Chapter 1

Triazole Modification of Coumarin Scaffolds by Copper (I)-Catalyzed Huisgen 1, 3-Dipolar Cycloaddition (Click Chemistry) Reaction: An Innovative Pathway towards Peptidomimetic Fluorophores

1.1. Drug discovery and natural products

Synthetic organic chemistry has always been a pivotal role in the highly integrated and multidisciplinary process of drug development. Drug discovery is a complex process of identification of a drug molecule starting from the proposal of biological target to the launching of the final product while passing through different phases taking overall 12-15 years and costing approximately $1-2 billion\(^1\). A large number of molecules will pass through the early stages from basic research to lead discovery but a very few reaches to the clinical development phase to attain drug status\(^2\). Lead discovery involves the process of intensive research to develop a drug-like small molecule capable of passing through the next phases to become a marketed medicine. This process of development started in the 20\(^{th}\) century when scientists discovered that drug action is caused by the specific interaction of drug molecule with biological targets rather than by the action of some mystical “power of life”. This idea started a new era in the field of pharmacology and many bioactive compounds were extracted from the crude mixtures of plant extracts and elucidated their chemical structure\(^3\). Classical examples include morphine (the active
agent in Opium), quinine, reserpine, cocaine, ephedrine, digoxin (a heart stimulant originating from flower *Digi-talislanata*) etc\(^3,4\).

**Figure 1.1.** Drug discovery process from target ID and validation through to filing of a compound and the approximate time scale for these processes. FDA, Food and Drug Administration; IND, Investigational New Drug; NDA, New Drug Application\(^1\).

Historically, most of the new drugs are originated from plant extracts through the identification of active ingredients. These drugs are not derived by using all parts of a specific plant; instead they are extracted from different parts. For example, extraction of drugs like cinnamon, quinidine, quinine etc. are carried out from the barks of certain plants. Reserpine and atropine are derived from the roots and castor oil, colchicine, morphine, strychnine, theobromine etc. are obtained from the extraction of seeds. Atropine, caffeine, cocaine, digoxin, and pilocarpine are obtained from the leaves of certain plants.

With the development of classical pharmacology, scientists go behind natural products or extracts to deliver chemical libraries of synthetic small molecules, so as to screen in intact cells or whole organisms to identify substances that have a desirable therapeutic effect. This led to the large scale synthesis of natural product derived molecules. But the development of natural product based drugs is problematic due to many reasons including the synthetic difficulties as well as the inherent nature of the resulting candidate. On the synthetic
point of view, drug discovery based on natural products is generally slow, costly, and hindered by complex syntheses. To overwhelm this situation, researchers go behind molecules which could mimic the natural product or Peptidomimetics.

1.2. Peptidomimetics

Peptides are the biological switches which modulate the biological functions in organisms including complex interaction with targets\(^5\). They are placed in between classical organic drug molecules and giant biopharmaceuticals. The molecules which mimic peptides are known as Peptidomimetics\(^6\). Even though many natural product based drugs are available, the area remains still underexploited due to several reasons. To overcome all the limitations, researchers paid their attention to design the mimetics and are optimized to get the best pharmacokinetic properties\(^7\). As a result, small molecules are designed to get a peptide like structural feature which could mimic the complex interaction of natural peptides with biological targets. These modified structural moieties with improved bioactivity are then called as peptidomimetics. The design and development of peptidomimetics involves the introduction of structural features analogues to that of original peptides to make it favorable for its biological action.

Scheme 1.1. Development of pyrrolidine based Peptidomimetic from parent peptide

1.3. Peptidomimetics in Drug discovery

The central point in drug discovery is the molecular mimicry which leads to the peptidomimetic chemistry\(^8\). The need for peptidomimetics arises due to the ineffectiveness of natural peptides in drug action. Natural products have complex molecular structures, with cyclic semi-rigid scaffolds, several chiral centers, more than five H-bond donors, more than ten H-bond acceptors, more than five rotatable C-C bonds, a large polar surface area, and a molecular weight above 500. Combined effect of all these factors may lead to moderate levels of bioavailability. The main problem associated with natural peptides is their poor stability. The peptide backbone should be sufficiently stable until it reaches the target. But in many cases, it easily gets hydrolyzed by peptidases in the gastrointestinal tract. Those one which can resist this hydrolysis will fail to cross the blood-brain barrier due to poor transport properties. In most cases, this will happen if the peptide is having a large size or high molecular weight. Another problem is the flexibility of peptide back bone. A more flexible structure causes interaction with multiple receptors in addition to the targeted protein which eventually causes unwanted side effects\(^9\). Therefore modifications are necessary to improve the bioavailability, receptor selectivity and other pharmacokinetic properties. Structure-activity relationship studies reveal that the biological activity can be altered by the chemical modifications\(^9\).

The design of a peptidomimetic structure needs the understanding of basic idea about peptide-target interaction. This interaction mainly depends on the nature of ligands used in
peptidomimetic. Cross examination of the peptide will result in the alterations of the side chains. While designing a new peptidomimetic molecule, the weak points of natural peptide which causes the inefficiency of the drug action must be known. At the same time, the key structural part responsible for the biological action should also be identified. And then the weak points are replaced with appropriate functionalities like hydroxyl group, amino group or sometimes some heterocyclic functionality. Literature reports suggest that incorporation of heterocyclic functionality to the peptidic structure could improve the biological properties. Such side chain activation will improve the binding affinity as well as selectivity at the same time reduces the unwanted side effects which will eventually lead to better therapeutic effects.

Introduction of such pharmacophoric groups to the basic core structure makes the molecule a peptidomimetic. This idea has altered the paradigms of the modern drug discovery process and has resulted in the industrial production of drugs by various research groups and acetyl salicylic acid was the first drug in that category. Since then many drugs were introduced to add to the drug chemical space and past century has witnessed the creation of many drugs which have now become a part of the contemporary therapeutic arsenal. Propranolol (1964), cimetidine (1975), captopril, simvastatin (1998) etc. to name a few.
The observation that some molecules, sometimes a part of molecules are effective at interacting with proteins and altering the course of disease led to the concept of “privileged scaffolds” in medicinal chemistry. In many cases several structural fragments or scaffolds are responsible for the activity of the specific drug. Or in other words, the specific pharmacological activity which controls the interaction of the drug with a target protein is caused due to the structural subunit present in the bioactive molecule making the compound as an efficient drug. These pharmacophoric groups are termed as privileged scaffolds. So the strategy based on these privileged scaffolds make the drug discovery process a readily exploitable one. In short, development of a peptidomimetic core structure with the incorporation of privileged structures to improve the binding affinity as well as selectivity without unwanted side effects will lead to better therapeutic effects.
1.4. Privileged scaffolds

The concept of “privileged medicinal structures or scaffolds” involves the wise exploitation of molecular frameworks with inherent potential for biological activity\textsuperscript{17}. In order to bind with more than one receptors, high affinity ligands are to be developed from core structures. The term was initially coined by Ben Evans\textsuperscript{18} of Merck research group during their work on benzodiazepines and has recently emerged as one of the guiding principles of modern drug discovery. They observed that benzodiazepines, which are a class of drugs powerful for treating anxiety is especially effective at altering the course of disease. They were also active against various other types of proteins and thereby can be used for other different disease classes. Consequently, benzodiazepins was the first to be described as privileged.

Evans described the term ‘privileged structures’ as simple structural subunits present in several drugs, with distinctive therapeutic uses, or affinities to several different receptors. So, it is clear that privileged scaffolds are those structural units which can act as good ligands for undruggable targets through appropriate functional group modifications\textsuperscript{19}. Compound libraries designed based on privileged scaffolds are exhibiting enhanced drug-like properties as well as good drug leads. After benzodiazepins, many more privileged scaffolds were discovered. Examples include 7-oxo-4-thia-1-azabicyclo[3.2.0]heptane ring present in penicillin-G, cyclopenteneperhydrophenanthrene present in several natural hormones such as testosterone and synthetic drugs like prednisolone, a synthetic glucocorticoid\textsuperscript{20}. Other important pharmacophoric units or privileged scaffolds include chalcone\textsuperscript{21}, isoflavone, 1, 4-benzopyrone\textsuperscript{22}, coumarin\textsuperscript{23}, quinoline ring\textsuperscript{24},
isoquinoline, indole\textsuperscript{25}, pyrrolidine etc. These privileged scaffolds either alone or along with other heterocyclic subunits can bind with multiple biological targets\textsuperscript{26}. Among the oxygen heterocycles, coumarin based privileged structural motifs are present in several natural and synthetic compounds and are recognized as a central active scaffold widely found in many natural products.

![Figure 1.3. Representative structures of natural privileged scaffolds](image)

### 1.5. Coumarin as a privileged scaffold

Coumarins are lactones of $2H$-1-benzopyran-2-one. They were first reported and isolated in the 1820’s and form an elite class of natural products\textsuperscript{27}. They are widely distributed in plants and occur as secondary plant metabolites. They owe their class name to ‘Coumarou’, the vernacular name of the Tonka bean (*Dipteryx odorata* Willd, Fabaceae) from which it is isolated. Their basic structure consists of a pyrone ring fused with benzene rings, with the pyrone carbonyl group at position 2\textsuperscript{28}.

![Figure 1.4. Structure of coumarin](image)
The coumarin chemistry began in early 20th century with the isolation of warfarin, a potent anti-coagulant. From then, many biologically active coumarins have been synthesized and made available in the market by the scientific community. This list includes acenocoumarol (anticoagulant), armillarisinA (antibiotic), hymecromone (choleretic and antispasmodic), carbochromen (coronary disease), phenprocoumon (anticoagulant) and novobiocin (antibiotic) etc. Coumarin compounds are characterized by strong pharmacological activity, low toxicity and side effects, fewer drug resistance, high bioavailability, better curative effects etc. These properties enable them to act as efficient therapeutics for various diseases. Coumarin scaffolds also obey Lipinski's rule of five and exhibit excellent cell membrane permeability, which are considered as the primary characteristics of an efficient drug29

![Structures of clinically used coumarin derivatives.](image_url)

**Figure 1.5.** Structures of clinically used coumarin derivatives.
The coumarin scaffold represents one of the key structural subunits for the discovery of new drug candidates. The recent decades have witnessed the emergence of coumarins (either fused or linked with heterocycle derivatives) as an efficient privileged scaffold in both medical and materials chemical research reflecting the importance of these compounds. Their structural variability enables them to achieve a special place in the realm of natural products and synthetic organic chemistry and as a consequence, many research groups have worked on coumarin chemistry to develop fused or heterocycle linked coumarin moieties as privileged scaffolds. They belong to a large family of heterocycles and have been extensively studied both in biochemical and pharmaceutical field due to their broad spectrum of pharmacological activities. Through large substituent tolerance, it forms weak interactions with a plethora of receptors and enzymes in the body. Various interactions like hydrogen bonding, electrostatic, van der Waals, \( \pi-\pi \), hydrophobic and metal coordination.
with active sites in the body are responsible for its huge biological
properties such as anti cancer, antipsychotic, antibacterial, antitumor, anticholinergic, antimicrobial activities etc. Also, they
have an important role in plant biochemistry and physiology, acting as
enzyme inhibitors and precursors of toxic substances. They are also
involved in the actions of plant growth hormones and growth
regulators, the control of respiration, photosynthesis, as well as defense
against infection.

1.5.1. Anticoagulant activity

Coumarin derivatives are prime oral anticoagulants. They
exhibit therapeutic effect by acting as competitive inhibitors in the
coagulation cascade pathway. They inhibit the function of vitamin K
which is required for the biosynthesis of prothrombin. Thus, coumarins
exhibit a desired therapeutic effect of anticoagulation by controlling
blood fluidity and removal of toxic effect of bleeding. The minimal
structural requirements for the anticoagulant activity of the coumarin
derivative are the presence of an intact 4-hydroxycoumarin residue and
a carbon chain in position 3. Classical examples of such anticoagulants include Warfarin (1.33) and Dicoumarol (1.34).

![Figure 1.7. Warfarin and Dicoumarol; parent compounds of anticoagulant activity](image)
Due to the narrow therapeutic index and plausible side effects, many research groups have worked on deriving other anticoagulant agents derived from coumarin. For example, Popowycz et al, reported such a warfarin modified family of C3 (linear and branched) alkyl-4-hydroxycoumarins, which led to the identification of the derived compounds as potential anticoagulants.42

\[
\begin{align*}
&\text{HO} \quad 1.35 \\
&\text{RMgBr,THF, 0°C to rt} \\
&4-\text{OHcoumarin,FeCl}_3,\text{CH}_2\text{Cl}_2, \\
&100°C,\mu\text{W,1h} \\
&\text{OH} \quad R \\
&\text{1.36}
\end{align*}
\]

Scheme 1.2. Synthesis of warfarin modified anticoagulants

Another warfarin modified compound has reported by Sashidhara et al in which various benzocoumarin amide based scaffolds were reported as orally active anti-thrombotic agents. These molecules are advantageous over aspirin since they exhibited both anti-platelet and anti-coagulant activity.

\[
\begin{align*}
&\text{R} \\
&\text{1.37}
\end{align*}
\]

Figure 1.8. Structure of novel benzocoumarin amide derivative

1.5.2. Antimicrobial activity

Coumarin molecules are also effective against microbes like bacteria, fungi etc. Earlier, naturally occurring coumarins like Novobiocin, Coumermycin A1 and Chlorobiocin were found to be an unprecedented class of antibiotics; but, their application is limited
owing to their relatively weak activity towards Gram-negative bacteria, poor water solubility and side effects and are therefore not used in clinic. To overcome this, Zhou et al synthesized and studied the antibacterial as well as antifungal activities of new coumarin-based 1,2,4-triazole derivatives against four Gram-positive bacteria (Staphylococcus aureus, MRSA, Bacillus subtilis and Micrococcus luteus), four Gram-negative bacteria (Escherichia coli, Proteus vulgaris, Salmonella typhi and Shigelladysenteriae) as well as three fungi (Candida albicans, Saccharomyces cerevisiae and Aspergillus fumigatus) by two-fold serial dilution technique\textsuperscript{44}.

![Coumarin-triazole systems](image)

\textbf{Figure 1.9.} Coumarin-triazole systems

The significance of this study relies on the presence of a 1, 2, 4-triazole ring to improve the properties to manifold. Among the three representative molecules, one with two triazole moieties (1.39) has the maximum antimicrobial effect and the structure (1.40) without a triazole ring has the least activity. This in turn depends on other structural parameters like the type of the linker, and the lengths of aliphatic chains which markedly influence their antimicrobial efficacy.

1.5.3. Antiviral activity

Viruses are the main agents responsible for various disease classes like influenza (seasonal, pandemic), smallpox, dengue, chikungunya etc. they are also involved in chronic diseases in the form
of human immunodeficiency virus (HIV), hepatitis B and C viruses (HBV and HCV, respectively), coronaviruses (Middle east respiratory Syndrome), MERS; severe acute respiratory syndrome (SARS), viral hemorrhagic fevers (Ebola) etc. Many of these diseases have global, societal and economic impact related to unexpected illnesses and deaths as well as troubling day-to-day normal life activities. Recent emergence of newer epidemics like H1N1 influenza, Ebola, Zika virus etc. are also a major threat to public health. Coumarins, owing to its high affinity and specificity to bind with different molecular targets, are considered as efficient antiviral agents. They are featured with a planar aromatic ring connected to the lactone group which being a hydrogen bond acceptor, facilitates protein-ligand binding and are therefore, used for the discovery of orally bioavailable non-peptidic antiviral agents.

Figure 1.10. Different classes of coumarins effective against various diseases
1.5.4. Anticancer activity

Among various disease classes, cancer attracts special attention of pharmaceutical researchers all over the world since it becomes the second leading cause of death in humans according to World Health Organization (WHO). It is a threat for both the developed and undeveloped countries being affected by both men and women. In women, it attacks mainly in the form of breast cancer. Because of the depth of this issue, many studies have done on this disease and many synthetic chemists are working on to deliver drugs that can wipe out this from earth. Unfortunately, there is no anticancer agent available now that has 100% efficacy without side effects. Therefore, there is a huge thrust across the globe to develop new chemotherapeutic drugs which would have maximum efficacy with specific mechanism of action to overcome the difficulties associated with the present clinically used drugs\textsuperscript{47}.

Towards this goal, many research groups have worked on various molecules to be used against cancer. Coumarin, owing to its broad range of physiological, bacteriostatic and anti-tumour and also due to its excellent cytotoxic activities, becomes an ideal choice for the development of new drug molecules in clinical use. This has resulted in an increased interest in coumarin based anticancer research and many molecules have been put forward. An additional advantage of coumarin based molecules is that it can also be used to treat the side effects caused by radiotherapy. Grotz, et al. reported that a coumarin/troxerutine combination therapy is effective for protection of salivary glands and mucosa in patients undergoing head and neck
radiotherapy\textsuperscript{48} indicating that this combination has a favorable effect in the treatment of radiogenic sialadenitis and mucositis.

Therefore use of derivatives with a hybrid structure containing more number of pharmacophores will be effective in action and many such are reported which include coumarin substituted thiazolyl-3-aryl-pyrazole-4-carbaldehydes (1.41 and 1.42) reported by Vedula et al\textsuperscript{49}, coumarin-3-sulfonamides (1.43) reported by Reddy et al.\textsuperscript{50} to name a few.

\textbf{Figure 1.11.} Coumarin substituted thiazolyl-3-aryl-pyrazole-4-carbaldehydes

\textbf{Scheme 1.3.} Synthetic scheme of coumarin-3-sulfonamides

The pharmacophores can be introduced with the aid of a linker to have better activity. The presence of a linker moiety can modulate the physicochemical properties of the total molecular frame work which will eventually result in the modulation of biological activities\textsuperscript{51}. For example, Shaimaa et al reported the development of coumarin linked to a 5 membered or 6 membered ring via a methylene thio
linkage (1.44 & 1.45). These molecules have excellent anticancer activity.\textsuperscript{52}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image12.png}
\caption{Coumarin-heterocycle diad spaced by methylene linker}
\end{figure}

1,2,3-Triazoles are important heteroaromatics with substantial biological activities like anti-tumour, anti-HIV, antiallergic, antifungal etc. They have been used as building blocks for bioconjugates\textsuperscript{53} and are also part of various anticancer agents. For example, heterocycle fused 1, 2, 3-triazoles reported by Yan et al\textsuperscript{54} showed excellent cytotoxicity against a panel of human tumor cell lines.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image13.png}
\caption{Triazole fused antitumor agent}
\end{figure}

Based on the importance of triazole as an efficient linker as well as better pharmacophore, Sinha et al synthesized triazolyl coumarins having potential theranostic activities. The term theranostic implies to molecules which play a dual role in the field of both diagnosis and therapy. A series of coumarin based target specific probes decorated with 1, 2, 3-triazoles were synthesized through
fluorogenic 1, 3-dipolar cycloaddition between azides and alkynes (DBCO) and are observed to be efficient fluorescent probes for cancer therapy\textsuperscript{55}.

**Scheme 1.4.** Synthetic scheme of triazolyl coumarins

Even though a large number of new molecules are reported and are active against cancer cell lines, the recent problem associated with them is their efficacy crisis due to the acquired resistance of these drugs. To overcome this, researchers introduce structure based design strategy in which hybrid molecules containing more than one pharmacophores are being developed. In this sense, it is desirable to synthesize molecules with more pharmacophores linked through a spacer.

As mentioned here, a recent trend in coumarin chemistry is the development of multi-target drugs decorated with two or more
pharmacophores having complementary biological activities so as to improve the result to manifold. This hybrid approach has been received much attention from medicinal chemists and considerable efforts have made towards the design and synthesis of drugs with improved druggability.

1.6. Coumarins in optical applications

In addition to the biological applications, coumarins are widely used as additives in food, perfumes, cosmetics etc. Recent developments in this area resulted in the exploration of coumarin moiety for its optoelectronic applications. They are effective fluorophores and are present in a large class of fluorescent dyes. They are extensively used as emission layers in organic light-emitting diodes (OLED), optical brighteners, fluorescent labels, probes, caging agents, anion sensors, cation sensors and also as neutral molecules. Due to the importance of coumarin as a privileged scaffold, many researchers have extensively worked on its chemistry to explore the optical properties by derivatization. The wide range of size, shape, and hydrophobicity that the coumarin molecule can adopt helps them to be useful as fluorescent probes of heterogeneous environments, such as supramolecular host cavities, micelles, polymers and solids. The “switch on” (increased fluorescence) or “switch off” (decreased fluorescence) behavior of coumarins upon inclusion in a specific cavity or region of a heterogeneous system makes them ideal for this purpose.
The reasonable stability as well as the ease of synthesis also makes them attractive for developing excellent fluorescent brightening agents and dyes\textsuperscript{67}. Recently a choice of fluorescent bioimaging probes has been developed as medical diagnostic tools. The optical properties of coumarins are influenced by the substitution pattern\textsuperscript{68}.

Coumarins with electron donor group at the 7-position and electron acceptor groups like benzothiazole, benzimidazole or benzoazole ring at the 3-position are generally considered as having good fluorescence properties\textsuperscript{69}. Based on this, Sanap et al reported the synthesis of a coumarin diad spaced by ethylenic link with one of the coumarin ring substituted with a 7-diethylamino group (acting as donor part) and the other coumarin ring with substituents at 7 and 8 positions (acting as acceptor part).

Scheme 1.5. Schematic representation for the synthesis of coumarin diad spaced with ethylenic linker

The ethylenic linker here ensures the conjugation in the molecule, thus providing improved efficiency to the molecule. 1, 2, 3-triazoles are another examples for a linker which, in addition to increase the fluorescence, also provide structural rigidity to the molecule. These heterocyclic structures are easily introduced by the straight-forward approach of click reactions.
Sivakumar et al. reported such a fluorogenic click reaction between 3-azido coumarins (which are nonfluorescent) and terminal alkynes to yield intensely fluorescent 1, 2, 3-triazole products. The small size, biocompatible nature and ease in synthesis make coumarins an ideal choice for the synthesis of fluorescent dyes through fluorogenic reactions. The reaction has significance in bioconjugation and bioimaging applications due to the inertness of both azides and alkynes.\textsuperscript{70}

\textbf{Figure 1.14}. Synthetic scheme depicting triazole as a highly fluorescent linkage in fluorogenic click reactions.

Most of the compounds presented here are 3-substituted derivatives indicating that their activity depends on the substituent’s position. From these studies, it is pretty recognizable that the optical properties of coumarin scaffold is mainly influenced by the substitution at 3-and/or 7 positions.\textsuperscript{71} Also, the importance of triazole ring cannot be neglected. It can also influence the biological as well as optical properties of the coumarin scaffolds. The hybrid structure also plays an important role in generating the properties. Hence, it can be inferred that adoption of a synthetic strategy in which the coumarin moiety is used as a pharmacophore as well as a fluorophore either
alone or linked with other heterocycles to deliver a multifunctional single frame work will be a fascinating area to find out new pathways.

The introduction of privileged scaffolds like coumarin prompted the drug discovery process to run faster. It simplified the complex pathway to reach in the final drug molecule. To improve the process furthermore, medicinal chemistry researchers has developed new approaches and methods. This new approaches include increase of structural diversity and decrease of number of steps in a reaction path.

1.7. Synthetic methodologies for biologically important molecules

Structural complexity is an important criterion in drug discovery because complex structures can interact with biological targets in a more convenient way. Improvement of structural complexity through molecular diversity will lead to the production of a large collection of biologically relevant structural frame works decorated with privileged structures. Introduction of more than one privileged scaffolds in a molecule will improve its biological activity to multiplex. This method of molecular hybridization strategy resulted in a rattling explosion in the field of drug discovery process for the development of multi-functional molecules through the introduction of more privileged scaffolds in a single molecular frame work. Such molecules will have multiple biological activities, modified selectivity with different modes of action and also have reduced side effects with improved pharmacokinetics and oral bioavailability. In general, the appended privileged scaffolds on a molecular skeleton determine the surface map and electron density distribution of the total molecular
surface. Such complex structures can provide a wealth of information in three dimensional spaces.

This fact led the scientists to use the Diversity Oriented Synthesis (DOS) for the development of complex structures so as to fasten the drug discovery programmes. This concept was introduced in 2000 by Schreiber\textsuperscript{72} which involves short reaction sequences to result in the final product in a pre-planned strategy. Molecules obtained through diversity oriented synthesis can interact with surrounding space in three dimensions and are characterized by high complexity content. An important property of DOS is that the final products, even though they are derived from a common starting material, are structurally and functionally different. Straight forward approach and relatively short synthetic effort complements DOS.

In general, a diversity-oriented synthesis involves the deliberate, simultaneous and efficient synthesis of more than one target compound in a diversity-driven approach to answer a complex problem\textsuperscript{73}. In drug research, instead of a large number of molecules; we need structurally diverse set of molecules even if it is in minor amounts. The DOS strategy follows a forward approach from simple starting materials to complex final products in contrast to the retrosynthetic method where complex molecules are broken down to get the simple starting materials\textsuperscript{74}.

A well defined structural diversity is achieved through the variation in various aspects like the building blocks, stereochemistry, functional groups and most importantly the molecular frame work\textsuperscript{73}. 

\hspace{1cm} 23
Methods like Multicomponent reactions (MCRs), complexity-generating reactions, branching pathways etc are usually used to increase the structural diversity and complexity.

To simplify the concept, DOS can be divided in to three based on the nature of product or the method of synthesis. Appendage diversity, stereochemical diversity and scaffold diversity (Fig. 1.15). In appendage diversity, different appendages are introduced to a common skeletal structure. Here, all the molecules will have a common molecular shape resulting in displaying similar chemical information. Overall, the molecules show limited diversity. The stereo chemical diversity refers to the stereochemical attachment of substituents. Stereospecific reactions results in different stereoisomeric products. The most important and relevant diversity is the scaffold diversity which leads to a library of compounds with different molecular skeletons. This diversity is achieved by changing the reagents used in a particular reaction. A well established DOS method consists of all these three approaches.

![Diagram of molecular diversity](image)

**Figure 1.15.** Three fundamental levels of molecular diversity
The building blocks to improve the structural diversity are introduced with the help of stepwise reaction sequences. But, this traditional classical method of synthesis schemes involves long steps consuming more time and often are expensive. As an alternative to solve this problem, multicomponent reaction method (MCRs) was introduced to the chemical space. Multicomponent reactions are considered as a near perfect tool for scaffold development through the efficient construction of functionally diverse structural scaffolds. The development of newer methods to carry out MCRs is a challenging endeavor. Through the MCR method, many heterocyclic drug-like molecules with structural diversity and complexity can be generated with minimum number of steps thereby facilitating lead identification in drug discovery programme. The straight forward approach of MCRs favors the synthesis of drug molecules to proceed through an easy and cost effective pathway avoiding complex strategies.

1.7.1. Multicomponent reaction chemistry

Multicomponent reactions (MCRs) are familiar to synthetic chemists for over 100 years. By definition, multicomponent reactions are special types of chemical transformations in which three or more starting materials react to form a product where the essential parts of the reactants must be seen in the newly formed product\textsuperscript{76}. They are promising, interesting field of chemistry, because of the ability to synthesize structurally complex molecules in a one pot, fast, efficient and time saving manner. Easiness in obtaining large array of structurally diverse compounds suitable for high throughput screening makes MCRs a very important tool in modern drug discovery process.
They offer the advantage of multiple carbon-carbon and/or carbon-hetero atom bond formations in a single operation. By concurrently forming several new bonds in a one-pot procedure and with high atom economy, MCRs provide rapid access to chemically diverse structures in an operationally simple manner. As a consequence, many large pharma companies are now focusing on MCR chemistry to develop new drug candidates. To supplement them, leading organic-chemistry laboratories are now focused on developing protocols to obtain stereo controlled MCR products by introducing novel catalysts and reaction conditions.

**Figure 1.16.** Multi-step vs. Multicomponent synthesis of same compound

In an MCR, all the starting materials react together to form a complex product within a short period of time avoiding sequential complex procedures (Fig.1.16). Therefore, MCRs can be considered as a convergent synthetic method where the simultaneous addition of all the starting materials, reagents and catalysts in an ordered manner leads to the final product. Figure 1.16 clearly indicates the easiness of MCRs to practice. Here, several bonds are formed in a single step which is the key criteria of efficiency of a chemical reaction and
therefore they are observed to be having high bond forming efficiency (BFE). Also, an optimal MCR should possess many starting materials in addition to high variability in inputs.\textsuperscript{79}

As mentioned earlier, the MCR chemistry began in the 19\textsuperscript{th} century with the preparation of the “benzoylazotide” from bitter almond oil and ammonia via benzaldehyde and hydrogen cyanide.\textsuperscript{80} This is considered as the oldest MCR. The work was reported by Laurent and Gerhardt in the year 1838. Subsequently many name reactions has been contributed to the MCR space like Strecker synthesis\textsuperscript{81}, Hantzsch synthesis of dihydropyrimidine as well as pyrroles\textsuperscript{82}, the Biginelli reaction affording 3, 4- dihydropyrimidin-2(1H)-ones\textsuperscript{83}, Mannich reaction\textsuperscript{84} etc. The first example of MCR in natural product synthesis is the Robinson synthesis of alkaloid tropinone.\textsuperscript{85} The reaction was reported in 1917.

Various multicomponent reactions are known today including isocyanide based MCRs, MCRs based on organoboron compounds, free radical mediated MCRs, and metal catalyzed MCRs. Among these, the isocyanide based MCRs are the most important and versatile ones and are therefore the most exploited also. Many marine natural products contain isocyanide group. The reason for the importance of isocyanide based MCRs is that the isocyanides exhibits resonance between its tetravalent and divalent carbon forms. The two most important isocyanide-based multicomponent reactions are the Passerini 3-component reaction to produce $\alpha$-acyloxycarboxamides and the Ugi 4-component reaction, which yields the $\alpha$-acylaminocarboxamides.
1.7.2. Isocyanide Based MCRs-A Source of Molecular Diversity

Isocyanides or isonitriles are stable organic compounds with a strange odour. The isocyanide chemistry\textsuperscript{86} began with the effort of Lieke (in 1859) for the formation of allyl isocyanide from allyl iodide and silver cyanide. But the field remained under explored until Ugi and co-workers started working on isocyanides in 1958\textsuperscript{87}. Eight years after Lieke’s discovery, Gautier formed alkyl isocyanides and in the same time Hofmann introduced the formation of isocyanides from primary amines, chloroform, and potassium hydroxide. For a whole century, only twelve isocyanides had been produced. One of the first MCRs using isocyanides was the Passerini reaction.

The Chemistry of isocyanides is based on three properties: $\alpha$-acidity, the $\alpha$ addition and its tendency of radical formation\textsuperscript{88}. The isocyanide group differ fundamentally from other functional groups because of the unusual valence structure and reactivity. They are the only class of organic compounds with a formally divalent carbon C\textsuperscript{II}. In exothermic reactions, C\textsuperscript{II} is oxidized to C\textsuperscript{IV}. This unusual carbon center undergoes reactions with electrophiles and nucleophiles, and also participates in radical reactions\textsuperscript{89}. Due to the peculiarity of this terminal carbon atom, they are tolerant to a wide range of transformations. This was already noted in 1892 by Nef.

![Scheme 1.6. Representation of Passerini reaction](image-url)
Mario Passerini was an early pioneer in the field of isocyanide chemistry and he developed a reaction in which aldehyde or ketone reacts with the isocyanide and a carboxylic acid in order to form an $\alpha$-acyloxyamide with a new stereocenter\(^9\). The reaction has excellent atom economy since every single portion of the educts are utilized and incorporated into the product and a new stereocenter is formed.

![Scheme 1.7. Mechanism of Passerini reaction](image)

The driving force is the oxidation of C\(^{\text{II}}\) to C\(^{\text{IV}}\) leading to more stable compounds\(^9\). In Passerini reaction, carbonyl compounds, carboxylic acids, and isocyanides afforded $\alpha$-acyloxycarboxamide in a one-pot procedure. The additional advantages of this reaction is that the intermediates can be further transformed by the Diels Alder reactions, Heck cyclizations, Ring Closing Metathesis (RCM), dipolar cycloadditions, nucleophilic aromatic substitutions and other reactions to generate a number of heterocyclic structures. The reaction can be used to produce a large number of libraries which can then be tested with enzymes or living organisms to find new active pharmaceutical substances.
1.7.3. The Ugi Reaction-An easy Platform for Natural Product Synthesis

Even though a variety of MCRs are available, each is characterized by compounds with similar skeletons with difference only in substituent types. A much great variation of MCRs is known only after the exploration of isocyanide chemistry. Three decades later, after the introduction of Passerini reaction, Ivar Ugi entered to this field and worked on isocyanide chemistry. He observed that an amine could also be included giving a four-component reaction of a carbonyl component, a carboxylic acid, an amine and the isocyanide and thereby introduced an extension of Passerini reaction\(^\text{94}\). However the Ugi reaction is much more versatile and is famous for its wide utility for the formation of rapid assembly of functionalized intermediates. A marking feature of Ugi-4CR is its skeletal diversity since it involves more educts than in conventional\(^\text{95}\) reactions. In the Ugi reaction the components combine reversibly to form the acylimidate. This intermediate undergoes irreversible Mumm rearrangement to form the product with a new stereocenter. The reaction is usually carried out in single step with polar protic solvents like methanol to yield a \(\alpha\)-N-acylamino amide.

![Scheme 1.8. Representation of Ugi 4-component reaction](image)

The chemical diversity of Ugi products\(^\text{96}\) results from the endless combination of the educts. Ketones or aldehydes can participate as the
carbonyl component. Carboxylic acids may be substituted with HN₃, HNCO, HNCS, HNCSe, H₂S₂O₃ or H₂Se₂, a thiocarboxylic acid phenol or water (the reaction between aqueous formaldehyde, 2,6-xylyl isocyanide and diethylamine affords the famous local anesthetic Xylocain; A.B. Astra, Sweden). Suitable amine components include primary or secondary amines, ammonia or equivalents such as hydrazine, hydrazides, urea, semicarbazide, monohydrazones, sulfonamides, and hydroxylamine. The reaction can also be varied by incorporating two of the components into a single molecule, for example amino acids, cyclic imines or keto acids. The Ugi reaction then yields cyclic products. There are reports on the use of carbonic acid monomethyl ester as a single component generated in situ by reacting carbon dioxide with methanol (MeOH) (employed as the solvent). Finally, the synthetic power of the Ugi reaction means that it represents the shortest track to the final product with a diverse structure. However, in order to reach at the designed molecule, post condensation modifications are necessary. The additional advantages of this reaction is that the intermediates can be further transformed by the Diels Alder reactions, Heck cyclizations, Ring Closing Metathesis (RCM), dipolar cycloadditions, nucleophilic aromatic substitutions and other reactions to generate a number of heterocyclic structures.

Synthetic chemists are always trying to develop newer methodologies that allow the easy access to large databases of compounds. Therefore recent efforts are focused on developing methods for the easy generation of carbon-hetero atom linkages. The process should be reliable and involves less number of steps. An ideal
synthetic procedure is featured with rapidity, efficiency, versatility and selectivity to produce molecules. Search for such a near perfect reaction methodology ended up in the development of click chemistry.

1.7.4. **Click chemistry-An expeditious synthetic protocol for developing highly efficient linear peptidomimetic entities**

Mimicking nature for its useful applications forms the primary basis of all chemistry\(^9\). A striking feature of nature’s favorite molecules or natural products is the presence of carbon-heteroatom links rather than usual carbon-carbon bonds. However, the synthesis of natural products required complicated procedures for the construction of large carbon skeletons involving protection and deprotection chemistry. The expensive linear synthetic methodology usually resulted in low final yields and the method was often difficult to scale up especially for C-C bond forming steps. Also in drug discovery, it was difficult to modify the lead structure and perform the structure activity relationship studies (SAR). In spite of this, the demand for new and effective drugs is still increasing. Because of all these, the pharmaceutical industry is facing an escalating challenge to design safer and more effective medicines. The strategy of medicinal chemists in their research is to discover new chemical entities that resemble the existing drugs with respect to key physicochemical and biological properties. This resulted in a continuous and aggressive search for simple, efficient and rapid methods for the generation of biologically active libraries of molecules useful for lead optimization and drug discovery.
1.7.4.1. **Definition of click chemistry**

The term click chemistry refers to a set of near perfect conditions which makes the chemical process an ideal and green one. It is a quantitative and selective approach on reaction conditions which reduces the cost of overall production and environmental impacts and thus gains a greener look. And hence this reaction is used as an acceptable method to develop complex molecules in simple way thus producing large number of bio conjugates including peptides, proteins, polysaccharides, and even entire viruses and cells. It is an extension as well as a complementary technology for the existing methods for drug discovery by mimicking traditional pharmacophores, drugs and natural products through the choice of appropriate building blocks.

The term “Click chemistry” was introduced in 2001 by Sharpless as a philosophical concept of designing simple reactions. As aforementioned, it is not a single reaction, but a massive term which involves a group of reactions which takes place under some defined conditions. By definition, Click Chemistry is a general term that identifies a group of chemical transformations with a number of attractive features including excellent functional-group tolerance, high yields and good selectivity under mild experimental conditions. Sharpless defined it as a “Strategy for the rapid and efficient assembly of molecules with diverse functionality enabled by a few nearly perfect reactions, it guarantees reliable synthesis of the desired products in high yield and purity”.

Even though this concept is reported to the synthetic field very recently, it gained quick recognition as a powerful
tool for the modification of existing molecules rather than preparing new, diverse leads and this dominating trend still continues.

Sharpless identified four main classes of reactions which come under this broad definition. The first category of reactions includes nucleophilic ring opening reactions of strained heterocyclic electrophiles such as epoxides, aziridines, aziridiniumions, and episulfoniumions etc. The non-aldol type carbonyl chemistry reactions form the next category which includes reactions like the formation of ureas, thioureas, aromatic heterocycles, oxime ethers, hydrazones, and amides. The third category of reactions involve additions to carbon-carbon multiple bonds especially oxidative cases such as epoxidation, dihydroxylation, aziridination, and sulfenyl halide addition, Michael additions of Nu-H reactants etc. Cycloadditions of unsaturated species forms the last category of reactions. This library includes 1, 3-dipolarcycloaddition reactions, the Diels-Alder family of transformations etc. Among these, the Huisgen 1, 3-dipolar cycloaddition reaction between an organic azide and terminal (or activated) alkyne yielding 1,2,3-triazoles are observed to be the “cream of the crop” reaction.

1.7.4.2. 1,2,3- Triazoles

As mentioned earlier, the generation of 1,2,3-triazole through click chemistry is as an important method in chemical biology. Triazoles are important and pharmacologically significant scaffolds which contribute a lion’s share to the pharmacologically active drug molecular library. The triazole sub unit is a core structural component
in an array of drug categories such as antimicrobial, anti-inflammatory, analgesic, antiepileptic, antiviral, antineoplastic, antihypertensive, antimalarial, local anaesthetic, antianxiety, antidepressant, antihistaminic, antioxidant, antitubercular, anti-Parkinson’s, antidiabetic, antiobesity and immunomodulatory agents\textsuperscript{103}. Orthogonality of azide and alkyne function to a wide variety of other functional groups and reaction conditions are also promising.

Triazoles are usually generated through a 1, 3-dipolar cycloaddition between azides and alkynes. The unanalyzed reaction between azide and alkynes required higher temperature to perform the reaction and yield a mixture of 1, 4 and 1, 5-triazole region isomers. The widely accepted reaction is the copper (I) catalyzed synthesis of triazole to result in the 1, 4-regio isomer solely\textsuperscript{104}. Later, the ruthenium catalyzed reaction was introduced by Fokin and Jia which afforded the 1, 5 triazole product only\textsuperscript{105}.

\textbf{Scheme 1.9.} Synthetic route for the generation of Triazoles.
1, 2, 3-triazoles mimic amide bonds with respect to the atom distances and electronic properties\textsuperscript{106} (Fig. 1.17). Both are possessed with hydrogen bond donor and acceptor atoms as well as have approximately equal $R^1$-$R^2$ distances. The dipole moment of triazole ring is much higher than the amide bond which enhances the peptide bond mimicry due to the enhancement in hydrogen bond donor-acceptor ability. This property makes the triazole ring as an active pharmacophore rather than merely acting as a neutral linkage. With the introduction of the copper catalyzed chemistry known as CuAAC reaction, this synthesis became very useful in various field of medicinal chemistry including drug discovery, modification of sugars, polymer and material chemistry etc. in addition to the CuAAC conditions, a number of experimental improvements were introduced with novel catalysts, use of microwave conditions, in situ azide formation, continuous flow processing, copper-free systems etc.\textsuperscript{107}.

![Figure 1.17. Similarity between 1, 2, 3-triazole ring and amide bond](image)

Thus, Click chemistry is now become one of the most powerful tools in drug discovery, chemical biology, and proteomic applications and became a promising strategy in the search for new pharmaceutical
lead structures. Many researchers have used click chemistry as a synthetic tool for the generation of pharmacologically valuable drugs.

1.8. Conclusion

The wide range of biological as well as physical properties makes coumarins an attractive scaffold in the hand of an organic chemist. Many studies have been already done based on the bio and photo properties of coumarin moiety and as a result; many are already characterized to evoke a particular biological activity. However, the challenge of design and synthesis of new coumarin hybrid derivatives with specific activity towards undruggable pharmacological targets and define their mechanism of action and potential side effects to achieve new therapeutic drugs is still remaining. Several novel compounds with biological and chemical properties are possible through proper backbone derivatization and screening. For which, an easy and straight forward synthetic strategy is required to get functionalized coumarins in a less number of synthetic steps and great efforts has been focused towards this goal by various researchers all over the world. By which, new methodologies are put forward to increase the structural complexity so as to facilitate the construction of new coumarin derivatives with applications in both material and medicinal chemistry. Synthesis of hybrid structure with pharmacophores including a triazole ring will pave a new way towards the development of coumarin based peptidomimetic fluorophores with interesting properties. The study presented in the subsequent chapters are mainly focused on the development of coumarin derived scaffolds as new potential lead compounds for drug discovery as novel anti-cancer agents and
fluorescent probes for bio-imaging. A schematic of the complete thesis is depicted below.
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