance) and their antagonistic effects on the M_3receptor. Some of the newer agents, however, such as risperidone and its metabolite paliperidone, ziprasidone, lurasidone, aripiprazole, asenapine and iloperidone have clinically-insignificant effects on the M_3 receptor and appear to carry a lower risk of insulin resistance. Whereas clozapine, olanzapine and quetiapine (indirectly via its active metabolite, norquetiapine) all antagonise the M_3 receptor at therapeutic-relevant concentrations.[64]

Recent evidence suggests a role of the α_1 adrenoceptor and 5-HT_{2A} receptor in the metabolic effects of atypical antipsychotics. The 5-HT_{2A} receptor, however, is also believed to play a crucial role in the therapeutic advantages of atypical antipsychotics over their predecessors, the typical antipsychotics.[65]

A study by Sernyak and colleagues found that the prevalence of diabetes in atypical antipsychotic treatments was statistically significantly higher than that of conventional treatment.[49] The authors of this study suggest that it is a causal relationship the Kabinoff et al. suggest the findings only suggest a temporal association.[49] Kabinoff et al. suggest that there is insufficient data from large studies to demonstrate a consistent or significant difference in the risk of insulin resistance during treatment with various atypical antipsychotics.[49]

Comparison table of adverse effects

Comparison of side effects for atypical antipsychotics

Generic Name	**Weight gain**	**Metabolic Effects**	**EPS**	**High prolactin**	**Sedation**	**Hypotension / Orthostasis**	**QTc**
Amisulpride	+	+	+	++	-	-	+++
Aripiprazole	0-10%[66]	0-10%[66]	10-20%[66]	-[66]	10-20%[66]	0-10%[66]	-
Asenapine	0-10%[66]	20%[66]	0-10%[66]	0-10%[66]	10-20%[66]	0-10%[66]	+
Blonanserin	+/-	-	++	+	+/-	-	+
Clozapine	20-30%[66]	0-15%[66]	-[66]	-[66]	>30%[66]	20-30%[66]	+
Iloperidone	0-10%[66]	0-10%[66]	0-10%[66]	-[66]	10-20%[66]	0-10%[66]	++
Lurasidone	-[66]	-[66]	>30%[66]	-[66]	20-30%[66]	-[66]	+
Melperone	+	+	+/-	-	+/++	+/++	++

Olanzapine	20-30%[66]	0-15%[66]	20-30%[66]	20-30%[66]	>30%[66]	0-10%[66]	+
Paliperidone	0-10%[66]	-[66]	10-20%[66]	>30%[66]	20-30%[66]	0-10%[66]	+/- (
Perospirone	?	?	>30%[68]	+	+	+	?
Quetiapine	20-30%[66]	0-15%[66]	10-20%[66]	-[66]	>30%[66]	0-10%[66]	++
Remoxipride[69]	+/-	-	-	-[61]	-	+/-	?
Risperidone	10-20%[66]	0-10%[66]	20-30%[66]	>30%[66]	>30%[66]	0-10%[66]	+
Sertindole	++	+/-	-	++	-	+++	+++
Sulpiride	+	+	+	+++	-	+++	+
Ziprasidone	0-10%[66]	0-10%[66]	0-10%[66]	-[66]	20-30%[66]	0-10%[66]	++

Discontinuation

The British National Formulary recommends a gradual withdrawal when discontinuing antipsychotics to avoid acute withdrawal syndrome or rapid relapse.[71] Symptoms of withdrawal commonly include nausea, vomiting, and loss of appetite.[72] Other symptoms may include restlessness, increased sweating, and trouble sleeping.[72] Less commonly there may be a feeling of the world spinning, numbness, or muscle pains.[72] Symptoms generally resolve after a short period of time.[72]

There is tentative evidence that discontinuation of antipsychotics can result in psychosis.[73] It may also result in reoccurrence of the condition that is being treated.[74] Rarely tardive dyskinesia can occur when the medication is stopped.[72]

Pharmacology

The atypical antipsychotics integrate with the serotonin (5-HT), norepinephrine (α, β), and dopamine (D) receptors in order to effectively treat schizophrenia.

D_2 Receptor: Hyperactive dopaminergic activity on D_2 receptors in the mesolimbic pathway is responsible for the positive symptoms of schizophrenia (hallucinations, delusions, paranoia). After taking an antipsychotic, antagonism of D_2 receptors occurs throughout the entire brain, leading to a number of deleterious side effects from D_2 receptor antagonism throughout the entire dopamine pathway system. Unfortunately, it's not possible to affect D_2 receptors only in the mesolimbic pathway.[75][Stahl AP Explained 1 - 1] Fortunately, 5-HT_{2A} receptor antagonism reverses these side effects to some extent.[Stahl AP Explained 1 - 2] Reducing D_2 dopaminergic activity in the mesolimbic pathway also results in an anhedonic effect, reducing pleasure, motivation, and the salience of one's life experience. In the mesocortical pathway to the DLPFC and VMPFC, endogenous D_2 receptor dopamine activity is sometimes low in schizophrenia, resulting in cognitive, affective, and, broadly, the negative symptoms of schizophrenia. D_2 receptor antagonism here further compounds these problems. In the nigrostratial pathway, D_2 receptor antagonism results in extrapyramidal symptoms. If this antagonism occurs long enough, symptoms of EPS may become permanent, even if antipsychotic use is discontinued. In the tuberoinfundibular pathway, D_2 receptor antagonism results in elevated prolactin. If prolactin levels become high enough, hyperprolactinaemia may occur, resulting in sexual dysfunction, weight gain, more rapid demineralization of bones, and possibly galactorrhea and amenorrhea.[Stahl AP Explained 1 - 1]

5-HT_{2A} Receptor: When serotonin is released on to postsynaptic 5-HT_{2A} receptors, the dopamine neuron is inhibited, thus acting as a brake on dopamine release.[Stahl AP Explained 1 - 2] This brake is disrupted through action of a 5-HT_{2A} antagonist, which disinhibits the dopamine neuron, stimulating dopamine release. The result of this is that dopamine competes with antipsychotic D_2 antagonistic action at D_2 receptors, thereby reducing antagonistic binding there and eliminating or lowering D_2 antagonistic effects in several pathways of the dopamine system.[Stahl AP Explained 1 - 2] In the nigrostratial pathway, it reduces EPS. In the tuberoinfundibular pathway, it reduces or eliminates prolactin elevation.[Stahl AP Explained 1 - 3] Dopamine release in the mesolimbic pathway from 5-HT_{2A} antagonism does not appear to be as robust as in the other pathways of the dopamine system, thereby accounting for why atypical antipsychotics still retain part of their efficacy against the positive symptoms of schizophrenia through their D_2 antagonism.[Stahl AP Explained 1 - 3] When 5-HT_{2A} antagonistic agent particles occupy 5-HT_{2A} receptors in the mesocortical pathway and in the prefrontal cortex, the negative symptoms of schizophrenia, affective symptoms, and cognitive deficits and abnormalities are treated and reduced.[Stahl AP Explained 1 - 3] Furthermore, 5-HT_{2A} receptor antagonism blocks the serotonergic excitation of cortical pyramidal cells, reducing glutamate release, which in turn lowers hyperactive dopaminergic D_2 receptor activity in the mesolimbic pathway, reducing or eliminating the positive symptoms of schizophrenia.[Stahl AP Explained 1 - 3][76][77]

Some effects of 5-HT_{1A} receptor activation include decreased aggressive behavior/ideation,[78] increased sociability, and decreased anxiety and depression.[*non-primary source needed*] 5-HT_{2C} activation blocks dopamine and inhibits norepinephrine release. Blockade of the 5-HT_{2C} receptor increases serotonin, releasing norepinephrine and dopamine within the brain.[75] But neuronal reuptake of norepinephrine is limited sharply by some antipsychotics, for example ziprasidone. Increased norepinephrine can cause increased glucose levels, which is to say blood sugar levels.[79][80][81] Increased blood sugar levels by increased norepinephrine causes hunger in many humans, which is why weight gain occurs with some antipsychotics if the norepinephrine is not inhibited.[82][83][84][85][86] Inhibition of norepinephrine stabilizes mood in humans.[87] 5-HT_6 receptor antagonists improve cognition, learning, and memory.[88] The 5-HT_7 receptor is very potent for the mitigation of bipolar conditions and also yields an antidepressant effect. The antipsychotics asenapine,[89] lurasidone,[90][91] risperidone,[92] and aripiprazole[93] are very potent at the 5-HT_7 receptor. Antagonistic affinity for the H_1 receptor also has an antidepressant effect. H_1 antagonism blocks serotonin and norepinephrine reuptake. Patients with increased histamine levels have been observed to have lower serotonin levels.[94] However, the H_1 receptor is linked to weight gain. To have partial agonism at the 5-HT_{1A} receptor can yield absence of weight gain in an antipsychotic. This is very relevant for ziprasidone,[95][96] but it creates a risk for a prolonged QTc interval.[97][98] On the other hand, blockade of the 5-HT_3 receptor removes the risk for a prolonged QTc interval,[90] but then creates a larger risk for weight gain. Relation to the 5-HT_3 receptor increases caloric uptake and glucose,[99] which is seen in clozapine and olanzapine.[100][101] Other ways for dopamine to resolve is to have agonism at both the D_2 receptor and 5-HT_{1A} receptor, which normalizes the dopamine level in the brain. This occurs with haloperidol and aripiprazole.

Whether the anhedonic, loss of pleasure and motivation effect resulting from dopamine insufficiency or blockade at D_2 receptors in the mesolimbic pathway, which is mediated in some part by antipsychotics (and despite dopamine release in the mesocortical pathway from 5-HT_{2A} antagonism, which is seen in atypical antipsychotics), or the positive mood, mood stabilization, and cognitive improvement effect resulting from atypical antipsychotic serotonergic activity is greater for the overall quality of life effect of an atypical antipsychotic is a question that is variable between individual experience and the atypical antipsychotic(s) being used.[75]

Terms

Inhibition. Disinhibition: The opposite process of inhibition, the turning on of a biological function. Release: Causes the appropriate neurotransmitters to be discharged in vesicles into the synapse where they attempt to bind to and activate a receptor. Downregulation and Upregulation.

Binding profile

Note: Unless otherwise specified, the drugs below serve as antagonists/inverse agonists at the receptors listed.

Generic Name[102]	D_1	D_2	D_3	D_4	5-HT_{1A}	5-HT_{1B}	5-HT_{2A}	5-HT_{2C}	5-HT_6	5-HT_7	α_1	α_2	M_1	M_3	H_1
Amisulpride	-	++++	++++	-	-	-	-	-	-	++/+	-	+/-	-	-	-
Aripiprazole	+	++++ (PA)	+++ (PA)	+ (PA)	+++ (PA)	+	+++	++ (PA)	+	+++ (PA)	++/+	+	-	-	++/+
Asenapine	+++	+++	++++	+++	+++ (PA)	+++	++++	++++	++++	++++	+++	+++	-	-	+++
Blonanserin	-	++++	++++	+	-	?	+++	+	+	+/-	+ (RC)	+ (RC)	+	?	-
Cariprazine		++++ (PA)	+++++ (PA)		++++ (PA)		+++	++		++	++		-	-	+++
Clozapine	++	++	++	+++	++ (PA)	++/+	++++	++++	+++	+++	++++	+++	++++	+++	++++
Iloperidone	+	+++	+++	++	+ (PA)	+	+++	+	++	+	++++	+++/++	-	-	+++
Lurasidone	+	++++	++	++	+++ (PA)	?	++++	+/-	?	++++	-	+++/++	-	-	-

Melperone	?	++	++++	++	+(PA)	?	++	+	-	++	++	++	-	-	++
Olanzapine	+++	+++	+++	+++	+(PA)	++	++++	+++	+++	++	++	++	++++	++++	++++
Paliperidone	++	+++	+++	++	+(PA)	+++/++	++++	+	-	++++/+++	+++	+++	-	-	+++/++
Quetiapine	+	++/+	++/+	+	++/+(PA)	+	+	+	++	+++/++	++++	+++/++	++	+++	++++
Risperidone	+	+++	++	+++	+(PA)	++	++++	++	-	+++/++	+++/++	++	-	-	++
Sertindole	?	+++	+++	+++	++/+(PA)	++	++++	++++	+++	++	++++/+++	+	-	-	++/+
Sulpiride	?	++++	++++	+++	-	-	-	-	-	-	-	-	-	-	-
Ziprasidone	+++/++	+++	+++	+++/++	+++(PA)	+++(PA)	++++	+++(PA)	++	+++	+++/++	++	-	-	++
Zotepine	+++/++	+++	++++/+++	+++	++(PA)	+++	++++	++++(RC)	++++	++++/+++	+++	+++/++	++(RC)	++(RC)	++++

Legend:

No Affinity or No Data

- Clinically Insignificant

+	Low
++	Moderate
+++	High
++++	Very High
+++++	Exceptionally High
PA	Partial Agonist
RC	Cloned Rat Receptor

Pharmacokinetics

Atypical antipsychotics are most commonly administered orally.[51] Antipsychotics can also be injected, but this method is not as common.[51] They are lipid-soluble, are readily absorbed from the digestive tract, and can easily pass the blood–brain barrier and placental barriers.[51] Once in the brain, the antipsychotics work at the synapse by binding to the receptor.[103] Antipsychotics are completely metabolized in the body and the metabolites are excreted in urine.[104] These drugs have relatively long half-lives.[51] Each drug has a different half-life, but the occupancy of the D2 receptor falls off within 24 hours with atypical antipsychotics, while lasting over 24 hours for the typical antipsychotics.[61] This may explain why relapse into psychosis happens quicker with atypical antipsychotics than with typical antipsychotics, as the drug is excreted faster and is no longer working in the brain.[61] Physical dependence with these drugs is very rare.[51] However, if the drug is abruptly discontinued, psychotic symptoms, movement disorders, and sleep difficulty may be observed.[51] It is possible that withdrawal is rarely seen because the AAP are stored in body fat tissues and slowly released.

HistoryThe first major tranquilizer or antipsychotic medication, chlorpromazine (Thorazine), a typical antipsychotic, was discovered in 1951 and introduced into clinical practice shortly thereafter. Clozapine (Clozaril), an atypical antipsychotic, fell out of favor due to concerns over drug-induced agranulocytosis. Following research indicating its effectiveness in treatment-resistant schizophrenia and the development of an adverse event monitoring system, clozapine re-emerged as a viable antipsychotic. According to Barker (2003), the three most-accepted atypical drugs are clozapine, risperidone, and olanzapine. However, he goes on to explain that clozapine is usually the last resort when other drugs fail. Clozapine can cause agranulocytosis (a decreased number of white blood cells), requiring blood monitoring for the patient. Despite the effectiveness of clozapine for treatment-resistant schizophrenia, agents with a more favorable side-effect profile were sought-after for widespread use. During the 1990s, olanzapine, risperidone, and quetiapine were introduced, with ziprasidone and aripiprazole following in the early 2000s. The atypical anti-psychotic paliperidone was approved by the FDA in late 2006.[*citation needed*]

The atypical antipsychotics have found favor among clinicians and are now considered to be first-line treatments for schizophrenia and are gradually replacing the typical antipsychotics. In the past, most researchers have agreed that the defining characteristics of atypical antipsychotics are the decreased incidence of extrapyramidal side effects (EPS)[123] and an absence of sustained prolactin elevation.[61]

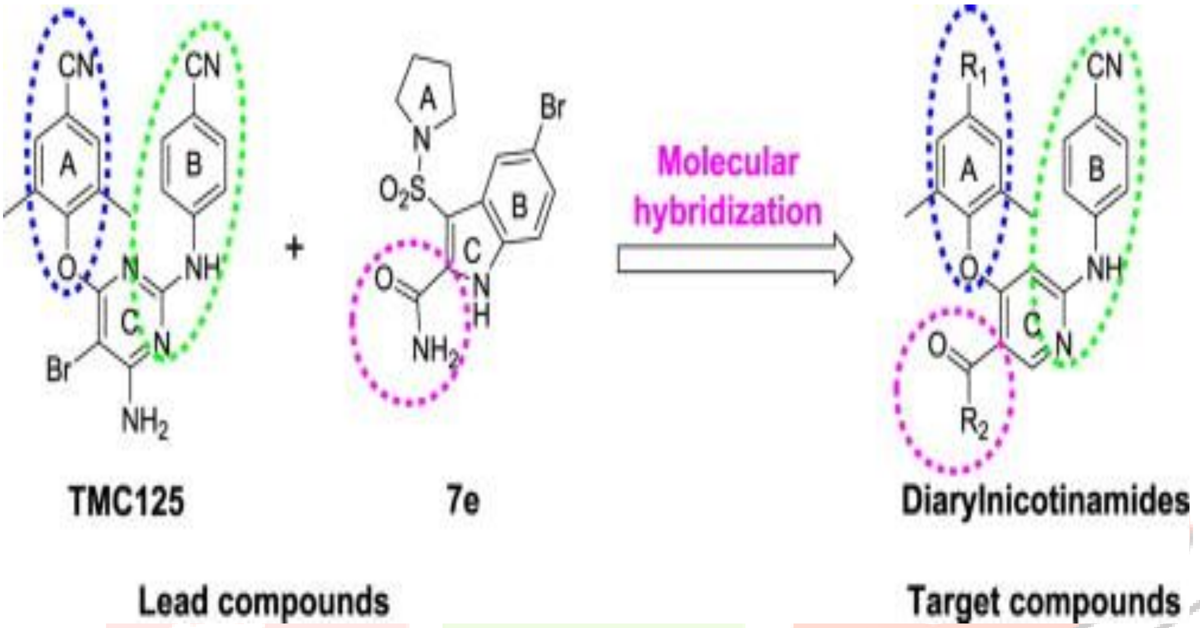
The terminology can still be imprecise. The definition of "atypicality" was based upon the absence of extrapyramidal side effects, but there is now a clear understanding that atypical antipsychotics can still induce these effects (though to a lesser degree than typical antipsychotics).[124] Recent literature focuses more upon specific pharmacological actions and less upon categorization of an agent as "typical" or "atypical". There is no clear dividing line between the typical and atypical antipsychotics therefore categorization based on the action is difficult.[61]

More recent research is questioning the notion that second-generation antipsychotics are superior to first generation typical anti-psychotics. Using a number of parameters to assess quality of life, Manchester University researchers found that typical antipsychotics were no worse than atypical antipsychotics. The research was funded by the National Health Service (NHS) of the UK.[125] Because each medication (whether first or second generation) has its own profile of desirable and adverse effects, a neuropsychopharmacologist may recommend one of the older ("typical" or first generation) or newer ("atypical" or second generation) antipsychotics alone or in combination with other medications, based on the symptom profile, response pattern, and adverse effects history of the individual patient.

Society and culture

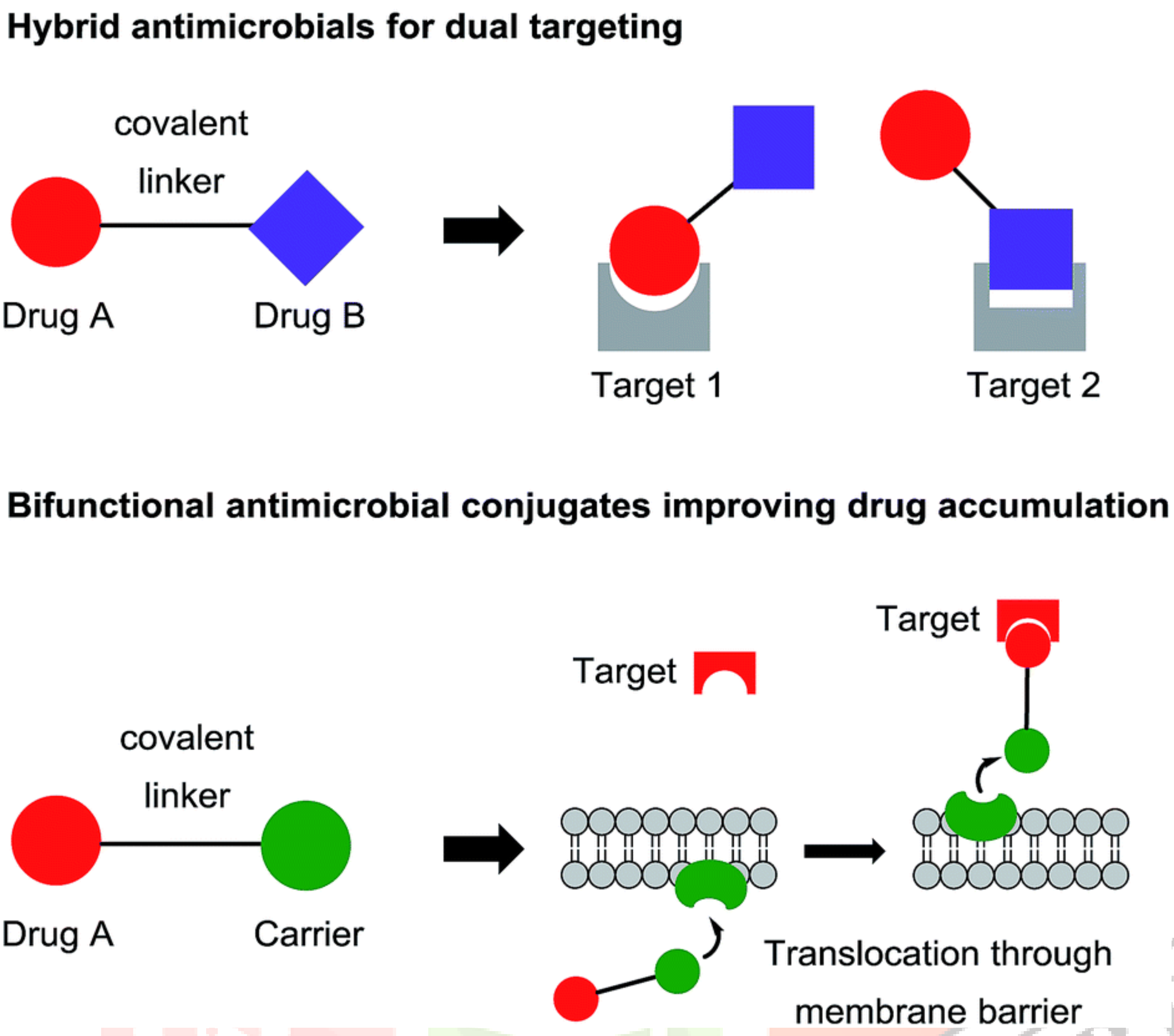
Between May 2007 and April 2008, 5.5 million Americans filled at least one prescription for an atypical antipsychotic.[41] In patients under the age of 65, 71% of patients were prescribed an atypical antipsychotic to treat Schizophrenia or Bipolar Disorder where this dropped to 38% in patients aged 65 or above.[41]

G1 .Anti HIV revolution

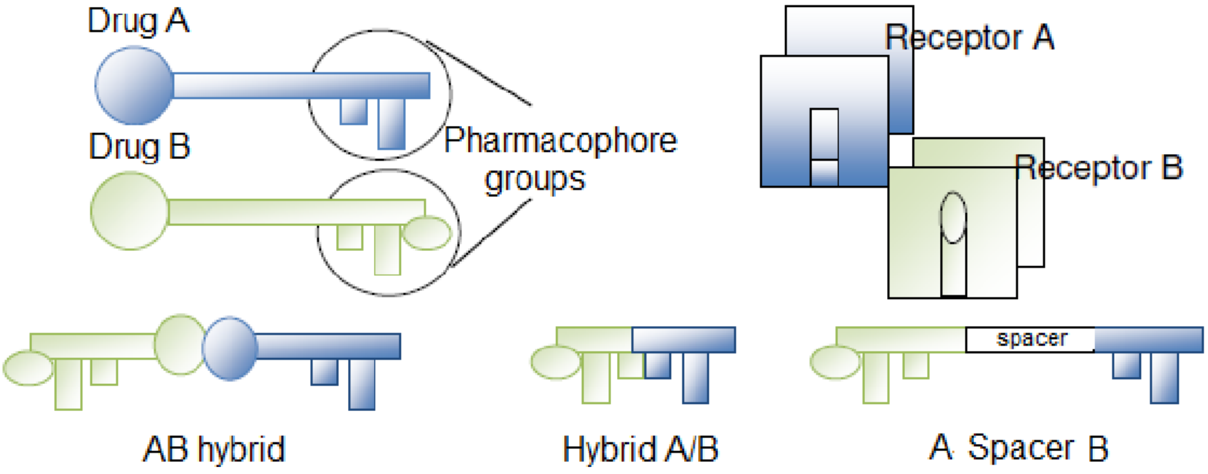


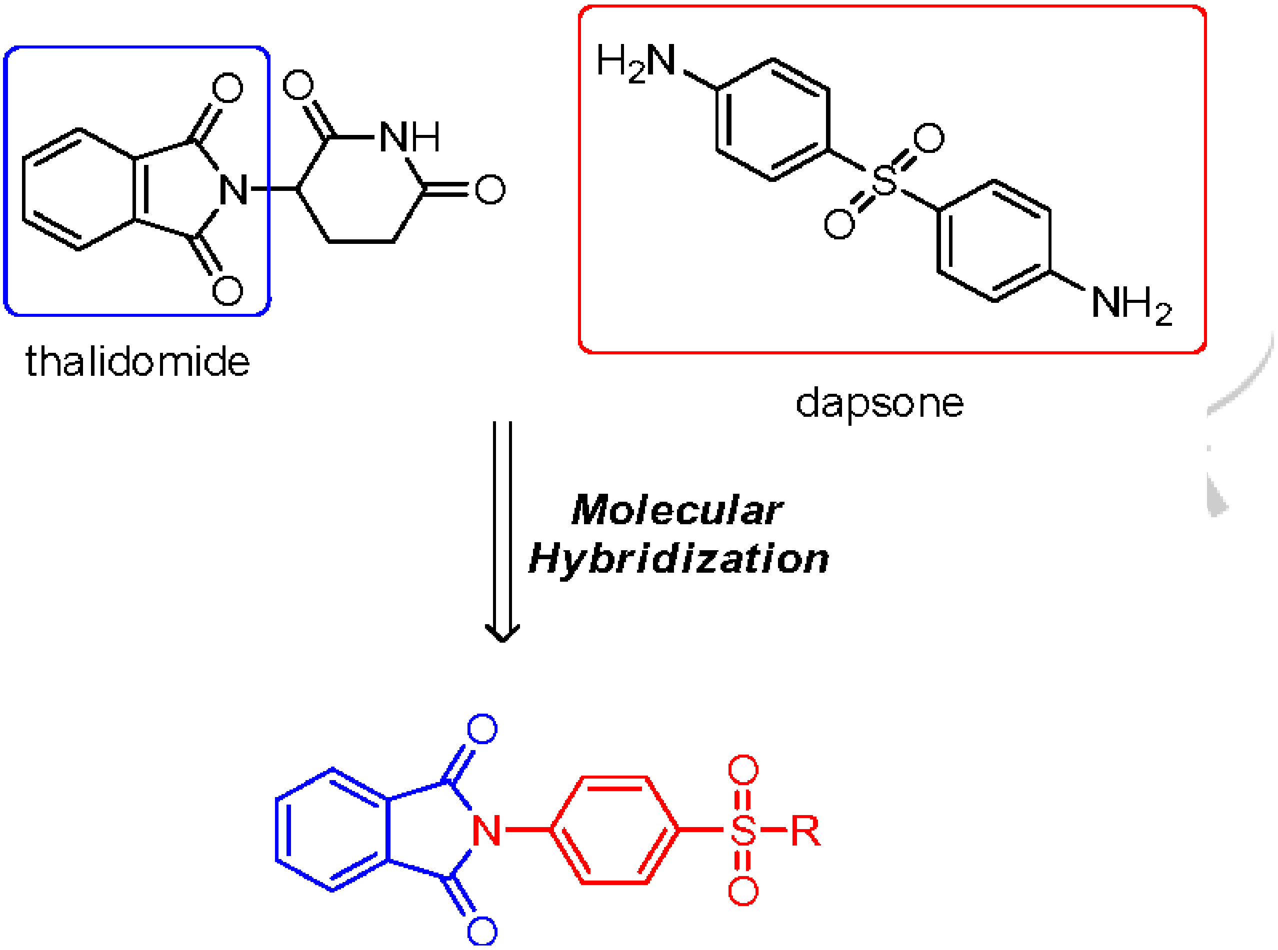
G2.Antimicrobial drug development

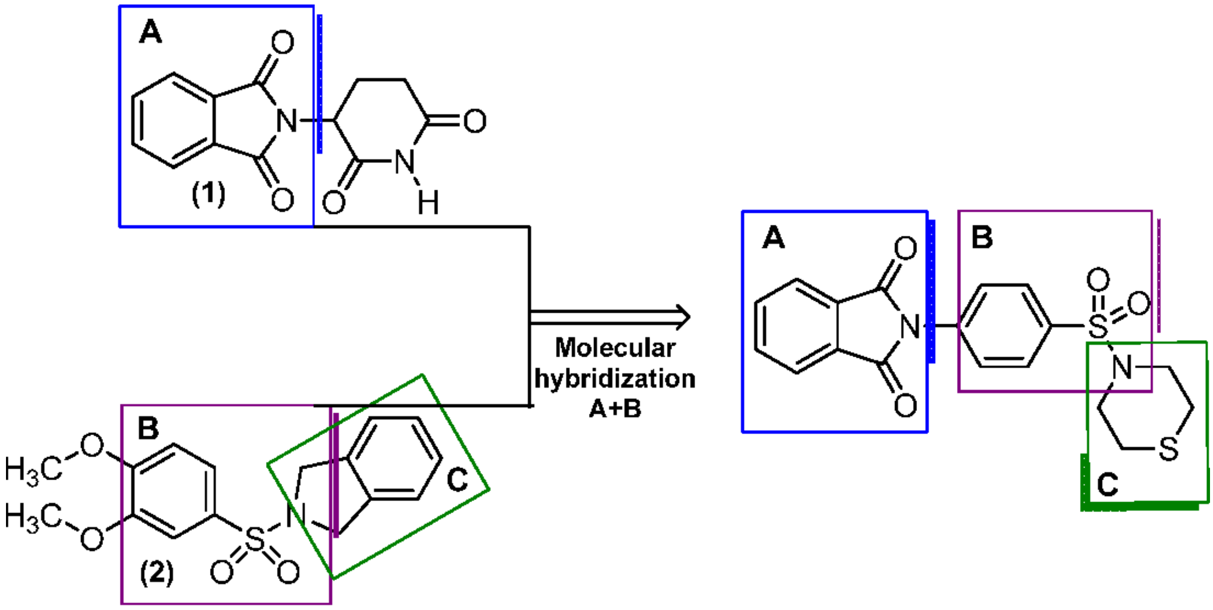
Hybridization approach in antimicrobial drug development

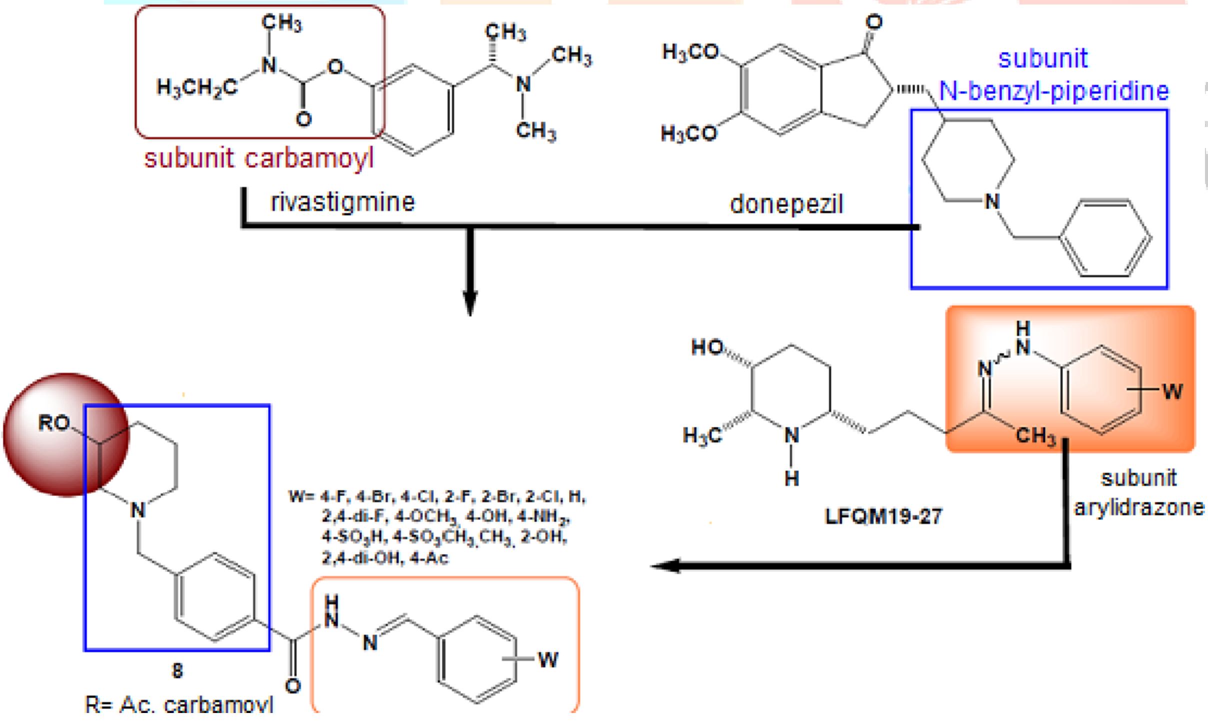


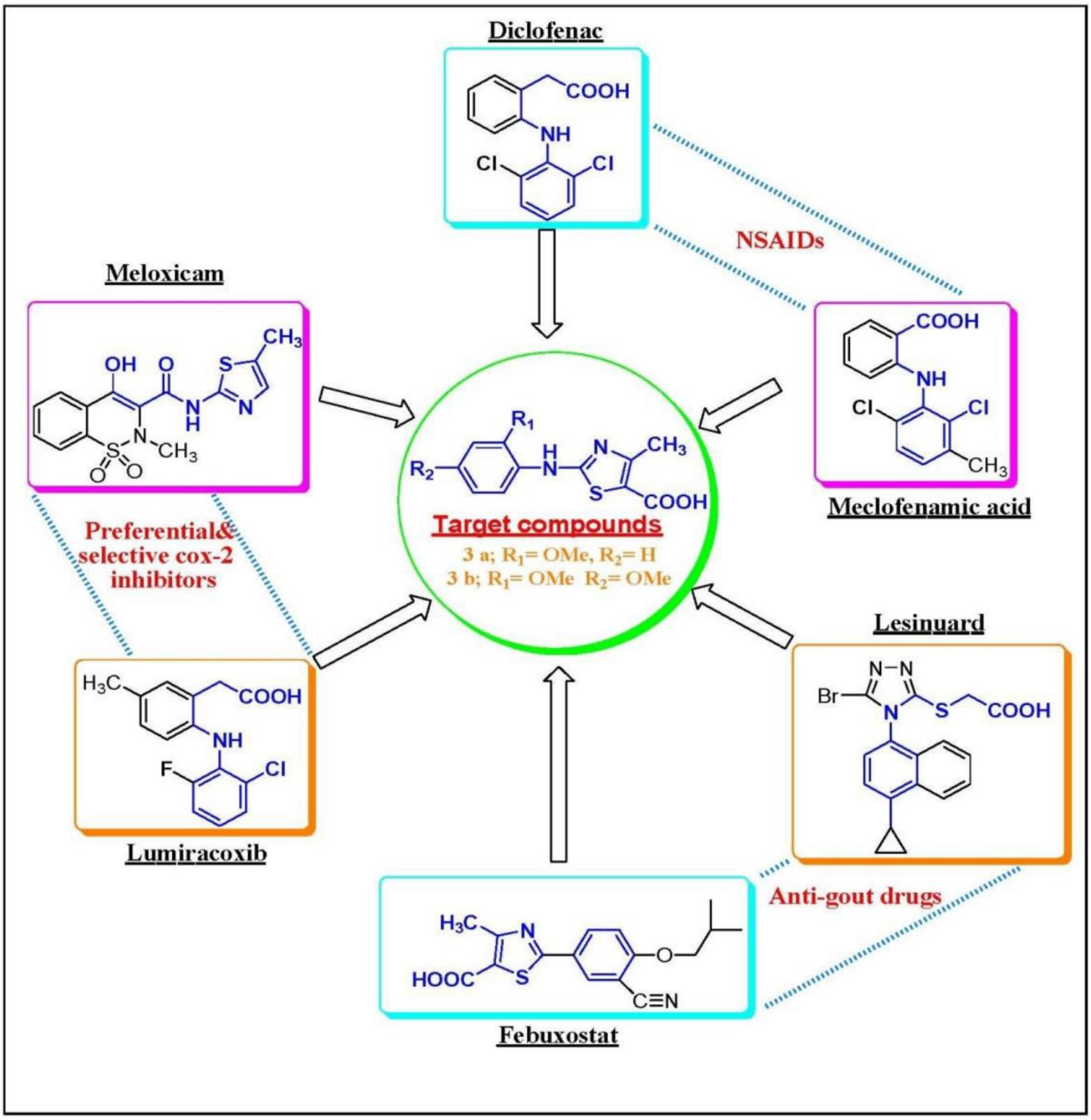
G3.ANTI-INFLAMMATORY Drug Design:

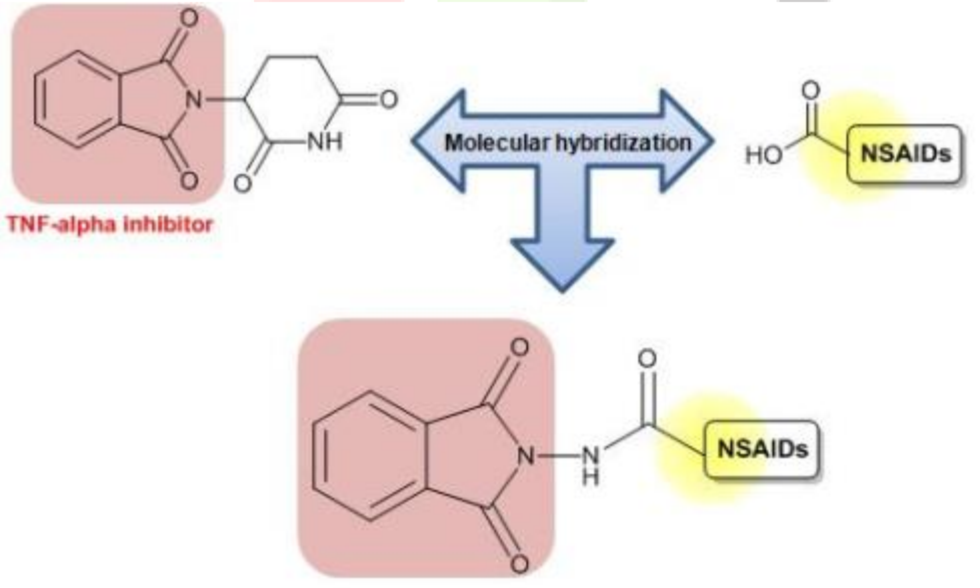

Drug A
Drug B
Pharmacophore groups
Receptor A
Receptor B
spacer
AB hybrid
Hybrid A/B
A Spacer B


O
O
O
O
N
NH
thalidomide
H$_2$N
S
O
O
NH$_2$
dapsone
Molecular Hybridization
O
O
N
O
O
S
R


A
(1)
B
C
(2)
H_3C
Molecular
hybridization
A+B
A
B
C

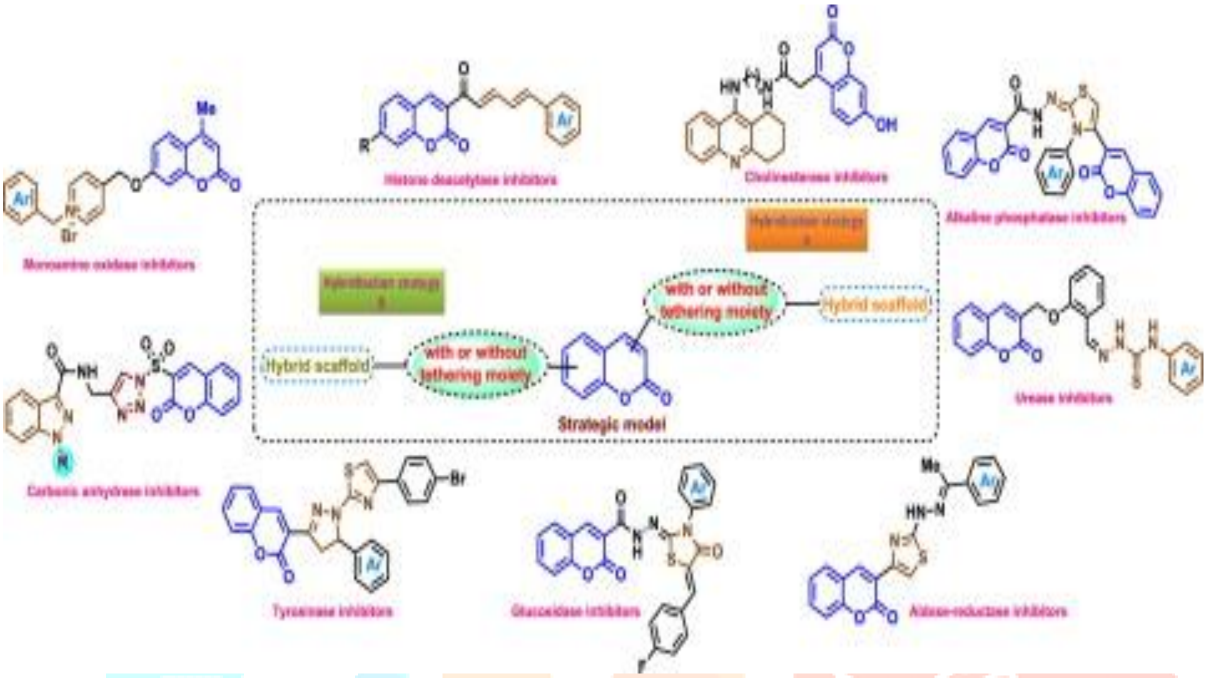

subunit carbamoyl
rivastigmine
donepezil
subunit
N-benzyl-piperidine
LFQM19-27
subunit
arylidrazone
W= 4-F, 4-Br, 4-Cl, 2-F, 2-Br, 2-Cl, H, 2,4-di-F, 4-OCH3, 4-OH, 4-NH2, 4-SO3H, 4-SO3CH3 CH3, 2-OH, 2,4-di-OH, 4-Ac
8
R= Ac, carbamoyl





NSAID with COX and TNF-alpha inhibition properties

G4. Therapeutic Drug Design:



K. SIMILAR DESIGN DISEASE AGENTS

Just like as discussed in the previous section for example CARDIOACTIVE AGENTS,

ANTI-TUMORAL AGENTS we can similarly develop the design the drug structure like

ANALGESIC, ANTI-INFLAMMATORY AND ANTITHRO-MBOTIC AGENTS ANTI HIV revolution Antimicrobial drug THERAPUTIC Drug

ANTI-INFECTIOUS AGENTS, ANTIDIABETIC AND NEUROACTIVE AGENTS etc.

L.Subcategories

Actually all the following Drugs sub categories may be designed and synthesised by the application of molecular hybridization approach

This category has the following 11 subcategories, out of 11 total.

A

- ▶ Anti-tuberculosis drugs (30 P)
- ▶ Antibiotic resistance (2 C, 29 P)
- ▶ Antileprotic drugs (13 P)

C

- ▶ Cell envelope antibiotics (4 C)
- ▶ Combination antibiotics (22 P)

E

- ▶ Eukaryotic selection compounds (10 P)

N

- ▶ Nucleic acid inhibitor antibiotics (6 C, 2 P)

P

- ▶ Protein synthesis inhibitor antibiotics (6 C, 7 P)

S

- ▶ Steroid antibiotics (1 P)

T

- ▶ Thiopeptides (5 P)

Σ

- ▶ Antibiotic stubs (244 P)

Pages in category "Anti-tuberculosis drugs"

The following pages are in this category, out of 30 total. This list may not reflect recent changes .

A

- Amikacin

B

- Bedaquiline
- User:BrianF4/sandbox

C

- Capreomycin
- Cycloserine

D

- Delamanid
- Diarylquinoline

E

- Ethambutol
- Ethambutol/isoniazid
- Ethionamide

F

- Furonazide

G

- Gatifloxacin

I

- Isoniazid

K

- Kanamycin A

L

- Linezolid

M

- Methaniazide
- Methaniazide/thioacetazone
- Moxifloxacin

P

- Pretomanid
- Prothionamide
- Pyrazinamide

R

- Rifabutin
- Rifalazil
- Rifampicin

- Rifampicin/isoniazid/pyrazinamide

S

- SQ109
- Streptomycin

T

- Terizidone
- Thioacetazone
- *Tuberculomucin Weleminsky*

Pages in category "Antibiotics"

The following pages are in this category, out of approximately 240 total. This list may not reflect recent changes
.

- Antibiotic

.

- Template:Use of antimicrobials

A

- A22 (antibiotic)
- A23187
- Acetic acid/hydrocortisone
- Acetoxycycloheximide
- Aciduliprofundum
- Actaplanin
- Actinorhodin
- Acyldepsipeptide antibiotics
- Agglomerin
- Alazopeptin
- Albomycin
- Allicin
- Allyl isothiocyanate
- Althiomycin
- Ambazone
- Aminocoumarin
- 4-Aminosalicylic acid
- Animal Drug Availability Act 1996
- Anthramycin
- Antibacterial soap

- Antibiosis
- Antibiotic sensitivity testing
- Antibiotic synergy
- *Antibiotic tolerance*
- Antibiotic use in dentistry
- Antimicrobial pharmacodynamics
- Antimicrobial spectrum
- Antimycin
- Antimycin A
- Antimycobacterial
- Aphidicolin
- Aplasmomycin
-
-
-
-
-
-
-
-
-
-
-
- Archaeocin
- Arsphenamine
- Ascofuranone
- Aspergillic acid
- Atrop-abyssomicin C
- Auranthine
- Avenanthramide
- Avibactam

B

- Bacteriostatic agent
- Bafilomycin
- Bambermycin
- Beauvericin
- Bezlotoxumab
- Bicyclomycin
- Blasticidin A
- Bottromycin
- Brilacidin
- Broad-spectrum antibiotic

C

- C-1027
- Caprazamycin
- Carbomycin
- Cefoperazone/sulbactam
- Cell envelope antibiotic
- Ceragenin
- Chartreusin

- Chloroxine
- Chromomycin A3
- Citromycin
- Clofoctol
- Clorobiocin
- CMV423
- Combination antibiotic
- Coprinol
- Coumermycin A1
- Curvularin

D

- Dalfopristin
- Daptomycin
- DB-2073
- Deflectin
- Delvotest
- The Demon Under the Microscope
- 17-Dimethylaminoethylamino-17-demethoxygeldanamycin
- Distamycin

E

- Echinomycin
- Efflux (microbiology)
- Endiandric acid C
- Enediyne
- Enzybiotics
- Erythromycin/sulfafurazole
- Esperamicin
- Etamycin
- Extended-spectrum penicillin

F

- (6S)-6-Fluoroshikimic acid
- Fosfomycin
- Fosfomycin/tobramycin
- Fosmidomycin
- Friulimicin
- Furazolidone
- Fusidic acid/betamethasone valerate

G

- Gepotidacin
- Gliotoxin
- Glycyrrhizol
- Gramicidin S
- GSK 299423
- Guanacastepene A

H

- Hachimycin
- Halicin
- Halocyamine
- Hedamycin
- Herbimycin
- Hexamethylenetetramine
- Hitachimycin
- Hydramacin-1

J ,Jadomycin

K

- Kalafungin
- Katanosin
- Kendomycin
- Kettapeptin
- Kidamycin
- Klebsazolicin

L

- Lactivicin
- Landomycins
- Lasalocid
- Leptomycin
- Linopristin
- Lipoglycopeptide
- Lipopeptide
- List of antibiotics

M

- Macromomycin B
- Maduropeptin
- Magic bullet (medicine)
- Mannopeptimycin glycopeptide
- Marinone
- MC21-A
- MC21-B
- Medicinal fungi
- Melafix
- Mercer protocol
-
-
-
- Methaniazide/thioacetazone
- Methylenomycin A
- Methylenomycin B

- Modern Meat
- Moenomycin family antibiotics
- Moromycins
- Mupirocin
- Mycosubtilin
- Myriocin
- Myxopyronin

N

- Naphthomycin A
- Narasin
- Narrow-spectrum antibiotic
- Neopluramycin
- Neosalvarsan
- Neothramycin
- Netropsin
- Nifuroxazide
- Nifurquinazol
- Nigericin
- Nitrofurantoin
- Nitrofurazone
- Nocathiacin I
- Nojirimycin
- Nonribosomal peptide
- Novobiocin
- NXL103

O

- Oxacephem

P

- Pen-Strep
- Penicillium rubens
- Peptaibol
- *Persister cell*
- Phytoalexin
- Alpha-Pinene
- Plantazolicin
- Platensimycin
- Plectasin
- Pluramycin A
- Polyoxins
- Pristinamycin IA
- Production of antibiotics
- Pulvinone
- Pyocyanase
- Pyocyanin
- Pyoluteorin
- Pyrenocine
- Pyrrolobenzodiazepine

Q

- Questiomycin A
- Quinupristin

R

- Ramoplanin
- Raphanin
- Reuterin
- Ridinilazole
- Ristocetin
- Ritipenem
- Roseophilin

S

- Salinomycin
- Salinosporamide A
- Saptomycin
- Saquayamycins
- Sceptrin
- SCH-79797
- Seraticin
- Shishijimicin A
- Sideromycin
- Skin secretions (human)
- SmeT
- Solithromycin

(previous page) (next page)

Media in category "Antibiotics"

The following 8 files are in this category, out of 8 total.

- Biological Target Prediction of Bioactive Molecules Based on Minimum Structures Identification.

DNA gyrase and topoisomerase IV identification in 4-pyridone group

Fungicides and bactericides classification.

Large ribosomal subunit identification in (1R)-propanol group.

Large ribosomal subunit identification in 3-glutarimidyl group.

Large ribosomal subunit identification in cytosine group.

Small ribosomal subunit identification in (4aRS,5aRS)-Sancycline group.

Small ribosomal subunit identification in 2,4(or 5)-diaminocyclohexanol group.

Pages in category "Antileprotic drugs"

The following pages are in this category, out of 13 total. This list may not reflect recent changes.

A

- Acedapsone
- *Antileprotic drugs*

C

- Clofazimine

D

- Dapsone
- Desoxyfructo-serotonin
- Ditophal
- Diucifon

E

- Ethionamide

L

- Leprostatic agent

P

- Promin

R

- Rifampicin
- Rifapentine

S

- Solasulfone

for this category is ***Cell envelope antibiotic***.

Subcategories

This category has the following 4 subcategories, out of 4 total.

A- Antimicrobial peptides

B

- ▶ Beta-lactam antibiotics (4 C, 9 P)
- ▶ Beta-lactamase inhibitors (10 P)

G

- ▶ Glycopeptide antibiotics (14 P)

Pages in category "Eukaryotic selection compounds"

The following 10 pages are in this category, out of 10 total. This list may not reflect recent changes (learn more).

B

- Bialaphos
- Blasticidin S
- Bleomycin

G

- G418
- Glufosinate

H

- Hygromycin B

N

- Nourseothricin

P

- Phleomycin
- Puromycin

Z

- Zeocin

The main article for this category is ***Nucleic acid inhibitor***.

Subcategories

This category has the following 6 subcategories, out of 6 total.

E

- ▶ Enediynes (11 P)

N

- ▶ Nitroimidazole antibiotics (9 P)

Q

- ▶ Quinolone antibiotics (1 C, 10 P)

R

- ▶ Rifamycin antibiotics (7 P)
- ▶ RNA polymerase inhibitors (2 C, 5 P)

S

- ▶ Sulfonamide antibiotics (40 P)

Pages in category "Nucleic acid inhibitor antibiotics"

The following 2 pages are in this category, out of 2 total. This list may not reflect recent changes (learn more).

*

- Nucleic acid inhibitor

K

- Kinamycin

Subcategories

This category has the following 6 subcategories, out of 6 total.

A

- ▶ Aminoglycoside antibiotics (33 P)
- ▶ Amphenicols (5 P)

L

- ▶ Lincosamide antibiotics (4 P)

O

- ▶ Oxazolidinone antibiotics (7 P)

P

- ▶ Pleuromutilin antibiotics (6 P)
- ▶ Polyketide antibiotics (3 C, 13 P)

Pages in category "Protein synthesis inhibitor antibiotics"

The following 7 pages are in this category, out of 7 total. This list may not reflect recent changes (learn more).

B

- Blasticidin S

E

- Epimerox

F

- Fusidic acid

G

- Girolline

O

- Odilorhabdin

P

- Protein synthesis inhibitor
- Puromycin

Pages in category "Steroid antibiotics"

This category contains only the following page. This list may not reflect recent changes (learn more).

F

- Fusidic acid

Pages in category "Thiopeptides"

The following 5 pages are in this category, out of 5 total. This list may not reflect recent changes (learn more).

- Thiopeptide

C

- Cyclothiazomycin

L

- Lactocillin

N

- Nosiheptide

T

- Thiostrepton

Pages in category "Antibiotic stubs"

The following 200 pages are in this category, out of approximately 244 total. This list may not reflect recent changes (learn more).

(previous page) (next page)

- Template:Antibiotic-stub

A

- Actinorhodin
- Aditoprim
- AGG01
- Alatrofloxacin
- Almecillin
- Aminopenicillin
- Amphenicol
- Angucyclines
- Antistaphylococcal penicillins
- Arylomycin A2
- Astromicin
- Auranthine
- Avibactam
- Azalide
- Azamulin
- Azidamfenicol
- Azlocillin

B

- Bacampicillin
- Balofloxacin
-
-
-
-
-
-
-
- Bekanamycin
- Benzathine phenoxymethylpenicillin
- Besifloxacin
- Betamipron
- Biapenem
- Blasticidin A
- Brodimoprim

C

- C-1027
- Cadazolid
- Caprazamycin
- Carbacephem
- Carboxypenicillin
- Carfecillin
- Carindacillin
- Carumonam
- Cefacetrile
- Cefaloglycin
- Cefalotin
- Cefaparole
- Cefapirin

- Cefatrizine
- Cefazaflur
- Cefazedone
- Cefbuperazone
- Cefcapene
- Cefclidin
- Cefdaloxime
- Cefetamet
- Cefluprenam
- Cefmatilen
- Cefmenoxime
- Cefmetazole
- Cefminox
- Cefonicid
- Cefoperazone/sulbactam
- Ceforanide
- Cefoselis
- Cefozopran
- Cefpimizole
- Cefpiramide
- Cefpirome
- Cefprozil
- Cefroxadine
- Cefsumide
- Cefteram
- Ceftezole
- Ceftiofur
- Ceftiolene
- Ceftizoxime
- Cefuzonam
- Cell envelope antibiotic
- Cephalosporin C
- Cephamycin
- Chromomycin A3
- Ciclacillin
- Clavanin B
- Clofoctol
- Clometocillin
- Clomocycline
- Clorobiocin
- Cloxacillin
- Corbomycin
- Cosmomycin B
- Coumermycin A1

D

- Danofloxacin
- Dehydrocurvularin
- Dibekacin
- Dihydrostreptomycin
- 17-Dimethylaminoethylamino-17-demethoxygeldanamycin

E

- Echinomycin
- Eperezolid
- Epicillin
- Etamycin

F

- Flomoxef
- Flopristin
- (6S)-6-Fluoroshikimic acid
- Flurithromycin
- Fosmidomycin
- Fusafungine

G

- Gardimycin
- Garenoxacin
- GSK 299423
- Guanacastepene A

H

- Hachimycin
- Halocidin
- Halocyamine
- Hetacillin

I

- Ibafloxacin
- Imipenem/cilastatin/relebactam
- Isepamicin

J

- JNJ-Q2

K

- Kettapeptin
- Kidamycin
- Kitasamycin

L

- Lactocillin
- Latamoxef
- Lefamulin
- Lenapenem
- Linopristin
- Lividomycin
- Lomefloxacin

- Loracarbef
- Lugdunin

M

- Macbecin
- Mannopeptimycin glycopeptide
-
-
-
-
-
-
-
- Meclocycline
- Metacycline
- Metampicillin
- Methaniazide
- Methaniazide/thioacetazone
- Methylenomycin A
- Methylenomycin B
- Mezlocillin
- Micronomicin
- Miocamycin
- Monobactam

N

- Naphthomycin A
- Narrow-spectrum antibiotic
- Neamine
- Nemonoxacin
- Nifuroxazide
- Nifurtoinol
- Niphimycin
- Nocardicin A
- Nocathiacin I
- Nojirimycin
- Nourseothricin
- Nucleic acid inhibitor

O

- Orbifloxacin
- Oudemansin A
- Oxacephem
- Oxolinic acid

P

- Panipenem
- Panipenem/betamipron
- Pazufloxacin
- Pecilocin
- Penamecillin
- Penimepicycline

- Pheneticillin
- Phleomycin
- Pipemidic acid
- Pirlimycin
- Piromidic acid
- Pivampicillin
- Plectasin
- Pleuromutilin
- Posizolid
- Pristinamycin IA
- Pristinamycin IIA
- Pristinamycin IIB
- Propicillin
- Pyrenocine

R

- Radezolid
- Ramoplanin
- Ranbezolid
- Relebactam
- Ribostamycin
- Ritipenem
- Rokitamycin
- Rolitetracycline
- Roseophilin
- Rosoxacin
- Rufloxacin

S

- Saquayamycins
- Sarafloxacin
- Sceptrin
- Seraticin
- Sitafloxacin
- Solasulfone
- Staphefekt
- Streptoduocin
- Streptogramin
- Streptolydigin
- Streptomyces aureofaciens
- Streptovaricin
- Styelin A
- Sulbactam
- Sulbenicillin
- Sulfabenzamide
- Sulfachlorpyridazine

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-
-
- Sulfacytine
- Sulfadimidine
- Sulfadoxine
- Sulfafurazole
- Sulfalene
- Sulfamazone
- Sulfamerazine
- Sulfamethizole
- Sulfamethoxypyridazine
- Sulfametomidine
- Sulfametrole
- Sulfamoxole
- Sulfanitran
- Sulfaperin
- Sulfaphenazole
- Sulfathiazole
- Sulfathiourea
- Sulfisomidine
- Surotomycin
- Sutezolid

T

- Tachyplesin
- Talampicillin
- TAN-1057 A
- TAN-1057 C
- Tazobactam
- Terephtyl
- Tetroxoprim
- Thiamphenicol
- Tiacumicin
- Tiamulin
- Ticarcillin/clavulanic acid
- Tigemonam
- Tosufloxacin
- Troleandomycin

U

- Urauchimycin
- Ureidopenicillin

V

- Valnemulin
- Verdamicin
- Virginiamycin S1

- Viridicatumtoxin A
- Viridicatumtoxin B

X

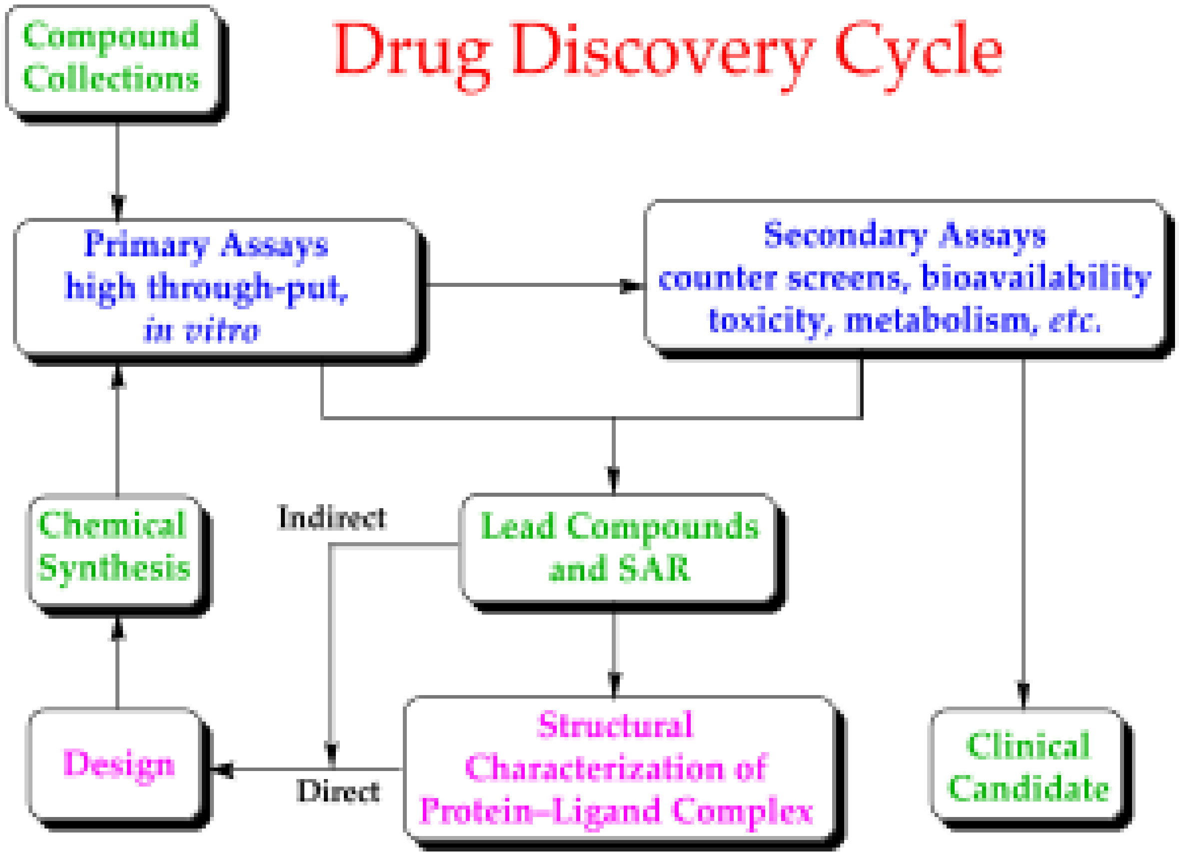
- Xibornol

Y

- Yaequinolone J1

H. .Drug Discovery Cycle (Process Maximization):

1. **Drug targets.**
2. **Rational Drug discovery**
3. **Computer-aided drug design**
4. **4Types**
    - **4.1 Ligand-based**
    - **4.2 Structure- based**
        - **4.2.1Binding site identification**
        - **4.2.2Scoring functions.**
5. **Examples.**
6. **Case Studies**
7. **Criticism**



Drug design, often referred to as **rational drug design** or simply rational design, is the inventive process of finding new medications based on the knowledge of a biological target.[1] The drug is most commonly an organic small molecule that activates or inhibits the function of a biomolecule such as a protein, which in turn results in a therapeutic benefit to the patient. In the most basic sense, drug design involves the design of molecules that are complementary in shape and charge to the biomolecular target with which they interact and therefore will bind to it. Drug design frequently but not necessarily relies on computer modelling techniques. This type of modelling is sometimes referred to as **computer-aided drug design**. Finally, drug design that relies on the knowledge of the three-dimensional structure of the biomolecular target is known as **structure-based drug design**. In addition to small molecules, biopharmaceuticals including peptides and especially therapeutic antibodies are an increasingly important class of drugs and computational methods for improving the affinity, selectivity, and stability of these protein-based therapeutics have also been developed.

The phrase "drug design" is to some extent a misnomer. A more accurate term is ligand design (i.e., design of a molecule that will bind tightly to its target). Although design techniques for prediction of binding affinity are reasonably successful, there are many other properties, such as bioavailability, metabolic half-life, side effects, etc., that first must be optimized before a ligand can become a safe and efficacious drug. These other characteristics are often difficult to predict with rational design techniques.

Nevertheless, due to high attrition rates, especially during clinical phases of drug development, more attention is being focused early in the drug design process on selecting candidate drugs whose physicochemical properties are predicted to result in fewer complications during development and hence more likely to lead to an approved, marketed drug. Furthermore, in vitro experiments complemented with computation methods are increasingly used in early drug discovery to select compounds with more favourable ADME (absorption, distribution, metabolism, and excretion) and toxicological profiles.

Drug targets

A biomolecular target (most commonly a protein or a nucleic acid) is a key molecule involved in a particular metabolic or signalling pathway that is associated with a specific disease condition or pathology or to the infectivity or survival of a microbial pathogen. Potential drug targets are not necessarily disease causing but must by definition be disease modifying.[10] In some cases, small molecules will be designed to enhance or inhibit the target function in the specific disease modifying pathway. Small molecules (for example receptor agonists, antagonists, inverse agonists, or modulators; enzyme activators or inhibitors; or ion channel openers or blockers will be designed that are complementary to the binding site of target. Small molecules (drugs) can be designed so as not to affect any other important "off-target" molecules (often referred to as antitargets) since drug interactions with off-target molecules may lead to undesirable side effects. Due to similarities in binding sites, closely related targets identified through sequence homology have the highest chance of cross reactivity and hence highest side effect potential.

Most commonly, drugs are organic small molecules produced through chemical synthesis, but biopolymer-based drugs (also known as biopharmaceuticals) produced through biological processes are becoming increasingly more common In addition, mRNA-based gene silencing technologies may have therapeutic applications.

Rational drug discovery

In contrast to traditional methods of drug discovery (known as forward pharmacology), which rely on trial-and-error testing of chemical substances on cultured cells or animals, and matching the apparent effects to treatments, rational drug design (also called reverse pharmacology) begins with a hypothesis that modulation of a specific biological target may have therapeutic value.

In order for a biomolecule to be selected as a drug target, two essential pieces of information are required. The first is evidence that modulation of the target will be disease modifying. This knowledge may come from, for example, disease linkage studies that show an association between mutations in the biological target and certain disease states. The second is that the target is "druggable". This means that it is capable of binding to a small molecule and that its activity can be modulated by the small molecule[.

Once a suitable target has been identified, the target is normally cloned and produced and purified. The purified protein is then used to establish a screening assay. In addition, the three-dimensional structure of the target may be determined.

The search for small molecules that bind to the target is begun by screening libraries of potential drug compounds. This may be done by using the screening assay (a "wet screen"). In addition, if the structure of the target is available, a virtual screen may be performed of candidate drugs. Ideally the candidate drug compounds should be "drug-like", that is they should possess properties that are predicted to lead to oral bioavailability, adequate chemical and metabolic stability, and minimal toxic effects.-Several methods are available to estimate drug likeness such as Lipinski's Rule of Five and a range of scoring methods such as lipophilic efficiency. Several methods for predicting drug metabolism have also been proposed in the scientific literature.

Due to the large number of drug properties that must be simultaneously optimized during the design process, multi-objective optimization techniques are sometimes employed- Finally because of the limitations in the current methods for prediction of activity, drug design is still very much reliant on serendipity and bounded rationality.

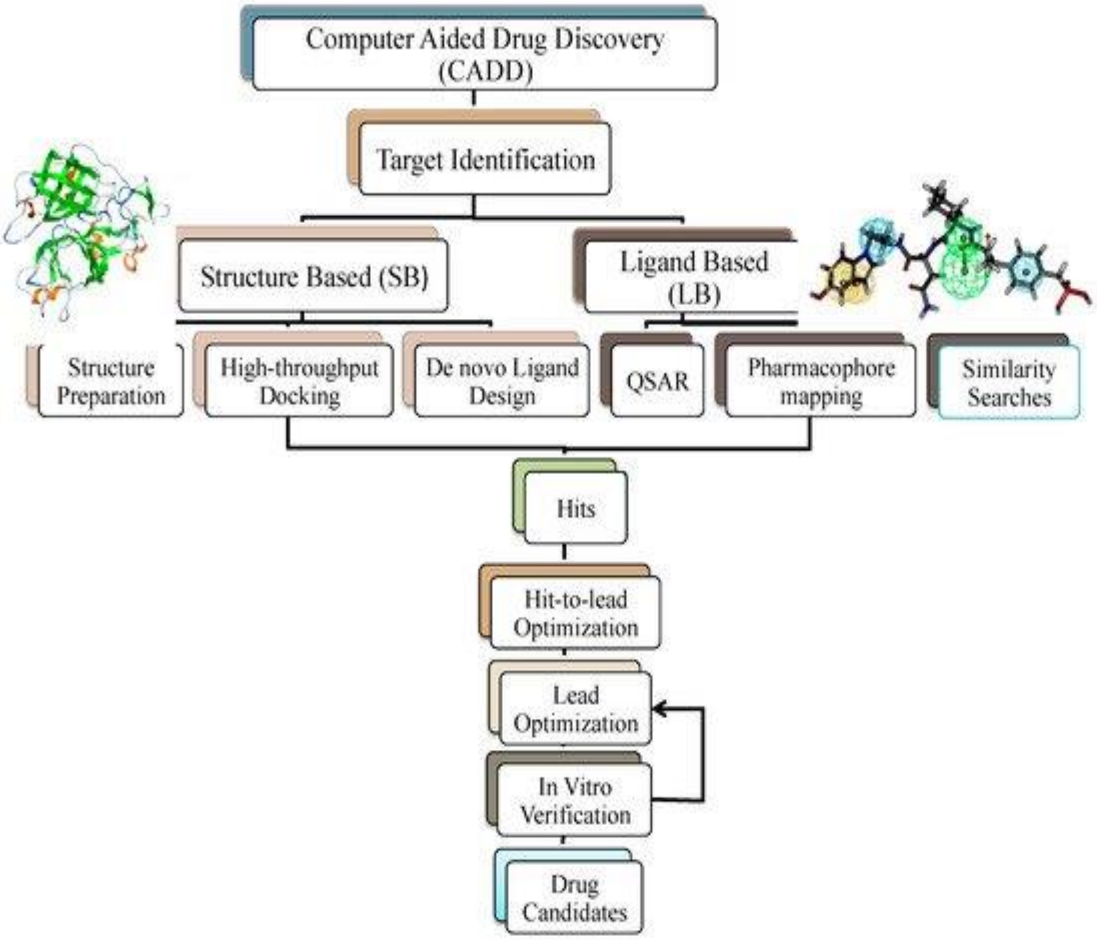
Computer-aided drug design

The most fundamental goal in drug design is to predict whether a given molecule will bind to a target and if so how strongly. Molecular mechanics or molecular dynamics is most often used to estimate the strength of the intermolecular interaction between the small molecule and its biological target. These methods are also used to predict the conformation of the small molecule and to model conformational changes in the target that may occur when the small molecule binds to it.

Semi-empirical, ab initio quantum chemistry methods, or density functional theory are often used to provide optimized parameters for the molecular mechanics calculations and also provide an estimate of the electronic properties (electrostatic potential, polarizability, etc.) of the drug candidate that will influence binding affinity.

Molecular mechanics methods may also be used to provide semi-quantitative prediction of the binding affinity. Also, knowledge-based scoring function may be used to provide binding affinity estimates. These methods use linear regression, machine learning, neural nets or other statistical techniques to derive predictive binding affinity equations by fitting experimental affinities to computationally derived interaction energies between the small molecule and the target.

Ideally, the computational method will be able to predict affinity before a compound is synthesized and hence in theory only one compound needs to be synthesized, saving enormous time and cost. The reality is that present computational methods are imperfect and provide, at best, only qualitatively accurate estimates of affinity. In practice it still takes several iterations of design, synthesis, and testing before an optimal drug is discovered. Computational methods have accelerated discovery by reducing the number of iterations required and have often provided novel structures.



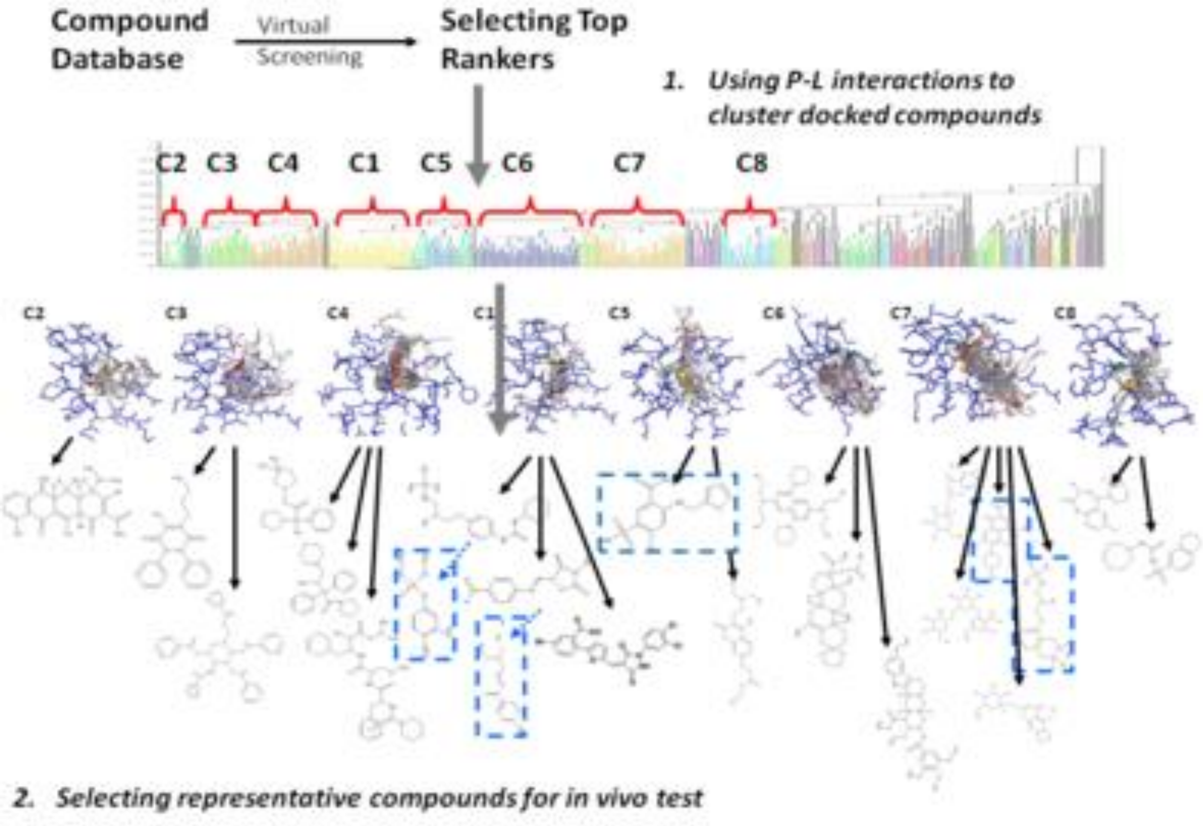
Drug design with the help of computers may be used at any of the following stages of drug discovery:

1. hit identification using virtual screening (structure- or ligand-based design)
2. hit-to-lead optimization of affinity and selectivity (structure-based design, QSAR, etc.)
3. lead optimization of other pharmaceutical properties while maintaining affinity

In order to overcome the insufficient prediction of binding affinity calculated by recent scoring functions, the protein-ligand interaction and compound 3D structure information are used for analysis. For structure-based drug design, several post-screening analyses focusing on protein-ligand interaction have been developed for improving enrichment and effectively mining potential candidates:

- Consensus scoring
  - Selecting candidates by voting of multiple scoring functions
  - May lose the relationship between protein-ligand structural information and scoring criterion
- Cluster analysis

- Represent and cluster candidates according to protein-ligand 3D information
- Needs meaningful representation of protein-ligand interactions.



There are two major types of drug design. The first is referred to as **ligand-based drug design** and the second, **structure-based drug design**.

Ligand-based

Ligand-based drug design (or **indirect drug design**) relies on knowledge of other molecules that bind to the biological target of interest. These other molecules may be used to derive a pharmacophore model that defines the minimum necessary structural characteristics a molecule must possess in order to bind to the target.[35] In other words, a model of the biological target may be built based on the knowledge of what binds to it, and this model in turn may be used to design new molecular entities that interact with the target. Alternatively, a quantitative structure-activity relationship (QSAR), in which a correlation between calculated properties of molecules and their experimentally determined biological activity, may be derived. These QSAR relationships in turn may be used to predict the activity of new analogs.

Structure-based

Structure-based drug design (or **direct drug design**) relies on knowledge of the three dimensional structure of the biological target obtained through methods such as x-ray crystallography or NMR spectroscopy.[37] If an experimental structure of a target is not available, it may be possible to create a homology model of the target based on the experimental structure of a related protein. Using the structure of the biological target, candidate drugs that are predicted to bind with high affinity and selectivity to the target may be designed using interactive graphics and the intuition of a medicinal chemist. Alternatively various automated computational procedures may be used to suggest new drug candidates.

Current methods for structure-based drug design can be divided roughly into three main categories.[39] The first method is identification of new ligands for a given receptor by searching large databases of 3D structures of small molecules to find those fitting the binding pocket of the receptor using fast approximate docking programs. This method is known as virtual screening. A second category is de novo design of new ligands. In this method, ligand molecules are built up within the constraints of the binding pocket by assembling small pieces in a stepwise manner. These pieces can be either individual atoms or molecular fragments. The key advantage of such a method is that novel structures, not contained in any database, can be suggested. A third method is the optimization of known ligands by evaluating proposed analogs within the binding cavity.

Binding site identification

Binding site identification is the first step in structure based design.[17][43] If the structure of the target or a sufficiently similar homolog is determined in the presence of a bound ligand, then the ligand should be observable in the structure in which case location of the binding site is trivial. However, there may be unoccupied allosteric binding sites that may be of interest. Furthermore, it may be that only apoprotein (protein without ligand) structures are available and the reliable identification of unoccupied sites that have the potential to bind ligands with high affinity is non-trivial. In brief, binding site identification usually relies on identification of concave surfaces on the protein that can accommodate drug sized molecules that also possess appropriate "hot spots" (hydrophobic surfaces, hydrogen bonding sites, etc.) that drive ligand binding.

Scoring functions

Main article: Scoring functions for docking

Structure-based drug design attempts to use the structure of proteins as a basis for designing new ligands by applying the principles of molecular recognition. Selective high affinity binding to the target is generally desirable since it leads to more efficacious drugs with fewer side effects. Thus, one of the most important principles for designing or obtaining potential new ligands is to predict the binding affinity of a certain ligand to its target (and known antitargets) and use the predicted affinity as a criterion for selection.[44]

One early general-purposed empirical scoring function to describe the binding energy of ligands to receptors was developed by Böhm. This empirical scoring function took the form:

where:

$\Delta G_{BINDING} =$

$(\Delta G_0 + \Delta G_{hb}\, \Delta G_{hb\text{-}BOND} + \Delta G_{ionic} \sum \text{IONIC-INT} + \Delta G_{lip} \left| A \right| + \Delta G_{rot}\, \text{NROT})$

-
-
-
-
-
- ΔG_0 – empirically derived offset that in part corresponds to the overall loss of translational and rotational entropy of the ligand upon binding.
- ΔG_{hb} – contribution from hydrogen bonding
- ΔG_{ionic} – contribution from ionic interactions
- ΔG_{lip} – contribution from lipophilic interactions where $|A_{lipo}|$ is surface area of lipophilic contact between the ligand and receptor
- ΔG_{rot} – entropy penalty due to freezing a rotatable in the ligand bond upon binding

- desolvation – enthalpic penalty for removing the ligand from solvent
- motion – entropic penalty for reducing the degrees of freedom when a ligand binds to its receptor
- configuration – conformational strain energy required to put the ligand in its "active" conformation
- interaction – enthalpic gain for "resolvating" the ligand with its receptor.

A more general thermodynamic "master" equation is as follows

$\Delta G_{BINDING} = \text{-ve } RT \ln Kd.$

$Kd=[Ligand][Receptor]/[Complex]$

$\Delta G_{BINDING} = \Delta G \text{ desolvation} + \Delta G \text{ motion} + \Delta G \text{ configuration} + \Delta G \text{ interaction} + \Delta G \text{ others.}$

where:

- desolvation – enthalpic penalty for removing the ligand from solvent
- motion – entropic penalty for reducing the degrees of freedom when a ligand binds to its receptor
- configuration – conformational strain energy required to put the ligand in its "active" conformation
- interaction – enthalpic gain for "resolvating" the ligand with its receptor

The basic idea is that the overall binding free energy can be decomposed into independent components that are known to be important for the binding process. Each component reflects a certain kind of free energy alteration during the binding process between a ligand and its target receptor. The Master Equation is the linear combination of these components. According to Gibbs free energy equation, the relation between dissociation equilibrium constant, K_d, and the components of free energy was built.

Various computational methods are used to estimate each of the components of the master equation. For example, the change in polar surface area upon ligand binding can be used to estimate the desolvation energy. The number of rotatable bonds frozen upon ligand binding is proportional to the motion term. The configurational or strain energy can be estimated using molecular mechanics calculations. Finally the interaction energy can be estimated using methods such as the change in non polar surface, statistically derived potentials of mean force, the number of hydrogen bonds formed, etc. In practice, the components of the master equation are fit to experimental data using multiple linear regression. This can be done with a diverse training set including many types of ligands and receptors to produce a less accurate but more general "global" model or a more restricted set of ligands and receptors to produce a more accurate but less general "local" model

Examples

A particular example of rational drug design involves the use of three-dimensional information about biomolecules obtained from such techniques as X-ray crystallography and NMR spectroscopy. Computer-aided drug design in particular becomes much more tractable when there is a high-resolution structure of a target protein bound to a potent ligand. This approach to drug discovery is sometimes referred to as structure-based drug design. The first unequivocal example of the application of structure-based drug design leading to an approved drug is the carbonic anhydrase inhibitor dorzolamide, which was approved in 1995.

Another important case study in rational drug design is imatinib, a tyrosine kinase inhibitor designed specifically for the *bcr-abl* fusion protein that is characteristic for Philadelphia chromosome-positive leukemias (chronic myelogenous leukemia and occasionally acute lymphocytic leukemia). Imatinib is substantially different from previous drugs for cancer, as most agents of chemotherapy simply target rapidly dividing cells, not differentiating between cancer cells and other tissues.

I. Medicinal chemistry and pharmaceutical chemistry

Contents

Medicinal chemistry and **pharmaceutical chemistry** are disciplines at the intersection of chemistry, especially synthetic organic chemistry, and pharmacology and various other biological specialties, where they are involved with design, chemical synthesis and development for market of pharmaceutical agents, or bio-active molecules (drugs).

Compounds used as medicines are most often organic compounds, which are often divided into the broad classes of small organic molecules (e.g., atorvastatin, fluticasone, clopidogrel) and "biologics" (infliximab, erythropoietin, insulin glargine), the latter of which are most often medicinal preparations of proteins (natural and recombinant antibodies, hormones, etc.). Inorganic and organometallic compounds are also useful as drugs (e.g., lithium and platinum-based agents such as lithium carbonate and cisplatin as well as gallium).

In particular, medicinal chemistry in its most common practice—focusing on small organic molecules—encompasses synthetic organic chemistry and aspects of natural products and computational chemistry in close combination with chemical biology, enzymology and structural biology, together aiming at the discovery and development of new therapeutic agents. Practically speaking, it involves chemical aspects of identification, and then systematic, thorough synthetic alteration of new chemical entities to make them suitable for therapeutic use. It includes synthetic and computational aspects of the study of existing drugs and agents in development in relation to their bioactivities (biological activities and properties), i.e., understanding their structure-activity relationships (SAR). Pharmaceutical chemistry is focused on quality aspects of medicines and aims to assure fitness for purpose of medicinal products.[3]

At the biological interface, medicinal chemistry combines to form a set of highly interdisciplinary sciences, setting its organic, physical, and computational emphases alongside biological areas such as biochemistry, molecular biology, pharmacognosy and pharmacology, toxicology and veterinary and human medicine; these, with project management, statistics, and pharmaceutical business practices, systematically oversee altering identified chemical agents such that after pharmaceutical formulation, they are safe and efficacious, and therefore suitable for use in treatment of disease.

Subcategories

This category has the following 8 subcategories, out of 8 total.

C

- ► Chemicals in medicine

Drug discovery.

- ► Drug discovery

J **Subcategories**

This category has the following 4 subcategories, out of 4 total.

C

- ► Clinical pharmacology
- ► Drug discovery companies

H

- ► HIV vaccine research

M

- ► Mathematical chemistry

- ► Medicinal chemistry journals

M

Pages in category "Medicinal chemistry journals"

The following pages are in this category, out of 20 total. This list may not reflect recent changes (learn more).

A

- ACS Medicinal Chemistry Letters
- Annals of Clinical Biochemistry
- Anti-Cancer Agents in Medicinal Chemistry

B

- Bioorganic & Medicinal Chemistry
- Bioorganic & Medicinal Chemistry Letters

C

- ChemMedChem
- Clinica Chimica Acta
- Clinical Biochemistry
- Clinical Chemistry and Laboratory Medicine
- Current Medicinal Chemistry
- Current Topics in Medicinal Chemistry

E

- European Journal of Medicinal Chemistry

F

- Future Medicinal Chemistry

J

- Journal of Enzyme Inhibition and Medicinal Chemistry
- Journal of Medicinal Chemistry
- Journal of Trace Elements in Medicine and Biology

M

- MedChemComm
- Medicinal Chemistry Research
- Mini-Reviews in Medicinal Chemistry

O

- The Open Clinical Biochemistry Journal

- ► Medicinal inorganic chemistry

Pages in category "Medicinal inorganic chemistry"

The following pages are in this category, out of 6 total. This list may not reflect recent changes (learn more).

A

- Artificial metalloenzyme

B

- Bioinorganic chemistry
- Bioorganometallic chemistry

C

- Cisplatin

M

- Metalloprotein

P

- Platinum-based antineoplastic

- ▶ Medicinal radiochemistry

N

Category:Medicinal radiochemistry

From Wikipedia, the free encyclopedia

Jump to navigationJump to search

Subcategories

This category has the following 2 subcategories, out of 2 total.

P

- ▶ PET radiotracers

R

- ▶ Radiopharmaceuticals

Pages in category "Medicinal radiochemistry"

The following pages are in this category, out of 18 total. This list may not reflect recent changes.

A

- ATC code V09

F

- Flortaucipir (18F)
- Fluciclovine (18F)
- Fludeoxyglucose (18F)
- Fluorine-18
- Fluorodeoxyglycosylamine
- Fluoroestradiol F-18

H

- George de Hevesy

I

- Isotopes in medicine

M

- Metabolic trapping

N

- Nuclear medicine

P

- PET for bone imaging
- PET radiotracer
- Positron emission tomography

R

- Radioactive tracer
- Radiopharmaceutical
- Radiopharmacology

T

- Technetium-99m

- ► Natural products

P

Subcategories

This category has the following 7 subcategories, out of 7 total.

- ► Natural products by elemental composition

A

- ► Alkaloids
- ► Animal products

B

- ► Biomolecules

E

- ► Essential oils

P

- ► Plant products

T

- ► Terpenes and terpenoids

Pages in category "Natural products"

The following pages are in this category, out of 13 total. This list may not reflect recent changes.

- Natural product

A

- Agglomerin
- Ambergris

D

- Dicerandrol C

F

- Natural foods

M

- Mycofactocin

P

- Phomoxanthone
- Phomoxanthone A
- Phomoxanthone B
- Phoslactomycin B

R

- Ribosomally synthesized and post-translationally modified peptides
- Römpp Encyclopedia Natural Products

S

- Salinosporamide

- ► Pharmacology

Σ

Subcategories

This category has the following 26 subcategories, out of 26 total.

B

- ► Biology and pharmacology of chemical elements
- ► Biopharmaceuticals
- ► Breakthrough therapy

C

- ► Chemopreventive agents
- ► Chirality
- ► Pharmacological classification systems
- ► Clinical pharmacology

D

- ► Drug brand names
- ► Drug delivery devices
- ► Drug discovery
- ► Drug resistance
- ► Drugs

L

- ► Pharmacology literature

N

- ► Neuropharmacology

P

- ► Pharmaceutical industry
- ► Pharmaceutical isolates
- ► Pharmacodynamics
- ► Pharmacognosy
- ► Pharmacokinetics
- ► Pharmacological societies
- ► Pharmacologists
- ► Pharmacy
- ► Psychopharmacology

R

- ► Routes of administration

S

- ► Sex and drugs

Σ

- ► Pharmacology stubs

Pages in category "Pharmacology"

The following pages are in this category, out of 57 total. This list may not reflect recent changes .

- Pharmacology

A

- Absorption (pharmacology)
- Active ingredient
- Autopharmacology

B

- Bioassay
- Biological target
- British Approved Name

C

- Chirality
- Chirality (chemistry)
- Classical pharmacology
- Clinical pharmacology
- CM-4620
- Coinduction (anesthetics)
- Combination therapy

D

- Department of Pharmacology, University College London
- Drug titration
- Drug vectorization

E

- E7070
- Excretion

F

- Functional analog (chemistry)

G

- Ginsenoside Rb1
- Graduate of Pharmacy
- Guide to Pharmacology

H

- Hallucinogenic plants in Chinese herbals
- Hill equation (biochemistry)
- Homochirality

I

- International nonproprietary name
- Irwin screen

J

- John J. Abel Award

M

- Master's in Pharmacology
- Mechanism of action
- Micro-atmosphere method

N

- Nonsteroidal

O

- Octanol-water partition coefficient
- Osmotic-controlled release oral delivery system

P

- Peripherally acting μ-opioid receptor antagonist
- Pharmaceutical industry
- Pharmacoepidemiology
- Pharmacogenomics
- Pharmacoinformatics
- Pharmacotoxicology
- Photopharmacology
- Pleiotropy (drugs)
- Polymer-protein hybrid
- Polypharmacology
- Proteolysis targeting chimera

Q

- Quantitative systems pharmacology

R

- Receptor theory
- Ro05-4082

S

- Safety pharmacology
- Sex and drugs
- Sheild Professor of Pharmacology
- Systems pharmacology

T

- Tissue selectivity
- Toxicity

U

- Uniformity of content

V

- Venoms in medicine

Media in category "Pharmacology"

The following 11 category,

- Biological Target Prediction of Bioactive Molecules Based on Minimum Structures Identification.

  DNA gyrase and topoisomerase IV identification in 4-pyridone group.

  Fungicides and bactericides classification.

  Large ribosomal subunit identification in (1R)-propanol group.

  Large ribosomal subunit identification in 3-glutarimidyl group.

  Large ribosomal subunit identification in cytosine group.

  Small ribosomal subunit identification in (4aRS,5aRS)-Sancycline group.

  Small ribosomal subunit identification in 2,4(or 5)-diaminocyclohexanol group.

  Sterol 14α-demethylase identification in 1,2,4-triazol-1-yl group.

  Sterol 14α-demethylase identification in imidazol-1-yl group.

  Target prediction process.

- ► Medicinal chemistry stubs

Subcategories

This category has only the following subcategory.

- ► Pharmacology stubs

Pages in category "Medicinal chemistry stubs"

The following pages are in this category, out of 4 total. This list may not reflect recent changes,.

- Template:Medicinal-chem-stub

C

- Cantarella
- 2-Carboxy-D-arabitinol 1-phosphate

G

- Geroprotector

Category:Chemicals in medicine

From Wikipedia, the free encyclopedia

Subcategories

This category has the following 8 subcategories, out of 8 total.

A

- ► Anti-infective agents (5 C, 8 P)

C

- ► Chemical substances for emergency medicine (1 C, 40 P)

D

- ► Drugs (43 C, 52 P, 10 F)

H

- ► Human drug metabolites (2 C, 124 P)

M

- ► Medical isotopes (2 P)

P

- ► PET radiotracers (24 P)
- ► Prodrugs (13 C, 395 P)

R

- ► Radiopharmaceuticals (3 C, 56 P)

Pages in category "Chemicals in medicine"

The following 6 pages are in this category, out of 6 total. This list may not reflect recent changes (learn more).

I

- Isotopes in medicine

M

- Metacresol purple
- Metals in medicine

P

- PET radiotracer
- Medication

R

- Radiopharmaceutical

This list may not reflect recent changes,

- Drug discovery

*

-
-
- List of drugs by year of discovery

0–9

- 5-HT2C receptor agonist
- 5-HT3 antagonist

A

- Discovery and development of ACE inhibitors
- Anabaseine
- Angiokinase inhibitors
- Discovery and development of angiotensin receptor blockers
- Antitarget
- Applicability domain

B

- Bcr-Abl tyrosine-kinase inhibitor
- Discovery and development of beta-blockers
- Discovery and development of beta2 agonists

C

- Cannabinoid receptor antagonist
- CCR5 receptor antagonist
- Center for Pharmaceutical Research and Innovation
- ChemAxon
- Chemical library
- Chemical similarity
- Cheminformatics
- Cheminformatics toolkits
- Chemogenomics
- Classical pharmacology

- Clinical trial
- Combinatorial chemistry
- Compound management
- Cost of drug development
- COVID-19 drug development
- Critical Path Institute
- Discovery and development of cyclooxygenase 2 inhibitors

D

- Dilution assay
- Discovery and development of dipeptidyl peptidase-4 inhibitors
- Discovery and development of direct thrombin inhibitors
- Dirty drug
- Discovery and development of 5α-reductase inhibitors
- Discovery and development of antiandrogens
- Discovery and development of bisphosphonates
- Discovery and development of cephalosporins
- Discovery and development of direct Xa inhibitors
- Discovery and development of integrase inhibitors
- Discovery and development of statins
- DNA-encoded chemical library
- Docking (molecular)
- Drug design
- Drug development
- Drug repositioning
- Druggability
- Druglikeness
- Dynamic combinatorial chemistry

E

- Eroom's law

F

- Fragment-based lead discovery

G

- GABAA receptor positive allosteric modulator
- Discovery and development of gastrointestinal lipase inhibitors
- Discovery and development of gliflozins
- GLOBAL
- Glycorandomization

H

- High-content screening
- High-throughput screening
-
-
-

-
-
-
-
- History of general anesthesia
- History of neuraxial anesthesia
- History of pharmacy
- Hit selection
- Hit to lead
- Discovery and development of HIV-protease inhibitors

I

- Immunomodulatory imide drug
- Influenza Antiviral Drug Search

L

- Latent semantic structure indexing
- Lead compound
- LeDock
- Ligand efficiency
- Christopher A. Lipinski
- Lipinski's rule of five
- Lipophilic efficiency
- Derek Lowe (chemist)

M

- Metalloprotease inhibitor
- Melatonin receptor agonist
- NMDA receptor
- Minor Use Animal Drug Program
- Molecular phenotyping
- MTOR inhibitors

N

- Discovery and development of neuraminidase inhibitors
- Nicotinic agonist
- NK1 receptor antagonist
- Nocebo
- Discovery and development of non-nucleoside reverse-transcriptase inhibitors
- Discovery and development of NS5A inhibitors
- Discovery and development of nucleoside and nucleotide reverse-transcriptase inhibitors
- Number needed to harm
- Number needed to treat

O

- Utako Okamoto
- Onasemnogene abeparvovec
- OpenPHACTS

- Orphan drug

P

- Pharmaceutical bioinformatics
- Pharmacoinformatics
- Pharming (genetics)
- Phenotypic screening
- Discovery and development of phosphodiesterase 5 inhibitors
- Phosphoinositide 3-kinase inhibitor
- Preclinical development
- Project 523
- Protein mimetic
- Protide
- Discovery and development of proton pump inhibitors
- Psychedelic therapy

Q

- Quantitative structure–activity relationship

R

- Research chemical
- Retro screening
- Retrometabolic drug design
- Reverse pharmacology
- RNA-targeting small molecule drugs

S

- SAMPL Challenge
- Small molecule
- Southern Research
- Steroidal aromatase inhibitor

T

- Target protein
- Targeted drug delivery
-
-
-
-
- Therapy
- TNO intestinal model
- Discovery and development of triptans
- Discovery and development of TRPV1 antagonists
- Discovery and development of tubulin inhibitors
- Tufts Center for the Study of Drug Development

U

- Universal flu vaccine

V

- Venoms in medicine

- Virtual screening

Pages in category "Medicinal chemistry"

The following pages are in this category, out of 73 total. This list may not reflect recent changes

- Medicinal chemistry

A

- ADME
- Agonist
- Antitarget
- Applicability domain

B

- Binding coefficient
- BindingDB
- Bioavailability
- Bioisostere
- Jeannette Brown

C

- Center for Pharmaceutical Research and Innovation
- Cholinergic crisis
- Clinical pharmaceutical chemistry
- Conformation–activity relationship
- List of cosmetic ingredients
- Craig plot

D

- Dienyl radical
- Discovery and development of phosphodiesterase 5 inhibitors
- Docking (molecular)
- Drug carrier
- Drug class
- Drug design
- Druglikeness

E

- Endogenous agonist
- Enzyme inducer
- Enzyme inhibitor
- Enzyme repressor
- Extrafarma

F

- First pass effect
- Matthew Fuchter

G

- Galenic formulation
- Glycorandomization
- Grimm's hydride displacement law

H

- Host-directed therapeutics

I

- Immunomodulatory imide drug

L

- Lead compound
- LeDock
- Ligand efficiency
- LigandScout
- Lipinski's rule of five
- Lipophilic efficiency

M

- Mechanism of action
- Mode of action
- Molecular binding
- Molecular oncology
- Peggoty Mutai

N

- New chemical entity
- Nocebo

P

- Parallel artificial membrane permeability assay
- Pan-assay interference compounds
- Partition coefficient
- Peng Sixun
- Pharmaceutical formulation
- Pharmacodynamics
- Pharmacophore
- Pharmacy
- Photopharmacology
- Polar surface area
- Prototype drug

Q

- Quantitative structure–activity relationship

R

- Radiopharmaceutical

- Radiopharmacology
- Research chemical

S

- Scale (chemistry)
- Semisynthesis
- Structure–activity relationship

T

- Targeted covalent inhibitors
- Targeted drug delivery
- Therapy
- Toxicokinetics

V

- Venoms in medicine
- Anabella Villalobos

Y

- Wendy Young

Media in category "Medicinal chemistry"

The following 10 categories are in this category, out of 10 total.

- Biological Target Prediction of Bioactive Molecules Based on Minimum Structures Identification.

DNA gyrase and topoisomerase IV identification in 4-pyridone group.

Large ribosomal subunit identification in (1R)-propanol group.

Large ribosomal subunit identification in 3-glutarimidyl group.

Large ribosomal subunit identification in cytosine group.

Small ribosomal subunit identification in (4aRS,5aRS)-Sancycline group.

Small ribosomal subunit identification in 2,4(or 5)-diaminocyclohexanol group.

Sterol 14α-demethylase identification in 1,2,4-triazol-1-yl group.

Sterol 14α-demethylase identification in imidazol-1-yl group.

Target prediction process.

Subcategories

This category has the following 8 subcategories, out of 8 total.

A

- ► Anti-infective agents

C

- ► Chemical substances for emergency medicine

D

- ► Drugs

H

- ► Human drug metabolites

M

- ► Medical isotopes

P

- ► PET radiotracers
- ► Prodrugs

R

- ► Radiopharmaceuticals

Pages in category "Chemicals in medicine"

The following 6 pages are in this category, out of 6 total. This list may not reflect recent changes (learn more).

I

- Isotopes in medicine

M

- Metacresol purple
- Metals in medicine

P

- PET radiotracer
- Medication

R -Radiopharmaceutical

In The Path of Drug Discovery

Discovery is the identification of novel active chemical compounds, often called "hits", which are typically found by assay of compounds for a desired biological activity.[4] Initial hits can come from repurposing existing agents toward a new pathologic processes,[5] and from observations of biologic effects of new or existing natural products from bacteria, fungi,[6] plants,[7] etc. In addition, hits also routinely originate from structural observations of small molecule "fragments" bound to therapeutic targets (enzymes, receptors, etc.), where the fragments serve as starting points to develop more chemically complex forms by synthesis. Finally, hits also regularly originate from *en-masse* testing of chemical compounds against biological targets, where the compounds may be from novel synthetic chemical libraries known to have particular properties (kinase inhibitory activity, diversity or drug-likeness, etc.), or from historic chemical compound collections or libraries created through combinatorial chemistry. While a number of approaches toward the identification and development of hits exist, the most successful techniques are based on chemical and biological intuition developed in team environments through years of rigorous practice aimed solely at discovering new therapeutic agents.

Hit to lead and lead optimization

Further chemistry and analysis is necessary, first to identify the "triage" compounds that do not provide series displaying suitable SAR and chemical characteristics associated with long-term potential for development, then to improve remaining hit series with regard to the desired primary activity, as well as secondary activities and physiochemical properties such that the agent will be useful when administered in real patients. In this regard, chemical modifications can improve the recognition and binding geometries (pharmacophores) of the candidate compounds, and so their affinities for their targets, as well as improving the physicochemical properties of the molecule that underlie necessary pharmacokinetic/pharmacodynamic (PK/PD), and toxicologic profiles (stability toward metabolic degradation, lack of geno-, hepatic, and cardiac toxicities, etc.) such that the chemical compound or biologic is suitable for introduction into animal and human studies.

Process chemistry and development

The final synthetic chemistry stages involve the production of a lead compound in suitable quantity and quality to allow large scale animal testing, and then human clinical trials. This involves the optimization of the synthetic route for bulk industrial production, and discovery of the most suitable drug formulation. The former of these is still the bailiwick of medicinal chemistry, the latter brings in the specialization of formulation science (with its components of physical and polymer chemistry and materials science). The synthetic chemistry specialization in medicinal chemistry aimed at adaptation and optimization of the synthetic route for industrial scale syntheses of hundreds of kilograms or more is termed process synthesis, and involves thorough knowledge of acceptable synthetic practice in the context of large scale reactions (reaction thermodynamics, economics, safety, etc.). Critical at this stage is the transition to more stringent GMP requirements for material sourcing, handling, and chemistry.

Synthetic analysis

The synthetic methodology employed in medicinal chemistry is subject to constraints that do not apply to traditional organic synthesis. Owing to the prospect of scaling the preparation, safety is of paramount importance. The potential toxicity of reagents affects methodology.[3][8]

Structural analysis

The structures of pharmaceuticals are assessed in many ways, in part as a means to predict efficacy, stability, and accessibility. Lipinski's rule of five focus on the number of hydrogen bond donors and acceptors, number of rotatable bonds, surface area, and lipophilicity. Other parameters by which medicinal chemists assess or classify their compounds are: synthetic complexity, chirality, flatness, and aromatic ring count.

☑

Structural analysis of lead compounds is often performed through computational methods prior to actual synthesis of the ligand(s). This is done for a number of reasons, including but not limited to: time and financial considerations (expenditure, etc.). Once the ligand of interest has been synthesized in the laboratory, analysis is then performed by traditional methods (TLC, NMR, GC/MS, and others).

Training

Medicinal chemistry is by nature an interdisciplinary science, and practitioners have a strong background in organic chemistry, which must eventually be coupled with a broad understanding of biological concepts related to cellular drug targets. Scientists in medicinal chemistry work are principally industrial scientists (but see following), working as part of an interdisciplinary team that uses their chemistry abilities, especially, their synthetic abilities, to use chemical principles to design effective therapeutic agents. The length of training is intense, with practitioners often required to attain a 4-year bachelor's degree followed by a 4-6 year Ph.D. in organic chemistry. Most training regimens also include a postdoctoral fellowship period of 2 or more years after receiving a Ph.D. in chemistry, making the total length of training range from 10-12 years of college education. However, employment opportunities at the Master's level also exist in the pharmaceutical industry, and at that and the Ph.D. level there are further opportunities for employment in academia and government. Many medicinal chemists, particularly in academia and research, also earn a Pharm.D (doctor of pharmacy). Some of these PharmD/PhD researchers are RPhs (Registered Pharmacists).

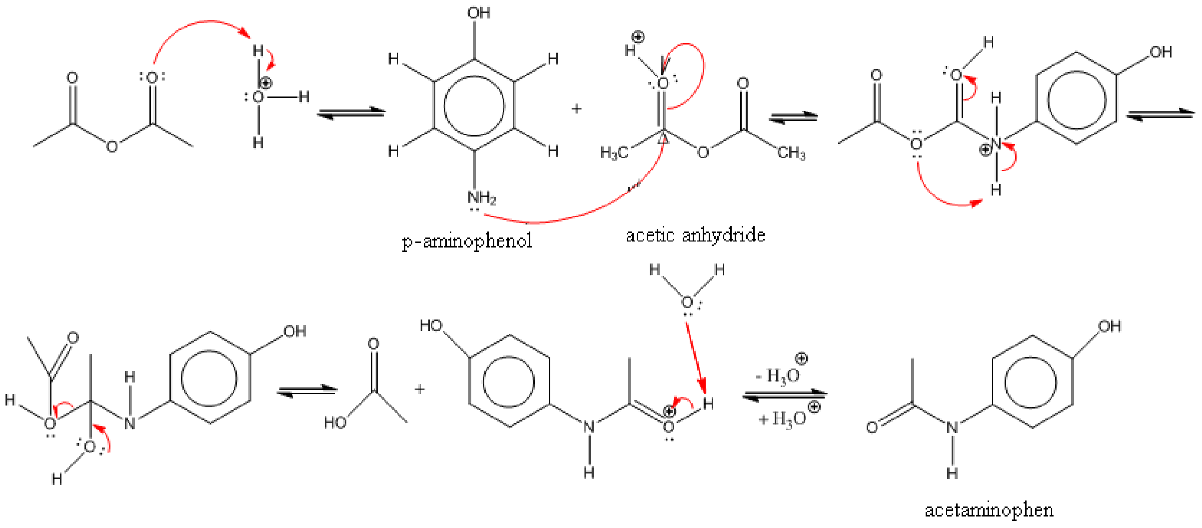
Graduate level programs in medicinal chemistry can be found in traditional medicinal chemistry or pharmaceutical sciences departments, both of which are traditionally associated with schools of pharmacy, and in some chemistry departments. However, the majority of working medicinal chemists have graduate degrees (MS, but especially Ph.D.) in organic chemistry, rather than medicinal chemistry,[9] and the preponderance of positions are in discovery, where the net is necessarily cast widest, and most broad synthetic activity occurs.

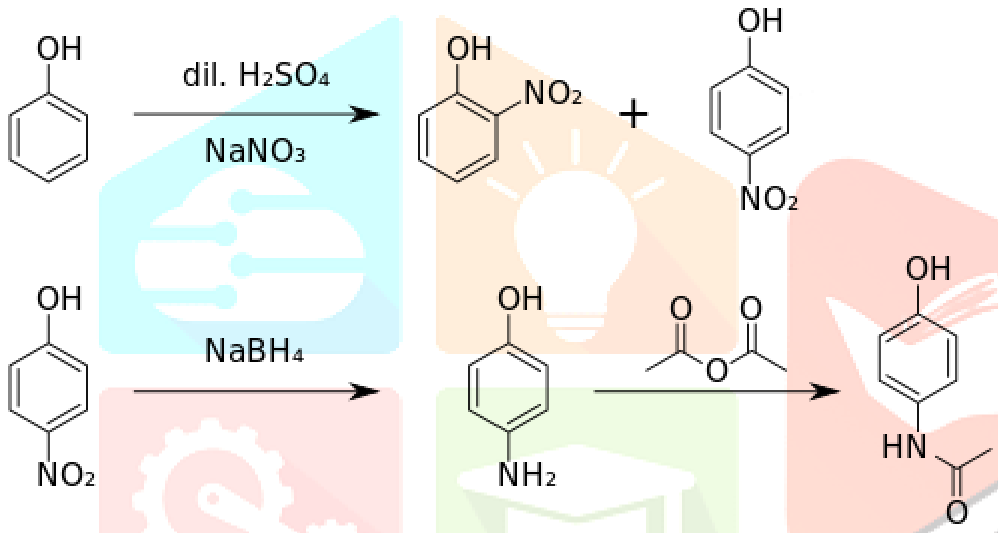
In discovery of small molecule therapeutics, an emphasis on training that provides for breadth of synthetic experience and "pace" of bench operations is clearly present (e.g., for individuals with pure synthetic organic and natural products synthesis in Ph.D. and post-doctoral positions, ibid.). In the medicinal chemistry specialty areas associated with the design and synthesis of chemical libraries or the execution of process chemistry aimed at viable commercial syntheses (areas generally with fewer opportunities), training paths are often much more varied (e.g., including focused training in physical organic chemistry, library-related syntheses, etc.).

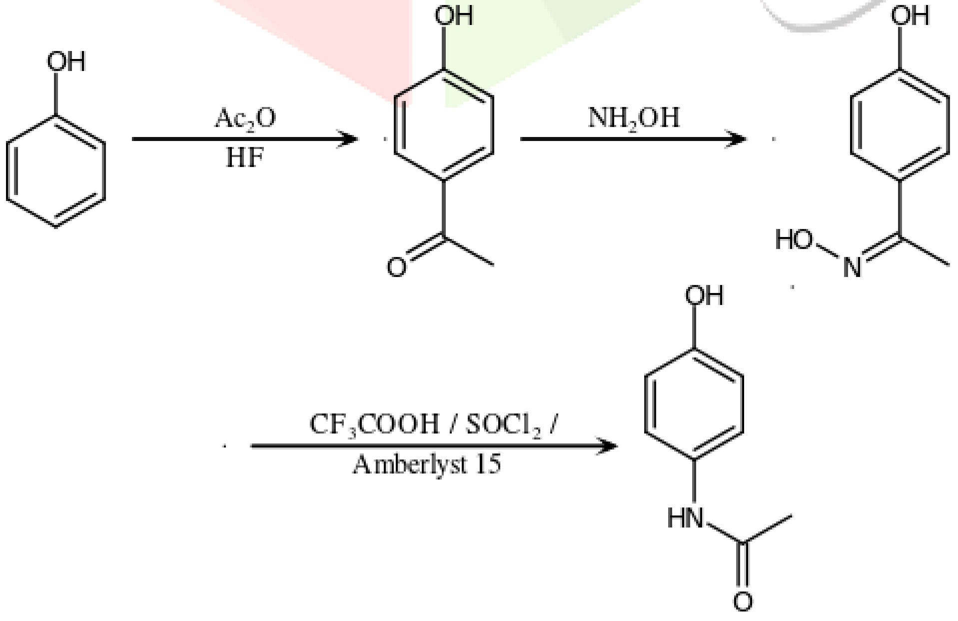
As such, most entry-level workers in medicinal chemistry, especially in the U.S., do not have formal training in medicinal chemistry but receive the necessary medicinal chemistry and pharmacologic background after employment—at entry into their work in a pharmaceutical company, where the company provides its particular understanding or model of "medichem" training through active involvement in practical synthesis on therapeutic projects. (The same is somewhat true of computational medicinal chemistry specialties, but not to the same degree as in synthetic areas.

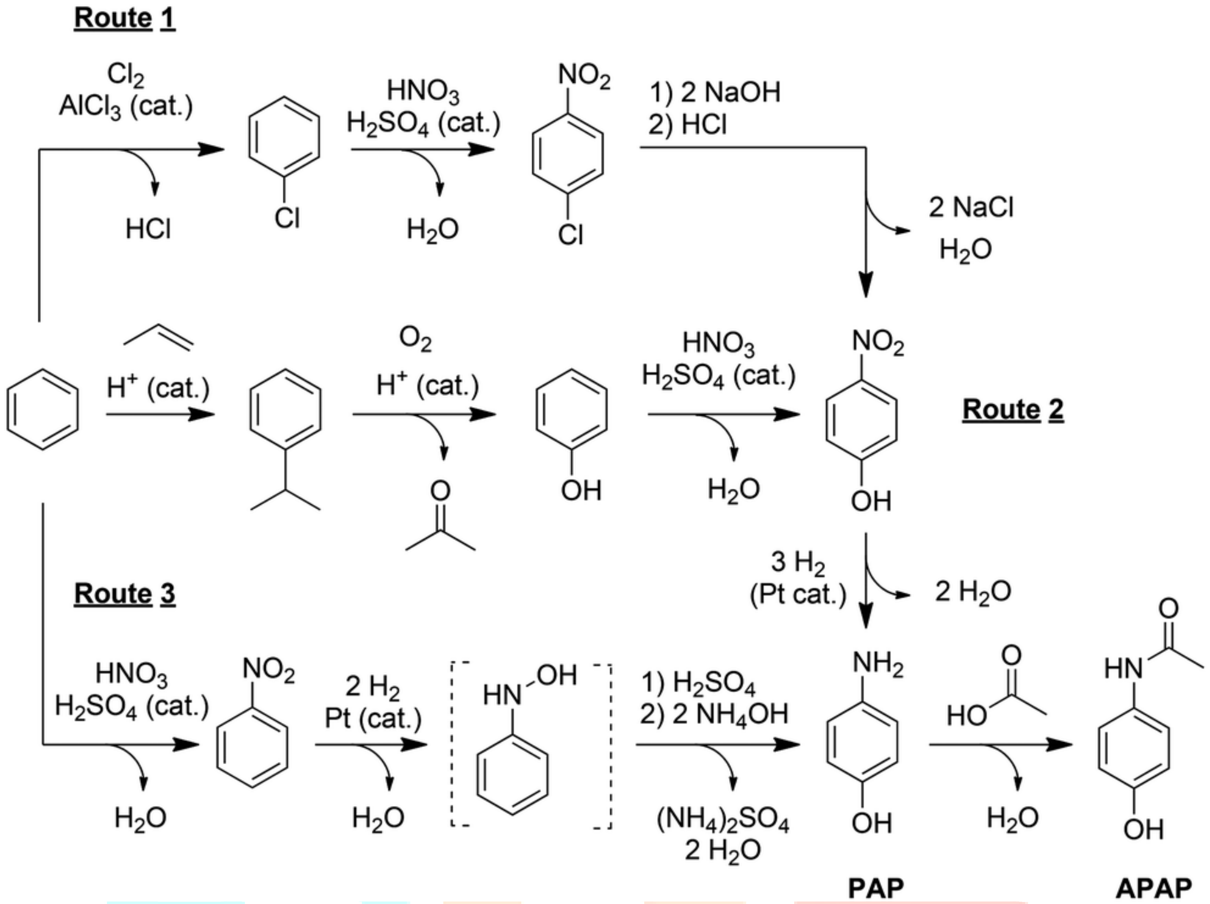
O. Although Common synthetic Routes Of Common Drugs like paracetamol and Ibuprofen and discussion in the light of green chemistry Where Atom economy, E -factor is also matter of concern, modern drug design is based on Host guest and MCR:

1.Synthetic routes of paracetamol





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Cl2
AlCl3 (cat.)
HCl
Cl
HNO3
H2SO4 (cat.)
H2O
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1) 2 NaOH
2) HCl
2 NaCl
H2O
H+ (cat.)
O2
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O
OH
HNO3
H2SO4 (cat.)
H2O
NO2
OH
Route 2
3 H2
(Pt cat.)
2 H2O
Route 3
HNO3
H2SO4 (cat.)
H2O
NO2
2 H2
Pt (cat.)
H2O
HN
OH
1) H2SO4
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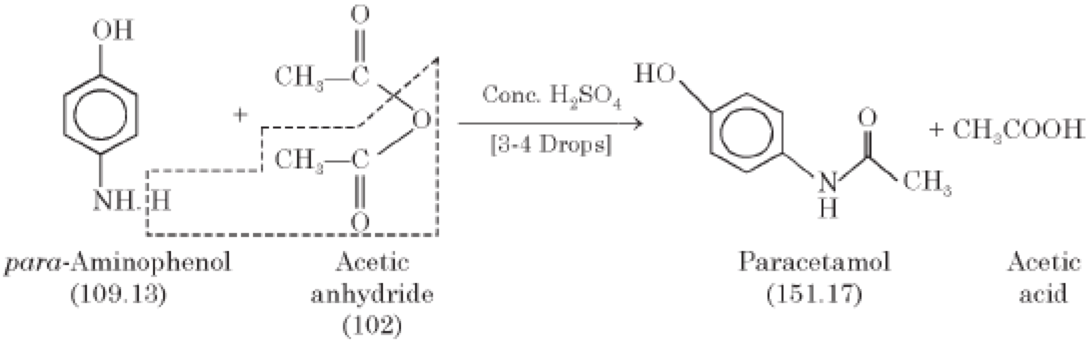
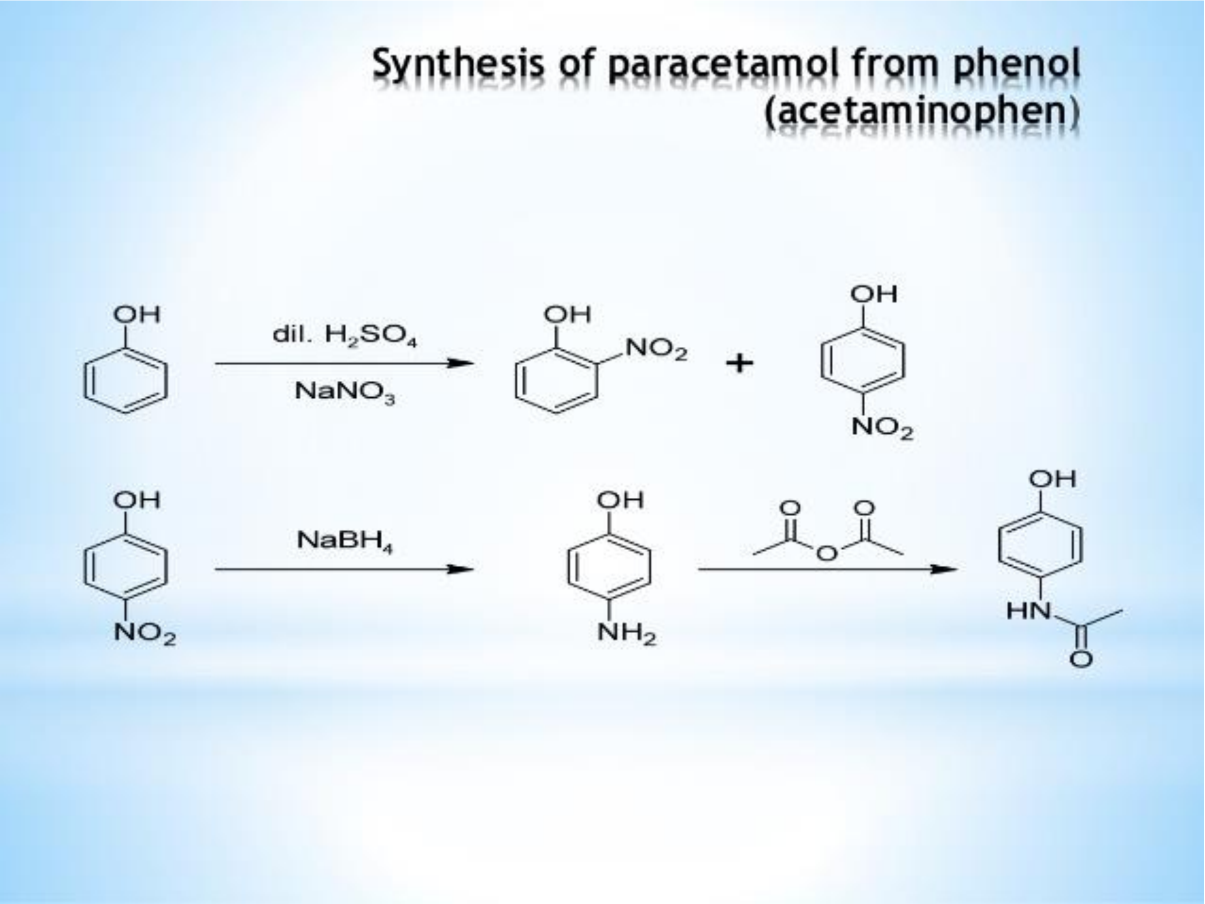
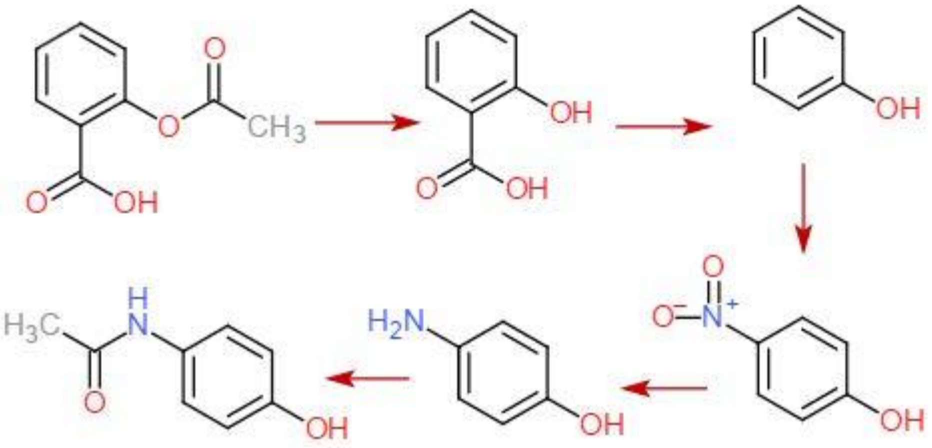
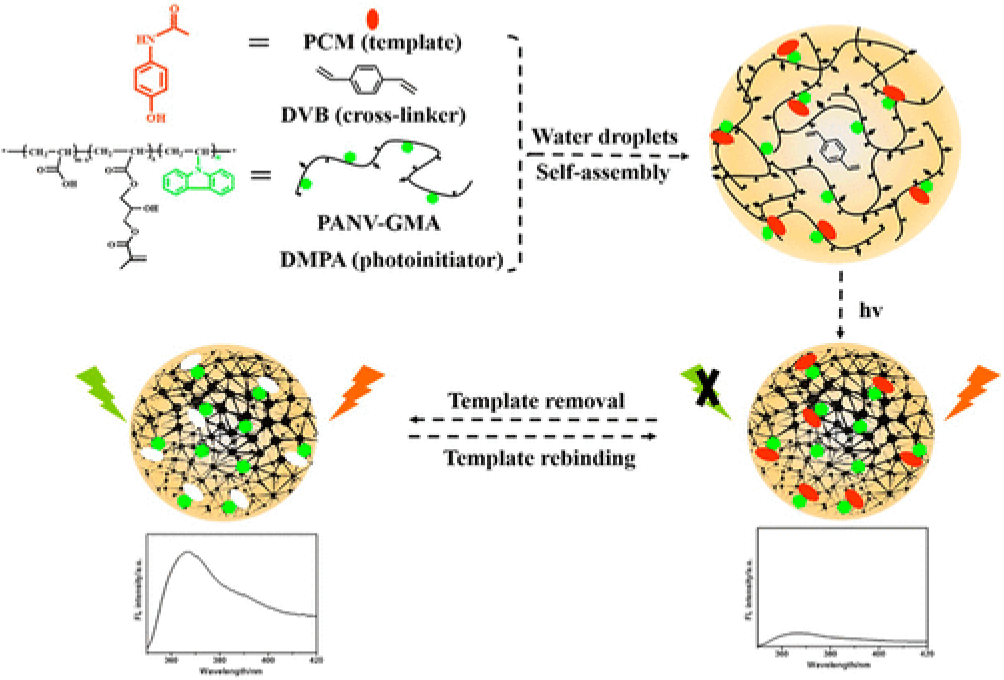

OH
NH. H
O
CH3—C
O
CH3—C
O
+
Conc. H2SO4
[3-4 Drops]
HO
O
N
H
CH3
+ CH3COOH
para-Aminophenol
(109.13)
Acetic
anhydride
(102)
Paracetamol
(151.17)
Acetic
acid



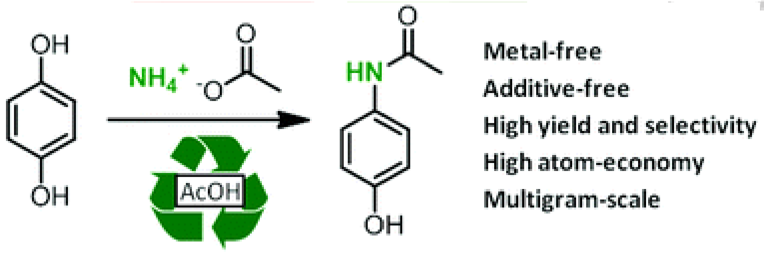
Synthesis of paracetamol from phenol (acetaminophen)

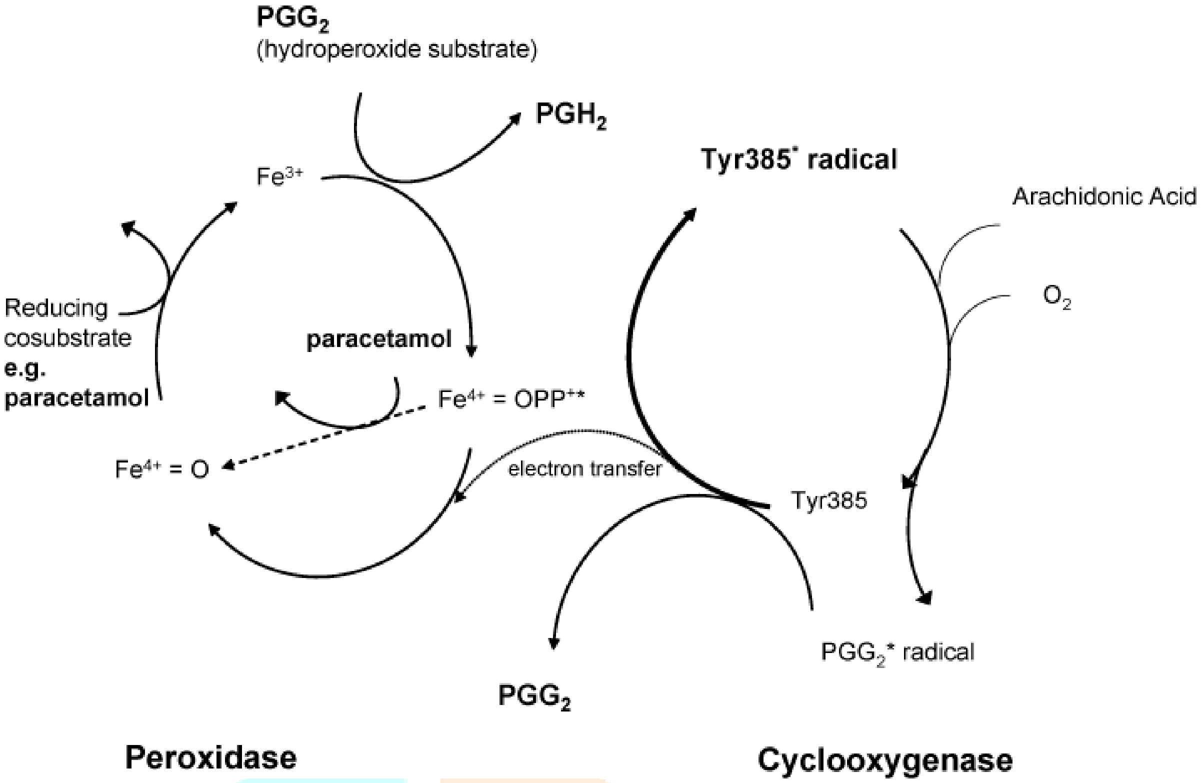


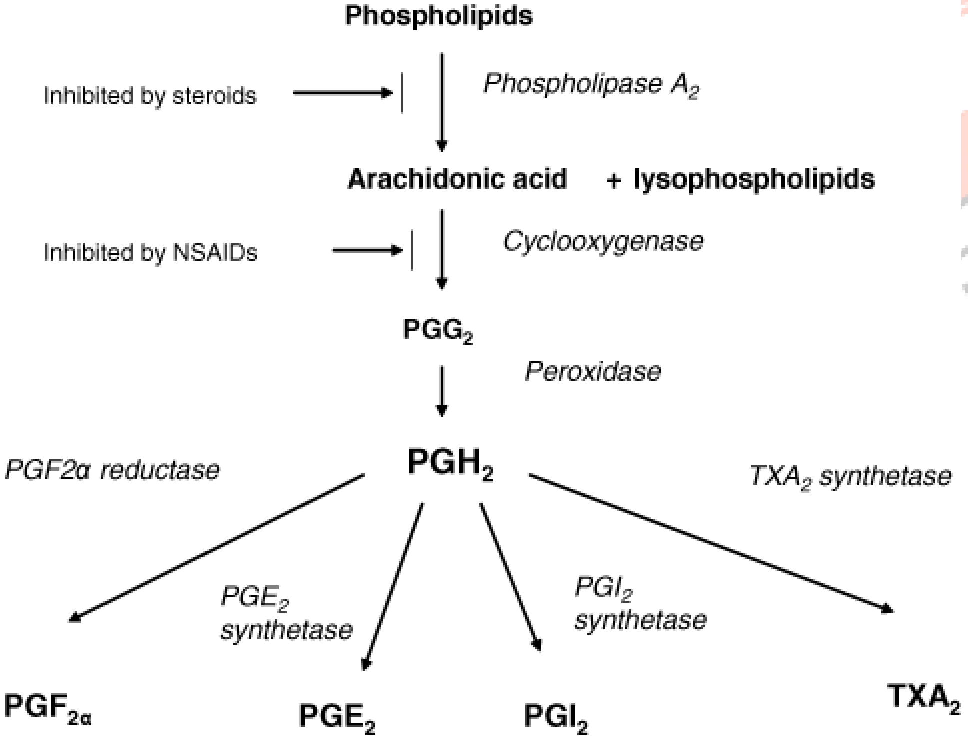


1a. Green Synthesis of Water-Compatible Fluorescent Molecularly Imprinted Polymeric Nanoparticles for Efficient Detection of Paracetamol

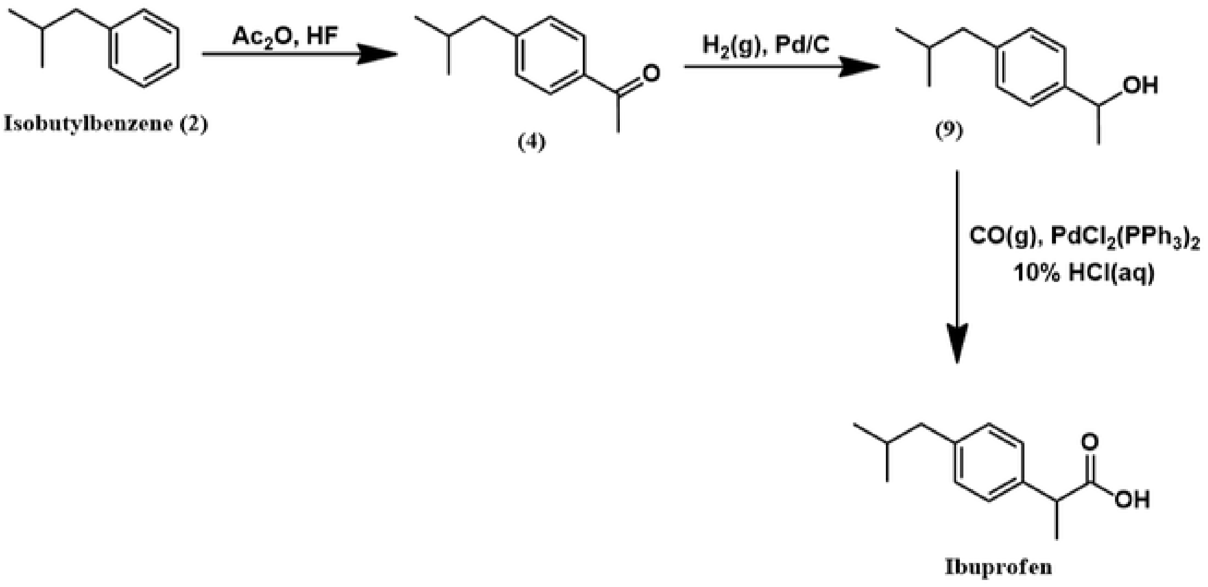
In this work, one-pot green synthesis of a novel kind of water-compatible fluorescent molecularly imprinted polymer nanoparticle for selective optosensing of paracetamol was developed via macromolecular assembly of an amphiphilic fluorescent copolymer and in situ photo-cross-linking. Amphiphilic photo-cross-linkable and fluorescent copolymers containing carbazole groups were synthesized and could coassemble with paracetamol (PCM, template molecule) and photoinitiator in aqueous solution. The obtained photo-cross-linkable fluorescent nanoparticles were then cross-linked triggered by UV-irradiation, generating the hydrophilic and fluorescent molecularly imprinted polymer nanoparticles (FMIP nanoparticles) in aqueous media. The whole procedure was carried out at mild working condition, which is facile, green, and energy-saving. The resulting FMIP nanoparticles showed high selectivity toward PCM and obvious fluorescence quenching, induced by template-binding, in water and were a kind of efficient fluorescent chemosensor for the sensing of PCM. A wide linear range over PCM concentration from 4 to 1000 μM with a detection limit of 1.0 μM has been demonstrated using FMIP nanoparticles as chemosensor. Moreover, a rapid response of less than 2 min has been demonstrated. Finally, such a chemosensor based on FMIP nanoparticles was also successfully employed for the detection of PCM in commercial PCM tablets as well as urine samples.

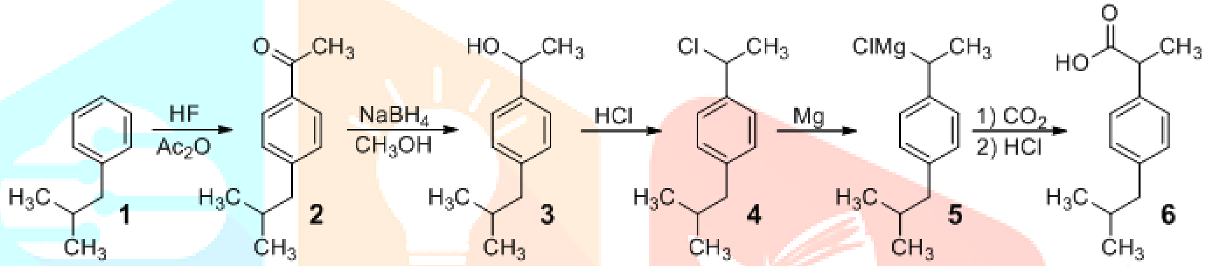






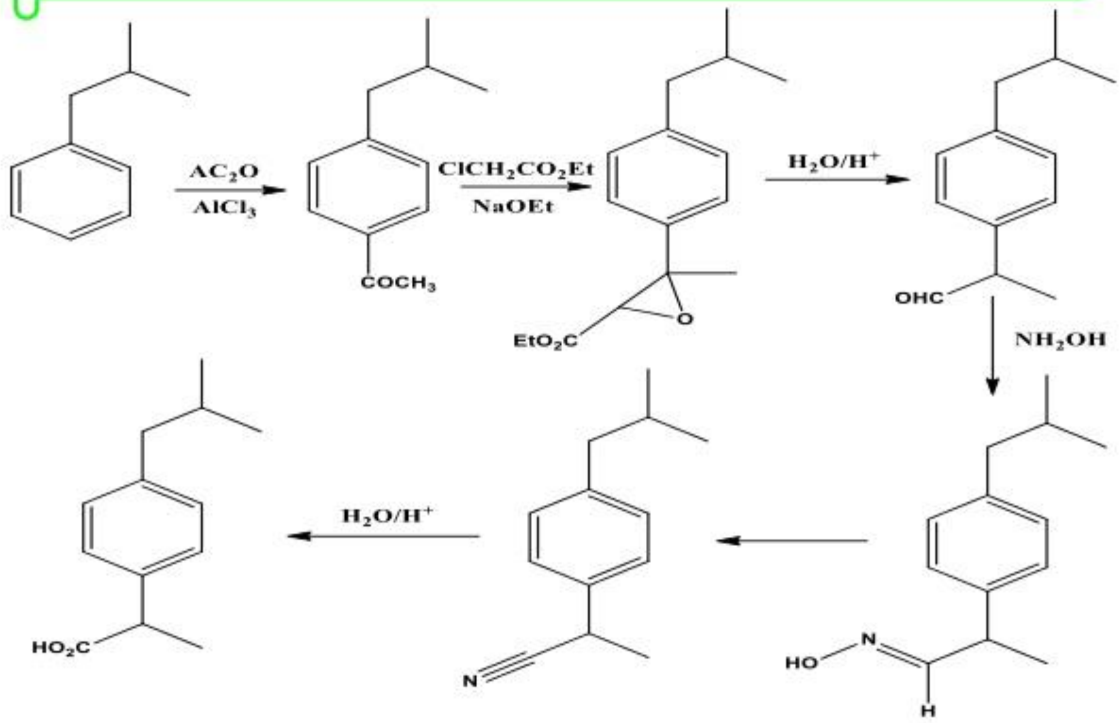
2.Synthesis Of Ibuprofen:



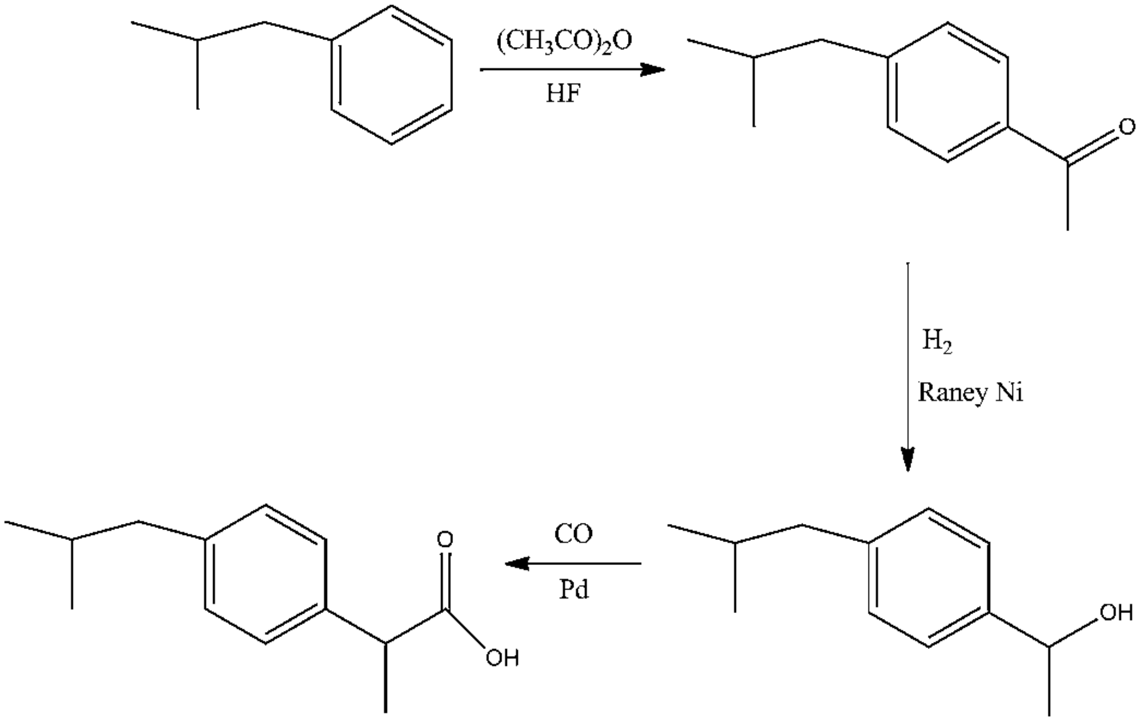


Scheme 2. Synthesis of ibuprofen

Old route for the preparation of Ibuprofen



SUSTAINABLE/GREEN SYNTHESIS



method for synthesizing ibuprofen. A previously published green synthetic route to ibuprofen

3.The twelve principles of Green Chemistry:

1. Prevention Try not to make waste, then you do not have to clean it up.

2. Atom economy The final product should aim to contain all the atoms used in the process.

3. Less hazardous chemical synthesis Wherever it is possible, production methods should be designed to make substances that are less toxic to people or the environment.

4. Designing safer chemicals Chemical products should be designed to do their job with minimum harm to people or the environment.

5. Safer solvents When making materials try not to use solvents or other unnecessary chemicals. If they are needed then they should not be harmful to the environment in any way.

6. Design for energy efficiency The energy needed to carry out a reaction should be minimized to reduce environmental and economic impact. If possible, processes should be carried out at ambient temperatures and pressures.

7. Use of renewable feedstocks A raw material should be renewable wherever possible.

8. Reduce derivatives Try not to have too many steps in the reaction because this means more reagents are needed and more waste is made.

9. Catalysis Reactions that are catalysed are more efficient than un catalysed reactions.

10. Design for degradation When chemical products are finished with, they should break down into substances that are not toxic and do not stay in the environment.

11. Real-time analysis for pollution prevention Methods need to be developed so that harmful products are detected before they are made.

12. Inherently safer chemistry for accident prevention Substances used in a chemical process should be chosen to minimise the risk of chemical accidents, including explosions and fire.

4.ATOM ECONOMY: A Measure of the Efficiency of a Reaction

Efficiency of a Reaction

Percentage Yield

Although the efficiency of a reaction can be measured in many ways, by far the most common way is to calculate the yield (percentage yield). Students are often required, especially in laboratory, to determine the theoretical yield based upon the limiting reagent and then to calculate the percentage yield based upon the ratio of the actual yield/theoretical yield X 100. In general organic chemists consider yields of 90% or better as excellent while 20% or less are poor.

Theoretical yield = (moles of limiting reagent)(stoichiometric ratio; desired product/limiting reagent)(MW of desired product)

Percentage yield= (actual yield/theoretical yield) X 100

In order to illustrate the calculation of the percentage yield (and the measurement of the efficiency of a reaction) consider the following acid promoted nucleophilic substitution reaction. A typical procedure[1] for this reaction begins with dissolving

Equation 1a

$$\underset{1}{CH_3CH_2CH_2CH_2OH} + \underset{2}{NaBr} + \underset{3}{H_2SO_4} \longrightarrow \underset{4}{CH_3CH_2CH_2CH_2Br} + \underset{5}{NaHSO_4} + \underset{6}{H_2O}$$

1.33 g of sodium bromide (2) in 1.5 mL of water, followed by addition of 0.80 mL of 1-butanol (1) and 1.1 mL (2.0 g) of concentrated sulfuric acid (3). The following Reagents Table (Table 1) and Desired Product Table (Table 2) can then be

Table 1 Reagents Table

Reagent	MW	Weight Used (g)	Moles Used	Theoretical Moles Needed	Density	Bp (°C)
1 C_4H_9OH	74.12	0.80	0.0108	0.0108	0.810	118
2 NaBr	102.91	1.33	0.0129	0.0108		
3 H_2SO_4	98.08	2.0	0.0200	0.0108	1.84	

Table 2 Desired Product Table

Compound	MW	Theoretical Yield (Moles)	Theoretical Yield (Grams)	Actual Yield (Grams)	% Yield	Density	Bp (°C)

4 C_4H_9Br	137.03	0.011	1.48 (100%)	1.20	81	1.275	101.6

setup. Dividing the weight of each reactant that is used, by the molecular weight of the reactant, gives the number of moles of each reagent used. From the stoichiometry of the reaction (Equation 1a) it is clear that one mole of each reactant is required to produce one mole of product (1-bromobutane) and since 1-butanol (0.0108 mole) is used in the smallest amount it is the limiting reagent. Calculation of the (as shown below) theoretical yield of 1-bromobutane gives 1.48 g. This means that using the

Theoretical yield = (moles of limiting reagent)(stoichiometric ratio; desired product /limiting reagent)(MW of desired product)
= (moles of 1-butanol)(stoichiometric ratio; 1-bromobutane/1-butanol)(MW of 1-bromobutane)
=(0.0180 mole)(1 mole / 1 mole)(137.03 g/mole)
=1.48 g

above quantities of reagents the maximum amount (assuming 100% yield) of 1-bromobutane that can be produced is 1.48 g. In fact no reaction ever proceeds with 100% yield due to such factors as the formation of side products, incomplete conversion of the starting materials, loss upon workup of the reaction mixture, and loss upon isolation and purification of the desired product. This reaction typically produces actual yields of 1-1.2 g. Assuming that the actual yield is 1.20 g calculation of the % yield is as follows. Thus 81% of the theoretical yield is actually isolated, which is a very respectable yield, that would please most chemists.

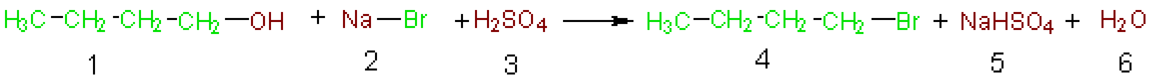
Percentage yield= (actual yield/theoretical yield) X 100
= (1.20 g/1.48 g) X 100 = 81%

Atom Economy in a Substitution Reaction

As indicated previously most chemists have traditionally measured the efficiency of a reaction by the percentage yield, however this only tells part of the story. If one considers the above reaction where a total of 4.13 g of reactants (0.8 g of 1-butanol, 1.33 g of NaBr and 2.0 g of H_2SO_4) was used, and that at best this reaction will only yield 1.48 g of the desired product, the question might be asked "what happens to the bulk (4.13 g -1.48 g = *2.7 g*) of the mass of reactants?". The answer is they end up in side products ($NaHSO_4$ and H_2O) that may be unwanted, unused, toxic and/or not recycled/reused. The side products are oftentimes treated as wastes and must be disposed of or otherwise treated. At best only 36% (1.48 g/4.13 g X 100) of the mass of the reactants end up in the desired product. If the actual yield is 81% then only 29% (.81 X .36 X 100) of the mass of the reactants actually ends up in the desired product!

In an effort to foster awareness of the atoms of reactants that are incorporated into the desired product and those that are wasted (incorporated into undesired products), Barry Trost developed the concept of ***atom economy***.[2] In 1998 Trost was awarded a Presidential Green Chemistry Challenge Award for the concept of atom economy. In light of the concept of atom economy, the above acid promoted nucleophilic substitution must now be reconsidered. In Equation 1b we have illustrated the atom economy of this reaction by showing all of the reactant atoms that are incorporated into the desired product in green,

Equation 1b



while those that are wasted are shown in brown. Likewise the atoms of the desired product are in green and the atoms composing the unwanted products are in brown. Table 3 provides another view of the atom economy of this reaction. In columns 1 and 2 of this table, the formulas and formula weights (FW) of the reactants are listed. Shown in green (columns 3 and 4) are the atoms and weights of the atoms of the reactants that are incorporated into the desired product (4), and shown in brown (columns 5 and 6) are the atoms and weights of atoms of the reactants that end up in unwanted side products. Focusing on the last row of this table it can be seen that of all the atoms of the reactants (4C, 12H, 5O, 1Br, 1Na and 1S) only 4C, 9H, and 1Br are utilized in the desired product and the bulk (3H, 5O, 1Na, 1S) are wasted as components of unwanted products. This is an example of poor atom economy! A logical extension of Trost's concept of atom economy is to calculate

Table 3 Atom Economy of Equation 1

Reagents Formula	Reagents FW	Utilized Atoms	Weight of Utilized Atoms	Unutilized Atoms	Weight of Unutilized Atoms
1 C_4H_9OH	74	4C,9H	57	HO	17
2 NaBr	103	Br	80	Na	23
3 H_2SO_4	98	——	0	2H,4O,S	98
Total 4C,12H,5O,BrNaS	275	4C,9H,Br	137	3H,5O,Na,S	138

the ***percentage atom economy***.[3] This can be done by taking the ratio of the mass of the utilized atoms (137) to the total mass of the atoms of all the reactants (275) and multiplying by 100. As shown below this reaction has only 50% atom economy.

% Atom Economy = (FW of atoms utilized/FW of all reactants) X 100
= (137/275) X 100 = 50%

Thus at best (if the reaction produced 100% yield) then only half of the mass of the reactants would be incorporated into the desired product while the rest would be wasted in unwanted side products.

A. "E-Factor as a Green Chemistry Metric":

Throughout green chemistry, there are a number of ways to determine if one method of making a product is better than another. One such metric is called the E-Factor or the Environmental Impact Factor. The E-factor is the measure of the amount of a waste generated while making a product. Define E-factor E-factor = mass of waste ÷ mass of product Introduce the phrase "The Goal is Zero"

Manufacturing Processes We have established that chemists make things. The process by which they make things is just as important to think about as the actual product that they make. At the end of a process, you end up with your final product (what you intend to make), but waste is usually generated as well. As green chemists, we want to reduce or eliminate waste as much as possible, yet still make a good performing product (crown). Let's get started making your green chemistry crown. Introduce Today's Experiment & Safety: In this activity, we are working with safe materials which will not require you to wear gloves and safety glasses, but please practice safety when handling scissors and do not put small items in your mouth. As green chemists, today you will make your own "Green Chemistry Crowns" and learn how scientists determine how wasteful a process is using the Environmental Impact Factor (E-Factor). You will learn how to calculate the E-Factor of your crown making process.

5."Application of supramolecular chemistry in the Bio-world related to drug design strategy."

5a.Introductions:

Supramolecular chemistry refers to the study of supramolecular assemblies. Traditional chemistry generally focuses on the covalent bonding but supramolecular chemistry monitored by weak interactions of noncovalent bonds; exist extensively in numerous vital biological processes. The origin of supramolecular chemistry was from the living biological systems to some extent. The study of biological systems often provides the motivation behind supramolecular chemical research

5b. Applications in Biologically derived units:

(A) The extremely strong complexation between avidin and biotin is instrumental in blood clotting, and has been used as the recognition motif to construct synthetic systems.

(B) The binding of **enzymes** with their cofactors has been used as a route to produce modified enzymes, electrically contacted enzymes, and even photo switchable enzymes.

(C) DNA has been used as both a structural and functional unit in synthetic supramolecular systems. DNA has been used as both a structural and functional unit in synthetic supramolecular systems. The use of Supramolecular Chemistry principles of various biological macromolecules and processes. For instance, the important breakthrough that processes. For instance, the important breakthrough that allowed elucidation of the double – helical structure of DNA consists of two separate stands of nucleotides connected through Hydrogen- bonds by AGCT link i.e. Adenine, Guanine, Cytosine, and Thiamine. U=Uracil is extra component for RNA.

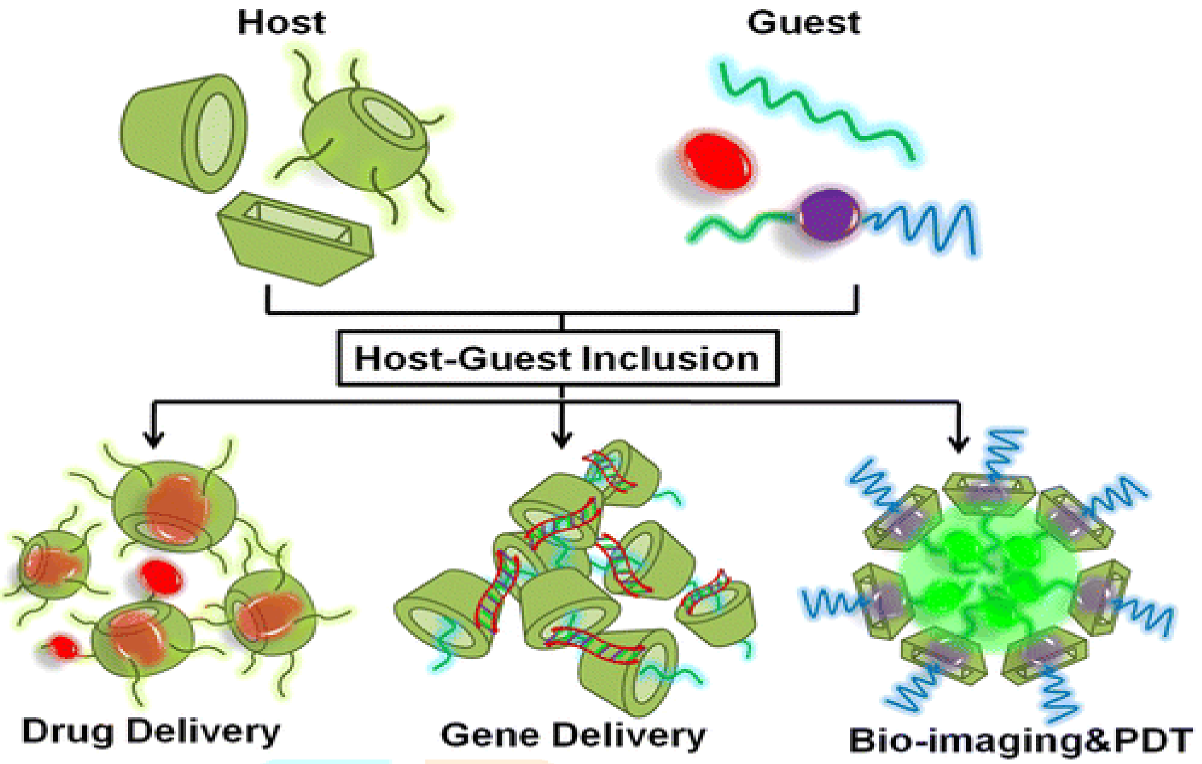
(D)Many synthetic supramolecular systems are derived to copy functions of biological systems. These biomimetic architectures can be used to learn about both the biological model and synthetic implementation. Examples include photoelectron chemical systems, catalytic systems, protein design and self-replications.

(E) The idea of supramolecular chemistry, has successfully explained the cause of Cancer cell development. Which will show the right path of new generation bio-drug discovery also for the treatment purposes.

(F) Nature(biological systems)-inspirations coming from the concept of life as a whole or total existence or 3D-shape consists of several cells in Biological creatures like animals, human beings itself could able to explain by the self-assembly process of supra molecular chemistry, which tells about the disorder systems of pre-existing components under thermodynamic equilibrium conditions organised into an ordered aggregate as consequence of non-covalent interactions among the components themselves, without external direction.

(G) Ultimately supra molecular materials have some unique features, some of them are (i) they are encoded with giga bytes of data, (ii) they can self-replicate,(iii) they are well built in error correction, (iv) they are information of storage. These unique features equate with the structures and functions of human brain.

5c. Biomedical Applications of Supramolecular Systems Based on Host–Guest Interactions:



Because of the above-mentioned advantages along with good biocompatibility or low toxicity of certain molecules and materials used, supramolecular systems have been widely utilized in the biological field. The recent research progress of self-assembly systems for the fabrication and application of bioinspired materials from the view of biomimetic chemistry.

Among various noncovalent interactions under the definition of supramolecular chemistry, host–guest interaction based on macrocyclic molecules is a very important phenomenon that has been extensively investigated. Through such host–guest inclusion, two or more chemical moieties can be integrated together in a facile and reversible manner, providing vast possibilities for the construction of novel supramolecular structures. During the past few decades, a series of macrocyclic molecules and their derivatives have been developed, including calixarenes (CAs), crown ethers, cyclodextrins (CDs), cellophanes, cucurbit[*n*]urils (CBs), pillar[*n*]arenes, and so on. These macrocyclic molecules are regarded as the hosts, possessing the cavities to encapsulate the guests. Usually, external property of the host molecules favours the interaction with surrounding solvent, while the internal features of their cavities facilitate the guest inclusion via hydrophobic interactions, hydrogen-bonding interactions, electrostatic interaction, specific molecular shape or size matching, etc. The most common case is to encapsulate hydrophobic guest molecules into hydrophobic cavities of macrocyclic molecules in aqueous solution. Such host–guest inclusion has relatively high stability, providing reliable and robust connection for the fabrication of supramolecular systems. For instance, the binding constant (K) for CD-based host–guest complexes can reach $10^4\ M^{-1}$, and that of CB-based host–guest complexes can be as high as $10^{15}\ M^{-1}$. Among these macrocyclic molecules, CAs, CDs, and CBs have attracted an increasing popularity, especially for their applications in biomedical field. One major reason is that these macrocyclic molecules are basically friendly to the ***biological environment and exhibit good biocompatibilities***. Another reason is that the host–guest complex formation based on these macrocyclic molecules is a facile and reversible process, which provides the feasibilities to design stimuli-responsive supramolecular systems.

Taking account of a large amount of scientific publications regarding the biological applications of supramolecular chemistry, this Review mainly discusses the fabrication and biomedical applications of supramolecular systems based on host–guest interactions. This interesting topic has gained a lot of attention on account of the specificity and versatility of the host–guest interactions. This Review covers three major macrocyclic host molecules, including CAs, CDs, and CBs. The specific biomedical applications of the host–

guest systems discussed contain several leading directions, that is, drug delivery, gene delivery, drug/gene code livery, bio imaging, and photodynamic therapy (PDT).

During recent years, only a few studies about the applications of simple host–guest complexes between CDs and drugs in biomedical field were reported. For instance, β-CD and its derivative, 2-hydroxypropyl-β-CD, were used to improve the water solubility of delta-8-tetrahydrocannabinol to enhance its ocular bioavailability. On the other hand, many new types of CD-based nanomaterials were reported by either using supramolecular self-assembly to form nano sized aggregates or combining with other functional materials to afford novel nano hybrids, which paved new ways for the exploration of CDs in biomedical applications. Among these supramolecular nano systems, the host–guest inclusion between CDs and drug molecules is still essential for the drug delivery. For example, anticancer drug paclitaxel (PTX) was encapsulated into β-CD. The host–guest complex (β-CD: PTX = 1:1) was further self-assembled into biodegradable capsules, which were used to treat breast and throat cancer cells.

5d. Conclusions:

Although the Supra molecular Chemistry is a multi-disciplinary field, it has wide nature (biological Systems) – inspiration and it is the basis of life. For its importance in the bio world this specific branch is developing fast in the thousand number of probable research fields and with its wide applications correlated with the modern world.

What is Supramolecular Chemistry?

MULTIDISCIPLINARY FIELD NATURE (biological systems) - inspiration ORGANIC and INORGANIC CHEMISTRY – building blocks (supramolecular synthons) PHYSICAL CHEMISTRY – methods to study and understand their properties

The ultimate supramolecular material?

(i)Held together by many specific hydrogen bonds, π - π stacking, etc. Encodes gigabytes of data

(II)Can Self-Replicate Built-in Error Correction

(III) Information Storage

(IV) Is the basis of life

But also:

Molecular Devices

Supramolecular Photochemistry

Electronic Switches

Dendrimers

Top-Down (current technology).

Continued reduction in size of bulk semiconductor devices

optical, ultra-violet, ion-beam, electron-beam lithography Bottom-Up (molecular scale electronics). Design of molecules with specific electronic function Design of molecules for self-assembly into supramolecular structures

Connecting molecules to the macroscopic world

Man-made synthesis (e.g. carbon nanotubes)

Top-Down (current technology).

Continued reduction in size of bulk semiconductor devices ,optical, ultra-violet, ion-beam, electron-beam lithography Bottom-Up (molecular scale electronics). , Design of molecules with specific electronic function . Design of molecules for self-assembly into supramolecular structures , Connecting molecules to the macroscopic world Man-made synthesis (e.g. carbon nanotubes)

Nature of Supramolecular Interactions Driving Forces for the Formation of Supramolecular Structures Covalent bond energies: C-O bond 340kJ / mol 1.43Å C-C bond 360kJ / mol 1.53Å C-H bond 430kJ / mol 1.11Å C=C bond 600kJ / mol 1.33Å C=O bond 690kJ / mol 1.21Å

Compared to most non-covalent interactions these are: • Very high energies • Very short distances Highly dependant on orientation hydrophobic interaction

Ion - Ion Interactions Can be a very strong bond - even stronger then covalent bonds in some cases. Can be an attractive or a repulsive force.

Non-directional force, Long range (1/r)

Highly dependant on the dielectric constant of the medium

Nature of Supramolecular Interactions : Halogen Bonding Halogen atoms iodine, bromine, chlorine and even fluorine can function as Lewis acids and engage in electron donor-acceptor interactions with atoms with lone pairs such as nitrogen, oxygen, phosphorus and sulfur. BI…X-

Halogen bonding may involve dihalogenes X2 and X-Y as well as organic halides

The strength of the donor-acceptor interaction depends on the polarizability of the halogen atom, decreases in the order: I > Br > Cl (> F)

Nature of Supramolecular Interactions : Van der Waals Forces

Strength of interaction is essentially a function of the surface area of contact. The larger the surface area the stronger the interaction will be.

Regardless of other interactions found within a complex there will almost always be a contribution from vdw. This is what drives molecules to eliminate spaces or vacuums and makes it difficult to engineer porous or hollow structures and gives rise to the phrase "Nature abhors a vacuum".

Nature of Supramolecular Interactions : CLOSE PACKING IN THE SOLID STATE Conformers allowing maximum intermolecular interactions, even very weak (which do not play a role in solution)

„Empty" space: crystal pores and channels -> inclusion crystals

TETRIS analogy

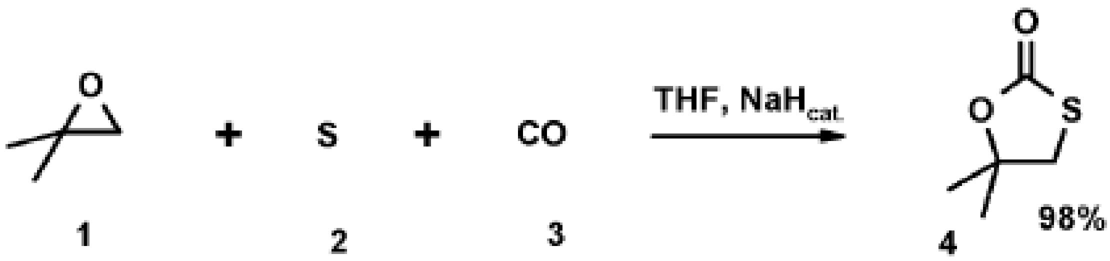
MOLECULAR CHEMISTRY – covalent bonds formation SUPRAMOLECULAR CHEMISTRY – "non-covalent bond" formation

J. M. Lehn: „Supramolecular chemistry is the chemistry of the intermolecular bond, covering the structures and functions of the entities formed by the association of two or more chemical species" F. Vögtle: „In contrast to molecular chemistry, which is predominantly based upon the covalent bonding of atoms, supramolecular chemistry is based upon intermolecular interactions, i.e. on the association of two or more building blocks, which are held together by intermolecular bond"

6. Even **Multicomponent Reactions (MCRs) could be utilized for the implementation of molecular hybridization for different drug design synthetic route:**

- **Multicomponent Reactions (MCRs) are general defined as reactions where more than two starting materials to react to form a product, incorporating essentially all of the atoms of the educts.**
- **Generally, there are different classification schemes of MCRs possible, e.g. according to the reaction mechanisms, the components involved, or the intrinsic variability.**

- **Variability of Multicomponent Reactions(1)**
- **For example, recently Sonoda et al. described a three component reaction (3-CR) as below:**



This reaction is not very useful to prepare large combinatorial libraries of compounds, since there is only one variable starting material, the epoxide, whereas the other two starting materials are fixed in all reactions. So this 3-CR constitutes a MCR of low variability.

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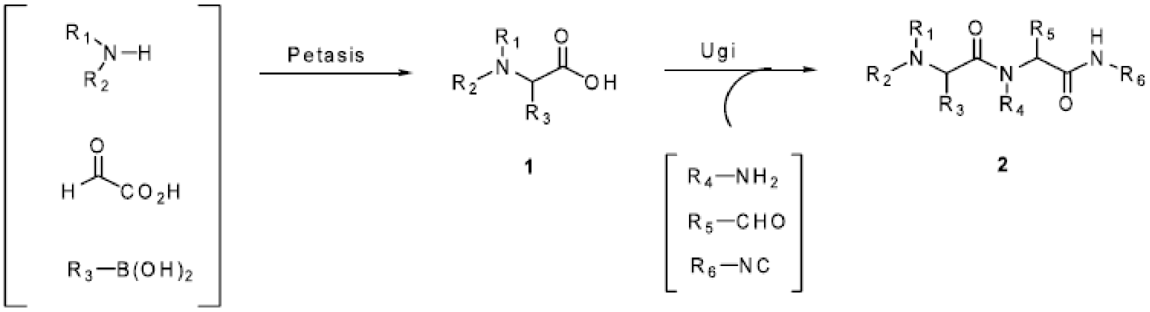
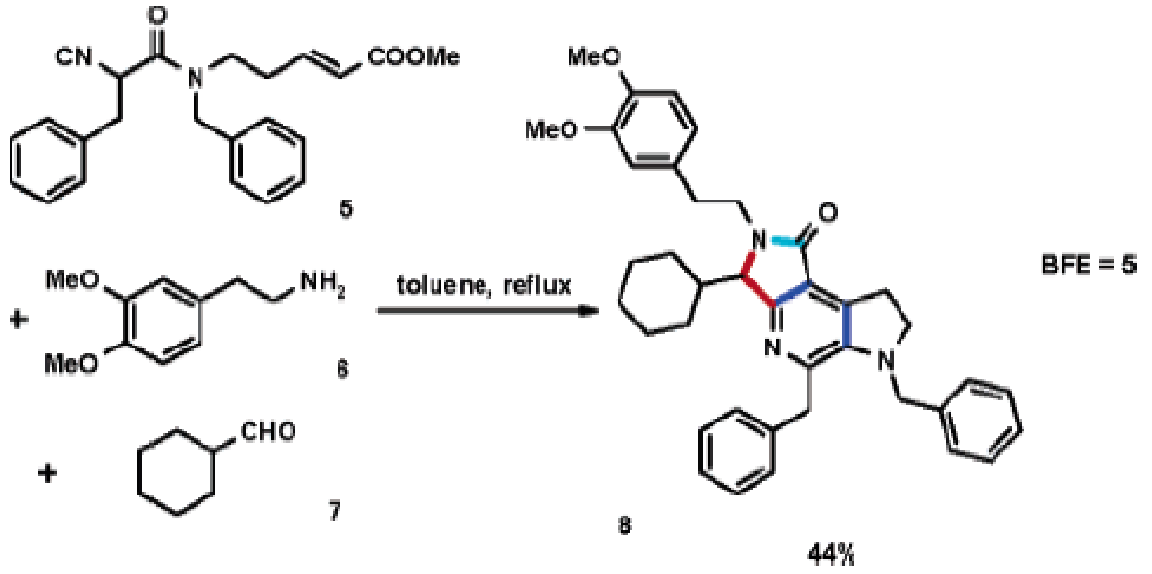
Variability of Multicomponent Reactions(2)

- **On the other hand, a recent publication introduces the union of two highly variable MCRs, the Petasis and Ugi reaction as below:**



-
- **Since both reactions use starting material which are commercially available in very large quantities. Theoretically, this combination of MCRs spans a chemical space of greater than 1000╳200╳500╳1000╳1000=10^{14} small molecules. This constitutes a combination of MCRs of very high variability.**

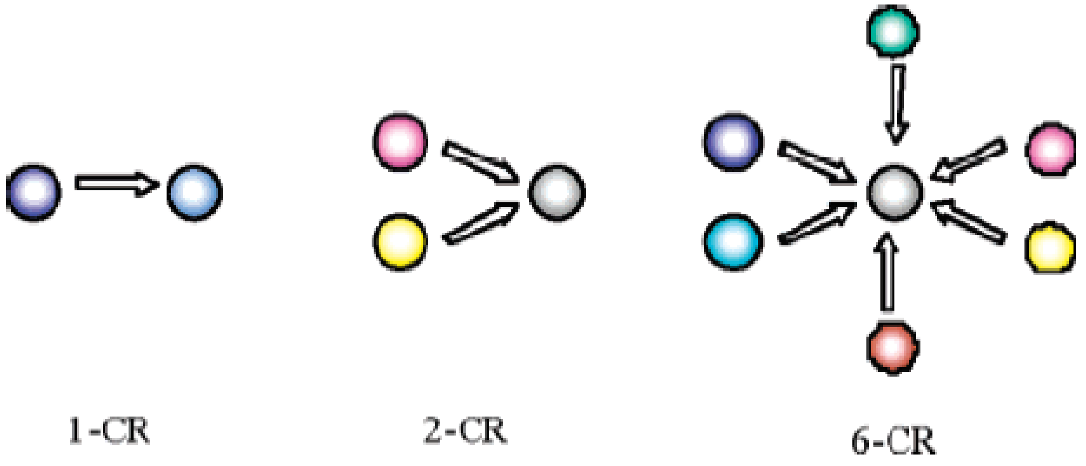
Efficiency of Multicomponent Reactions



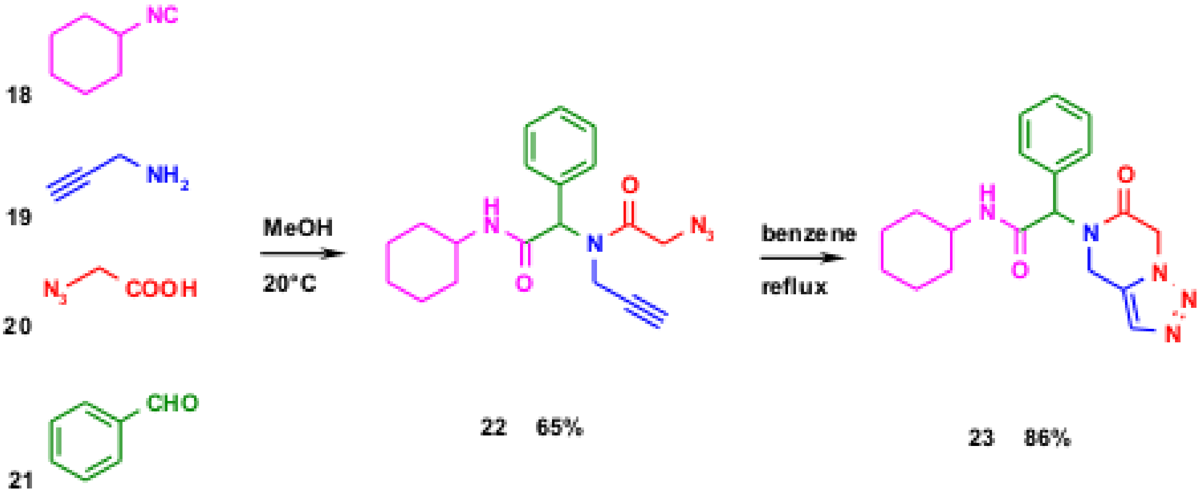
During this reaction, three C-C bonds and two C-N bonds are formed

Convergency of Multicomponent Reactions(1)

- **Unlike the usual stepwise formation of individual bonds in the target molecule, the utmost attribute of MCRs is the inherent formation of several bonds in one operation, ideally without isolating the intermediates, changing the reaction conditions, or adding further reagents.**
- **In a MCR, several starting materials assemble to form complex products. Thus, one can call MCRs convergent reactions, in analogy to a convergent synthesis and in contrast to a divergent multistep synthesis.**



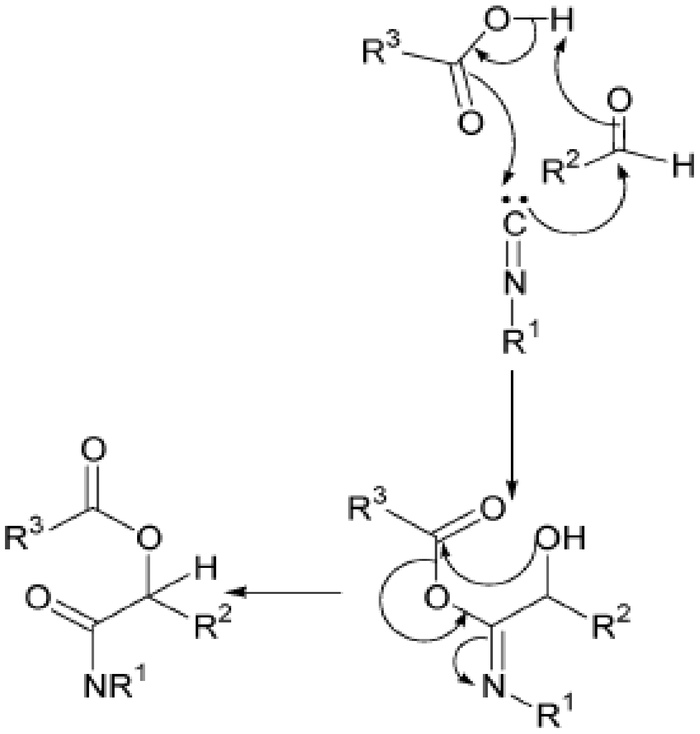
- **Convergency of Multicomponent Reactions(2) For example: Triazole-fused piperazines can be synthesized by the sequence U-4CR followed by [3+2] cycloaddition.**
- **For example: Triazole-fused piperazines can be synthesized by the sequence U-4CR followed by [3+2] cycloaddition.**



IMCRs

- **Hundreds of MCRs have been described over the years.**
- **Special subclasses are isocyanide based MCRs (IMCRs). They are particularly interesting because they are more versatile and diverse than the remaining MCRs.**
- **Today most MCRs chemistry performed with isoyanide relates to the classical reactions of Passerini and Ugi.**

Passerini and Passerini Reaction

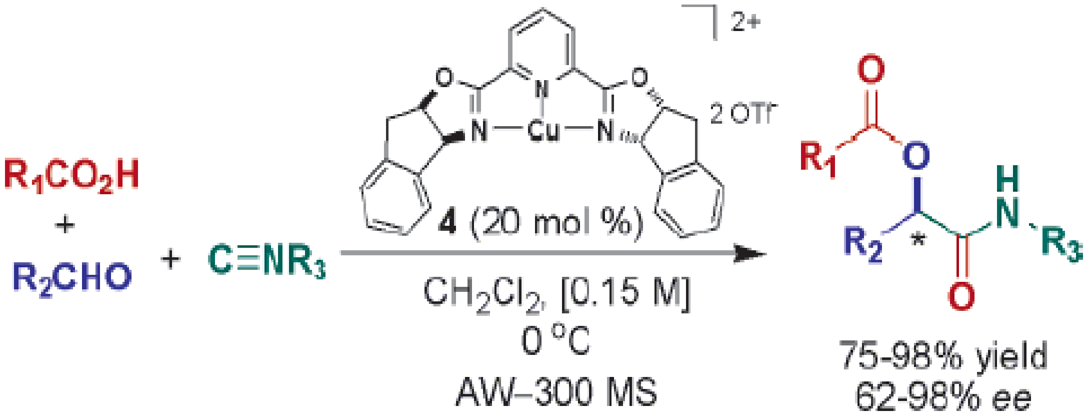


Mechanism of Passerini Reaction

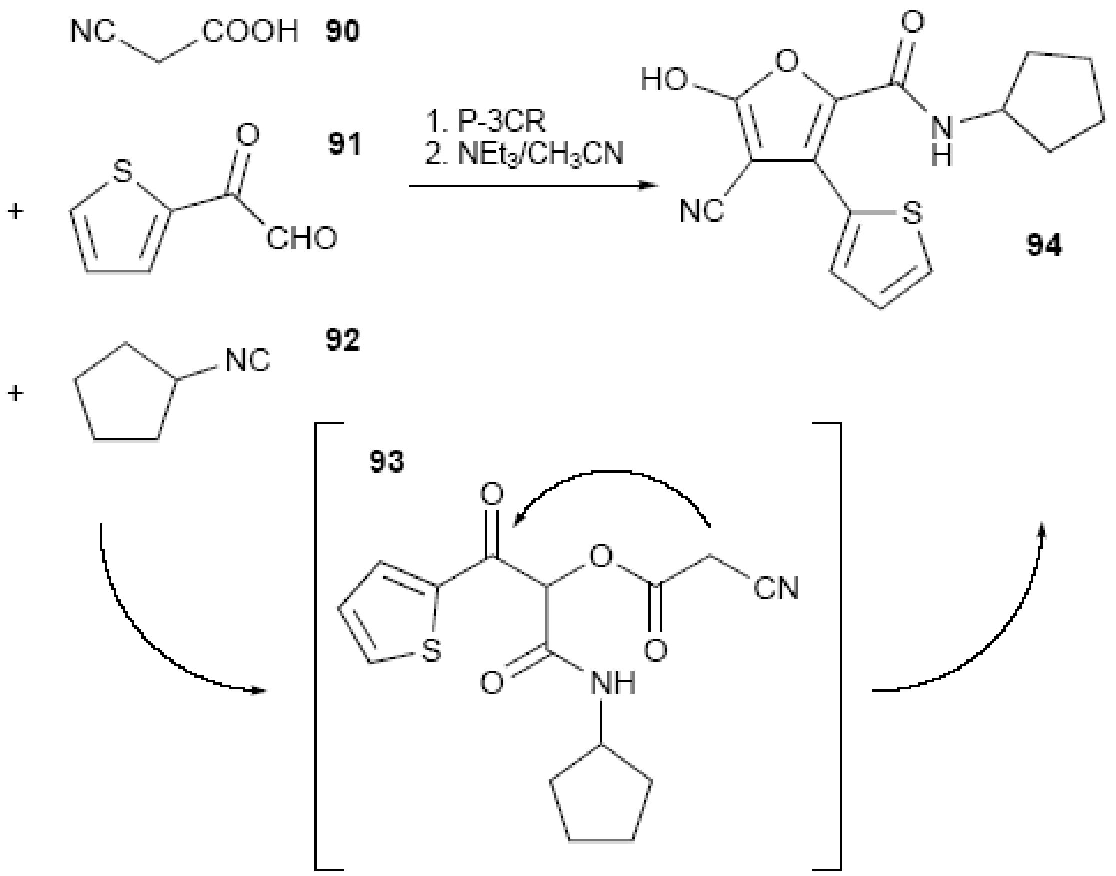
- **Mario Passerini (1881-1962), the inventor of the most significant isocyanide based MCRs.**

Stereocontrol in Passerini Reaction

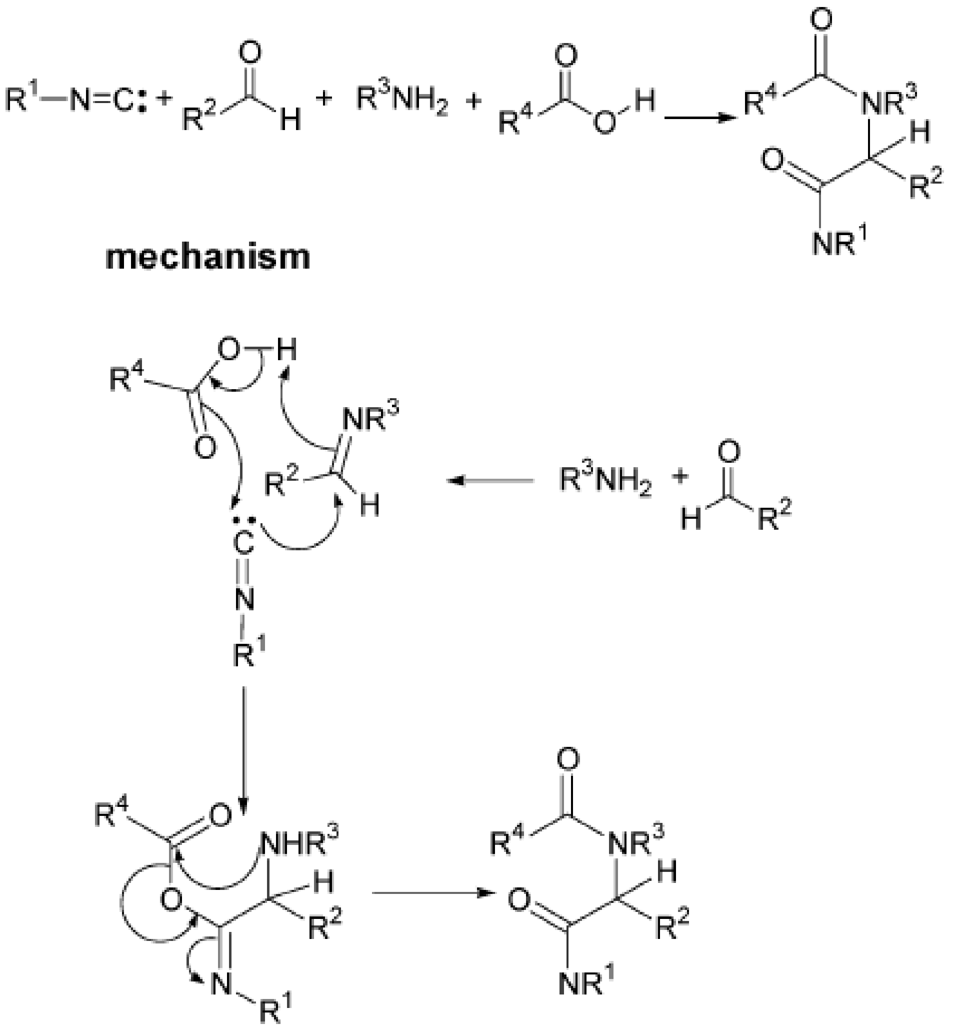
- **Stereochemically pure compounds are of uttermost importance in IMCRs. During the Passerini reaction, typically a new stereocenter is formed, resulting in racemic products in the absence of stereoinduction.**
- **Recently , Schreiber and co-workers described the use of amounts of pybox Cu(II) complex to perform P-3CRs. Sixteen examples were described between 60 to 98%ee and 75-98% chemical yield.**



Cyclic Variation of the P-3CR as follows



Ugi Reaction(1)

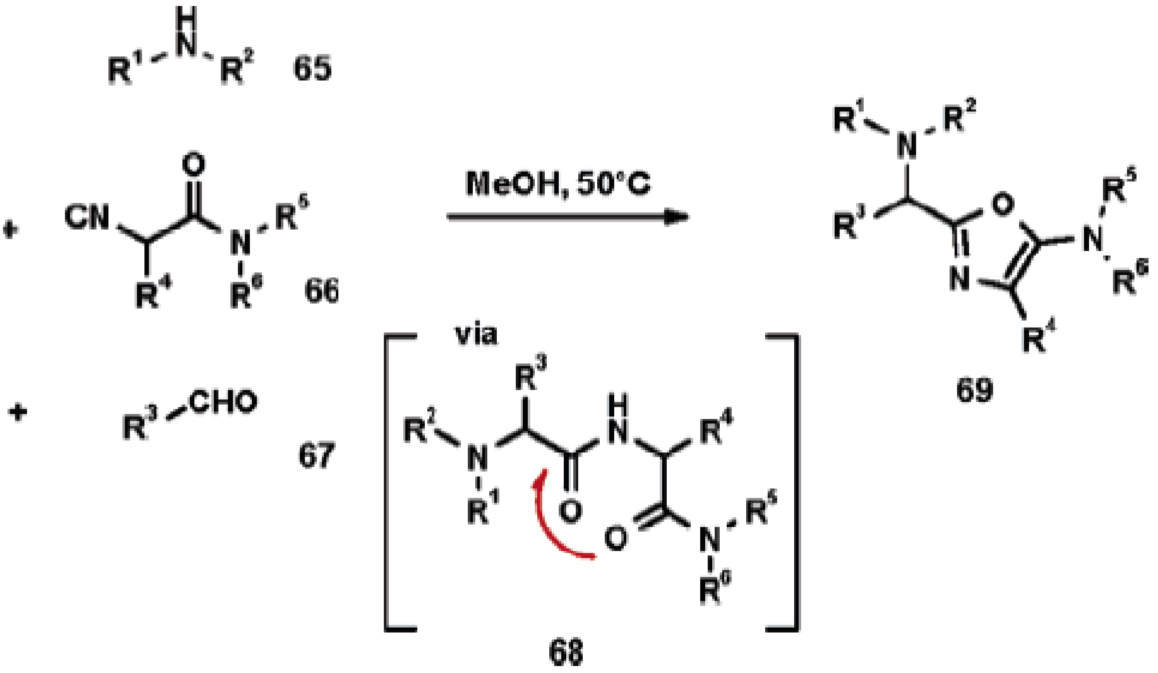


Ugi Reaction (U-4CR)

- **Ivar Karl Ugi (1930-2005), the inventor of the most significant isocyanide based MCRs.**

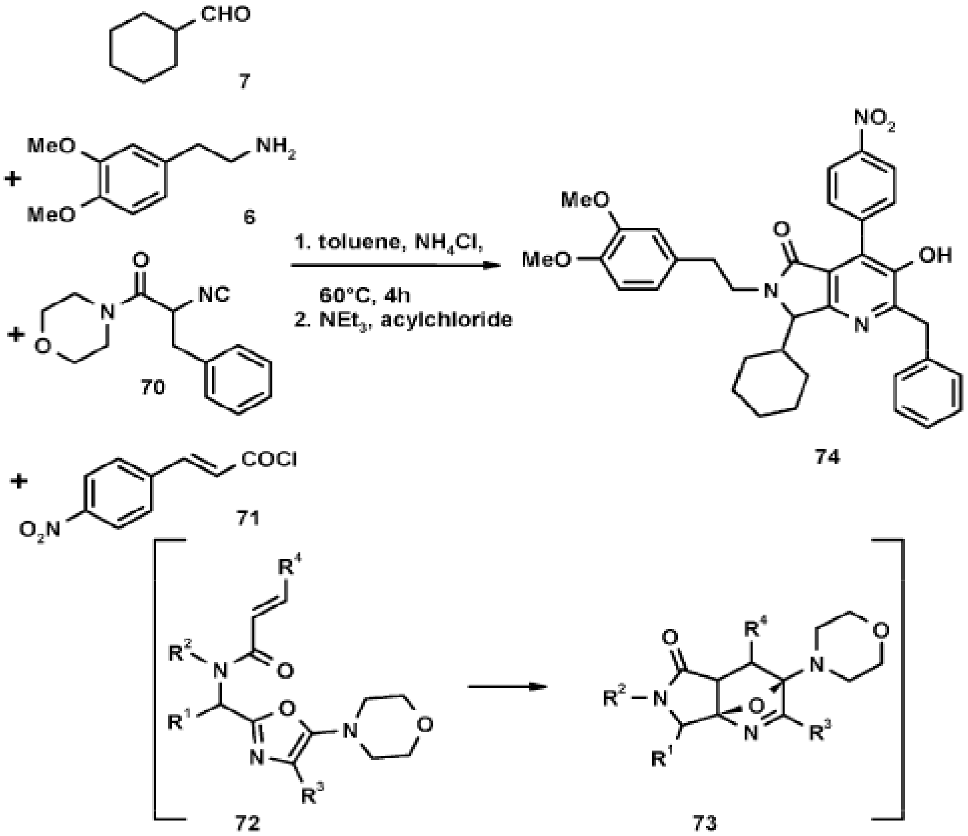
U-3CR of Isocyanoacetamides(1)

- **Isocyanoacetic amides, aldehydes, and primary or secondary amines react in the usual way in a U-3CR.**

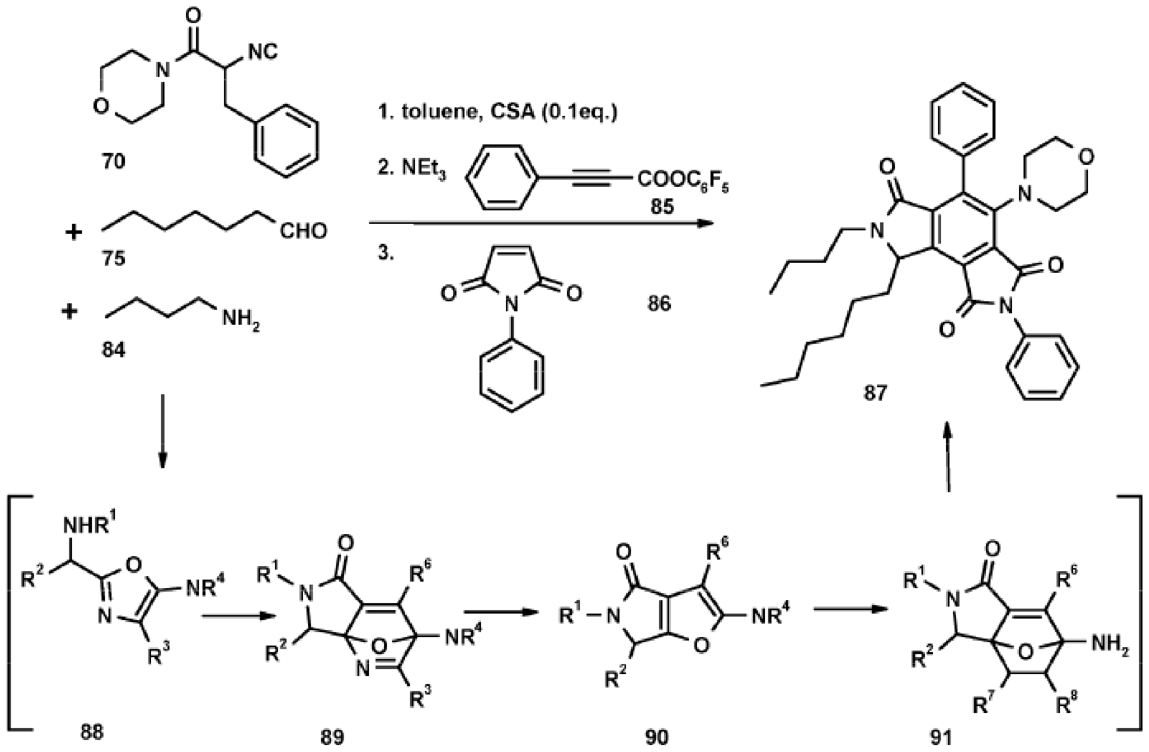


U-3CR of Isocyanoacetamides(2)

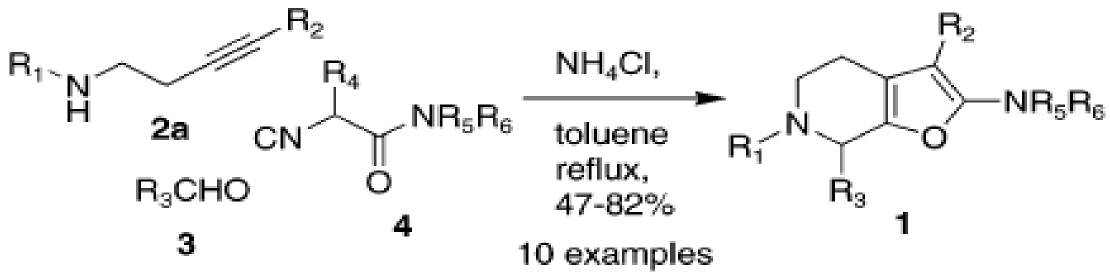
- **Pyrrolo-[3,4]pyridin-5-ones 74 in good yield through a domino process involving a three-component condensation, an intermolecular acylation to 72, an intramolecular Diels-Alder cycloaddition to 73, and a retro-Michael cycloreversion to 74.**

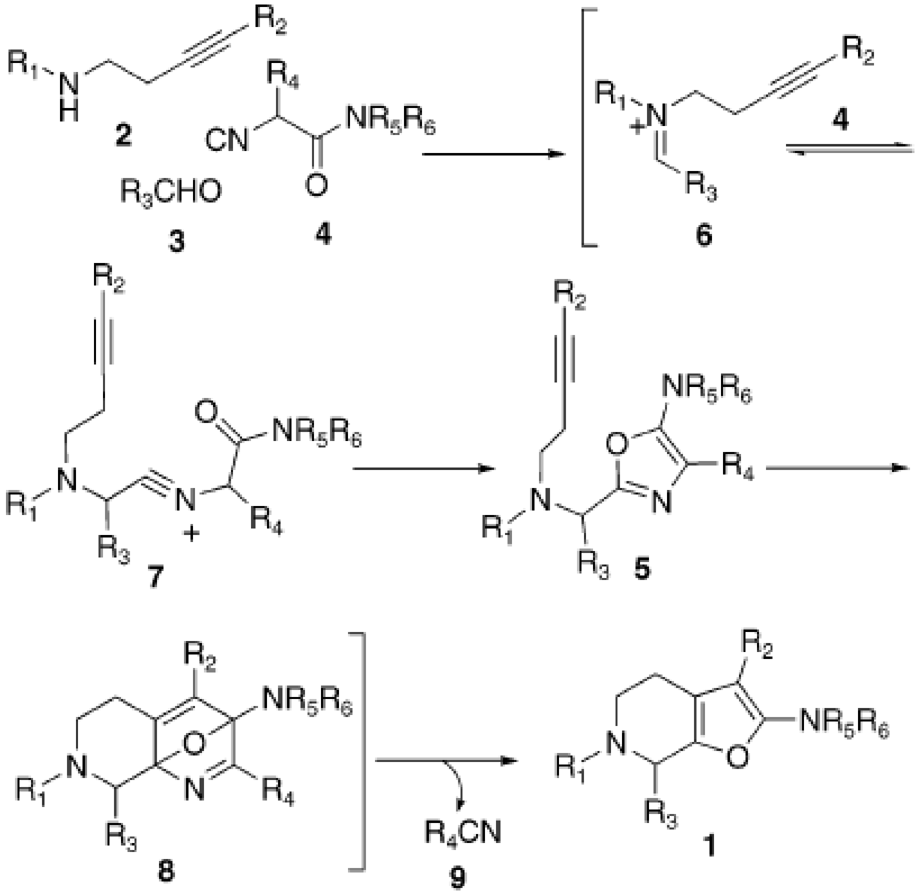


U-3CR of Isocyanoacetamides(3)as follows

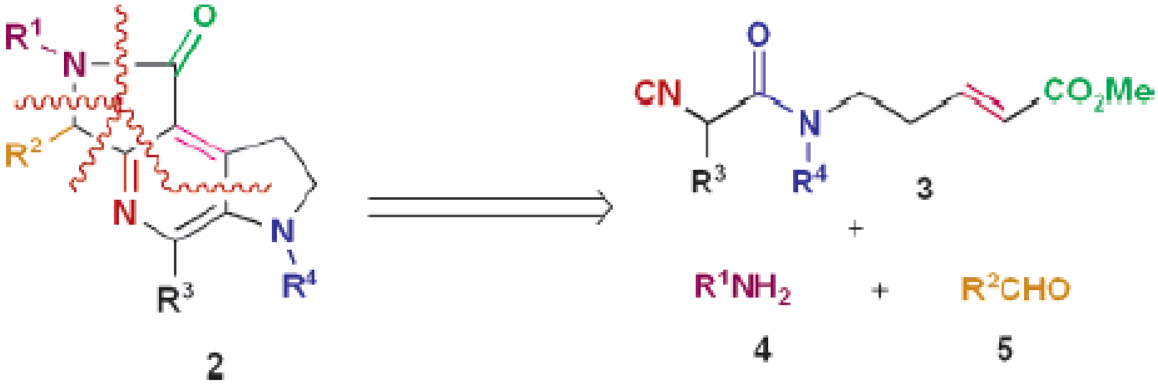


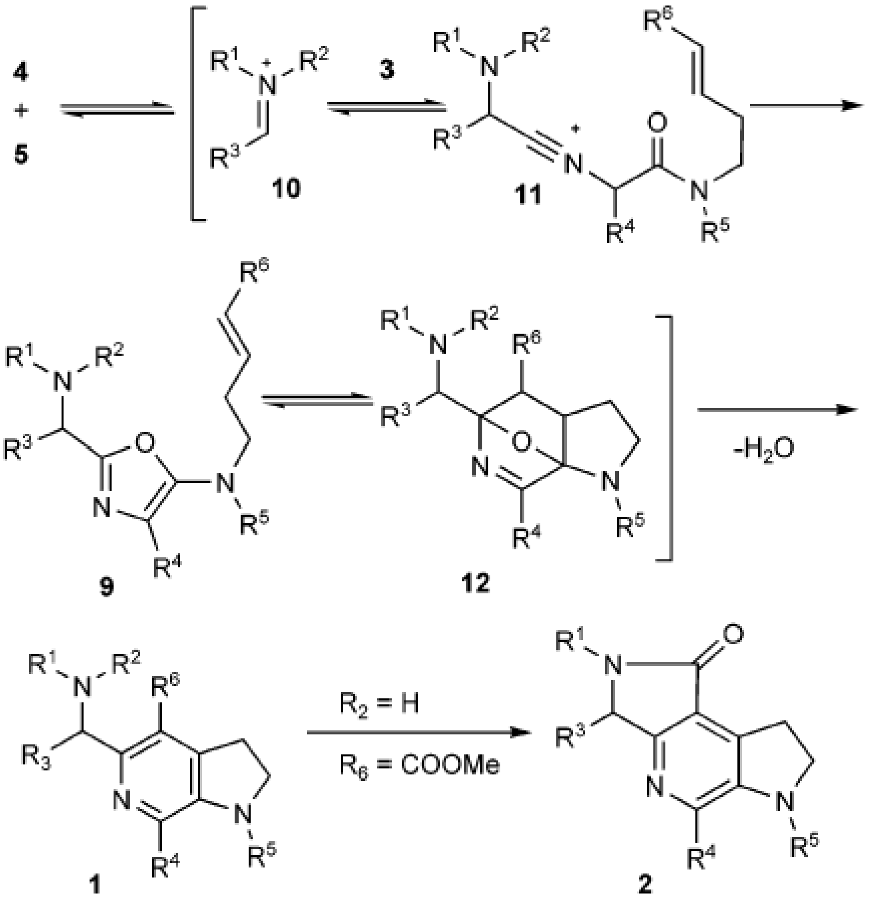
U-3CR of Isocyanoacetamides(4)





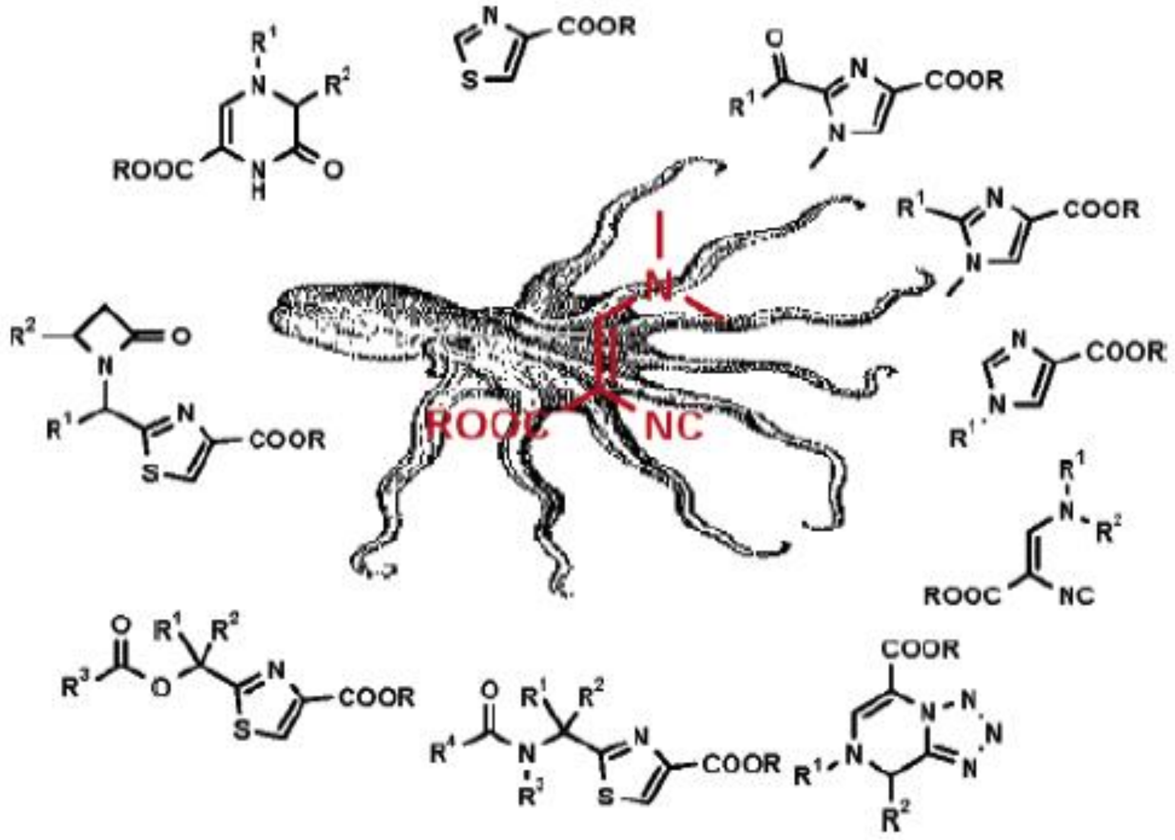
U-3CR of Isocyanoacetamides(5)





IMCRs of 3,3-(Dimethylamino)-2-isocyanoacrylates

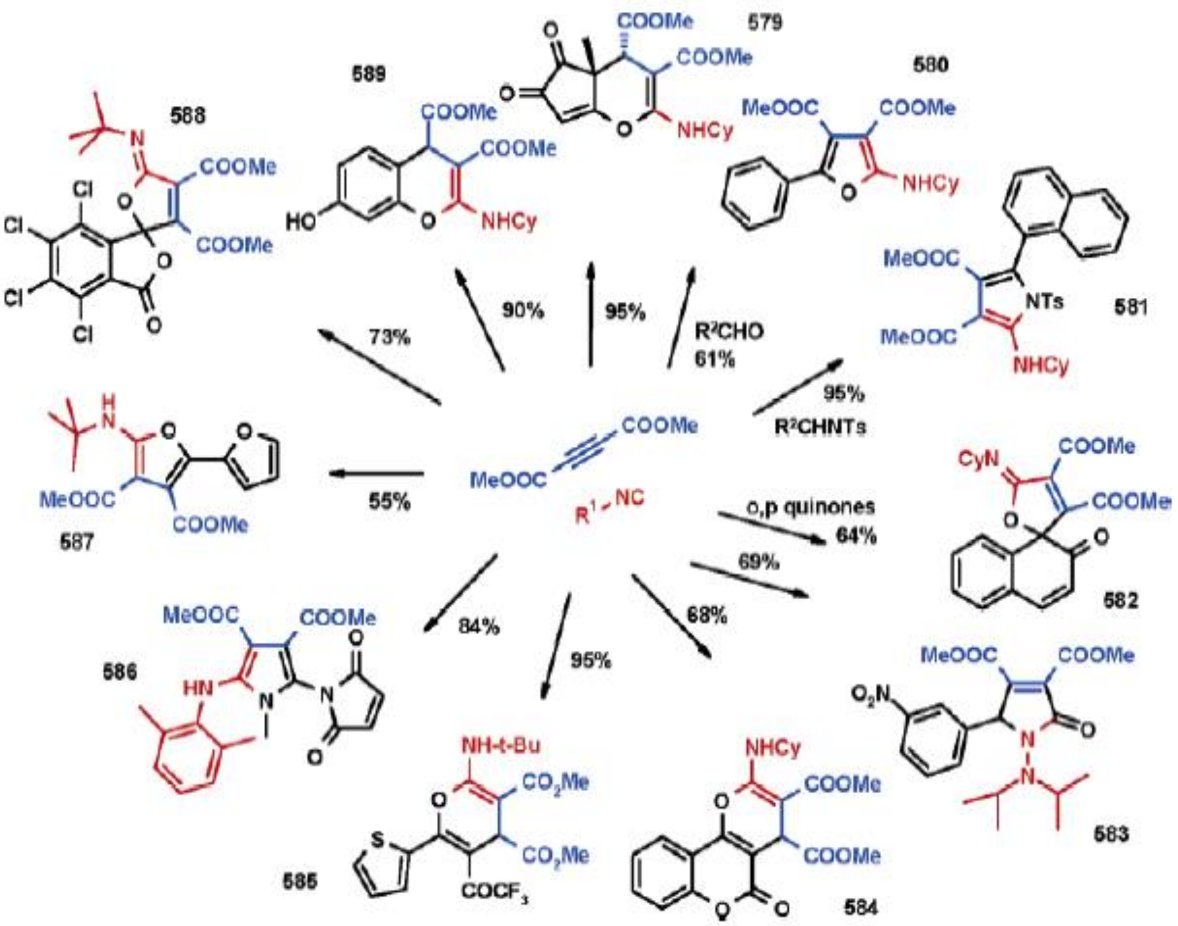
- **The highly functionalized 3-(dimethylamino)-2-isocyanoacrylate is a versatile starting material for diversity generating synthesis of multiple scaffolds and their libraries, e.g., imidazoles, thiazoles, ketopiperazines, and bicylic tetrazolopiperazines.**



- **Octopus chemistry**

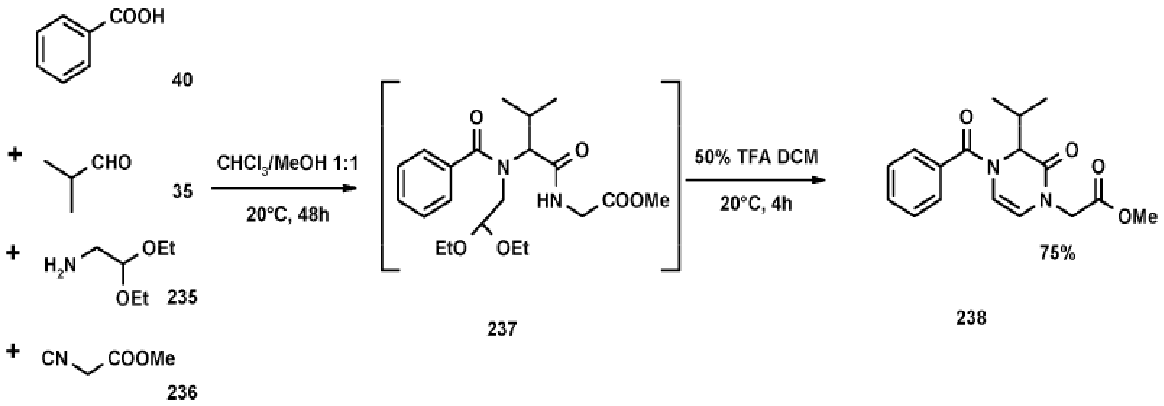
IMCRs of DMAD

In 1969, Winterfeld first described the reaction of isocyanides and acetylene compounds. DMAD and isocyanides with a third component undergo a variety of cycloadditions to form diverse heterocyclic scaffolds

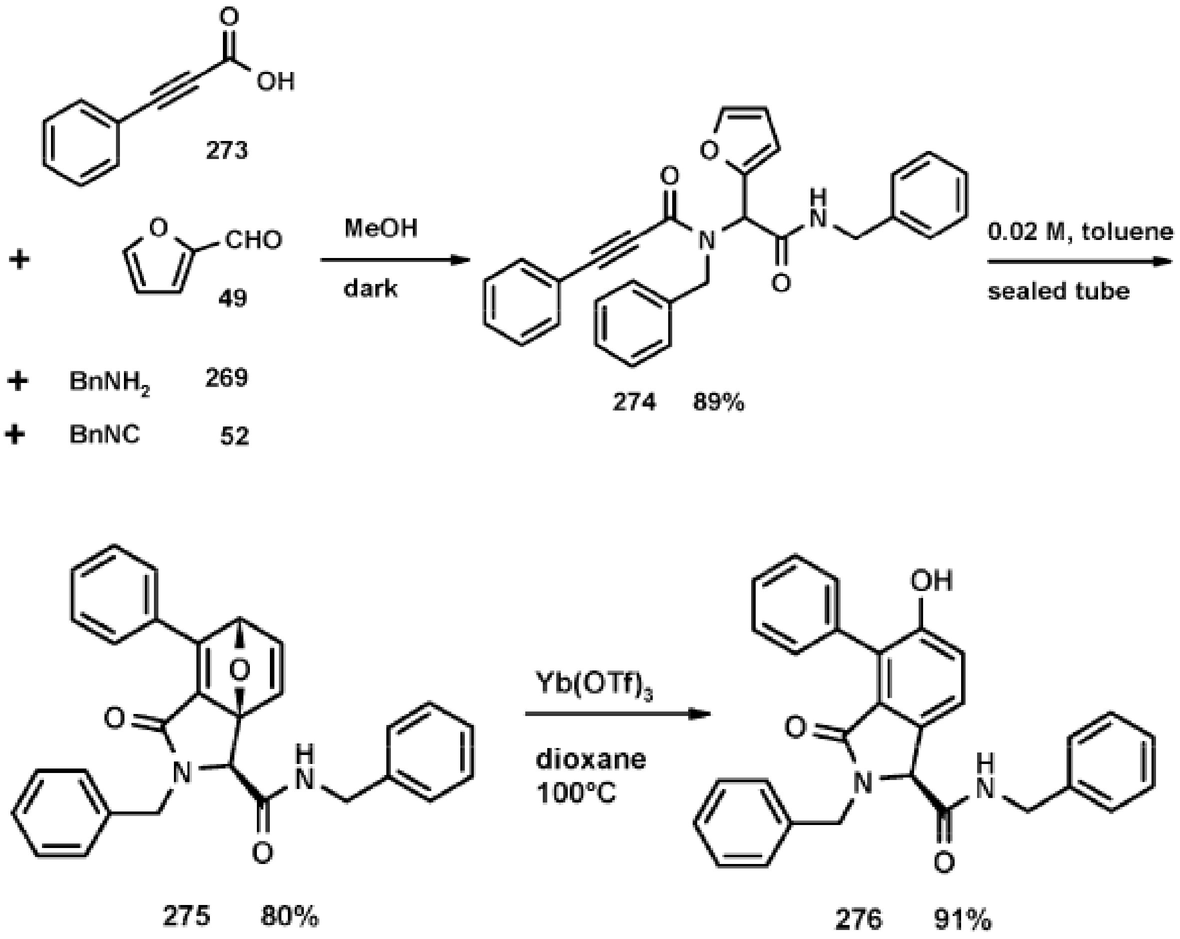


IMCR/Cyclic Condensations

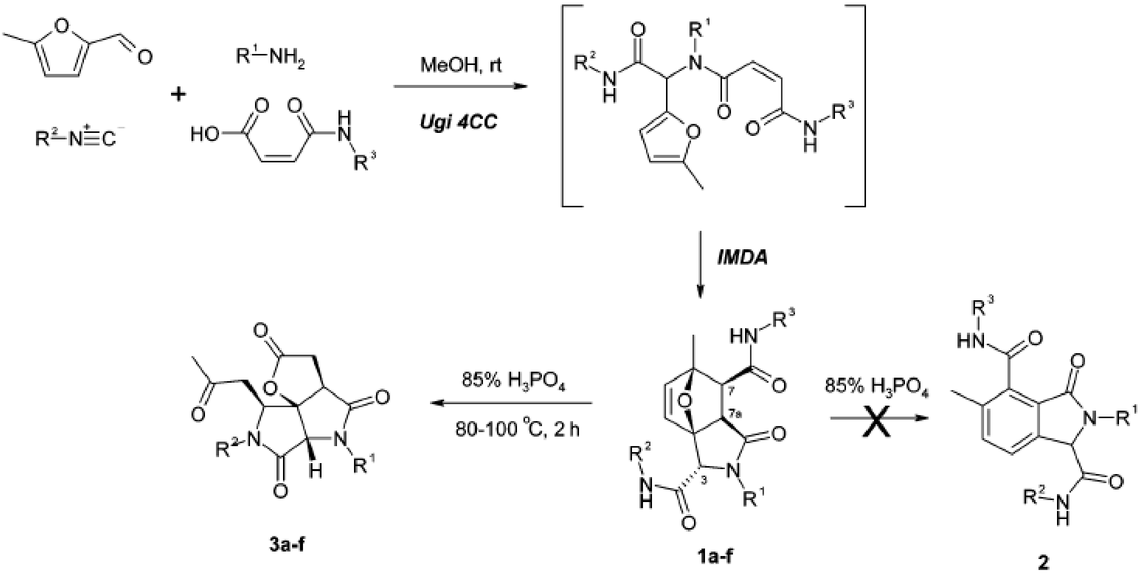
- **U-4CR of Amino Acetaldehyde Diethylacetal and Subsequent Acid Catalyzed condensation-Cyclization.**



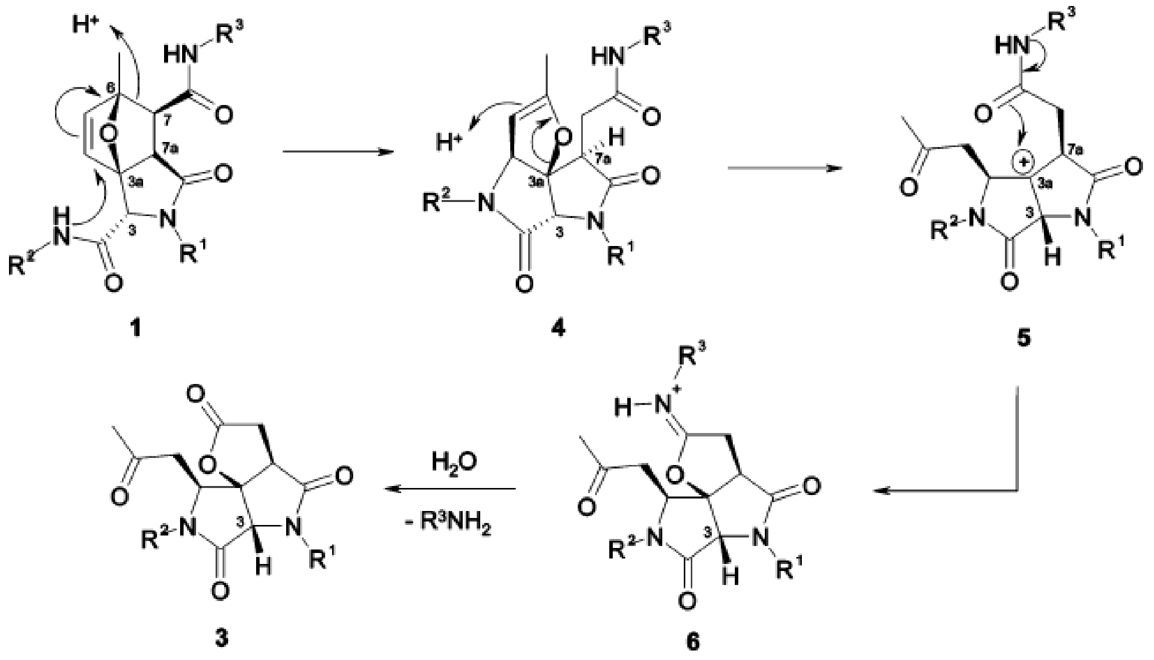
IMCRs Followed by a Cycloaddition



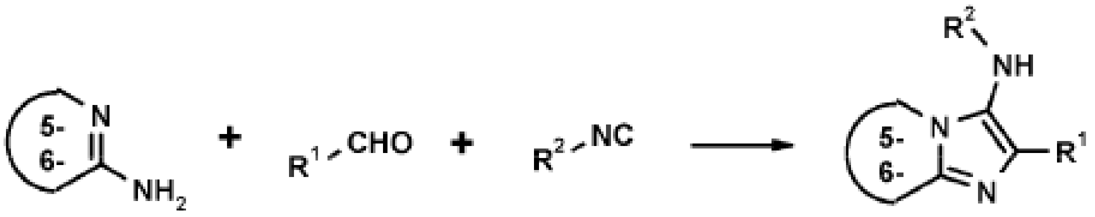
Ugi-4CR/Intramolecular Diels-Alder Reaction(1)



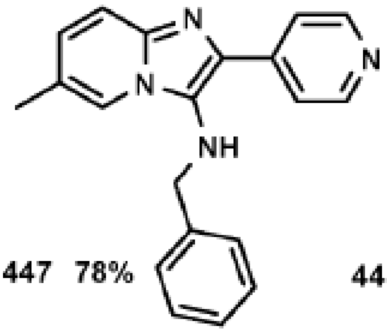
Ugi-4CR/Intramolecular Diels-Alder Reaction(2)



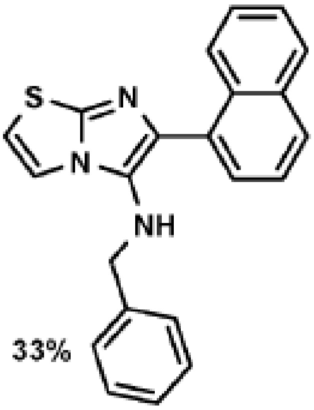
Groebcke Reaction



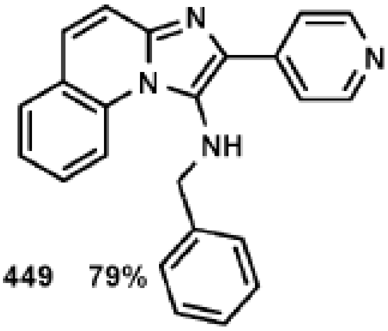
443 444 445 446



447 78%



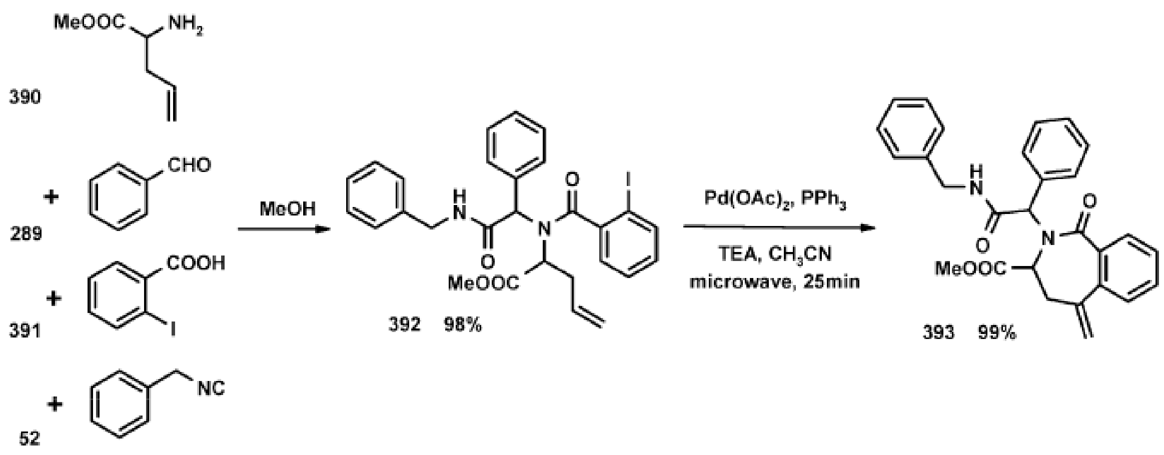
448 33%



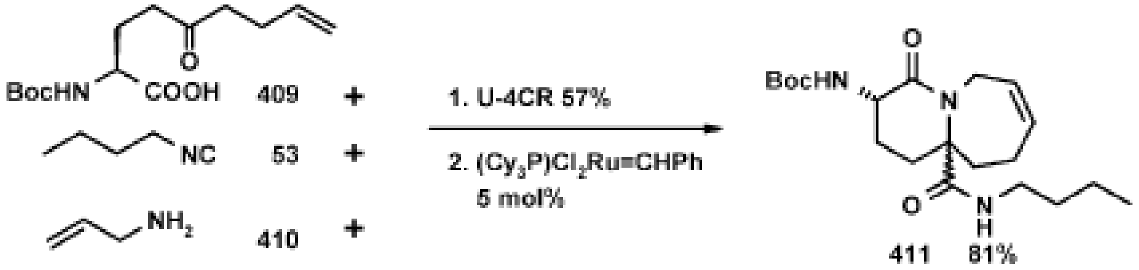
449 79%

- **Fused 3-Aminoimidazoles are broadly accessible by U-3CR of isocyanides, aldehydes, and five-or six-membered imidine aromatics.**

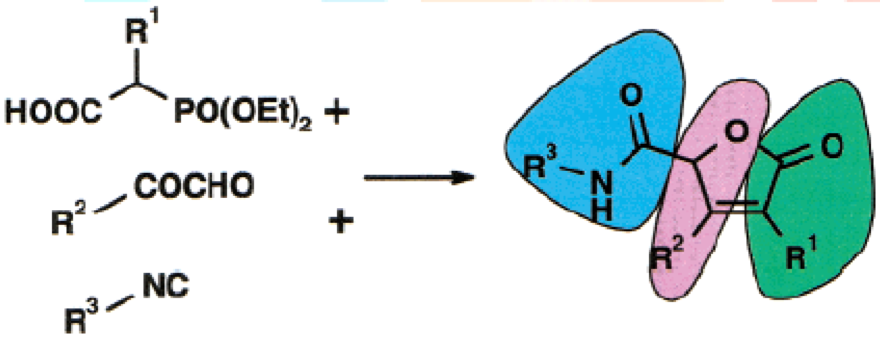
IMCR/Heck Reaction

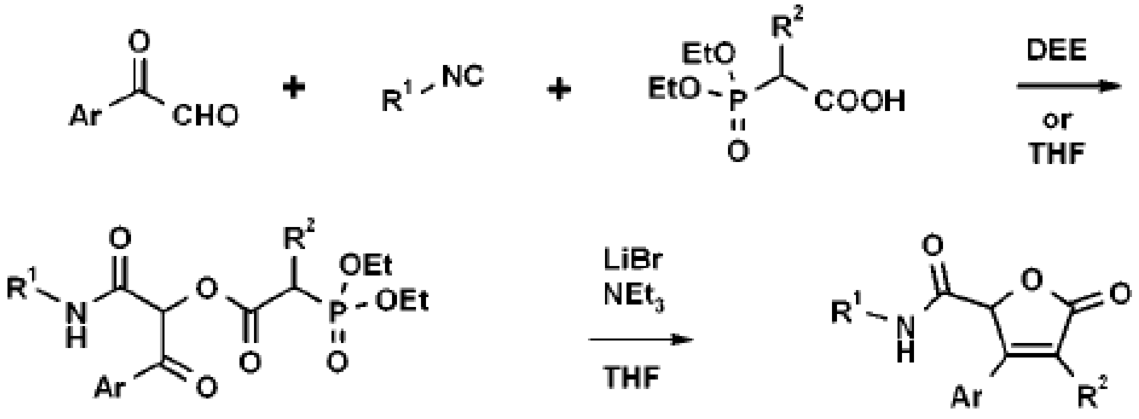


IMCR/RCM

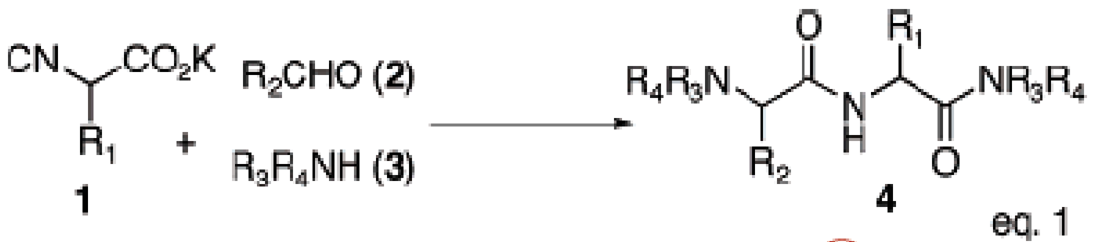


IMCR/Wittig Reaction

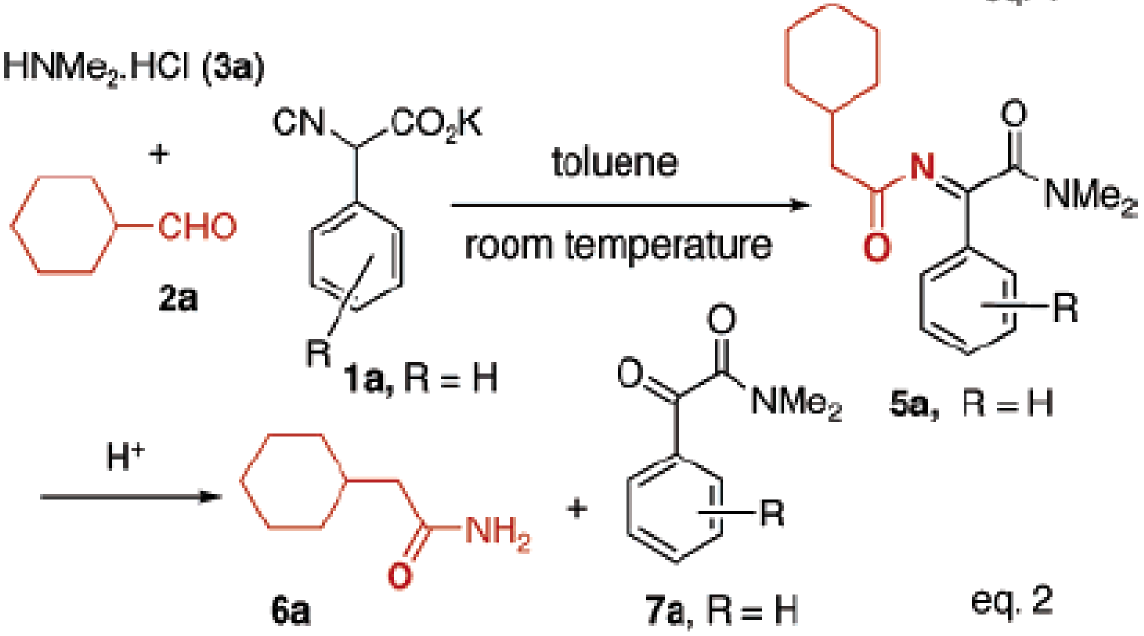




One –Carbon Homologation of Aldehyde to Amide(1)

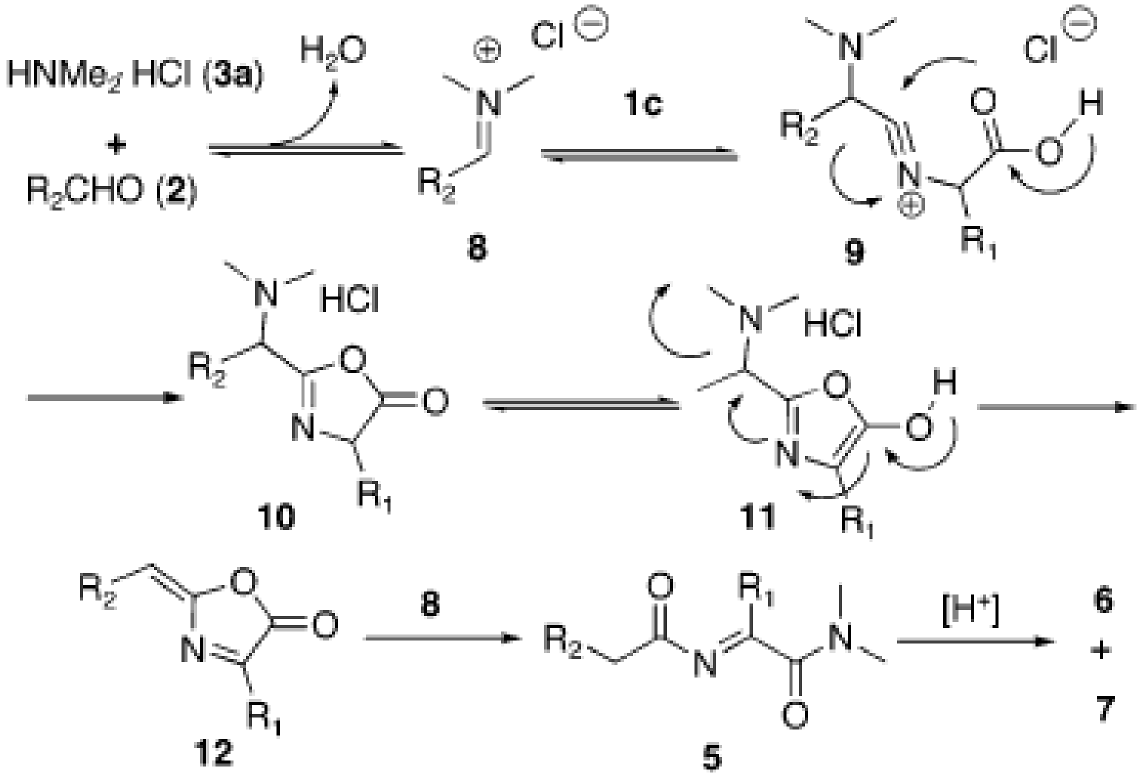


eq. 1

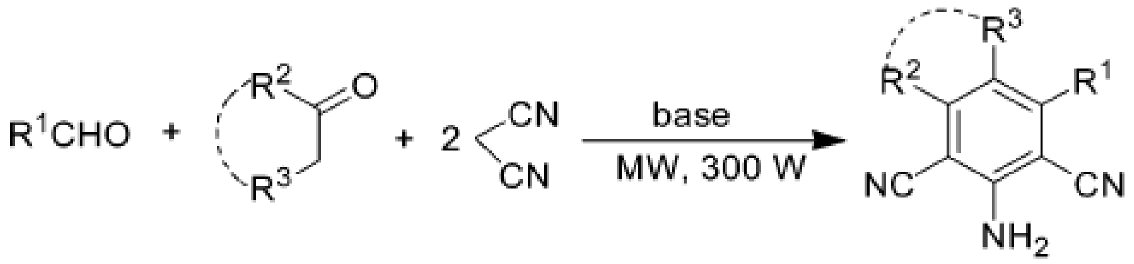


eq. 2

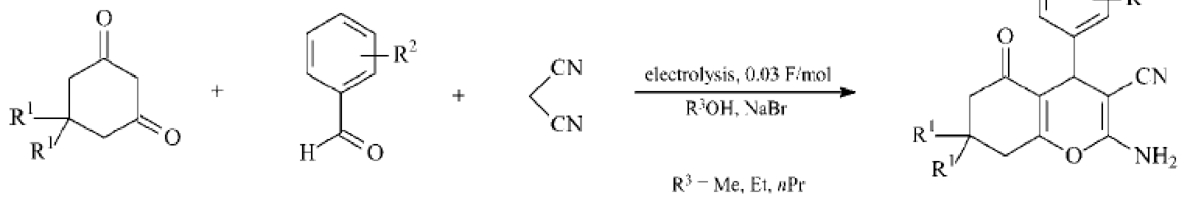
One –Carbon Homologation of Aldehyde to Amide(2)



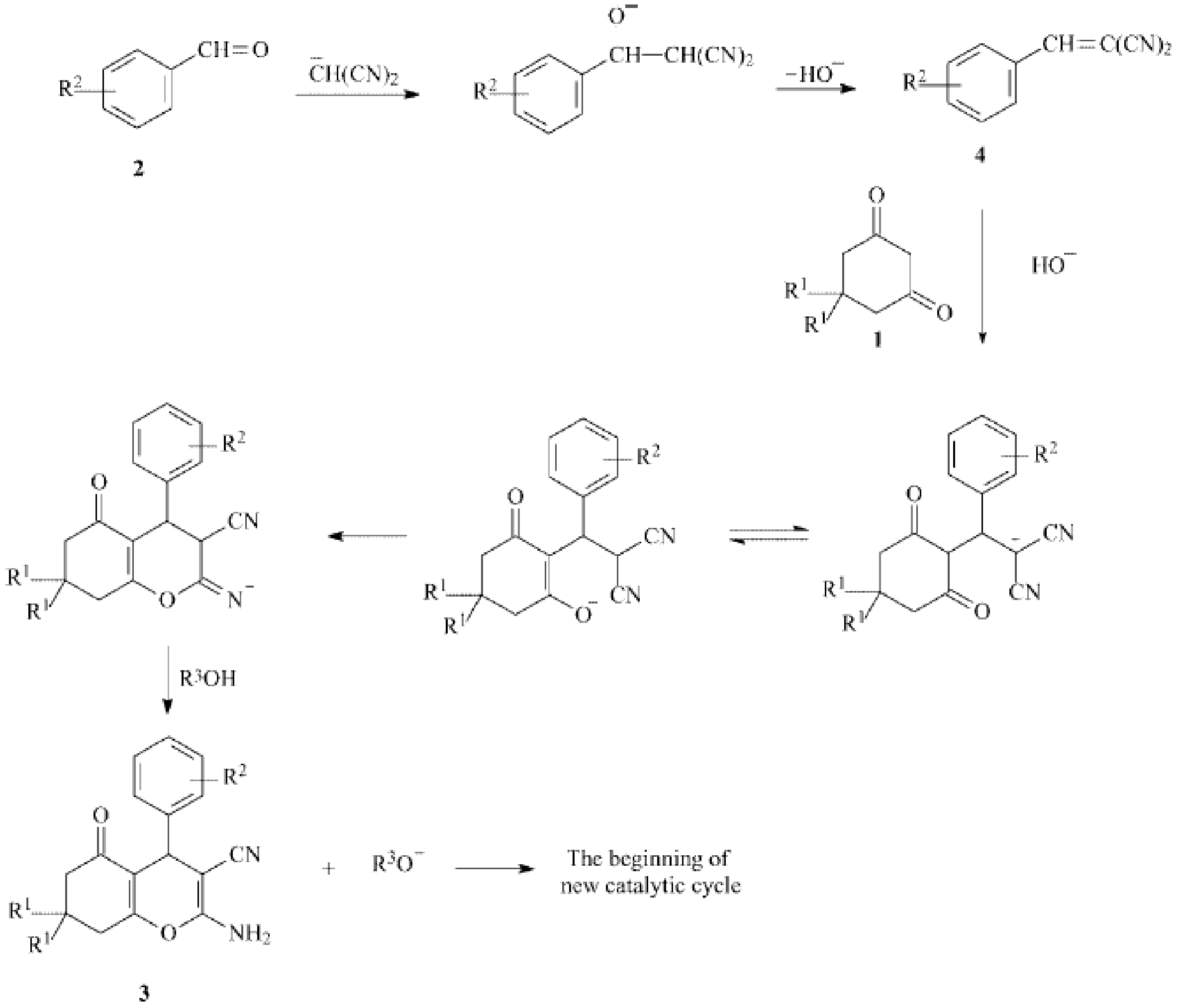
MCR/Malononitrile(1)



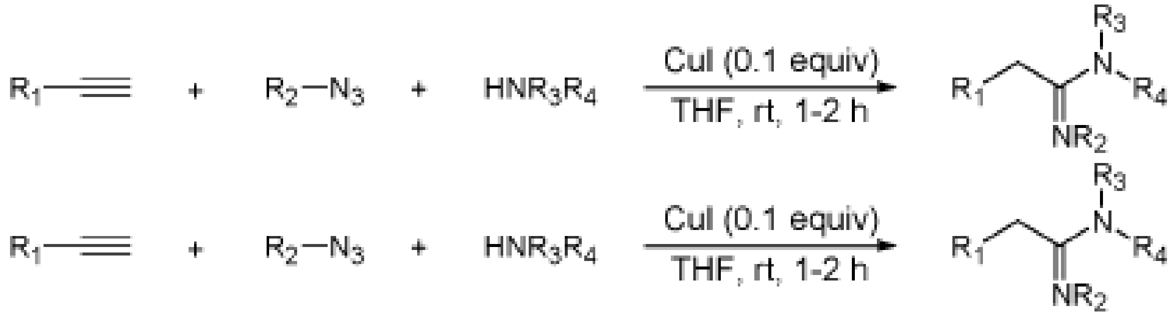
MCR/Malononitrile(2)



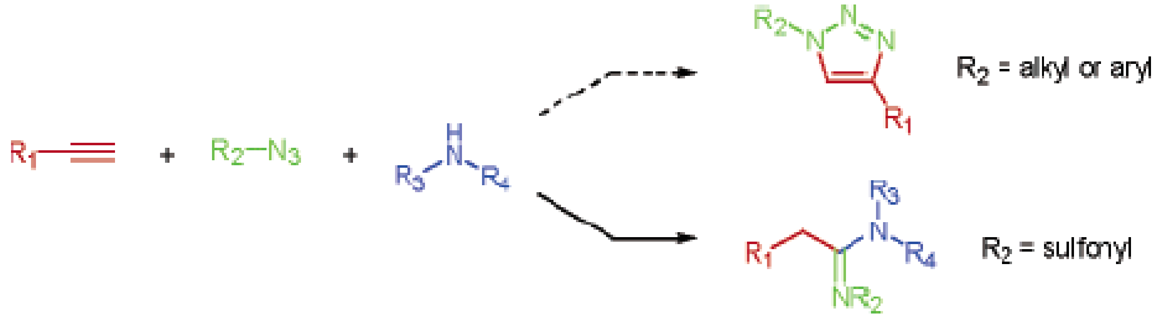
MCR/Malononitrile(3)



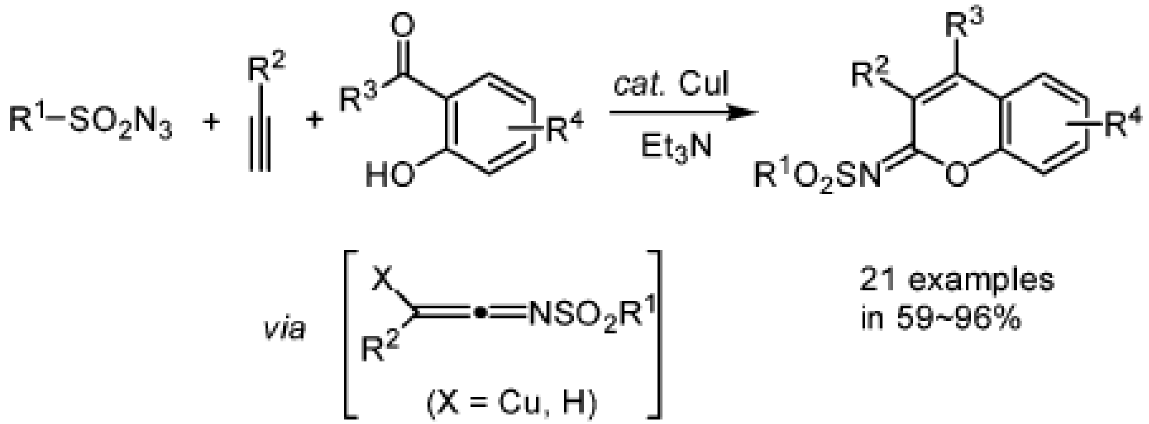
MCR/Sulfonyl Azide(1)



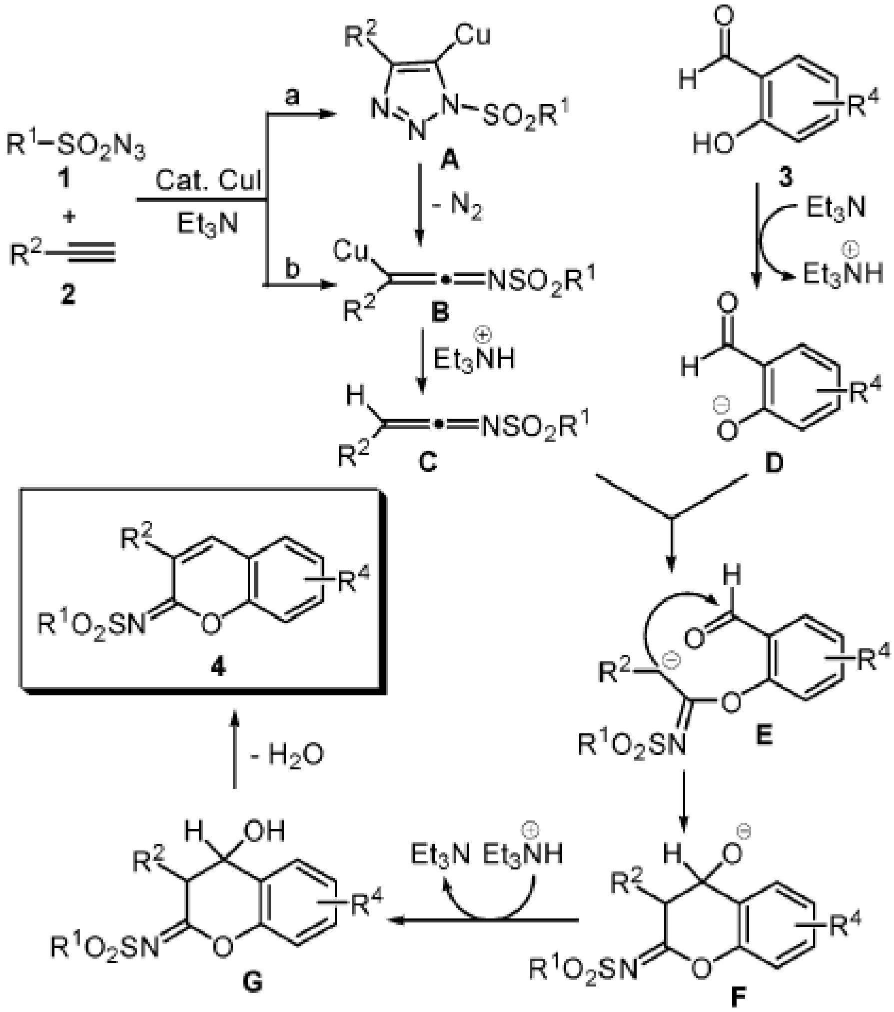
MCR



MCR/**Sulfonyl Azide(2)**

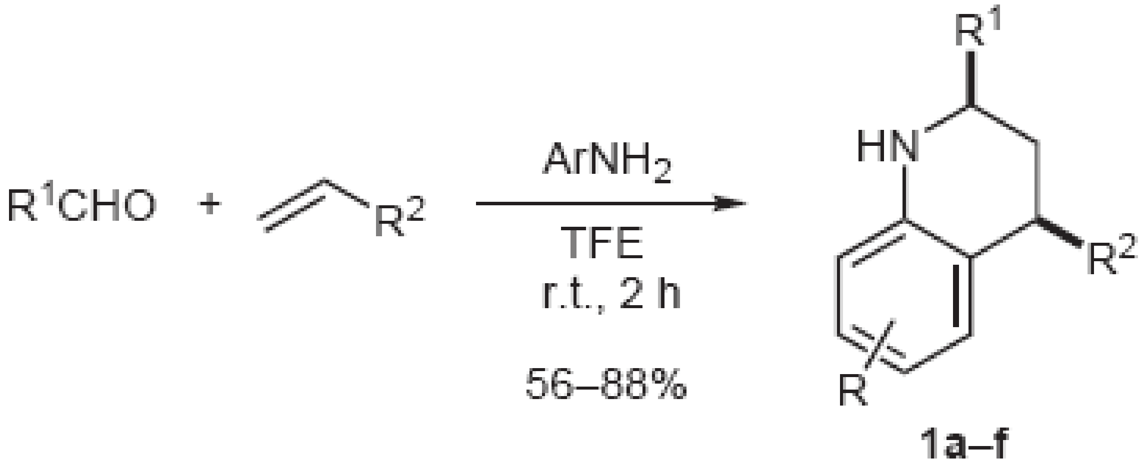


MCR/Sulfonyl Azide(3)

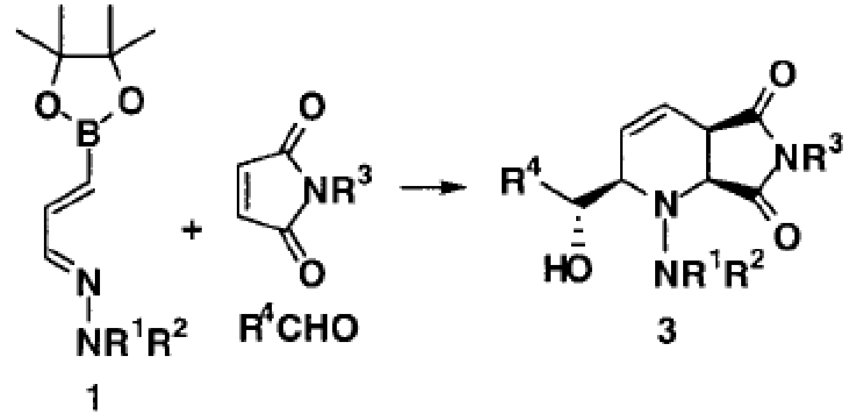


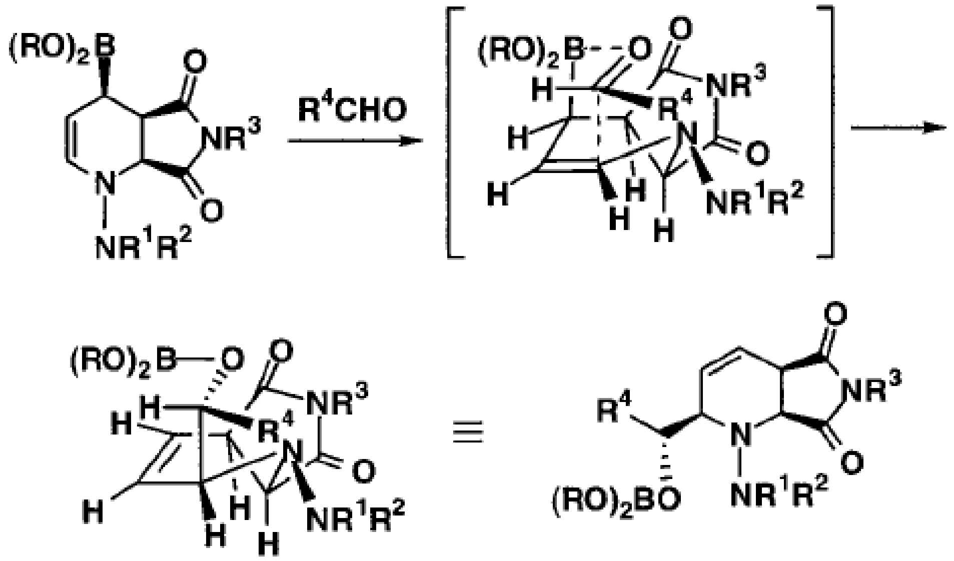
MCR/Sulfonyl Azide(3

MCR/aza-Diels-Alder Reaction(1)

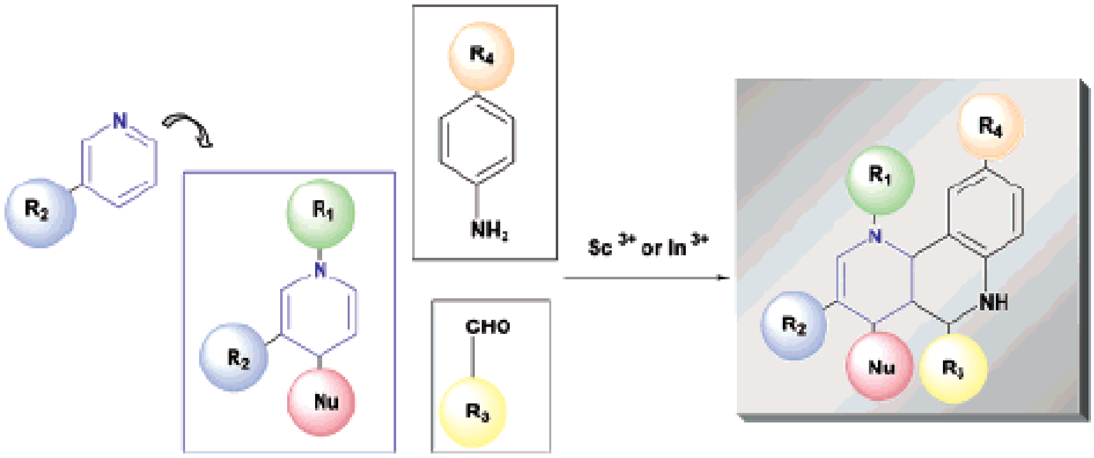


MCR/aza-Diels-Alder Reaction(2)

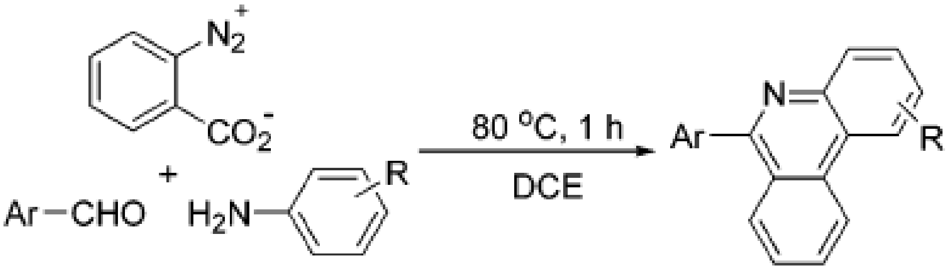


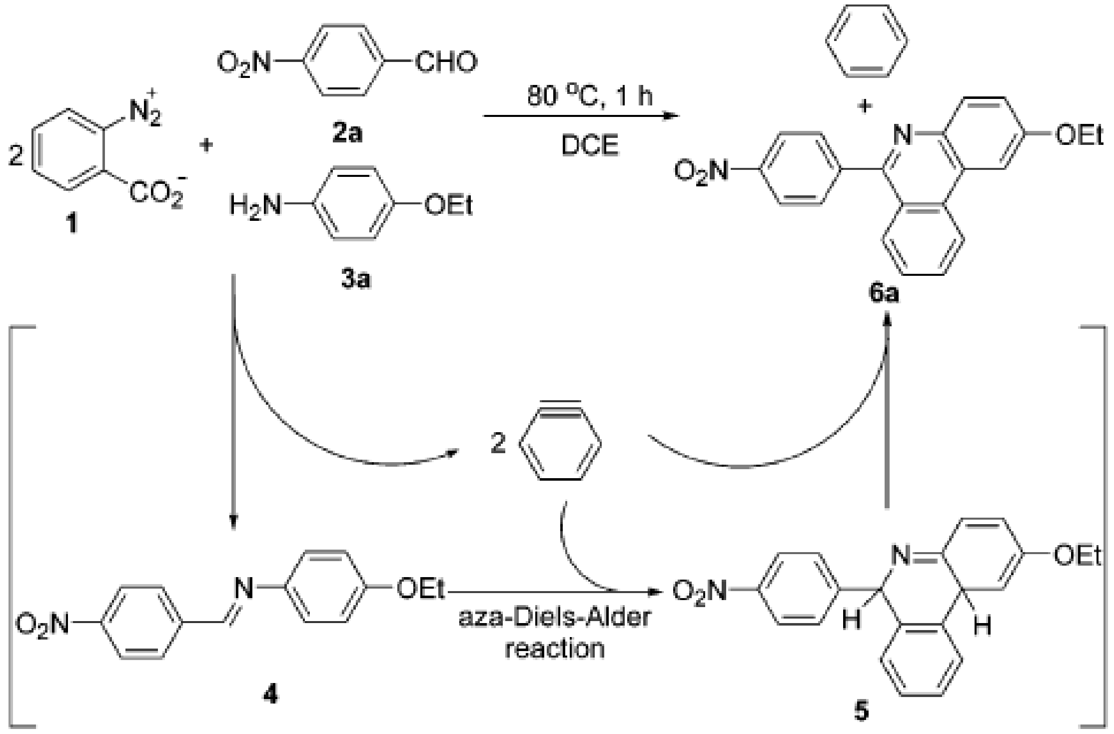


MCR/aza-Diels-Alder Reaction(3)



MCR/aza-Diels-Alder Reaction(4)





Out look

- **Hardly a field in organic chemistry has evolved faster over only a couple of years than MCRs. However, the very large MCR space of >10^{20} is only explored marginally.**
- **Future research in the area of MCRs will include the discovery and design of novel MCRs, combinations of MCRs and traditional reactions, experimental improvements in solid-and liquid-phase, enantio-and diastereo-selective variations, and more and more applications in drug discovery, material science, bioconjugates, and agrochemical compounds.**

P.. Consideration Hybridization assay

In this article we tried to demonstrate the versatility of the molecular hybridization approach as structural modification strategy useful in the design of new optimized ligands and prototypes with new molecular architectures. New chemical classes were discovered coming from the combination of pharmacophore sub-unities of known prototypes, resulting frequently, in more potent and selective hybrid derivatives. In some cases, this strategy was exploited for the modulation of undesirable secondary effects and, in other ones, it was used to combine two distinct pharmacological profile in only one molecule, characterizing new potential dual-acting drugs, able of reproducing the effect of associations of more than one therapeutic agent. The molecular hybridization strategy is particularly interesting for the development of new prototypes for the treatment of physiopathology whose treatment is under research by scientists.

A **hybridization assay** comprises any form of quantifiable hybridization *i.e.* the quantitative annealing of two complementary strands of nucleic acids, known as nucleic acid hybridization

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2Sandwich hybridization assay

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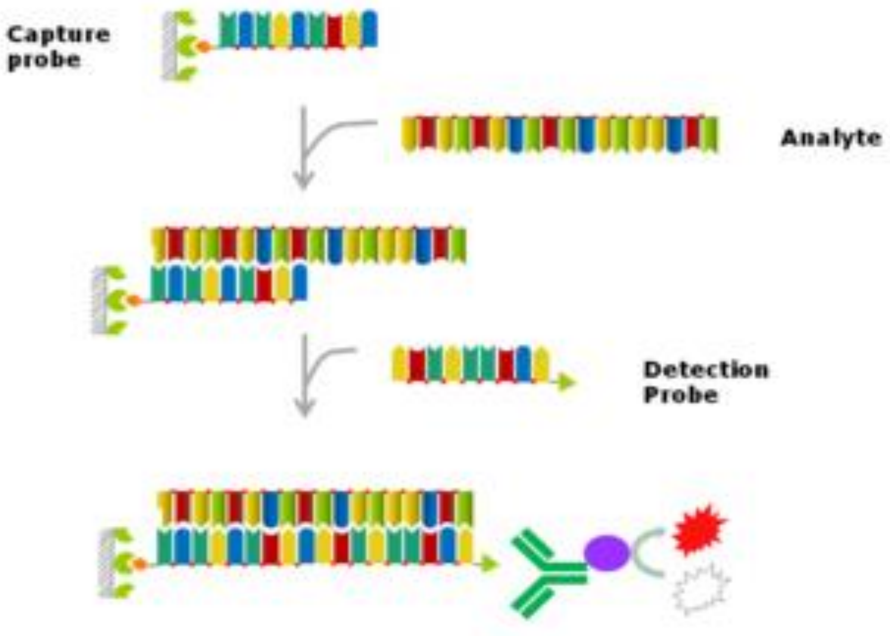
Overview

In the context of biochemistry and drug development, a hybridization assay is a type of Ligand Binding Assay (LBA) used to quantify nucleic acids in biological matrices. Hybridization assays can be in solution or on a solid support such as 96-well plates or labelled beads.

Hybridization assays involve labelled nucleic acid probes to identify related DNA or RNA molecules (i.e. with significantly high degree of sequence similarity) within a complex mixture of unlabelled nucleic acid molecules. Antisense therapy, siRNA, and other oligonucleotide and nucleic acid based biotherapeutics can be quantified with hybridization assays.

Signalling of hybridization methods can be performed using oligonucleotide probes modified in-synthesis with haptens and small molecule ligands which act homologous to the capture and detection antibodies. As with traditional ELISA, conjugates to horse radish peroxidase (HRP) or alkaline phosphatase (AP) can be used as secondary antibodies.

Sandwich hybridization assay



Sandwich hybridization assay

In the sandwich hybridization ELISA assay format, the antigen ligand and antibodies in ELISA are replaced with a nucleic acid analyte, complementary oligonucleotide capture and detection probes.

Generally, in the case of nucleic acid hybridization, monovalent salt concentration and temperature are controlled for hybridization and wash stringency, contrary to a traditional ELISA, where the salt concentration will usually be fixed for the binding and wash steps (i.e. PBS or TBS). Thus, optimal salt concentration in hybridization assays varies dependent upon the length and base composition of the analyte, capture and detection probes.

Competitive hybridization assay

The competitive hybridization assay is similar to a traditional competitive immunoassay. Like other hybridization assays, it relies on complementarity, where the capture probe competes between the analyte and the tracer–a labelled oligonucleotide analog to the analyte.

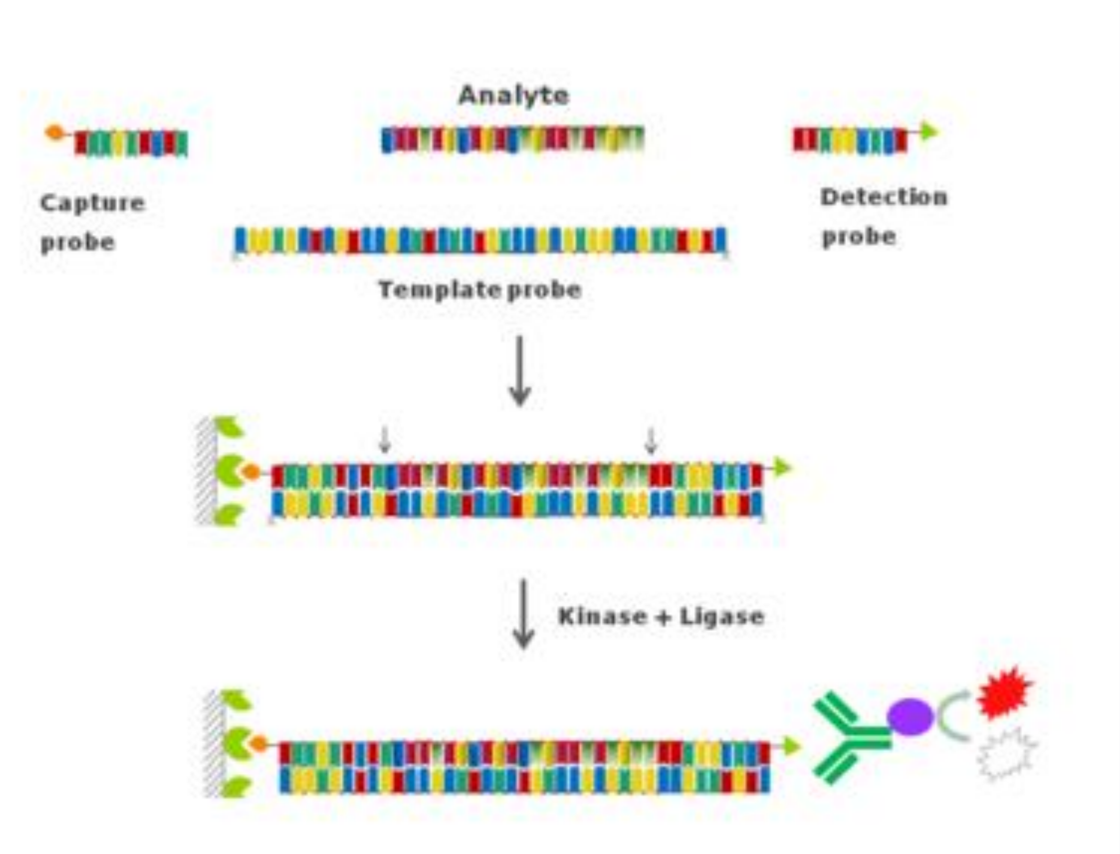
Q.Hybridization-ligation assay

In the hybridization-ligation assay a template probe replaces the capture probe in the sandwich assay for immobilization to the solid support. The template probe is fully complementary to the oligonucleotide analyte and is intended to serve as a substrate for T4 DNA ligase-mediated ligation. The template probe has in addition an additional stretch complementary to a ligation probe so that the ligation probe will ligate onto the 3'-end of the analyte. Albeit generic, the ligation probe is similar to a detection probe in that it is labelled with, for example, digoxigenin for downstream signalling. Stringent, low/no salt wash will remove un-ligated products.

The ligation of the analyte to the ligation probe makes the method specific for the 3'-end of the analyte, ligation by T4 DNA ligase being much less efficient over a bulge loop, which would happen for a 3' metabolite N-1 version of the analyte, for example. The specificity of the hybridization-ligation assay for ligation at the 3'-end is particularly relevant because the predominant nucleases in blood are 3' to 5' exonucleases.

One limitation of the method is that it requires a free 3'-end hydroxyl which may not be available when targeting moieties are attached to the 3'-end, for example. Further, more exotic nucleic acid chemistries with oligonucleotide drugs may impact upon the activity of the ligase, which needs to be determined on a case-by-case basis.

Dual ligation hybridization assay



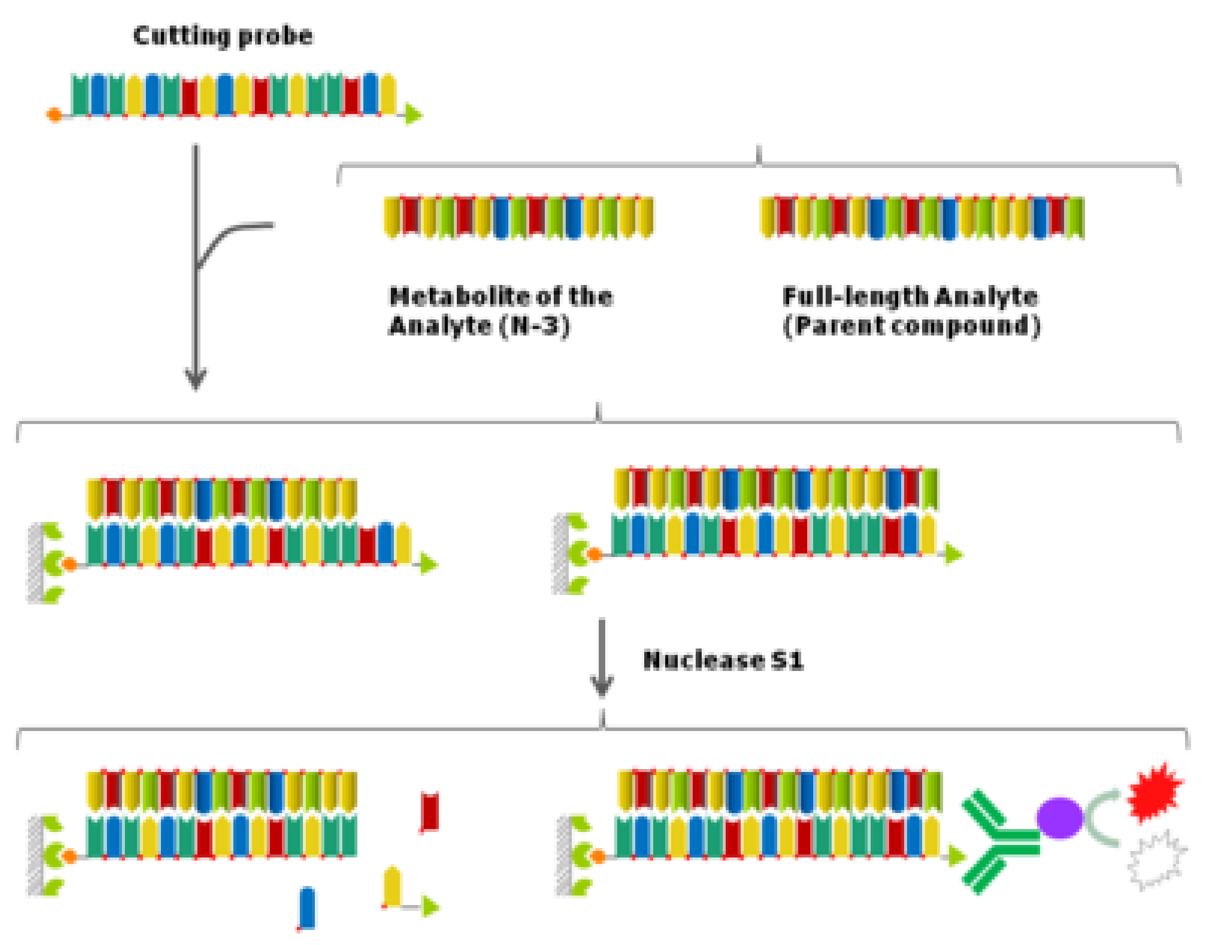
Dual Ligation Hybridization Assay

The dual ligation hybridization assay (DLA)extends the specificity of the hybridization-ligation assay to a specific method for the parent compound. Despite hybridization-ligation assay's robustness, sensitivity and added specificity for the 3'-end of the oligonculeotide analyte, the hybridization-ligation assay is not specific for the 5' end of the analyte.

The DLA is intended to quantify the full-length, parent oligonucleotide compound only, with both intact 5' and 3' ends. DLA probes are ligated at the 5' and 3' ends of the analyte by the joint action of T4 DNA ligase and T4 polynucleotide kinase. The kinase phosphorylates the 5'-end of the analyte and the ligase will join the capture probe to the analyte to the detection probe. The capture and detection probes in the DLA can thus be termed ligation probes. As for the hybridization-ligation assay, the DLA is specific for the parent compound because the efficiency of ligation over a bulge loop is low, and thus the DLA detects the full-length analyte with both intact 5' and 3'-ends. The DLA can also be used for the determination of individual metabolites in biological matrices.

The limitations with the hybridization-ligation assay also apply to the dual ligation assay, with the 5'-end in addition to the 3'-end requiring to have a free hydroxyl (or a phosphate group). Further, T4 DNA ligases are incompatible with ligation of RNA molecules as a donor (i.e. RNA at the 5' end of the ligation). Therefore, second generation antisense that comprise 2'-O-methyl RNA, 2'-O-methoxyethyl or locked nucleic acids may not be compatible with the dual ligation assay.

Nuclease hybridization assay



Nuclease hybridization assay

The nuclease hybridization assay,[7][8] also called S1 nuclease cutting assay, is a nuclease protection assay-based hybridization ELISA. The assay is using S1 nuclease, which degrades single-stranded DNA and RNA into oligo- or mononucleotides, leaving intact double-stranded DNA and RNA.

In the nuclease hybridization assay, the oligonucleotide analyte is captured onto the solid support such as a 96-well plate via a fully complementary cutting probe. After enzymatic processing by S1 nuclease, the free cutting probe and the cutting probe hybridized to metabolites, *i.e.* shortmers of the analyte are degraded, allowing signal to be generated only from the full-length cutting probe-analyte duplex.

The assay is well tolerant to diverse chemistries, as exemplified by the development of a nuclease assay for morpholino oligonucleotides.[9]

This assay set-up can lack robustness and is not suitable for validation following the FDA's guidelines for bioanalytical method validation. This is demonstrated by an absence of published method that have been validated to the standards outlined by the FDA for bioanalytical methods.

K.Hybrid Capture-Based Next Generation Sequencing and Its Application to Human Infectious Diseases

This review describes target-enrichment approaches followed by next generation sequencing and their recent application to the research and diagnostic field of modern and past infectious human diseases caused by viruses, bacteria, parasites and fungi.

Introduction

The development of next-generation sequencing (NGS) approaches has revolutionized human clinical research because of its ability to rapidly generate large volumes of sequencing data per run, with a concomitant decrease of sequencing costs (Shendure and Ji, 2008). Unbiased ultra-deep sequencing of complex samples is now accessible, although bioinformatics analyses may still be long and tedious. This issue is particularly problematic in the field of infectious disease diagnostic, where the rapid identification and functional characterization of a particular pathogen is critical for the clinical management of infected patients. So far, polymerase chain reaction (PCR) has been the gold standard method for the clinical diagnosis of infectious diseases (Edwards and Gibbs, 1994). This approach, which is based on the amplification of a generally short and conserved genomic region, can provide information on the presence/absence and abundance of a targeted microbial pathogen. PCR has numerous advantages, such as low cost, rapid processing and results acquisition, automation, sensitivity and specificity. However, and precisely because of its high specificity, PCR may not detect microorganisms whose sequences are too divergent from those targeted by the primers and probes designed.

In addition, PCR will provide only partial information on the genetic diversity, genotype, functional potential, nutritional requirements as well as virulence or antibiotic-resistance. Such information, that could only be retrieved from whole genome sequencing (WGS), usually requires culture of the pathogen, which can be unsuccessful in the majority of cases (and particularly for viruses and other intracellular organisms which need host cells), can take several weeks for fastidious microorganisms or can be prevented by early administration of antimicrobial drugs. The power of NGS might thus be of particular interest in that cases for reconstructing full genomes of pathogens directly from nucleic acids extracted from clinical samples. However, due to the low pathogen/nucleic acid ratio in these complex biological samples, NGS may fail to detect/reconstruct genomes from pathogens present in low copy numbers in the sample. To overcome these limitations, capture methods, such as hybridization capture followed by NGS sequencing (also called hybrid-capture sequencing or target-enrichment sequencing) applied directly on human clinical samples have been developed (Mamanova et al., 2010). These approaches allow retrieving large genomic fragments to complete genomes with high sequencing coverage, which facilitate downstream investigations, such as phylogenetics, evolution, epidemiology, and drug resistance. In this review, we will briefly describe the different principles of hybridization capture coupled with NGS, its early developments on human genetic studies and applications in the recent years to the study of present and past human infectious diseases (Gasc et al., 2016) directly from biological samples.

Overview of the Experimental Procedure and Applications

Next-generation sequencing hybridization-based capture is an approach directly applied after nucleic acid extraction and library preparation (Figure 1). Fragmented shotgun libraries are denatured by heating and subjected to hybridization with DNA or RNA single-stranded oligonucleotides (called also ‘probes’ or ‘baits’) specific to the region of interest (Kozarewa et al., 2015). RNA baits are preferable, because RNA:DNA duplexes are better in term of hybridization efficiency and stability, compared to DNA:DNA hybrids (Lesnik and Freier, 1995). Non-specific unbound molecules are washed away, and the enriched DNA is eluted for NGS (Kozarewa et al., 2015). The hybridization between DNA libraries and baits can be carried out in solution or on a solid support. In “solid-phase,” DNA probes are bound to a solid support, such as a glass microarray slide (Albert et al., 2007; Okou et al., 2007), where in “solution-capture,” free DNA or RNA probes are biotinylated allowing them to isolate the targeted fragment-probe heteroduplexes using magnetic streptavidin beads (Gnirke et al., 2009). So far, there is no standardize protocol for target enrichment processes and several adjustments can be made on library fragment size, sample fragmentation, cleanup procedures, number of PCR amplification cycles, and/or the hybridization duration. Detailed protocols are described in the work of Mamanova et al. (2010) along with values on specificity, sensitivity and reproducibility of each tested procedure. The ultimate step of NGS hybridization-based capture is the sequencing of the enriched nucleic acids and bioinformatic analyses of the reads. The last process usually includes steps of trimming (for adapter sequences, low quality and duplicate reads), mapping of the remaining reads on reference genomes for pathogen detection and identification, and/or assembly into contigs for genome reconstruction (Figure 1). The information provided by the genome can further be explored to investigate the genetic diversity (strain genotyping, variant detection), epidemiology, evolutionary history, transmission networks, and/or antimicrobial resistance of the target pathogen(s).

Early Developments of Hybrid-Capture Strategies: Human Genetic Studies on Modern and Ancient Samples

Target-enrichment strategy using hybrid capture was originally developed for human genomic studies for which it was used to capture and sequence the entire human exome. This genomic technique, also called exome sequencing (or whole exome sequencing) was first applied by using an array-based hybrid capture method in 2007 (Hodges et al., 2007). In this study, the authors developed six customized NimbleGen arrays to capture about 180,000 coding exons with overlapping 60–90-nt probes allowing an average enrichment of exon DNA sequences of 323 folds. Whole exome sequencing using capture arrays has proven its usefulness in identifying rare variants and mutations causing disease (Choi et al., 2009; Ng et al., 2009). The limitations of this technique include the need to design an array and a relatively large amount of DNA To overcome some of the weaknesses of the previous method, Gnirke et al. (2009) have developed an in-solution hybrid capture method for human whole exome sequencing. To do so, biotinylated RNA baits of 170 bases in length were constructed, targeting 5,565 human protein-coding exons. In this study, authors have demonstrated the possibility to perform hybrid selection in solution. Following this, many targeted human exome in-solution enrichment methods for NGS have been developed, including those commercialized by Illumina[1] and Agilent Genomics[2] (Chen et al., 2015a,b). In-solution capture for exome sequencing turned out to be an effective approach applied to discover the causal mutation of rare Mendelian disorders (Shearer et al., 2010; Martignetti et al., 2013; Nectoux et al., 2015; Rousseau-Nepton et al., 2015), of complex disorders (Poultney et al., 2013; Guipponi et al., 2014; Griesi-Oliveira et al., 2015; Pérez-Serra et al., 2015), mitochondrial disorders (Calvo et al., 2012; Gai et al., 2013) and more recently of the screening of potential genetic mutation of patients suffering from cancer (Sikkema-Raddatz et al., 2013; Drilon et al., 2015; Xie et al., 2016; Rozenblum et al., 2017; Xu et al., 2017; Clark T.A. et al., 2018; Schrock et al., 2018).

The power of hybridization capture has been also successfully used to study human ancient DNA (aDNA) preserved in ancient human remains. Indeed, in ancient human samples, DNA is highly fragmented (thus a shotgun fragmentation step is usually not required) and dominated by a large contamination of environmental and bacterial DNA, which poses a limitation in shotgun aDNA sequencing experiment (Knapp and Hofreiter, 2010). Another characteristic of aDNA is cytosine deamination on the ends of DNA fragments.

Library construction can be done directly on the double stranded DNAs (dsDNAs) or single stranded DNAs (ssDNAs) and may include cytosine deamination removal by the use of a damage treatment step with uracil DNA glycosylase and/or endonuclease VIII (Briggs et al., 2010). The first genetic marker analyzed in human paleogenetic studies was mitochondrial DNA (mtDNA) because of its higher copy number in the cell than nuclear DNA. Probe hybridization assays used biotinylated DNA or RNA probes targeting the two hypervariable segments of the mtDNA control region (CR) (Briggs et al., 2009; Krause et al., 2010; Maricic et al., 2010; Enk et al., 2013; Kihana et al., 2013; Templeton et al., 2013; Eduardoff et al., 2017; Loreille et al., 2018). Another uniparental marker, the Y-chromosome DNA (Y-DNA), was also used to study aDNA. As each cell possesses only one copy of the Y chromosome, the hybridization capture was carried out to enrich specific genomic regions of the Y chromosome both on solid support (Fu et al., 2013) and in solution (Cruz Dávalos et al., 2017). However, targeting mitochondrial DNA or Y chromosome involves discarding a large proportion of potentially informative sequences present in autosomal DNA. For this reason, Carpenter et al. (2013) reported a new capture-based method, called whole-genome in-solution capture (WISC), using modern DNA as bait covering the entire human genome. This method was applied to 12 ancient human DNA libraries and showed an enrichment of 6 to 159 folds of the sequence mapping to the human genome with enrichments of 2 to 13 folds for unique fragments (Carpenter et al., 2013). As for modern human genetic studies, commercial kits targeting mitochondrial DNA, custom loci, or entire nuclear genomes, such as those developed by Arbor Bioscience (myBaits®[3]) are now employed in the genetic sequencing of ancient DNA (Enk et al., 2014; Lindo et al., 2017).

M.Applications of Target-Enrichment Sequencing to Human Infectious Diseases

Parasites and Fungi

The first application of hybrid selection method for infectious diseases was in the field of human parasitology research(Melnikov et al., 2011; Table 1). To overcome the low proportion of *Plasmodium falciparum* sequences relative to that of their human host, authors have proposed to adapt in-solution NGS hybrid capture method to enrich this pathogen. This protocol has been tested in both mock mixtures composed of 99% human DNA and 1% *Plasmodium* but also in *P. falciparum* clinical samples. For this purpose, synthetic 140 bp oligos labeled with biotin were designed to capture exonic regions of the *P. falciparum* genome, whereas 250 bp oligos were constructed to target the entire genome. Processed and unprocessed samples were then sequenced with an Illumina technology. In the mockmetagenome, sequencing of the hybrid-selected samples yielded between 37 to 44-fold enrichment of the parasite DNA. In the human clinical sample, Illumina sequencing showed that at least 5.9% of reads mapped to *Plasmodium*, but no data was provided regarding the percentage of *Plasmodium* reads obtained without hybrid capture (Melnikov et al., 2011). However, this first study highlighted the good performance of NGS hybrid capture to sequence parasite genome from human clinical samples. In 2012, other studies confirmed the good performance of in-solution hybrid capture to enrich *P. falciparum* (Smith et al., 2012) sequences and *P. vivax* (Bright et al., 2012).

Fungi are also a major cause of human diseases that can be particularly serious in immunocompromized patients or in patients hospitalized for serious diseases (Pfaller and Diekema, 2010). For example, systemic infections with *Candida albicans* in immunocompromized patients result in mortality rates of about 50% (Pfaller and Diekema, 2010).

The prevention, diagnosis and therapy of fungi infections remain very difficult and comprehension of transcriptional regulation between fungal pathogens and host is an important step to identify potential novel targets for drug development (Pfaller and Diekema, 2010). Again, the limitations of host and pathogen transcriptome analysis lie in the low proportion of fungal RNA present in the total extracted RNA. The use of specific enrichment procedures before RNASeq analysis has then been proposed as an alternative method to overcome the problem of low fungus/host RNA ratio. For this purpose, Amorim-Vaz et al. (2015) have designed a set of 55,342 biotinylated 120 bp-RNA probes covering 6,094 *C. albicans* ORFs. cDNA libraries were established using SureSelect (Agilent) after extraction of RNA from mice kidney or *Galleria mellonella* larvae infected with *C. albicans*, and were subjected to capture with biotinylated probes before Illumina HiSeq sequencing. Results showed up to a 1670-fold enrichment of *C. albicans* reads in a given biological sample and a detection of more than 86% of its genes. Many genes that have been regulated in *in vivo* infection experiments have functions that have not yet been characterized and will require further research to understand their role during infection (Amorim-Vaz et al., 2015).

Bacteria

In bacteriological research and diagnostic, targeted capture strategies prior to sequencing could be a powerful tool in the management and therapeutics of patients with infectious disease. Indeed, the rapid identification of antimicrobial resistance is essential for a rapid and effective treatment. Regarding *Mycobacterium tuberculosis*, current methods of screening for antimicrobial resistance, which are based on the culture of the organism from sputum samples before sequencing, can take up to several weeks.

To overcome these limitations, Brown et al. have proposed to use oligonucleotide enrichment technology to capture *M. tuberculosis* genome sequences directly from positive smear sputum samples (Brown et al., 2015; Table 1). Whole genome baits (120-mer RNA baits) were designed to span the entire positive strand of the *H37Rv M. tuberculosis* reference genome and synthesized by Agilent Technologies. The authors demonstrated the reliability of targeted sequencing to recover and sequence, in less than 96 h, nearly complete genomes directly from 81% (21/26) smear positive sputa but also its robustness to identify the genotype and resistance determinants of all samples that were previously tested positive samples.

This study emphasizes the use of hybrid selection target enrichment that could allow personalized antimicrobial treatment in multidrug-resistant tuberculosis (Brown et al., 2015). Other studies have used biotinylated baits spanning entire genomes for high-resolution strain genotyping directly from clinical samples. Indeed, discrimination of *Chlamydia trachomatis* serovars from genital samples would facilitate the study of population structures and modes of transmission (Christiansen et al., 2014) while genomic data from uncultured *Neisseria* *meningitidis* not grown in the case of invasive meningococcal would allow increased surveillance of vaccine antigens and studies on possible vaccine deficiencies (Clark S.A. et al., 2018).

Viruses

In viral research and diagnostic laboratories, viral WGS is also essential for the detection of drug resistance and the development of novel treatments and vaccines (Houldcroft et al., 2017). In this domain, the first study that demonstrated the effectiveness of target capture technology for reconstructing full herpesvirus genomes from complex biological samples was proposed by Depledge et al. (2011) (Table 1). In this study, 120-mer RNA baits generating a 2× coverage for Varicella-Zoster Virus (VZV), a 5× coverage for Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated Herpesvirus (KSHV), were synthetized and hybridized with DNA extracted from a range of clinical samples including blood, saliva, vesicle fluid, cerebrospinal fluid, and tumor cell lines.

Full-length herpes virus genomes were reconstructed at high read depth for the 13 samples tested and generated further studies on the structure and diversity of the viral population (Depledge et al., 2011). Following this study, the capture of whole genomic hybrids made it possible to study the genomic diversity of eight new complete EBV genomes isolated from biopsy specimens of primary nasopharyngeal carcinomas (Kwok et al., 2014), 37 Zika virus genomes (ZIKV) samples out of 66 attempts (Metsky et al., 2017), 453 complete genomes (with >90% genome coverage and >100-fold read depth) of different norovirus genotypes from 509 stool samples (Brown et al., 2016) and to achieve sufficient coverage for *de novo* genome assembly and detection of single nucleotide variants of Lassa virus (LASV) from ultra-low input samples (Matranga et al., 2014).

This approach has been also used to characterize other clinically relevant viruses, such as hepatitis C virus (HCV) (Thomson et al., 2016), varicella zoster virus (Depledge et al., 2014), human herpesvirus 7 (HHV-7) (Donaldson et al., 2013, 7) and the herpes simplex virus 1 and 2 (HSV-1 and HSV-2) (Greninger et al., 2018). Hybrid capture associated with shotgun sequencing could also be performed using a combination of several viral species used as baits. Indeed, Wylie et al. (2015) developed ViroCap, a panel of probes designed to enrich nucleic acid from 34 families of DNA and RNA viruses (190 viral genera and 337 species) that infect vertebrate hosts, except human endogenous retrovirus. These probes were tested both on a pool of 14 clinical samples, which tested positive for a viral infection, and on eight samples from young children with fever, also positive for one or more viruses. Libraries were sequenced before capture (pre-capture) and following capture using ViroCap (post-capture). Combining results from both experiments, 32 viruses were detected (11 additional in the post-captured samples), including diverse DNA and RNA viruses (with genomes ranging from 5–161 kb) with genomic coverage >80% for 16 of the 32 genomes.

Several complete genomes were reconstructed and belonged to Human bocavirus 1, Human parvovirus B19, human adenovirus B (type 3), human adenovirus C (type 1), KI polyomavirus, sapovirus, and human astrovirus 1. Finally, although ViroCap cannot detect viral sequences that are completely novel, its design, which includes neighbor genomes of reference sequences, allows variants with nucleotide sequence identity as low as 58% to be identified (Wylie et al., 2015). The same year, VirCapSeq-VERT, a virome capture sequencing platform targeting 207 viral taxa infecting vertebrates was described (Briese et al., 2015). VirCapSeq-VERT allowed reduction of background human DNA and a 100 to 10,000 fold enrichment in viral reads when compared with other enrichment procedures such as treatment with nucleases or RiboZero rRNA depletion (Briese et al., 2015). In 2018, a similar approach called ViroFind was designed to target 535 DNA and RNA viruses, which are known to infect humans or cause zoonoses. This in-solution target enrichment was applied to the brain biopsy samples of five patients with progressive multifocal leukoencephalopathy (PML) (Chalkias et al., 2018).

It allowed the description of highly complex Polyoma virus JC populations as well as the detection of large genetic divergence among variants, with some of these mutations conferring viral fitness advantages (Chalkias et al., 2018)

Lastly, other applications of target-enrichment sequencing have been described, such as the study of viral genome integrations within the human genome. This approach was powerful and efficient to identify Merkel Cell Polyomavirus (MCPyV) insertion sites on DNA extracted from formalin-fixed and paraffin-embedded tissue from Merkel cell carcinoma (Duncavage et al., 2011). It also allowed to analyze retroviral genomes integrated within host genomic DNA in case of human T-cell leukemia virus type-1 (HTLV-1) and human immunodeficiency virus type-1 (HIV-1) infections (Miyazato et al., 2016).

Paleomicrobiology

Paleomicrobiology is an emerging research field dedicated to the detection, identification and characterization of microorganisms (bacteria, viruses, and parasites) in ancient specimens. Elucidating past infectious diseases can lead to a better understanding of the temporal and geographical distributions of infected individuals, the introduction of microorganisms into human populations, the host-pathogen relationships but also the genetic evolution of the microorganisms (Drancourt and Raoult, 2005). The main limitations of paleomicrobiological studies concern the degradation of ancient DNA (aDNA) and the risk of contamination by modern DNA (Riviera-Perez et al., 2016). Target-enrichment prior to sequencing is therefore a particularly relevant tool in this context for genomes study. The first two studies using targeted enrichment in paleomicrobiology have investigated genetic changes and virulence factor of *Yersinia pestis*, the causal agent of the second plague pandemics (Black Death, 14–17th centuries) (Bos et al., 2011; Schuenemann et al., 2011; Table 1). To this end, an array-based enrichment using probe targeting either the full *Y. pestis* chromosome or *pestis*-specific virulence plasmids was applied directly after the DNA extraction from ancient bones (Schuenemann et al., 2011, 1) and/or teeth (Bos et al., 2011; Schuenemann et al., 2011)

Using targeted DNA capture approach combined with high-throughput sequencing, the authors reconstructed 99% of the pPCP1 plasmid sequence (Schuenemann et al., 2011) and a draft genome of *Y. pestis* (Bos et al., 2011) with the molecular damages typically associated with aDNA. Comparisons with modern genomes did not identify any significant genetic variation that could explain the differences between the ancient and modern forms of the disease (Schuenemann et al., 2011). More recently, three other draft genomes of *Y. pestis* have been recovered from individuals who died during the first plague pandemics (the Plague of Justinian, 6–8th centuries) in two different rural sites in southern Germany (Wagner et al., 2014; Feldman et al., 2016).

Genetic characterization showed that these 3 drafts derived from a single Justinianic strain which is unique and harbors novel substitutions and structural polymorphism (Wagner et al., 2014; Feldman et al., 2016). Finally, target enrichment sequencing also allowed the reconstruction of new *Y. pestis* strains from Bronze Age individuals (~3,800 BP) (Spyrou et al., 2018) providing further datas into the early stages of *Y. pestis* genome evolution including on genomic characteristics supporting flea-borne transmission in rodents or humans (Spyrou et al., 2018). Finally, target-enrichment sequencing approaches in the paleomicrobiological research field have not been exclusively applied to the study of ancient plague pandemics, but have also allowed genomic investigation of ancient *Mycobacterium tuberculosis* (Bos et al., 2014), *M. leprae* (Schuenemann et al., 2013), *Variola virus* (Duggan et al., 2016), *P. falciparum* (Marciniak et al., 2016), and *Treponema pallidum* (Schuenemann et al., 2018) in human remains.

Conclusion

Target-enrichment sequencing is an efficient approach that allows large fragments and even entire sequences of the genome of targeted microorganisms to be reconstructed directly from modern and ancient complex biological samples containing a low pathogen/host nucleic acid ratio. The information provided by the genome can be used to explore the genetic diversity, epidemiology, evolutionary traits, transmission networks, host-pathogen interactions or antimicrobial resistance of the target pathogen or its variants.

The main current limitations of democratizing target-enrichment sequencing in clinical diagnostic laboratories are its elevated cost, the high expertise required for library preparation and the necessary time to generate biotinylated probes from reference genomes, which hampers a rapid response to an emerging pathogen.

Above all, it is not suitable for the detection and characterization of completely novel microorganisms, including viruses whose emergence may represent one of the main threats to human health in the near future.

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