Indian Journal of Pharmaceutical Chemistry and Analytical Techniques (IJPCAT)
ISSN: 3049-3765 | Vol. 02, Issue-04, July-August, 2026, pp. 01-27
https://ijpcat.com/1

Click Chemistry in Peptide-Based Drug Discovery: Recent Advances, Emerging Applications, and Future Perspectives in Medicinal Chemistry

Click Chemistry in Peptide Drug Discovery

Maniteja Gorikapudi[1*], K. Sampath Kumar[2]

[*1] Department of Chemistry, Malla Reddy college of engineering for women, maisammaguda, Hyderabad, Telangana 500100.

[2]Department of Pharmaceutics, Malla Reddy Institute of Pharmaceutical Sciences, Malla Reddy Vishwavidyapeeth (Deemed to be University), Suraram, Hyderabad -500055, Telangana, India.

***Corresponding Author E-mail: manitejagp@gmail.com**

Abstract:

Click chemistry has fundamentally transformed modern medicinal chemistry by providing a versatile, high-yielding, and chemoselective strategy for assembling complex molecular architectures under mild reaction conditions. Since its introduction, this synthetic approach has become an indispensable tool for the rapid construction of biologically active molecules, particularly in peptide-based drug discovery, where structural precision, metabolic stability, and synthetic efficiency are critical determinants of therapeutic success. Among the various click reactions, copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC) has gained widespread recognition because of its exceptional regioselectivity, functional-group tolerance, and ability to generate stable 1,2,3-triazole linkages that effectively mimic peptide and amide bonds. These attributes have significantly expanded opportunities for developing peptide therapeutics with improved pharmacological performance and enhanced resistance to enzymatic degradation. This review critically examines the scientific evolution of click chemistry and its expanding role in peptide engineering, drug design, and pharmaceutical innovation. Particular emphasis is placed on the mechanistic basis of major click reactions, triazole-mediated peptide modification, peptide cyclization, stapling strategies, and the development of therapeutics for cancer, infectious diseases, inflammatory disorders, and other clinically significant conditions. Recent advances in bio-orthogonal chemistry, targeted drug delivery, molecular imaging, Nano medicine, and bio molecular conjugation are also discussed to illustrate the broad translational impact of click chemistry across contemporary biomedical research. In addition, the review highlights the emerging convergence of click chemistry with artificial intelligence, machine learning, and computational drug discovery, emphasizing how predictive molecular design, reaction optimization, and virtual screening are reshaping medicinal chemistry workflows. Current limitations, including catalyst-associated toxicity, scalability, manufacturing challenges, and regulatory considerations, are critically evaluated alongside promising solutions that support sustainable and clinically translatable pharmaceutical development. By integrating foundational concepts with recent scientific advances, this review provides a comprehensive perspective on the present status of click chemistry and outlines future directions for its application in peptide therapeutics, precision medicine, and next-generation drug discovery.

Keywords: Click chemistry; Peptide therapeutics; Triazole chemistry; Medicinal chemistry; Drug discovery

Received: March 10, 2026 **Revised: April 19, 2026** **Accepted: May 28, 2026** **Published: Aug., 01 2026**

DOI: https://doi.org/10.64062/IJPCAT.Vol2.Issue4.1

https://ijpcat.com/index.php/1/issue/archive

1. INTRODUCTION

The development of innovative therapeutic agents remains one of the most demanding undertakings in pharmaceutical research, with increasing pressure to reduce discovery timelines while improving molecular selectivity, safety, and clinical success rates. Conventional medicinal chemistry frequently relies on multistep synthetic pathways that require extensive optimization, consume substantial resources, and often produce modest yields. These challenges have stimulated the search for synthetic methodologies that enable rapid molecular diversification while maintaining high reaction efficiency and compatibility with biologically relevant functional groups. Among the transformative advances introduced during the past two decades, click chemistry has emerged as one of the most influential approaches for the modular construction of complex molecular architectures[1-3].

The concept of click chemistry was introduced in 2001 to describe a family of reactions that proceed with exceptional efficiency, high chemo-selectivity, broad functional-group tolerance, and minimal by-product formation under relatively mild reaction conditions[4]. The philosophy underlying click chemistry emphasizes simplicity, reliability, and reproducibility, allowing molecular fragments to be assembled in a predictable manner without extensive purification. These characteristics distinguish click reactions from many conventional synthetic approaches and have facilitated their widespread adoption across medicinal chemistry, chemical biology, materials science, nanotechnology, and bio-molecular engineering[5]. Among the reactions included within the click chemistry framework, the copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC) represents the most extensively investigated and widely implemented methodology[6]. CuAAC enables the rapid formation of 1,2,3-triazole rings with remarkable regioselectivity and consistently high yields. The resulting triazole linkage possesses outstanding chemical and metabolic stability, resistance to enzymatic degradation, and favorable electronic characteristics, making it an attractive structural motif for therapeutic design. Triazoles also function as bio-isosteric replacements for amide, ester, and disulfide linkages, thereby improving molecular stability while preserving or enhancing biological activity[7-9]. These properties have established click chemistry as a versatile platform for generating structurally diverse compound libraries suitable for biological screening and lead optimization.

Peptide-based therapeutics have become an increasingly important class of medicines because of their high target specificity, predictable pharmacology, and comparatively favorable safety profiles. Advances in peptide engineering have expanded their application to oncology, infectious diseases, metabolic disorders, autoimmune conditions, and neurological illnesses[10]. Nevertheless, naturally occurring peptides often exhibit limited clinical utility because of rapid proteolytic degradation, conformational flexibility, poor membrane permeability, short systemic half-life, and restricted oral bioavailability[11]. Numerous chemical modification strategies have therefore been investigated to overcome these limitations, including cyclization, stapling, backbone modification, PEGylation, lipidation, and incorporation of non-natural amino acids. Among these approaches, click chemistry has gained particular importance because it enables site-selective peptide modification while preserving biological function and providing enhanced structural stability[12-14].The incorporation of triazole bridges

into peptide structures has demonstrated several advantages over traditional covalent linkages. Triazoles can mimic peptide bonds while introducing increased rigidity, improved metabolic stability, and enhanced resistance to enzymatic cleavage. Click-mediated cyclization has also proven effective in stabilizing secondary structures, improving receptor binding affinity, and increasing biological half-life. These attributes have supported the development of peptide analogues with improved pharmacokinetic and pharmacodynamic profiles across diverse therapeutic areas. Recent investigations have reported encouraging outcomes for triazole-containing antimicrobial peptides, anticancer peptide conjugates, enzyme inhibitors, receptor-targeted ligands, vaccine constructs, and multifunctional drug-delivery systems[15-18].

Beyond peptide modification, click chemistry has substantially influenced the broader landscape of pharmaceutical innovation. The methodology has enabled efficient synthesis of small-molecule libraries, antibody-drug conjugates, polymeric drug carriers, dendrimers, liposomes, nanoparticles, imaging probes, radiopharmaceuticals, and biomaterials designed for targeted therapeutic applications[19-22]. The emergence of bio-orthogonal click reactions, particularly strain-promoted azide-alkyne cycloaddition (SPAAC) and inverse-electron-demand Diels-Alder reactions, has further expanded the utility of click chemistry by allowing selective molecular conjugation within living biological systems without requiring cytotoxic metal catalysts. These advances have opened new opportunities for in vivo imaging, targeted drug delivery, molecular diagnostics, and precision medicine.

Recent developments in computational chemistry and artificial intelligence have further transformed click chemistry-driven drug discovery. Machine learning algorithms, molecular language models, graph neural networks, and generative artificial intelligence can now prioritize synthetically accessible compounds, predict physicochemical and pharmacokinetic properties, estimate reaction outcomes, and identify promising therapeutic candidates before laboratory synthesis[23-26]. Integration of these computational approaches with click chemistry has the potential to reduce experimental burden, improve lead optimization, and accelerate translational research from molecular design to preclinical evaluation. Although several studies have independently explored computational chemistry, peptide engineering, or click reactions, comprehensive integration of these disciplines remains comparatively underdeveloped, highlighting an important opportunity for future research.

Despite remarkable scientific progress, several challenges continue to limit broader implementation of click chemistry in peptide drug development. Copper-associated cytotoxicity, optimization of catalyst-free bio-orthogonal reactions, scalability of complex peptide conjugation, manufacturing costs, regulatory considerations, and translation from laboratory synthesis to industrial production remain active areas of investigation. Furthermore, standardization of reaction protocols, improved predictive computational tools, sustainable catalytic systems, and environmentally responsible manufacturing practices are increasingly recognized as priorities for the next generation of pharmaceutical innovation. This review critically examines the evolution, mechanistic principles, and pharmaceutical applications of click chemistry with particular emphasis on peptide-based drug discovery. The discussion synthesizes current knowledge regarding CuAAC and emerging bio-orthogonal reactions, triazole-mediated peptide engineering, therapeutic applications across major disease areas, advances in targeted drug delivery and molecular imaging, and the growing influence of artificial intelligence in accelerating click chemistry-enabled medicinal chemistry. The review also identifies current limitations, unresolved research questions, and future directions that are expected to shape the continued integration of click chemistry into precision therapeutics and next-generation pharmaceutical development.

2. Principles and Historical Evolution of Click Chemistry

2.1 Emergence of Click Chemistry as a Paradigm in Modern Synthetic Chemistry

The remarkable expansion of medicinal chemistry during the late twentieth century created an increasing demand for synthetic methodologies capable of producing structurally diverse molecules with greater efficiency, reliability, and reproducibility. Conventional organic synthesis, although highly versatile, frequently involved lengthy multistep procedures, low overall yields, extensive purification, and poor compatibility with sensitive biological molecules. These limitations became increasingly evident as pharmaceutical research shifted toward high-throughput screening, combinatorial chemistry, chemical biology, and bio molecular engineering. The need for reactions that could rapidly assemble complex molecular structures without compromising functional-group integrity led to the development of a fundamentally different synthetic philosophy known as click chemistry[1-3].

Click chemistry was formally introduced in 2001 by K. Barry Sharpless and colleagues, who proposed that a limited family of highly efficient chemical reactions could serve as universal tools for assembling molecular building blocks.[4] Rather than emphasizing structural complexity through elaborate synthetic sequences, the click chemistry concept advocated the rapid formation of stable covalent bonds using reactions that are experimentally simple, highly selective, insensitive to oxygen or water, and capable of proceeding under mild conditions with minimal purification requirements. This conceptual framework represented a significant departure from traditional synthetic strategies by prioritizing modularity, predictability, and synthetic practicality.

The term "click" was intentionally chosen to describe molecular assembly in a manner analogous to fastening two compatible components together with precision and reliability. Instead of relying on numerous protecting-group manipulations and sequential functionalization steps, click chemistry promotes the direct coupling of pre-functionalized molecular fragments possessing mutually reactive groups. Consequently, the methodology substantially reduces synthetic complexity while simultaneously improving reproducibility and scalability, characteristics that have become increasingly valuable for pharmaceutical development and translational research[5].

2.2 Fundamental Chemical Principles Governing Click Reactions

Although click chemistry encompasses several distinct reaction classes, all click reactions are expected to satisfy a common set of physicochemical and practical criteria that distinguish them from conventional synthetic methodologies. These principles were established to maximize reaction efficiency while minimizing operational complexity and environmental burden.

A characteristic click reaction should exhibit: exceptionally high reaction yield; rapid reaction kinetics; broad functional-group tolerance; excellent chemo selectivity; stereo chemical reliability; compatibility with aqueous or physiologically relevant environments; production of benign or easily removable by-products; simple reaction conditions without extensive purification; thermodynamic favourability leading to essentially irreversible product formation; and Applicability across diverse molecular architectures[4-6]. Collectively, these features enable efficient coupling of structurally diverse molecular fragments while preserving sensitive functional groups commonly encountered in peptides, proteins, nucleic acids, carbohydrates, polymers, and biologically active small molecules. Unlike many classical condensation reactions that compete with hydrolysis or require strict exclusion of moisture, click reactions generally proceed efficiently under ambient laboratory conditions. This operational simplicity has facilitated their widespread adoption not only within synthetic organic chemistry but also across medicinal chemistry, biomaterials science, nanotechnology, diagnostic imaging, and chemical biology.

2.3 Thermodynamic and Kinetic Basis of Click Chemistry

The remarkable efficiency of click reactions arises from the favorable interplay between reaction thermodynamics and kinetics. Most click reactions involve the formation of highly stable covalent bonds accompanied by substantial decreases in Gibbs free energy, thereby

driving reactions toward nearly quantitative completion. Simultaneously, the activation barriers remain sufficiently low to permit rapid bond formation under relatively mild temperatures without compromising selectivity[7]. From a kinetic perspective, click reactions minimize competing side reactions because the participating functional groups exhibit exceptional orthogonality toward surrounding chemical functionalities. This orthogonality permits selective bond formation even in chemically complex environments containing alcohols, amines, thiols, carboxylic acids, esters, and amides. Such selectivity is particularly advantageous during peptide synthesis and bio-molecular conjugation, where preservation of native molecular architecture is essential. The formation of thermodynamically stable products also contributes to the excellent reproducibility of click reactions. Once formed, the resulting triazole or related heterocyclic products display remarkable resistance toward hydrolysis, oxidation, enzymatic degradation, and metabolic transformation, characteristics that enhance their pharmaceutical utility.

2.4 Historical Development of Click Chemistry

Although the conceptual framework of click chemistry was introduced in 2001, its historical roots extend considerably further. The Huisgen 1,3-dipolar cycloaddition reaction, reported during the 1960s, demonstrated that azides and alkynes could react to form triazole rings. However, the original reaction required elevated temperatures and produced mixtures of regioisomeric products, thereby limiting its synthetic utility[8]. A major breakthrough occurred in 2002 when two independent research groups led by Tornøe, Meldal, and Sharpless demonstrated that copper(I) catalysis transformed the classical Huisgen cycloaddition into a rapid, regioselective reaction yielding exclusively the 1,4-disubstituted triazole under mild conditions[9-10]. This copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC) rapidly became the archetypal click reaction because it combined outstanding reaction efficiency with exceptional selectivity and operational simplicity.

Subsequent years witnessed rapid expansion of click chemistry into multiple scientific disciplines. Bio-orthogonal chemistry emerged through the development of catalyst-free strain-promoted azide-alkyne cycloaddition (SPAAC), enabling selective molecular conjugation within living systems without copper-associated toxicity[11]. Additional click methodologies, including thiol-ene reactions, thiol-yne reactions, inverse-electron-demand Diels-Alder cycloaddition, and sulfur (VI) fluoride exchange (SuFEx); further broadened the scope of click chemistry, providing researchers with complementary strategies for constructing increasingly sophisticated molecular systems.

The impact of these developments was formally recognized in 2022, when the Nobel Prize in Chemistry was awarded to K. Barry Sharpless, Morten Meldal, and Carolyn R. Bertozzi for the development of click chemistry and bio-orthogonal chemistry. This recognition underscored the profound influence of click chemistry on contemporary chemical synthesis and biomedical research.

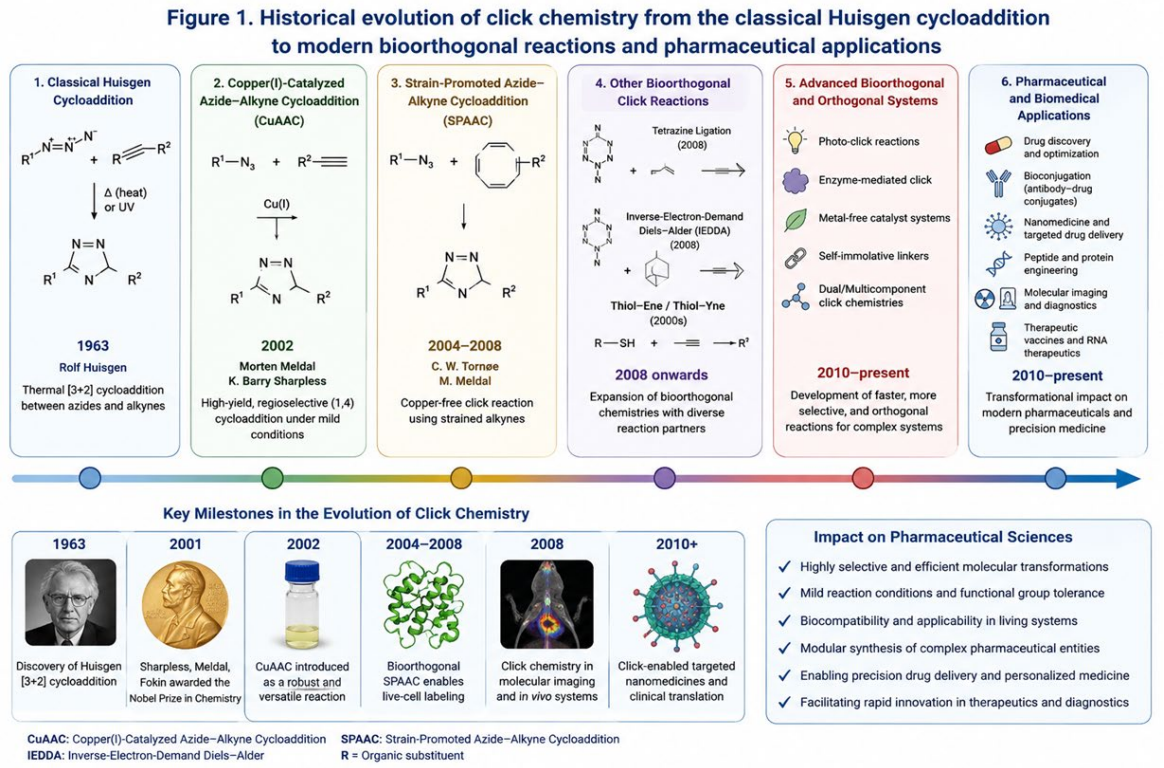

Figure 1. Historical evolution of click chemistry from the classical Huisgen cycloaddition to modern bio-orthogonal reactions and pharmaceutical applications.

Figure 1 represents the timeline illustrating major milestones, including the discovery of the Huisgen cycloaddition, introduction of the click chemistry concept, development of CuAAC, emergence of bio-orthogonal chemistry, expansion into peptide engineering and Nano medicine, integration with artificial intelligence-assisted molecular design, and recognition through the 2022 Nobel Prize in Chemistry.

Table 1. Major milestones in the historical evolution of click chemistry and their significance in medicinal chemistry.

Year	Scientific milestone	Principal contribution	Pharmaceutical significance
1960s	Huisgen 1,3-dipolar cycloaddition	Foundation of azide-alkyne cycloaddition chemistry	Basis for future click reactions
2001	Introduction of the click chemistry concept	Defined modular, high-yield synthetic philosophy	Accelerated medicinal chemistry
2002	Copper(I)-catalyzed azide-alkyne cycloaddition	Highly regioselective triazole synthesis	Rapid drug scaffold construction
2004-2010	Expansion into biomolecular conjugation	Protein and peptide modification	Targeted therapeutic design
2010-2020	Development of bio-orthogonal chemistry	Catalyst-free in vivo conjugation	Imaging and precision medicine
2020-Present	Integration with AI and computational chemistry	Predictive molecular design and reaction optimization	Next-generation drug discovery

2.5 Pharmaceutical Importance of Click Chemistry

The pharmaceutical significance of click chemistry extends well beyond synthetic convenience. The methodology has fundamentally changed the way medicinal chemists design, optimize, and functionalize therapeutic molecules. The ability to assemble structurally diverse analogues rapidly enables efficient exploration of structure-activity relationships while reducing the synthetic effort traditionally associated with lead optimization. Triazole-containing compounds generated through click chemistry frequently demonstrate enhanced metabolic stability, improved receptor affinity, greater aqueous solubility, and increased resistance to enzymatic degradation compared with their non-click-derived counterparts[12-15].

Click chemistry has also facilitated the development of antibody-drug conjugates, peptide therapeutics, targeted nanoparticles, radiopharmaceuticals, polymer-drug conjugates, nucleic acid delivery systems, and multifunctional imaging probes. More recently, the integration of computational chemistry, machine learning, and artificial intelligence with click chemistry has created opportunities for predictive reaction planning, virtual library generation, and rapid identification of synthetically accessible drug candidates, thereby strengthening the role of click chemistry within contemporary precision medicine.

3. Classification of Click Reactions

Click chemistry has evolved from a single synthetic concept into a broad collection of highly selective chemical transformations that satisfy the fundamental criteria proposed for efficient molecular assembly. Although these reactions differ in their mechanistic pathways and catalytic requirements, they share common characteristics, including rapid reaction kinetics, high product yields, excellent chemo-selectivity, operational simplicity, and broad compatibility with structurally diverse substrates[16-18]. Their collective contribution has transformed medicinal chemistry by enabling efficient synthesis of drug candidates, bio-molecular conjugates, diagnostic probes, polymeric materials, and targeted therapeutic systems.

The major classes of click reactions currently employed in pharmaceutical research include:

- Copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC)
- Strain-promoted azide-alkyne cycloaddition (SPAAC)
- Thiol-ene reactions
- Thiol-yne reactions
- Inverse-electron-demand Diels-Alder (IEDDA) reactions
- Sulfur (VI) fluoride exchange (SuFEx)
- Tetrazine ligation and other emerging bio-orthogonal reactions

Each reaction possesses distinct mechanistic features, substrate scope, and translational applications that determine its suitability for specific pharmaceutical objectives.

3.1 Copper (I)-Catalyzed Azide-Alkyne Cycloaddition (CuAAC)

The copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) remains the most extensively investigated member of the click chemistry family and is widely regarded as the benchmark against which other click reactions are compared. Developed independently by Meldal and by Tornoe and Sharpless in 2002, this reaction transformed the classical Huisgen 1,3-dipolar cycloaddition into a highly regioselective, rapid, and experimentally accessible synthetic methodology[9-10-19].

In CuAAC, a terminal alkyne reacts with an organic azide in the presence of a catalytic copper(I) species to generate a stable 1,4-disubstituted 1,2,3-triazole. Copper (I) coordinates with the terminal alkyne to produce a copper acetylide intermediate, which subsequently reacts with the azide through a concerted cycloaddition pathway. The resulting metallacyclic intermediate undergoes protonation and catalyst regeneration, ultimately yielding the triazole product with exceptional regioselectivity.

Unlike the original thermal Huisgen reaction, which produces mixtures of regioisomeric triazoles at elevated temperatures, CuAAC proceeds efficiently at ambient temperature with near-quantitative conversion and excellent reproducibility. The reaction

tolerates a broad range of functional groups, including alcohols, amines, esters, amides, carboxylic acids, thiols, carbohydrates, nucleic acids, peptides, proteins, polymers, and nanoparticles, thereby enabling molecular coupling without protecting-group manipulation[20].

The remarkable stability of the triazole ring has contributed substantially to the pharmaceutical significance of CuAAC. Triazoles exhibit strong resistance toward hydrolysis, oxidation, enzymatic degradation, and metabolic transformation while maintaining favorable electronic characteristics that frequently improve ligand-receptor interactions. Consequently, CuAAC has become an indispensable strategy for constructing enzyme inhibitors, receptor ligands, antimicrobial agents, antiviral compounds, anticancer therapeutics, peptide conjugates, antibody-drug conjugates, radiopharmaceuticals, and multifunctional nanomedicines[21-23].

Beyond drug synthesis, CuAAC has revolutionized chemical biology through selective bio-molecular labeling, proteomics, glycobiology, molecular imaging, and biosensor development. The modular nature of the reaction allows independent optimization of individual molecular fragments before their final assembly, thereby facilitating rapid structure-activity relationship studies and medicinal chemistry optimization.

Despite these advantages, CuAAC possesses certain limitations. The requirement for copper catalysis introduces concerns regarding catalyst-associated cytotoxicity, oxidative stress, interference with biological systems, and the need for rigorous purification when products are intended for in vivo applications. These challenges have motivated the development of catalyst-free bio-orthogonal alternatives that retain the synthetic efficiency of CuAAC while eliminating metal-associated toxicity[24].

Figure 2. Mechanistic pathway of copper (I)-catalyzed azide–alkyne cycloaddition (CuAAC)

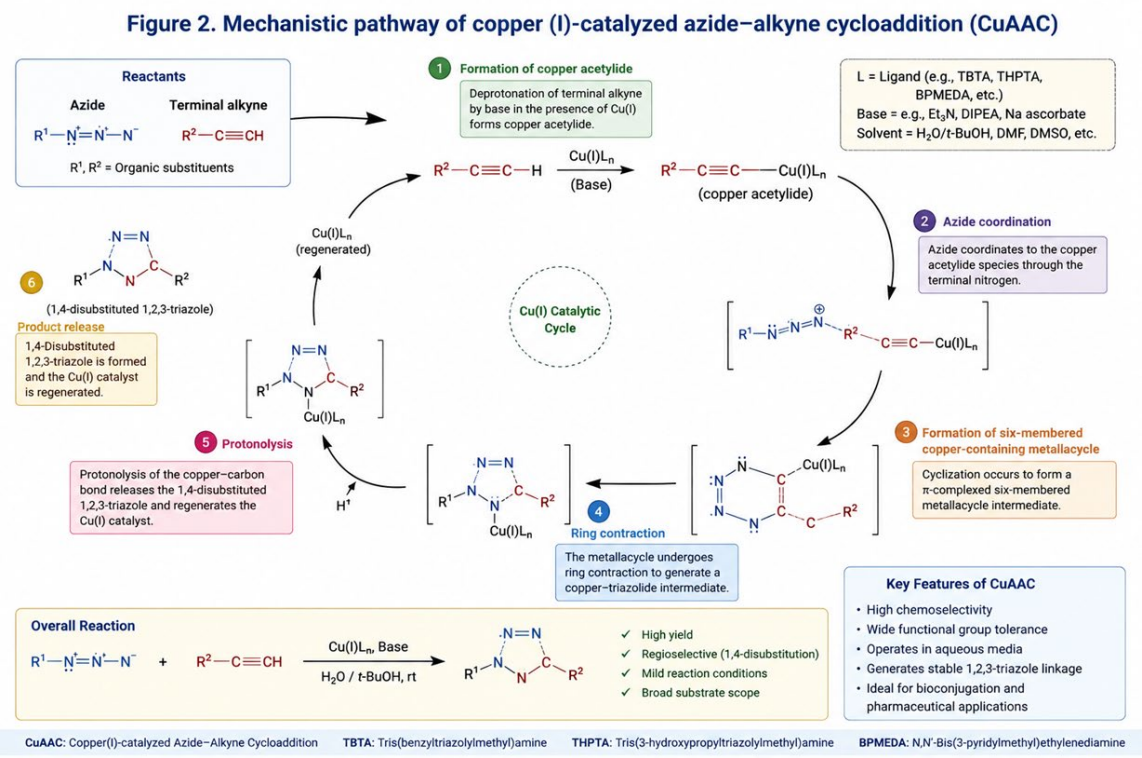

Figure 2. Mechanistic pathway of copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC).

The schematic Figure 2 illustrates activation of the terminal alkyne by copper (I), formation of the copper acetylide intermediate, cycloaddition with the organic azide, generation of the metallacyclic intermediate, and regioselective formation of the 1,4-disubstituted 1,2,3-triazole following protonation and catalyst regeneration.

3.2 Strain-Promoted Azide-Alkyne Cycloaddition (SPAAC)

Although CuAAC represents one of the most efficient synthetic methodologies in medicinal chemistry, its dependence on copper catalysis restricts direct application within living biological systems. Copper ions may induce oxidative damage, interfere with

intracellular biochemical processes, and exhibit cytotoxicity at concentrations required for efficient catalysis. These limitations stimulated the development of catalyst-free bio-orthogonal click reactions capable of proceeding under physiological conditions[25].

A major advance was achieved through the introduction of strain-promoted azide-alkyne cycloaddition (SPAAC), pioneered by Carolyn Bertozzi and colleagues. SPAAC employs highly strained cyclic alkynes, such as cyclooctynes, whose ring strain substantially lowers the activation energy for cycloaddition with azides. As a consequence, the reaction proceeds spontaneously without requiring metal catalysts while maintaining excellent selectivity and compatibility with living systems[26].

The absence of catalytic metals has made SPAAC particularly valuable for applications involving intact cells, living organisms, tissue engineering, and molecular imaging. The reaction exhibits rapid kinetics in aqueous environments and demonstrates exceptional orthogonality toward naturally occurring biomolecules. Consequently, SPAAC has become one of the principal bio-orthogonal reactions used for selective labeling of proteins, glycans, nucleic acids, lipids, extracellular vesicles, and therapeutic nanoparticles.

In pharmaceutical research, SPAAC has enabled site-specific conjugation of monoclonal antibodies, peptide therapeutics, oligonucleotide constructs, liposomal formulations, polymeric nanoparticles, and imaging probes without compromising biological activity. The methodology has proven especially useful in targeted drug delivery, where selective conjugation under physiological conditions is essential for preserving molecular function and minimizing off-target modification[27].

Despite its biological advantages, SPAAC also presents certain limitations. The synthesis of strained cyclooctyne derivatives is generally more demanding than preparation of conventional terminal alkynes, and some cyclooctyne reagents exhibit limited stability or comparatively high production costs. Furthermore, reaction rates may vary depending on the structural characteristics of substituted cyclooctynes and the accessibility of azide groups within complex biological environments.

Nevertheless, the unique combination of catalyst-free operation, excellent chemoselectivity, and compatibility with living systems has established SPAAC as one of the most important bio-orthogonal reactions currently available for pharmaceutical and biomedical research.

Table 2. Comparative characteristics of CuAAC and SPAAC reactions used in pharmaceutical research.

Characteristic	CuAAC	SPAAC
Catalyst requirement	Copper(I) catalyst	No catalyst required
Reaction conditions	Mild laboratory conditions	Physiological conditions
Regioselectivity	Excellent	Excellent
Product	1,4-Disubstituted triazole	Triazole derivative
Functional-group tolerance	Very high	Very high
Compatibility with living systems	Limited by copper toxicity	Excellent
Typical applications	Drug synthesis, peptide conjugation, polymers, medicinal chemistry	Bio-orthogonal labeling, molecular imaging, targeted drug delivery, live-cell applications
Major limitation	Catalyst-associated cytotoxicity	Cost and synthesis of strained alkynes

The evolution from CuAAC to SPAAC illustrates the progressive refinement of click chemistry from an efficient synthetic methodology toward highly selective reactions that can be performed directly within complex biological environments. This transition has significantly expanded the applicability of click chemistry in precision therapeutics, molecular diagnostics, regenerative medicine, and targeted pharmaceutical delivery.

3.3 Thiol-Ene Click Reactions

Thiol-ene chemistry has emerged as one of the most versatile members of the click chemistry family because of its operational simplicity, high atom economy, and broad applicability in both materials science and pharmaceutical research. Unlike azide-alkyne cycloaddition, thiol-ene reactions proceed through a radical-mediated addition of a thiol group across a carbon-carbon double bond, resulting in the formation of a stable thioether linkage[28]. The reaction can be initiated thermally, photochemically, or by radical initiators under relatively mild conditions, making it particularly suitable for modifying sensitive biomolecules and polymeric systems.

The mechanistic pathway begins with the generation of a thiyl radical from the thiol precursor. The thiyl radical rapidly adds across the carbon-carbon double bond to form a carbon-centered radical, which subsequently abstracts a hydrogen atom from another thiol molecule, regenerating the thiyl radical and propagating the reaction. This step-growth mechanism minimizes chain transfer reactions and contributes to excellent control over molecular architecture[29].

One of the principal advantages of thiol-ene chemistry is its tolerance toward oxygen and moisture, characteristics that simplify experimental procedures compared with many conventional radical polymerization methods. The reaction also demonstrates minimal side-product formation, excellent functional-group compatibility, and high conversion efficiency, allowing selective modification of peptides, proteins, polysaccharides, lipids, dendrimers, hydrogels, and polymeric drug carriers.

Within peptide chemistry, thiol-ene reactions have been widely employed for site-specific functionalization of cysteine residues. Since cysteine is comparatively rare in protein sequences, its selective modification enables controlled conjugation of fluorophores, polyethylene glycol (PEG), therapeutic peptides, antibodies, carbohydrates, and targeting ligands without significantly altering protein structure or biological activity[30].

Pharmaceutical applications of thiol-ene chemistry continue to expand rapidly. The methodology has facilitated the fabrication of injectable hydrogels for controlled drug release, tissue-engineering scaffolds, mucoadhesive delivery systems, implantable biomaterials, wound dressings, and stimuli-responsive polymeric networks. The high spatial and temporal control achievable through photoinitiated thiol-ene reactions has further enhanced their utility in three-dimensional bioprinting and regenerative medicine.

Despite these advantages, several challenges remain. Radical initiation may require specialized photoinitiators or ultraviolet irradiation, while oxygen inhibition can occasionally influence polymerization kinetics under certain experimental conditions. Furthermore, the availability of appropriately positioned thiol groups may limit site-selective modification of some biomolecules. Nevertheless, continued advances in visible-light photochemistry and catalyst development are expected to further expand the biomedical applications of thiol-ene reactions.

3.4 Thiol-Yne Click Reactions

Thiol-yne chemistry represents a logical extension of thiol-ene reactions in which thiol groups react with carbon-carbon triple bonds instead of alkenes. This methodology has attracted considerable interest because each alkyne can undergo two sequential thiol addition reactions, permitting significantly higher functional-group density than thiol-ene chemistry[31].

The reaction mechanism initially resembles thiol-ene chemistry. Following radical generation, the thiyl radical adds across the carbon-carbon triple bond to generate a vinyl sulfide intermediate. This intermediate remains sufficiently reactive to undergo a second thiol addition, ultimately yielding a bis-thioether product. The ability to perform two consecutive addition reactions provides opportunities for constructing densely functionalized molecular architectures that would be difficult to achieve using conventional synthetic methodologies.

From a pharmaceutical perspective, thiol-yne chemistry offers several important advantages. The increased functional-group density permits preparation of highly cross-linked polymeric networks exhibiting improved mechanical strength, enhanced chemical stability,

and tunable degradation profiles. Consequently, thiol-yne reactions have been extensively investigated for preparing nano particulate drug carriers, injectable biomaterials, implant coatings, responsive hydrogels, and multifunctional polymeric therapeutics.

The reaction also provides an efficient strategy for preparing multivalent drug-delivery platforms capable of simultaneously incorporating therapeutic agents, imaging probes, targeting ligands, and diagnostic molecules within a single construct. Such multifunctional systems are increasingly important in precision medicine because they combine therapeutic delivery with real-time monitoring of drug distribution and treatment response.

Although thiol-yne chemistry offers greater molecular versatility than thiol-ene reactions, optimization of reaction stoichiometry is often required to achieve complete double addition. Control of stereochemistry may also become more complex because intermediate vinyl sulfides can exist as geometric isomers before the second addition step proceeds. Continued refinement of catalyst systems and photochemical initiation methods has substantially improved reaction efficiency while reducing undesirable side reactions.

Figure 3. Mechanistic comparison of thiol–ene and thiol–yne click reactions

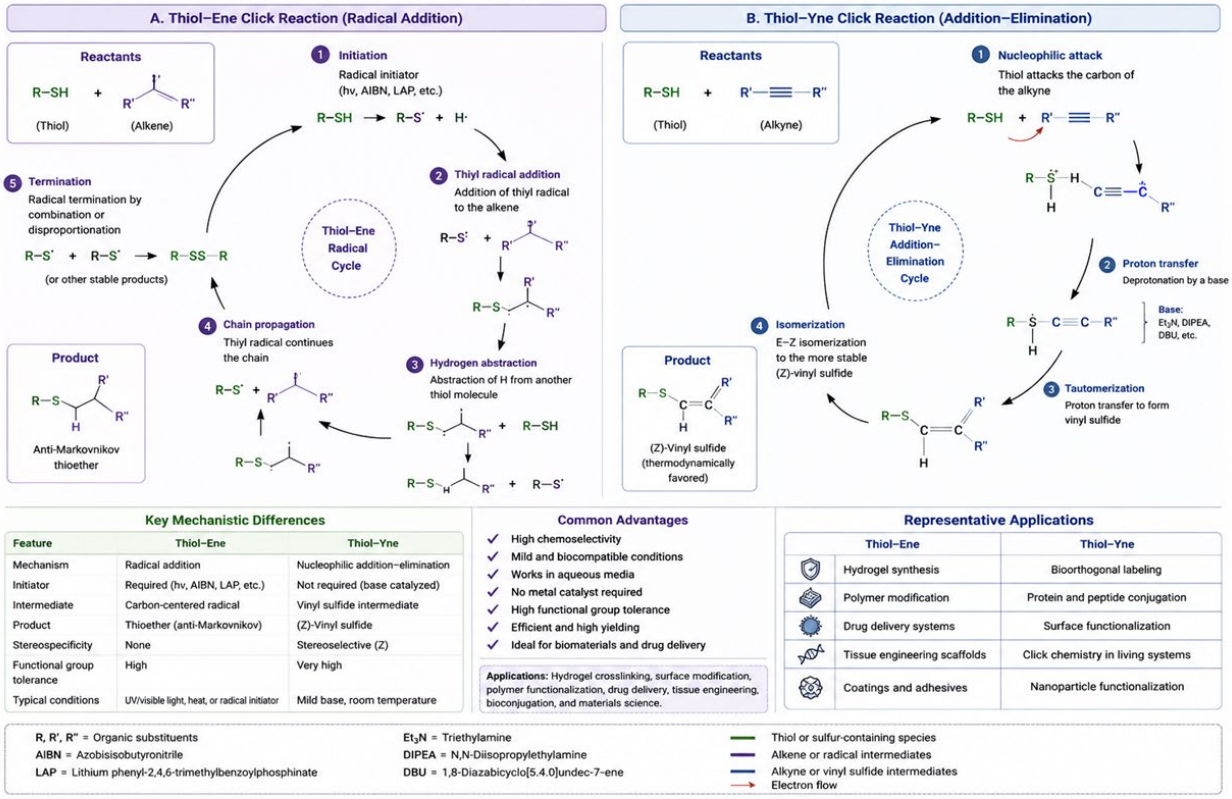

Figure 3. Mechanistic comparison of thiol-ene and thiol-yne click reactions.

Figure 3 represents Radical-mediated addition pathways illustrating thiyl radical generation, sequential addition to carbon-carbon double or triple bonds, propagation through hydrogen abstraction, and formation of stable thioether-linked products. The schematic highlights the increased functional-group density achievable through thiol-yne chemistry relative to thiol-ene reactions.

3.5 Inverse-Electron-Demand Diels-Alder (IEDDA) Click Chemistry

Among the newest developments in bio-orthogonal chemistry, the inverse-electron-demand Diels-Alder (IEDDA) reaction has emerged as one of the fastest click reactions currently available. Unlike classical Diels-Alder cycloadditions, which involve electron-rich dienes and electron-deficient dienophiles, IEDDA reactions proceed between electron-deficient tetrazines and strained alkenes or alkynes[32].

The reaction exhibits exceptionally rapid kinetics, often several orders of magnitude faster than many other bio-orthogonal reactions, while maintaining outstanding selectivity

under physiological conditions. Importantly, no metal catalyst is required, thereby eliminating concerns regarding catalyst-associated toxicity during biological applications.

The mechanism begins with a [4+2] cycloaddition between the tetrazine ring and the strained alkene, producing a dihydropyridazine intermediate. Subsequent extrusion of molecular nitrogen provides a strong thermodynamic driving force, resulting in formation of highly stable products with excellent reaction efficiency.

IEDDA chemistry has significantly expanded opportunities for molecular imaging, targeted drug delivery, in vivo diagnostics, antibody-drug conjugates, radionuclide labeling, and controlled activation of prodrugs. Because the reaction proceeds rapidly at extremely low reactant concentrations, it is particularly suitable for selective labeling of biomolecules within living organisms, where competing biological reactions could otherwise reduce reaction efficiency.

Recent pharmaceutical research has employed IEDDA chemistry for pre-targeted imaging of malignant tumors, selective activation of cytotoxic drugs at disease sites, engineering of multifunctional nanoparticles, and controlled release of therapeutic payloads. The exceptionally fast reaction kinetics have also facilitated time-resolved biological investigations requiring rapid molecular labeling.

Limitations primarily involve synthesis of highly strained dienophiles and tetrazine derivatives, which may require specialized synthetic procedures. Nevertheless, continued development of stable tetrazine analogues and improved dienophile design is steadily increasing the translational potential of this reaction.

3.6 Sulfur (VI) Fluoride Exchange (SuFEx) Click Chemistry

Sulfu (VI) fluoride exchange (SuFEx) chemistry represents one of the most recent additions to the click chemistry family and has substantially expanded the chemical diversity accessible through modular synthesis. Introduced as a second-generation click reaction, SuFEx utilizes sulfur (VI)-fluoride functional groups as highly selective electrophilic connectors capable of forming stable sulfur-containing linkages under mild reaction conditions[33].

The reaction generally involves sulfur (VI)-fluoride compounds, such as sulfonyl fluorides or fluorosulfates, reacting with suitable nucleophiles including phenols, alcohols, or amines. Although sulfur (VI)-fluoride bonds remain remarkably stable under physiological conditions, they undergo highly selective exchange reactions in the presence of appropriate nucleophiles, thereby enabling controlled molecular assembly.

SuFEx chemistry has gained increasing attention within medicinal chemistry because sulfur-containing functional groups frequently display favorable pharmacological characteristics, including enhanced metabolic stability, improved aqueous solubility, and prolonged biological half-life. Furthermore, sulfonyl fluoride moieties have proven valuable as covalent warheads for selective enzyme inhibition, particularly against serine hydrolases and related protein families.

Applications of SuFEx now extend to preparation of enzyme inhibitors, covalent therapeutics, polymeric biomaterials, molecular imaging probes, and functional nanomaterials. The methodology has also been incorporated into diversity-oriented synthesis, allowing efficient construction of structurally complex molecular libraries suitable for high-throughput pharmaceutical screening. Although SuFEx remains a comparatively young field relative to CuAAC, continued methodological improvements, catalyst development, and expanding substrate diversity suggest that sulfur (VI)-based click chemistry will play an increasingly important role in future medicinal chemistry and precision therapeutics.

Table 3. Comparative characteristics of major click reactions employed in medicinal chemistry and pharmaceutical research.

Click reaction	Reaction partners	Catalyst requirement	Major product	Principal pharmaceutical applications	Major advantages	Key limitations

CuAAC	Azide + terminal alkyne	Copper(I)	1,4-Triazole	Drug synthesis, peptide conjugation, medicinal chemistry	Excellent regioselectivity, high yield	Copper-associated cytotoxicity
SPAAC	Azide + strained cyclooctyne	None	Triazole	Bio-orthogonal labeling, molecular imaging	Catalyst-free, compatible with living systems	Expensive strained alkynes
Thiol-ene	Thiol + alkene	Radical initiator or light	Thioether	Hydrogels, biomaterials, protein modification	Mild conditions, high efficiency	Radical initiation required
Thiol-yne	Thiol + alkyne	Radical initiator or light	Bis-thioether	Multifunctional polymers, drug delivery	High functional-group density	Reaction optimization needed
IEDDA	Tetrazine + strained alkene	None	Pyridazine derivative	Live-cell imaging, antibody conjugates, pretargeted therapy	Extremely rapid kinetics	Specialized reactants
SuFEx	Sulfur(VI)-fluoride + nucleophile	Mild catalyst (when applicable)	Sulfur(VI) linkage	Covalent inhibitors, medicinal chemistry	Stable sulfur linkages, high selectivity	Developing methodology

3.7 Comparative Perspective

The progressive diversification of click chemistry has produced a complementary toolkit of highly selective reactions, each optimized for distinct pharmaceutical applications. CuAAC remains the preferred methodology for routine synthetic medicinal chemistry because of its robustness and exceptional reliability. SPAAC and IEDDA reactions have become indispensable for bio-orthogonal chemistry owing to their compatibility with living systems. Thiol-ene and thiol-yne reactions provide versatile strategies for engineering polymeric biomaterials and drug-delivery platforms, whereas SuFEx chemistry continues to expand opportunities for covalent drug design and sulfur-based medicinal chemistry. Rather than competing technologies, these reactions should be regarded as complementary synthetic platforms whose combined application enables increasingly sophisticated approaches to drug discovery, bio-molecular engineering, precision therapeutics, and translational pharmaceutical research.

4. Copper (I)-Catalyzed Azide-Alkyne Cycloaddition (CuAAC): Mechanism, Synthetic Strategies, and Pharmaceutical Applications

4.1 Introduction

Among the diverse family of click reactions, the copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC) has become the defining transformation of modern click chemistry. Since its independent development by Meldal and by Tornøe and Sharpless in 2002, CuAAC has profoundly influenced synthetic organic chemistry, medicinal chemistry, chemical biology, and pharmaceutical sciences[19–21]. The exceptional efficiency of this reaction arises from its ability to produce 1,4-disubstituted 1,2,3-triazoles rapidly, regioselectively, and in consistently

high yields under relatively mild experimental conditions. These characteristics have established CuAAC as one of the most reliable synthetic methodologies available for constructing structurally complex molecules intended for biomedical applications.

Unlike conventional cycloaddition reactions that frequently require elevated temperatures, prolonged reaction times, and extensive purification procedures, CuAAC proceeds efficiently in aqueous or mixed solvent systems while exhibiting remarkable tolerance toward a broad range of functional groups. Consequently, the methodology has become indispensable for synthesizing peptide conjugates, enzyme inhibitors, carbohydrate derivatives, polymeric therapeutics, nucleic acid conjugates, antibody-drug conjugates, radiopharmaceuticals, and multifunctional nanomedicines[22-24]

The significance of CuAAC extends beyond synthetic convenience. The reaction provides a modular platform for joining independently optimized molecular fragments while preserving their structural integrity and biological function. This modularity has greatly accelerated lead optimization, structure-activity relationship studies, and diversity-oriented synthesis within pharmaceutical research.

4.2 Chemical Basis of CuAAC

CuAAC is based on the cycloaddition between an organic azide and a terminal alkyne in the presence of a catalytic copper (I) species. The reaction results in the formation of a five-membered aromatic 1,2,3-triazole ring through a highly regioselective process that almost exclusively yields the 1,4-disubstituted triazole isomer[25].

From a medicinal chemistry perspective, the triazole ring possesses several desirable physicochemical characteristics. The aromatic heterocycle exhibits exceptional resistance toward hydrolysis, oxidation, and enzymatic degradation while maintaining favorable electronic properties that frequently enhance molecular recognition by biological targets. Triazoles also function as effective bioisosteres for amide bonds, peptide bonds, esters, and disulfide bridges, enabling medicinal chemists to improve metabolic stability without substantially altering pharmacological activity[26].

Another important feature of CuAAC is its remarkable orthogonality. The azide and terminal alkyne functional groups remain essentially inert toward most naturally occurring biological functionalities, allowing selective bond formation even in chemically complex environments containing alcohols, amines, thiols, carboxylic acids, phosphates, carbohydrates, and nucleic acids. This exceptional selectivity has been fundamental to the success of CuAAC in chemical biology and bio-molecular engineering.

4.3 Mechanistic Pathway

The catalytic cycle of CuAAC has been extensively investigated through experimental and computational studies. Although mechanistic refinements continue to emerge, the generally accepted pathway involves several coordinated elementary steps that collectively contribute to the remarkable efficiency of the reaction.

Initially, copper (I) coordinates with the π-electron cloud of the terminal alkyne, facilitating deprotonation and formation of a copper acetylide intermediate. This activation substantially increases the nucleophilicity of the alkyne carbon atoms while lowering the activation energy required for cycloaddition.[27]

The activated copper acetylide subsequently interacts with the terminal nitrogen atom of the organic azide, producing a six-membered copper-containing metallacyclic intermediate. Progressive rearrangement of this intermediate ultimately generates the aromatic triazole ring through intramolecular cyclization. Finally, protonation of the triazolyl-copper intermediate regenerates the active copper catalyst while releasing the desired 1,4-disubstituted triazole product.

The catalytic participation of copper (I) fundamentally alters the reaction pathway relative to the uncatalyzed Huisgen cycloaddition. Instead of producing mixtures of regioisomeric products, CuAAC directs cyclization almost exclusively toward formation of the pharmacologically valuable 1,4-disubstituted triazole with excellent reproducibility.

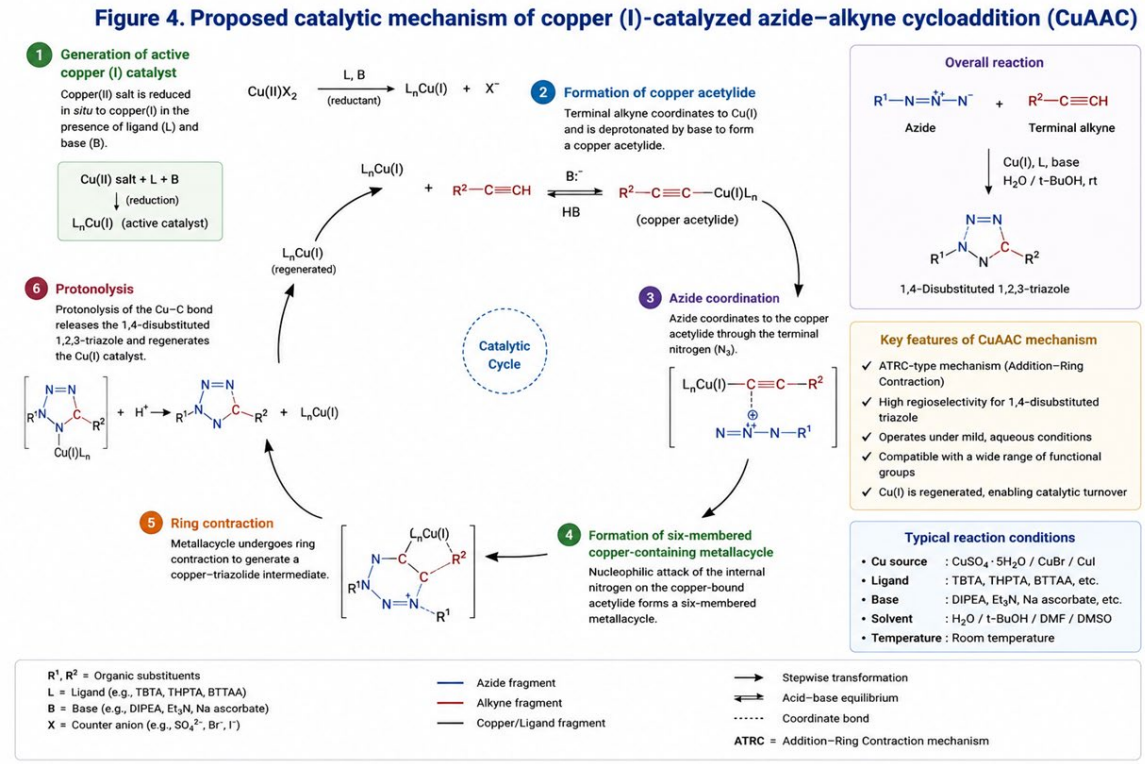

Figure 4. Proposed catalytic mechanism of copper (I)-catalyzed azide-alkyne cycloaddition.

Figure 4 represents the Sequential catalytic cycle illustrating (A) copper(I) coordination with the terminal alkyne, (B) formation of the copper acetylide intermediate, (C) interaction with the organic azide, (D) generation of the metallacyclic intermediate, (E) triazole ring formation, and (F) catalyst regeneration following protonation. The mechanism highlights the origin of regioselective formation of 1,4-disubstituted triazoles.

4.4 Factors Influencing Reaction Efficiency

Although CuAAC is recognized for its robustness, several experimental variables influence reaction efficiency and product quality.

Copper Source

Catalytic activity depends strongly on the oxidation state and stability of the copper species employed. Copper(I) salts generally provide the highest catalytic efficiency; however, copper(II) salts combined with reducing agents such as sodium ascorbate are frequently used because of their greater stability during storage and handling. In situ reduction continuously generates catalytically active copper (I), thereby maintaining efficient reaction kinetics throughout the synthesis.[28]

Solvent System

CuAAC demonstrates remarkable solvent flexibility. Water, ethanol, methanol, dimethyl sulfoxide, tert-butanol-water mixtures, acetonitrile, and numerous mixed solvent systems have all been successfully employed. Aqueous reaction media are particularly attractive for bio-molecular conjugation because they preserve the structural integrity of peptides, proteins, carbohydrates, and nucleic acids.

Temperature

Most CuAAC reactions proceed efficiently at ambient temperature. Moderate heating may accelerate slower reactions involving sterically hindered substrates, whereas elevated temperatures are generally unnecessary because of the intrinsically favorable reaction kinetics.

Ligands and Stabilizing Agents

Nitrogen-containing ligands, including tris (triazolylmethyl) amine derivatives, are frequently incorporated to stabilize copper (I), minimize catalyst disproportionation, and improve reaction efficiency in aqueous environments. Ligand selection becomes particularly

important during bio-conjugation reactions where catalyst stability directly influences product yield and biological compatibility.

4.5 Advantages of CuAAC in Medicinal Chemistry

The widespread adoption of CuAAC within pharmaceutical sciences reflects the convergence of several highly desirable synthetic characteristics.

First, the reaction exhibits exceptional chemoselectivity, allowing selective coupling of azides and terminal alkynes even in the presence of numerous competing functional groups. This minimizes undesired side reactions and reduces purification requirements.

Second, CuAAC consistently provides excellent reaction yields, frequently approaching quantitative conversion. High reaction efficiency improves synthetic economy while facilitating large-scale pharmaceutical manufacturing.

Third, the methodology displays broad substrate compatibility. Small organic molecules, peptides, proteins, nucleic acids, carbohydrates, dendrimers, liposomes, polymeric nanoparticles, metallic nanoparticles, antibodies, and imaging probes can all be functionalized using the same fundamental reaction principle.

Fourth, the resulting triazole linkage contributes favorable physicochemical properties including metabolic stability, enhanced hydrogen-bonding capability, increased conformational rigidity, and improved resistance toward enzymatic degradation. These characteristics often translate into improved pharmacokinetic performance of therapeutic molecules.

Finally, CuAAC supports modular synthesis. Individual pharmacophores may be independently synthesized, optimized, and subsequently joined during the final stages of drug development. This flexibility substantially accelerates medicinal chemistry optimization while facilitating efficient exploration of structure-activity relationships.

Table 4. Principal advantages of copper (I)-catalyzed azide-alkyne cycloaddition in pharmaceutical research.

Characteristic	Pharmaceutical relevance
High regioselectivity	Formation of a single major triazole isomer
Excellent chemoselectivity	Minimal interference from biological functional groups
Mild reaction conditions	Compatible with sensitive biomolecules
High reaction yield	Improved synthetic efficiency
Broad substrate scope	Applicable to peptides, proteins, carbohydrates, polymers, and nanoparticles
Stable triazole linkage	Improved metabolic and chemical stability
Modular synthesis	Facilitates rapid medicinal chemistry optimization
Excellent reproducibility	Suitable for laboratory and industrial production

4.6 Limitations and Current Challenges

Despite its considerable strengths, CuAAC is not without limitations. The most frequently cited concern involves residual copper ions, which may induce oxidative stress, interfere with protein function, catalyze biomolecular degradation, or exhibit cytotoxicity during biological applications. Consequently, extensive purification is often required when CuAAC products are intended for therapeutic use or in vivo investigation[29].

Additional challenges include catalyst removal during large-scale manufacturing, optimization of reaction conditions for sterically hindered substrates, and prevention of copper-mediated oxidation of sensitive amino acid residues. Although numerous ligand systems have been developed to minimize these effects, catalyst-associated toxicity remains one of the principal motivations for the continued development of catalyst-free bio-orthogonal click reactions such as SPAAC and IEDDA chemistry.

Recent advances in heterogeneous copper catalysts, recyclable catalytic systems, continuous-flow synthesis, microreactor technology, and green chemistry approaches have substantially improved the sustainability and industrial applicability of CuAAC. These

innovations are expected to facilitate broader pharmaceutical implementation while reducing environmental impact and manufacturing costs.

4.7 CuAAC in Peptide-Based Drug Design

The application of copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC) has transformed peptide medicinal chemistry by providing a robust and highly selective strategy for introducing structural modifications that improve the pharmaceutical performance of peptide therapeutics. Although peptides possess remarkable target specificity and favorable safety profiles, their therapeutic translation is frequently limited by rapid enzymatic degradation, conformational flexibility, poor membrane permeability, and short systemic half-life. Conventional chemical modification techniques often require multiple protection-deprotection steps and may compromise biological activity. CuAAC offers an efficient alternative by enabling site-specific conjugation of peptide fragments under mild reaction conditions while preserving sensitive functional groups and biological integrity[30-32].

One of the most important applications of CuAAC in peptide engineering is the incorporation of **1,2,3-triazole linkages** as bioisosteric replacements for peptide bonds and disulfide bridges. Triazole rings possess exceptional chemical and metabolic stability, resistance to hydrolysis, and favorable electronic characteristics that enable them to mimic the geometry of naturally occurring peptide bonds while improving resistance to proteolytic degradation. Consequently, triazole-containing peptide analogues frequently exhibit prolonged circulation time, enhanced receptor affinity, and improved pharmacokinetic properties compared with their unmodified counterparts[33].

CuAAC has also become a valuable tool for **peptide cyclization**, a strategy widely employed to reduce conformational flexibility and stabilize bioactive secondary structures. Cyclized peptides generally display greater receptor selectivity, improved metabolic stability, and increased biological potency because structural preorganization minimizes the entropic penalty associated with target binding. Click-mediated cyclization offers excellent regioselectivity and can be performed without interfering with other functional groups present within complex peptide sequences. The resulting cyclic peptides have demonstrated promising therapeutic potential across oncology, endocrinology, immunology, and infectious disease research.

Another rapidly expanding application involves **peptide stapling**, in which CuAAC is used to create intramolecular covalent bridges that stabilize α-helical conformations. Stapled peptides exhibit enhanced cell permeability, increased resistance toward proteolytic enzymes, prolonged biological half-life, and improved intracellular target engagement. Such properties have stimulated considerable interest in developing stapled peptide therapeutics targeting protein-protein interactions that are traditionally considered difficult to modulate using small molecules.

Beyond structural stabilization, CuAAC facilitates the conjugation of peptides with polyethylene glycol (PEG), carbohydrates, fluorophores, radionuclides, lipids, antibodies, nanoparticles, and targeting ligands. These modifications improve pharmacokinetic behavior, tissue distribution, diagnostic capability, and therapeutic selectivity while maintaining the biological activity of the parent peptide. Consequently, CuAAC has become an indispensable methodology for developing multifunctional peptide therapeutics suitable for precision medicine.

4.8 Applications in Anticancer Therapeutics

Cancer remains one of the leading causes of mortality worldwide, creating an urgent need for therapeutic strategies that combine improved efficacy with reduced systemic toxicity. Click chemistry has emerged as an important enabling technology for anticancer drug discovery by supporting the rapid synthesis of structurally diverse compound libraries, targeted conjugates, multifunctional nanocarriers, and peptide-based therapeutics[34-36].

The modular nature of CuAAC allows medicinal chemists to independently optimize pharmacologically active fragments before joining them through stable triazole linkages. This

strategy has facilitated the development of hybrid anticancer agents that combine multiple mechanisms of action within a single molecular framework. Triazole-containing analogues have demonstrated improved inhibition of kinases, proteases, DNA topoisomerases, and other molecular targets implicated in tumor progression.

Peptide-based anticancer therapeutics have particularly benefited from click chemistry. Tumor-targeting peptides can be conjugated with cytotoxic drugs, imaging probes, radionuclides, or immune-modulating agents using CuAAC, thereby improving selective accumulation within malignant tissues while minimizing exposure of healthy organs. Such conjugates have shown enhanced therapeutic indices in preclinical investigations and have stimulated further development of targeted cancer therapies.

Click chemistry also plays an important role in the preparation of **antibody-drug conjugates (ADCs)**. Site-specific conjugation through CuAAC improves the homogeneity of ADC preparations by producing defined drug-to-antibody ratios and reducing batch-to-batch variability. Improved conjugation precision contributes to enhanced pharmacokinetics, greater therapeutic consistency, and reduced off-target toxicity.

Nanotechnology represents another major area of application. CuAAC enables precise functionalization of liposomes, polymeric nanoparticles, dendrimers, and inorganic nanocarriers with tumor-targeting ligands such as folic acid, RGD peptides, transferrin, aptamers, and monoclonal antibodies. Such multifunctional nanocarriers improve tumor localization through both passive and active targeting mechanisms while enabling simultaneous drug delivery and molecular imaging.

Furthermore, CuAAC has facilitated the development of stimuli-responsive nanomedicines capable of releasing therapeutic payloads in response to tumor-specific conditions such as acidic pH, elevated glutathione concentrations, or enzyme overexpression. These advances illustrate how click chemistry contributes not only to molecular synthesis but also to sophisticated therapeutic engineering for precision oncology.

4.9 Applications in Antimicrobial and Antiviral Drug Development

The rapid emergence of multidrug-resistant bacterial pathogens and newly emerging viral infections has intensified efforts to identify innovative antimicrobial strategies. Click chemistry has become an increasingly valuable platform for developing structurally diverse antimicrobial and antiviral agents with improved biological activity and reduced susceptibility to resistance mechanisms[37-39].

Triazole-containing antimicrobial compounds synthesized through CuAAC frequently exhibit enhanced chemical stability, improved membrane penetration, and favorable pharmacokinetic characteristics. The modular nature of the reaction permits rapid generation of compound libraries in which individual pharmacophoric fragments can be systematically modified to optimize antimicrobial potency and selectivity. Such diversity-oriented synthesis accelerates structure-activity relationship studies and facilitates identification of promising lead compounds.

In peptide therapeutics, CuAAC enables conjugation of antimicrobial peptides with polymers, lipids, nanoparticles, and targeting molecules to improve stability and prolong biological activity. Cyclization through triazole formation has also been shown to increase resistance toward proteolytic degradation while preserving membrane-disruptive mechanisms responsible for antimicrobial efficacy.

Within antiviral drug discovery, click chemistry has supported the synthesis of nucleoside analogues, protease inhibitors, polymerase inhibitors, neuraminidase inhibitors, and viral entry inhibitors. Triazole scaffolds frequently improve molecular rigidity and optimize intermolecular interactions with viral enzymes, thereby enhancing inhibitory activity against diverse viral pathogens.

Click chemistry has also facilitated preparation of vaccine conjugates and antigen-delivery systems by enabling efficient coupling of peptides, proteins, carbohydrates, and

immune-stimulatory molecules. Site-specific conjugation contributes to improved antigen presentation, enhanced immunogenicity, and more reproducible vaccine manufacturing.

Recent investigations have further explored CuAAC for preparing multifunctional antiviral nano carriers capable of combining therapeutic delivery with diagnostic imaging, targeted cellular uptake, and controlled drug release. Such integrated systems represent promising approaches for combating future viral outbreaks through precision antiviral therapy.

4.10 CuAAC in Nano medicine and Targeted Drug Delivery

Nanotechnology has become an integral component of modern pharmaceutical research by enabling controlled drug release, improved pharmacokinetics, enhanced tissue targeting, and reduced systemic toxicity. The success of nano medicine depends largely on precise surface functionalization, reproducible conjugation chemistry, and preservation of nanoparticle stability-requirements that are exceptionally well addressed by CuAAC[40-42].

CuAAC provides a highly selective method for attaching targeting ligands, peptides, antibodies, aptamers, carbohydrates, polyethylene glycol, fluorophores, radionuclides, and therapeutic molecules onto nanoparticle surfaces. Because azide and alkyne groups exhibit excellent orthogonality toward most biological functionalities, nanoparticle functionalization can be performed without disrupting encapsulated drugs or sensitive biomolecular components.

Polymeric nanoparticles, liposomes, dendrimers, mesoporous silica nanoparticles, gold nanoparticles, magnetic nanoparticles, and lipid nanoparticles have all been successfully engineered using CuAAC-mediated surface modification. These functionalized nanocarriers demonstrate improved colloidal stability, prolonged systemic circulation, enhanced cellular uptake, and greater target specificity.

An important application involves active targeting**,** where nanoparticles are decorated with receptor-specific ligands capable of recognizing molecular markers overexpressed on diseased tissues. Peptides targeting integrins, folate receptors, transferrin receptors, HER2 receptors, and prostate-specific membrane antigen have been successfully conjugated using CuAAC, improving accumulation at pathological sites while reducing nonspecific biodistribution.

CuAAC has also facilitated development of **theranostic nanoplatforms** that integrate diagnostic imaging with therapeutic delivery. By simultaneously attaching imaging probes and pharmacologically active compounds to the same nanoparticle, researchers can monitor drug distribution, evaluate therapeutic response, and personalize treatment strategies in real time.

Emerging applications include multifunctional nano carriers capable of sequential drug release, stimulus-responsive delivery, combination chemotherapy, gene therapy, immunotherapy, and CRISPR-based genome editing. These sophisticated systems illustrate the expanding contribution of click chemistry to precision medicine and next-generation pharmaceutical technology.

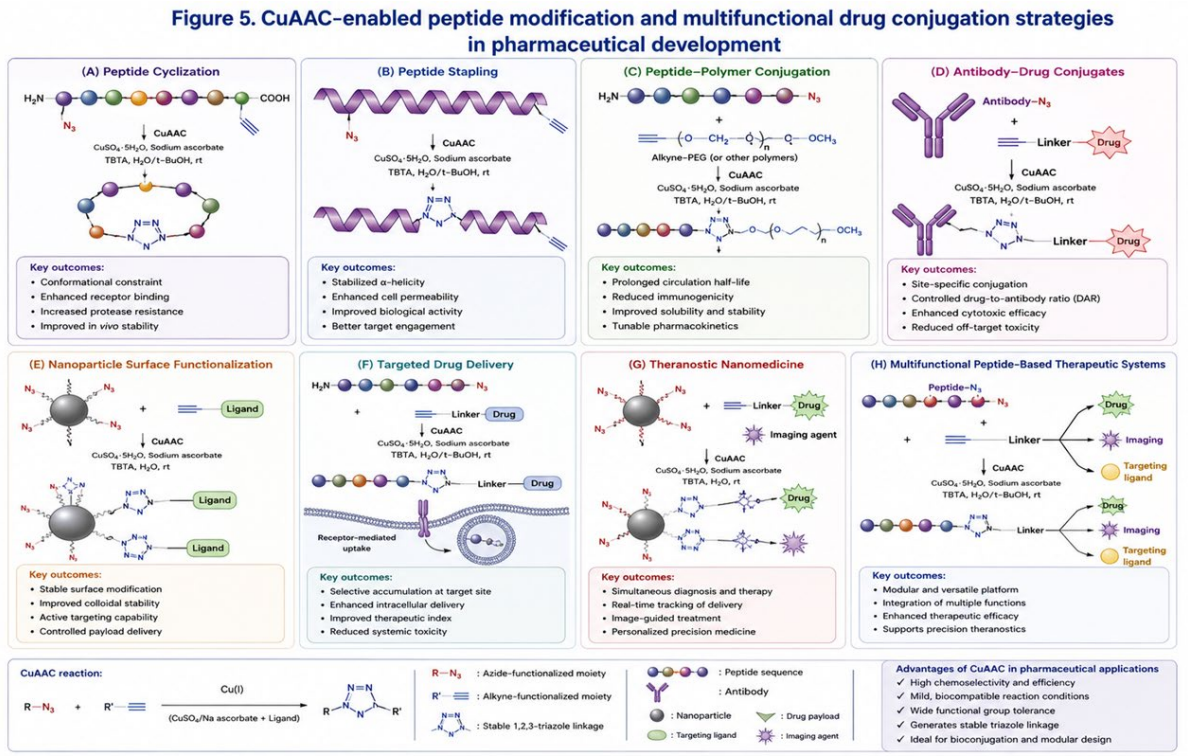

Figure 5. CuAAC-enabled peptide modification and multifunctional drug conjugation strategies in pharmaceutical development.

Schematic representation in Figure 5 of CuAAC-mediated applications illustrating (A) peptide cyclization, (B) peptide stapling, (C) peptide-polymer conjugation, (D) antibody-drug conjugates, (E) nanoparticle surface functionalization, (F) targeted drug delivery, (G) theranostic nanomedicine, and (H) multifunctional peptide-based therapeutic systems generated through modular click chemistry.

Table 5. Therapeutic applications of copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC) in contemporary pharmaceutical research.

Therapeutic area	CuAAC strategy	Representative application	Major pharmaceutical advantages
Peptide therapeutics	Triazole incorporation and cyclization	Stabilized peptide analogues	Improved metabolic stability, enhanced receptor affinity
Anticancer therapy	Drug conjugation and hybrid molecule synthesis	Antibody-drug conjugates, peptide-drug conjugates	Increased selectivity, reduced systemic toxicity
Antimicrobial therapy	Triazole scaffold engineering	Antimicrobial peptides and small molecules	Enhanced potency and resistance to enzymatic degradation
Antiviral therapy	Nucleoside and peptide conjugation	Viral enzyme inhibitors and vaccine constructs	Improved antiviral activity and molecular stability
Nanomedicine	Nanoparticle surface functionalization	Targeted liposomes, dendrimers, polymeric nanoparticles	Enhanced drug delivery, prolonged circulation, active targeting
Molecular imaging	Conjugation of fluorophores and radionuclides	PET, SPECT, fluorescence imaging	High labeling efficiency and imaging sensitivity

Precision medicine	Multifunctional bioconjugation	Theranostic systems and personalized therapeutics	Simultaneous diagnosis and targeted treatment
Drug delivery	Ligand-mediated targeting	Receptor-specific nanocarriers	Improved tissue specificity and controlled drug release

Copper (I)-catalyzed azide-alkyne cycloaddition has evolved from a highly efficient synthetic reaction into a foundational technology that underpins modern peptide engineering, targeted drug design, nanomedicine, molecular imaging, and precision therapeutics. Its exceptional selectivity, modularity, and broad substrate compatibility have enabled the development of structurally sophisticated therapeutic platforms that would be difficult to achieve using conventional synthetic approaches. Continued integration of CuAAC with advanced biomaterials, computational molecular design, and intelligent drug delivery systems is expected to further expand its influence on translational pharmaceutical research and future therapeutic innovation.

5. Discussion

The present review demonstrates that click chemistry has evolved from a highly efficient synthetic methodology into a multidisciplinary platform that now underpins several aspects of modern pharmaceutical research, including medicinal chemistry, peptide engineering, targeted drug delivery, nanomedicine, molecular imaging, and precision therapeutics. Since the introduction of the click chemistry concept by Sharpless and co-workers in 2001, continuous methodological innovations have transformed simple modular reactions into sophisticated bio-orthogonal technologies capable of operating within complex biological systems. As illustrated in Figure 1, the historical evolution of click chemistry reflects a progressive transition from the classical Huisgen cycloaddition to CuAAC, catalyst-free bio-orthogonal reactions, and their subsequent integration into contemporary drug discovery and biomedical engineering. The major milestones summarized in Table 1 further emphasize how each technological advancement has expanded the pharmaceutical relevance of click chemistry while improving synthetic efficiency and biological applicability.

One of the principal strengths of click chemistry lies in its ability to overcome several longstanding challenges associated with conventional organic synthesis. Traditional medicinal chemistry frequently involves multistep synthetic pathways characterized by lengthy reaction sequences, extensive purification procedures, poor atom economy, and limited compatibility with sensitive biological molecules. In contrast, click reactions provide highly efficient molecular assembly under relatively mild conditions with excellent chemoselectivity, broad functional-group tolerance, and consistently high reaction yields. These properties have significantly accelerated lead optimization, diversity-oriented synthesis, and structure-activity relationship investigations. The mechanistic characteristics summarized in Figure 2 clearly explain why CuAAC has become the benchmark click reaction, whereas the catalytic features discussed throughout Section 4 illustrate its exceptional reliability for pharmaceutical synthesis.

The comparative evaluation of major click reactions demonstrates that no single reaction is universally applicable; rather, each possesses unique characteristics that make it suitable for specific biomedical applications. CuAAC remains the preferred methodology for routine medicinal chemistry because of its outstanding regioselectivity and synthetic robustness, whereas strain-promoted azide-alkyne cycloaddition and inverse-electron-demand Diels-Alder reactions have become indispensable for bio-orthogonal applications in living systems. Similarly, thiol-ene and thiol-yne reactions have substantially expanded opportunities for polymer engineering, hydrogel fabrication, biomaterials development, and regenerative medicine. The mechanistic comparison presented in Figure 3 and the comprehensive comparison summarized in Table 3 collectively demonstrate how the diversification of click

chemistry has produced complementary reaction platforms rather than competing technologies. This mechanistic diversity provides medicinal chemists with considerable flexibility for selecting reaction systems according to substrate compatibility, reaction environment, biological constraints, and intended pharmaceutical application.

Among the various click methodologies, CuAAC continues to occupy a central position in pharmaceutical sciences because of its exceptional balance between reaction efficiency, reproducibility, and structural versatility. The proposed catalytic cycle illustrated in Figure 4 explains the molecular basis for the remarkable regioselectivity and high reaction efficiency that distinguish CuAAC from the classical thermal Huisgen cycloaddition. Formation of stable 1,2,3-triazole linkages not only simplifies molecular assembly but also introduces pharmacologically advantageous structural features, including enhanced metabolic stability, improved hydrogen-bonding capability, increased conformational rigidity, and resistance to enzymatic degradation. These characteristics have established CuAAC as one of the most reliable synthetic methodologies for preparing structurally complex therapeutic molecules.

The review further demonstrates that peptide-based drug discovery represents one of the most significant beneficiaries of click chemistry. Peptide therapeutics possess remarkable biological specificity but frequently suffer from rapid proteolytic degradation, conformational instability, poor membrane permeability, and limited oral bioavailability. CuAAC provides highly selective approaches for peptide cyclization, stapling, PEGylation, polymer conjugation, and site-specific functionalization while preserving biological activity. These modifications enhance structural rigidity, improve receptor-binding affinity, prolong systemic circulation, and increase resistance to enzymatic degradation. The diverse peptide engineering strategies summarized in Figure 5 illustrate the remarkable versatility of CuAAC for constructing multifunctional peptide therapeutics, while the broad range of pharmaceutical applications presented in Table 5 underscores its importance in advancing peptide-based precision medicine.

The application of click chemistry to oncology represents another major area of pharmaceutical innovation. Modern anticancer drug development increasingly emphasizes molecular selectivity, targeted delivery, and reduced systemic toxicity. CuAAC has enabled the construction of hybrid anticancer agents, peptide-drug conjugates, antibody-drug conjugates, and multifunctional nanotherapeutics with well-defined molecular architecture and improved pharmacokinetic characteristics. Site-specific conjugation through triazole formation minimizes structural heterogeneity while facilitating reproducible manufacturing of complex therapeutic systems. Furthermore, click chemistry supports the development of theranostic platforms that combine therapeutic delivery with molecular imaging, thereby enabling simultaneous diagnosis, treatment, and therapeutic monitoring. The integrated conjugation strategies depicted in Figure 5 exemplify how modular click chemistry has transformed conventional cytotoxic drug development into sophisticated targeted therapeutic engineering.

The increasing prevalence of antimicrobial resistance and recurrent viral outbreaks has further underscored the importance of modular synthetic methodologies that can rapidly generate structurally diverse anti-infective agents. CuAAC has accelerated antimicrobial and antiviral drug discovery through efficient synthesis of triazole-containing scaffolds, peptide conjugates, vaccine constructs, and multifunctional nanocarriers. The ability to rapidly assemble focused chemical libraries facilitates systematic optimization of biological activity while reducing synthetic complexity. In addition, click chemistry has facilitated the development of peptide-based antimicrobial agents with enhanced proteolytic stability and prolonged biological activity, thereby addressing several limitations of conventional peptide therapeutics.

Nanomedicine has emerged as another area in which click chemistry provides substantial pharmaceutical value. Surface functionalization of nanoparticles requires highly selective conjugation methods that preserve nanoparticle integrity while enabling the controlled attachment of targeting ligands, therapeutic agents, imaging probes, and responsive

polymers. CuAAC fulfils these requirements through exceptionally efficient bio-conjugation chemistry, enabling the construction of multifunctional nanocarriers with predictable structural organization and excellent physicochemical stability. As illustrated in Figure 5, click chemistry facilitates the assembly of targeted nanoparticles, peptide-functionalized carriers, antibody-drug conjugates, and theranostic systems capable of integrating multiple therapeutic and diagnostic functions within a single pharmaceutical platform. Correspondingly, Table 5 demonstrates that CuAAC applications now extend well beyond synthetic chemistry to encompass precision drug delivery, molecular imaging, vaccine engineering, and personalized therapeutics.

An important theme emerging throughout this review is the progressive convergence of click chemistry with computational medicinal chemistry and artificial intelligence. Although the fundamental principles of click chemistry are rooted in synthetic organic chemistry, contemporary pharmaceutical research increasingly integrates machine learning, molecular modeling, virtual screening, and automated reaction optimization to accelerate drug discovery. Artificial intelligence provides opportunities for predicting reaction outcomes, identifying synthetically accessible pharmacophores, optimizing physicochemical properties, and prioritizing compounds before experimental synthesis. When combined with the modularity of click chemistry, these computational approaches substantially reduce experimental workload while improving lead optimization efficiency. The integration of predictive computational tools with highly reliable click reactions represents a major step toward data-driven medicinal chemistry and precision pharmaceutical development.

Despite its remarkable achievements, several scientific and translational challenges continue to influence the broader implementation of click chemistry. Copper-associated cytotoxicity remains an important consideration for CuAAC during biological applications, particularly where complete catalyst removal cannot be guaranteed. Large-scale manufacturing, catalyst recycling, regulatory standardization, environmental sustainability, and cost-effective industrial production also require continued methodological refinement. Furthermore, although bio-orthogonal reactions have significantly improved compatibility with living systems, their widespread clinical translation will depend upon simplified synthesis of strained reactants, comprehensive toxicological evaluation, and robust regulatory validation. Addressing these limitations will require sustained collaboration among synthetic chemists, pharmaceutical scientists, biomedical engineers, clinicians, and regulatory authorities.

Another emerging priority involves the implementation of green chemistry principles throughout click chemistry-enabled pharmaceutical manufacturing. Development of recyclable catalysts, aqueous reaction systems, continuous-flow synthesis, solvent minimization, energy-efficient reaction conditions, and environmentally sustainable manufacturing technologies will become increasingly important as industrial adoption expands. Simultaneously, advances in automated synthesis, digital medicinal chemistry, laboratory robotics, and artificial intelligence-assisted reaction optimization are expected to further enhance manufacturing reproducibility while reducing environmental impact.

Overall, the evidence synthesized in this review clearly indicates that click chemistry has evolved far beyond its original role as a synthetic reaction. It now represents a comprehensive molecular engineering platform that supports the rational design of peptide therapeutics, targeted drug conjugates, multifunctional nanomedicines, molecular imaging agents, biomaterials, and precision therapeutic systems. The historical progression summarized in Figure 1, the mechanistic foundations illustrated in Figures 2-4, the multifunctional pharmaceutical applications presented in Figure 5, and the comparative analyses provided in Tables 1-5 collectively demonstrate the remarkable breadth of contemporary click chemistry. Continued interdisciplinary integration of synthetic chemistry, chemical biology, computational science, nanotechnology, and precision medicine is expected to further expand the clinical impact of click chemistry, positioning it as one of the most influential enabling technologies in next-generation pharmaceutical research and therapeutic innovation.

6. Conflict of Interest

The authors declare that they have no known financial interests, personal relationships, commercial affiliations, or institutional associations that could have influenced the preparation, interpretation, or presentation of this review. The manuscript was developed solely through critical analysis and synthesis of the published scientific literature, and no commercial entity had any role in the conception, literature selection, and interpretation of evidence, manuscript preparation, revision, or decision to submit the work for publication. The authors further confirm that there are no competing intellectual property interests, consultancy agreements, honoraria, stock ownership, patents, or funding-related conflicts that could be perceived as influencing the objectivity or scientific integrity of this review.

7. Acknowledgements

The authors gratefully acknowledge the contributions of the global scientific community whose pioneering investigations in click chemistry, medicinal chemistry, peptide science, chemical biology, and pharmaceutical research have established the knowledge base that made this review possible. The authors sincerely appreciate the efforts of researchers who have advanced the understanding of modular synthetic methodologies and their applications in drug discovery, targeted therapeutics, biomaterials, nanomedicine, and precision medicine.

The authors also acknowledge the support of their respective institutions for providing an academic environment that encouraged critical evaluation of the scientific literature and facilitated the preparation of this comprehensive review. The constructive contributions of peer reviewers, editors, and the broader scientific community, whose published work has continually strengthened this rapidly evolving field, are also sincerely appreciated.

This review was prepared through an independent and comprehensive appraisal of published scientific evidence with the objective of presenting a balanced and up-to-date overview of recent developments in click chemistry and its expanding role in peptide-based drug discovery and pharmaceutical sciences. The authors express their appreciation to all investigators whose original research has contributed to the continued advancement of this important interdisciplinary field.

8. Conclusion

Click chemistry has emerged as a transformative platform in modern medicinal chemistry, evolving from a powerful synthetic methodology into an indispensable tool for pharmaceutical research and drug discovery. Its modularity, high chemoselectivity, operational simplicity, and exceptional reaction efficiency have enabled the rapid construction of structurally complex molecules while overcoming many limitations of conventional synthetic approaches. Among the available click reactions, copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) remains the most widely applied because of its excellent regioselectivity, broad substrate compatibility, and ability to generate stable 1,2,3-triazole linkages that enhance the pharmacological and physicochemical properties of therapeutic molecules.

This review highlights the pivotal role of click chemistry in advancing peptide-based drug discovery through peptide cyclization, stapling, bioisosteric substitution, and site-specific bio-conjugation, leading to improved peptide stability, receptor affinity, metabolic resistance, and therapeutic efficacy. In addition, click chemistry has significantly accelerated the development of targeted anticancer agents, antimicrobial and antiviral therapeutics, multifunctional Nano medicines, antibody-drug conjugates, and intelligent drug-delivery systems. The emergence of catalyst-free bio-orthogonal reactions has further expanded the applicability of click chemistry to in vivo molecular engineering, molecular imaging, regenerative medicine, and precision therapeutics by enabling highly selective reactions under physiological conditions.

Despite remarkable progress, challenges related to catalyst removal, scalable manufacturing, regulatory acceptance, and long-term biological safety remain important considerations for successful clinical translation. Future advances are expected to arise from the integration of click chemistry with artificial intelligence, computational drug design, automated synthesis, advanced biomaterials, and precision medicine, enabling faster development of safer and more effective therapeutics.

Overall, click chemistry has become a cornerstone technology in contemporary pharmaceutical sciences, bridging synthetic chemistry with translational medicine. Continued interdisciplinary innovation is expected to further expand its impact on peptide therapeutics, targeted drug delivery, nanomedicine, and personalized healthcare, establishing click chemistry as a key driver of next-generation drug discovery and pharmaceutical development.

References

1. Kolb, H. C., Finn, M. G., & Sharpless, K. B. (2001). Click chemistry: Diverse chemical function from a few good reactions. *Angewandte Chemie International Edition, 40*(11), 2004–2021. https://doi.org/10.1002/1521-3773(20010601)40:11<2004::AID-ANIE2004>3.0.CO;2-5
2. Moses, J. E., & Moorhouse, A. D. (2007). The growing applications of click chemistry. *Chemical Society Reviews, 36*(8), 1249–1262. https://doi.org/10.1039/B613014N
3. Hein, J. E., & Fokin, V. V. (2010). Copper-catalyzed azide–alkyne cycloaddition (CuAAC) and beyond: New reactivity of copper (I) acetylides. *Chemical Society Reviews, 39*(4), 1302–1315. https://doi.org/10.1039/B904091A
4. Rostovtsev, V. V., Green, L. G., Fokin, V. V., & Sharpless, K. B. (2002). A stepwise Huisgen cycloaddition process: Copper(I)-catalyzed regioselective ligation of azides and terminal alkynes. *Angewandte Chemie International Edition, 41*(14), 2596–2599. https://doi.org/10.1002/1521-3773(20020715)41:14<2596::AID-ANIE2596>3.0.CO;2-4
5. Meldal, M., & Tornøe, C. W. (2008). Cu-catalyzed azide–alkyne cycloaddition. *Chemical Reviews, 108*(8), 2952–3015. https://doi.org/10.1021/cr0783479
6. Tornøe, C. W., Christensen, C., & Meldal, M. (2002). Peptidotriazoles on solid phase: Copper(I)-catalyzed 1,3-dipolar cycloadditions of azides and alkynes. *Journal of Organic Chemistry, 67*(9), 3057–3064. https://doi.org/10.1021/jo011148j
7. Agalave, S. G., Maujan, S. R., & Pore, V. S. (2011). Click chemistry: 1,2,3-Triazoles as pharmacophores. *Chemical Asian Journal, 6*(10), 2696–2718. https://doi.org/10.1002/asia.201100432
8. Tron, G. C., Pirali, T., Billington, R. A., Canonico, P. L., Sorba, G., & Genazzani, A. A. (2008). Click chemistry reactions in medicinal chemistry: Applications of the 1,2,3-triazole linkage. *Medicinal Research Reviews, 28*(2), 278–308. https://doi.org/10.1002/med.20107
9. Bonandi, E., Christodoulou, M. S., Fumagalli, G., Perdicchia, D., Rastelli, G., & Passarella, D. (2017). The 1,2,3-triazole ring as a bioisostere in medicinal chemistry. *Drug Discovery Today, 22*(10), 1572–1581. https://doi.org/10.1016/j.drudis.2017.05.014
10. Fosgerau, K., & Hoffmann, T. (2015). Peptide therapeutics: Current status and future directions. *Drug Discovery Today, 20*(1), 122–128. https://doi.org/10.1016/j.drudis.2014.10.003
11. Craik, D. J., Fairlie, D. P., Liras, S., & Price, D. (2013). The future of peptide-based drugs. *Chemical Biology & Drug Design, 81*(1), 136–147. https://doi.org/10.1111/cbdd.12055

12. El-Sagheer, A. H., & Brown, T. (2010). Click chemistry with DNA. *Chemical Society Reviews, 39*(4), 1388–1405. https://doi.org/10.1039/B901971P
13. Sletten, E. M., & Bertozzi, C. R. (2009). Bioorthogonal chemistry: Fishing for selectivity in a sea of functionality. *Angewandte Chemie International Edition, 48*(38), 6974–6998. https://doi.org/10.1002/anie.200900942
14. Prescher, J. A., & Bertozzi, C. R. (2005). Chemistry in living systems. *Nature Chemical Biology, 1*(1), 13–21. https://doi.org/10.1038/nchembio0605-13
15. White, C. J., & Yudin, A. K. (2011). Contemporary strategies for peptide macrocyclization. *Nature Chemistry, 3*(7), 509–524. https://doi.org/10.1038/nchem.1062
16. Lau, Y. H., de Andrade, P., Wu, Y., & Spring, D. R. (2015). Peptide stapling techniques based on different macrocyclization chemistries. *Chemical Society Reviews, 44*(1), 91–102. https://doi.org/10.1039/C4CS00246F
17. Bird, G. H., Mazzola, E., Opoku-Nsiah, K., Lammert, M. A., Godes, M., Neuberg, D. S., & Walensky, L. D. (2016). Biophysical determinants for cellular uptake of hydrocarbon-stapled peptide helices. *Nature Chemical Biology, 12*(10), 845–852. https://doi.org/10.1038/nchembio.2153
18. Hoyle, C. E., & Bowman, C. N. (2010). Thiol–ene click chemistry. *Angewandte Chemie International Edition, 49*(9), 1540–1573. https://doi.org/10.1002/anie.200903924
19. Bertozzi, C. R. (2011). A decade of bioorthogonal chemistry. *Accounts of Chemical Research, 44*(9), 651–653. https://doi.org/10.1021/ar200193f
20. Debets, M. F., van Berkel, S. S., Dommerholt, J., Dirks, A. T. J., Rutjes, F. P. J. T., & van Delft, F. L. (2011). Bioconjugation with strained alkenes and alkynes. *Accounts of Chemical Research, 44*(9), 805–815. https://doi.org/10.1021/ar200059z
21. Nwe, K., Brechbiel, M. W. (2009). Growing applications of "click chemistry" for bioconjugation in contemporary biomedical research. *Cancer Biotherapy & Radiopharmaceuticals, 24*(3), 289–302. https://doi.org/10.1089/cbr.2008.0616
22. Lallana, E., Riguera, R., & Fernández-Megía, E. (2011). Reliable and efficient procedures for the conjugation of biomolecules through Huisgen azide–alkyne cycloadditions. *Angewandte Chemie International Edition, 50*(38), 8794–8804. https://doi.org/10.1002/anie.201101019
23. Liang, L., & Astruc, D. (2011). The copper(I)-catalyzed alkyne–azide cycloaddition (CuAAC) "click" reaction and its applications. *Coordination Chemistry Reviews, 255*(23–24), 2933–2945. https://doi.org/10.1016/j.ccr.2011.06.028
24. Hong, V., Presolski, S. I., Ma, C., & Finn, M. G. (2009). Analysis and optimization of copper-catalyzed azide–alkyne cycloaddition for bioconjugation. *Angewandte Chemie International Edition, 48*(52), 9879–9883. https://doi.org/10.1002/anie.200905087
25. Agard, N. J., Prescher, J. A., & Bertozzi, C. R. (2004). A strain-promoted [3+2] azide–alkyne cycloaddition for covalent modification of biomolecules in living systems. *Journal of the American Chemical Society, 126*(46), 15046–15047. https://doi.org/10.1021/ja044996f
26. Jewett, J. C., & Bertozzi, C. R. (2010). Cu-free click cycloaddition reactions in chemical biology. *Chemical Society Reviews, 39*(4), 1272–1279. https://doi.org/10.1039/B901970G
27. Debets, M. F., van der Doelen, C. W. J., Rutjes, F. P. J. T., & van Delft, F. L. (2010). Azide: A unique dipole for metal-free bioorthogonal ligations. *ChemBioChem, 11*(9), 1168–1184. https://doi.org/10.1002/cbic.201000116

28. Hoyle, C. E., Lowe, A. B., & Bowman, C. N. (2010). Thiol-click chemistry: A multifaceted toolbox for small molecule and polymer synthesis. *Chemical Society Reviews, 39*(4), 1355–1387. https://doi.org/10.1039/B901979K
29. Lowe, A. B. (2014). Thiol–ene "click" reactions and recent applications in polymer and materials synthesis. *Polymer Chemistry, 5*(17), 4820–4870. https://doi.org/10.1039/C4PY00339J
30. Chudasama, V., Maruani, A., & Caddick, S. (2016). Recent advances in the construction of antibody–drug conjugates. *Nature Chemistry, 8*(2), 114–119. https://doi.org/10.1038/nchem.2425
31. Lowe, A. B. (2010). Thiol–yne chemistry: A powerful route to functional materials. *Polymer Chemistry, 1*(1), 17–36. https://doi.org/10.1039/B9PY00216B
32. Blackman, M. L., Royzen, M., & Fox, J. M. (2008). Tetrazine ligation: Fast bioconjugation based on inverse-electron-demand Diels–Alder reactivity. *Journal of the American Chemical Society, 130*(41), 13518–13519. https://doi.org/10.1021/ja8053805
33. Dong, J., Krasnova, L., Finn, M. G., & Sharpless, K. B. (2014). Sulfur(VI) fluoride exchange (SuFEx): Another good reaction for click chemistry. *Angewandte Chemie International Edition, 53*(36), 9430–9448. https://doi.org/10.1002/anie.201309399
34. Kolb, H. C., & Sharpless, K. B. (2003). The growing impact of click chemistry on drug discovery. *Drug Discovery Today, 8*(24), 1128–1137. https://doi.org/10.1016/S1359-6446(03)02933-7
35. Singh, M. S., Chowdhury, S., & Koley, S. (2016). Advances of azide–alkyne cycloaddition-click chemistry over the recent decade. *Tetrahedron, 72*(35), 5257–5283. https://doi.org/10.1016/j.tet.2016.05.044
36. Thirumurugan, P., Matosiuk, D., & Jozwiak, K. (2013). Click chemistry for drug development and diverse chemical–biology applications. *Chemical Reviews, 113*(7), 4905–4979. https://doi.org/10.1021/cr200409f
37. Bozorov, K., Zhao, J., & Aisa, H. A. (2019). 1,2,3-Triazole-containing hybrids as leads in medicinal chemistry. *Bioorganic & Medicinal Chemistry, 27*(16), 3511–3531. https://doi.org/10.1016/j.bmc.2019.06.005
38. Zeng, Q., Li, Z., & Dong, G. (2020). Click chemistry in antimicrobial drug discovery and development. *European Journal of Medicinal Chemistry, 202*, 112512. https://doi.org/10.1016/j.ejmech.2020.112512
39. Talele, T. T. (2018). Opportunities for tapping into three-dimensional chemical space through a quaternary carbon. *Journal of Medicinal Chemistry, 61*(6), 2166–2210. https://doi.org/10.1021/acs.jmedchem.7b00788
40. Shi, J., Kantoff, P. W., Wooster, R., & Farokhzad, O. C. (2017). Cancer nanomedicine: Progress, challenges and opportunities. *Nature Reviews Cancer, 17*(1), 20–37. https://doi.org/10.1038/nrc.2016.108
41. Conde, J., Doria, G., & Baptista, P. (2012). Noble metal nanoparticles applications in cancer. *Journal of Drug Delivery, 2012*, 751075. https://doi.org/10.1155/2012/751075
42. Vamathevan, J., Clark, D., Czodrowski, P., Dunham, I., Ferran, E., Lee, G., Li, B., Madabhushi, A., Shah, P., Spitzer, M., & Zhao, S. (2019). Applications of machine learning in drug discovery and development. *Nature Reviews Drug Discovery, 18*(6), 463–477. https://doi.org/10.1038/s41573-019-0024-5