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**Review Article**

# An Insight Into Click Chemistry

**D. Varaprada[1], D. Sonia[1], Fharin Begum[1], M. Dharani[1], K. Blessi Priaynaka[2]***

[1]*Department of Pharmaceutical Chemistry, Osmania University*
[2]*Department of Pharmaceutical Chemistry, Kakatiya University*

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## ABSTRACT

Click chemistry has revolutionized the field of drug discovery and delivery by providing a modular and efficient approach to synthesizing complex molecules. This review article provides an overview of click chemistry, its classification, and applications in drug discovery and delivery, with a focus on nanoparticles and polymer synthesis. We discuss the principles and types of click reactions, including CuAAC, SPAAC, and others, and their advantages in synthesizing biologically active compounds. We also explore the applications of click chemistry in drug discovery, including target identification, lead optimization, and library synthesis. Furthermore, we highlight the role of click chemistry in drug delivery, including the design of prodrugs, targeted delivery systems, and diagnostic agents, with a special emphasis on nanoparticles and polymer synthesis. We discuss how click chemistry has enabled the development of novel nanoparticles and polymers with tailored properties for drug delivery and targeting. Finally, we discuss the future perspectives and challenges of click chemistry in drug discovery and delivery. This review aims to provide a comprehensive understanding of click chemistry and its potential to revolutionize drug discovery and delivery".

## INTRODUCTION

In 2001, K Barry Sharpless of The Scripps Research Institute introduced the concept of "click chemistry," which is a kind of chemistry that creates substances rapidly and consistently by joining small units together. This is modelled after nature, which likewise creates substances by assembling tiny modular pieces. It's an idea that borrows from nature. The definition of click chemistry is the process of creating substances by using heteroatom links (C-X-C) to join small units 1.Click chemistry builds complex molecules from olefins, heteroatom linkers, and electrophiles using only the most dependable reactions. 2 For processes or reactions to be considered within the context of CC, Sharpless et al., established strict requirements, stating that they had to be "modular reactions, wide in scope, produce high chemical yields, provide only inoffensive byproducts that

***Corresponding Author:** K. Blessi Priaynaka
**Address**: *Department of Pharmaceutical Chemistry, Kakatiya University*
**Email** ✉: blessipriyanka@kakatiya.ac.in
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could be easily separated by non-chromatographic methods (like crystallization or distillation) or no need of re-purification, with readily available starting materials"3 The thiol-ene reaction, strain-promoted azide-alkyne cycloaddition reaction (SPAAC), Cu-catalyzed azide-alkyne cycloaddition reaction (CuAAC), and inverse electron demand Diels-Alder reaction (IEDDA) are the main processes in click chemistry, an effective chemo-selective synthesis method for coupling molecular fragments under mild reaction conditions.These properties are attained by using a high thermodynamic force, typically greater than 20 kcal mol_1. Every reaction is extremely selective and proceeds quickly to completion (also known as "spring loaded")4. With the wide range of click reaction applications in polymer sciences being warmly welcomed and regarded as an instant success, this intriguing idea appears to be the perfect response to the needs of contemporary scientists working in research areas. Click chemistry's biomedical applications, particularly in the pharmaceutical sciences, are a growing area of interest. One reason is that linker chemistry is essential to many research fields, including drug delivery and nanomedicine. Probes must be attached to biological therapies in order to make detection and assessment easier. To deliver drugs in vivo, nanoscale delivery vehicles must be put together. The drug(s) and targeting moieties must be loaded or affixed to delivery systems. These are extremely laborious tasks to complete because of all the limitations5.

## CLASSIFICATION OF CLICK CHEMISTRY REACTIONS

### 1. Cycloaddition click reactions:

Cycloaddition reactions occur when two molecules having π bonds combine to generate a new cyclic molecule by forming two additional σ bonds.

**Azide–alkyne Huisgen 1, 3-dipolar cycloaddition:**

- cycloaddition that yields a 1, 2, 3-triazole.
- Reaction is faster and selective when alkyne is substituted with an electron withdrawing group7.

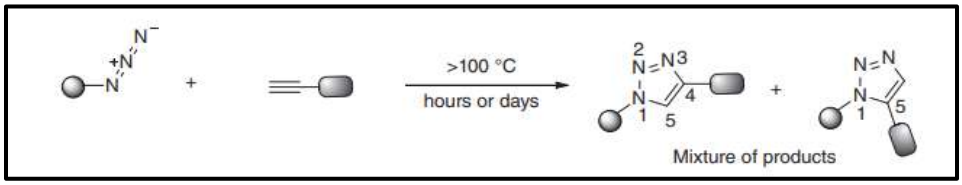



**Figure1.Huisgen 1,3-dipolar cycloaddition between alkynes and azides7.**

**Copper-Catalyzed Azide–Alkyne Cycloaddition (CuAAC) Click Reaction:**

- In the presence of copper [Cu(I)], an alkyne–azide reaction takes place between an organic azide and a terminal alkyne to produce a 1,4 disubstituted 1,2,3 triazole (Fig. 1), in contrast to the high-temperature noncatalyzed reaction, which develops into create a combination of the regioisomers of 1,4- and 1,5-triazoles.
- The frequency of this response is greater than 107. Multiplied by the traditional response, which indicates that at room temperature, it works effectively3

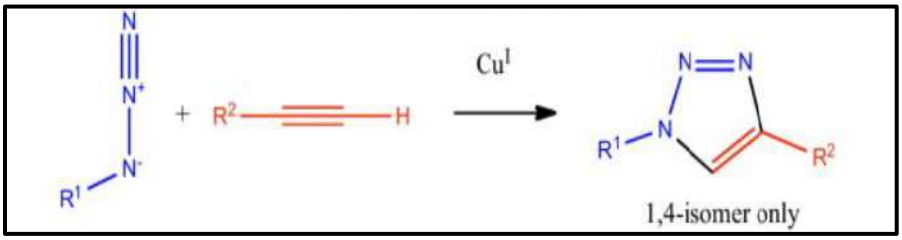



**Figure2. Copper-Catalyzed Azide–Alkyne Cycloaddition (CuAAC) Click Reaction3.**

**Ruthenium-Catalyzed Azide–Alkyne Cycloaddition (RuAAC) click reactions:**

- Ruthenium catalyzed 1,3 -dipolar azide-alkyne cycloaddition (RuAAC) results in 1,5 -triazole ,and both terminal and internal alkynes participate in the reaction3.

**Strain-Promoted Azide–Alkyne Cycloaddition (SPAAC) reactions:**

- The release of substantial ring strain of nearly 18kcal/mol in the cyclooctyne was the driving force for this reaction. The possibility of using this strain promoted azide alkyne cycloaddition (SPAAC)reaction as a click reaction for bioconjugation7.
- An alternative means of activating alkynes for catalyst-free cycloaddition with azides: Ring strain. The reaction between cyclooctyne,the smallest of the stable cyclo alkynes- and phenyl azide proceeded like an explosion to give a single product-The triazole.8

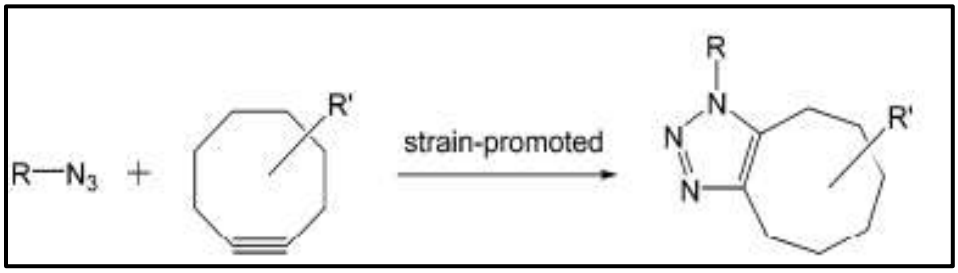



**Figure 3. Strain-promoted (3+2) cycloaddition of azides and cyclooctynes8.**

**2. Thiol-based click reaction:**

- Thiols react with a wide range of substrates and with a number of Functional groups. Thiols are easily available conjugation tools.
- Thiol–Ene radical click reaction:
- The reaction can be initiated either by using a radical initiator or directly by irradiating thiols with a UV source, preferably at 254nm.
- Irradiation of thiols promotes homolysis of the S-H bond resulting in the formation of a thiol radical9.

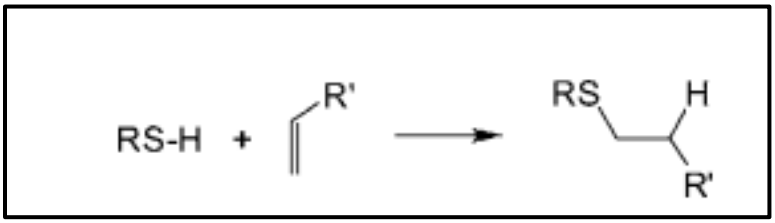



**Figure 4.Hydrothiolation of c=c bond9.**

**Nucleophilic addition click reactions of thiols:**

- Thiols &thiolate anions are nucleophiles in nature.The nucleophilic attack of thiols on electrophilic substrates like isocyanates, epoxides, and halides is the basis for many click reactions.
- The rates of reactions are dependent on the substrates and their inherent ability to attack by thiols and thiolates ions1.

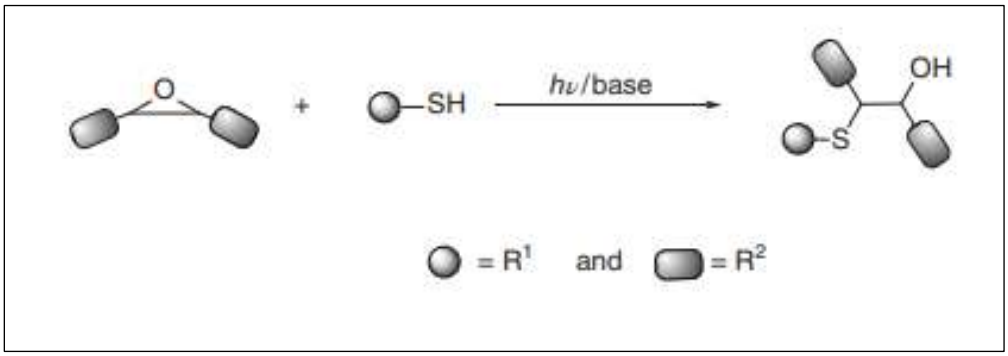



**Figure 5.Thiol–epoxide click reaction7.**

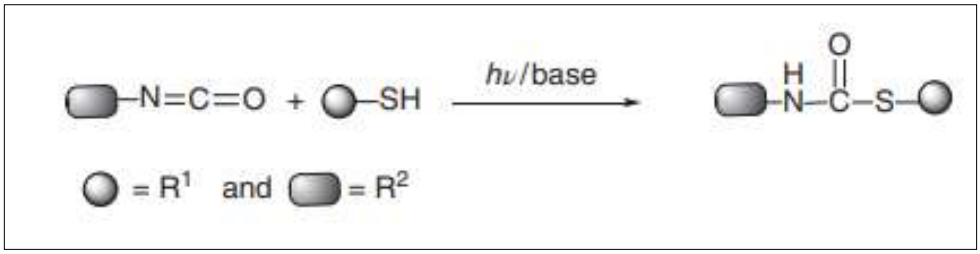



**Figure6.Thiol–isocyanate click reaction7..**

**2. Miscellaneous click reactions:**

- Nucleophilic ring-opening reactions:
- This classification composed of strained heterocyclic electrophile openings such as epoxides, aziridines, cyclic sulphates, aziridinium ions, episulfonium ions, etc5


**Figure 7. Nucleophilic ring-opening reactions6**

- Non-aldol carbonyl chemistry:This classification involves the synthesis of ureas, hydrazones, thioureas, aromatic heterocycles, oxime ethers, amide etc.
- Aldol carbonyl reactions have low thermodynamic driving forces, resulting in extended reaction durations and the production of side products, and so can't be termed click reactions5.

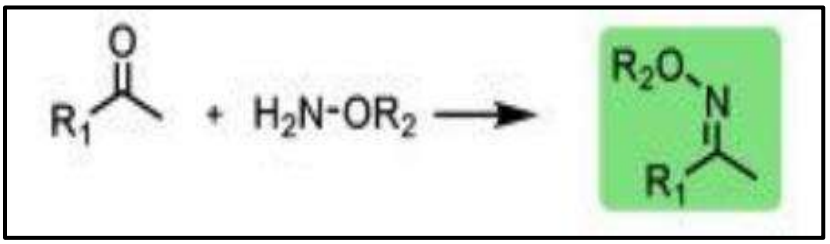



**Figure 8. Non-aldol carbonyl chemistry.(6).**

- Addition to carbon-carbon multiple bonds:
- This classification including epoxidations, aziridinations, dihydroxylations, sulfonyl halide additions, nitrosyl halide additions, and some Michael additions. (5).

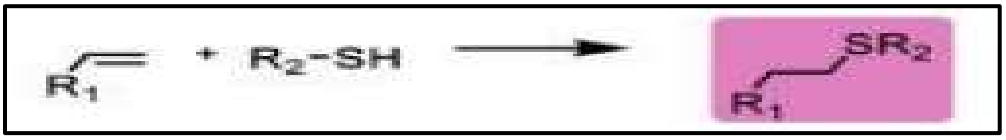



**Figure 9. Addition to carbon-carbon multiple bonds 6.**

## THE GROWING APPLICATIONS OF CLICK CHEMISTRY IN PHARMACEUTICAL SCIENCE

In Pharmaceutics click chemistry used for everything from drug discovery , drug delivery and optimisation to biological system detection, including nucleotides, proteins, and entire organisms . Combinatorial chemistry has aided the difficult lead process in recent years. Identification and optimisation, which depend on the accuracy of particular responses used to a unique configuration of chemical bonds. By simplifying the synthesis process constructing bricks for unique chemical entities, click chemistry has greatly benefited the overall procedure of developing drugs (NMEs). It has aided in lead optimisation andidentification.Expanded and improved traditional drug discovery techniques, however it hasn't completely taken their place6.

### Click Chemistry Approaches for Drug Discovery and Development

- High throughput screening
- Fragment based drug discovery
- Peptide based drug design
- Development of Enzyme Inhibitors

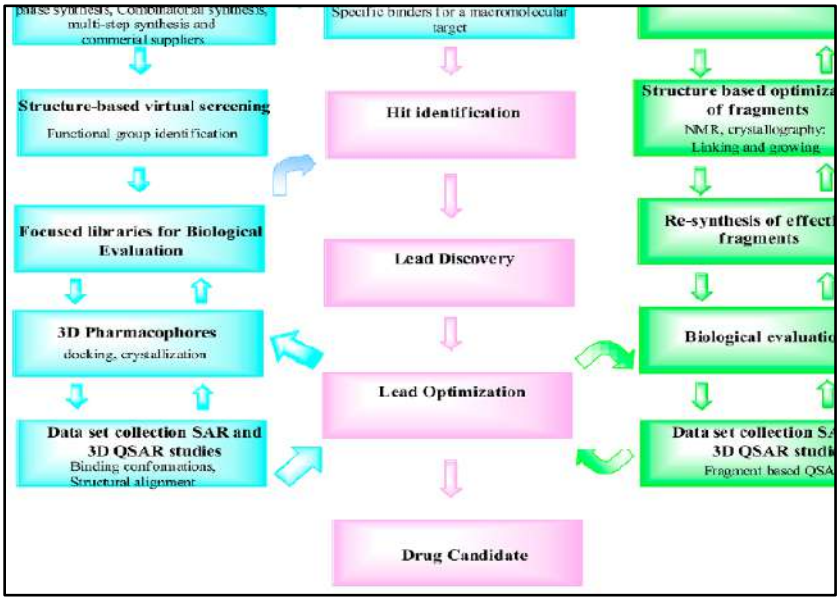



**Figure 10. Workflow of the process of hit-to-lead optimization from click chemistry and drug candidate selection.FBDD, SAR, and QSAR studies are critical components of this complex approachs. FBDD,fragment-based drug design; QSAR, quantitative SAR; SAR, structure−activity relationship10.**

## HIGH THROUGHPUT SCREENING:

A tried-and-true method for finding lead drugs in pharmaceutical and biotechnology companies is high-throughput screening, or HTS. is currently employed in academics for both basic and applied research. It entails using automation, miniature assays, and extensive data analysis to screen huge chemical libraries for activity against biological targets. The tools available for lead discovery today are growing and working in tandem with one another: structure-based design, virtual screening and access to a vast array of external chemistry sources (including isolated natural products), targeted screening for closely related target classes, and cellular assays that are becoming more and more valuable in comprehending the effects of

compounds. These tools all serve as valuable supplements to HTS in terms of offering entry points for lead optimization. Effective lead discovery strategies will distinguish drug discovery 6. Click chemistry and high-throughput enzyme assay technologies, such microarrays, transformed the drug development process's lead discovery and lead optimisation stages. Click chemistry-assembled small molecule libraries have proven effective in producing distinct inhibitor and activity-based fingerprints of significant enzymes. In the future, these fingerprinting techniques can help identify and characterise new subclasses of enzymes and possibly solve the problem of the functional convergence of enzymes on a larger scale. Click chemistry and its innovative versions, along with potent technologies like microarrays and other creative characterisation approaches, will continue to drive the rapid advancements in high-throughput screening10.

**FRAGMENT BASED DRUG DISCOVERY:**

As per FBDD, the free restricting energy of a protein-ligand is represented by its atomic parts. For worked on restricting liking, the principal pieces that stick to the protein framework are changed after the revelation of a little particle section that adheres to the protein framework of interest, and the lead structure is streamlined. Despite the fact that various proteins have a few restricting pockets, most inhibitor revelation focuses on the dynamic district. Auxiliary or allosteric restricting destinations, then again, frequently include selectivity along with power. As a result of its inconceivably adaptable and able receptive nature, click chemistry is one of the most amazing conceivable procedures for the making of "fragment-based inhibitors" in this circumstance. As a result of the snap response's proficiency and water similarity, the framed mixtures might be assessed for hindrance immediately without the requirement for decontamination6.

**Dynamic template -assisted approaches in FBDD:**

To resolve the issue of the distinguishing proof of low-affinity fragments and physiologically proficient dynamic linkages related with FBDD, dynamic template-assisted methodologies have been proposed by scientists. In every such methodology, there is a substance response ,reversible or irreversible/enzymatic or nonenzymatic, which is taken advantage of for the best fragment combination. In click reactions, azide and alkyne groups are joined onto fragments that collaborate well with the chemical, and are chosen to some extent connect with the enzymatic restricting site. Accordingly, cycloaddition among alkyne and azide interfaces the fragments to shape the final inhibitor 10. This outcomes in a quality/protein-explicit drug organization that contains the encoded combination of two or on the other hand more bio orthogonally divided prodrugs that can be controlled all the while or successively for in vivo self-get together of the medication by means of target-driven component. Subsequently, low-molecular weight drugs with profoundly beneficial pharmacological properties can be accomplished and issues connected with plasma level biodistribution can be limited for clinical application. In this way, with the help of Click Chemistry drug arrangements of firmly related however divided prodrugs can be intended to interface explicitly with atomic targets, like protor qualities, bringing about customized therapeutics10.. As an outcome, little biomolecules in medications with much wanted pharmacological qualities might be created, and issues related with "plasma level bio distribution" can be decreased for clinical use. Subsequently, utilizing click science, Drug definitions containing basically indistinguishable yet "fragmented prodrugs" can be made to interrelate directly with biological foci, whether proteomic or genomic

foci, coming about in uniquely custom- made biosimilar6.

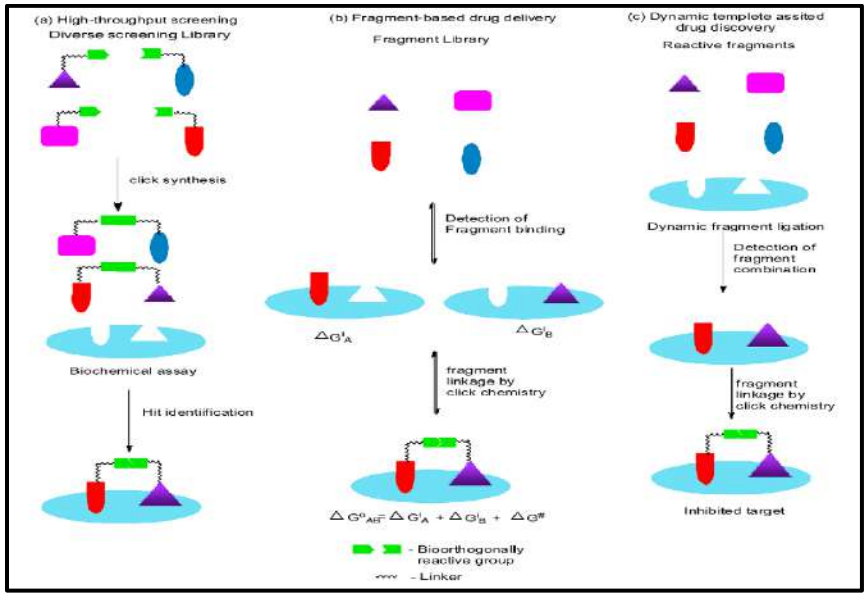



**Figure 11.Concepts in lead discovery. (a) High-throughput screening (HTS). A wide range of chemical compounds are gathered and examined in relation to the intended drug target.(b) Fragment-based lead discovery. The binding of small molecular fragments to the protein is detected. Low-affinity fragments can be linked to provide high-affinity ligands. The binding constant KAB is an exponential function of the binding energy. (c) Dynamic strategies in fragment-based drug discovery. Reactive fragments are incubated with the protein and form specific combinations of fragments on the protein template, which facilitates fragment detection and linkage to a new ligand10.**

## PEPTIDE BASED DRUG DESIGN:

Peptide-based drugs are becoming an important component of the pharmaceutical drug market, peptides are less immunogenic and have the potential to penetrate into organs and tissues owing to their smaller size. The utilization of present day engineered procedures has decisively sped up the advancement of peptide drugs, like solid phase peptide synthesis and native chemical ligation. In 2001, an exceptionally chemoselective and stereospecific Cu(I) catalyzed [3+2] cycloaddition reaction, frequently referred to as "click chemistry", was brought about by Sharpless et al., and, Meldal and colleagues and has enormously improved admittance to synthetic space of peptide-based components11..

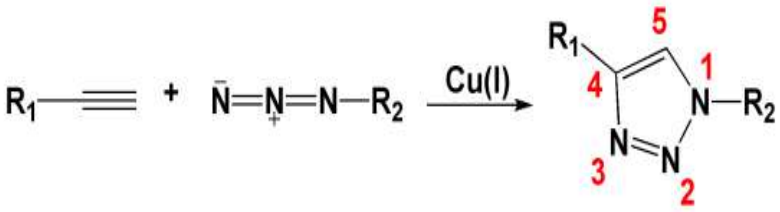



**Figure 12. Cu(I)catalyzed Huisgen 1,3-dipolar cycloaddition reaction11.**

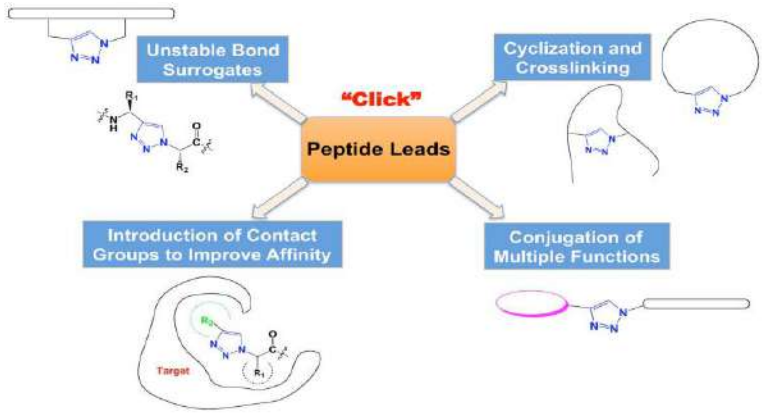



**Figure13. Types of click chemistry applications in peptide-based drug discovery11.**

Click chemistry has been extensively utilized in peptide-based drug research. Because of structural similarity it has been utilized to present a proxy of amide and disulfide bonds. This is exceptionally valuable for peptide change to build the metabolic stability. Because of the selectivity, productivity, and gentle response condition of the click reaction, it is a helpful method for connecting groups, for example, peptide fragments also, functional groups, and to accomplish peptide cyclization (side-chain to side-chain or go to tail)11.

**CLICK CHEMISTRY IN THE DEVELOPMENT OF ENZYME INHIBITORS:**

Enzymes are helpful objectives for various afflictions, including tumours, diabetes, neurodegenerative sicknesses, tuberculosis, and various other difficult ailments, and hence may display a fundamental role in treating such issues. With a rising figure of patients experiencing such terminal sicknesses, click chemistry is affecting compound union and improvement in an all the more rapidly and successfully way, giving new procedures for intensifies library screening Through fragment-based enzyme inhibitor synthesis, synthetic scientists have had the option to deliver libraries of different sorts of molecules. Each enzyme inhibitor's average design thanks to extraordinarily click chemistry particular and effective reaction properties3.

**Protein-Tyrosine Phosphatase Inhibitors:**

Protein tyrosine phosphatases (PTPs, protein tyrosine phosphatase class enzyme) comprise a significant class of signalling enzymes that catalyzes the dephosphorylation of phosphotyrosine residues in a protein substrate. Among them, PTP1B (protein tyrosine phosphatase 1B, nonreceptor phosphotyrosine PTP) has been recognized as the significant regulator of both insulin and leptin signalling pathways. Breaking down of PTP1B prompts different human disease like cancer, diabetes, obesity, and inflammation1.

1. Zhang and colleagues arranged a profoundly powerful and selective mPTPB inhibitor utilizingnovel, double click chemistry system. The most intense mPTPB inhibitor fromthis approach has a Ki value of 160nm and a >25-increase selectivity for mPTPB over 19 other protein tyrosine phosphataseinhibitor.(fig14)10.
2. Zhou and collaborators as of recently reported a powerful and specific mPTPB inhibitor with profoundly cellular activity, from a combinatorial library of bidentate benzofuran salicylic acid derivatives gathered by click Chemistry.(fig15).10.
3. Xie et al., reported new PTP inhibitor elements by essentially"clicking" alkynyl amino acids onto different azido sugar formats. Triazolyl glucosyl, galactosyl, and

mannosyl serine furthermorethreonine derivatives were productively synthesized through click reaction, one of which was distinguished as a powerful and selective PTP1B inhibitor against a panel of homologous PTP1B. (fig16) 10.

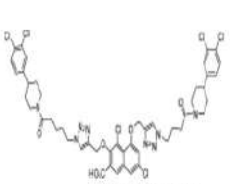


Figure 14.

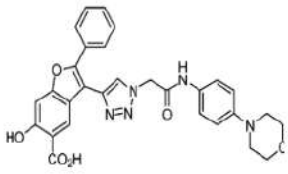


Figure 15.

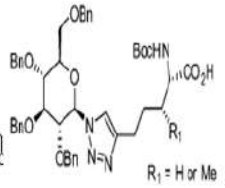



Figure 16.

**Chemical structures of protein tyrosine phosphatase inhibitor synthesized via click chemistry10.**

**Protein Kinase:**

Kinases are broadly perceived as significant medication targets engaged with numerous seriousillnesses, like malignant growth and diabetes. Protein kinase phosphorylating proteins assume an essential part in cell signal transduction, and numerous infections are described by irregularities in a kinase or its expression level. Protein kinases (PK) are a group of proteins that are engaged with controlling the capability of different proteins through the phosphorylation of hydroxyl groups of serine and threonine amino acid residues on these proteins. PK proteins are thus initiated by signs such as expansions in the centralization of diacylglycerol or Ca2+. Consequently, PK compounds assume significant parts in a few signal transduction overflows. Consequently, a critical part of medication revelation endeavour's has made protein kinases as essential targets10.

1. Liskamp et al., utilized click chemistry for the synthesis of bisubstrate-based kinase inhibitors utilizing arginine deposits highlighting acetylene or azide moieties in their side chain.Created bisubstrate-based kinase inhibitor was tried for affinity and selectivity toward three profoundly homologous PKC isozymes. The subsequent inhibitor showed further developed affinity and a profoundly interesting shift in selectivity toward PKCteta with IC50 = 0.17 ± 0.029 μm (fig17)10.

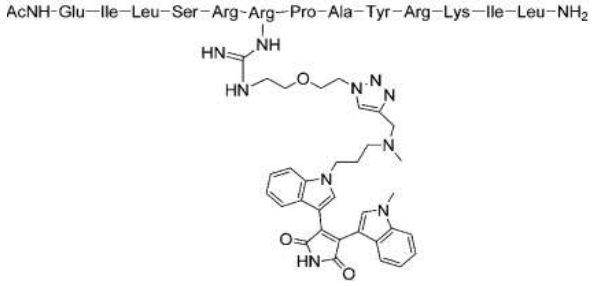



**Figure 17. Structures of the protein kinase inhibitors produced by click chemistry10.**

**Transferase:**

Transferase is an enormous class of enzymes, which play a significant role in numerous biological processes. Transferase inhibitors are potential drug targets for numerous diseases like malignant growth, and many viral and bacterialinfection diseases1.

**Nicotinamide Phosphoribosyl transferase Inhibitors:**

Interfering with NAD levels might lead to cell death of those cells that have a high usage rate of this pyridine nucleotide, that is, tumoural cells with a high division rate.Eukaryotic cellshave a few components to replenish NAD, including a denovo combination pathway from the amino acid

tryptophan and atleast two reusing pathways. One of these pathwaysdepends on the enzyme nicotinamide phosphoribosyltransferase(NMPRTase) that changes over nicotinamide into nicotinamidemononucleotide (NMN) that is subsequently changed over completely toNAD by NMN adenylyltransferase (NMNAT). NMPRTase isthe objective forthe small molecule inhibitor FK866 (APO866) that has been displayed to induce apoptosis in tumoral cells and ina similar setting to bring down essentially NAD levels10. Colombano et al., as of recently detailed copper-catalyzed [3 + 2] cycloaddition among azides and alkynes to synthesize 185 novel analogs. The most promising compound showed an IC50 for cytotoxicity in vitro of 3.8 ± 0.3 nm and an IC50 for NAD consumption of 3.0 ± 0.4 nm. Compound presents a 2-aminobiphenyl aromatic group, was the possible inhibitor of nicotinamide phosphoribosyl transferase. (fig18)1.

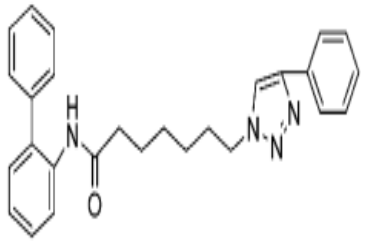


**Figure18. Chemical structures of transferase inhibitor synthesized via click chemistry1.**

**Glycogen phosphorylase Inhibitors:**

It is a key regulatory enzyme present in most mammals, catalyzes the phosphorolysis of glycogen main-chain, linked through an a-1, 4-glucosidic bond, to glucose-1- phosphate (Glc-1-P) that is subsequently converted to a-Dglucose1.

- Xie et al., reported the synthesis and biological assessment of glucoconjugates of oleanolic acid, connected by either a triazole moiety or an ester function, as novel inhibitors of glycogenphosphorylase. Several synthesized triterpene−glycoside conjugates exhibited modest inhibitory action against RMGPa. Compound showed the great inhibition with anIC50 value of 1.14 μM. Structure−activity relationship (SAR) analysis of these inhibitors was likewise examined, and conceivable binding methods of compound were investigated by moleculardocking simulations. (fig19)1.
- Cheng et al., synthesized a few dimers of oleanolic acid by utilizing amide, ester, or triazolelinkage with click chemistry. The produced oleanolic acid derivatives were tested against the glycogen phosphorylase of rabbit muscle.Oleanolic acid was as an found to be an inhibitor of glycogen phosphorylase. Among the synthesized homologous series ofcompounds, analogue was found to be the mostpotent inhibitor against rabbit muscle glycogen phosphorylase(RMGPa), and showed an IC50 worth of 2.59 μM.(fig20)10.

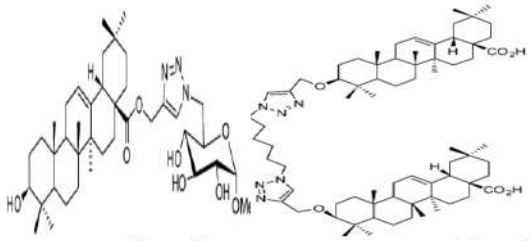


Figure 19.



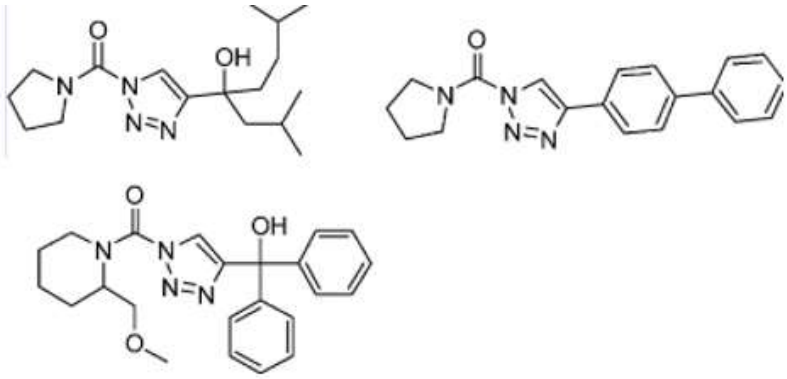
Figure 20.

**Chemical structures of glycogen phosphorylase inhibitors synthesized via click chemistry10.**

**Serine hydrolase Inhibitors:**

Mammalian serine hydrolases are almost similarly partitioned into two major subgroups of ∼125 serine proteases, for the most part from the chymotrypsin and trypsin class, andanother ∼110 "metabolic" enzymes, for the most part from the α,βhydrolase class (Pfam class task AB_hydrolase (CL0028)), that hydrolyze metabolites, peptides, or posttranslational ester and thioester changes on proteins.Due to the biological significance of serine hydrolases, clinically approved drugs target individuals from this enzyme class to treat diseases like obesity, diabetes, microbial diseases,and Alzheimer's disease10.

- Cravatt et al,. as of recently fostered a click chemistrythat produced triazole urea derivatives with ultra powerful inhibition for serine hydrolase with in vivo activity. Rapidlead optimization by click chemistry empowered synthesis andcomepetitive activity based profiling distinguished 1, 2, 3-triazoleureas that specifically inhibit enzymes from assorted branchesof the serine hydrolase class. The triazole urea inhibitorswere exceptionally intense inhibitors against their separate serinehydrolase targets in mouse T-cell proteomes (fig21a,b,c) 1.




Chemical structures of serine hydrolase inhibitors synthesized via click chemistry10.

**Cysteine and Serine Protease Inhibitors:**

cysteine proteases are fundamental in regulation of physiological cycles and disease proliferation, and the proteases play significant jobs in treatment of cardiovascular illnesses, oncology osteoporosis, and joint inflammation. They control apoptosis, MHC class II immunological responses, prohormone processing, and extracellular matrix remodeling—all of which are critical for the growth of bones in humans10.

- Ellman and his associates as of recently utilized click science for the synthesis of 1,4-disubstituted-1,2,3-triazole cruzain inhibitor analogs from aryloxymethyl ketone azide and enantiomerically unadulterated propargyl amine. 1,4-Disubstituted1,2,3-triazole analogs were screened against T. cruzi-tainted C3H mice, and tetrafluorophenoxymethyl ketone inhibitor was viewed as the most strong restraint against T. cruzi cysteine protease with IC50 = 3.1 μM and Ki = 0.10 ± 0.03 μM.(fig22) 1.
- Freire and his colleagues as of recently revealed a little library of 25 triazole/tetrazole-based sulfonamides had been synthesized, further considered for their inhibitory activity in against to thrombin, trypsin, tryptase, and chymase. The triazole-based sulfonamides inhibited thrombin more productively than the tetrazole partners. Especially, compound showed solid thrombin restraint (Ki = 880 nM) and huge selectivity against other human-related serine proteases

class enzymes like trypsin (Ki = 729 μM).(fig23) 10.

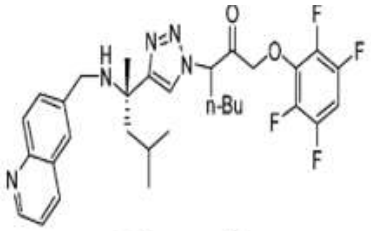


Figure 22

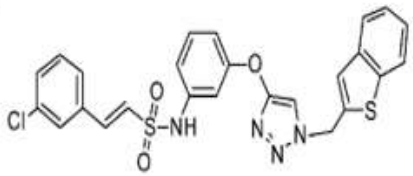


Figure23

Chemical structures of serine and cysteine protease inhibitors synthesized via click chemistry10.

**Metalloproteinase Inhibitors:**

Proteolytic enzymes called matrix metalloproteinases (MMPs) are involved in a variety of physiological and pathological processes. Overactivation of these Enzymes brings about tissue degradationdelivering a wide cluster of diseases, for example, rheumatoid joint pain, osteoarthritis, cancer development and metastasis, various sclerosis, congestive cardiovascular breakdown, and others. Accordingly, MMP inhibitors are contender for restorative specialists to battle anumber of infections10.

Ramos and his Colleagues as of recently utilized click science for the synthesis of another series of MMP2 (lattice metalloproteinase-2) inhibitors utilizing fragment based drug design approach. A click science reaction was utilized to associate the azide to lipophilic alkynes chose to interact specifically with the S1′ subunit of MMP2.(fig24a,b) 1.

2. Forino and colleagues detailed a small molecule MMP-click inhibitor library outfitted with rhodanine as the zinc-bindingscaffold. The inhibition tests uncovered reasonably powerfulinhibitors. against MMP7 and MMP13 over other MMPs (IC50 = 6.5 μM against MMP.(fig25) 1.

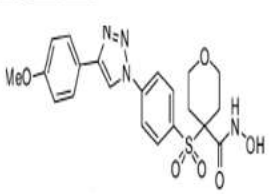


Figure 24(a)

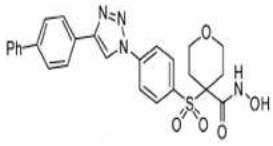


Figure 24(b)

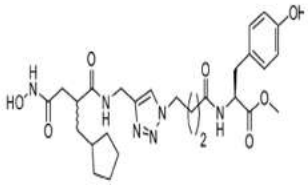


Figure25

Chemical structures of metalloproteinase inhibitors generated via click chemistry10..

**Aspartic Protease Inhibitors:**

Aspartic proteases playsignificant role in a several diseases like AIDS (HIVprotease), neoplastic issues (cathepsin D and E), Malaria(plasmepsins), and Alzheimer disease (β and γ secretase).Expanded articulation of human lysosomal cathepsin D isrelated with various obsessive circumstances includingneoplastic issues and fiery diseases10.

- Carlier and his colleagues as of recently utilized click sciencefor the preparation of β-site APPcleaving enzymes 1 (BACE1)inhibitors from 120 diminished amide isostere inhibitors utilizing ahigh-throughput in situ screening convention. Compound showed most strong restraint of BACE1 when compared withthe synthesised homologues with IC50 = 2.0 μM.(fig26) 10.
- Yao and colleagues revealed a click gathering of azido precursors of AfBPs of plasmepsins in malarial parasites with a library of aromatic

azides, which prompted the discovery of compound that showed great hindrance against each of the four PMs and parasite development in infected RBCs with great membrane porousness and least cytotoxicity with EC50 =1.04 μM against aspartic protease.(fig27) 10.

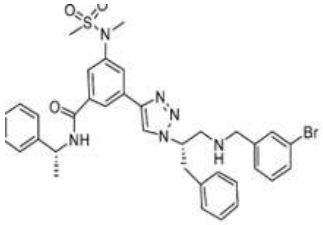


Figure 26

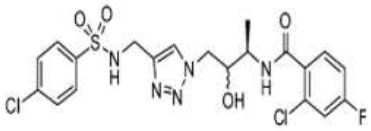


Figure 27

**Chemical structures of aspartic protease inhibitors generated via click chemistry10.**

**Oxidoreductase Inhibitors:**

Zhu and his colleagues fostered an effective technique for the quick development of 108 compounds involving click science for monoamine oxidases-A/B hindrance. The fingerprint of inhibitory action toward monoamine oxidases-A/B against this library was obtained and, four hit compounds were distinguished as specific inhibitors toward monoamine oxidases-A (MAO-A). Compound was the most powerful inhibitor of MAO-An amongthe four hit compounds with IC50 of 0.83 μM.(fig28) 1

Novel cinnamoyl and caffeoyl clusters were synthesized bydifferent Cu(I)- catalyzed [1,3]-dipolar cycloadditions, and their anti- 5-lipoxygenase inhibitory activity was tested. Caffeoyl cluster showed a improved 5-lipoxygenase (LO-5) inhibitory activity when contrasted with caffeic acid, with caffeoyl tetramer showing the great LO-5 inhibitory activity with IC50 = 0.66 μM.(fig29) 1.

3. Oyelere and colleagues synthesized a small library of SAHAlike hydroxamates utilizing click science. The amide bond in SAHA was supplanted with a triazole ring. Both the linker chain length and the aromatic ring were fluctuated, and series HDAC inhibitors were synthesised. Hindrance measures revealed a several HDAC inhibitor) with further developed strength as contrasted with SAHA.(fig30) 1.

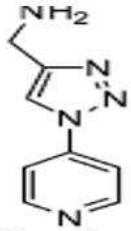


Figure 28

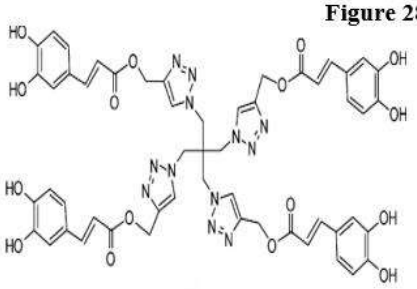


Figure 29

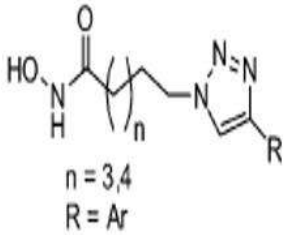



Figure 30

Chemical structures of Oxidoreductase inhibitors generated via click chemistry10.

**Glycosidase Inhibitors:**

Ferreira and his colleagues reported the synthesis of a series of 4-substituted

1,2,3-triazoles formed with sugars, including D-xylose, Dgalactose, D-allose, and D-ribose. These mixtures were evaluated for α-glucosidase inhibitory activity utilizing yeast maltase (MAL12) as a model enzyme. Methyl-2,3-Oisopropylidene-β-D-ribofuranosides, like the 4-(1-cyclohexenyl)- 1,2,3-triazole derivative were among the most active mixtures, making an appearance to 25-overlaphigher inhibitory intensity than the mind complex oligosaccharide acarbose with IC50 = 3.8 ± 0.5 μM.(fig31) 1.

Linhardt and his colleagues reported that a small library of 1,2,3-triazole-connected sialic acid derivatives was synthesized utilizingclick science. These novel sialic acid derivatives were then, assessed aspotential neuraminidase inhibitors utilizing a 96-wellplate fluorescence measure. Compound showed powerful restraint action against Neuraminidase with an IC50 value of 17 μM.(fig32) 10.

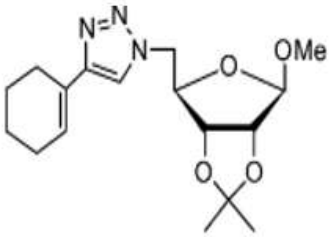



**Figure 31**

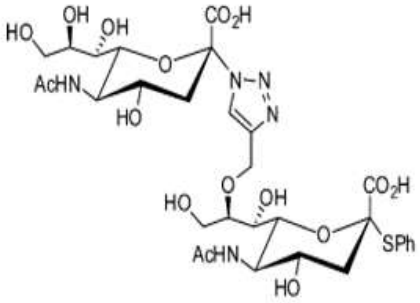



**Figure 32**

Chemical structures of glucocerebrosidase inhibitors synthesized via click chemistry10.

**In Situ Click Chemistry:**

Throughout the last few years, novel systems for the revelation of lead compounds based on click chemistry have been examined where the target moiety is effectively involved in the synthesis of its own inhibitorycompound. These fragment based techniques, also called target guided synthesis(TGS), are a subset of the conventional combinatorial methodology, where the biological target (either protein or DNA) is effectively involved in the selection of ligands assembled from a pool of smaller fragments3..

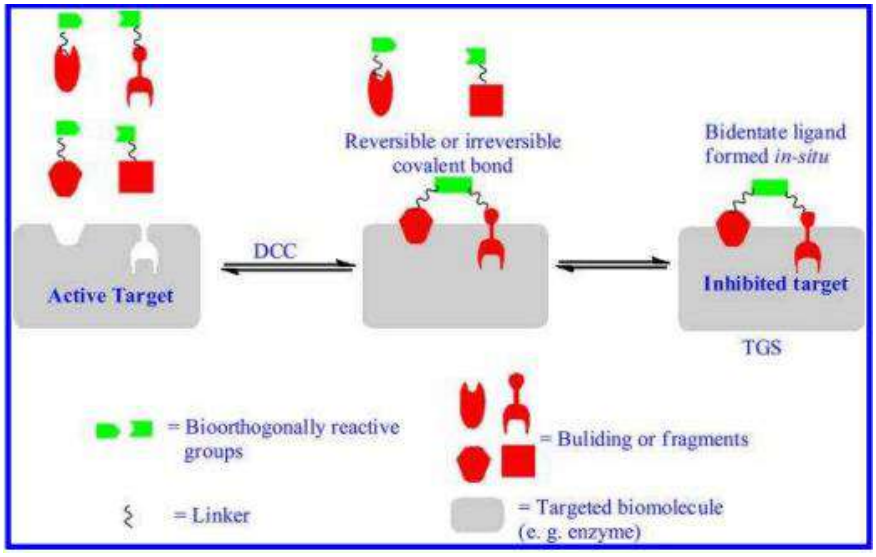



**Figure 33. In situ click chemistry used for the development of enzyme inhibitors10.**

The utilization of the click chemistry reaction in TGS has been named "click chemistry in situ". Representative examples of in situ click chemistry are depicted below. In principle, the "click chemistry" way to deal with drug design is applicable to practically any target. If it is possible to use a pure enzyme, the capability of which can be estimated effectively, then the methods are more easier to apply. In any case, Finn focuses on, "the in situ approach has the virtue of being possibly valuable when these conditions were not met." Assuming that everything works impeccably,one might envision managing a set of pieces of a medication to a patient and having the medication assemble itself at the ideal site (e.g., a cancer) because of the particular nature of the target Further improvement work with enzyme inhibitors is presently underway in many research groups, on an assortment of disease including AIDS, malignant growth, anthrax, and Huntington's disease10.

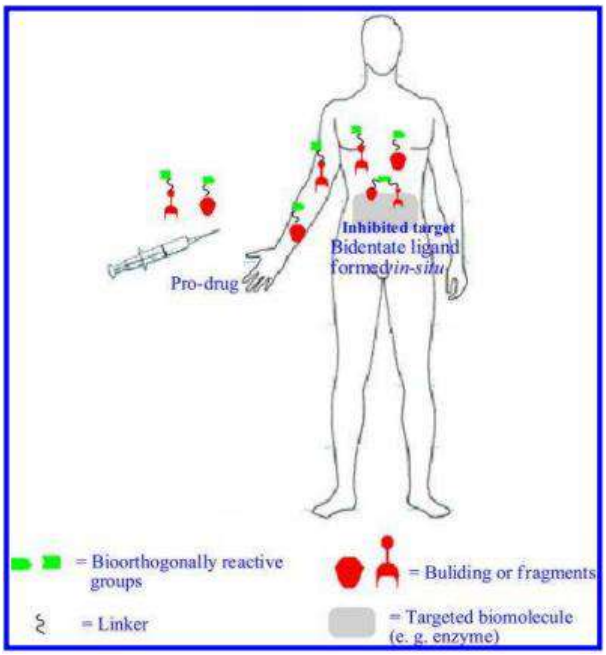



**Figure 34. In situ click chemistry used for drug development10.**

The slow reaction between inactivated alkynes and azides is vital in the utilization of this reaction to TGS. Sharpless and Finn utilized the alkyne-azide click reaction (AAC) in a template-directed development of a enzyme inhibitor, the enzyme playing the conceptual role of the cucurbituril host. The utilization of AAC reactions in TGS has been named 'CC in situ' (click chemistry in situ).

The properties of this irreversible reaction, which make it well suited for lead discovery, include:

a. extremely thermodynamically favorable reaction
b. reaction doesn't involve any third-party participants, like catalysts or different reagents
c. bioorthogonality (i.e., the two azides and alkyne are inert in vivo and, can survive in biological conditions).

The enzyme acetylcholinesterase (AChE) was chosen as the first target for in situ click chemistry because it is an essential part of neurological function, specifically in Alzheimer's diseases, and for the structure of its active site. Sharpless et al., synthesized 98 possible inhibitors from 16 building blocks by using the in situ click approach to AChE.It was predicted that a triazole link made between two of these small-molecule inhibitors properly designed with integral alkyne and azide functionalities could shape in situ, to create another bivalent inhibitor of the enzyme3.

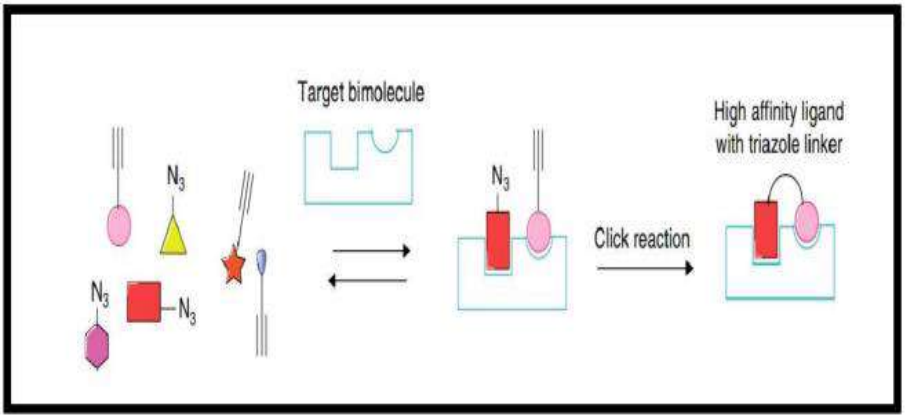



**Figure 35. The process of In situ click chemistry12.**

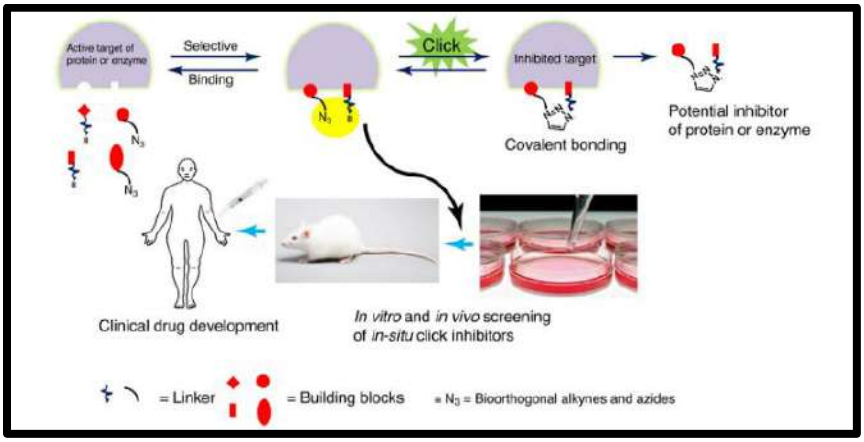



**Figure 36.In situ click chemistry employed to generate protein or enzyme inhibitors and drugs12.**

**CLICK CHEMISTRY APPLICATIONS IN DRUG DELIVERY:**

- Click Chemistry In Polymer Based Drug Delivery System
- Block Copolymer Synthesis
- Click Chemistry and Polymeric Micelles
- Linear Multifunctional Polymeric Delivery Systems
- Bioconjugation
- Dendrimer Synthesis
- Click Chemistry In Nanoparticle Delivery Synthesis
- Gold Nanoparticles
- Magnetic nanoparticle
- Liposomes
- ClickIn Bioorthogonal Chemistry

**CLICK CHEMISTRY IN POLYMER BASED DRUG DELIVERY SYSTEM:**

Polymer therapies is a broad phrase that encompasses polymeric pharmaceuticals, polymer-drug conjugates, polymer-protein conjugates, polymeric micelles to which drugs are covalently attached, and multi-component polyplexes being developed as non-viral vectors. All subclasses use unique water-soluble polymers, either as the bioactive itself or as an inert functional element of a multifunctional construct for better drug, protein, or gene delivery." "Click chemistry" or "click reaction" refer to polymer synthesis and/or modification. These works are broadly divided into five categories: block copolymer synthesis, linear multifunctional copolymer synthesis, dendrimer synthesis, polymer network synthesis, and polymer homologous modification. In addition to greatly boosting product yields, most of these click chemistry applications dramatically simplified the synthetic routes and purification procedures5.

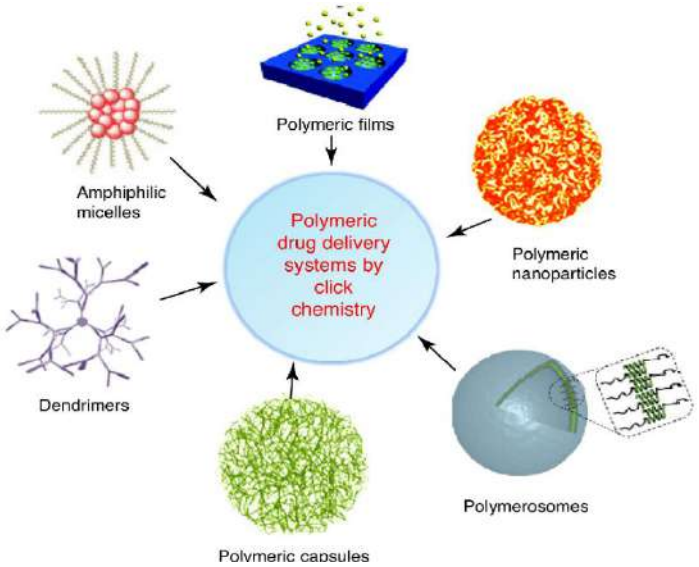



**Figure 37. Click chemistry-based polymeric drug delivery systems3.**

**CLICK CHEMISTRY IN BLOCK COPOLYMER SYNTHESIS:**

Click chemistry has become synonymous with linker chemistry because to its extraordinarily high reaction efficiency and tolerance for a wide range of functional groups.It is one of the most efficient techniques to combine two substances, and it has been used numerous times to link well-defined homopolymers to make block copolymers. Van Camp et al., have published a synthetic method for a variety of amphiphilic copolymer structures using ATRP and the HDC reaction. Using a modular approach, polymers having alkyne and azide functions [e.g., poly (1-ethoxyethyl acrylate) and poly(acrylic acid)] were first produced using ATRP. After that, they "clicked" together to produce block copolymers. Likewise, Opsteen and colleagues reported on the click chemical synthesis of polystyrene (PS), poly (tert-butyl acrylate) (PtBA), and poly(methyl acrylate) (PMA) block copolymers . The three homo polymer blocks were produced by ATRP using an initiator containing a triisopropylsilyl (TIPS) protected acetylene, and the terminal bromides were then changed to azides. Block copolymer synthesis's second strategy has been given new life by click chemistry. Block copolymers can be formed by joining any two homopolymer blocks together using click chemistry. This makes it possible to synthesize a variety of copolymers with extremely distinctive features rapidly and easily via combinatorial block copolymer synthesis, which has the potential to advance the pharmaceutical sciences significantly. Gillies and colleagues made an intriguing contribution when they described a similar technique for attaching dendrons to polymer vesicles with the goal of increasing the availability of peripheral ligands for multivalent interactions with biological targets5.

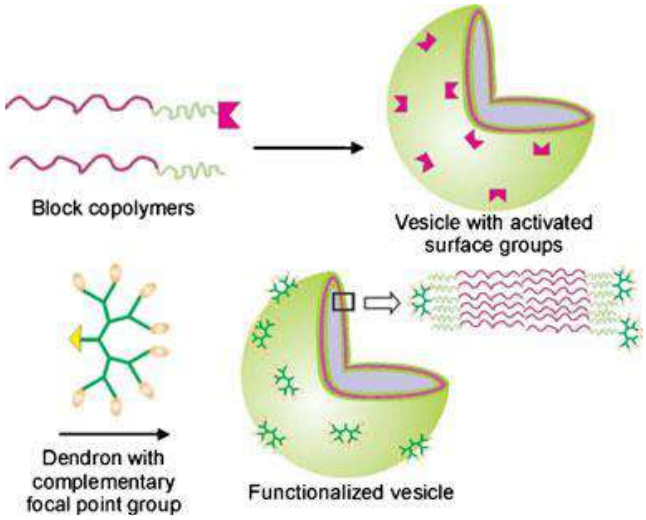



**Figure 38. A general strategy for functionalizing vesicles' surfaces with dendritic wedges13**

## CLICK CHEMISTRY AND POLYMERIC MICELLES:

Amphiphilicblock copolymers are commonlyused in the pharmaceutical industry to create polymeric micelles that serve as delivery vehicles for medicinal, imaging, or diagnostic compounds .Although polymericmicelles are simple and effective delivery systems that have been tested in clinical trials for many malignancies, they still face a number of problems, including drug stability andrelease control. The group reported synthesizing well-defined core crosslinked polymeric micelles using multi-functional dendritic crosslinkers, which is a novel approach to stabilizing polymeric micelles. In order to create micelles that were click-ready for crosslinking, amphiphilic diblock copolymers of poly(acrylic acid)-b-poly(styrene) (PAA-b-PS) were first functionalized with terminal alkynes throughout the hydrophobic polystyrene block segment.Reactions with complementary click-functionalized fluorescent dyes showed the availability and reactivity of the functional groups towards click chemistry in both of these crosslinked micelles. Analytical ultracentrifugation sedimentation studies verified that the fluorescent tags were covalently attached to the crosslinked micelle's shell or core5.

## CLICK CHEMISTRY AND LINEAR MULTI FUNCTIONAL POLYMERIC DELIVERY SYSTEMS:

N-(2-hydroxypropyl)methacrylamide (HPMA) copolymers, polyglutamate,and PEG are the most commonbiocompatible water-soluble polymers used for drug delivery.Liu et al., used click chemistry to create a linear multifunctional PEG as a new drug delivery system . PEG oligomer diol was first activated with phosgene and then capped with propargyl amine, resulting in acetylene termination. This was to be used as the polymer building block. The functionality building blocks included a diazide monomer, 2, 2-bis(azidomethyl)propane-1,3-diol, and a variety of functional derivatives.

The advantages of this approach over traditional synthetic polymeric delivery systems are as follows: no special reaction conditions or protection steps are required; oligopeptide sequences can be easily inserted between PEG and the acetylene functionality to allow biodegradability of the delivery system; the entire synthetic procedure is simple, modular, free from vinyl chemistry, and produces high yields. Furthermore, Liu et al., have managed to regulate the average molecular weight by incorporating some monoacetylene-functionalized PEG into the system5.

**BIOCONJUGATION:**

Click chemistry has made possible one of the mostremarkable uses of the novel, effective techniques for bioconjugation, which has grown to be one of the most employedtechniques to create cutting-edge biomedical applications.Cu-catalyzed azide-alkyl cycloaddition reaction (CuAAC), a common click reaction, has been developed to synthesize conjugates with a range of structures and functional groups in vitro14.

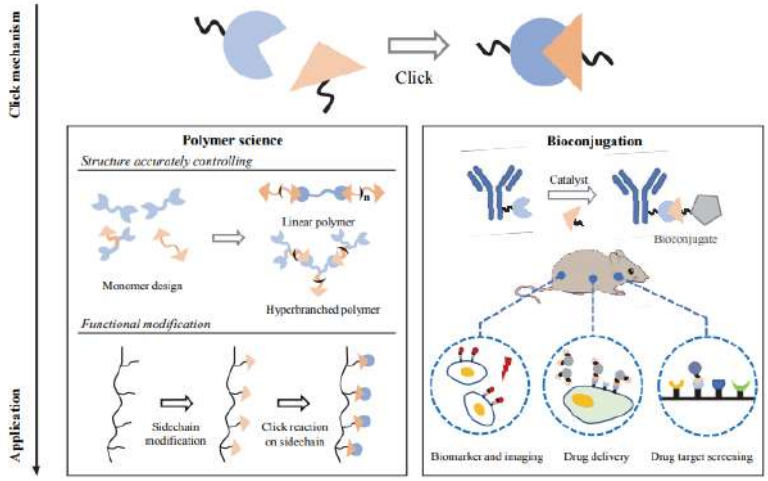



**Figure 39.The possible use of click chemistry in the biomedical and polymer science fields. In polymer synthesis, click chemistry may make precise structure control and adaptable function modification easier.It also brings new breakthroughs in bioconjugation, further applied to biomarker/imaging, drug delivery, and drug target screening fields14.**

**DENDRIMER SYNTHESIS:**

The synthesis of dendrimers can be significantly streamlined by click chemistry, increasing their affordability and utility.Gopin et al., reported the synthesis and evaluation of a single-triggered disassemble dendrimer as a potential platform for a multiprodrug , as an example of a dendrimer used for drug delivery. "A single cleavage at the dendrimer'score initiates a self-immolative chain fragmentation that releases all of these unique structural dendrimers' tail units". By conjugating azide-monoterminated PEG to the dendritic platform via click chemistry, the second generation of self-immolative dendrimers could be activated enzymatically and the hydrophobic drugs were prevented from aggregating5.

**CLICK CHEMISTRY AND NANOPARTICLE DELIVERY SYSTEM:**

Systems for delivering nanoparticular particles are carriers with sizes between 10 and 1000 nm. Delivery systems for imaging and drug/gene delivery have been thoroughly studied over the last few decades, including liposomes, magnetic nanoparticles, gold nanoparticles, micelles, and quantum dots . Because of their small size, they can effectively accumulate drugs at target sites by penetrating the vasculature through fissures. Additionally, drug targeting through nanoparticular delivery methods has a number of significant benefits. They lower the dosage of medications, lessen their adverse effects, shield them from deterioration, and improve their stability5.

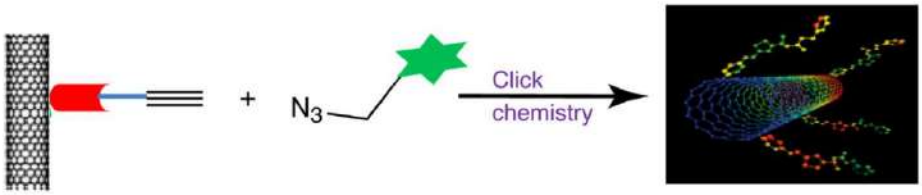



**Figure 40.Functionalization of carbon nanotubes by click chemistry3.**

**Gold Nanoparticles:**

Azide groups were added to the surface of firstly prepared gold nanoparticles to functionalize them. Additionally, a 30 kDa globular recombinant protein called lipase was altered to express a single terminal alkyne, and the nanoparticles were then allowed to interact with it. A lipase assay demonstrated that the lipases had maintained their enzymatic activity, and gel electrophoresis confirmed that multiple lipases had covalently attached to each nanoparticle, free of any nonspecific binding. By using a similar process, Fleming et al., were able to conjugate gold nanoparticles to a variety of alkynyl derivatives, such as ferrocene, aniline, and PEG. Using CuAAC, Rowan, Brust, and colleagues were able to effectively prepare functional enzyme-AuNP conjugates from water-soluble AuNP (CuSO4, ascorbic acid) decorated with azido and acetylene-functionalized Thermomyces lanuginosus lipase5.

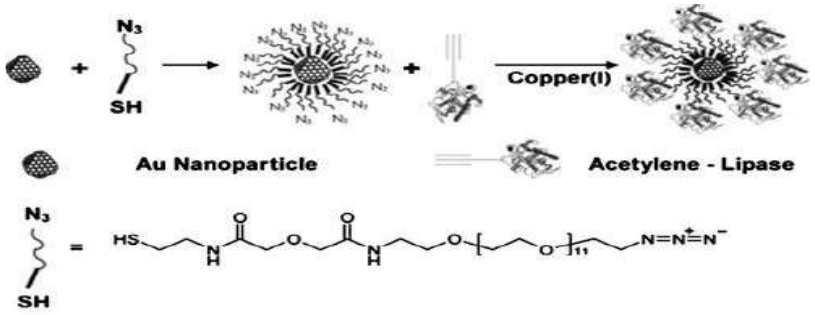



**Figure 41. Functionalization of AuNP with a lipase via CuAAC13.**

**Magnetic Nanoparticles:**

Magnetic nanoparticles are widely used in pharmaceutical sciences due to their biocompatibility, injectability, and specific accumulation in target tissues under a magnetic field . Several methods have been studied to functionalize magnetic nanoparticles. Several organic molecules were successfully attached to magnetic particles using the HDC reaction. These molecules included Tn antigen, flag peptide, biotin, 2,4-dinitrophenol (DNP), and maltose binding protein (MBP), all of which were functionalized with a terminal alkyne. In contrast, the magneticnanoparticles were functionalizedwith azides. White et al., used ligands with a phosphonic acid or carboxylic acid group at one end to bind to the surface of a γ-Fe2O3 nanoparticle and an azide or acetylene group at the other end for chemical modification, unlike Lin et al., Both benzyl azide and 5-chloropentyne were successfully conjugated using click chemistry. To demonstrate the strategy's versatility, an acetylene-terminated polymeric ligand was added to the nanoparticles5.

**LIPOSOMES :**

Cai and colleagues optimized the CuAAC conditions for liposome functionalization (74). They created physically robust clickable polymerized liposomes from polydiacetylene lipids that were efficientlydecorated with multiple functionalities (FITC, coumarin, and a GRGD peptide) without liposomedecomposition, which is typical of unsaturated liposomes under CuAAC conditions. The improved reaction conditions are

based on the use of a Cu (I)-chelating ligand [a tris(triazolylmethyl)amine derivative bearing tetra(ethylenglycol) side chains], which is highly soluble in water and insensitive to air, and has been shown to shorten reaction times with lower amounts of CuSO4 and sodium ascorbate. In order to functionalize polymersomes, van Hest and colleagues (Figure 42) reported the first use of CuAAC. They prepared azido-decorated polymersomes and how they bind to bioactive ligands like enhanced green fluorescent protein (EGFP), biotin, and a dansyl probe. In order to achieve this, ATRP created an amphiphilic PS-b-PAA and replaced azides with terminal bromide end groups. The resulting copolymers were incubated with aqueous solutions of alkyne functionalized ligands under standard CuAAC conditions (CuSO4, sodium ascorbate, TBTA, or BPDS) after being allowed to self-assemble into vesicular aggregates5.

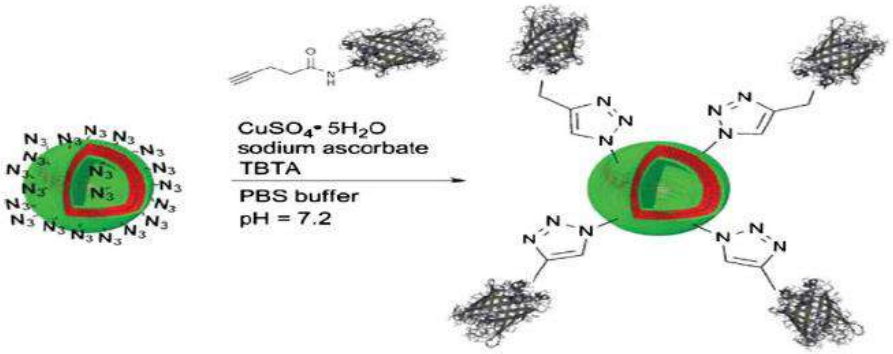



**Figure 42. Preparation of PS-b-PAA polymersomes with peripheral azide groups and their functionalization with Alk-EGFP. [TBAF (tetra-n-butylammonium fluoride)] 13.**

## CLICK IN BIOORTHOGONAL CHEMISTRY:

Understanding many biological structures and functions in the living system is critical for unlocking biology's secrets, but it remains extremely difficult due to the high complexity of biological process networks and wiring. The bioorthogonal nature of click chemistry components makes these reactions useful for selective labeling and detection of biological molecules in complex samples such as cellular extracts, and eventually in vivo. Such investigations are required to comprehend the complex nuances of spatial and temporal aspects of biomolecule localization and function within the cell. Staudinger ligation and azide-alkyne cycloadditions are effective methods for selective derivatization of various biomolecules, such as proteins, viruses, sugars, DNA, RNA, and lipids1.

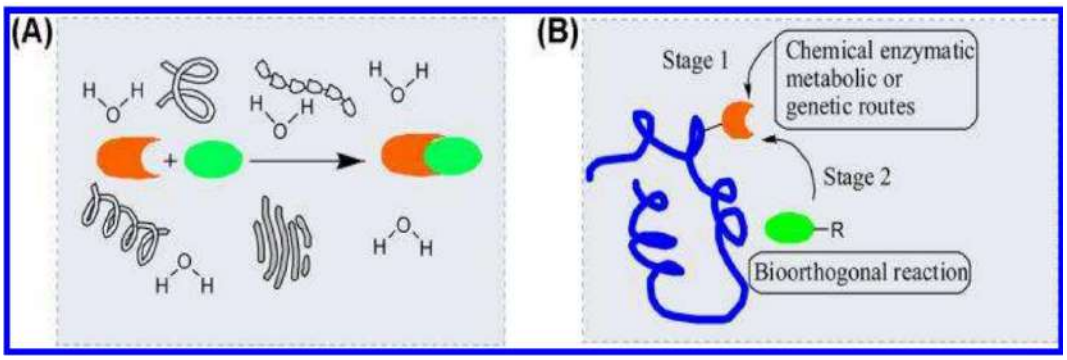



**Figure43. (A)(A) Bio-orthogonal reactions: completely selective and stable to external stimuli. (B) Bioconjugation employing bioorthogonal chemistry10.**

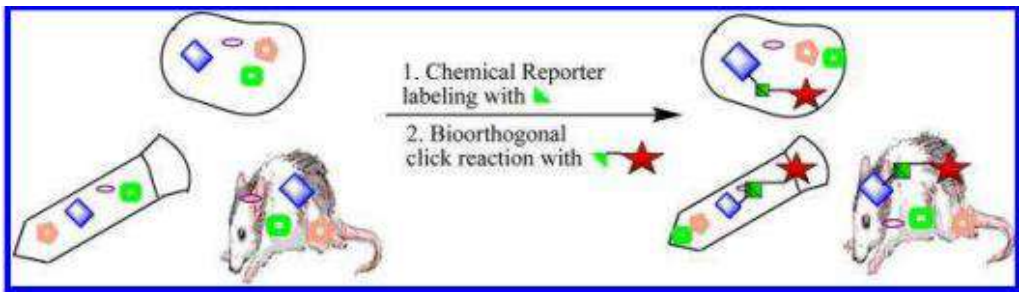



**Figure 44.Bioorthogonal-click chemistry reporter strategy. (1) Target biomolecules (blue square) are tagged in the presence of nontarget biomolecules (green triangle) in vitro or in vivo using a chemical reporter (triangle).**
**(2) The chemical reporter is then detected using bioorthogonal click chemistry to append a functional probe of interest (star) 10.**

## CONCLUSION :

Click chemistry has emerged as a transformative tool in drug discovery and development, offering a modular and efficient approach to synthesizing complex molecules. By harnessing the power of cycloaddition, thio-based, and miscellaneous reactions, researchers have unlocked new avenues for the development of novel therapeutics. The applications of click chemistry in drug discovery and development are vast, with significant impacts on high-throughput screening, fragment-based drug design, peptide-based drug design, and enzyme inhibitor development. Moreover, click chemistry has revolutionized drug delivery systems through the synthesis of nanoparticles and polymers, enabling targeted and controlled release. As research in click chemistry continues to evolve, its potential to drive innovation in drug discovery and development is boundless. By leveraging the versatility and efficiency of click chemistry, scientists can accelerate the development of life-saving drugs and improve patient outcome".

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