Review Article

Role of Click Chemistry in development of Biomedicines

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Abstract

The important purpose of medicinal chemistry is to incorporate compounds or study of compounds during the process of drug discovery or lead optimization. The recognition of these rapid synthetic methodologies should permitting the medicinal chemist to arrange a large amount of biologically active compounds in a very short period of time which facilitate the process of drug discovery and lead optimization. Click chemistry involved a set of reactions that are fast, simple, regiospecific, easy to purify, versatile, and give high product yields. Most of the reactions that fulfill the criteria are discussed, the Huisgen 1, 3-dipolar cyclo addition of azides and terminal alkynes has appear as a front-runner. It has number of applications in various fields of research areas, including pharmaceutical sciences, materials sciences, polymer chemistry. In this article, important aspects of the Click reactions are been reviewed, along with some of its applications in biomedicine. Bio conjugation, nanoparticle Polymer and pharmaceutical-related polymer chemistry is also been covered. Types, advantages and limitations of Click reaction are also been enclosed in this article.

Keywords: Click Chemistry, biomedicines, medicinal chemistry, bio-conjugation

Introduction

The importantpurposeof medicinal chemistry is to incorporate compounds or study of compounds duringthe process of drug discovery or lead optimization (Wenderet al.,1997).The recognition ofthese rapid synthetic methodologies should permitting the medicinal chemist to arrange a large amount of biologically active compounds in a very short period of time whichincrease the process of drug discoveryandlead optimization (Wenderet al.,1997). Even now, alongside being rapid, the key features of the ideal synthesis are efficiency, energetically, flexibility and selectivity. In 2001, the term "**Click Chemistry**"was initially conceive by Sharpless and team mates(kolb et al.,2001) to announce the reactionsexplain by a set of stringent criteria."The click reaction must be extensible,broard scope, give very high yeild, give only harmlessside-products that can be removed by non-chromatographic methods of separationand be stereospecific (but not enantioselective). The required process characteristicsinclude simple reaction conditions, readily accessibleprecursor and reagents, the use of non-solvents or a solvent that is benign i.e wateror easily removed, and simple product extractions." the most well-known reaction that has been modified to fulfil these standards is the1,3-dipolarcycloaddition, also known as Huisgen cycloaddition,joining an azide and a terminal alkyneaffording the 1,2,3-triazolea moiety to form 1,2,3 triazole derivatives. But Copper-catalyzed azide-alkyne cycloaddition **(CuAAC)** is more advantageous then 1,3-dipolar cycloaddition or Huisgen cycloaddition reaction(Kolb et al.,2004,Kolb and Sharplees 2003). As it known,1,2,3-triazole is a confidential platform in medicinal chemistry and compounds contains this structure have a broad ranges of biological activities, as demonstrated by the sanctioned drugs or approved drugs.(as mentioned in Figure.1) 1,4-Substituted-1,2,3-triazole has many roles in biologically active molecules: as a basic pharmacophore element, it engage in the synthesis of hydrogen bonding or hydrophobic; asa molecular

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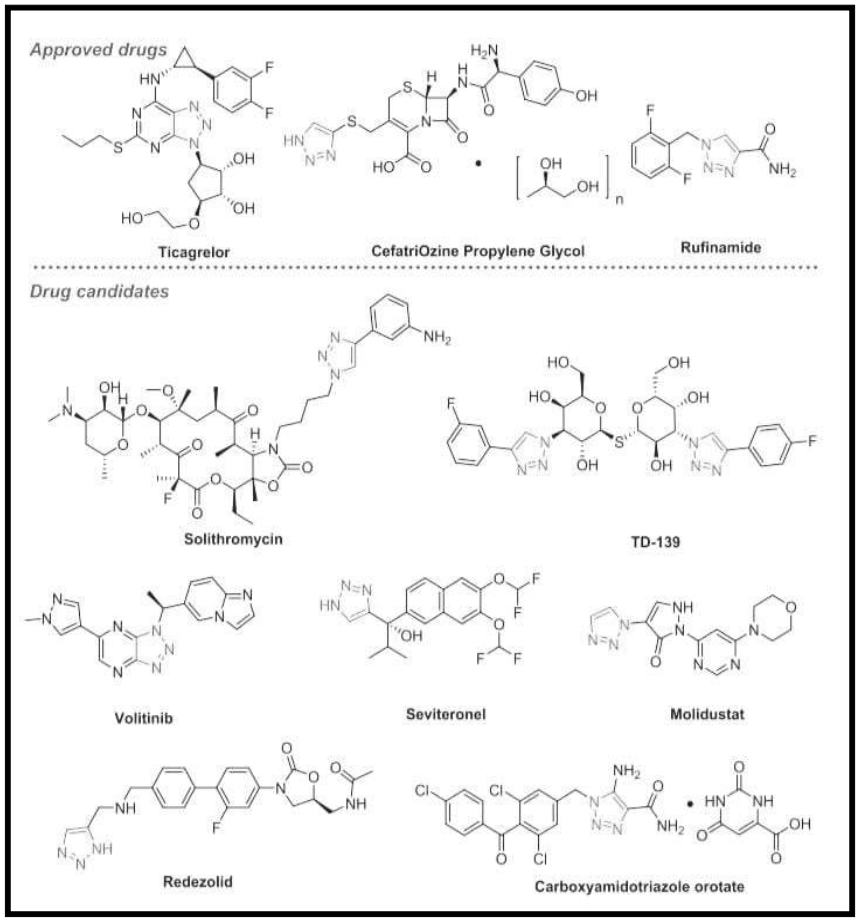

Figure 1. Approved drugs or drug candidates carrying 1,2,3-triazole structure

scaffold, it controls other pharmacophore elements to keep an active configuration; as a connecting group, it joins to conjugated molecules (Wang et al.,2016,Fang et al.,2015).

This reaction was discovered at the starting of the 20thcentury, but the potential of this reaction and its mechanism were announced in the 1960s byHuisgen et al(Huisgen et al.,1967).Click chemistry explains chemistry that customized to produced substances rapidly and reliably by joining small units together as nature does. The click Chemistry is designated as a fast,extensible, process-driven approach to non reversible connections of the substrates being involved in clickreactions. Click chemistry uses only themost authentic reactions to construct complex molecules from alkenes, electrophiles, and heteroatom linkers. The criteria for being classified as click chemisty accommodate yield close to 100% as well as a favoured and quicklytakes placenon reversible.As is well known, 1,2,3-triazole is a privileged scaffold inmedicinal chemistry and compounds containing this structurehave a broad spectrum of biological activities, as exemplified bythe approved drugs and Drug Candidates(Jingli et al.,2012)(as shown in Figure. 1).

The physicochemical properties of 1,2,3-triazoles

1,2,3-Triazoles are p-electron-deficient and shows both basicand acidic properties. Due to their poor basicity, the 1,2,3-triazole ring is not protonated at physiological pH. In addition,they possess a strong dipole moment (5.2 -- 5.6 Debye) (Purcell and Singer,1967),an aromatic character and a good hydrogen-bond-acceptingability. Furthermore, they are very stable to both metabolic (Chemama et al., 2009)and chemical degradations, being rather inert to severehydrolytic, oxidizing and reducing conditions, even at hightemperatures.(Jingli et al.,2012)

Classification of Click Reactions

Click chemistry contains a group of powerful linking reactions thatare simple to carried out, have high yields, require no or minimum purification, and are flexiblein joining multiple structures without any pre-requirements of protection. The four major class of click reactions which are mentioned below.(Figure.2)

Cycloadditions -primarily it is 1,3-dipolar cycloadditions, but also covershetero-Diels-Alder cycloadditions reactions (Kolb and Sharplees, 2003).

Nucleophilic ring-openings - In these, openings of strained heterocyclicelectrophiles, such as epoxides,aziridinium ions,aziridines,cyclic sulfates,episulfonium ions, etc occurs (Kolb and Sharplees,2003).

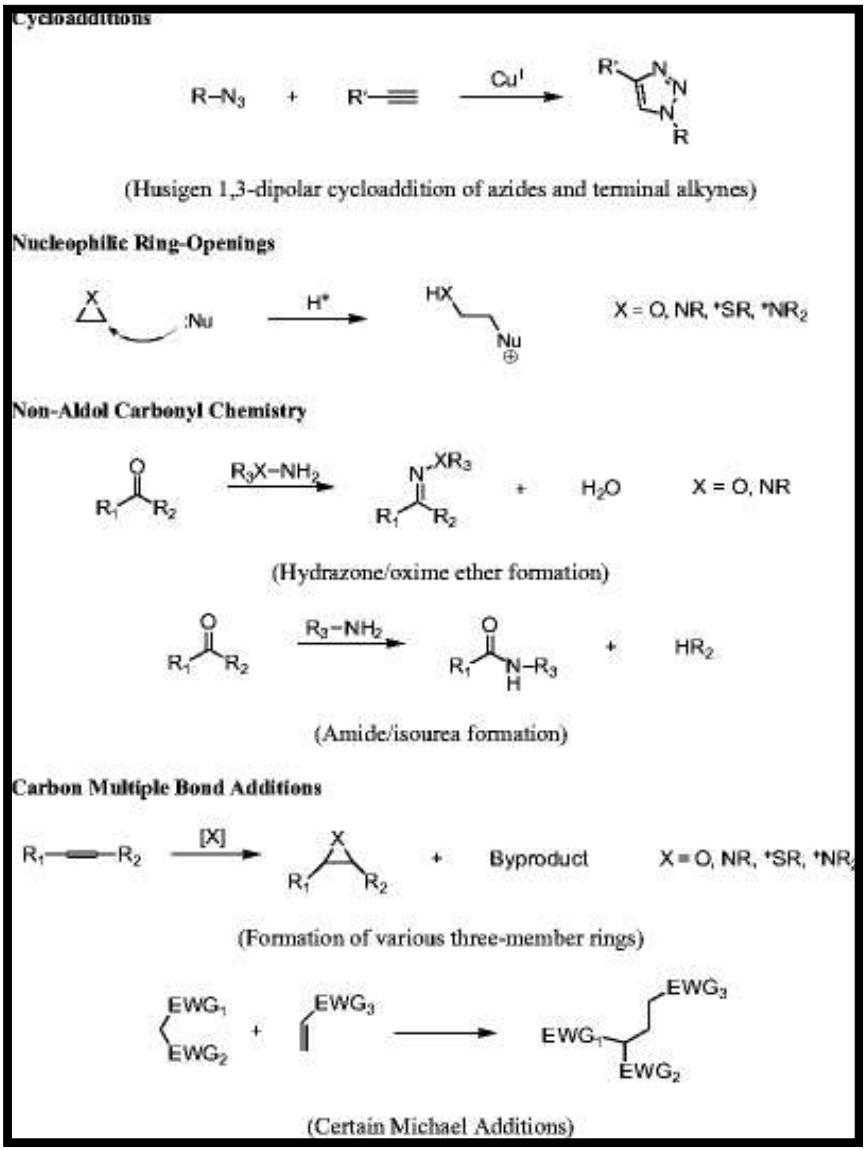

Figure 2. Major classifications of click chemistry reactions, with corresponding example.[Nu = nucleophile; EWG = electron withdrowing group.]

Carbonyl chemistry of the non-aldol compound- examples are formations of ureas,hydrazones, amides,thioureas, aromatic heterocycles,oxime ether,etc (Kolb and Sharplees,2001). Carbonyl reactions of the aldol have low thermodynamic driving forces, therefore they have longer reaction period and give bi products, and hence they can't beconsidered as click reactions (Kolb and Sharplees, 2001).

Additions to carbon-carbon multiple bonds - examples are, sulfenyl halide additions,aziridinations, dihydroxylations, epoxidations, nitrosyl halide additions and some Michael addition(Kolb and Sharpless,2003 , Kolb, Finn and Sharpless,2001). The most important class is cycloadditions, particularly the Copper-catalyzed Huisgen1,3-dipolar cycloaddition of terminal alkynes and azides to form 1,2,3-triazoles(Huisgen,1963), are mostly used. Based on the literature search mentioned earlier, almost allhas been found applicable in variousresearch areas.The pharmaceutical applications of this click reaction will be discussed.

Copper-catalyzed azide-alkyne cycloaddition

The chief example of reactions involved in click chemistry is an alkyne–azide reaction occurs in between an terminal alkyne and aorganic azides in the catalytic presence of copper [Cu(I)] to producethe 1,4disubstituted 1,2,3 triazole (Figure.3), which is in contrast to thenon-catalyzed reaction, which carried out at high temperatures range toform a equilibrium mixture of 1,4- and 1,5-triazole regioisomers.The reaction was reported simultaneously by the independent groups of Fokin and Sharpless(Rostovtsev et al.,2002)and Morten Meldal (Tomoe et al.,2002) (As shown in Figure 3a).Click reaction has been so successfulbecause of the fact that it produces quantitative yields, non-sensitive, allows orthogonal ligation and even polymerization(Meldal and

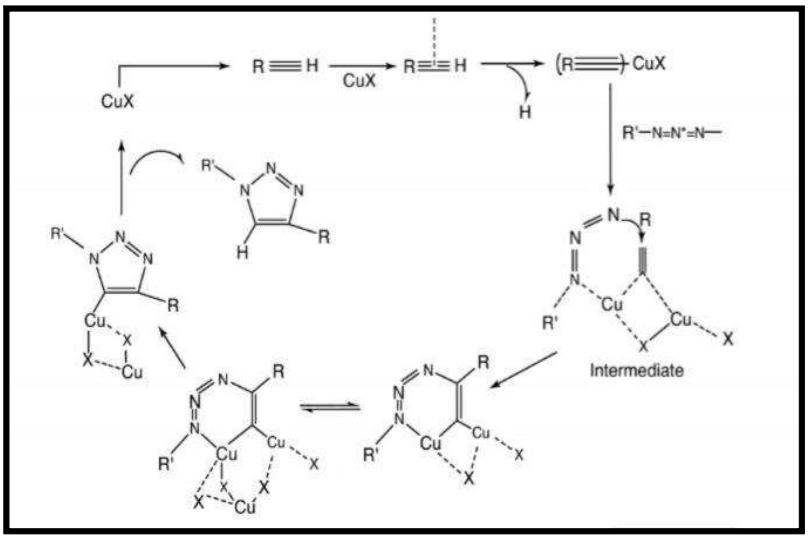

Figure 3. Mechanism of copper (Cu) mediated azide- alkyne click reaction.

Tomoe,2008). The forms triazole ring is chemically insensitive to oxidation, reduction and hydrolysis.The reaction involved copper species, the reaction is non-sensitive to most of the reaction conditions and can be carry out in aqueous or non aqueous and also in solid phase chemistry. If the reactants are not fully dissolved in medium, which are existsin a glassy state or aggregate(Baut et al.,2007, Hassane et al.,2006) but the reaction occurs with a good yield. The most importantcharacter that determines the yield is presence of Copper(Mendal and Tomoe,2008). The amount of the copper catalyst has to be maintained at a higher level during the reaction

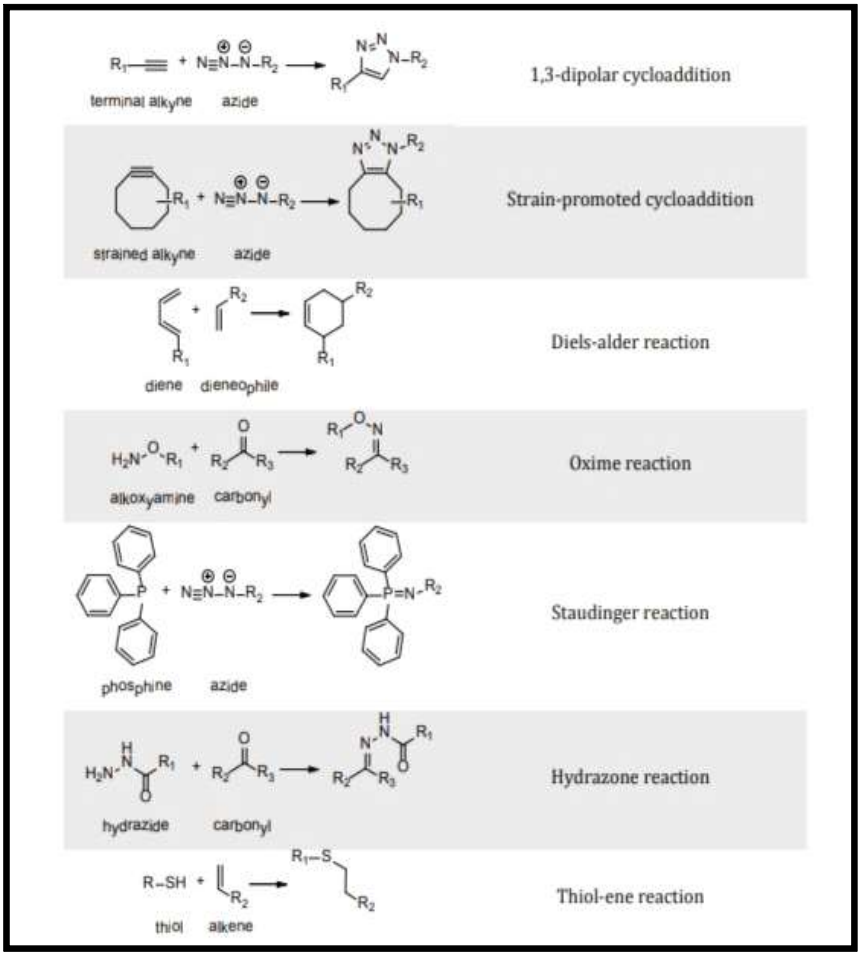

Figure 3a. List of chemical reaction which can be classified as click reaction

procedure. This is the reason why the preferred method for CuAAC is the use of a Cu(II) together with a reducing agent to provide Cu(I) in situ, for example with $CuSO_4$ and sodium ascorbate, two reactants which are inexpensive and Commonly available. In the catalytic presence of a reducing agent, the reaction is less susceptible to Oxidation so it is carried out in open air. Other sources of Cu(I) include copper halides such as Cuper bromide,Copper Iodide and Copper Chloride. But the success of the reaction when using these salts is depends on the introduction of an amine base such as N,N-Diisopropylethylamine or Triethylamine. The Cu(I) type generated with a Cu(II) source and a reducing agent rapidly forms the copper-acetylide needs to proceed the reaction. With copper halides, this complex is only formed after introduction of base. A disadvantage of using a direct source of Cu(I) is the require to work in a oxygen free environment. Even solid sources of Cu(0) including wire, powder or nanoparticles can be used to produce Cu(I) in aqueous conditions(Molten et al.,2006, Orgueira et al.,2005). Although, the active type is formed with a longer reaction times are required as well as higher amounts of copper. Ligands such as Tris(benzyltriazolylmethyl)amine can be work to increase the rate of reaction and also to stop oxygen from oxidizing Cu(I)(Chan,Sharplees,Fokin,2004). Also solid phase of copper on charcoal exist which facilitate removal of catalyst by simple filtration(Lipshutz and Taft,2006). Now, the 1,3-dipolar cycloaddition is themost used type, represented by the reaction between terminal acetylene and azide, which is called "the cream of the crop". The reaction was proposed by Mechael in 1893and studied in detail by Rolf Huisgen from 1960s to 1980s(Michael and Parkt,1893). After that, Medal et al(Tomoe,Christensen and Mendal,2002)and Sharpless et al(Rostovtsev et al.,2002) respectively exposed that this types of reactions could produce 1,4-triazole Particularly the catalytic presence ofCu^{+}. The production was as highas 91% and the reaction time could be minimise from 18 to 8 hrs.

Nucleophilic ring-opening reaction

Nucleophilic ring-opening reaction occurs simply because the reaction could evolved the tensile energy of the three-membered heterocyclic ring. The practicable reactantsinclude aziridines, cyclic sulfates, epoxy derivatives,, aziridine ions and sulfocarbenium ions, etc. In these compounds, epoxy derivatives and aziridine ionsare oftentimes used as substrates in reaction of click chemistry. They could forms the product having high stereo selectivity in alcohol/water mixture, or without any solvents. Nucleophilic ring-opening reaction also contains the Michael's addition of the α,β-unsaturated carbonyl compounds. Proceeds the reaction between biepoxyethan and benzylamine as an example, Sharpless et al (Kolb,Finn and Sharpless,2001)describe the formation of 1,4-diol product in methyl alcohol withyieldof 90%, and 1,3-diol product without any kind solvent with the yield of 94%.

Regioselective oxirane opening to produce constitutional isomers can be maintained by the reaction conditions as demonstrated by the nucleophilic opening of diepoxid(Seneci andPierfausto,2000)with benzylamine(Scheme 1). In the presence of methyl alcohol, the 1,4-diol is formed in 90% yield while in theabsenceof solvent, the 1,3-diol is formed in 94% yield. Formation of the first hydroxyl group is permit intramolecular activation of the remaining epoxide. This effect is less common when the reaction is occurred in protic solvents which permit for nucleophilic attack from a more stable chair conformation, leading to the 1,4-diol-product.

Non-aldol carbonylation reaction

Non-aldol carbonylation reaction, has been mostly used and proven to be reliable and valid which are classified as follow: (Raindlova et al.,2010)

a. aldehydes/ketones react with 1,3-diol to form 1,3-dioxolane;

b. production of hydrazone and oxime through the reaction of aldehydes with hydrazine/hydroxylamine;

c. the reaction of α-carbonyl aldehydes, β-carbonyl aldehydes, ketones withsome esters to get heterocyclic compounds.

Carbon-carbon multiple bonds addition reaction

Typical carbon-carbon multiple bonds addition reaction covers dihydroxylation reaction and aziridine reaction ,epoxy reaction,etc. Eg. under the catalytic reaction of osmium, alkene exhibit particular reaction activity in the amination and hydroxylation as well as dihydroxylation of alkene. In the presence of same concentration of ammonium halide, fast and nearly quantitative amination and hydroxylation could be completed at room temperature. α,β-unsaturated carboxylic acids and amine compounds shows high reaction activities(NIE Ji et al.,2015). In last few years, the reactions that do not required the catalysis of metal have induced great attention. This type of reactions could be good method for surface modification because of the advantages like higher rate of reaction, simple mechanism and minimum effect from oxygen(Nie et al.,2015). Thiol-ene reactions contains free radical-mediated and catalyst-initiated types.

Applications click chemistry in biomedicine

Medicinal chemistry & click chemistry

The triazole ring shows structural resemblance with the amide bond thus this group potentially have bioisosteric potential(Tron et al., 2008). Depends on the kind of substitution, the triazole ring either imitates a Z-amine bond or an E-amide bond. When the established ring is 1,4-disubstituted, as is the case in simple Azide-alkyne Huisgen cycloaddition (CuAAC) in catalytic reaction of copper, the triazole imitates a Z-amine bond. The lone pair of electron of the third nitrogen nearly resembles the electron of the oxygen

from carbonyl moiety in the amide bond. The C-H bond is polarized and because of that it can tak e a part in development of hydrogen bond resembles to the amide N-H bond.Ultimately, the electrophilicity and polarity of the fourth carbon is same as the carbonyl carbon. The dissimilarities between the triazole ring and the Z-amide bond is the enlarged distances between the substituents: in the amide-bond the substituents are two atoms apart, while in the triazole ring the substituents are three atoms apart. A advantage of the triazole ring is its stability towards the hydrolysis, which amides do not have. The inertness of the triazole ring and the simplicity, softness and rigidity of the reaction makes the CuAAC very acceptable for connecting two molecular entities, In cass of the development of homodimers, heterodimers and other kind of fragment-based drug discovery (Tron et al., 2008). The production of homodimers can be successful for increasing the biological activity if one molecule brings the second one nearer to its target. For this purpose, the triazole ring act as a connector or spacer between two active moieties increasing the whole molecule's activity by synernism (Hon et al.,2012). The path and flexibility of the linker, both of which influence the distance between the two monomers, are important for the activity of the dimeric compound. The production of heterodimers permit for the combination of different compounds with two distinct activities. In this case, the orthogonality andeffectiveness of click reaction is very helpful when dealing with chemically complex elements of molecules.

In situ click chemistry

In situ click chemistry using various combinatorial chemistry and structure based prospective; a large number of compounds can be Formed from a small set of precursors. Then all the formulated molecules required to be tested forpharmacological activity and toxicology to the lead, its optimization and terminally to provide the potent candidate.These are very rapidly occurring processes. The idea of in vitro combinatorial chemistry constitutes a variety of technologies where the

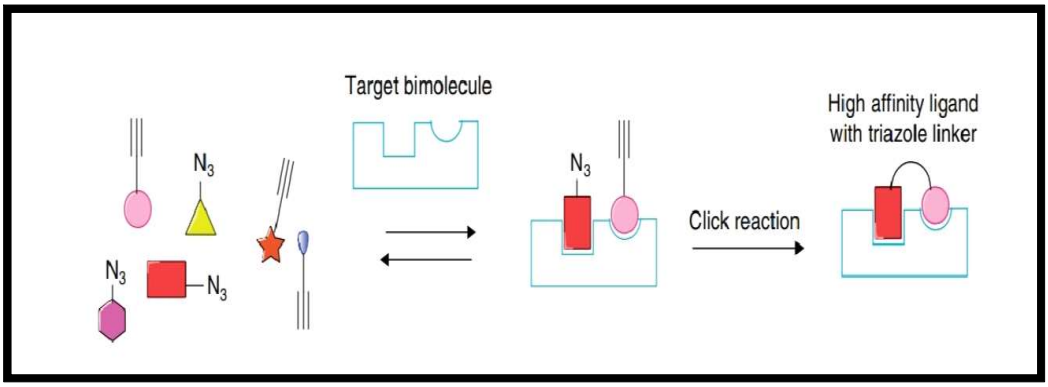

Figure 4. The process of In situ click chemistry

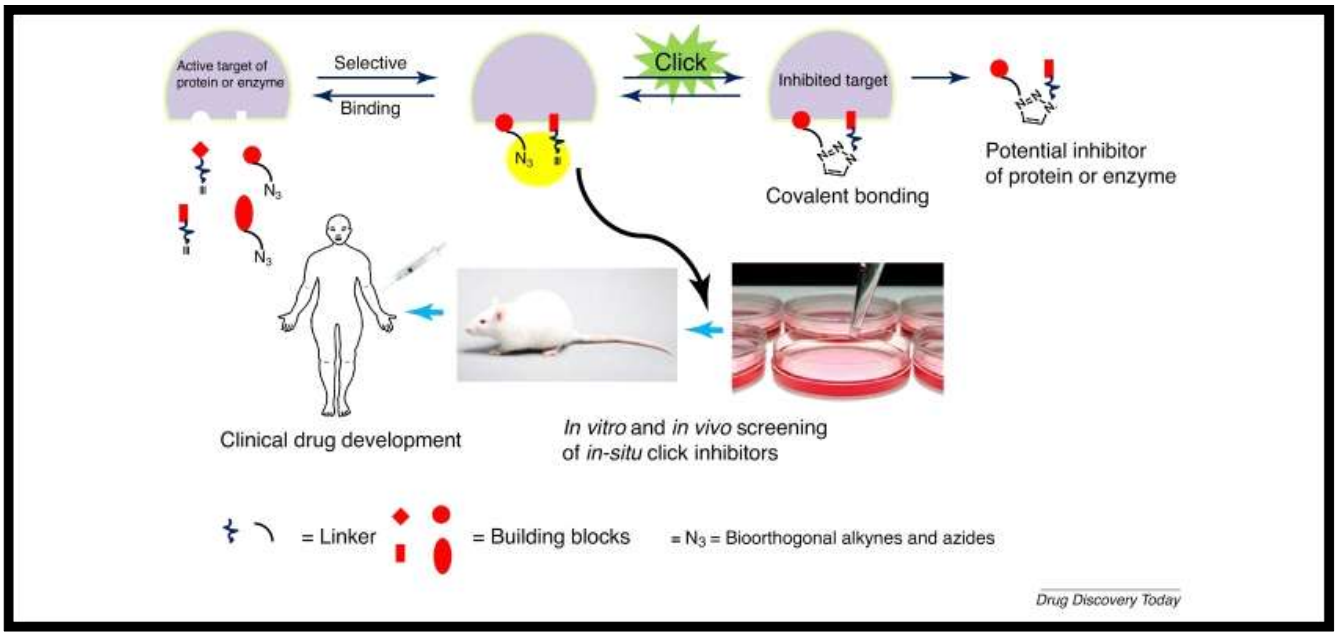

Figure 5. Schematic illustration of in situ click chemistry used to develop enzyme or protein inhibitors and drug development

selection and combination of novel molecules is carry out in one single process. In situ click chemistry, introduced by Sharpless, is a category of in vitro combinatorial chemistry(Lewis et al.,2002). Sharpless and coworkers used this perspective to find new inhibitors of acetylcholinesterase (AChE). The first target used for in situ click chemistry was the enzymeacetylcholinesterase (AChE), selected for its biologicalimportance as a key component of neuronal activities andtherefore a drug target(Tylor and Radic,1994) and for the structure of its active site.(as mentioned in figure.4). It was predicted that a triazole link is madebetween two of these small-molecule inhibitors competentlydesign with the complementary alkyne and azide functional groupscould form in situ, to formulate a novel bivalent inhibitor of the enzyme. Recently, Fokin et al. mixed CuAAC and in situ forthe formation of a highly potent and selective ligand for Lymnaeastagnalis acetylcholine binding protein (AChBPs). Their studysays that an in situ click reaction carried at the subunitinterfaces of the oligomeric protein, and, thus, this proposalcan be used as a tool for the identification of novel drug candidates for nicotinicACh receptor ligands. Recently, automated docking using theprogram AutoDock with protein pliability was used to designpotent non-covalent antagonist of AChE with a Kd of 100 fM. Thisproposal also permits additional conformational pliability inselected amino acid side chains on the target protein.(As mentioned in Figure 5)

The Huisgen cycloadditionreaction in between the azides and alkynes was chooses for this study. While azides and alkynes are spring loaded reactants but yet they have very slow reacting under physiological conditions if and only if they are activated; either by using any catalyst or by using a biological target which acts as template to bring the reactants close enough to react(Jingli et al.,2012). The inertness of the triazole ring and the simplicity, softness and rigidity of the reaction makes the CuAAC very acceptable for connecting two molecular entities, In cass of the development of homodimers, heterodimers and other kind of fragment-based drug discovery. The production of homodimers can be successful for increasing the biological activity if one molecule brings the second one nearer to its target. For this purpose, the triazole ring act as a linker or spacer between two active moieties increasing the whole molecule's activity by synernism.The path and flexibility of the linker, both of which influence the distance between the two monomers, are important for the activity of the dimeric compound. The production of heterodimers permit for the combination of different compounds with two distinct activities. In this case, the orthogonality and effectiveness of click reaction is very helpful when dealing with chemically complex elementsof molecules.

Click chemistry & nanomedicine

Click reactions which are highly effective and selective bring an huge utility to the area of nanomedicine(Lutz and Angew,2007). Nanodevices can frequently be breakable supramolecular constructionsand hard topurify or separate. Because of this click reactions which are easy to carry out and are very valuable. Eg, the CuAAC, which can be carry out at room temperature and in abroad variety of solvents and with stoichiometric amounts of reagents, has exhibit to be a versatile tool for constructing, functionalizing and cross-linking colloidal systems such as lipid, polymer and inorganic nanoparticles.(Lallana et al.,2012) In this manner, colloidal systems can be customized as drug delivery carrier's, contrast agents,gene carrier's, bioseparation anddiagnostics tools.(Lutz and Zarafshani,2008) Click chemistry can also be utilized to flat surfaces to functionalize self-builded monolayers by triazole linkages(Collman,Devraj and Chidsey,2004, Collman et al.,2006) which can be used in molecular catalysis,electronicsor biosensors.

Polymer chemistry & click chemistry

In the area of polymers, click chemistry helps remarkably in the development of product yields, however it is applied for polymer formation or functionalization(Binder and Sachsenhofer,2008). Also, click chemistry highly minimizes the difficulty and/or need for purificationand simplifiesthe artificial treatments for them selves.Clickable functionalities can be introduced into polymers in a number of categories(Patricia et al.,2010). Important functionality into the side chains can be incorporate by direct polymerization of a click-functional monomer, or by modificationof functional groups into alkynes or azides which can take part in click reactions(Lallanan et al.,2012). The functionalization of polymer side-chains by click chemistry has goes through the specialattentiveness as it would permit a popular type of polymer to be rapidly customized for specific needs through grafting of functional groups(Foumier et al.,2007). Although, the post-modification of polymer side-chains is irritating due to steric hindrance and that's where the efficacious and robust Azide-alkyne Huisgen cycloaddition (CuAAC) comes in to play.Another barrier that controlled by applying click reactions in polymer chemistry in the Formulation of block polymers and cyclic polymers. These kind of polymers are usually not available or difficult to formulate by ordinary polymerization techniques(Patricia et al.,2010, Fourmier et al.,2007). Due to the broad therapeutic potential of peptides, peptide-based polymers are of substantial interest for thedevelopment of drug delivery systems and plateform for tissue engineering(Timothy,1997,Matthew,Grigory and Neil,2007). The formation of such molecules is troublemaker because of the elaborate use of protecting groups and applying them by protein engineering is complicated. The CuAAC is very suitable technique to formulate polypeptide products as the triazole moiety is a known peptidomimetic. In same manner, click reactions have become a important tool for the

formation of glycopolymers(Gregory and Kakkar,2008).

Click chemistry & hydrogels

The cross-linking of polymers leads the way to the development of hydrogel materials whichare of special interest for drug delivery system and tissue engineering implementation.(Chelsea, Molly and Shoichet,2011)For the formulation of hydrogels by CuAAC two prospectives can be used:

1) a single step production method in which a versatile monomeric unit carrying both azide and alkynemoieties reacts with itself after addtion of Cu(I);

2) the mixing of two non identicalmonomeric units carrying either an alkyne or an azide accompanying by the addition of Cu(I) to start the cross-linking.(Fournier, Hoogenboon and Schubert,2007) The 'spring-loaded' capacity of the CuAAC causes hydrogel production to occur after some time and a higher degree of cross-linking can be obtained. The mechanical characteristics of hydrogels are dictated by the number of cross-links formed in between the polymer chains via non covalent bonds or covalent interactions. Thus the high rate of cross-links promotes the formation of hydrogels with superior mechanical characteristics. Firstly the hydrogels are formulate by using click chemistry by Assipov et al..(Dmitri and Hilborn,2006) These approaches can also be applied for the formulation of pure Poly Ethylene Glycol-base hydrogels, are describe by Hawker and it's co-workers.(Michael et al.,2006)The cross-link efficiency can be balance by differing both the polymer and/or catalyst concentration. Apart from this cross-linking artificial, natural polymers such as hyaluronic acid or collagen can be incorporate to mimic naturaltissues.

Click chemistry & dendrimers

The major challenge in dendrimer formulation lies in the development of new artificial methods for their major scale formulation and subsequent commercialization for biomedical applications. To prevent this matter, organic chemists have looked for the library of accessible chemical reactions to find out suitable alternative. Since it's initiation in 2001, the CuAAC between other types of reactions involved in click chemistry, has proven to be a important tool for stimulate the development of new methods in dendrimers formation. The first dendrimers formulated by CuAAC was initiated in 2004.(Wu,Fedman,Nugent et al.,2004) The steps required for dendrimer formulation need to be efficient and proceed simply in order to prevent the structural defects. The CuAAC meets up these needs to an exception. This kind of click reactions does not give any side-products and the Major product are obtained at yields more then 90%. This means that no excess of reactants are needed to complete the reaction procedure and it's purification. Also, the CuAAC is so flexible that it can be applied for dendrimer formulation by theconcurrent method, combination of asymmetrical dendrons and dendrimer functionalization.(Grigory and kakkar,2008) Some advantages of CuAAC covers its compatibility with water, simple work-up so it can be known as a green reaction.(Gregory and Kakkar,2010) The reaction can be monitored with Fourier Transform Infrared Spectroscopy (FTIR)by checking for the disappearance of the band to theazide. Varieties of click reactions which are orthogonal can fuse to construct dendrimers in an accelerated manner.(Marie and Malkoch,2012)This prospective depends on utilizing two distinct monomers (AB & CD). The chemoselective moeties in both monomers are selected for that functionality A Particularly reacts with D, and B with particularly C. Thus any non protection steps are removed and the number of reaction steps is reduced. This can be obtained by using both CuAAC and thiol-ene simultaneously. The dendrimers which are formed arecomposed ofheterogenous layers due to different kinds of chemical bonds and monomers.

Click chemistry & bioconjugation

The rigidity and resemblance to the amide bond of the triazole ring, incorporate with its higherstability in biological conditions makes it highly suitable as linker for bioconjugation.(Jingli et al.,2012)Bioconjugation is applied broadly for improvement in the aqueous solubility,for decreasing immunogenicity, increasing circulation time and stability of pharmaceuticals preparations and for tagging and labeling of biomolecules.(John E Moses and Adam D Moorhous,2007) The orthogonality of azides and alkynes for the number of types of functional groups in complex biomolecules makes the CuAAC a approved methodology for selective bioconjugation. The azide functionality is specially suited as it does not materialize in natural compounds. As an example, cowpea mosaic virus can be functionalized with either alkynes and azides due to the specific protein unit which includes one lysine and one cysteine. Subsequently, fluorescein dye derivatives can be linked through click chemistry. Product yield is 100% and various fluorophores can be attached.(QianWang et.al.,2003)Oligonucleotides are interesting molecules for gene therapy and as molecular probes. They can be labele at the 5'-end or at the 3'-end. Latest advances have made the incorporation of alkyne groups into the nucleobase viable without affecting the belonging of the oligonucleotides.

Advantages of click chemistry

I. The mixture has only the stable molecules.

II. The reaction has a very high yieldnear about 90-98%.

III. To composed a desired product in a simple and quantitative

procedure.

IV. Energetically highly favorable linking reaction.

V. The purification can be done on larger scale.

VI. The connection is chemoselective.

VII. Click reaction must be of broad scope, giving constantly higher yields with a variety of

Precursor.

VIII. It must be simple to perform, it is water and oxygen insensitive and has very cheap reactants.(Shirame,Bhosle and Raghunath,2009)

Click chemistry acts as a green approach

we have try to connect and compare green approach and click Chemistry, using the "Principles of Green Chemistry", by Anastas and Warner 1998 (Peabody, Peterson and Warner,2009)

Reduce derivatives: Click chemistry is known for it's superior selectivity and tolerance to

most of the functional groups.

Catalysis: In click chemistry only chemical or light catalysts are used.**Safe chemistry for accident prevention:** The utilization of azides in 1,3 dipolar cycloaddition reaction are reduced the chance chemical accident.

Design for energy efficiency: Click chemistry has many reactions which can be done without using too much heating.

Safer solvents: In click Chemistry water is used as a solvent.

High production rate: Huisgen cycloadditon addition reaction has high yield.

Limitations of Click Chemistry

The Huisgen-1,3-dipolar cycloaddition (HDC) reaction has a many oflimitations, Firstly, like with any cycloaddition, if the diene is more electron deficienttherefore it doesn't undergo the reaction. The energyof its ground state configuration is much low for it to interact with a the terminalalkyne. (dienophile) Similarly, the dienophile has also electron deficient. These positionsare highlyunlikely to carried and require functional groups which not commonly occurs inbiological systems. A more frequent limitations is alkyne homocoupling. Thistakes place when an alkyne reacts with another alkyne instead of the azide.(Christopher,Xin and Dong,2008)

A more frequently default is CuI saturation. This successfully prevents any azide functional groups by forming the complex andperforming displacement. The stability of number of azides may be a limitation. Obviously, if the ratio of nitrogenatoms to carbon atoms in an organic molecule increases, or it is equal to one then themoleculeis considered explosive and very dangerous. As an example, methyl azide oftendecomposes explosively and heavy-metal azides are more commonly used as detonators.(Brase et al.,2005)this isgenerally not a huge issue for pharmaceutical research, which tends to focus on largecompound with larger carbon chain.

Conclusion

As a easy, powerful, selective and quick method of synthesis in bio-medical research,that define as a set of dependablereactions that can be utilized to construct the novel pharmacophores in the aim offacilitating the process of drug discovery. At Present, the reaction of click chemistry hasbecames the synonymous of Huisgen 1,3-dipolarcycloaddition because it mostly used.Eventually, other reactions that Occupy the click criteria should be determined for their use towards the purification andsynthesis of biological activecompound in order to access greatest structural diversity.Chemical sensors, coupled with the click chemistry,has much more improved their performance, reproducibility and stability. Above all,to its modular design, great scope and reliance on extremelyshort succession of near-perfect reactions.

The application of click chemistry in bio-medical research is still in its prominent phase, and there are lots of issues to be resolved. Firstly, click chemistry required difinite functional groups to bring on, which needs the function of reactants, and could only occurs in severe conditions, thus restricts it's applications.The reaction between terminal alkyne and azide is Commonly used. There are numerous reports on thiol group-alkeneaddition reaction recently, but few descriptions about other reactions.In future, researchers still need to develop simple, advance and more efficient methods.

Other limitations involves bio-compatibility of 1,2,3-triazoles molecules. Truly, it was

first identifies over a century ago, not too much is known about their biological pathway.Theseparate toxicities of many 1,2,3-triazole-containing compounds are extensively

studied, but no generalization has been confirmed. This is very amazing since number of drug molecules aresynthesized by click chemistry and 1,2,3-triazoles have used as bioisostericsubstitutes of amide functional groups. Actually, the major purpose of clickchemistry is for drugdiscovery.

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