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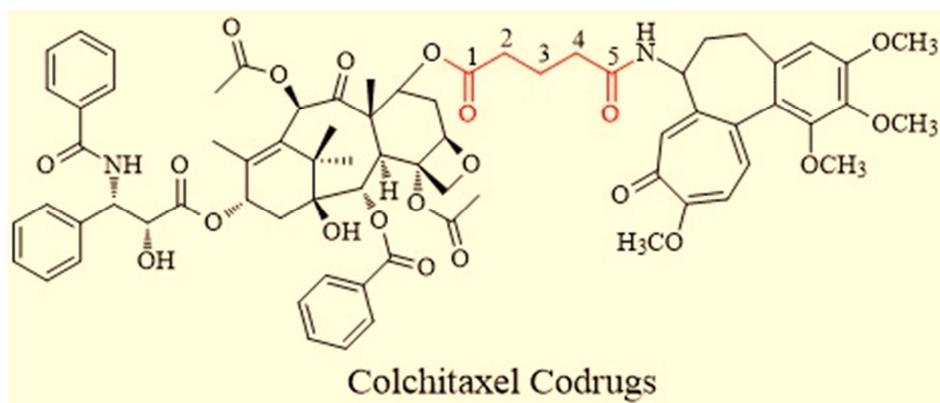
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## Colchicine Prodrugs and Codrugs: Chemistry and Bioactivities

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### Abstract

Antimitotic colchicine possesses low therapeutic index due to high toxicity effects in non-target cell. However, diverse colchicine analogs have been derivatized as intentions for toxicity reduction and structure-activity relationship (SAR) studying. Hybrid system of colchicine structure with nontoxic biofunctional compounds modified further affords a new entity in chemical structure with enhanced activity and selectivity. Moreover, nanocarrier formulation strategies have been used for colchicine delivery. This review paper focuses on colchicine nanoformulation, chemical synthesis of colchicine prodrugs and codrugs with different linkers, highlights linker chemical nature and biological activity of synthesized compounds. Additionally, classification of colchicine prodrugs based on type of conjugates is discussed, as biopolymers prodrugs, fluorescent prodrug, metal complexes prodrug, metal-labile prodrug and bioconjugate prodrug. Finally, we briefly summarized the biological importance of colchicine nanoformulation, colchicine prodrugs and codrugs.

**Keywords:** Colchicine, prodrugs, codrugs, biological activities.

### 1. Introduction

Colchicine (Fig.1), an alkaloid of secondary metabolite, has been known in 1820 after Pelletier and Caventou separated it from poison plant *Colchicum autumnale* [1, 2], rough extracts have been utilized as a part of treatment of acute gout for more than 2000 years[3, 4].

Colchicine is a lethal compound; it has an intense hostile to tumour property that bonds to tumour tubulin protein, designs antitumor-tubulin complex and forestalls tubulin

polymerization, prompting tumour cells demise [5, 6]. Today, alongside its utilization in gout [7], it is also utilized as a part of treatment familial Mediterranean fever [8, 9], and in inflammatory disorder [10, 11]. Colchicine has applications in various illnesses like cirrhosis of bile and liver, and amyloidosis [12, 13]. It is found to be used in plants to separate chromosomes at metaphase and for multiplication, and in animals as selective neurotoxin to study Alzheimer's dementia [14, 15].

Elucidation of the chemical structure of colchicine was a great achievement for scientists and their endeavours, particularly being developed for natural products chemistry. Zeisel began the investigation of colchicine structure (1883-1913), by determining the methoxy groups in colchicine. Windaus (1924) proposed a structure for colchicine based on phenanthrene ring framework, and in 1940, Cohen proposed the B ring that is 7-membered. Then, in 1954, Dewar assumed C-ring had an aromatic character with a carbonyl group (cycloheptatrienolone), and this was a critical advancement to term another class of tropolones chemistry. X-ray analysis affirmed the correct structure of colchicine by King in 1952, and was approved by their chemical degradation [16, 17].

The structure activity relationship (SAR) of colchicine binding to tubulin studied showed only compounds with (aS) axial chirality to be active. (-)-Colchicine was displayed to be active while (+)-colchicine 100-fold was less active. The trimethoxy moiety in ring (A) increased the affinity of colchicine binding to tubulin, and demethylation of ring (A) causes decrease of activity in the order of 1>2>3. A similar effect was observed for the methoxy moiety on tropolone ring (C). 2,3-methylenedioxy compound shows activity similar to colchicine. For ring (B), replacement of N-acetyl group with other alkyl amide results in no loss of activity, while N-alkyl groups are less active. The reversal of C-9/10 (iso compound) in ring C shows no activity at all and replacement of methoxy group on position 10 with group SMe or NR<sub>2</sub> results in increase of activity. The substitution of OH or H in position 10 showed decrease in activity but addition of ethyl substituents increased the activity. Conversion of tropolone ring to phenyl ring results in a slight decrease in activity [5, 18].

Capacity of colchicine to form tubulin-colchicine complex has been considered for anticancer activity [19], but high toxicity of colchicine limited its medical application in cancer therapy [20]. Synthesis of colchicine derivative by modifying the basic structure with substituents has been made to decrease its toxicity and increase its therapeutic properties [19, 21-23].

Recently, many efforts have been developed to form a hybrid system of colchicine and bioactive drugs, called conjugates system [24].

During the last decade, in the field of drug synthesis, about 10% has been approved as prodrug [25]. Prodrugs are chemically modified inactive forms of drug derivatives to be activated by a chemical or an enzymatic reaction *in vivo*. Prodrugs are established as a strategy to improve pharmaceutical, pharmacokinetic and/or pharmacodynamics properties of parent drugs and to increase the selectivity *in vivo*. The term prodrug is standard, but it also refers to a reversible or bioreversible derivatives, or biolabile drug carrier conjugates [25-28]. The term prodrug is generally used to optimize ADME properties (absorption, distribution, metabolism, and excretion), any modification on ADME required knowledge in the behaviour of physicochemical and biological of the drug [29].

The history of prodrugs started in 1899, when Schering introduced a design of prodrug for methenamine, which serves as a good example for site selectivity of prodrug, aspirin (acetylsalicylic acid) used in 1899 as sodium salicylate form with less irritation of the anti-inflammatory agent. Albert (1958) first used term of “pro-drug”, but in 1985 he apologized for using this term because the term “pre-drug” is more descriptive [30, 31]. For seven decades, many prodrugs have been developed, leading to an increase in the use of prodrugs in drug discovery [26, 28, 31-35].

Any reaction on drugs can change in its structure and overcome toxicity problems. So, toxicity can be reduced by altering the drug structure, but it should be accomplished by changing one or more ADME properties, in addition to the achievement of site selective drug delivery and targeting by using responsible endogenous enzymes for distribution and delivery of bioactive drugs into targeting site [36-38]. An advantage of prodrug design is that it can be faster and more feasible than looking into a new therapeutically active agent [37, 39, 40]. This review article will focus on colchicine conjugate, prodrug systems and nanoformulations, chemical synthesis of conjugates, type of conjugates, type of linker used and biological applications.

Prodrugs alter the toxicity of parent drugs. Thus, the development of prodrugs aims to improve [27]:

1. Pharmaceutical properties (pH) like solubility, chemical stability, taste, odor, decrease irritation and pain, and reduce any problems in pharmaceutical technology of the active agent.

2. Pharmacokinetic properties like oral and non-oral absorption, time profile, organ/tissue – selective delivery of active agent, and decrease the presystemic metabolism.
3. Pharmacodynamic properties like therapeutic index (lower toxicity), synthesis codrugs, and to activate the drugs to active agent.

## 2. Functional groups applied in prodrugs design

Prodrug design in some cases was made by coupling two pharmacologically active drugs, called codrugs, each drug act as promoiety to other, linked together with bioreversible covalent bond. In another case, molecular modification of the active drug like oxidation or reduction results in a new compound that can undergo metabolism or chemical transformation to produce an active agent [35].

Codrugs with promoiety properties use functional groups like [hydroxyl, carboxyl, phosphate, carbonyl group and amines] to link them [35]. The modification of these functional groups produced prodrugs such as carbamates, carbonates, esters, amides, oximes and phosphates [35, 41]. In addition, uncommon functional groups like ethers and amines were also used and the result was prodrugs with thioethers, thioesters, imines and N-mannich bases [35, 41], common functional groups used as linker in prodrugs is illustrated in Fig. 2.

## 3. Colchicine prodrug

Prodrug is a version of a chemically modified drug that could be activated after administration, preferably to target tumor cells, either by biotransformation or physico-chemical activation [35, 42, 43]. Prodrug is established to overcome unwanted properties of drugs and to optimize the ADME properties of the parent drug, thus improving solubility and chemical stability, which leads to facilitate oral absorption and selectivity in drug delivery [25-28]. Colchicine possesses a representative structure in drug discovery; it has a unique molecular platform, it binds to tubulin and inhibits the formation of microtubule that leads to hinder mitosis and cell death [12]. These properties have made it a useful drug in anticancer probe. However, colchicine has a limited use as an antitumor agent due to high toxicity, which affects normal neighbouring cells [19]. Synthesis of colchicine conjugates system started in 1978 when John Clark and Donita Garland studied the microtubule assembly in colchicine binding microtubule protein, resulting in the production of pro-drug fluorescein isothiocyanate-colchicine [44]. Then, colchicine prodrugs system has been developed and various conjugates compound have been reported, as illustrated below.

### 3.1 Fluorescent colchicine as prodrug

The first fluorescent colchicine (FC) reported was on 1978 [44], *John Clark and Donita Garland* prepared a colchicine conjugate with fluorescein core and isothiocyanate was used as linker. This conjugate maintains the specificity and biological activity of colchicine itself and provides a new strategy for relationships studying between cell functions and binding site of colchicine. Synthesis was started with deacetylation of colchicine using Wilson and Friedkin method to give isomers a mixture of deacetylcolchicine, iso-deacetylcolchicine and lumi-deacetylcolchicine, which were separated by thin-layer chromatography (TLC) in acetone/methanol solvent system. Fluorescein isothiocyanate was attached to deacetylcolchicine via condensation reaction and the product was purified with TLC (Fig. 3A). Excitation and emission fluorescein study showed no significant difference than fluorescein isothiocyanate, at maximum 520 nm wavelength emission, spectrum showed a 490 nm excitation maximum for fluorescein isothiocyanate compared to fluorescein (490 nm). At maximum 480 nm wavelength excitation, spectrum showed 521 nm emission maximum for fluorescein compared to fluorescein isothiocyanate (521 nm). Biological assay showed that fluorescein colchicine conjugate did not impair the activity and specificity of colchicine itself.

Toshiaki Hiratsuka and Toyoki Kato have reported the synthesis fluorescent derivative of *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-colcemid conjugate, (NBD-colcemid) [45]. Colcemid is colchicine structural analogue of *N*-deacetyl-*N*-methylcolchicine. Similar to colchicine, colcemid has high specificity and affinity to interfere with microtubules. Although NBD is fluorophore that has been used as fluorescent for studying protein conformational changes, and is highly soluble in low polarity solvents and low soluble in water, the excitation and emission maximum ranges are 460-480 and 520-550 nm, respectively. They synthesized the conjugate in one step by reaction of methylamino group colcemid with NBD-F, which only reacted with secondary amines. The excitation and emission spectrum showed that the maximum peak when NBD-colcemid bound to tubulin appeared at 465 and 530, respectively. Therefore, this conjugate is useful for studying the interaction of colcemid with tubulin, in addition to studying the relationship of cell function with colcemid-binding site (Fig. 3B).

One of the earliest studies on fluorescent colchicine conjugate was reported by Arnold et al. [46]. They synthesized two probes designated colchicine-504 and colchicine-646, with pH-independent and photo stable properties by coupling deacetyl colchicine with BODIPY

(Boron-Dipyrromethane) succinimidyl esters dyes in dimethylformamide (Fig. 3 B and D). BODIPY have properties like photo-nonbleaching, good quantum yield, green to red excitation wavelengths and insensitive to environment. The authors used fluorescence polarization to measure binding of conjugates to tubulin after the solubility of conjugates in Phosphate-buffered saline (PBS) was determined as 32  $\mu\text{M}$  for colchicine-504 and  $<10 \mu\text{M}$  for colchicine-646. Binding affinity of colchicine-504 and colchicine was higher than FC, at 5.8  $\mu\text{M}$  for colchicine-504 and 7.6  $\mu\text{M}$  for colchicine-646 compared to 14.5  $\mu\text{M}$  for FC. Biological assay studied on HeLa, HepG2, Raji and Vero cell lines showed colchicine-504 is a stronger tubulin inhibitor than colchicine-646 due to lower quantum yield and the cytotoxicity of conjugates was less than colchicine.

### 3.2 Biopolymer-colchicine as prodrug

Recently, Smith et al. [47] have reported the synthesis and *in vitro* evaluation of new peptide sequence-colchicine as prodrug with over 90 % purity. This peptide sequence includes Ala-Ala-Asn-Val-OH cleavable by cysteine protease legumain (mammalian asparaginyl (Asn) endopeptidase), which is a restricted substrate specific enzyme and found in endothelial and stromal cells as well as in tumour macrophage. This enzyme supports tumour progress by activating cathepsin B, H and L process. Although the author mentioned that legumain was found in colorectal cancer and in more aggressive tumour cells, at low pH, it is auto-activated from 56 kDa to 47/46 kDa intermediate forms, then converted to mature active legumain 36 kDa by other cysteine proteases. Both intermediate and mature forms are inhibited by endogenous type 2 cystatins. The synthesis started with connection tripeptide Ala-Ala-Asn with valine (Val) as linker. As this sequence has high lipophilicity, N on Ala terminal was protected with BOC protection group, followed by condensation reaction of tetrapeptide sequence with deacetylcolchicine in dimethylformamide (DMF) solvent. Deprotection of *N*-BOC was the last step using trifluoroacetic acid and dichloromethane. Purification of prodrug was done by preparative HPLC.

The authors also synthesized valyl colchicine in low yield (20%) by coupling deacetylcolchicine with protected *N*-BOC-Val-OH in dimethylformamide solvent, followed by *N*-BOC deprotection using trifluoroacetic acid in dichloromethane, the product was precipitated as yellow salt. Biological assay was made on M38L, HCT116, HEK293, M4C and SW620 colorectal cancer cell lines, which showed that the prodrug is more toxic to cells that contain more active legumain than others, in addition to cleavage of prodrug *in vitro* by

legumain at C-terminal of peptide sequence Ala-Ala-Asn-Val which released valyl colchicine as an active drug (Fig. S1-A). It was proposed that this strategy could be used in targeted cancer therapy.

Crielaard et al. have reported less toxic and vascular disruption agent of polymeric nanomedicine colchicine [48]. Polyethylene glycol (PEG<sub>5000</sub>) used to increase hydrophilicity and size of prodrug, hence helping in the penetration of tissues to target cells. Colchicine was first substituted with N-2-hydroxyacetyl moiety on N of C-ring as linker for prodrug, produced hydroxyl moiety of colchicine, then hydroxyl colchicine coupled with PEG acetic acid produced nanomedicine prodrug of polymeric colchicine with ester linker (Fig. S1B). The strategy to use polymeric nanomedicine as prodrug was to reduce the toxicity of colchicine and improve the solubility, which leads to a change in the tissue membrane and drug hydrolysed then delivered colchicine drug to the targeted site. Additionally, the authors have used N-2-hydroxypropinoyl moiety linker for prodrug alternative of N-2-hydroxyacetyl moiety [49]. Biological evaluation in vivo showed higher efficiency vascular disruption agent and less toxic of prodrug than colchicine itself, also using different linkers received the prodrugs to liposomes and the hydrolysis rate determined the kinetics of drugs in liposomes.

Johansson et al. have reported Glycopeptide dendrimers colchicine conjugate for drug delivery system [50]. Colchicine was loaded on glycopeptide dendrimers using thioacetamide linker (Fig. S2). Biological assay has shown that colchicine-dendrimer conjugate delivered colchicine as an active drug to the targeted site after degradation of intracellular without binding to tubulin, which leads to an increase cytotoxicity and selectivity of the active drug.

### *3.3 Colchicine-bioconjugate prodrugs*

Malysheva et al. have reported prodrug of colchicine with tubulizine bivalent product using triazole linker [51]. Tubulizine is used in mitosis process and is a fundamental component of cytoskeleton in eukaryotic cells besides being considered as a target site in cancer treatment. Synthesized reaction started with deacetylation of colchicine, again introduced to steglich reaction with azido-carboxylic acid which gave azidoamide colchicine. A prepared alkyne-tubulizine coupled with azidoamide colchicine in reaction of [3+2] cycloaddition (click chemistry reaction) using copper sulfate catalyst in tetrahydrofuran solvent gave a high yield colchicine-tubulizine prodrug (Fig. 4A and 4B). The authors have synthesized conjugates with several different lengths in order to study the effects of length on conjugate binding to

tubulin. In addition to trizole linker, polyethylene glycol has been used with 14 carbons atoms to linked colchicine and tubulizine. Cytotoxicity assay on HBL100 human mammary cell line has shown reverse relation of inhibition activity with linker length. Moreover, hydrophobicity plays an imperative role in activity.

Cathepsin B activated prodrug of 4-halocolchicine with dipeptide side chain has been reported by Yasobu et al. [52]. Dipeptide of phenylalanine and L-lysine were used based on Dubowchick's developed system by cathepsin B activation, enzyme overexpressed in tumor cells, prior to amidation reaction of amino acids, protection was handled on *N*-position L-lysine and amino position phenylalanine using allylchloroformate and carboxybenzyl. group respectively, followed by activation of carboxylic acid group with *N*-hydroxysuccinimide. Amino acids were bonded together in condensation reaction using NaHCO<sub>3</sub> in H<sub>2</sub>O/ dimethyl ether, which gave dipeptides at high yield. 4-fluorodeacetylcolchicine was attached to dipeptide carbonate to give high percentage of prodrug, followed by deprotection using Pd(PPh<sub>3</sub>)<sub>4</sub> (Fig. 4C). Biological assay on HCT116 human colorectal carcinoma cell line showed that prodrug was cleaved by cathepsin B and liberated active drug in *vitro* with double selectivity on tumor cells.

Another example on specific gene target prodrug was reported by van *Rossenberget al.* [53]. They synthesized targeted prodrug for hepatocyte gene receptor, by attaching colchicine with *di-N<sub>α</sub>,N<sub>ε</sub>*-(5-(2-acetamido-2-deoxy-β-D-galactopyranosyloxy)pentanomido) lysine ligand, K(GalNAc)<sub>2</sub>, a high affinity ligand for asialoglycoprotein receptor in liver cells. Disulfide bridge was used as linker and fluorescein isothiocyanate was connected to prodrug for confocal microscopy study. Biological assay has shown that the affinity of prodrug to receptor was 4.5 nM, and augmented polyplexed DNA transfection efficiency in parenchymal liver cells is 50 fold higher at concentration 1 nM. In addition, the prodrug was nontoxic and can be employed as vehicles to transfer hepatic nonviral gene (Fig. S3).

Another example of hepatocyte-targeted prodrugs with colchicine has been reported by Plourde et al. [54]. Colchicine was linked with asialoglycoprotein by disulfide linker in ratio 2 equivalent of colchicine to 1 equivalent of asialoglycoprotein, asialoglycoprotein used as vehicle to intracellular asialoglycoprotein receptor. Deacetylation of colchicine was made based on Raffauf et al.'s procedures gave isomeric mixtures of decetylcolchicine and deacetylcolchicine with ratio 1:1. Acylation of isomeric mixture with disulfide linker afforded chromatographic-separated isomers with 40% and 46% yield, respectively. Coupling

of drug with asialoglycoprotein (ASOR) done in two ways, deacetylcolchicine was attached to S-S linker followed by coupling with ASOR, and attachment of S-S to ASOR then coupled with deacetylcolchicine. Biological studies showed that the conjugate compound has destroyed microtubule cells, consequently conjugate was delivered to intracellular receptor and hydrolysis released the active drug into cell target.

Triazole linker containing prodrugs of colchicine has been reported by Kuznetsova et al. [55]. The authors have attached palmitic and oleic esters to colchicine and allocolchicine through triazole bridge at nano-sized level with 100 nm diameter. Prodrug formulation (Fig. S4-A) was formed to improve lipophilicity, reduce toxicity, and to deliver colchicine to the targeted site. Palmitic and oleic esters were used as transporter through cell membranes due to both esters possessing hydrophobic properties to lipid bilayer. Biological assays have shown lipophilic prodrugs have potential anti-proliferative activity; in addition, fast liposomal hydrolysis leads to rapid delivering of drug onto the targeted site.

An androgen receptor (AR) antagonist colchicine prodrug CCN was reported by Sharifi Nima et al. [56]. Bifunctional colchicine prodrug inhibits the function of AR in castrate-resistant prostate cancer (CRPC), using cyanonilutamide, in addition to tubulin function inhibition greater than colchicine. Rigid alkyne was used as linker to colchicine-cyanonilutamide (CCN), (Fig. S4-B). Cogan and Koch method was used to attach halo-alkyn-ol linker to cyanonilutamide, followed by coupling of deacetylcolchicine to alkynyl-cyanonilutamide by Castro's reagent (BOP) in dichloromethane. Biological evaluations have shown CCN prodrug with two functions: AR-binding activity and antitubulin activity. (The prodrug increases the concentration of AR protein in cytoplasm, in addition to toxicity of CCN greater than individual drugs). Bifunctional prodrug could be useful to focus on organic synthesis of new biological receptor ligand.

Fournier-Dit-Chabert et al. have reported colchicine-amino peptides prodrug named ICT2588 cleavable by matrix of metalloproteinase (MT-MMPs) active agent in tumor cells [57]. Colchicine was linked to peptide on 10-OCH<sub>3</sub>, the prodrug showed high selectivity after cleavage by MMPs and greater activity on tubulin binding site. (Fig. 5A) show the structure of prodrug and scissile bond of MT-MMPs.

Zhang et al. have reported bifunctional prodrug of colchicine and colchicine-suberanilohydroxamic acid (SAHA) prodrug [58]. SAHA is used as histone deacetylase (HDAC) inhibitor which results in accumulated HDAC and affects transcription and gene

expression, in addition to colchicine tubulin inhibitor. Structure of prodrug consists of colchicine as capping group, simple amide linker with different lengths and with hydroxamic acid as zinc-binding group (ZBG) (Fig. 5B). Biological evaluation showed potent dual inhibitor on HDAC and tubulin site.

Additionally, novel colchicine prodrugs with dual inhibitors for tubulin-HDAC have designed by X. Zhang et al., [59]. Benzamide has been used as HDAC zinc-binding group (ZBG), and linked to C7 of colchicine with amine or amide linkage (Fig. 5C). Biological evaluation of this hybrid system showed influential HDAC and tubulin inhibitor activity with potent cytotoxicity ( $IC_{50} = 2-105$  nM).

Thomopoulou, P. et al. have reported a new colchicinoids triazole-amino acid prodrug series [60]. The prodrug was synthesized by click-chemistry method, the colchicine has derived with triazole linker at C-7, then conjugated with several amino acids derivatives through 1,3-dipolar cycloaddition using Cu(I) as catalyst (Fig. S5). Biological evaluation of prodrug series against tumor cell lines including Hela, A549, and SK MES 1, showed a potent activity. The presence of benzyl, and/or halo-groups in amino acid derivatives increased compounds activity. Although, the  $IC_{50}$  cytotoxicity test showed a remarkable activity in three compounds ( $IC_{50} \leq 5$  nM). This results improved the ability of compound for tubulin depolymerization.

ZYN-linker thiocolchicine prodrugs have been reported by Baker et al. [61]. ZYN-Linker synthetic lipid-like molecules were designed to be inserted into cell membranes and to increase drug delivery to cells. pH-dependent hydrazone and imine linkers were used, three prodrugs were synthesized (Fig. S6) for SAR studies. In addition ZYN-linker was attached to thiocolchicine at different positions, one on ring A and another on C-7 position of ring B. Biological evaluation displayed that slow release of drug *in vitro* was less active (100 time) than individual drug, but it was established that the prodrug was able to block cell on metaphase division after 10 minutes of injection.

A library of colchicine neoglycoside prodrugs were synthesized by Ahmed A. et al. [62], the role for using neoglycoside that modified the ADMET (absorption, distribution, metabolism, excretion and toxicity ) property of drug by prompting its solubility, toxicity, pharmacology, recognition of target and action mechanism. Methoxyamine linker that was attached to deacetylcolchicine gave methoxyamine deacetylcolchicine, followed by conjugation with D-glucose in neoglycosylation reaction (chemoselective) using DMF/AcOH which yielded the

prodrug (Fig. S7). Biological study on cancer cell lines showed colchicine neoglycosides has less potency than normal colchicine at 10  $\mu\text{M}$  level, which is similar to Paclitaxel and Doxorubicin range.

Cauda et al. have reported a nano-sphere of lipid bilayers with colchicine loaded with diameter of 50 nm [63]; the sphere consists of supported lipid bilayer (SLB) functionalized with colloidal mesoporous silica (CMS), leading to a stable shell with hybrid system (SLB@CMS) as a factor to deliver drug in a successful way. Nano-sphere is employed to protect the drug before releasing it to the target site and decrease the toxicity of drug by the lipid bilayer boundary. The researchers prepared SLB on CMS using the method of solvent exchange. Biological evaluation displayed the efficiency of nano-sphere method for delivery of colchicine drug in an appropriate way through sphere diffusion in intracellular condition.

#### *3.4 Colchicine-metal complexes prodrug*

Bagnato et al. have reported a conjugate of cobalamin with colchicine [64]. Cobalamin, also known as vitamin B12, is a water-soluble derivative of corrin ring based on cobalt metal recognized by B12 transport protein, employed in metabolism of all human cells, specifically in synthesis and control of DNA. It is required for the formation of thymidine for DNA replication. The prodrug has been attached through hydrazine linker, where the linker is hydrolysable in lysosomal media. Synthesis started with deacetylcolchicine later derived with *p*-alkoxy-acetophenone on C-7 in the presence of Castro's reagent, cobalamin had been functionalized with hydrazide, and finally coupling of cobalamin with colchicine derivatives in methanol at room temperature gave prodrug (Fig. 6). The researchers also have synthesized a stable prodrug by attaching N,N'-deacetyl-colchicine-hemiglutaramide-NHS with CNCbl-5'-[(6-Amino)-hexylcarbamate]. Biological study revealed the cytotoxicity of prodrug against melanoma, brain and breast cancer cell lines with nanomolar values of  $\text{LC}_{50}$ , in addition to lower toxicity and side effects correlated to parent drug.

A theranostic agent conjugate of gadolinium (III) DOTA with colchicinic acid was reported by Kalber et al. [65]. This agent has the ability for continuous diagnostic and MRI contrast image at the same time. The conjugate synthesis started with the conversion of colchicine to colchicinic acid by treatment with 30%  $\text{H}_2\text{SO}_4$  at 100  $^\circ\text{C}$ , followed by condensation reaction of colchicinic acid with Tetraazacyclododecanetetraacetic acid (DOTA) in ester form to give DOTA-colchicinic acid. Finally complexation reaction with gadolinium chloride in water yielded Gd.DOTA-colchicinic acid (Fig. S8-A). Biological study on ovarian carcinoma cell

lines showed the ability of conjugate as a tubulin binding agent and competent MR image tracer, in addition to the ability to cause cell death after 24 h due to vascular disrupting agent at level millimolar range concentration.

One more Gd.DOTA-colchicine prodrug with different linker lengths of thiocyanate has been reported by Efthimiadou et al. [66]. The authors synthesized two prodrugs as Magnetic Resonance Image (MRI) contrast agents, starting with deacetylation of colchicine followed by amidation with 3-aminopropanoic acid, and finally coupling reaction of colchicine derivatives with *p*-SCN-Bn-DOTA-Gd gave conjugate Gd.DOTA-colchicine. The synthesis of the second prodrug was the same except amidation with 3-aminopropanoic acid method was dropped (Fig. S8-B). Compared to Gd-colchicinic acid prodrug, Gd-DOTA-colchicine showed micromolar cytotoxicity level against MCF-7 cells; however, relaxivity of colchicinic acid conjugate was less than colchicine conjugate, 13 and 3.83 mM<sup>-1</sup>s<sup>-1</sup> respectively. The length of prodrug plays an important role on cytotoxicity based on the comparison made above.

### 3.5 Colchicine-radiolabeled prodrug

To explore the metabolism, pharmacodynamics and actions of anti-cancer drugs, radiolabeling techniques were used, especially on colchicine conjugates. Korde et al. have reported the synthesis of two radiolabeled prodrug <sup>99m</sup>Tc-labeling of colchicine [67]. They used [<sup>99m</sup>Tc(CO)<sub>3</sub>(H<sub>2</sub>O)<sub>3</sub>]<sup>+</sup> and [<sup>99m</sup>Tc≡N]<sup>2+</sup> conjugated to colchicine to study multidrug resistance of P-glycoprotein during reversal binding of colchicine to P-glycoprotein. <sup>99m</sup>Technetium (I) carbonyl [<sup>99m</sup>Tc(CO)<sub>3</sub>(H<sub>2</sub>O)<sub>3</sub>]<sup>+</sup> is less sensitive to oxidation than <sup>99m</sup>Technetium (V), later required high concentration of ligand to form stable complexes, whereas [<sup>99m</sup>Tc(CO)<sub>3</sub>(H<sub>2</sub>O)<sub>3</sub>]<sup>+</sup> required low concentration of ligand in addition to having flexibility towards ligands of low size, charges and lipophilicity. [<sup>99m</sup>Tc≡N]<sup>2+</sup> possesses high stability and affinity to sulfur and phosphorus containing ligands. Synthesis of prodrug has started with deacetylation of colchicine to deacetylcolchicinic acid, next condensed with iminodiacetic acid and dithiocarbamate, finally complexation with [<sup>99m</sup>Tc(CO)<sub>3</sub>(H<sub>2</sub>O)<sub>3</sub>]<sup>+</sup> and [<sup>99m</sup>Tc≡N]<sup>2+</sup> gave radiolabeled prodrugs with high purity (Fig. 7). Biological study showed higher uptake of drugs in tumor cell lines and good pharmacokinetic *in vivo*.

Wang et al. have also reported [<sup>99m</sup>Tc(CO)<sub>3</sub>]<sup>+</sup> prodrug with colchicine [68]. *N*-(acetyloxy)-2-picolylamion (AOPA) has been to conjugated colchicine, then attached to [<sup>99m</sup>Tc(CO)<sub>3</sub>]<sup>+</sup> ligand using carbonyl-methylene linker (Fig. S9). <sup>99m</sup>Tc possesses significant physical

properties ( $t_{1/2} = 6$  h, 89% abundance) and low cost contributes to extensive radionuclide applications [69]. Biological evaluation revealed higher cell uptake but slow clearance observed in normal organs, leading to further development in linker or in ligand.

The authors have reported in 2013 a novel cationic and hydrophilic complex of  $[^{99m}\text{Tc}(\text{CO})_3(\text{PA-TZ-CHC})]^+$  prodrug with modified triazole linker to overcome slow clearance from normal organs [70]. Colchicine was deacylated then derivatized with 2-(2-azidoethoxy)ethyl to give azido colchicine derivative, click reaction of azido-derivative with *N*-(pyridin-2-ylmethyl)prop-2-yn-1-amine, which produced PA-TZ-colchicine as ligand for complexation. Finally, the attachment of ligand with  $[^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$  created the complex prodrug (Fig. S9). Biodistribution study showed good accumulation and higher uptake than  $^{99m}\text{Tc}(\text{CO})_3\text{-AOPA}$  colchicine, fast clearance from normal organs and the prodrug possesses hydrophilic and cationic properties which make it stable at room temperature.

One more  $^{99m}\text{Tc}$ -colchicine complex prodrug has been reported by Erfani et al. via hydrazinonicotinic acid (HYNIC) [71]. After deacetylation of colchicine, HYNIC was conjugated with deacetylcolchicine giving HYNIC-colchicine, while complexation of HYNIC-colchicine with  $^{99}\text{TcO}_4^-$  gave unstable  $^{99m}\text{Tc}$  HYNIC-colchicine, so the authors used ethylenediamine-*N,N*-diacetate/tricine coligand to support the complex stability (Fig. S10). Biodistribution revealed high accumulation in tumor cell and fast clearance from normal organs.

Zareneyrizi et al. have reported other  $^{99m}\text{Tc}$ -colchicine complex with ethylenedicysteine chelator for density appraisal of tumor microvascular. The complex prodrug showed in biodistribution is capable to control the effects of antiangiogenic and therapeutic during chemotherapy [72].

El-Azony et al. have reported colchicine radioiodination of  $^{125}\text{I}$  for medical applications [73]. Prodrug synthesis was achieved through electrophilic substitution reaction with colchicine using  $\text{Na}^{125}\text{I}$ , catalyst and oxidizing agent (Fig. S10). Purification and separation was controlled under HPLC, although not purified completely. Biodistribution revealed that radioiodinated colchicine is capable to be used in muscle imaging.

$^{131}\text{I}$ -radiosynthesized with SIB-PEG<sub>4</sub>-colchicine was reported by Zheng et al. [74]. Synthesis procedures started with deacetylcolchicine reacted with PEG in amidation reaction, which gave amino PEGylated colchicine  $\text{NH}_2\text{-PEG}_4\text{-colchicine}$ , followed by conjugation with *N*-

succinimidyl-3-iodobenzoate (SIB) and *N*-succinimidyl 3-(tri-*n*-butylstanny)benzoate (ATE) that produced SIB-PEG<sub>4</sub>-colchicine (cold reference) and ATE- PEG<sub>4</sub>-colchicine (precursor), finally electrophilic iododestannylation reaction of cold reference with the precursor gave lipophilic and pure [<sup>131</sup>I]SIB-PEG<sub>4</sub>-colchicine with moderate yield (Fig. 8A). Biodistribution showed poor uptake and retention of tumor cells but fast clearance from normal organs.

<sup>68</sup>Ga-labeled to DOTA-colchicine and NOTA-colchicine were reported by Satpati et al. using *p*-SCN-benzene linker [75], which showed hundredfold significant cytotoxicity on MCF-7 and T47D breast cancer cell lines, in addition to higher uptake on mice bearing fibrosarcoma tumors and fast clearance from normal organs (Fig. 8B and 8C).

Satpati et al. also reported <sup>90</sup>Y-labeled with DOTA-colchicine in high yield using isothiocyanate linker [76], complexation reaction employed in acidic media. DOTA was used in complexation due to bifunctional property, while <sup>90</sup>Y possesses high radioisotope energy, which is used in the treatment of large-sized tumor cells. Biodistribution showed high accumulation in tumor cells and fast clearance from normal organs.

Additionally, Satpati et al. reported that <sup>188</sup>Re(CO)<sub>3</sub>-colchicine complex [77], iminodiacetic acid (IDA) has been used as tridentate ligand, and reaction was carried out using CO gas and reducing agent (amino borane). Synthesis procedure was similar to <sup>99m</sup>Tc-colchicine-IDA (Fig. 8D). <sup>188</sup>Re(CO)<sub>3</sub>-colchicine-IDA possesses high uptake in tumor cells, in addition to high retention time in tumor, making it favourable for tumor therapy.

#### 4. Colchicine codrug

Codrug or 'mutual prodrug' is defined as two or more identical or non-identical drugs bonded *via* covalent chemical linkage. It differs from prodrug in having an active group, a cleavable linker coupled both drugs directly or indirectly. However, codrug has limitation in the selection of a specific functional group for linkage. The codrug possesses a good absorption rate prime with effortless delivery to target site. After being hydrolyzed in gastrointestinal level it provides two or more different drugs without any shifting in therapeutic properties and it is desirable to be released concomitantly at the target site. Another strategy of codrug has been built using a double drug, called hybrid drugs, where the two drugs are linked everlastingly without cleavable bond [78-80].

Codrugs can be classified into two groups based on spacer: directly linked or indirectly linked codrugs. The rule of spacer in active drugs is either to increase chemical stability or to control

the drug release, but codrugs have disadvantages in higher molecular weight and side effect associated in individual drugs. Therefore, the selection of spacer with some desired properties is also logical for codrug delivery system. In direct-coupling of codrug, the conjugation of both drugs was made by ester/amide bond and the most codrugs designed under this group [29, 81-84].

In the last decade, there has been a steady improvement in colchicine ADMET properties by the implementation of codrug strategy. Several colchicine codrugs systems have been developed. This section illustrates various colchicine codrugs that have been reported.

Zefirova et al. have reported synthesis of adamantane-colchicine codrug with depolymerization and clustering of tubulin [85]. Adamantane is used as an antiviral drug for flu and is also used as a key structural subunit for drug design. Adamantane was coupled with colchicine using an amide linker with different lengths for SAR study (Fig. S11-A). Biological assays have shown that cytotoxicity increased by increasing the length of linker, and substitution on adamantane plays an important role on tubulin clustering. The authors again have reported that the synthesis of colchicine-tubuloclustin codrug (n=5) emerged unusual clustering of tubulin [86].

Another colchicine codrug was synthesized as homodimer of colchicine and isocolchicine by Funaioli et al. [87]. Colchicine and isocolchicine was coupled on C-10 and C-9 substituent respectively in Semmelhack's reaction where bis(cyclooctadiene) nickel(0)  $\text{Ni}(\text{COD})_2$  was used as a catalyst in DMF solvent. Homodimer of 10,10'-bicolchicine and 9,9'-bisicolchicine (Fig. S11-B) linked by a single covalent bond at absolute configuration ( $R_a, 7S$ ), bisicolchicine produced as isomer of ( $R_a, 7S$ ) and ( $S_a, 7'S$ ). These have both hydrophilic and hydrophobic properties that made them easily soluble in polar and nonpolar solvents. UV and Circular Dichroism (CD) spectrums have shown that homodimer is solvent-dependent due to multichromophoric system and any changes in solvent mixtures changes the CD bands.

Bensel et al. have also reported the synthesis of homodimer of colchicine coupled on C-4 using methylene bridge linker [88]. The reaction was done with formaldehyde in  $\text{H}_2\text{SO}_4$  with heating at 50 °C, which gave homodimer colchicine at low yield (Fig. S11-B). Biological assay of 4,4'-bicolchicine on HL-60, KB-31 and KB-8511 human epidermoid cancer cell lines did not show any activity on cell lines, except for a small effect on HL-60 cells.

Bombuwala et al. have reported the synthesis of colchicine-paclitaxel codrug using glutamate linker [89]. Paclitaxel (taxol) has a different tubulin-binding site from colchicine, its bind on (+) and (-) ends of microtubule, colchicine binds on the (+) end. Paclitaxel promotes microtubule polymerization and assembly but prevents free tubulin in the cell. Synthesis was started with deacetylation of colchicine in three steps reaction using Lebeau method, followed by amidation on N-deacetylated with glutaric anhydride in dimethylformamide (DMF) solvent, which produced *N*-glutaryl-deacetylcolchicine. Paclitaxel has two hydroxyl groups; a protection was made on less active -OH using *tert*-butyldimethylsilyl (TBDMS) ether, then two drugs were coupled using Postema and co-workers procedure [90], deprotection of -OH was achieved by tetra-*N*-butylammonium fluoride (TBAF), which gave colchitaxel codrug (Fig. 9A). Colchicine-Paclitaxel (Colchitaxel) compound in bio-assay has shown partial properties as single compounds, which also had affected the + end protein cap that caused fragmentation and shortening of the + end.

In addition, paclitaxel have been coupled with colchicine analogue, thiocolchicine, using succinate linker [91]. Danieli et al. have reported production of three paclitaxel-thiocolchicine co-drugs with thiocolchicine linker connected at 10, 7 and 2-OH of paclitaxel (Fig. 9B-9D). The coupling was achieved via condensation reaction using 1,3-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP).

Triethylsilane (TES) was used as protection group. In the case of using 10-OH in connection with *N*-succinate deacetylthiocolchicine, 13 and 7-OH on paclitaxel was protected with TES, the same was with 13-OH connection and 7-OH connection, while other OH was protected to prevent its contribution in reaction synthesis. Biological assay showed that 7-O-paclitaxol-thiocolchicine and 10-O-paclitaxel-thiocolchicine compounds possess high cytotoxicity, 2-O-paclitaxel-thiocolchicine did not show any activity but had an obvious molecular target different from tubulin.

Another example on thiocolchicine codrug was also reported by Danieli et Al. [92], where thiocolchicine was coupled with podophyllotoxin using disulfide linker (Fig. 10A). Podophyllotoxin is a pharmacological precursor used for treatment of etoposide cancer [93]. The authors formed dynamic combinational library (DCL) of thiocolchicine-podophyllotoxin conjugate based on dynamic variant of combinational chemistry, which forms blocks of building of scaffolds that are in thermodynamic equilibrium and is capable to self-assemble with other complementary scaffolds. Linkers were synthesized from mercaptoacetic acid and

3-mercaptopropionic acid gave two of dithiocarboxylic acids, then condensation reaction was carried out between *N*-deacetylthiocolchicine and dithiocarboxylic acid, followed by coupling reaction with podophyllotoxin using DCC and DMAP in dichloromethane solvent. Biological assays in vitro have shown activity differing from parent drugs and had different biological target sites.

Namgoong et al. have reported the synthesis of several colchicine codrugs [94] possessing lower toxicity and high anticancer affinity properties. The authors synthesized codrugs in a single step by coupling deacetylcolchicine with carboxylic acids of drugs in the presence (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride), which gave prodrugs with yield of 70-80%.

Shen, Lihong, et al have reported synthesis a series of thiocolchicine (colchicine analogue) codrugs with uracil and 5-fluorouracil using amide linker [95]. Uracil is antitumor agent, also it's known as base of RNA [96]. 5-fluorouracil is one of anticancer drugs, has been used for treatment of several cancer cells including gastric head, neck, colorectal and stomach [97, 98]. Nonetheless, due to its side effect, it has limited in clinical application [99]. The synthesis started by conversion of colchicine into thiocolchicine in the presence of NaSCH<sub>3</sub>, followed by deacetylation of acetyl group on C-7 to give *N*-deacetylthiocolchicine, the final step was coupling of *N*-deacetylthiocolchicine with uracil and 5-fluorouracil-1-yl acetic acid via acylation reaction in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and 4-dimethylamino-pyridine (DMAP) in dry CH<sub>2</sub>Cl<sub>2</sub> (Fig. 10B). Biological evaluation showed that synthesized compounds a significance antitumor activity against BEL7402, A2780, A549 and MCF7 cell lines, more than colchicine and 5-fluorouracil itself.

A hybrid molecule of colchicine-pironetin analogue with ester linker have been reported by Vilanova et al. as gene expression inhibitor for *VEGF*, *hTERT* and *c-Myc* genes, which are important in the process of cancer generation [100]. Pironetin is an anticancer and tubulin inhibitor, able to pervert cell cycle progression by binding the tubulin at M-phase, specifically at Cys316 site of alpha tubulin [101]. VEGF gene used in angiogenic process, term to Vascular Endothelial Growth Factor. Whereas hTERT and c-Myc involved in telomerase activation. The biological evaluation showed codrug is being responsible for the activity (Fig. S12).

## 5. Nanoformulation of colchicine

Herbal drugs are mostly hydrophobic hence possess poorly absorbable property, this property leads to a greater dose with repeated administration during treatments result in a decrease in bioavailability and limitation in clinical use [102, 103]. Therefore, the trend to apply nanotechnology in herbal drug delivery has spread rapidly in the recent time [104].

Drug delivery system (DDS) is capable to increase drug bioavailability and reduce the repeated administration by providing site targeting selectivity and sustained drug release, in addition to consent increasing of the patient [105]. Meanwhile, several commercial drugs have been formulated and easily accessible in the market. Those formulations have been applied using several drug delivery strategies such as microemulsion, polymeric nanoparticles, liposomes, liquid crystal, and microspheres [106-108].

Several nanocarriers have been designed for colchicine drug delivery. Singh et al. have reported Elastic Liposomes to sustain colchicine delivery and to enhance their activity against gout disease [109]. Liposomes are nanoparticles cover lipid bilayer membranes surrounding an aqueous interior, the drug could be in the aqueous (hydrophilic) or in the lipid membrane (hydrophobic). Singh et al. have used rat skin for *in vitro* study of skin permeation of elastic liposomes compared to drug solution, they found that elastic liposomes increased the skin deposition (up to 12.5 fold). Moreover, the penetration and accumulation were up to 200  $\mu\text{m}$  compared to drug solution (up to 12  $\mu\text{m}$ ). The biological evaluation showed that elastic liposomes have better and sustained drug delivery with enhancement of site selectivity than the drug solution.

Additionally, Singh et al. developed elastic liposomes with cyclodextrin-colchicine complexes encapsulation, they prepared the complex using the freeze-drying method [110]. Transdermal flux was 6-fold higher than drug solution based on skin retention studies, and the amount of drug deposited in liposome drug complexes case was 12.4-fold better than in case of drug solution. Biological evaluation of rat skin showed that this nanocarrier is able to reduce the accumulation in inflammatory cells, decrease in leukocyte count and collagen deposition.

Shen et al. had improved a nanoemulsion carrier with eugenol as an enhancer for oral administration of colchicine [111]. The authors prepared the nanoemulsion formulation by mixing the oil phase with an aqueous phase containing colchicine drug under gentle stirring.

The oil phase contains isopropyl myristate, Tween80 as surfactant and eugenol as an enhancer. Additionally, the average particle size was approximately 41.2 nm. Oral administration method gave 2.1-fold higher in colchicine bioavailability than colchicine solution and the intestinal absorption of colchicine was enhanced by using of eugenol.

Recently, Bazylińska et al. have reported a new nanoformulation carrier for cancer therapy and imaging drug, colchicine was used as cytostatic drug and coumarin-6 as a fluorescent biomarker [112]. They prepared the nanoformulation by using core-shell encapsulation strategy, colchicine was encapsulated in palm oil phase and stabilized using diamidequate surfactant - N,N-dimethyl-N,N-bis[2-(N-alkylcarbamoyl) ethyl]ammonium methylsulfates (2xCnA-MS, n = 8,10,12). Biological evaluation using MCF-7/WT, A549 and MEWO cell lines showed a potential, excellent biocompatibility and sustainable delivery with cell imaging.

## 6. Future Perspectives

The development in nanomedicine is rapidly growing at this time, especially for controlled released drug and sustained delivery. Moreover, due to the high surface area, biocompatibility, biodegradability, and non-toxicity of graphene oxide (GO), many researchers have used GO as a nanocarrier for an anticancer drug with a high potential activity and better selectivity to the target site. Specifically, when it is fabricated in a hydrogel matrix with a biopolymer, such as carboxymethyl cellulose, sodium alginate, chitosan and guar gum, those biopolymers possess excellent properties including water-soluble, biocompatible, and non-toxic. Applying graphene oxide hydrogel matrix as a nanocarrier for colchicine drug delivery either by the chemical or physical method will provide sustained drug delivery and give better result for cancer treatment.

## 7. Summary

In summary, colchicine prodrugs and codrugs have become an attractive part in drug design and in improving ADMET properties of colchicine itself. Modification in colchicine structure leads to biological activity with various toxicity levels, which could be in substituents attachment or in incorporation with versatile drugs, biopolymers, bio-compounds, radiotopes and metal-complexes. Substituents-moiety like steric and chain length affects the activity of colchicine. Linker plays important roles in biological affinity of colchicine prodrugs, kind and length of linkers employed in colchicine delivery to target

cells. Conjugation of colchicine in nanosphere technology using solvent exchange method can be considered as an efficient technique in drug delivery system.

## 8. References

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**Short Forms**

*SAR*: structure activity relationship

*ADME*: absorption, distribution, metabolism and excretion

*FC*: fluorescent colchicine

*TLC*: thin-layer chromatography

*NBD-colcemid*: 7-nitrobenz-2-oxa-1,3-diazol-4-yl)-colcemid

*BODIPY*: boron-dipyrromethene

*Ala*: alanine

*Asn*: asparagine

*Val*: valine

*BOC*: tert-Butyloxycarbonyl

*DMF*: dimethylformamide

*PEG*: polyethylene glycol

*ASOR*: asialoglycoprotein

*AR*: androgen receptor

*CCN*: colchicine cyanonilutamide

*Bop*: (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (Castro's reagent)

*MT-MMPs*: membrane-type matrix metalloproteinases

*SAHA*: suberanolhydroxamic acid

*HDAC*: histone deacetylase

*ZBG*: zinc-binding group

*ADMET*: absorption, distribution, metabolism, excretion and toxicity

*SLB*: supported lipid bilayer

*CMS*: colloidal mesoporous silica

*MRI*: magnetic resonance image

*DOTA*: 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid

*AOPA*: N-(acetyloxy)-2-picolyamion

*PA-TZ-CHC*: N-(pyridin-2-ylmethyl)prop-2-yn-1-amine triazole colchicine

*HYNIC*: hydrazinonicoctinic acid

*HPLC*: high performance liquid chromatography

*SIB*: N-succinimidyl-3-iodobenzoate

*NOTA*: 1,4,7-triazacyclononane-1,4,7-trisacetic acid

*IDA*: iminodiacetic acid

*CD*: circular dichroism

*DCC*: 1,3-dicyclohexylcarbodiimide

*DMAP*: 4-(dimethylamino)pyridine

*TES*: triethylsilane

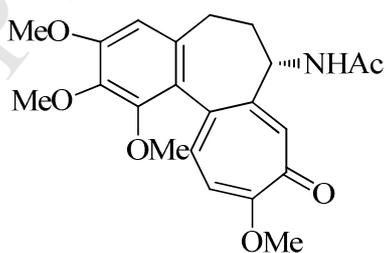
*VEGF*: vascular endothelial growth factor

*MCF-7/WT*: breast cancer cell lines

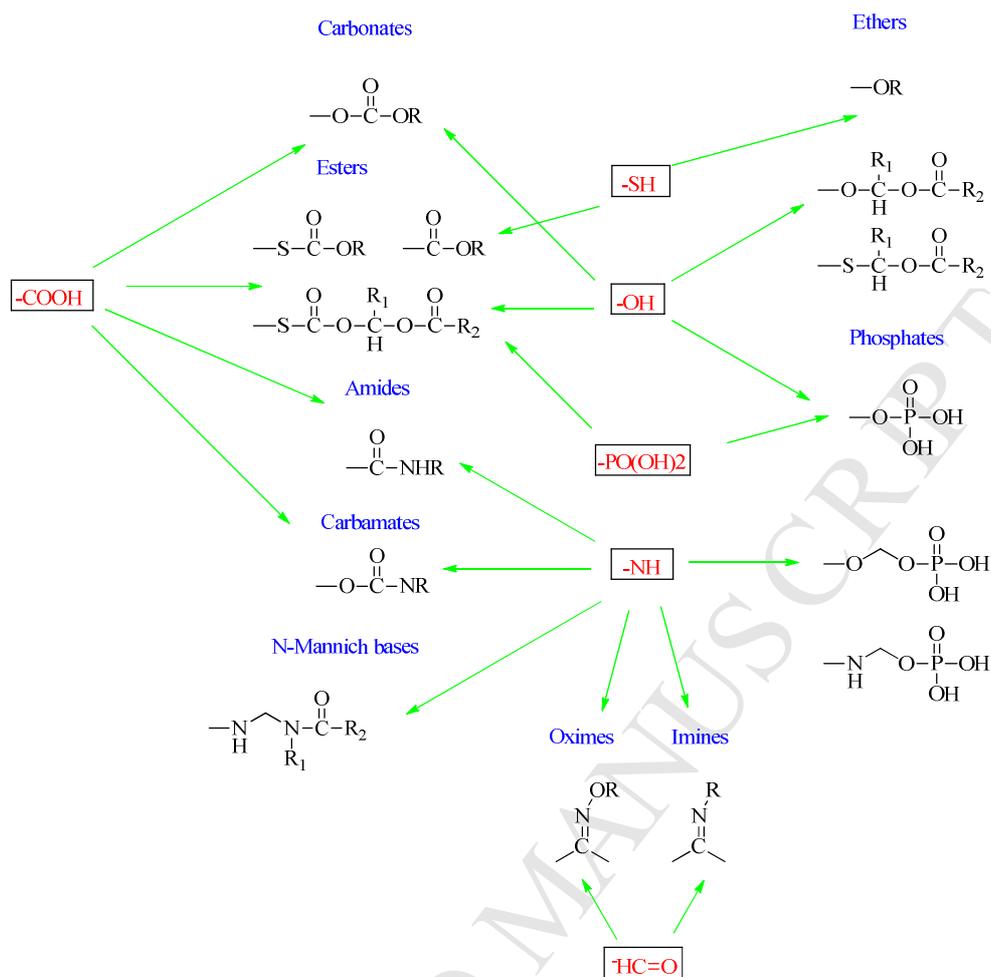
*A549*: alveolar basal epithelial cell lines

*MEWO*: skin melanoma cell lines

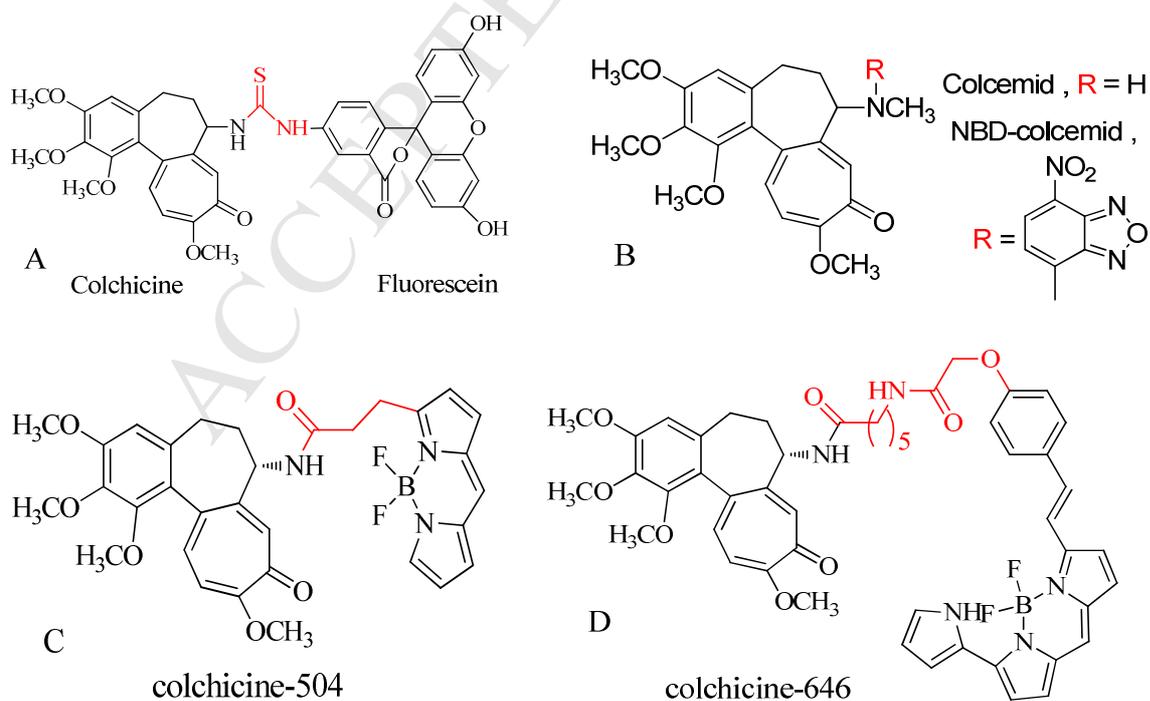
## List of Figures



**Fig. 1.** Colchicine structure.

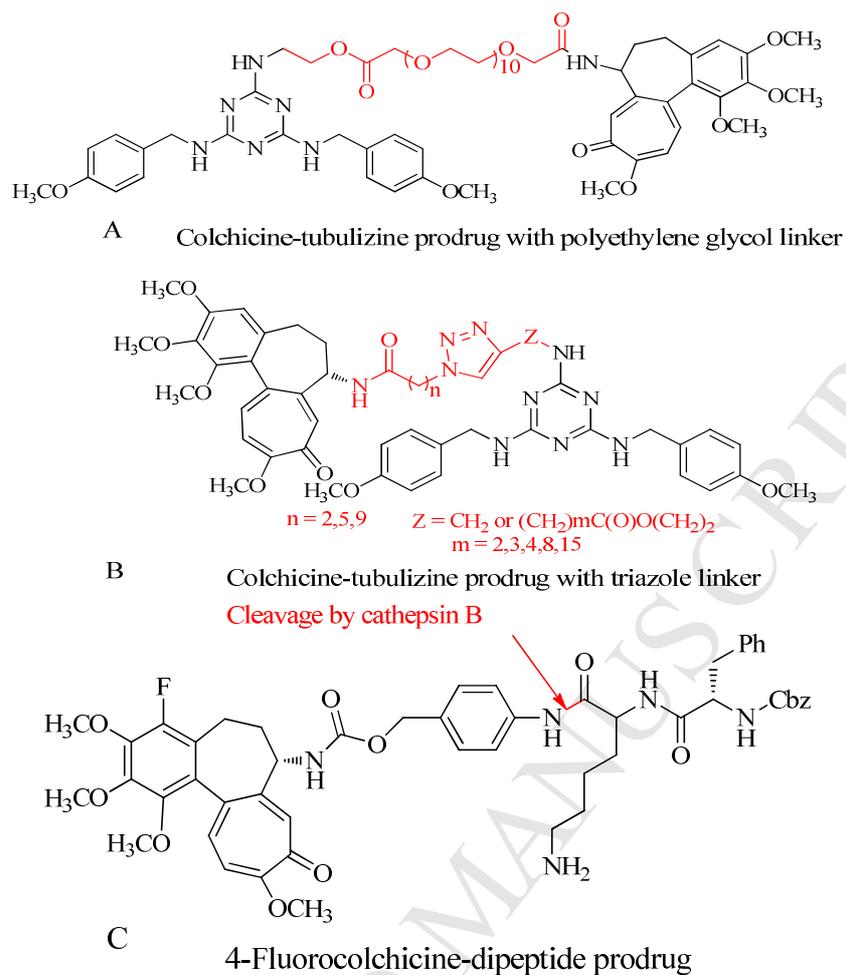


**Fig. 2.** Functional groups used as linker applied in prodrugs design.

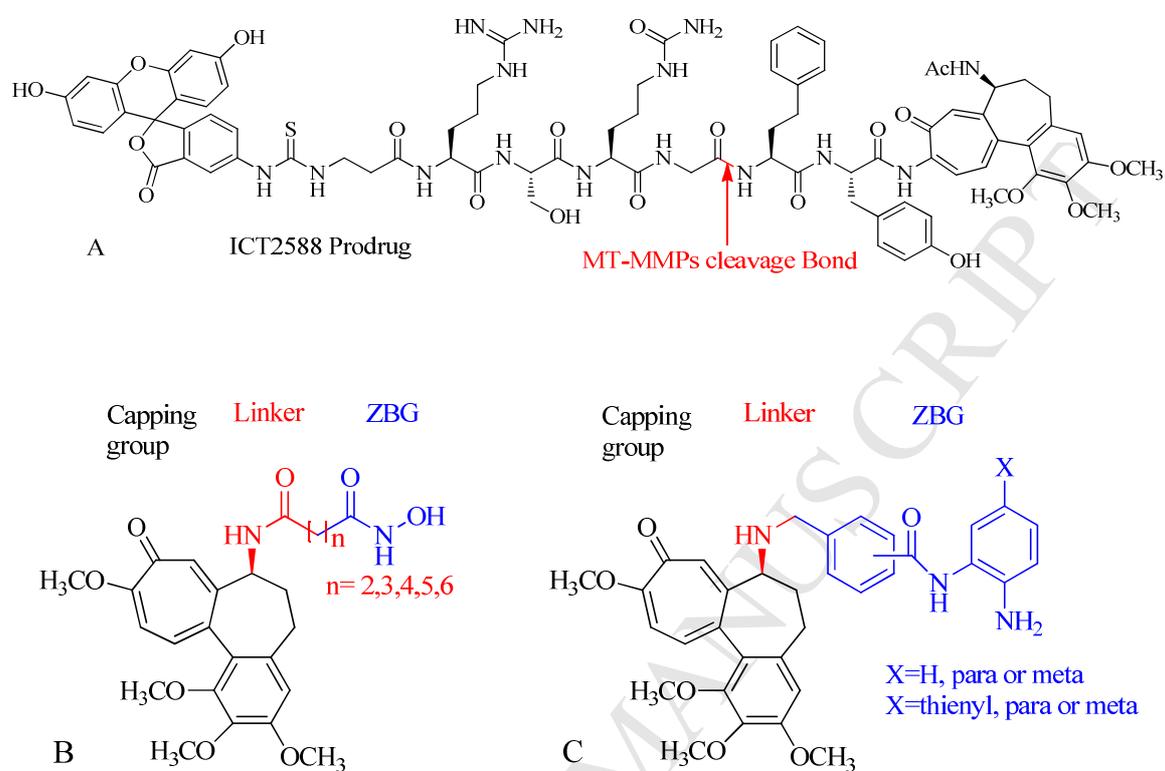


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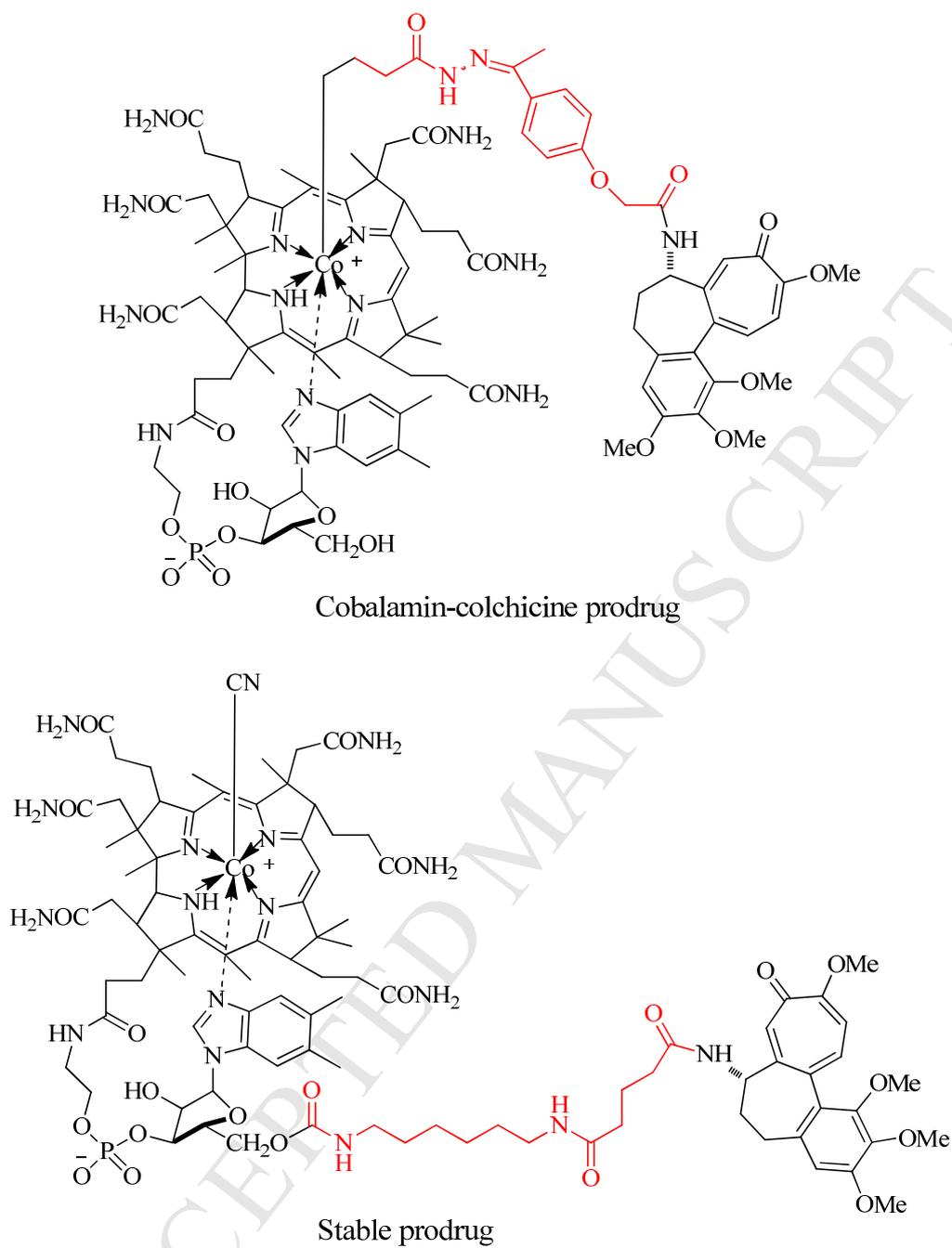
ACCEPTED MANUSCRIPT



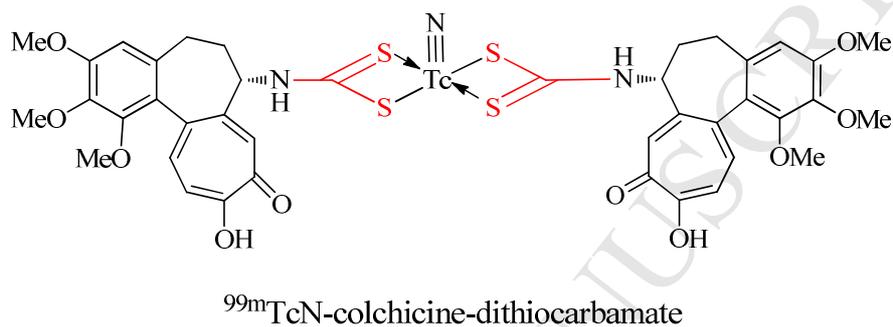
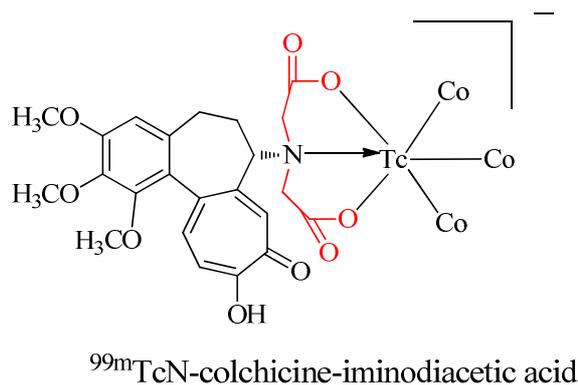
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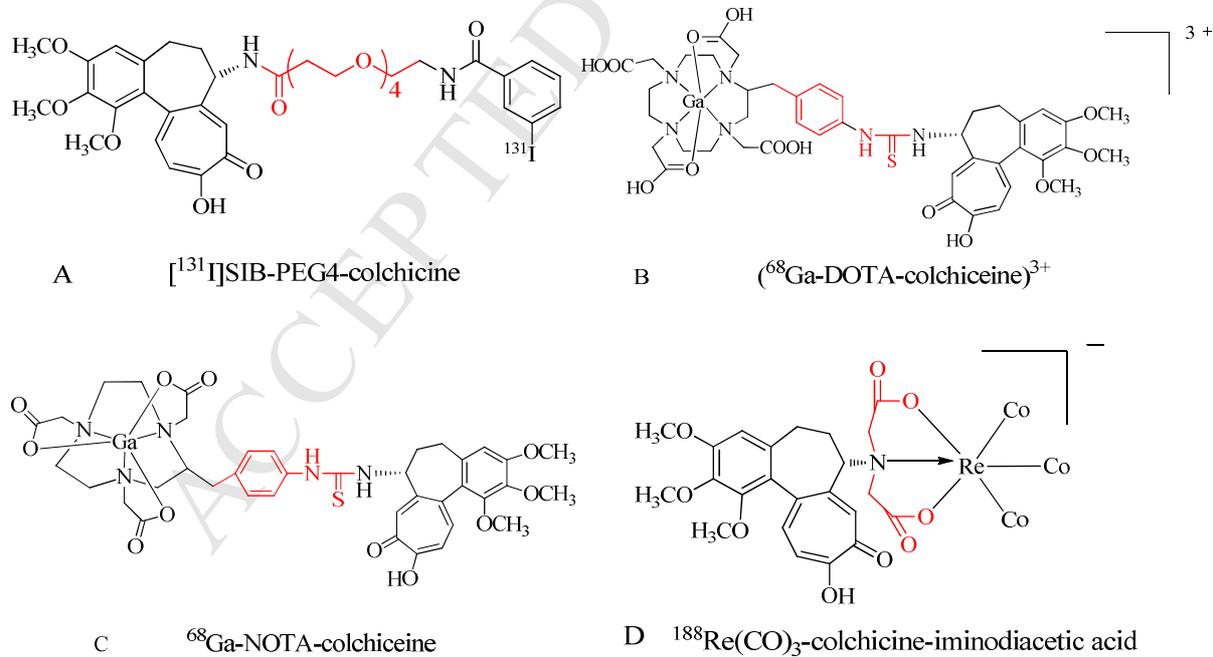
**Fig. 5.** Structure of ICT2588 prodrug (A). Structure of colchicine-SAHA prodrug (B). Structure of tubulin-HDAC dual inhibitors colchicine prodrug with amine linkage (C).



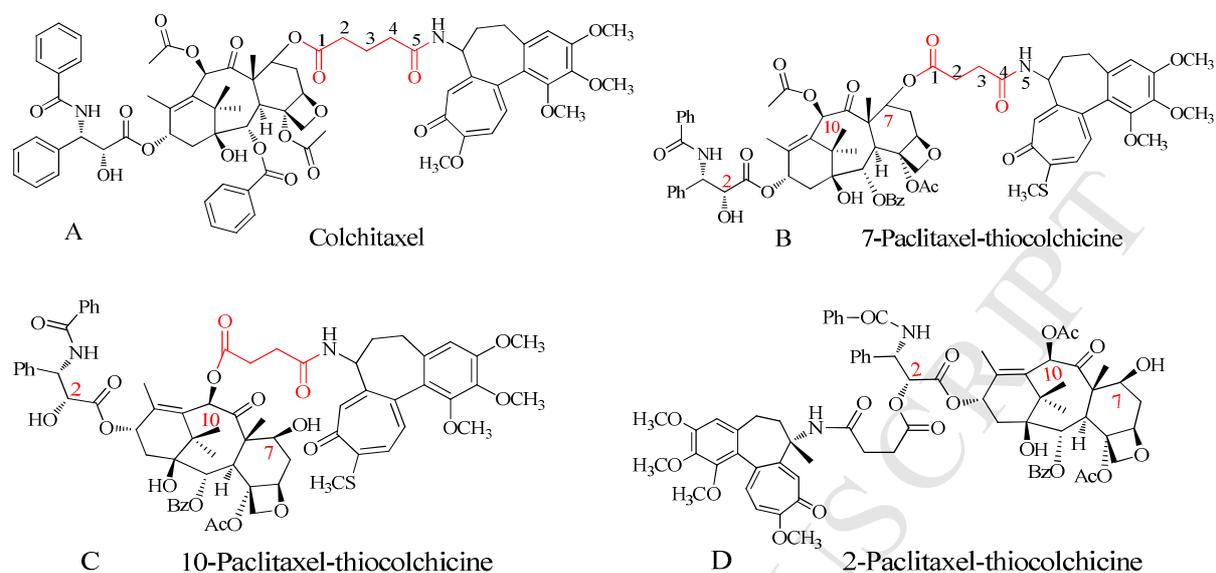
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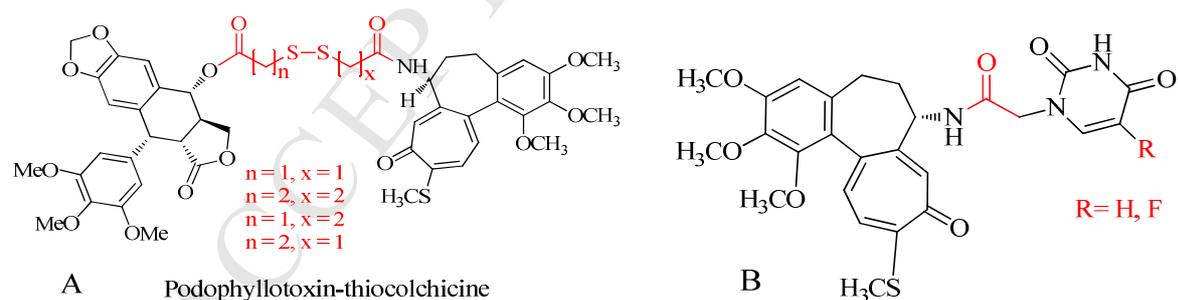
**Fig. 7.** Structure of  $^{99m}\text{Tc}$ -colchicine prodrugs.



**Fig. 8.** Structure of  $^{131}\text{I}$ SIB-PEG4-colchicine (A).  $^{68}\text{Ga}$ -labeled colchicine (B and C). Structure of  $^{188}\text{Re}(\text{CO})_3$ -colchicine-IDA prodrug (D).



**Fig. 9.** Structure of Colchitaxel codrug (A). Structure of paclitaxel-thiocolchicine (B, C and D).



**Fig. 10.** Structure of podophyllotoxin-thiocolchicine (A). Structure of Uracil and 5-Fluorouracil-thiocolchicine codrugs (B).

**Highlights**

- Colchicine is an antimetabolic agent with low therapeutic index due to high toxicity effects in non-target cell.
- Modified systems of colchicine prodrugs and codrugs improve the ADMET properties of colchicine itself.
- Structure of colchicine modified with substituents attachment or with incorporation with versatile drugs and bio-conjugates using appropriate linker.
- Linker plays important roles in biological affinity of modified systems.
- Prodrug and codrug designs convert colchicine drug into inactive forms and could be activated by an enzymatic or a chemical reaction *in vivo*.