

# Conjugates toward medicines

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## ABSTRACT

The more and more drugs in basic research, drug discovery, and drug development have been diverting to various types of structural perspectives, in which conjugated constructions emerge with remarkable functions superior to conventional drugs. These conjugated drugs include small molecules and macro compounds. The main difference between conjugated and conventional structural modifications is multi-functionalities. These multi-functionalities combine one or more bioavailable moieties: a drug

segment, a ligand segment, and a linker segment. The drug segment plays the critical therapeutic role, the ligand segment is in charge of the selectivity, and the linker segment connects the drug and the ligand together. The simplest conjugate would be bi-functionalized molecule that biologic properties has been changed upon intent design. This review will summarize the most recent advancements in the medicinal conjugations under development and at various other stages of development as they are investigated as for novel drugs, drug candidates, and drug leads.

**Key Words:** Conjugation; Linker; Multiple functions; Pre-drug; Drug delivery; Drug targeting; Antibody; Nano medicine; Aptamer

In drug discovery and development, it is impractical to test large quantities of drug candidates during clinic studies because that the expense of the clinic studies would be enormous. Only those possessing comprehensive properties as drugs should be moved to this stage, which is largely relied on structural modification or optimization. Conventional drugs are composed of single molecule or macro compound, or various size compounds in between. These drugs, such as active pharmaceutical ingredient (API), have to be subjected accordingly to delivery techniques. In the last few decades, more and more drugs in basic research, drug discovery, and drug development have been diverting to various types in structural perspective, in which conjugated constructions are emerging. In principle, a conjugated constructed drug is composed of three parts, the therapeutic part or the 'warhead', the additive segment or ligand, and the linker. The therapeutic part is the "warhead" for the conjugated constructed drug. The additive segment or ligand possesses specific functionalities upon the purpose of the designs. The linker tethers the first two moieties together. The linker can be classified as degradable or non-degradable. Degradable linkers are relatively chemically and enzymatically sensitive either out of the cell or in the cytoplasm or lysosomes, including pH responding hydrolysis or proteolytic dissociation, to cleave the therapeutic segment from the parent conjugate. In contrast, a non-degradable linker resulting no dissociation so that the entire compound behaves as a whole therapeutic entity.

## LITERATURE REVIEW

The features of degradable and non-degradable linker depend on whether the therapeutic agent remains stable when and after the conjugate binds to the target. The creation of a clinically successful conjugated medicine has been challenging. For the therapeutic to work well, each of the parts must be carefully considered in the molecular design (1). The most common bioconjugations are small molecule (such as vitamins, fluorescent dyes, cytotoxine, etc.) coupled to protein, oligonucleotides, carbohydrates, polymers, metals, or antibodies (2). A number of antibody-drug conjugates (ADC) used to target tumors named vaxculature, Brentuximab vedotin and Gemtuzumab ozogamicin are active areas of research in the pharmaceutical industry (3). In contrast to peptides and/or proteins, molecules like oligosaccharides, nucleic acids, synthetic polymers, carbon nanotubes, or polyethylene glycol used as bioconjugations are relatively inexpensive, but their cost are increasing rapidly (4). Bioconjugation has also gained importance in nanotechnology applications, such as in bioconjugated quantum dots (5,6). In this review conjugated molecules are classified into following sections in terms of conjugate medicine. It will be shown that conjugate distinguishes is not so strict in between but crossed related.

## CONJUGATION CHEMISTRY

The constitution of conjugate has been resolving by various of approaches in chemistry. Different linkers have been constructed to connect small molecule drugs to other small or large molecules. The conjugation chemistry regulates the comprehensive profiles of the characters, nevertheless, it is essential to the safety as well as efficacy of the conjugate. Antibody-drug conjugate has been used as medicine, and typically represents conjugate drugs. The requirement to design an ideal conjugate is it has to be relative stable throughout its active time before and after administration. The ligand binds to the surface of the target cell to ensure that the drug can be delivered to the initial site of interaction, and eventually internalized into the cell.

The most well developed approaches of conjugation chemistry are highly dependent on the type of functional groups on the back bone or core frame of the moieties. The functional groups provide sufficient flexibility and distant between the moieties so that not to interfere with the function of the therapeutic site. However, as a whole entity, the rigidity must be considered to ensure the synergistic or coincident interactions between the ligand and the receptor, or conjugate and the target.

In peptide and protein conjugation, cysteines and lysines are reacted with hydrazones, disulfides, and thioethers, because these functionalities can serve as good nucleophiles. In the developments of ADCs, more than half of the thiol groups of the cysteine residues, with one quarter conjugated through surface lysines (7), so does lysine residues, which make the conjugation heterogeneously.

The conjugating conditions are required to have a high conversion rate (>99%), faster, milder, with good solubility, particularly in biologic environments. Extensive studies have been performed on the conjugating conditions during the last few decades. On the other hand, the conjugated bonds must be easily cleaved whenever not needed *in vitro*, but the bonds must be stable enough to allow for controlled cleavage.

The emblematic reactive substances and intermediates in conjugation are summarized in Figures 1 and 2. Some of the common intermediates for coupling bears NHS-, and Sulfo-NHS-active esters. One of the advantages of these groups is the availability in aqueous solutions (Figure 1). Amide formation is the most conventional coupling approach along with primary and/or secondary amine nucleophile coupled to active esters, or cyanates, or carbonyl derivatives (Figure 1). Activated acyl derivatives of carboxylic acids can react with target nucleophilic groups to give the acetylated products listed below (Table 1).

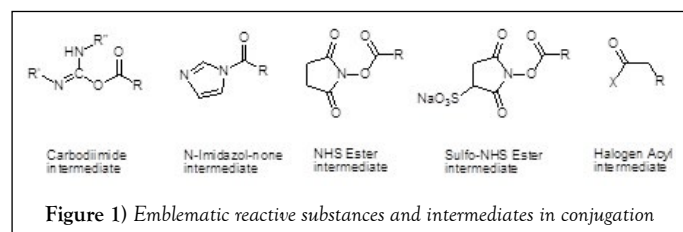
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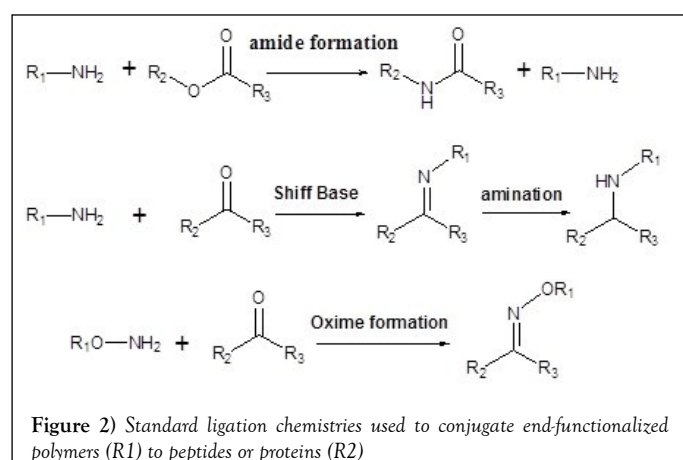


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In addition to conventional approaches, a few novel techniques for selective reactions resulting in homogeneous conjugated productions have been developed, providing additional options under different conditions (8), which have the potential to provide the products with improved therapeutic properties, particularly for macromolecules (9).

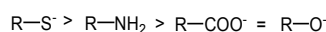
Among these, Staudinger ligation are a relatively new development that uses phosphorene, but its effects on biological processes is not clear (8). Click chemistry provides an even more convenient approach to conjugating because of its reaction conditions are more feasible both in aquatic and organic solvents. Nevertheless, this 2+3 cycloaddition creates triazole ring that may elicit immunogenic interfering (10) along with undesired antigenic.



Another valuable reaction in peptides or proteins is thioester-linked intermediate product undergoing subsequently a S→N-acyl rearrangement, giving a common amide bond at the reaction site. The ligation is highly specific and hence, only a single product is produced, even additional cysteine residues presented. This method typically requires a N-terminal cysteine at the ligation site, but recently it was demonstrated that auxiliary groups like N-ethanethiol, N-2-mercaptobenzylamine or N-(1-phenyl-2-mercaptoethyl) sulfhydryl can also be used (11-13). The auxiliary groups act as surrogates for the N-terminal cysteine and facilitate the chemical ligation, making ligation possible at a non-cysteine residue (11,14). Nucleophilics and additions molecules are summarized schematically below (Figure 3).

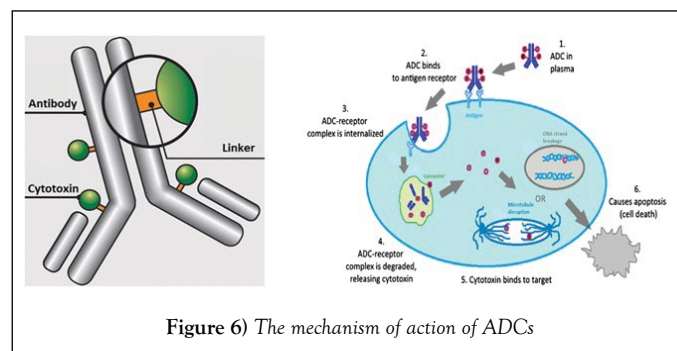
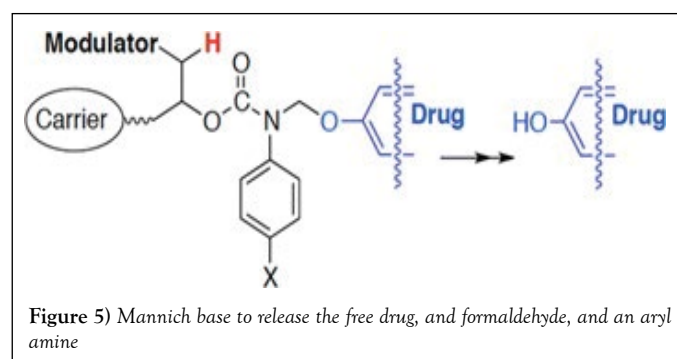
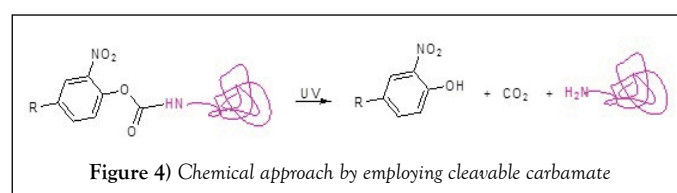
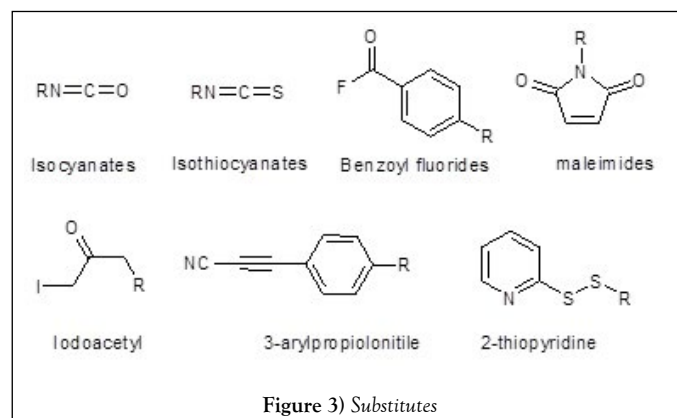
In actual application, selective N-terminal conjugation bases on the different reactivity of internal and terminal amino acids. Site-specific functionalization can be realized by enzymatic approach in terms of bioengineering, incorporated with unnatural amino acids, carbohydrates at desired positions to react with functional groups like azides, ketones, aldehydes, or alkyne (15), which are enable the use of novel conjugation chemistry, enzymatic ligation and the click chemistry for instance (16).

Nucleophilic strength:



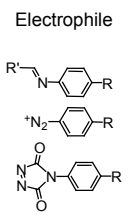
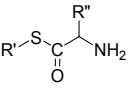
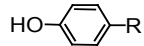
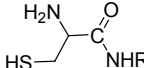
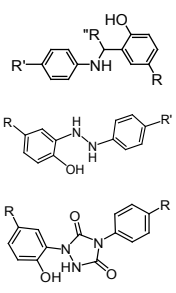
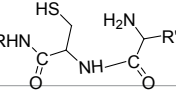
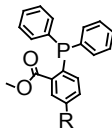

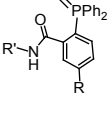
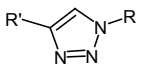
### SUMMARY OF LINKERS

Linkers not only act as connection, but they may also affect biological activities. Lipid chains show remarkable cell internalizing characters, because it is compatible with cell membranes and easily inserts into cell due to its high hydrophobicity. This forces the other parts of the conjugate to be internalized by way of diffusion. On the contrary, polyethylene Glycol (PEG) enhances hydrophilicity, and increase the water solubility of the conjugate. Both types of linkers may be flexible chains, and may impact the stability of active structure, i.e. insisting protease, nuclease. Linker chains with disulfide bond can be cleaved by internal cell glutathione or reductants. Ester bonds are biodegradable and they are easily hydrolyzed by enzymatic disassociation with lipase.



A new chemical approach by employing cleavable carbamate group extended the life time of the conjugate, which provides more options to design a conjugate (17), as showed in Figure 4. In this structure, linker undergoes  $\beta$ -elimination to release the active drug that could be small or big, it can be easily broken down by UV light. Such photo responsive conjugation empowers the development of novel method for drug discovery, disease diagnosis, and high-throughput screening. However, it requires amino group on the drug to introduce the carbamate, nevertheless, not all small molecules have amine. Carbonate and nitrile phenol ester had been then developed for a versatile cross-linker that can be used for a wide range of applications where photocleavage ensures clean and controllable release of biological active molecules from the conjugate. One profound design was adapted the similar mechanism; carbamate linkers can connect phenolic hydroxyl groups. The methylene is necessary in between the drug segment and the carbamate nitrogen that is also connected to an electron-withdrawing group to stabilize the carbamate structure from pH sensitive dissociation, and causes  $\beta$ -elimination, (Figure 5) intermediated a Mannich base to release the free drug, and formaldehyde, and an aryl amine (18,19).

**TABLE 1**  
**Emblematic reactive substances and intermediates in conjugation.**

Nucleophile	Substitute	Product	Medicating
R-NH <sub>2</sub> , R-NH-R, R-OH, R-SH, R-NHNH <sub>2</sub> , R-ONH <sub>2</sub>	Carbodiimide N-Imidazol- none, NHS Ester, Sulfo-NHS Ester, Halogen Acyl, Urea, Thoreau, Cyanates	Amides conjugates, Esters conjugates, Thioesters conjugates, Hydrazides of small molecules, Carbohydrates, Nucleotide and nucleosides	
	Aldehyde, Ketone	Oxime Diazenes, Diazenes Imines or Schiff Base	
Electrophile  Thioester and Cysteine terminated ligation 	Tyrosine Derivatives  	 	Pro-drug Targeting Delivery Imaging
Azide and Alkyl terminated 3+2 R-N <sub>3</sub> Staudinger ligation <sup>(7)</sup> Click Chemistry	 	 	Vaccine Therapy

## DISCUSSION

In drug development, it is not so fruitful to discover a bioactive molecule that may be a good candidate or lead compound in medicine development. One up-to-date solution that has been used during the last decade is conjugated medicines that consist of modified generic drugs with less drawbacks than the current treatments. In these conjugated medicines, modulators, linkers, and therapeutic heads are chemically connected through covalent bonds. The objectives to design these conjugates are summarized below. There may be no significant distinction between these classified categories, these compounds have crossed characteristics and activities.

### Pro-drugs

Pro-drugs are intensively modified therapeutic molecules that consist of multifunctionalities. These pro-drugs are coupled to at least one additional moiety to form a conjugate. These compounds are able to dissociate or release the therapeutic moiety after delivery to prevent the original therapeutic problem. As a consequence of this, these conjugates have been widely used in generic drug development. Conjugated functional groups may improve biological properties of the molecule, such as bioavailability, pharmacokinetics, pharmacodynamics, metabolism, stability, and targeting. Using anti-cancer drugs as an example, an antibody and cytotoxic reagent conjugated through a linker have specific impacts to the overall therapeutic efficacy, including the process of internalization and the efficiency of intracellular transferring of receptor-conjugate complex. In designing anti-cancer conjugate, the tumor specific metabolites result ADC dissociation. Trastuzumab emtansine is an example of such an anti-cancer prodrug, it is dissociated in the cytoplasm of tumor cell (20). So far, three ADCs were approved by FDA: Adcetris® (brentuximab vedotin developed by Seattle Genetics), Kadcyla® (ado-trastuzumab emtansine developed by Genentech), and ZEVALIN® (ibritumomab tiuxetan developed by Spectrum Pharmaceuticals) that was used to treat certain non-Hodgkin lymphomas, differs from the previous

two in that rather than delivering an anti-neoplastic payload to cancer cells, it delivers yttrium, a cytotoxic radioactive compound directly to the cancer cells. A fourth pro-drug, Inotuzumab ozogamicin, was approved by the European Commission, it is a monotherapy to treat adult patients suffered by refractory or relapsed CD22-positive B-cell precursor acute lymphoblastic. Inotuzumab ozogamicin has also been approved recently by FDA (21).

In recent years there have been about 40 ADCs in clinical trials (22). Lonza Group Ltd. (Basel, Switzerland) has been able to start large-scale production of antibody drug conjugates for an emerging class of products utilized mainly for the treatment of cancer. ADCs are made by coupling highly active chemical substances with a biotechnologically manufactured antibody (23). The mechanism of action of ADCs is that the antibody portion of an ADC hones onto a cell-surface antigen that is specific to a certain type of cancer cell. Upon binding, the ADC-antigen protein complex becomes internalized into the cancer cell. When the complex is degraded in the lysosome, it releases the cytotoxin which then binds to its target to cause cancer cell apoptosis (24). This mechanism is elucidated as Figure 6.

Cholic acid and peptides conjugates are one type of pre-drugs that are designed and investigated for peptide drug oral delivery from our study. These compounds have demonstrated that the ester bond linking the bi-functional moieties are internalized into the cancer cell membrane and then dissociated efficiently (25), suggesting that the peptide drugs may be administrated through the gastrointestinal track by employing specific transporter mediated delivery, such as cholic acid receptor. Similarly, another example of a pro-drug consists of lipid conjugated drugs in our project were able to penetrate into brain cells in an *in vivo* trial, on which (26) the conjugates are connected with lipid chains or cholesterol hydrophobic group, made up of 10 borane cages for positron capture therapy against brain cancer.

### Polymeric and nano medicines

Covalent attached synthetic polymers are another type of conjugates in

medicine. Nucleic acids, peptides, proteins, enzymes, carbohydrates biologic molecules, even viruses are most likely in the nano scale. Because of the similarities in macro molecular mass and geometric size, polymeric and nano medicines are classified as one family. Polymer therapeutic is used as a general term because its major character is bioactive. These large conjugates are not encapsulated, but connected covalently to the polymeric backbone in various rate and positions (27,28), thus they differ in molecular structures in apparently large range (29-31).

In contrast, thermosensitive nanocarriers are the “smart” drug delivery systems that have shown tremendous promise in the field of controlled drug delivery due to their special property. Thermosensitive nanocarriers have long circulative features, they can accumulate in pathological sites because of increased permeability and retention induced by attaching target ligands onto the surface of the nanocarriers, the conjugate dissociating rates can be adjusted in response to thermal variability of the environment. In this case, the therapeutic drug is encapsulated in the aggregated particles rather than covalently connected. These drugs can contain particles such as liposome, micelle, poly cross-linked matrix, or dendrimer matrix that are sensitive to temperature (32). Polymer bioconjugates can be typically classified into four main strategies or approaches: first, the coupling strategies, in which biological segments are covalently linked to preformed polymers using one or multiple reactive sites; second, the macroinitiator strategies, in which the synthetic polymer segment is grown *in situ* from a biological structure, third, the inverse approaches, in which the biomolecules are constructed on a preformed synthetic polymer, and the fourth, the monomer approaches, which rely on short polymerizable bio-segments (33-35).

Schematic illustration of the four principal strategies or approaches towards polymer bioconjugates nanotechnology is showed in Figure 7. These approaches are related via synthesis and usage of a wide range of nanomaterials. These nanomaterials are crystalline allotropic forms of carbon such as fullerene, nanotube, graphene, carbon, or nanodiamond. These materials represent an important source for medical applications (36-39).

#### Synthetic vaccine

Vaccines are traditionally produced from attenuated or deactivated organisms, which are subjected to constitute vaccine formulations for clinic treatment. If a vaccine is deemed successfully, it has to meet three important criteria, first, it must be able to elicit immune response, and provide long-lasting protection (40); second, it has to be safe and effective in vaccinations; third, it should be moderately stable (41). Additionally, a vaccine or an antigen has also to be delivered to particular immunogenic cells to elicit immune response. In the design of the delivery system, it has been considered to prevent induced possibility of diminishing immunity or immune tolerance (42). Since the mutations have been developing always in different pathogens, including cancer cells, accordingly the regeneration of the new antigens is necessary. Synthetic or artificial vaccine is considerable to be manipulated due to the molecular antigen enables to conjugate to carrier protein chemically, giving an opportunity to evade this problem. It sights climax of the progress that has been succeeded to evolve synthetic or artificial vaccines by using various molecular antigens. The approaches to design conjugate vaccines are adaptable by employing large molecules like peptide/proteins, carbohydrates or small compounds as antigens (43). A latent problem associated with synthetic vaccines would be that these vaccine conjugates may not need to be necessarily cleaved.

Carbohydrate has been well known as the major immunogenic epitope in extensive vaccines design. The molecular immune properties of glycoconjugate vaccines has heavily count on the structure of carbohydrate. A carbohydrate segment with a well-defined  $\beta$ -(1-3) glycan conjugates to the predetermined tyrosine or lysine residues onto the carrier protein CRM 197 is a successful anti- *Candida* glycoconjugate vaccine (10).

Higher molecular mass and larger geometric size make the conjugated molecules behave immunogenicity that can be designed rationally. On the assumption that the larger molecules as nanoparticles are able to target dendritic cells, and delivered to draining lymph nodes so that they can induct faster immune responses. It is the key to develop new generation vaccines to use conjugated structures inducing the antigen-specific cellular response.

Numerous antigens have been linked to small or larger ligands in order to develop vaccines that are modified chemically to introduce immunogenic moieties. The advantages of this type of vaccines are manipulative. It can be rationally designed, and synthesized chemically on a large scale with less cost. They can also be diversified in parallel and combinatory manners into potential drug candidates by widely screening.

Nanoparticle vaccine development involves using a template technique for the preparation of nanoparticles. Besides, nanoparticle conjugated antigen traffic systems have been used to develop new generation vaccines (44). Efforts are in the works to design glycosylated peptides and mini protein segment (Figure 8) synthetic vaccines composed of multivalent antigens or antigenic functional groups in the treatment against HIV-1(45-47).

#### Imaging reagents

In the diagnostic applications, fluorescent imaging of biological systems in the second near-infrared window can probe tissue at centimeter depths and achieve micrometer-scale resolution at depths of several millimeters. It has been reported that recombinant human SUMO E1 proteins have shown to be a probe to target overexpressing hEphA2 in cancer cells. Another conjugated compound has also been developed to serve as a specific optical imaging agent, so called Renilla luciferase variant 8 and green fluorescent protein (EGFP) (48). High levels of uptake of even simple PEGylated-CH1055 conjugated dyes have observed in brain tumors in mice, on which PEGylated tails acts a key role to help the drug cross the brain blood barrier. This compound applies also molecular imaging of tumors if connected to anti-EGFR antibodies (49).

Another type useful conjugate is nanoparticle magnetic resonance imaging (MRI) negative agent, its major effort is to increase cell-loading capacity but maintain less cytotoxicity. Recent reports have shown that synthesized gold/iron bimetallic nanoparticles have been able to deposited on the surface a matrix, its diameter is about 4 nm. Phantom MRI image has revealed these nanoparticles versatility of providing capabilities for conjugate diagnostics, therapeutic applications, as well as imaging contrast reagents (50,51). Precautions must be considered when trying to adopt heavy metals into nano-conjugates as medicines because of their metabolic and toxicity issues.

Poly ethylene glycol (PEG) coated living cells lead to reduced immune responses in blood transfusion or tissue transplantation. Such behavior was previously reported for red blood cells, T-lymphocytes or pancreas islets (52). In Figure 9, it was displayed that an islet surface modified by the coupling PEG that possesses an active terminal ester.

Synthetic strategy for modifying the surface of pancreas islets with poly (ethylene glycol) (PEG). Images A and B show the morphology of the cells

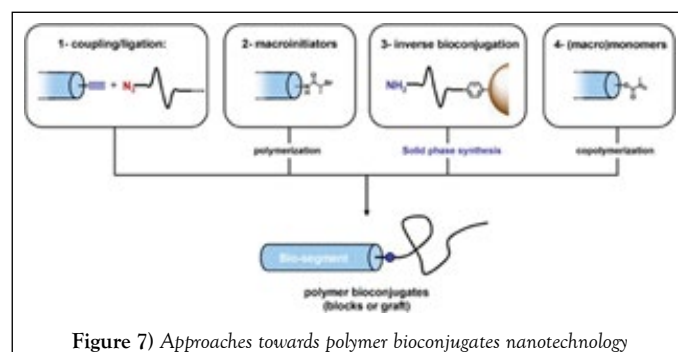


Figure 7) Approaches towards polymer bioconjugates nanotechnology

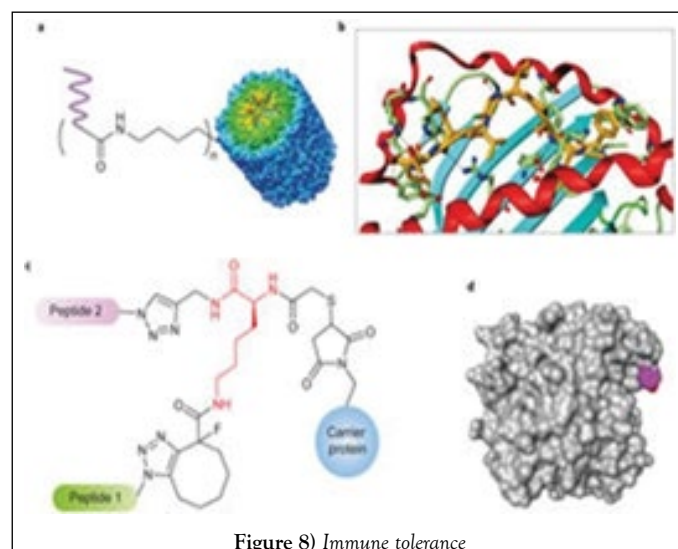


Figure 8) Immune tolerance

before and after PEGylation respectively. Image C shows a confocal laser scanning micrograph after conjugation with a fluorescein labeled PEG.

**Drug delivery**

Drug delivery is the vital application for conjugated compounds. Small nanoparticles made up of DNA conjugated by a complex of biodegradable poly  $\beta$ -amino ester with PEG can penetrate orthotopic brain tumors as well as healthy brain parenchyma rapidly, this rapid diffusion of DNA-loaded nanocomplexes have been observed in non-cancerous tissues *ex vivo*. It was demonstrated that the nanoconjugates evade adhesive trap in brain due to the dense of PEG coating (53). As we well know that pharmacokinetic properties can be dramatically improved by conjugation technique. It has been reported that homogeneous 8-loaded ADCs with drug linkers designed to minimize hydrophobicity has been administered without impairing antibody pharmacokinetics. The drug-antibody ratio (DAR) in the conjugate enhance *in vitro* efficacy, so does the plasma clearance. While it also reduces *in vivo* efficacy and exposure however. The hydrophobicity of this ADC is favorable to accelerate clearance, this function owes to modulation of drug-linker design, hydrophilic linkers and PEG connected ADCs with high DAR are superior *in vivo* study (54).

In a recently reported micelle formula approach, the drug warhead was not attached by a covalent bond. The matrix metalloproteinase (MMP)-responsive peptide GPLGVRGDG was introduced into the block copolymer via click chemistry, and ring-opening polymerization to prepare PEG-GPLGVRGDG-PDLLA. This complex was then self-assembled on to micellar nanoparticles (NPs) in load paclitaxel (PTX). The resulting complex was treated with MMP-2 which triggered dePEGylation due to cleavage of the peptide linkage. Moreover, the residual VRGDG sequences are retained on the surface of the NPs after dePEGylation, which facilitates in cellular uptake. The cytotoxicity of PTX loaded in the NPs against 4T1 cells was significantly enhanced (55) (Figure 10).

In the next bacatin generation molecules, the PTX wrapped liposomes composed of RGD-PEG-Chol were constituted by a method of film formation. The liposome size is about 100 nm; this leads to enhancement of cytotoxicity of paclitaxel to B16F10 cell significantly. Flow cytometry revealed the increasing cellular uptake of drug encapsulated in the liposome. Using peptide modified liposome provides a supplementary practicable approach to prepare these materials that are controllable in performances as new drug delivery method (56).

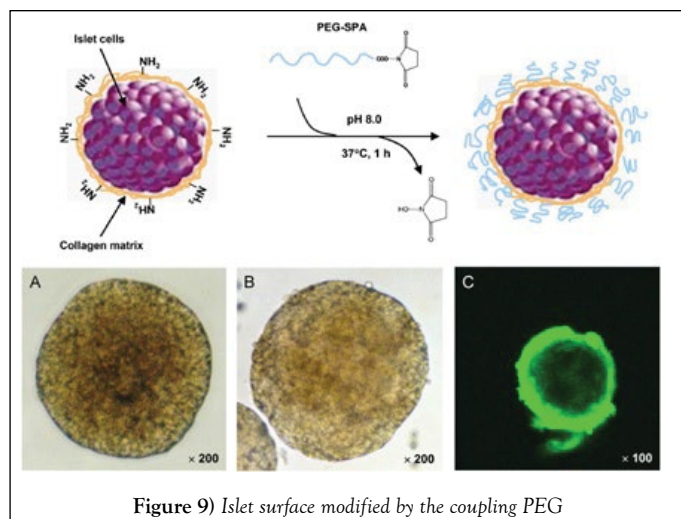


Figure 9) Islet surface modified by the coupling PEG

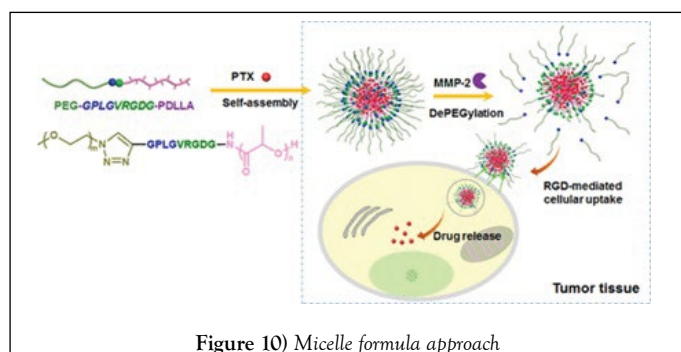


Figure 10) Micelle formula approach

**Drug targeting**

As described above, antibody-drug conjugates are exceedingly effective drugs designed for targeting therapy of cancer (57). Comparing with traditional chemotherapy, ADCs are more specific to cancer cells, and more safe to normal cells (58-61). These conjugates family has impeccable structures that refers to ligand based molecular design for conjugate medicines (62). Because the healthy cells are less severely affected it would be a good choice to against cancer by using these conjugated molecules (63,64). One more representative ADC is CD46 antibody connected to the microtubule inhibitor, monomethyl auristatin F. Figure 11 shows toxicity specifically against myeloma. This conjugate has also been revealed the potent to treat primary multiple myeloma *ex vivo* (65). The distinctive sizes of moieties in ADCs and the conjugating ratio and connecting sites can effect entire molecule's behavior. Mylotarg is the first ADC approved by FDA, which is a conjugate between a recombinant large IgG4 kappa antibody and a small cytotoxic antibiotic-calicheamicin via a bifunctional linker of N-acetyl-gamma calicheamicin. This conjugated molecule has about 50% of loaded antibody, five folds' moles of calicheamicin than antibody (66).

**Therapeutic reagents**

As full entity, protected and specific delivery of nucleic acids to malignant cells remains a highly desirable approach for cancer therapy. Therapeutic efficacy of a tenfibgen (TBG)-shell nanocapsule for tumor-directed delivery of single stranded DNA/RNA chimeric oligomers shows to target CK2 $\alpha\alpha'$  and xenograft tumors in mice. This encapsulated chimeric oligomer targets CK2 $\alpha\alpha'$  suggesting the development of a systemic treatment of prostate tumor with significant promise (67).

It was reported recently that the monoclonal antibody cetuximab targeting epidermal growth factor receptor (EGFR) was conjugated to cisplatin (cis-diamminedichloroplatinum), it was proven to treat brain tumors by targeting human EGFR. It behaves as a full drug and still can undergo dissociation at the binding position to release cisplatin (63).

Peptide-based drug is an another promising therapeutic approach for cancer, which can provide increased selectivity and decreased side effects as compared to current anticancer drugs. A daunorubicin- Gonadotropin-releasing hormone-III (GnRH-III) conjugate complex attached to the 8Lys of a GnRH-III derivative via an oxime linkage has proved to have significant benefits over free daunorubicin that typically has be administered at the maximum tolerated dose *in vivo* to have an antitumor effect on subcutaneously developed HT-29 colon tumors. GnRH-III peptide represents a suitable targeting moiety, in the treatment of hormone independent tumors that highly express GnRH receptors (Figure 12) (e.g. colon carcinoma) (68).

As a small conjugate, haluronan conjugates was designed to enhance cytotoxic drugs selectively penetrating hyaluronic acid (HA) receptor-expressing cancerous cells. As an example, HA-paclitaxel conjugate was used to treat intraperitoneal of ovarian cancer, it can be distributed all over connective, neural tissues, and epithelial (69).

In our study using neutron capture cancer therapy, boron 10 cluster-lipids and cholesterol conjugates were employed. The conjugates were efficiently transported into brain cell by undertaken liposome formulations (26). High boron content liposomes are promising antitumor effect for neutron capture therapy, which has developed the distearoyl boron lipid (DSBL) liposomes as a vehicle for neutron capture cancer therapy. Significant antitumor effect was observed in mice injected with Mercaptoundecahydrododecaborate (BSH)-encapsulating 10% DSBL liposomes even at the dose of 15 mg/kg. The tumor completely disappeared after three weeks of thermal neutron irradiation (70).

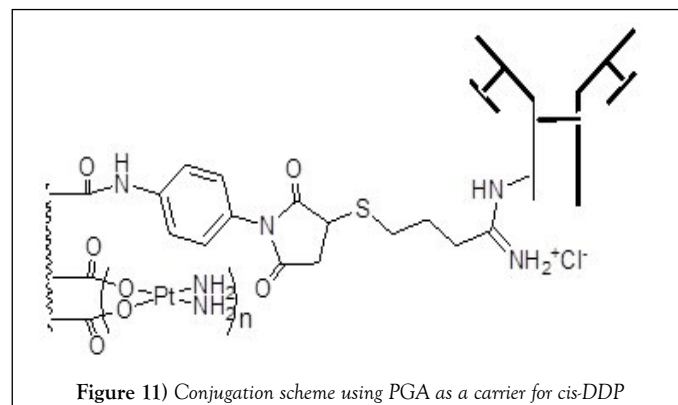
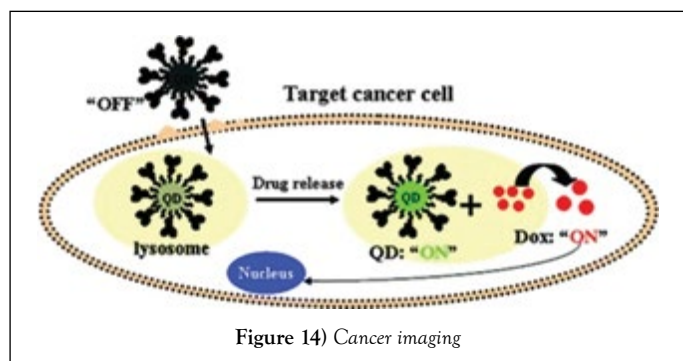
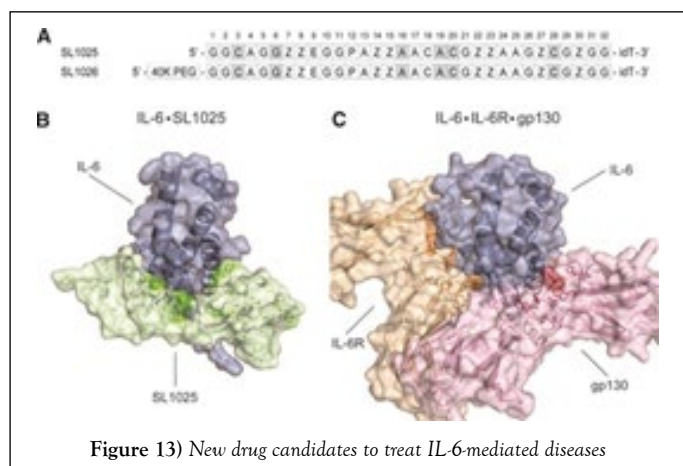
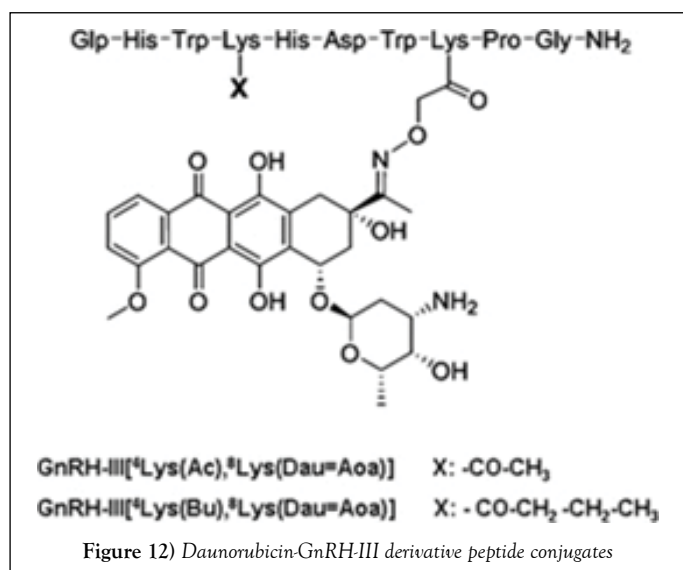


Figure 11) Conjugation scheme using PGA as a carrier for cis-DDP

Except for anti-cancer reagents, anti-microbial conjugate medicine is also critical in drug discovery and development. When a fungus *Penicillium citrinum* fermented mass was conjugated with silver to produce nano-bioconjugates (SNBCs), it is an eco-friendly process to synthesize a stable silver nanobioconjugate with antibacterial properties. It was found that SNBCs exert antimicrobial activity against Gram positive and Gram negative bacteria and fungus (71).

As mentioned before, lipopeptide is a new family of potent versatile bioconjugates acting with a variety of phytopathogens, and is considered environmentally acceptable. Lipopeptides are nontoxic, biodegradable, highly stable, ecofriendly, and nonpolluting biomolecules, therefore they have wide applications in plant disease management, in cosmetics, food preservation, as surfactants, anti-parasitic, anti-viral, and anti-tumor/cancer agents. This compound seems to be a promising biopesticides in agriculture as it can be used to replace harmful chemical pesticides and a potent tool to overcome chemical resistance of phytopathogens (72).

Interestingly, three types of Bacillus lipopeptides, Surfactins, Iturins and



Fengycins, have expressed antagonistic activities within a wide range of phytopathogens such as bacteria, fungi, and oomycetes. Both Fengycin and Iturin are active against fungal, however Surfactins are active against broad spectrum of bacteria, and they are classified to larvicidal agent (73). The first cyclic lipopeptide antibiotic Daptomycin was approved by FDA in US for the treatment of serious infections in blood and skin in 2003 (74). Now, it has been approved in more than 70 countries.

#### Aptamer or oligonucleotide

Oligoaptamers have attracted ample interest in the areas of nanotechnology, biology, and biochemistry, medicines over the past decade (6,75-77). One of the modification on monomeric building block is 2'-amino-LNA that has improved the therapeutic property of siRNA linked with aptamer (78-80), on which N-acetylgalactosamine and Galactose serve as binding ligands of asialoglycoprotein receptor (81,82).

In aptamer family, many conjugates in terms of 'Aptabody' have been developed for various medical applications. Among which, aptamer-hybrid nano-conjugate delivery technique was designed to selectively transport miRNA-29b to MUC1-expressing carcinoma cells. These novel aptamer nano-conjugates could serve for intracellular delivery of miRNAs to cancer cells to improve the therapeutic consequence of lung cancer (83,84). Another modified slow off-rate aptamer (SOMAmer) with high binding affinity (K<sub>d</sub>=0.2 nM) inhibits the signaling pathways of IL-6-dependent cell (85), it was screened from large random libraries. The structural properties are effected by hydrophobic functional groups including benzyl-, 2-naphthyl-, or 3-indolyl-carboxamide, linked at the 5-position of uridine via a carboxamide bond (86). The hydrophobicity plays essential role of interactions with target molecules, and accordingly to form fresh secondary and tertiary intramolecular structural motifs (87,88). Except for the improvement of binding affinities, SOMAmer enhances nuclease resistance, and the selection rates as well (89).

Similarly, SL105 and SL1026 are potent antagonists of the IL-6 signaling aptamer-small (PEG)

molecular conjugates, they represent new drug candidates to treat IL-6-mediated diseases as shown in Figure 13 (90).

A new developed conjugate, Dot-aptamer-doxorubicin (QD-Apt(Dox)) is so called quantum dot conjugate. It acts as cancer imaging, therapy, and sensing compound. A10 RNA aptamer conjugates with fluorescent QD, which recognizes the prostate specific membrane antigen (PSMA) on the extracellular domain, targets the imaging reagent (QD-Apt) to prostate cancer cells that express the PSMA protein (Figure 14).

Once QD-Apt then conjugates with cytotoxic drug, Dox, forms antineoplastic anthracycline drug and fluorescent multi-functional properties, in which, the double-stranded stem of the A10 aptamer acts targeting. The mechanism is the fluorescence resonance energy transfer within QD and Dox, and quench between Dox and aptamer. This multifunctional nanoparticle design is able to specifically deliver cytotoxicity to prostate cancer cells, and sense the fluorescence of QD for either cancer imaging, or therapy (91).

#### CONCLUSION

In this review, conjugated drugs have been classified as a group of medicines or new generation of medicine candidates from the standpoints of chemistry and the medications.

In the conjugated drugs, the original therapeutic compounds or new discovery leads may be administered by using advanced conjugate formulating techniques. Combining with chemical modification and rational design of the conjugation, the efficacies and safety of new and generic drugs will be potentially enhanced. Particularly small molecular conjugated drugs have certified that their modifications do not affect the core structures in terms of warheads. Because of multi functionality, the advantages of a conjugate drug may not simply only improve chemical, biologic and physic properties, but also create simultaneous interaction mechanisms which may highly benefit the efficacy of the drug from various perspectives.

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