Review Article

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The Use of Click Chemisty in Drug Development Applications

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ABSTRACT

Click chemistry is defined as powerful, reliable, and selective reactions that assemble small building blocks via heteroatom linkages. Click reactions are a modular strategy that is a practical and convenient approach to chemical transformations. This strategy has been a promising approach for challenging processes such as mild reaction conditions, easy purification of intermediates, and imaging of drug targets. These advantages have added a new dimension to drug discovery and development in pharmaceutical sciences. Click reactions are interesting reactions that are currently studied in drug development studies, bioconjugation, polymer and material chemistry, and drug delivery systems. In this review, important highlights of the copper-catalyzed Huisgen 1,3-dipolar cycloaddition reaction, which is recognized to be the gold standard of click chemistry because of its biocompatibility and reliability, and the use of the selectively formed 1,2,3-triazole in drug development studies are discussed.

Keywords: copper catalysis, triazole, drug design, 1,3-dipolar cycloaddition, bioconjugation

1. Introduction

Pharmaceutical chemistry deals with the synthesis, design, and structure elucidation of biologically active drug candidates. Researchers may experience all kinds of difficulties in the synthesis and purification steps. For this purpose, it is aimed to design reactions that are easy to synthesize and easy to purify.

Click chemistry (CC) is a modular strategy that is a practical and useful approach for chemical transformations. Click chemistry is concerned with the formation of substances by combining small, selective, and modular building blocks with heteroatom connections (C-X-C) (Figure 1).

The term CC was first independently introduced by Sharpless and Meldal [1, 2]. In 2001, Sharpless listed a set of criteria in its definition of CC. This transformation according to this definition*: "The reaction must be modular, give very high yields, wide in scope, generate only inoffensive by-products that can be removed by non-chromatographic methods, and be stereospecific (but not necessarily enantioselective). The required process characteristics include easily available starting materials and reagents, the use of no solvents or a solvent that is benign (mainly water) or easily removed, simple reaction conditions, and simple product isolation."* To fulfill these criteria, it is characteristic that the thermodynamic energy of the reaction is greater than 20 kcal/mol. The reaction must be fast and selective due to the formation of a single type of product [3].

2. Material and Methods

2.1. The Classifications of Click Chemistry Reactions

Click reactions can be broadly categorized into five different types (i) cycloaddition reactions (Huisgen 1,3-dipolar cycloaddition, Diels–Alder reaction), (ii) nucleophilic ring-opening reactions of strained heterocyclic electrophiles (aziridines, aziridinium ions and epoxides), (iii) non-aldol carbonyl chemistry (oximes, ureas and hydrazones) and (iv) additions to carbon–carbon multiple bonds (oxidative reactions such as aziridation, sulfenyl halide additions, epoxidation, hydroxylation, nitrosyl, thiol–ene and Michael additions) (v) sulfur (VI) fluoride exchange (SuFEx) reactions [4, 5].

Among the reactions regarded as click reactions Huisgen Azide−Alkyne 1,3-Dipolar Cycloaddition to give 1,2,3-triazoles is the most prominent example of a click reaction [6].

2.2. Cu(I)-Catalysed Huisgen 1,3-Dipolar Cycloaddition Click Reaction of Terminal Alkynes and Azide

In the 1,3-Dipolar Huisgen (HDC) reaction, both alkyne and azide are not reactive under physiological conditions. High temperatures are required for the reaction to take place. These two reactants react easily at high temperatures, but the reaction is an exothermic reaction and an activation energy of 25-26 kcal/mol is required. Therefore, this reaction carried out without a catalyst is slow and non-selective. As a result of the reaction, a mixture of 1,4 and 1,5 disubstituted 1,2,3-triazole products is formed [6, 7].

Cu(I) is the ideal catalyst for Cu(I)-catalyzed azide-alkyne reaction (CuAAC) reactions (7). The copper(I) catalyzed process is 10^7 times quicker than the uncatalyzed reaction, according to Sharpless and Meldal's separately published research. CuAAC gives 1,4-disubstituted 1,2,3- triazoles in high yields. The reaction can take place at 20-50 °C (8). Sharpless reported that Cu(I) obtained by the reduction of copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) with ascorbic acid is an effective catalyst for azide-alkyne conjugation reactions [9].

Sodium ascorbate is the most extensively used molecule for reducing Cu(II). The main advantages are cost savings, shorter reaction times, and increased tolerance to aqueous environments [10]. Hydrazine and tris(2-carboxyethyl)phosphine (TCEP) are also used as reductants in click reactions, but these agents may reduce Cu(II) to Cu(0) [8]. The second method is to add Cu(I) straight into the media. Cu(I) salts such as CuI, CuBr, and $CuOTf \cdot C_6H_6$ show strong

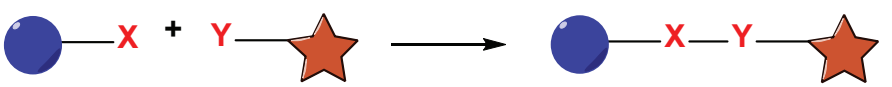

Figure 1. Schematic Representation of Click Reaction

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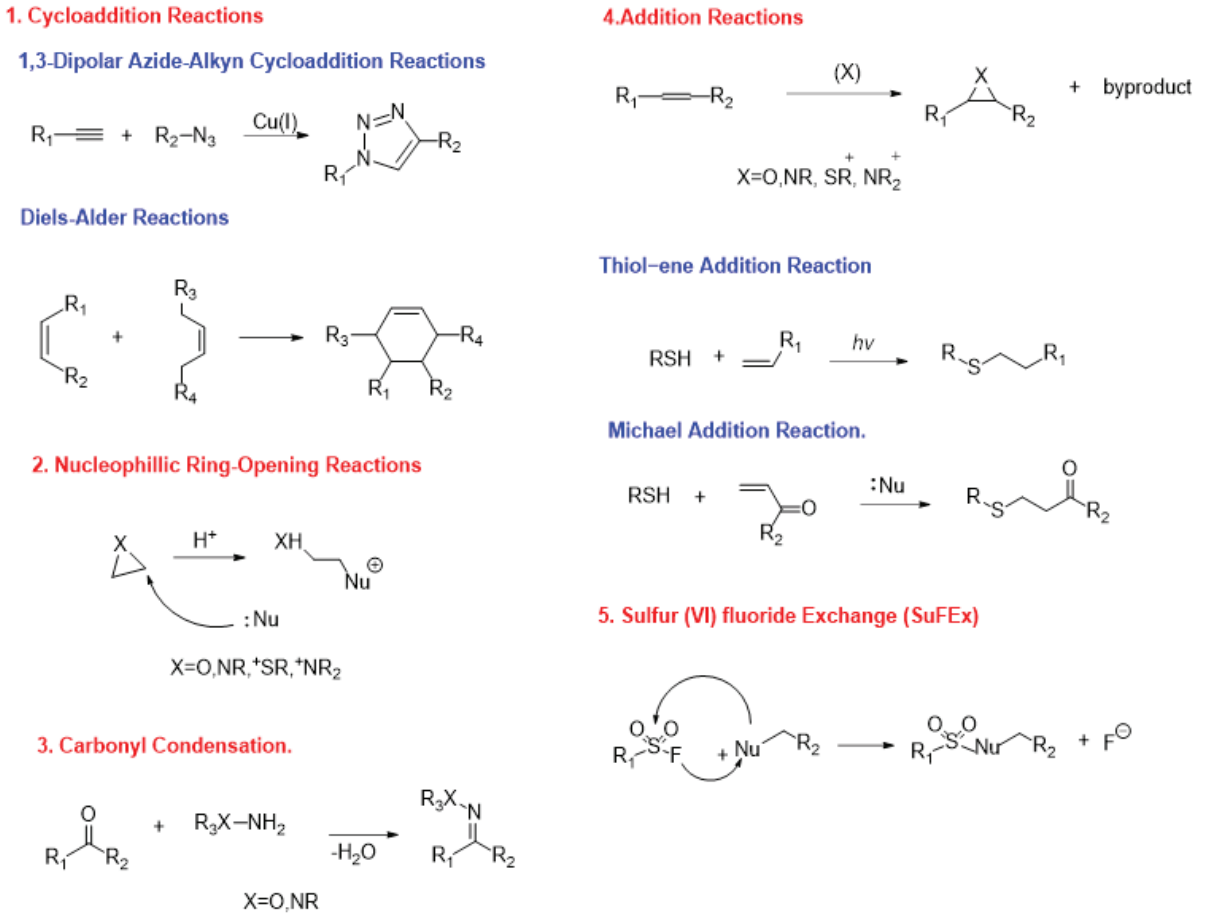

Figure 2. Types of click reactions

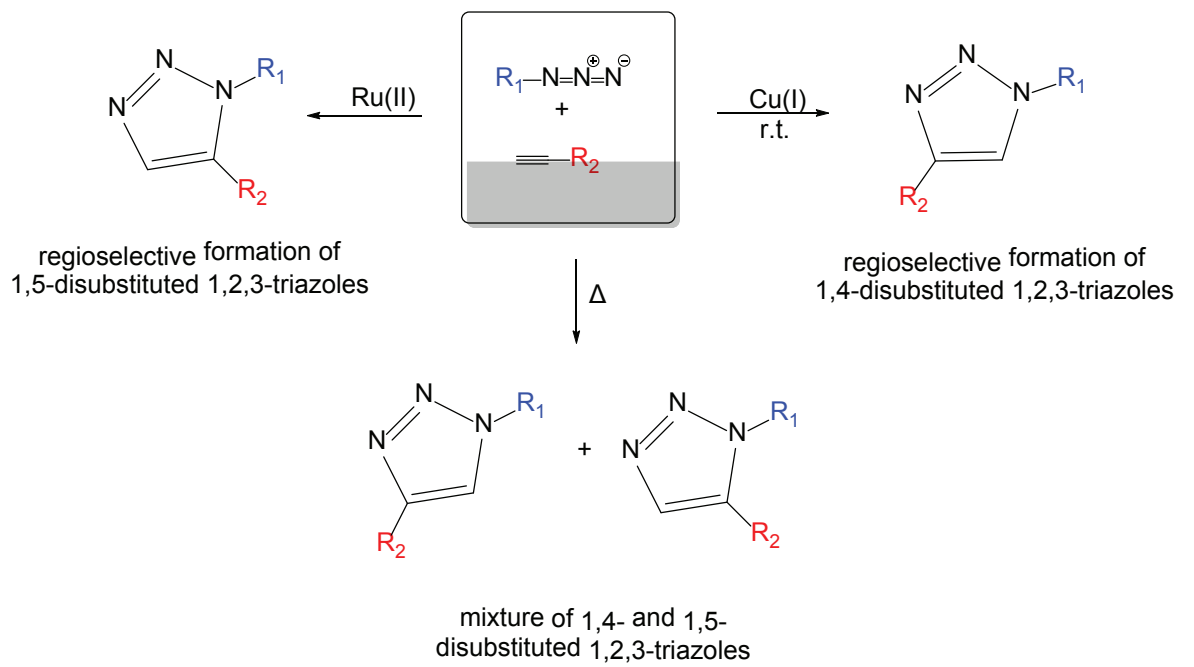

Figure 3. The1,3-dipolar cycloaddition between azides alkynes

catalyzing effects in solvents. When using Cu(I) salts in solvents, a tertiary amine base like 2,6-lutidine or diisopropylethylamine (DIPEA) is typically added [11]. This is primarily explained by the need for a base to deprotonate the Cu-alkyne π complex and produce copper acetylide [12]. Another efficient catalyst for cycloaddition processes is ruthenium ion. The [Cp*RuCl] Cp*RuCl(PPh_3)$_2$, Cp*RuCl(COD), and Cp*RuCl-(NBD) are the most frequently utilized catalysts complexes. It has been noted that internal alkynes are effectively linked with organic azides to create 1,4,5-trisubstituted 1,2,3-triazoles (Figure 4) in cycloaddition processes using ruthenium catalysts. At temperatures between room temperature and 80°C, reactions are carried out in THF dioxane or any other nonprotic solvent using a %1-2 mol catalyst. It has been observed that Ru-catalysed reactions can react with internal alkynes azides in good yields to obtain 1,4,5-trisubstituted triazoles [8].

In click reactions, new catalytic systems can also be made from other metals such as silver, nickel, iron, and platinum, as these provide simple experimental conditions and good yields of triazole-based products [4].

The fast supply, low cost, and chemical inertness of the solvents to the reactants and products generated in the reaction are critical considerations in click chemistry when choosing a solvent. Sharpless reported in 1999 that the best classes of click reactions proceed most rapidly and with the highest efficiency when only water is present in the medium, not water or water-solvent mixtures [11]. The most commonly used solvents in click chemistry are tetrahydrofuran, *t*-butanol, toluene, dichloromethane, acetonitrile, ethanol, dimethylformamide, and dimethyl sulphoxide. In addition, it is desired to use less toxic, recyclable, and environmentally friendly solvents by click chemistry criteria. In this context, green solvents such as water, glycerol, γ-valerolactone, lactic acid, and 2-methyl-THF are preferred by researchers [4].

CuAAC is a powerful, stable, and practical technique for creating substituted triazoles and building chemical libraries. Azide and alkyne compounds are essentially inactive and bio-orthogonal towards biological and aqueous media so the reaction offers a wide range of biological applications in pharmaceutical research [13].

3. The Use of 1,2,3-Triazole Group in Drug Development Studies

1,2,3-Triazoles are one of the most significant families of nitrogen-containing heterocycles and act as biocompatible binders as they can readily associate with biological targets through bonds such as hydrogen bonding, dipole-dipole interactions and van der Waals forces [14].

1,2,3-Triazoles are stable against hydrolysis, metabolic degradation, and redox conditions under acidic or basic conditions. These substances can form H-bonds and π-π stacking interactions. Despite being very stable, the aromatic ring of 1,2,3-triazoles becomes more reactive when attached to electronegative groups [15].

1,4-Disubtituted 1,2,3-triazole has multiple functions in bioactive molecules. Due to their bioisosteric effects, strong dipole values, and nature of the heteroatoms, among other properties, triazole units are a substantial moiety in medicinal chemistry. Numerous medical drugs consist of 1,2,3-triazole scaffold (Figure 5), and research in medicinal chemistry has focused on their analogs [16].

In the following subsections, we will discuss the applications of click chemistry in drug synthesis and development studies.

3.1. 1,2,3-triazoles as isosteres of the functional groups

Bioisosteric substitution is the replacement of functional groups of the molecule with groups with

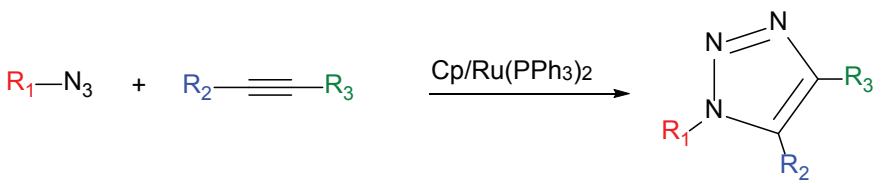

Figure 4. Formation of 1,4,5-trisubstituted-1,2,3-triazole in the existence of Cp*Ru catalysts

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similar biological features, used in drug design to promote the synthetic availability, potency, and drug-like qualities of a compound and to enter new chemical fields [17].

Among the most widespread amide bond isosteres are 1,2,3-triazoles. Indeed, their structural properties allow for an appropriate overlap with the amide-binding moiety despite certain variances in the total dipolar moment and distance between the substituents [16].

Phillips *et al.* synthesized 1,2,3-triazole hybrids by replacing the amide group of linezolid with a click reaction (Figure 6).

The obtained derivatives were assessed for their antibacterial activity against antibiotic-sensitive isolated gram-positive bacteria and 5-(4-methyl-1,2,3-triazolylmethyl oxazolidinone series demonstrated stronger in vitro activity than linezolid [18].

1,2,3-Triazoles are isosteres of heteroaromatic compounds and double bonds. Genazzani et al. synthesized hybrids bearing 1,2,3-triazole structure instead of the double bond in resveratrol compound by CuAAC click reaction. According to the initial data, it was thought that derivatives containing some activities of the primary molecule Resveratrol could be synthesized with this approach [19].

3.2. 1,2,3-Triazoles as linkers

Under physiological circumstances, this 1,2,3-triazole moiety is highly stable. The 1,2,3-triazole com-

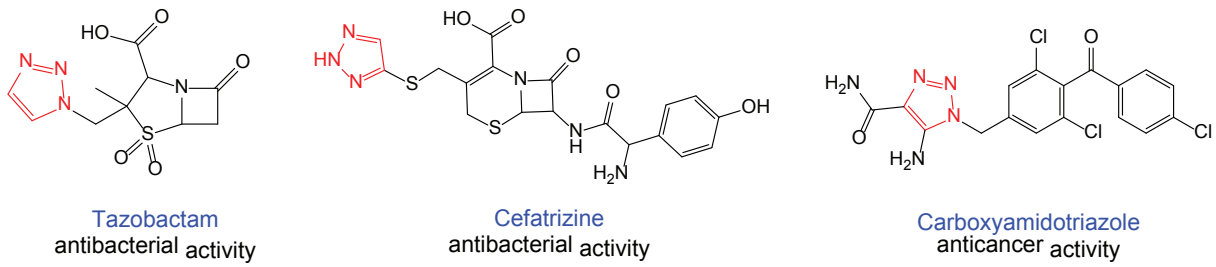

Figure 5. Some drugs containing the 1,2,3-triazole structure

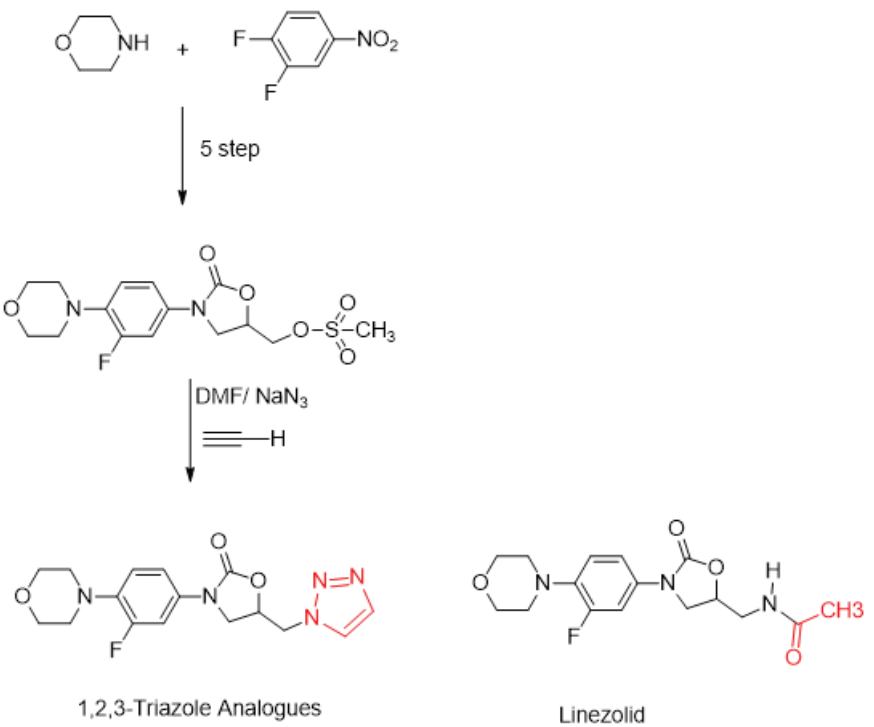

Figure 6. Structures of Linezolid and Triazole Analogues

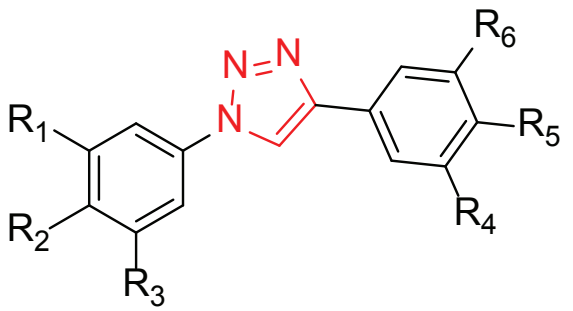

Figure 7. Structure of triazole-modified resveratrol hybrid

ponent is a suitable binder because of its stability [20].

By Roy *et al.*, a novel series of chimeric molecules were synthesized. These were made by combining polyphenols like epicatechin and catechin with antibacterial monocyclic-lactams using click chemistry and a 1,2,3-triazole linker (Figure 8). The synthesized derivatives exhibited different levels of antibacterial activity against *Escherichia coli*, some showed weak activity while others showed good activity. It also exhibited moderate activity to inhibit RNAase. Thus, previously ineffective monobactams were successfully sensitized against *E. coli* after conjugation with epicatechin /catechin [21].

3.3. Bioconjugation

The theory of bioconjugation, which involves the formation of covalent bonds between synthetic labels and biomolecular frameworks, spans a wide field of science at the intersection between chemistry and molecular biology [22]. It incorporates biocompatible chemical processes that quickly, simply, and selectively insert substrates into biomolecules. The development of click chemistry has opened new avenues for bioconjugation applications which involve the covalent attachment of two biomolecules, usually proteins or peptides, with a functional substance such as a drug, polymer, or fluorophore [23].

Copper-catalyzed azide-alkyne cycloaddition (CuAAC) is the archetypal click reaction and a breakthrough technique in bioconjugation. This is because 1,2,3-triazoles are ideal binders. They are highly soluble in water and make *in vivo* application much easier. The 1,2,3-triazole ring, the selected by-product of Cu-catalyzed click chemistry, possesses significant physicochemical characteristics that increase the stability and effectiveness of the bioconjugate.

The application of CuAAC to generate fluorogenic compounds has been extensively studied (Figure 9) as it would be a powerful new tool for monitoring bio-molecules in the cell [8].

The triazole ring can be linked to the conjugate by hydrogen bonding and dipole interactions, modulating its bioactivity. In addition, the triazole ring is metabolically stable and resistant to enzymatic and hydrolysis degradation, thus improving the pharmacokinetic properties of bioconjugates [24].

First, in 2003, Sharpless and Finn applied the click reaction to modify purified Cowpea mosaic virus

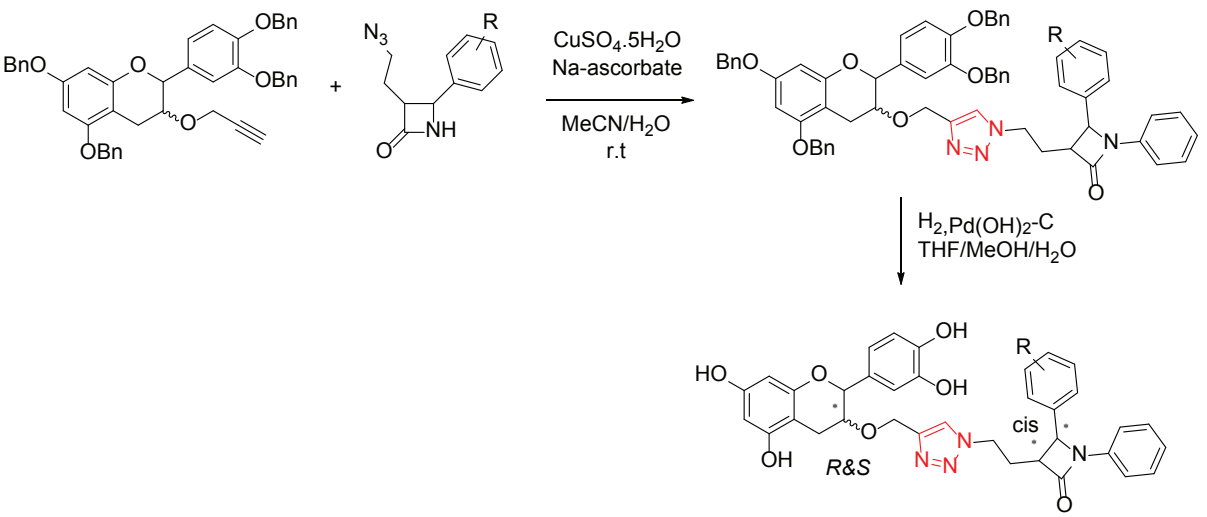

Figure 8. Synthesis of triazole-catechin and epicatechin hybrids

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(CPMV) vesicles with a fluorescent probe [25]. Terminal azides or alkynes were modified to the capsid of the virus, then a fluorescent probe functionalized with the azide or alkyne was successfully attached to the capsid surface via triazole formation [26].

The click reactions also have their difficulties, especially in the areas of pharmaceutical sciences. The most noticeable challenge is that it demands a Cu(I) catalyst. Although copper is needed to fulfill the function of the human body, excessive intake can cause to toxicity. Therefore, the copper must be fully removed and excised so the click reaction can be applied *in vivo* [27]. Although the advantages of the CuAAC reaction have rapidly stimulated interest in using this approach in biological systems, the use of CuAAC chemistry for such applications has been restricted by the discovery that copper ions are hazardous to cells and living organisms. Furthermore, as the majority of these non-Cu(I) transition-metal catalyzed cycloadditions must still take place in organic solvents, their biological applications are severely limited [28].

The copper-free click reaction, recognized as strain-promoted azide-alkyne cycloaddition (SPAAC), was introduced as an alternative to address the limitations of CuAAC in biological environments. Despite producing a variety of potentially isomeric products (Figure 10), this azide-cyclooctene (3+2) cycloaddition was shown to function rather effectively in the absence of Cu(I) [29].

In 2004, Carolyn R. Bertozzi et al. exploited the high reactivity of cycloalkynes, especially highly strained cyclooctynes, as a remedy for the realization of click chemistry in biological systems.

The absence of Cu(I) catalyst in the environment improves biocompatibility, but the reaction rate is considerably slower than CuAAC [24]. Bio-orthogonal click reactions have been employed in the field of molecular imaging as a noteworthy application for labeling and visualizing biomolecules in living systems (Figure 11). It was developed as a faster alternative to Staudinger ligation and the first generations reacted more than sixty times faster. The bio-orthogonality of the reaction allowed the Cu(I)-free click reaction to be implemented in mice, cultured cells, and live zebrafish [29].

These investigations have resulted in the recognition and development of a wide variety of reactions applicable to bio-orthogonal chemistry. As a result, bio-orthogonal click chemistry has been applied in the design of enzyme inhibitors and receptors, various macromolecular materials (gels, polymers, etc.), ligands, sensing elements, photo stabilizers, diagnostic and herbicides, corrosion and corrosion inhibitors, pharmaceuticals, biomacromolecule conjugates, tissue regeneration matrices, herbicides and in the mapping of complex biological processes. [30].

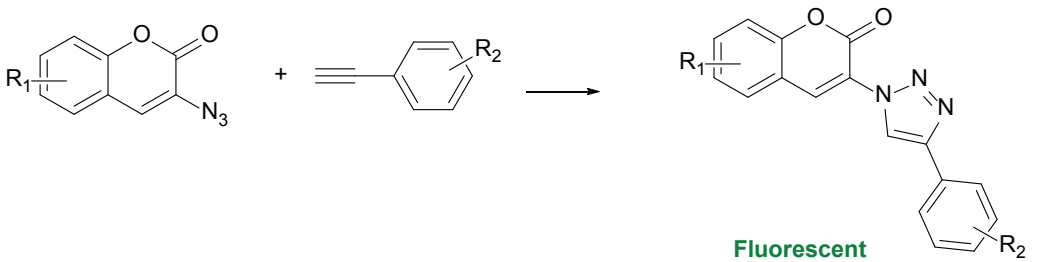

Figure 9. Fluorogenic probe synthesized via CuAAC.

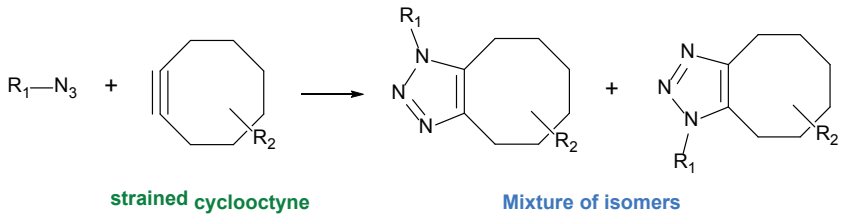

Figure 10. Strain-promoted azide-alkyne cycloaddition reactions

4. Conclusion

Click reactions have a wide range of advantages such as easy to perform and purify, high reaction yield, regioselective, tolerant to standard biological conditions and most functional groups, and mild environmental conditions. The Cu(I)-catalyzed azide-alkyne cycloaddition reaction is now the most extensively studied of these efficient transformation processes. Unlike the 1,3-dipolar Huisgen reaction, CuAAC, which can take place at high temperatures, Cu salts were used as catalysts to increase the reaction rate and yield. Furthermore, unlike the 1,3-Dipolar Huisgen reaction, the copper-catalyzed reaction is a selective reaction with the formation of only 1,4-disubstituted 1,2,3-triazole molecule. The 1,2,3-triazole molecule formed as a result of the CuAAC reaction is a structure frequently seen in drug molecules due to its advantages such as being compatible with many functional groups, stable under physiological conditions, and aromatic. Due to all these properties, the use of the amide group of the 1,2,3-triazole molecule as a bioisoster of aromatic and double bonds and as a good binder has been encountered in synthesis studies. This reaction is applied in drug development studies, bioconjugation, polymer and material chemistry, and drug carrier systems. However, the CuAAC reaction is not perfect in all aspects.

Although many advantages, there are limitations to consider, as well. The most major limitation is the toxicity of copper to the living cells. For this reason, scientists have designed an alternative method, a strain-assisted azide-alkyne cycloaddition (SPAAC) reaction without a copper catalyst. This synthesis pathway is the basis of bio-orthogonal chemistry. Especially after the Nobel Prize in chemistry in 2022, bio-orthogonal click chemistry is of rising interest in tissue engineering studies, imaging methods for the diagnosis and treatment of diseases, and the development of pharmaceuticals and biomedical tools.

In conclusion, click chemistry reactions are anticipated to increasingly gain favor and make valuable contributions to fields such as pharmaceutical sciences and various other areas of research.

Conflict of Interest

The author/editor has no conflicts of interest, financial or otherwise, to declare.

Statement of Contribution of Researchers

Concept –B.D., G.K., M.A.; Design – B.D., G.K.; Supervision – G.K., M.A.; Data collection – B.D., G.K.; Data interpretation – B.D., G.K., M.A.; Literature Search – B.D., G.K.; Writing – B.D., G.K., M.A.; Critical Reviews — G.K., M.A.

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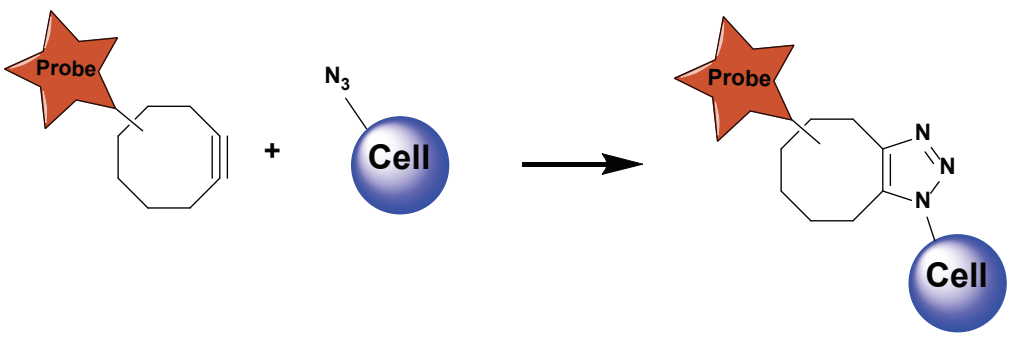

Figure 11. SPAAC application in living systems

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