



Click Chemistry Complexes As A Platform For Biological Application: A Review

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Abstract

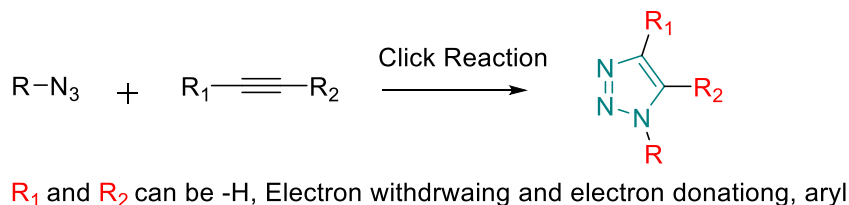
The 1,2, 3-triazole moiety is the main biomolecule formed from the chemistry of clicks. Click chemistry is cycloaddition reaction including Copper or ruthenium catalyzed with azide-alkyne and resulting in five membered rings. Click chemistry and its complexes are utilized as anti-cancer, anti-microbial, anti-tuberculosis, anti-viral, anti-diabetic, anti-malarial, anti-leishmanial and neuroprotective agents and also in fluorescent technology. Biological targets required a method containing the linker feature like 1,2, 3-triazoles moiety and a new set of 1,2,3-triazole consisting hybrids and conjugates. The present review summarizes developments over the last few years in application complexes of 1,2, 3-triazole analogue. Scientists of organic fields, clinical chemistry, photochemistry, and pharmacology will benefit from this study. This review offers essential knowledge to the interested researchers.

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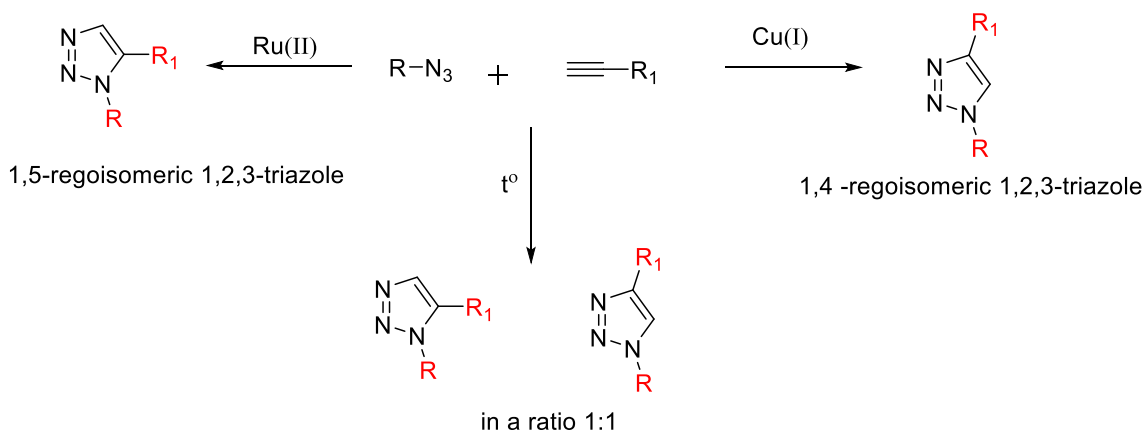
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1. Introduction

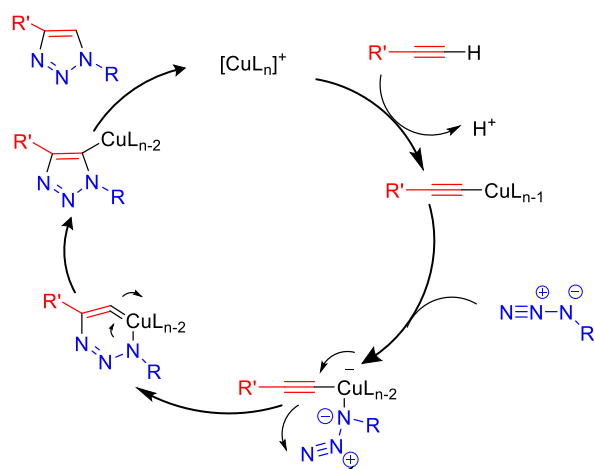
Sharpless is first one who coined the term of click chemistry in 2001 [1]. It refers to the 1,3-dipolar or Huisgen cycloaddition involving copper (I) as a catalyzed, an alkyne and an azide moiety react together to produce 5- membered cycle called 1,4-disubstituted triazole, Scheme 1. This reaction is given great yields, generated only inoffensive byproducts that can be isolated by non-chromatographic tools, the product is stereo-specific. They can often occur in aqueous solvents, resulting more environmentally - friendly than other reactions.

**Scheme 1.** Click chemistry reaction

Michael in 1893 is the first one who described the cyclisation reaction [2]. Later, Huisgen in the 1960s reported the 1,3-dipolar cycloaddition, after that, it was named by Huisgen himself [3]. A catalyst does not used in the earlier reaction, reaction conditions was requiring quite harsh, and gives a mixture of the 1,4- and 1,5- regioisomers due to this reaction was considered not click reaction until 2002 when the that Meldal and Tornøe discovered used Cu(I) salts as catalysts for the click reaction in organic solvents, the rate of reaction was increased, and only the 1,4-regioisomer was formed [4]. At the same time, Sharpless independently developed the same reaction but using aqueous conditions and using a copper (II) salt with a reducing compound, such as sodium ascorbate, to produce the active copper(I) species in situ [5]. In 2005, same researcher also announced to form of the 1,5-disubstituted triazole isomer by reaction between azides and end part of alkynes and using ruthenium cyclopentadienyl complexes as catalysis (RuAACc reaction) [6-7]. Previous report illustrated that Ni^{2+} [8], Pd^{2+} , Pt^{2+} [9] and Au^{+1} were consider as less efficient catalysts [10-11]. Copper catalyzed azide-alkyne cycloaddition (CuAAC) considered as a type of Huisgen 1, 3-dipolar cycloaddition and an important branch of click reaction, which is including different type of reaction such as thiolene, oxime, Diels-Alder, Michael addition and pyridyl sulphide reactions [12].

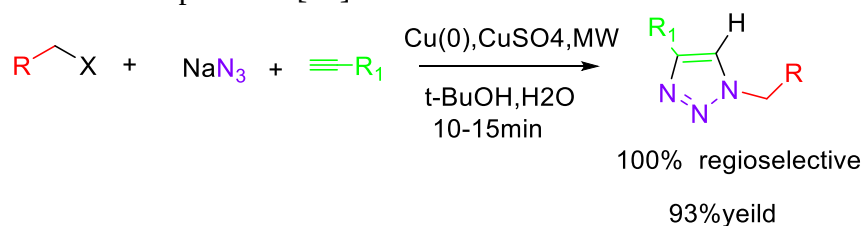
**Scheme 2.** The route of cycloaddition Coppercatalyzedazidealkyne -reaction (CuAAC) [13]

Cycloaddition (CuAAC) be known as a reaction between a terminal alkyne and an aliphatic azide in the presence of a catalyst such as copper to produce 1, 4,4-disubstituted 1,2, 3,3-triazoles as a product. Click reaction has been improving the quality of yielding, a facile, and selective, via allow the two molecular building blocks in organic reactions to connection under serious reaction conditions to produce few or no byproducts [14]. The mechanism of the click reaction supposed to proceed through a copper acetylide and a copper metallic cycle. However, there are several proposed mechanisms. In the first proposed mechanism shown in Scheme 3, the copper is proposed to coordinate to the π -bond of the alkyne. The terminal proton is then removed, and then the copper acetylide is formed. The azide molecule then coordinates to the copper. Intermolecular attack resulting that the alkyne connected to the azide, facilitated by the charge on the copper. The six-membered copper metallacycle then undergoes ring contraction. And then a proton exchanges with the copper to produce the triazole ring, and restores the copper catalyst [15].



Scheme 3. Mechanism proposed of CuAAC reaction [16]

There have been several attempts over the last decade to develop the click reaction by using non-traditional energy sources such as Microwave Radiation, Ultrasound and photo induced reactions compare with conventional system [5]. The probe is well known as an effective tool for enhancing the proceeding rate of reaction for several chemical transformations. Recently, A few Cu(I)coupled chemical click using microwave approach have been reported. A Copper (I) catalysis was used for microwave activation to boost cycloaddition between azide & terminal alkynes under solvent-free conditions to provide its relative 44-substituted 1, 2, 3-triazolyl [17]. Appukkuttan P. et al. were successfully enabling to reduce the associated reaction time from hours to minutes and develop the quality of yielding when using different alkyl halides and alkynes containing different functionalities when illuminated with microwave heating [18]. The component of azide is produced in situ from its corresponding halides, due to capture via copper (I) acetylides, resulting product that relative of 1, 4-disubstituted 1, 2, 3-triazoles. Significantly, when finishing both steps of this process under microwave irradiation reaction time lead to limited and easy filtration that used to isolate the products [19].



Scheme 4. Synthesis of 1,4-disubstituted 1,2,3-triazole

It was found that this method could reduce the time response by effectively generating 1,4-disubstituted-1, 2, 3-triazoles as main yield, which usually crystallize out of the mixture of reaction and do not need further purification [20]. This technique would prevent separation or taking off due to potentially unstable small organic azide, with result a triazole products in their pure form via this a method [21-22]. This current review will emphasis mainly on the recent literature of click reaction and their applications in the field of clinical chemistry, in specific on use of 1,2,3-triazole moiety and its complexes as active biomolecules. Since this is an extremely fast developing area, this review provides the interested researchers with important knowledge.

2. Method of Synthesis 1, 2, 3-triazoles

1,2,3-triazoles is the an essential product for a click reaction, this reaction is consisted of the CuI as a catalyzed. 1,4-substituted 1, 2,3-triazole products that forms in this reaction [1]. The path for click

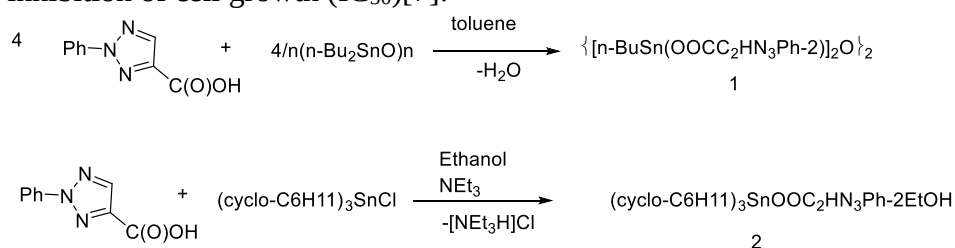
reaction is shown in Scheme 1, the contribution of organic azides with alkynes molecule in presence of Cu(I)-catalysed is the principle route of Huisgen 1,3-dipolar cycloaddition, to form only 1,4-regioisomeric 1,2,3-triazoles. Non-catalytic thermal pathway is known when ruthenium catalysts are explored as alternative to Cu(I) for this reaction and the major products is 1,5-regioisomeric 1,2,3-triazoles that produces from this reaction [2]. Number of experiment for synthesis 1H-1, 3,2,3-triazoles including Cu-AAC have been successful and developed, using different copper source. CuSO₄ or Cu(OAc)₂ as the source of copper which using by Sharpless and co-workers in Cu-AAC reaction. These copper compounds were used in combination with sodium ascorbate as a reducing agent to produce Cu(I) in situ in an aqueous environment [3]. Since previous publication, alternative sources of copper have been exploited upon to the specific environment of reaction, for example when the CuAAC reaction was carried out inorganic solvents (e.g. CH₃CN, DMF, DMSO, THF). Desirable source of copper in this case is CuI. This reaction effectively works in both aqueous and organic solvent to produce selectivity product which can be isolated with simple filtration. 1,2,3-triazole is tolerant of numerous functional group, triazole component is stable and resistant to the oxidation, reduction and hydrolysis reaction. Yoo *et al* illustrated that a catalytic quantity of CuI effect on the formation of the desired 1,4-disubstitued-1,2,3-triazole in organic conditions [4]. However, it was found that presence of a base is one of important requirement in this reaction in order to boost the generation of an intermediate copper acetylide[5]. Most CuAAC often produce triazole with coordination to copper metals, this problem can sorted by extracting copper from the reaction media by extraction with aqueous ammonia solution or ethylenediaminetetraacetic acid (EDTA) solution [6].

2.1 Biological application of Click Chemistry Complexes

Complexes based metal are broadly used in chemistry and in many of diseases, consisting cancer chemotherapy. Researchers have become more interested in the complexes chelating the triazole moiety because of their potential to inhibit tumor growth in living cells.

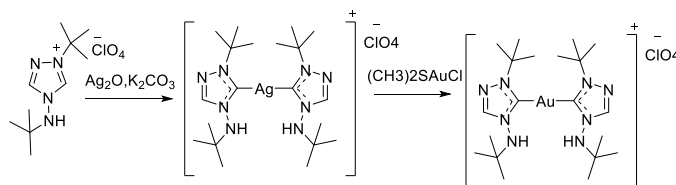
2.2 Click chemistry complexes as anticancer

One of these attempts are synthesized by Laijin Tian *et al.* who characterized two organotin complexes bearing 22-phenyl-11,22,3-triazole-44-carboxylicacid, the prepared complexes have general formal (n-Bu₂ Sn(OOCC₂H₃NPh-22)]₂O)₂(1) and (c-C₆H₁₁)₃ Sn (OOCC₂ H₃NPh-22)(C₂ H₅cOH) (2), these complexes were assessed against HeLa cells (human cervical cancer cell line), CoLo (colon carcinoma cell) and MCF-77 (human breast cancer cell line) there sults of bioassay was illustrated that the two compounds have more cytotoxicity than cis-platin against above mentioned cancerous cells and exhibited less than 50% inhibition of cell growth (IC₅₀)[7].

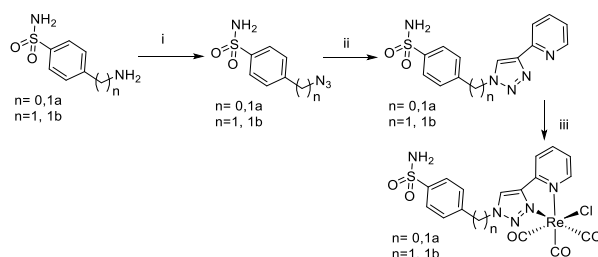


Scheme 4. Synthesis compound 1 and 2

Serebryanskaya and co-worker synthesized gold (I) complexes containing aminotriazole-based N-heterocyclic carbene and the complexes was evaluated as anti-cancer activity against HT-29 & MDA-MB231 cancer cell lines. The complex based gold exhibited anticancer activity with low micromolar range (1-2 μM) of IC₅₀ value. Hydrophilic nature of the side chain amino group rather than α amino group can overcome solubility problems and has ability to interact with receptor binding site. The results revealed that gold complex has toxic properties against HT-29 and MDA-MB231 cancer cell lines, also gold complex has potent ability to inhibit the activity of the thioredoxin reductase enzyme [8].

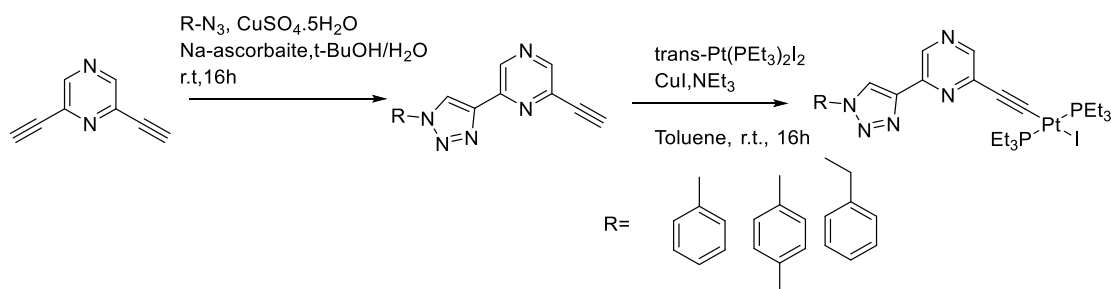


Scheme 5. The route of synthesis gold complexes



Scheme 6. the route of ligands Synthesised 3a/3b and its relative complexes of rhenium tricarbonyl bearing benzenesulfonamide 4a/4b. Conditions and catalysts: (i) 1a, NaNO_2 , NaN_3 , $\text{HCl}/\text{TfHF}/\text{DMF}$ (v/v/v: 1:1:1), 0 – 25 °C, one night (93%) or 1b, NaN_3 , Tf_2O , $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, K_2CO_3 , CH_2Cl_2 , 0–25 °C, 18hr (85%); (ii) $\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$, sodium ascorbate, CH_3CN , 45 °C, 18h, (3a: 69%, 3b: 61%); (iii) $[\text{Re}(\text{CO})_3\text{Cl}]$, 65 °C, meoh, 12hr., (4a: 67%, 4b: 66%).

A new type of organo-platinum complexes that chelating with a triazole ring were designed, synthesised, and characterized by Khushwant S. and coworkers. The MTT cell viability assay was used to investigate the cytotoxicity of these compounds against three cell lines (MG-63, MD-MB-2311, and HDF). The finding showed that the novel organo-platinum complexes containing 1,2,3-triazole are three times more effective as antiproliferative agents than cisplatin against human osteosarcoma (MG-63) and human breast cancer (MDAMB-231).

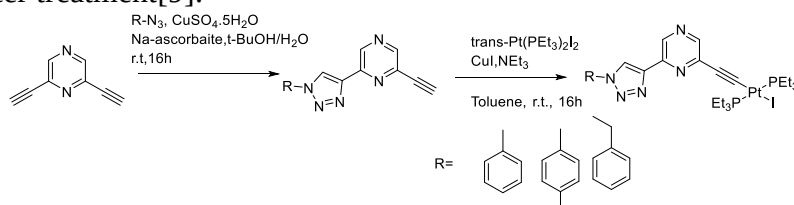


Scheme 7. Synthesis of platinum complexes

Alfonso and coworker characterization, biological activity and described the strategy of synthesis a new Pt (II) cationic complexes with square-planar geometry, which consisting glucoconjugated triazole ligand s4Pt-py and compared with the finding of its corresponding five-coordinate complexes which having the identical triazole ligand 3Pt. The work illustrated that the complexes with five-coordinate square-planar

show less stability than their relative ones. Moreover, even though that the complexes with square planar geometry are less toxic than the latter ones, they exhibit a good selectivity.

At the same time, Yassine Aimene *et al* prepared two new rhenium complexes of bidentate 2-Pyridyl-1, 2,3,3-triazole ligands (3a & 3b) bearing 4-substituted benzene sulfonamide moiety following standard click reaction method, the general formula of compounds 4a and 4b $[\text{ReCl}(\text{CO})_3(\text{L})](\text{L} \frac{1}{4} \text{33a or 33b})$. These complexes are completely described via spectroscopic technique (IR, NMR, MS, UV-vis), Elemental analysis, X-ray diffraction & theoretical studies operated by DFT and TD-DFT methods. Especially, an uncommon cis analogue was adapted to the Pyridine with the triazole rings of 3b in the solid phase that stems from intermolecular hydrogen bonds. Both ligands and complexes exhibited inhibitory activity when examined against carbonic anhydrase isoform IX and a great affinity (K_i of 2.8 nM) for ligand 3a via preliminary assays. These results demonstrated that compound 4b displayed a noticeable selectivity in contrast to hCA IX over the off-target shCA I and hCA II, 4b compound could be candidate as anticancer treatment[9].



Scheme 8. Synthesis of platinum complexes

Alfonso and coworker characterization, biological activity and described the strategy of synthesis a new Pt (II) cationic complexes with square-planar geometry, which consisting gluco conjugated triazole ligand s4Pt-py and compared with the finding of its corresponding five-coordinate complexes which having the identical triazole ligand 3Pt. The work illustrated that the complexes with five-coordinate square-planar show less stability than their relative ones. Moreover, even though that the complexes with square planar geometry are less toxic than the latter ones, they exhibit a good selectivity.

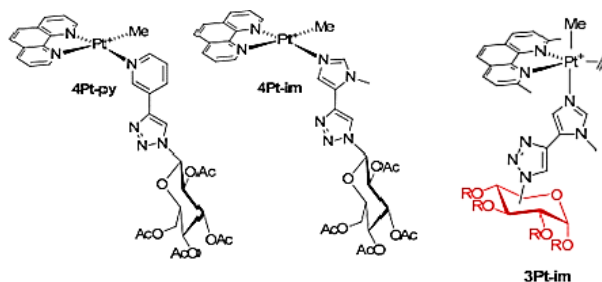


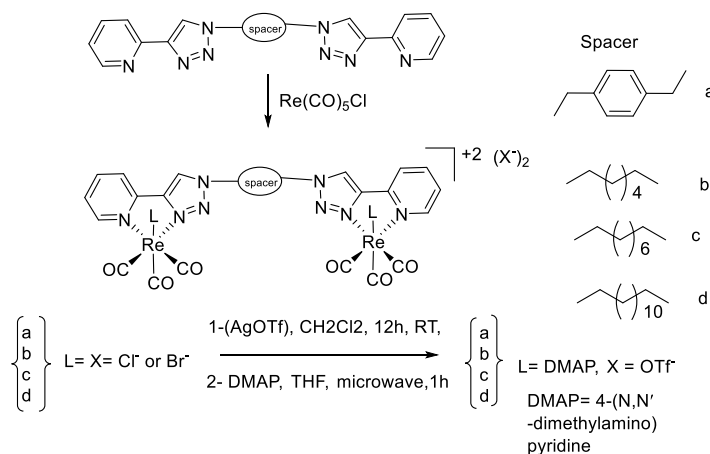
Figure 1. Pt(II) complexes coordination with glucoconjugated triazole ligands

Also it was found that the complexes with square planar are more stable compared with the five coordination species. This study demonstrated that the properties of antitumor agents depended on the coordination geometry [10].

2.3 Click chemistry complexes as antimicrobial

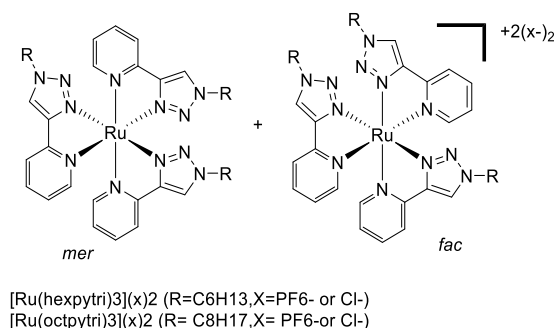
Derivatives of 1,2,3-triazole Complexes exhibited a significant antibacterial activity and antimicrobial compared with corresponding free 1,2,3-triazole derivatives. A new series of mono- and di-*fac*-rhenium tricarbonyl bearing 2-Pyridyl-1,2,3-triazole complexes with different aliphatic as well as aromatic

substituents are synthesized by Sreedhar V. *et al* in yields between (46–99 %). ^1H and ^{13}C NMR spectroscopy, infrared spectroscopy, UV-visible spectroscopy, high-resolution electrospray mass spectrometry, and elemental analyses were used to characterize the new complexes. The structure of Re (I) complexes was confirmed by X-ray crystallography in solid phase. Antimicrobial activity for the family of the mono- and di-rhenium (I) complexes and its corresponding 2-pyridyl-1,2,3-triazole were assessed in vitro for both Gram positive (*Staphylococcus aureus*) & Gram negative (*Escherichia coli*) microorganisms. The results showed that the most Re (I) complexes were active against *Staphylococcus aureus* and that the cationic rhenium (I) complexes were more active than the related neutral systems. Also, the minimum inhibitory concentrations (MIC) for all the complexes were modest (i.e. 16–1024 mg mL⁻¹) in all cases)[11].

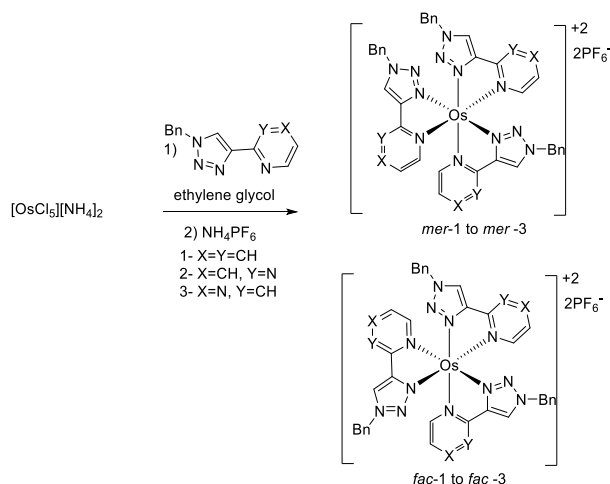


Scheme 9: Di-rhenium (I) complexes

At the same time, a library of ruthenium (II) complexes contain three ligands of 2-(1-R-1H-1,2,3-triazol-4-yl) Pyridine forming by cycloaddition reaction (R-pytri) with different aromatic (R = phenyl and benzyl substituents) & aliphatic (R = butyl, hexyl, octyl, dodecyl, and hexadecyl) substituents were prepared with excellent yield. The complexes of $[\text{Ru}(\text{R-pytri})_3]^{2+}(\text{X}^-)_2$ (where $\text{X}^- = \text{PF}_6^-$ or Cl^-) were prepared (figure 2). The finding showed that the R-pytri ligands and their relative complexes $[\text{Ru}(\text{R-pytri})_3]^{2+}$ (where $\text{X} = \text{PF}_6^-$ & R = hexyl or n-octyl) have a good antimicrobial activity against *Staphylococcus aureus* and have no action against Gram-negative *Escherichia coli* bacteria. After converted to the water-soluble chloridesalts, the important active $[\text{Ru}(\text{Rpytri})_3]^{2+}$, $[\text{Ru}(\text{hexpytri})_3]^{2+}$ and $[\text{Ru}(\text{octpytri})_3]^{2+}$ were tested against a wide range of pathogenic bacteria. These complexes exhibited great efficiency against a range of Gram-positive dyes (low inhibitory concentration (MIC) = 1–8 $\mu\text{g/mL}$) while the result exhibited that these complexes were much less active against Gram-negative bacteria (MIC = 16–128 $\mu\text{g/mL}$). Great interestingly, in some types, Ru(II) click complexes evidenced great active (MIC = 4–8 $\mu\text{g/mL}$) compare with the gentamicin control (MIC = 16 $\mu\text{g/mL}$) against two strains of methicillin-resistant *S. aureus* (MRSA) (MR 4393 and MR 4549). Cytotoxicity procedures on human dermal keratinocyte and Vero (African green monkey kidney epithelial) cell lines proposed that the compounds were only moderate toxic at concentrations well over the MIC values[11].

**Figure 2.** Ruthenium complexes

Kirsty L. Smitten *et al.* investigated that the antimicrobial ability of the specific analogue of Os (II) complexes that containing 1, 2,3-triazole ligands which belongs to a family of water-soluble homoleptic Scheme 1, the finding is exhibited that the meridional analogue of these geometry structure great increase inhibition of Gram positive bacteria compared with facial structural of analogue. The results were demonstrated that the remarkable ability of Os (II) complexes in (photo) as a drug in antimicrobial applications [12].

**Scheme 10:** The structure of the Osmium complexes

2.4 Click chemistry complexes for imaging living cells

Click chemistry has been utilized in developing a variety of multifunctional molecules and a useful platform in the tailoring and design bio-component exploitation in various biological applications. Bio imaging labeling is one of the essential tools for diagnosis and therapy of tumor in living cells. Heterocyclic compounds have been used in various applications. Recently, photo application has been more interesting by researcher, and five-membered ring containing nitrogen atoms is one of the important heterocyclic compound that suitable for clinical application due to the unique properties. Specifically, these compounds including 1,2,3-triazole core in their structure [13]. Heavy metal complexes involving metal-to-ligand charge-transfer transition (3MLCT) exhibit better advantageous photo physical properties (such as relatively long lifetimes and significant stokes shifts for easy separation of excitation and emission) compare with pure organic fluorophores, phosphorescent [14]. One of great significant reports of emissive click reaction in this subject is the study by Takumi Ishizuka *et al.* who developing a new

protocol for multicolor imaging of stain chromosomal DNA by click chemistry technique alternative to the traditional dye method. This strategy including prepared cores that served as light-up reporters to stain chromosomal DNA depend on click reaction, visualized the clear chromosomes in multicolor. This applied strategy in fluorescence in situ hybridization (FISH) was used to identify the telomere DNA at the end of the chromosome with high sensitivity and specificity, this work show that the click reaction enables direct imaging or observation the protocols of the chromosome during division of cell. These findings propose that the methods can be widely used for imaging chromosomes and may help as a new rout to deep study chromosome and genetic diagnostics. The fluorescence was quenched by the azido molecule of profluorophores, while, the azide alkyne of click reaction can reduce the quenching, leading to a bright luminescent. This approach offers a hyper-resolution to image the chromosomes as a single and enabling to be visualized and no required to wash the rest of unreacted fluorescence dye [15].

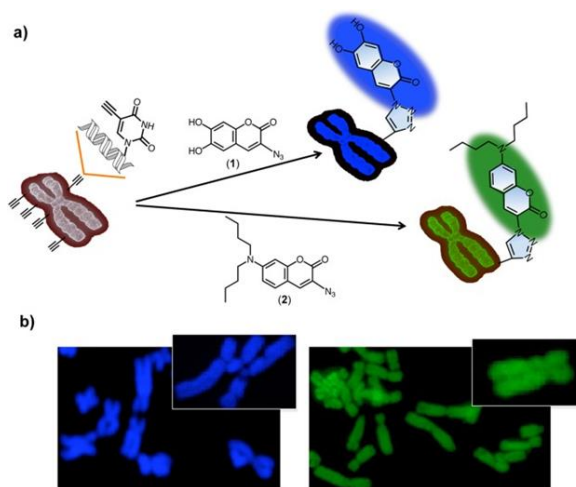
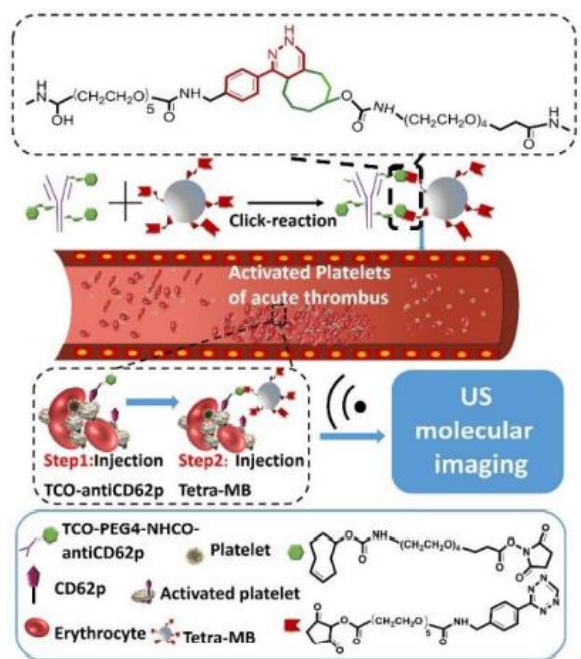


Figure 3. Chromosome imaging by using two pro-fluorophore.

In another major study in this area is the study by T. Wang *et al* who described a rapid and effective, highly sensitive approach to achieve activated-platelet-specific CD62p targeted thrombus ultrasound molecular imaging, this approach including two steps. At the beginning step, they treated with TCO-antiCD62p, and then bind and then reacted with thrombus. Second step, an elaborately of fabricated tetrazine-tagged microbubbles (Tetra-MBs) were added, resulting rapidly and selectively reaction with TCO- antiCD62p pre-treated thrombus through subsequent click chemistry by which the Tetrazine modified microbubbles (Tetra-MBs) could be captured specifically and rapidly. Tetra-MBs demonstrated great long-term physiological stability to preserve the ability to detect changes in the thrombus in real-time. The tetrazine probe based on bioorthogonal reactions are shown in Scheme 10[16].



Scheme 11. Schematic diagram of click reaction through pretargeting and bioorthogonal chemistry

Xie *et al.* presented a new probe for imaging the sialoglycans biosynthesis in brain tumor of mouse helping by a liposome-assisted bioorthogonal reporter (LABOR). This protocol consisted that Copper-free click chemistry conjugated the incorporated azides with fluorescent probes and allowed for imaging the brain sialoglycans in living mice. 9-Azido sialic acid cannot cross the blood–brain-barrier (BBB) via a metabolic process, thus, a liposome carrier was used to carry derivatives of click to the brain cell. The molecule may enter the brain and contribute to brain metabolic glycoengineering after insert 9-azido sialoglycans then loaded by liposomes. As a consequence, the additional azido groups improved the novel sialic acid synthesized in the brain section. The LABOR approach may use as a probe dynamic sialylation to distinguish the brain parts by performing pulse–chase process [17].

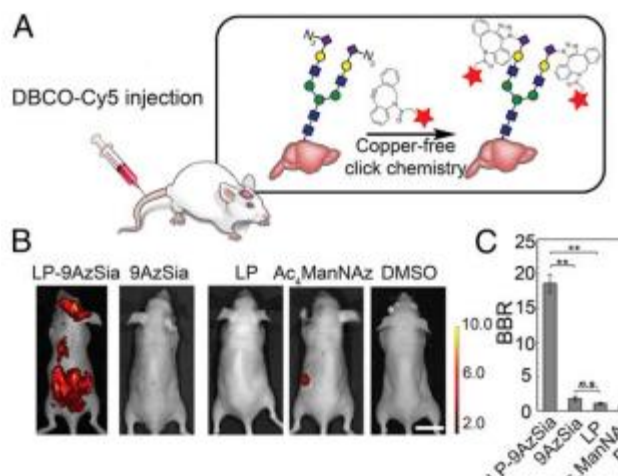


Figure 4. luminescence imaging of sialoglycans in brain of mouse via click chemistry in vivo

The application of click reaction has been widely used in vitro and vivo for labeling living cells due to its favorable characteristics especially biocompatibility and great specificity with a good reaction rate. Thus, many different techniques have been improved including developing the effective labeling agent by changing their structure, Tetraacetyl-Nazidoacetylmannosamine (Ac4ManNAz) is one of the most broadly studied metabolic cell-labeling reagents. Ac4ManNAz can be usually metabolized in the cell through sialic acid biosynthesis to azido sialic acid on the cell membrane, and the cell then labeled through click chemistry after treated with dibenzocyclooctyne (DBCO)-bearing reagents. A group of researchers have been developed the (Ac4ManAz) from microbubbles (MBs) for (cancer targeted- labeling) or (labeling targeted cancer cell) and visualized the assistance of targeted Ultrasound (US). Targeted US pulses could have boosted the breakdown of Ac4-MB with liberated and metabolic expression of azidosugars within the tumor which causes substantially elevated the tumor accumulation of dibenzocyclooctyne DBCOCy5 by 65% compared to the group without US treatment via the Click reaction. Unlike antibody drug conjugate and nanoparticle targeting ligand conjugate based targeting strategies which improved the cellular uptake rather than the overall tumor/organ accumulation ratio of drugs or drug delivery systems, the tumor-organ accumulation ratio of therapeutic agents can enhance by this strategy. This probe Ac4-MB including targeted US could be a simple but powerful tool for in vivo cancer diagnosis and cancer treatments [18].

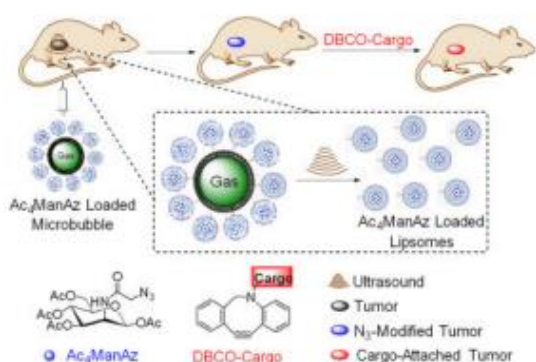
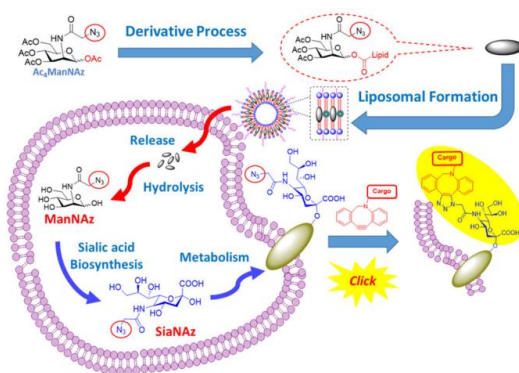


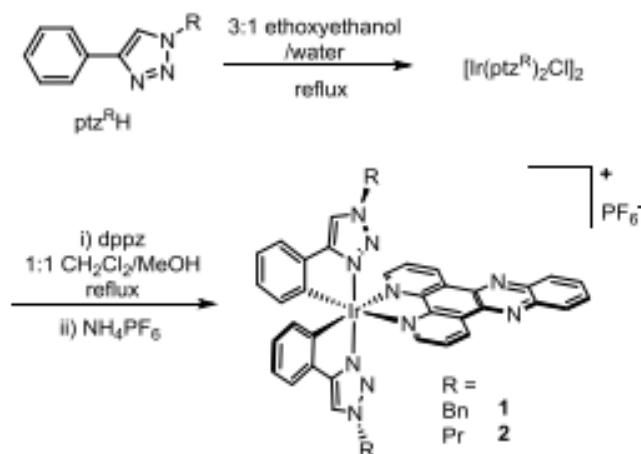
Figure 5. the probe Ac4-MB including targeted US

To be increase the chemical stability and cell labeling performance of Ac4ManNAz (AAM), the novel synthesized of AAM consisting diverse carbon chain length esters at the C-1-OH of Ac3ManNAzOH for hydrophobic lipids were prepared (Scheme 12). Li Shen *et al* synthesized two novel liposomal azido mannosamine lipids with hexanoic (C6) or dodecanoic (C12) ester tails alternative to acetyl (C2) ester on the anomeric hydroxyl group of Ac3ManNAzOH. This new agent used to test the cell labeling efficiency via Cu-free click chemistry. Confocal microscopy results showed that after treatment with DBCO-Cy5, the fluorescence on the cell membrane was produced by all cells treated with lipid-loaded liposomes of Ac3ManNAzOH compared with PBS as the negative control and free AAM as the positive control. It was found that after extending the length of the ester on an omeric carbon, the labeling were highly efficient[19].



Scheme12. The route of Liposomal azido Mannosamine Lipids on metabolic cell labeling and imaging

S Sreedharan and coworkers deeply investigated two novel biscyclometalated complexes with formula $[\text{Ir}(\text{ptzzR})_2(\text{dppzz})]^+$ (dppzp = dipyrrophenazine, ptzRH = 4-phenyl-1-benzyl-1,2,3-triazole (1+) and 44-phenyl-1-propyle-1,2,3-triazoles (2+)). These complexes hexafluorophosphate salts have been fully characterized. The structure of complexes nitrate salt was recorded by X-ray technique. The chloride salts of the complexes were investigated by DNA binding properties, the uptake of cellular was approved by A2780 and MCF7 celllines. Two complexes illustrated great intensity of phosphorescence due to binding to DNA, demonstrating the contribution of the dppz ligand and, produce that they are monocations, the complexes show substantial DNA binding affinity. These complexes are penetration to live cancer cell lines which confirmed by optical microscopy studies which display cytosol and luminescence. Colocalization studies using commercial probes show great pearson coefficients with mitotracker dyes confirming that the novel complexes specifically localized on mitochondria [20].



Scheme13. the route of synthesis iridium complexes

Although, there are many benefits of fluorescence imaging, including simple handling and high resolution, but its short penetration depth make it restricted to apply in medical imaging. Thus, the scientists have many attempts to exploit click chemistry in vivo through labeling methods, consisting PET /SPECT, MRI, and ultrasound, which are favorite in medical aspect. The MRI technique has been used in a wide range of medical application such as imaging the organs inside body using gadolinium contract agent. Andre A. *et al.* developed new probe including a strained cyclooctyne TMDIBO linked, via a

hydrophilic lysine linker, to a gadolinium DOTA chelate that is TMDIBO–Lys–Gd (2; Figure 7). This TMDIBO–Lys–Gd probe exhibited good water- solubility. In vitro and in vivo, the metabolically labeled cell-surface glycans on tumor cells could be imaged (labeled) by this probe. Also, it can used to image another organ such as the pancreas, spleen, kidney, liver, and gut. The result revealed that most tissues displayed only low levels of TMDIBO-Lys-Gd of non-specific retention, and a major N-azidoacetyl-galactosamine based contrast was detected during two hours after administration of the probe[21].

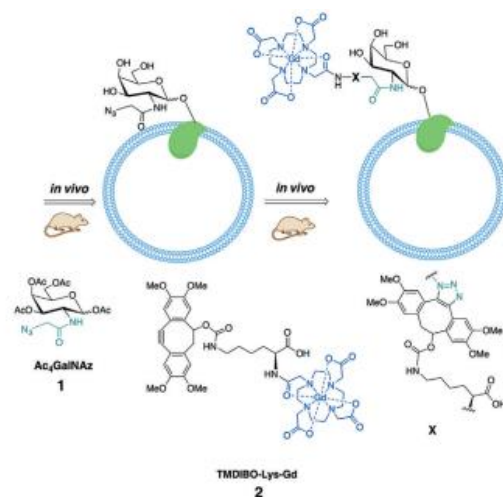


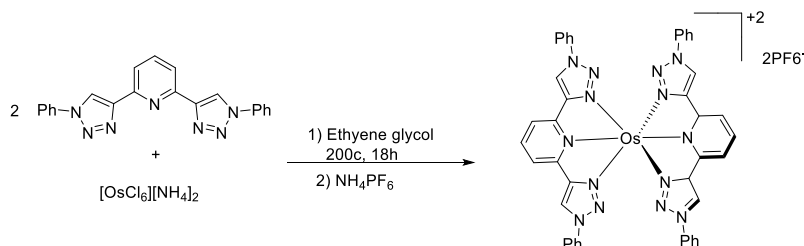
Figure 6. MR imaging of 2LL2 tumor of glycans via click chemistry in vivo

An effective therapeutic tool is transplantation of cell or therapy depend on cell which dealing with different diseases, involving intractable infections. In recent times, trials of Clinical for cell treatment or stem cells like mesenchymal stem cells (MSCs) have been documented [46-47]. Generally, the preferred therapeutic result of transplanted cells has not been completed because the low engraftment speed with lack post-transplantation existence times. Currently, the functionalizing the cell has been interesting by many researchers to improve the therapeutic effects. Also, limitations in a vivo cell tracking methods resulting less understanding about the fate of biodistribution of transplanted cells. Thus, most of researchers have many attempts to develop this method.

In the last decay, the click complexes have improved in cellular imaging and one of the most important studies have reported by Salem A. E. *et al.* who synthesized and characterized the complex [Os(btzpy)₂][PF₆]₂ (1, btzpy = 2,2,6,6-tetrakis(11-phenyl-11,2,3,3-tetrazol-4-yl)pyridine). Imaging study showed that complex 1^{Cl} readily go into the cancer cell lines HeLa and found that detected U₂OS cells with mitochondrial staining leading to the intense emission for imaging even at low concentrations as 1 μM. Also chronic toxicity study shows low toxicity in HeLa cells with LD₅₀ >100 μM. Osmium(II) complexes 1 could be used as a promising theranostic agents for anticancer activity [22].

To updating of previous study, anovel series compounds[Os(1bpy)₃– 1n(pytz)n][PF₆]₂ (bpy = 2,2,2'-bipyridyl, pptz = 11-benzyl- 14-(pyrid-22-yl)-1,2,2,3-tetrazoles, 1n = 0, 22n = 1, 3n = 2, 4n = 3) were synthesized with characterized bySalem A. E.Omar *et al.* This method including prepared complexes 3 in the harsh conditions and particularly mild preparative route via an Osmium (II) η⁶-arene precursor circumventing. All complexes display spectral absorption bands of remarkable intensity in the range of 500–700 nm. The results found that the homoleptic complex 4 is abright emitter (λ_{max,em} = 614 nm) with a relatively high quantum yield of emission of ~40% in deoxygenated acetonitrile solutions at room temperature. Confocal microscopy shows that complex 4 isalready entered into cancer celllines (HeLa and

EJ) with apparent lysosomal and endosomal localization, while toxicity findings illustrated that the compounds have low toxicity. It was suggested that these complexes therefore provide an excellent platform for improvement of efficient luminescent cellular imaging agents with advantageous photophysical properties that enable excitation and emission in the biologically transparent region of the optical spectrum[23].



Scheme 14. Os(II) complexes Synthesised 1 $[\text{Os}(\text{btzy})_2][\text{PF}_6]_2$

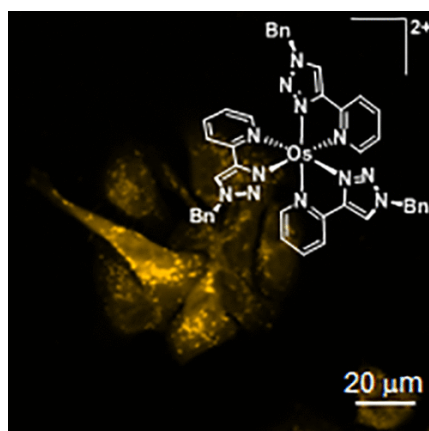


Figure 7. $[\text{Os}(\text{bpy})_3\text{n}(\text{pytz})\text{n}][\text{PF}_6]_2$ for imaging cellular

2.5 Click Chemistry complexes as Delivery of a Drug for targeting and therapy Diseases

Currently, the effective tissue-targeting tool is the drug that derivative or carrying by click chemistry due to hyper specificity, speed reaction rate, and rigidity, so it was explored as drug delivery for targeting beside treatment of diseases. Number of scientists have shown that labeling approach is used metabolic glycoengineering and click reaction which is appropriate with the visualization of glycans *in vivo*. Click chemistry involving different uses like tissue-targeted delivery of imaging agents with anti-cancer agents. Specific drug delivery for tumor organ or cancer cell is greatly needed in cancer therapy due to boost therapeutic efficiency with reduce side symptoms. Oh *et al.* described an effective click chemistry probe to synthesize aptamer-polymer hybrids (APHs), by coupling cell targeting a ptamers to block copolymers and in an inactive state that secure a therapeutic payload. The APH enters the host cell through endocytosis upon recognizing the targeted cell-surface marker, at which point the payload is activated to be released into the cytoplasm. Targeted breakdown the tumor cells with doxorubicin is established after visualizing this process with coumarin stain. Importantly, it is possible to generalize this process to produce APHs that directly target various surface markers[24].

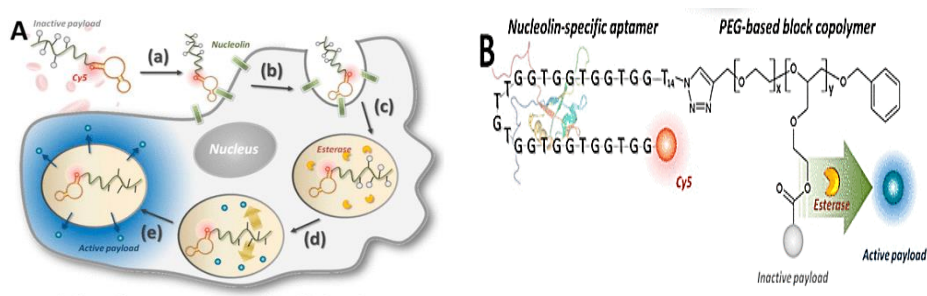
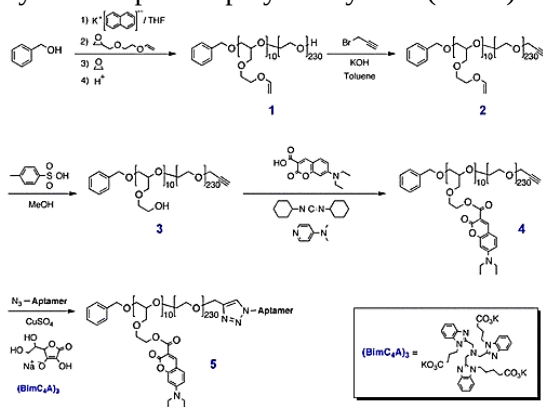


Figure 8. Synthesis aptamer-polymer hybrids (APHs) via click chemistry



Scheme 15. The route of synthesis aptamer-polymer hybrids (APHs)

Wang *et al* investigated diagnosis of tumor approach exploiting metabolic glycol engineering and DBCO drug conjugate, to promote the proliferation of treatment in tumors. This process including the synthesis (synthesized) of the DBCO valinecysteine-doxorubicin conjugate (DBCO-VC-Dox) after degenerated by a cancer-over expressing cathepsin B protease and then liberation of Dox. For the introduction of azide groups into the tumor cell, DCL-AAM was intravenous (IV) inserted by syringe into tumorbearing mice every day for three days (Figure10) [41]. After third dose the DBCO-VC-Dox was intravenous injected directly into DCL-AAMor PBS-treatedmice. As a sequence the quantity of DBCO-VC-Dox in the DCL-AAM-treated group was hyper compared with that of the PBS set in the tumor cell. However, no significantchanges were assigned in the normal tissue in terms of DBCO-VC-Dox accumulation. This work demonstrated that specifictumor labeled azide group increased drug accumulation in targeting tumors and the therapeutic efficiency of anticancer agents via the SPAAC methods [25].

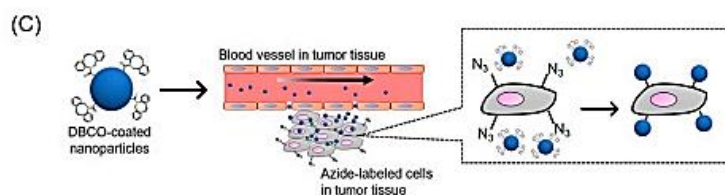
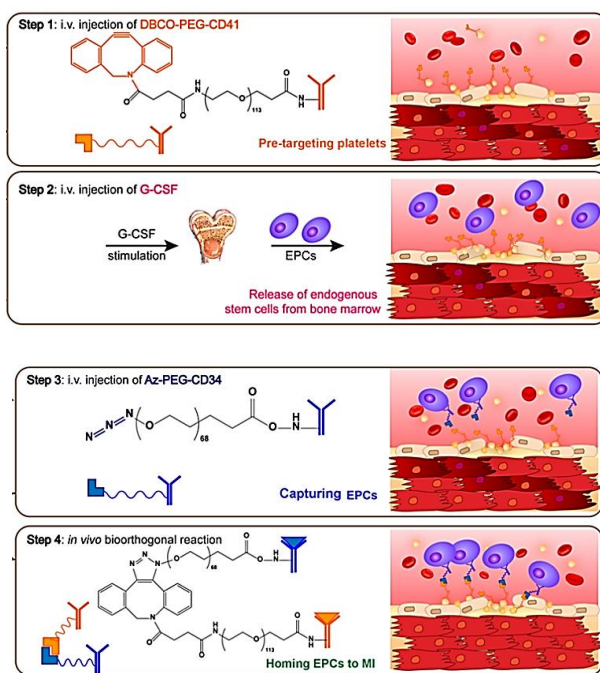


Figure 9. The approach of DBCO-VC-Dox in targeted tumors

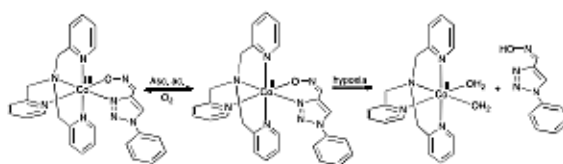
Recently, Li *et al.* developed a new probe based on click chemistry for cardiac cell treatment in vivo. It was designed to repair myocardial infarction (MI) by cell therapy, in this case the injury site in the heart

need a huge numbers of stem cells come to this section[26]. However, they were utilized two groups of antibodies, CD41 and CD34. Both antibodies are joined inside cells and binding platelets with endogenous stem cells. They improved CD41 antibody with PEG & DBCO, and injected it into MI mice intravenously. After that, DBCO/PEG/CD41 binds platelets in blood by their homing ability, which accumulates on the MI region. The mice were then controlled a granulocyte colony stimulating factor to activate and elevate endothelial progenitor cells (EPCs). Azide with PEG-modified CD34 (Az-PEG-CD34) were injected intravenously as well. They connects DBCOPEGCD41 by SPACC in vivo in the MI tissue region, and inducts EPCs to the region for therapy (scheme15). DiR labeled EPCs amount stored in the heart was more increased than five-fold by injection of DBCO-PEG-CD41 and Az-PEG-CD34. Click reaction based pre-targeting route showing improved therapy in term of enhanced fibrosis with increased CD34+cells in the MI section in vivo [27].



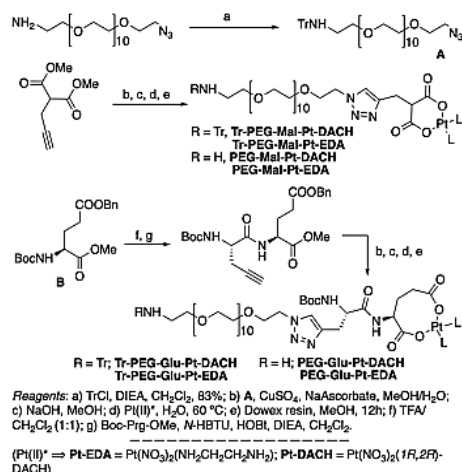
Scheme 16. pre targeting click chemistry/ bioorthogonal chemical reaction

A new approach was developed by de Souza and coworkers using click chemistry. This protocol consisted of synthesis three new complexes of cobalt(III) bearing triazoleligand (E)-11-phenyl-11H-1,2,3-triazole -44- carbaldehydeoxime (HTz) with TPA, py2en and py2enMe2 as prodrugs for hypoxia-activated drug delivery (scheme 16). It has been illustrated that involving of anti-cancer drug to cobalt (III) complexes could minimize their toxic effects. In a hypoxic environment the oxidation state of cobalt (III) reduced to cobalt (II), the active particle is liberated with recycled to its active form in order to destroy the surrounding cells. This report revealed that the ancillary ligand effect on the activity of drugs and complexes become more stable. This work is rationally developed with new application of click reaction based metals which used as chemotherapy in vivo [28].



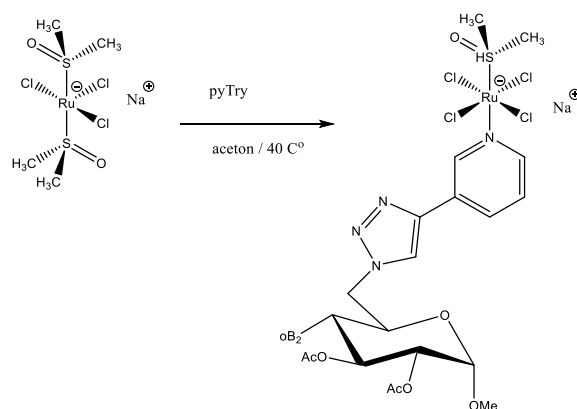
Scheme 17: predictable reaction of 1 and ascorbic acid under hypoxic/ normoxic

Chemotherapeutic drug design is one of the main challenges faced by researchers in biomedical research due to ability of this drug to diagnosis and therapy the tumor cells. Alessandra et al. designed and synthesized four new complexes of Pt (II) (called, PEGGluPt-EDA, PEG-Glu-Pt-DACH, PEG-Mal-Pt-EDA and PEG-Mal-Pt-DACH) with investigated a general protocol from covalently bind them to iron oxide nanoparticles. New complexes of platinum are specially designed to adjust its stability inside endosomal pH to liberate Pt(II) ions and at the same time the protons contribution into the endosomes. This study proved the penetration and the toxic effect of the nano conjugates by using two species of tumor cells and compared these result with the typical drugs and commercially available cisplatin. The cellular findings reveal that the penetration the Pt(II) complexes conjugated to the nanoparticles is always enhanced compared to that of free Pt(II) complexes and it is even great when the uptake is performed in a magnet of section experiment with amagnet placed beneath the cell dish. Among the four MNP platinum complexes developed, Pt-MNP nanoconjugates having Pt linked through a glutamate ligand as the chelating dicarboxylate and having EDA as an unexchangeable bidentate ligand in their structure were the most effective anti-tumoral complexes[29].



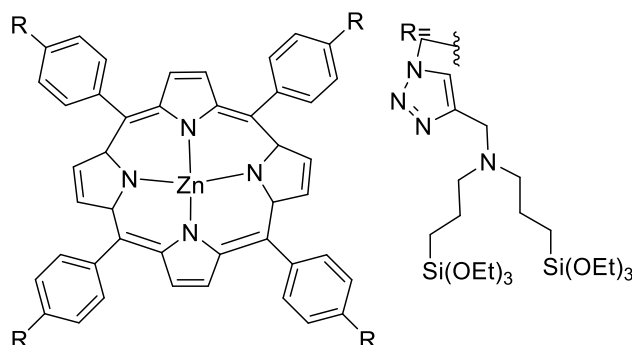
Scheme 18. the synthesis of PEGGLUPtDACH, PEGGIUPtEDA, PEGMalPtDACH and PEGGIUPtEDA complexes

Lately, Walsby's group prepared a new NAMIA analogues as a delivery system. This study suggest to deliver complexes of ruthenium in the deep aqueous compartment of liposomes and to assess the biological activity of two NAMI –A like pyridine analogue and it was revealed that the growth of lipophilicity boosts toxicity in vitro. Specifically, in this work it was exploiting two pyridine isomers of the sodium-compensated analogue of NAMIA, Na[trans-RuCl₄(pyridine)(DMSO)](RuPy) and Na[transRuCl₄(PyTry)(DMSO)] (RuPyTry) (Figure 18). The synthesized Ru-complex bearing pyridine ligand is functionalized with a completely protected sugar moiety to enhance the biocompatibility and increase the ability to penetrate the cell membrane due to the lipophilic moiety in the last step of preparation. In addition, the multiple points of interaction as H-bond donor or acceptor and monitoring the hydrophilic possessions can be afford or added by carbohydrates. The biological data revealed that the ruthenium (III) complex exhibit higher cytotoxic ability than cisplatin. Moreover, the lipoRuPyTry preparation is selective toward the malignant cells also the loading in the liposome allows that metal center acts inside the cell fixed the crossing membrane problem and confirmed that the Ru-complexes could have a potential anticancer activities or Ru-complexes is a promising anticancer drug [30].



Scheme 20. Synthesis of ruthenium complexes bearing the sugar molecule

As to improvement the delivery drug system, mesoporous organosilica NPs were explored for the delivery a Zn(II)-porphyrin analogues (scheme 19). Zn(II)-porphyrin was covalently entrapped in the NPs, and doxorubicin that was loaded into the NP pores by adsorption [31]. The finding displayed potent two-photon fluorescence-imaging and efficient anticancer abilities, but its application in phototherapy was not mentionedit [32].



Scheme 19. Zn(II) complexes bearing triazole ring delivered by delivery system

3. Conclusion

In summary, this review describes the role of 1,3-dipolar cycloaddition of alkynes & azides, as a potential synthetic tool with its applications in nearly all areas of modern chemistry and clinical and preclinical pharmaceutical research. Click reactions are easy to perform under ambient conditions, give high reaction yields, are regiospecific, and are tolerant to standard biological conditions and most of the functional groups. This review has also highlighted the potential uses of the 1,2,3-triazole complexes as a bioisostere and a linker for the covalently and rapidly coupling of diverse building blocks for the synthesis of different clinical therapeutic agents. We have also emphasized the enormous potential of the triazole-forming click reactions for generating compound libraries. The click reaction and its complexes are promising to use as candidate drug to be more effective and (less) toxic than cisplatin to treat tumor cell.

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Conflict of Interest

None of the authors has any conflict of interest

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