Available on line www.eijppr.com

International Journal of Pharmaceutical and Phytopharmacological Research

ISSN (Online) 2249 – 6084

ISSN (Print) 2250 – 1029

Int.J.Pharm.Phytopharmacol.Res. 2012, 1(5): 322-331

(Review Article)

Click Chemical Reactions:An Emerging Approach and Its Pharmaceutical Applications

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Received on: 21/03/2012 ***Accepted on:*** 24/04/2012

ABSTRACT

Click chemistry provides reactions which are simple, fast, stereospecific, give product in high yields and show many other desirable characteristics. These reactions involve linking of the reactant blocks by formation of carbon heteroatom bond between them. Out of the reactions which satisfy click reaction criteria, copper catalysed 1,3-dipolar cycloaddition of alkyne and azide (HDC reaction) has emerged as a perfect click reaction. Some click reactions which can proceed in absence of metal catalyst (metal free click chemistry) are also being developed. Click chemical reactions have wide applications in many areas of pharmaceutical sciences such as synthesis of lead discovery libraries, polymer therapeutics, drug delivery systems and bioconjugation etc.

Key Words: Click chemical reactions, Metal free click chemistry, Pharmaceutical Applications, Polymer Therapeutics, Bioconjugation.

INTRODUCTION

Drug designing and drug synthesis are very important steps in drug development. Once the drug with particular structure, having desirable properties is designed the main skill lies in actual synthesis of the designed drug. An ideal synthetic method for drug synthesis is the one which is simple, has few steps and can be used effectively at small as well as large scale. The concept of Click Chemistry introduced by Hartmuth C. Kolb and K. Barry Sharpless[1] seems to provide reactions which help us to develop such simple method for synthesis of varieties of compounds. According to Kolb and coworkers, current methods of drug discovery which are based on the conventional chemistry to create compounds with many carbon-carbon bonds and complex structures were not efficient to discover new drugs. In case of any drug, its function is more important than its chemical structure. So giving more importance to the biological function of the drug they stated that "all searches must be restricted to the molecules that are easy to make" and from this approach thus emerged the concept of Click Chemistry. It can be defined as an approach of developing the expanding set of powerful, selective and modular blocks that work reliably in both small and large scale applications. It basically involves preparation of substances quickly by joining small units together (Fig.1).

NEED OF CLICK CHEMISTRY

Conventional methods of drug synthesis involve formation of new carbon – carbon bond. But the reactions of formation of C-C bond do not have moderate thermodynamic driving force. So for such reactions to reach completion, extra energy has to be provided. So organic synthesis involving C-C bond formation becomes very complex and costly and is not very useful for drug synthesis. More the cost of the synthesis of drug more will be the cost of manufacturing and eventually final cost of the formulation will also increase. To reduce the cost and complexity of drug synthesis, Click Chemistry approach is useful. The click reactions are quick, simple and have many desirable characteristics. Click reactions mainly involve (C-X-C) i.e. carbon-heteroatom bond formation rather than C-C bond formation and hence they carry great potential to

simplify drug synthesis methods and thus ultimately fasten the drug discovery process.

IDEAL PROPERTIES OF CLICK REACTIONS

As the name suggests, click reactions are as quick as clicking two things together. A reaction is considered to be a click reaction when it shows following characteristics [2].

1. The reaction must be modular which means that reactants in the form of modules or blocks can be joined to yield desired product.
2. The reaction should be wide in scope i.e. it should have variety of applications.
3. It should give products in very high yield.
4. It should generate only inoffensive byproducts that can be removed by non-chromatographic methods like crystallization or distillation.
5. The reaction should be stereospecific (but not necessarily enantioselective).
6. The reaction must have sufficient thermodynamic force to reach completion; so extra energy need not be provided for completion of reaction.
7. The reaction conditions should be simple. It means reaction should be insensitive to oxygen and water.
8. Starting materials and reagents used must be readily available.
9. The process should use no solvent or solvent like water which is benign or which can be easily removed.
10. The click reaction product isolation must be easy .
11. The product must be stable under physiological conditions.

Most of the times water is used as a solvent in click reactions. The very best click reaction proceeds most rapidly and in the highest yield, not in water or water-co-solvent mixtures[3] but floating on water. Reaction between organic species can proceed faster in aqueous media than in organic media because the apparent rate constant of the reaction is higher when the reaction is carried out in aqueous solution[4]. Many of the times the reacting species and product formed are insoluble in water still the reaction proceeds faster. This is because when the organic molecules are poorly solvated in water their free energies are higher and hence the reactivity of reacting species is increased and which in turn increases the rate of the reaction[5]. Click reactions being exothermic in nature; water is the best solvent for them as it absorbs the heat produced during the reaction, due to its high heat capacity and has a convenient boiling temperature, both are useful for large scale processes. Some reactions like nucleophile addition to epoxide and aziridine electrophiles require the solvent which is capable of forming hydrogen bonds; so the water is suitable solvent for such reactions. If one of the two reacting species or solutes reacts with water then that side reaction which has lower driving force proceeds very slowly and the actual favourable reaction between those two species proceeds with very high speed when water is solvent. Water is also preferred as it is benign and cheaper as compared to other solvents.

TYPES OF CLICK CHEMICAL REACTIONS

Olefins are important compounds from click chemistry point of view as they generate starting materials of click reactions. Oxidative addition of heteroatoms to specifically placed olefinic sites gives variety of compounds such as epoxides, aziridines, episulfonium ions and aziridinium ions. These compounds have high energy and still they are stable and are perfect for click chemistry reactions[6-11].The click reactions are classified as follows:

1.Nucleophilic Ring Opening Click Reactions

Nucleophilic ring opening reactions of epoxides, aziridines (Fig.2), cyclic sulfates, cyclic sulfamidates, aziridinium ions and episulfonium ions are reliable, stereospecific, often highly regioselective and hence these reactions are one of the important click reactions. These reactions are mostly exothermic. Aziridines can be prepared by aziridination of olefins and also from epoxides and amino alcohol. Aziridinnium systems have high ring strain. Nucleophilic ring opening of these aziridines is facilitated by release of strain[12]. In aziridines the presence of electron withdrawing substituent on nitrogen atom activates the ring and then that aziridine easily reacts with the nucleophile to give ring opened products. In contrast, non activated aziridines are relatively inert towards nucleophiles[13]. But opening of unactivated aziridines can proceed readily in water with buffered azide and with hydrazine. Regioselectivity of such reactions mainly depends on the nature of nitrogen substituent, nature of solvent and reaction conditions.

2. Cycloaddition Click Reactions

Cycloaddition reactions involving heteroatoms such as hetero Diel-Alder reaction[14, 15] and 1, 3-Dipolar cycloadditions[16-18] are few important click reactions. These reactions provide five and six membered heterocycles simply by uniting the unsaturated reactants [19]. Cu (I)-catalyzed Huisgen 1,3-dipolar cycloaddition (HDC) of azides and terminal alkynes to form 1,2,3-triazoles [20] is such

important reaction which has wide applicability in synthetic and pharmaceutical chemistry (Fig.3).

3. Non Aldol Type Carbonyl Click Chemical Reactions

The reactions of formation of hydrazones, thiourea, ureas, oximes (Fig.4), ethers, aromatic heterocycles etc. come under this category [21, 22].

4.C—C Multiple Bond Addition Reactions

These are the reactions involving addition to the carbon–carbon multiple bonds which includes oxidation reactions such as epoxidation [23] (Fig.5), aziridination [24], dihydroxylation[25], etc.

Out of aforementioned click reactions HDC reaction of azides and terminal alkynes can be considered as a perfect click reaction with great utility. However the only drawback of this reaction is, copper which is used as a catalyst is cytotoxic in nature and hence efforts are being made to develop reactions that do not require metal catalyst and still show all beneficial characteristics of click reactions. These reactions are categorized as copper or metal free click reactions.

METAL FREE CLICK CHEMICAL REACTIONS [26]

These are classified as follows:

1. **Copper-Free [3+2] Cycloaddition Reactions with Azides**
   a) Reaction of azides with substituted cyclooctyne: This reaction is called as strain-promoted azide-alkyne [3+2] cycloaddition (SPAAC) reaction.
   b) Reaction of azides with activated alkynes.
   c) Reaction of azides with electron-deficient alkynes.
   d) Reaction of azides with arynes.

2. **Thiol-Based Click Reactions**
   a) Radical addition reaction between thiols and alkenes (thiol-ene radical addition) - It involves addition of thiol to alkene catalysed by UV light to give thioether.
   b) Michael addition of thiols.
   c) Nucleophilic substitution of thiols with amines.

3. **Diels–Alder Click Reactions**
   a) Reaction of tetrazines with alkenes.
   b) Reaction of anthracene with maleimide.
   c) Reaction of dithioesters with dienes.

Since most of the reactions mentioned above do not satisfy some of the conditions required for a click reaction, they are not categorized as perfect click reactions. Metal free click reactions have their own limitations. Most metal-free click reactions involve large complicated reactive groups such as cyclooctyne, pentafluorophenyl, dipyridyltetrazine and anthracene. The large size of the coupling units is disadvantageous for most applications. Hence they are not very important from application point of view. However thiol-ene radical addition reaction is the only simple metal free click reaction as popular as HDC reaction[26].

ADVANTAGES OF CLICK CHEMICAL REACTIONS

Because of peculiar characteristics, the click chemical reactions have many advantages. These are as follows:

1. Click reactions have high thermodynamic force so no extra energy needs to be provided.
2. Aspects such as simple reaction conditions, use of non-chromatographic methods for removal of byproducts and purification of product, use of readily available starting materials and reagents, etc. make the process economically efficient.
3. Solvent used is usually water which is environment friendly and cheap.
4. Click chemical reactions have wide applications in variety of fields.

APPLICATIONS OF CLICK CHEMICAL REACTIONS

1.Polymer Therapeutics

Polymer therapeutics involves polymer protein conjugates, drug polymer conjugates, polymeric drug delivery systems [27] and polymeric micelles to which the drug is covalently bound[28]. Click chemistry is widely used in many such areas of polymer therapeutics.

Synthesis of block copolymers

Block copolymers consist of two or more homopolymer subunits linked by covalent bonds and are of great pharmaceutical importance. Many methods are used in synthesis of these block copolymers. Since, Click reaction is one of the most efficient ways to join two substances, it is very efficiently used to link the homopolymers to form block copolymers. For example, amphiphilic copolymers were synthesized by combination of atom transfer free radical polymerization (ATRP) and HDC reaction. The two polymers which were to be linked one with alkyne functionality and the other with azide functionality were synthesized by ATRP [eg. Poly(1-ethoxy ethyl acrylate)and

poly(acrylic acid)]. Then they were clicked together to yield copolymer[29].Other block copolymers such as polystyrene, poly(tert butyl acrylate), poly(methyl acrylate) have also been synthesized by using click techniques [30].

Polymeric micelles

Polymeric micelles have great applications in areas such as drug delivery, drug targeting, drug solubilisation and controlled drug release. The formation of cross-links throughout the shell of polymer micelles offers stability to the nanostructured assemblies, by enhancing the weak interactions that facilitate polymer micelle existence [31]. Such cross linking can be achieved by using click chemical reactions.

Cross linked polymer micelles of poly(ethylene oxide)-block-poly(ε-caprolactone) (PEO-*b*-PCL) were synthesized using click chemical reaction. First the substituted monomer that is α-propargyl carboxylate-ε-caprolactone was synthesized. Ring-opening polymerization of α-propargyl carboxylate-ε-caprolactone with methoxy PEO as initiator and stannous octoate as catalyst was used to prepare PEO-*b*-poly(α-propargyl carboxylate-ε-caprolactone) (PEO-*b*-PPCL) block copolymer. The block copolymers were found to spontaneously associate in aqueous solution forming well-defined micelles. Stabilization of the micelles was obtained by cross-linking the core via click reaction between the azide group of tetraethylene glycol (bis)azide reagent and the alkyne group on the PPCL block in the presence of copper catalyst at room temperature[32].

Another most exciting example of use of click chemistry in polymeric micelles is synthesis of the hemoglobin-conjugated polymer micelles which can be used as the oxygen carriers [33]. In this, amphiphilic triblock copolymers were synthesized using monomethoxy capped poly(ethylene glycol) (MPEG), cyclic carbonic ester monomer including propargyl group (MPC) and L-lactide (LA). These copolymers could self-assemble into core–shell spherical micelles with propargyl groups on the surface. Azided hemoglobin (Hb) was conjugated with the micelles through click reaction to form Hb-bearing nano-micelles. The resulted nano-micelles had 100 nm diameter with 50–60 wt% of Hb content. The Hb-based nano-micelles showed appropriate stability and oxygen carrying capacity, and would have the potential to be used as the new type of artificial oxygen carrier.

Polymeric drug delivery systems

Linear multifunctional PEG can be used as polymeric drug carrier [34]. Liu et al.[35] synthesized linear multifunctional PEG by using click chemistry. In this, a short acetylene-terminated PEG was linked to 2, 2-bis(azidomethyl)propane-1,3-diol using HDC reaction in water at room temperature. High-molecular-weight PEGs with pendant hydroxyl groups were obtained. A prototype bone-targeting polymeric drug delivery system was also successfully synthesized based on this new method [35].

2.Drug Delivery Systems

Liposomes

Liposomes are widely used as drug carrier in drug delivery systems. The drug to be delivered can be attached to it at different sites available in the liposome. Delivery of agents to the reticuloendothelial system (RES) is easily achieved, since most conventional liposomes are trapped by the RES. For delivering agents to target organs other than RES, long-circulating liposomes are developed by modifying the liposomal surface [36]. Conjugation of drug or ligand to the surface of liposomes can be achieved by using HDC reaction. Conjugation of mannose ligands to surfaces of liposomes was done by click reaction [37]. In this a lipid anchor functionalized with alkyne moiety was first introduced into the liposomes. Then mannosyl residue functionalized with azide group was conjugated to the surface of the liposomes in a single step. This reaction gave mannosylated liposomes which can be used as vehicles to target specific cells, such as human dendritic cells [38].

Dendrimers

Dendrimers are highly branched, multivalent, globular macromolecules having applications in drug delivery, gene delivery etc [39].Click reactions can be used to synthesize these dendrimers. Copper catalysed HDC reaction has been used for designing the dendrimers and for functionalizing them at the periphery with desired molecules [40]. Dendrimers were also synthesized by using metal free click reactions. Dendrimers up to the fourth generation were successfully prepared by the divergent growth strategy using a combination of thiol-ene "click" reaction and traditional esterification reactions [41].

3.Bioconjugation

The highly reliable union of azide-acetylene in presence of Cu as a catalyst has wide applications in *In-vivo* and *In-vitro* bioconjugation. These are discussed below:

Bioconjugation to gold and magnetic nanoparticles

Gold nanoparticles are used in cancer cell diagnostics[42], labeling antibodies[43], in drug delivery systems [44] and other areas because of their unique physical properties[45]. Most of these

methods involve attachment of biological molecules to the nanoparticle. Such bioconjugation to the gold nanoparticle via covalent bond can be accomplished by using click reaction. Magnetic nanoparticles have many applications in medicine because they are biocompatible and have highly specific accumulation in target tissues under a local magnetic field[46]. Many organic molecules including 2, 4- dinitrophenol, flag peptides, biotin and maltose binding protein have been attached to magnetic nanopaticles using HDC reaction. In this reaction, magnetic nanoparticles were functionalized by azide moiety and the molecule to be attached was functionalized to contain a terminal alkyne moiety[47] and then they were clicked together.

Radiolabellling

It involves attaching drug to the radionucleotide and this enables us to track its distribution inside the body and its metabolic pathway [48]. Commonly used radioisotope is technetium-99m. To attach this radioisotope to various organic molecules many click reactions are reported [49].

Polysaccharides

Modifications of polysaccharides to improve their pharmacological properties can also be achieved by using click reactions. Libert et.al [50] modified the surface of cellulose using click reactions. Azide functional groups were introduced into cellulose by tosylation of secondary alcohol group followed by azidation with sodium azide. Then three alkyl containing low molecular weight compounds (methylpropiolate, 2-ethynylanaline and 3-ethynylthiophene) were mixed with three different strands of azide functionalized cellulose in presence of copper as a catalyst and sodium ascorbate.

Hafren et al. [51] also have reported preparation of modified cellulose using click reactions. In this terminal alkynes were introduced through secondary alcohol of cellulose using 5-hexynoic acid. Then it was allowed to react with 3-azidocoumarinin in presence of copper catalyst and this reaction gave coumarin cellulose compound.

Tagging of live organisms and proteins

Interestingly click reactions have also been used to tag live organisms and proteins. For example, Cowpea mosaic virus (CPVM) whose capsid is made of 60 copies of an asymmetric two protein unit enclosing RNA genome has been tagged by bioconjugation methods with 60 azides per virus particles. It was found that all the 60 azide groups reacted to form triazoles (yield being >95%), which was helpful for labeling the virus with fluorescein [52].

Activity based protein profiling (ABPP)

Proteomics is a field which studies the functions of thousands of eukaryotic and prokaryotic proteins. Various methods like 2D gel electrophoresis, protein microarrays analyze proteins based on their abundance; but ABPP is a method which analyses the function of enzymes within biological systems[53].

ABPP requires two elements:

a) A site directed reactive group which binds covalently and labels a specific family of enzymes.

b) Reporter tag (fluorescein/biotin) for detection and quantification of labeled enzymes.

The ABPP probes have less cell permeability due to bulky reporter tags. To overcome this, the reporter tag is substituted by small chemical groups (alkyne or azide) which do not obstruct the permeability of the probes into the cell. This orthogonal reporter tag is then attached to the probe with the help of Cu catalysed HDC reaction.

Labeling of DNA

Click reactions exhibit vast applications in oligonucleotide labeling with fluorescent dyes. For labeling of DNA metal free click chemistry is used since metals may cause DNA cleavage. The method utilizes 5′-dibenzocyclooctyne (DIBO)-modified oligonucleotides, prepared using a new DIBO phosphoramidite, which react with azides via copper-free, strain-promoted alkyne–azide cycloaddition (SPAAC). After undergoing polymerase chain reaction (PCR) DIBO-modified primers yielded “clickable” amplicons that could be tagged with azide-modified fluorophores [54].

4. Synthesis of Lead Discovery Libraries

The concept of click reactions is being extensively used for the synthsis of ‘lead discovery libraries’. These libraries contain lead compounds that have biological activity and their chemical structure is used as a starting point for chemical modifications that will improve their physicochemical characteristics. By applying the scientifics of click chemistry, each library compound is produced in few steps with the help of key building block reagents. For example, the Cu catalysed formation of 1,2,3-triazoles has recently been used to prepare functionalized resins for solid phase synthesis of a library of dopaminergic arylcarbamides [55].

LIMITATIONS OF CLICK CHEMISTRY REACTIONS

1.Alkyne homocoupling **:**

In case of HDC reaction sometimes alkyne combines with other alkyne molecule instead of azide to give a different product [56].

2.Use of copper as a catalyst can be a disadvantage because copper is cytotoxic in nature and its excessive intake can lead to conditions such as hepatitis, Alzheimer's disease, neurological disorders etc.[57].

CONCLUSION

The characteristics of click reactions such as simple reaction conditions, high yield of product, compatibility with human biological system have made 'Click Chemistry' an emerging area of research in pharmaceutical industry. It has widespread applications ranging from new drug delivery systems to lead discovery. Click reaction like HDC has various applications in organic synthesis and polymer synthesis. Efforts are being made to develop catalyst free click reactions. It can be firmly concluded that Click Chemistry has turned out to be an emerging area in chemical and pharmaceutical science owing to its diverse potential applications and greener nature.

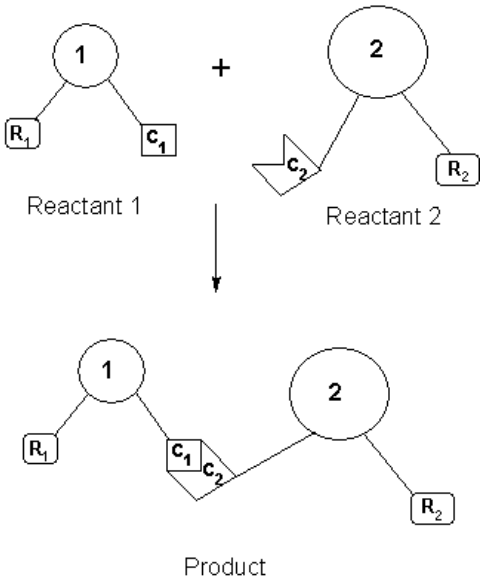

Figure-1: Schematic representation of a Click Chemical Reaction.

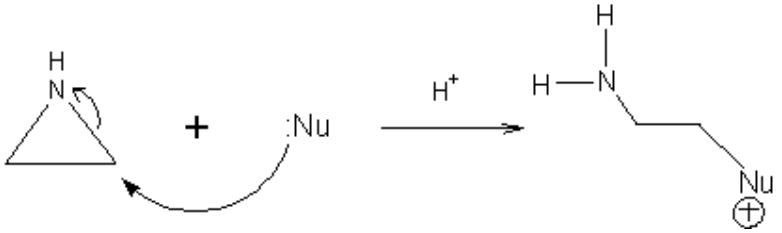

Figure-2: Nucleophilic ring opening of aziridine.

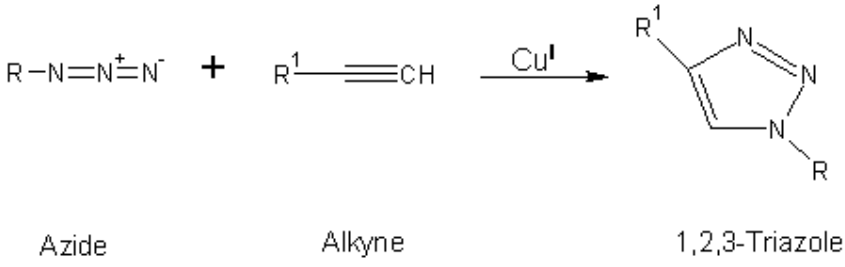

Figure-3: Huisgen 1,3-dipolar cycloaddition (HDC) reaction of azide and terminal alkyne.

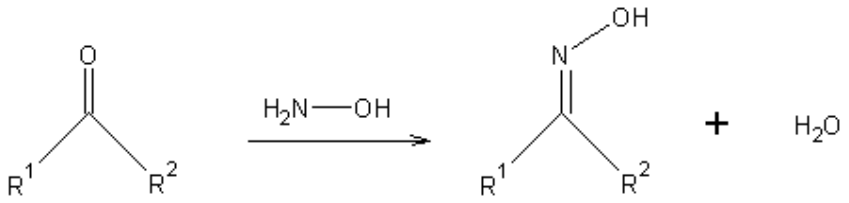

Figure-4: Formation of oxime.

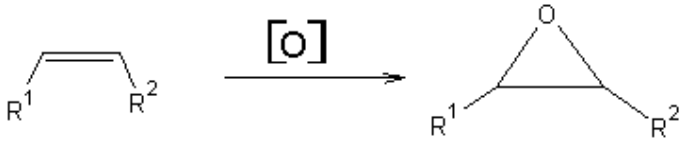

Figure-5: Epoxidation of alkyne.

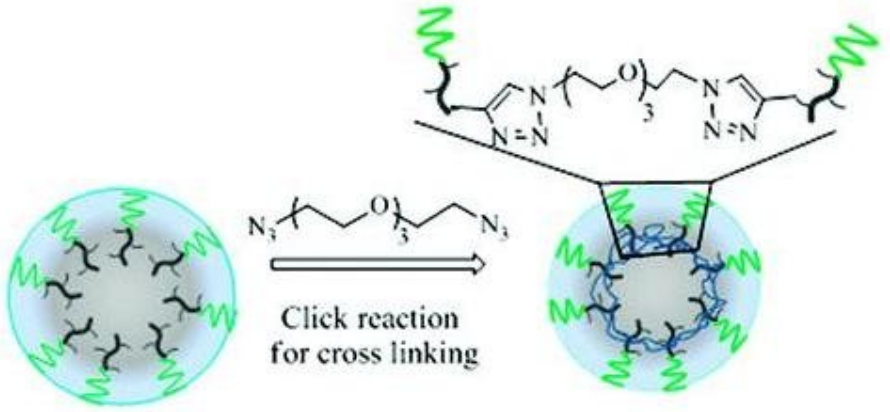

Figure-6: Stabilization of polymeric micelle by cross linking of core using click reaction.

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