RESEARCH ARTICLE

Anti-Cancer Efficacy of 6 Mercaptopurine Loaded Biotinylated Cellulose Nanowhiskers

Osamah N. Wennas[1]*, Mohammed H. Mohammed[2], Dhulfiqar Ali Abed[3,4]

[1]*Department of Pharmaceutical Chemistry, College of Pharmacy, Al-Zahraa University for women, Karbala, Iraq*
[2]*Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad, Baghdad, Iraq*
[3]*Department of Pharmaceutical Chemistry, College of Pharmacy, University of Babylon, Babylon, Iraq*
[4]*Department of Medicinal Chemistry, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, United States*

Received: 24th August, 2021; Revised: 16th October, 2021; Accepted: 28th October, 2021; Available Online: 25th December, 2021

ABSTRACT

6 Mercaptopurine (6 MP) is an important drug used for cancer treatment. However, 6 mercaptopurine has many disadvantages including low water solubility, limited and variable bioavailability, and severe side effects. This work's objective was to optimize the delivery of 6 MP by targeting it to the solid cancer tissues using an endogenous receptor system and glutathione-sensitive α, β unsaturated linker. Two 6 MP prodrugs were prepared, cellulose nanowhiskers (CNW) were used as a nanocarrier for the preparation of 6 MP-CNW-biotin (compound 12), using glutathione (GSH) sensitive linkage between the CNW and 6 mercaptopurine. The second prodrug, 6 MP-biotin (compound 9), was prepared by conjugating 6 mercaptopurine to the biotin directly using glutathione sensitive linker to evaluate the CNW role. The release assay was done in-vitro and it showed that just 11.4% of 6 mercaptopurine was released from compound 12 under glutathione at the micromolar concentration (100 μM), while the accumulative release reached 78.2% under 10 mM glutathione concentration. The in vitro cytotoxic effect was evaluated by 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay, the results showed that compound 9 has a greater inhibitory effect on cell lines with biotin overexpression (HepG2 and MCF-7) and low inhibitory effect on biotin negative cell line (CHO). Compound 12 showed activity comparable to 6 mercaptopurine against HepG2 and MCF-7, and lower inhibitory effect on CHO.

Keywords: Anticancer, 6 mercaptopurine, Cellulose nanowhiskers, Biotin, Glutathione.

International Journal of Drug Delivery Technology (2021); DOI: 10.25258/ijddt.11.4.62

How to cite this article: Wennas ON, Mohammed MH, Abed DA. Anti-Cancer Efficacy of 6 Mercaptopurine Loaded Biotinylated Cellulose Nanowhiskers. International Journal of Drug Delivery Technology. 2021;11(4):1478-1483.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Cancers represent the second most common cause of death after cardiovascular diseases in many countries.[1] Chemotherapy occupies an important position compared with other treatment methods, despite many advantages of cancer chemotherapy like potent inhibition of metastasized cancers and rapid onset of action, etc., many disadvantages are also apparent.[2,3]

6 Mercaptopurine (6 MP, 1, Figure 1) is widely used as an antileukemic in clinics.[4] 6 mercaptopurine suffers, however, from poor water solubility (0.124 mg/mL),[5] erratic oral absorption, and short plasma half-life (0.5–1.5 hour),[6] incomplete and variable bioavailability (10%–50%),[7] as well as severe bone marrow suppression and liver toxicity.[8] To solve these problems, some structural analogs and derivatives of 6 mercaptopurine have been synthesized, like S-(6-purinyl)-L-cysteine (2), S-allylthio-6-mercaptopurine (3) and 2-((7H-purin-6-yl)disulfanyl)-5-(pyridin-4-yl)-1,3,4-oxadiazole (4).[9-11] The delivery of 6 MP using nanocarrier based on the chemical release was also investigated, like the conjugation of six mercaptopurine via a glutathione-sensitive carbonyl vinyl sulfide linkage to carboxymethyl chitosan (5).[12] Nanomedicine has emerged as an important strategy for the target delivery of therapeutic and diagnostic agents during the past years. Cellulose nanowhiskers (CNW), called also cellulose nanocrystals (CNCs) have many characteristics that make them interesting nanosize carriers, cellulose nanowhiskers are biocompatible and non-toxic;[13] have a

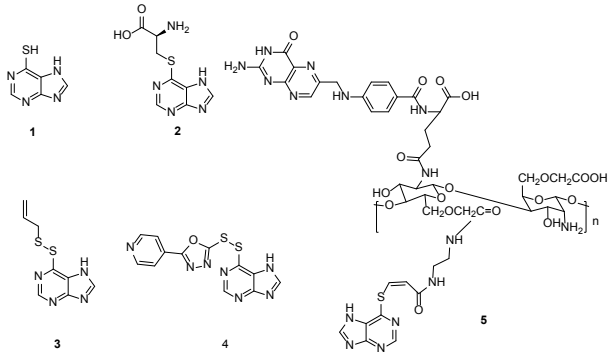


Figure 1: Chemical structures of 6 MP and some 6 MP prodrugs

**Author for Correspondence: osamawennas@gmail.com*

prolonged half-life.[14] The surface chemistry of CNW can be easily changed by the modification of the hydroxyl groups.[15]

The tripeptide, γ-l-glutamyl-l-cysteinyl-glycine, is known as glutathione (GSH).[16] It is available in micromolar concentrations in the blood plasma (2–20 μM) and millimolar range in the mammalian cells (0.5–10 mM), and its concentration in the cancer cells is higher than the concentration in the normal cells.[17] The different concentrations of GSH can be used to trigger the release of the drugs at the target sites, especially for the prodrugs containing disulfide bonds or α,β unsaturated carbonyl groups.[18,19]

The main obstacle of the anticancer agents' clinical applications is their cytotoxic effects on the normal cells.[20] Smart drug delivery systems with tumor-targeting units that primarily attack the malignant cells have been developed to avoid these problems.[21] Biotin has many advantages over other cancer-targeting units. Biotin has higher tumor specificity, low molecular weight, relatively simple and stable biochemical structure, low cost, lack of immunogenicity, and significant difference in expression levels of the biotin receptor on the normal and cancer cells' surfaces.[20]

The aim of this study is to prepare a GSH-responsive nanoparticle with the ability of active tumor targeting. The design of this system is based on CNW as a carrier, 6 mercaptopurine as an effector unit, and biotin as a guiding moiety. Finally, the cytotoxicity of the nano-conjugates on HpeG2, MCF7, and CHO cells was evaluated and compared with 6 mercaptopurine prodrug without the carrier (CNW), and the *in vitro* release property was studied.

MATERIALS AND METHODS

Materials

6 mercaptopurine and biotin were obtained from Hangzhou Hyper Chemicals Limited, China, Cellulose nanowhiskers were obtained from Maine University, United States of America. Propiolic acid, EDC-HCl, glutathione, and NHS were purchased from Sigma–Aldrich.

Characterization of compounds 6–9, and 11

Fourier transform infrared spectroscopy: FTIR spectra were done using Shimadzu device. NMR: NMR spectra (^{1}H & ^{13}C) were recorded on Vraian NMR system, Inova 500 MH. Electrospray ionization mass spectrometry: ESI-MS was done using Agilent device, electrospray ionization was performed in positive mode and the mass range was 115–2000 m/z.

Characterization of Nanoparticles

Determination of Biotin and 6 Mercaptopurine Content

The biotin content conjugated to the CNW was determined by elemental analysis depending on the Nitrogen% using LECO elemental analyzer, Model: TruSpec Micro. The 6 mercaptopurine content was determined using Carry 100 UV-visible spectrophotometer at 311 nm.

Dynamic Light Scattering (DLS)

The particle size and Zeta potential were measured using a Horiba device (model: SZ100). All samples were measured at 0.01 wt.% shortly after sonication in a water bath (output: 100 Watts).

Chemical Synthesis

The synthesis of 3- ((7H-purin-6-yl) thio) acrylic acid, PTA (compound 6)

6 mercaptopurine (1.02 g, 6.0 mmole) was dissolved in 50 mL of methanol containing 1.188 g (22.0 mmole) of CH_3NaO. Propiolic acid (0.370 mL, 6.0 mmole) was subsequently added. The reaction mixture was refluxed for 18 hours. After that, 8 mL of distilled water was added to quench the reaction. To obtain the product, 1M HCl was added to get a white precipitate. The purification of the product was done by filtration, redissolving by adding 1M NaOH, reprecipitation using 1M HCl, then washing with D.W., and allowed to dry to get compound 6 as a white solid, 64% yield. ^{1}HNMR (DMSO-*d6*, 500 MHz) δ 13.71 (s, 1H), 12.77 (s, 1H), 8.77 (d, J = 10.2 Hz, 1H), 8.56 (s, 1H), 6.29 (d, J = 10.1 Hz, 1H). ^{13}CNMR (DMSO-*d6*, 125 MHz) δ 167.80, 154.58, 151.81, 144.72, 137.54, 117.18. ESI-MS calculated for $C_8H_6N_4O_2S$ $(M+H)^+$ 223.03, found 223.2.

Synthesis of Compound 7

Biotin (3 g, 12.3 mmole) was suspended in 300 mL of methanol containing 2 g of Amberlite IR-120 resin, and the mixture was stirred at R.T. for 24 hours. The mixture was filtered to remove the resin and the product was obtained by evaporating the solvent to get compound 7 as a white solid, 89% yield. ^{1}HNMR (DMSO-*d6*, 500 MHz) 6.45 (s, 1H), 6.37 (s, 1H), 4.34 – 4.28 (m, 1H), 4.13 (m, 1H), 3.58 (s, 3H), 3.11 (dd, J = 4.8, 3.3 Hz, 1H), 2.82 (dd, J = 12.3, 5.2 Hz, 1H), 2.58 (d, J = 12.4 Hz, 1H), 2.30 (t, J = 7.4 Hz, 2H), 1.67 – 1.41 (m, 4H), 1.34 (m, 2H). ^{13}CNMR (DMSO-*d6*, 125 MHz) δ 173.77, 163.21, 61.51, 59.67, 55.82, 51.65, 40.32, 33.56, 28.47, 28.43, 24.94. ESI-MS calculated for $C_{11}H_{19}N_2O_3S$ $(M+H)^+$ 259.11, found 259.2.

Synthesis of Compound 8

Compound **7** (1.032g, 4 mmole, 1.00 equivalent) was suspended in 16 ml of methanol containing hydrazine (2 mL, 40 mmole, 10.0 equivalent). The suspension was stirred for 24 hours at R.T. The mixture was filtered to and then washed with chloroform to get compound **8** as a white solid, 92% yield. ^{1}HNMR (DMSO-*d6*, 500 MHz) δ 8.92 (s, 1H), 6.42 (s, 1H), 6.36 (s, 1H), 4.31 (dd, J = 7.8, 5.0 Hz, 1H), 4.14 (m, 3H), 3.11 (m, 1H), 2.82 (dd, J = 12.3, 5.1 Hz, 1H), 2.58 (d, J = 12.3 Hz, 1H), 2.03 (t, J = 7.3 Hz, 2H), 1.65 – 1.42 (m, 4H), 1.32 (m, 2H). ^{13}CNMR (DMSO-*d6*, 125 MHz) δ 172.04, 163.22, 61.53, 59.69, 55.88, 33.72, 28.71, 28.51, 25.72. ESI-MS calculated for $C_{10}H_{19}N_4O_2S$ $(M+H)^+$ 259.12, found 259.2.

Synthesis of Compound 9

Compound 6 (896 mg, 4 mmole) was dissolved in 30 mL of DMSO, then EDC-HCL (1.152 mg, 6 mmole) and NHS (688 mg, 6 mmole) were added. The mixture was stirred at R.T. for 3 h to activate the carboxyl group. Then 1.032 g (4 mmole) of compound 8 was added, and the reaction mixture was stirred at R.T. for 24 hours. The precipitation of the product was done by adding of 250 mL acetone. The purification of the product was done by washing with 0.1 N hydrochloric

acid, sodium bicarbonate 5%, and D.W. and then dried to get the title compound as a yellow powder, yield 64%. ^{1}HNMR (DMSO-*d6*, 500 MHz) δ 13.65 (s, 1H), 10.21 (s, 1H), 9.98 (s, 1H), 8.77 (d, J = 3.1 Hz, 1H), 8.67 – 8.50 (m, 2H), 6.52 (s, 1H), 6.43 (m, 2H), 4.364– 4.32 (m, 1H), 4.17 – 4.13 (m, 1H), 3.07 (m, 1H), 2.81 (m, 1H), 2.57 (d, J = 12.3 Hz, 1H), 2.16 (t, J = 7.3 Hz, 2H), 1.67-141 (m, 4H), 1.35 (m, 2H). ^{13}CNMR (DMSO-*d6*, 125 MHz) δ 171.54, 164.61, 163.37, 151.82, 144.83, 133.42, 116.86, 79.57, 61.62, 59.72, 55.85, 33.47, 28.57, 28.47, 25.53. ESI-MS calculated for $C_{18}H_{23}N_8O_3S_2$ $(M+H)^+$ 463.13, found 463.2.

Synthesis of Carboxylated CNW (CNW-COOH, Compound 10)

About 2.04 g, i.e., 12.6 mmole of equivalent anydroglucose unit of cellulose nanowhiskers were suspended in water (200 ml) and sonicated for 5 min. TEMPO (59.5 mg, 0.376 mmole) and sodium bromide (648 mg, 6.3 mmole) were added to CNW suspension. Then, 15.6 mL of sodium hypochlorite solution, corresponding to 6.3 mmole of sodium hypochlorite was slowly added to the suspension. The reaction pH was kept at about 10.5 using sodium hydroxide (0.5 M). After 45 minutes, the reaction was terminated by the addition of methanol (2 mL), and the pH was adjusted to 7 with 0.5 M HCl. The oxidized cellulose nanowhiskers were dialyzed against distilled water. The concentration of the suspension was determined by the gravimetrical method.

Synthesis of PTA Hydrazide (Compound 11)

In 40 mL of DMF, Compound 6 (444 mg, 2 mmole) was dissolved. Then HOBt (324 mg, 2.4 mmole) was added followed by 460 mg (2.4 mmole) of EDC-HCl and the reaction mixture was stirred at R.T. for 2 hours. The activation of the carboxylic acid group and the formation of active HOBt ester was checked by TLC. The activated HOBT ester was added slowly by inverse addition to 5 mL of DMF containing 4 mmole of hydrazine and 0.05 mL of cyclohexene. The temp. was kept during the addition at 0°C. The reaction completion was reached at the end of the addition. The precipitation of the product was done by adding 100 mL of D.W. The product was obtained by filtration and then excessive washing with distilled water and then allowed to dry to get compound 11 as an off-white solid, 78% yield. ^{1}HNMR (DMSO-*d6*, 500 MHz) δ 13.64 (s, 1H), 9.45 (s, 1H), 8.79 (s, 1H), 8.54 (s, 1H), 8.46 (d, J = 10.1 Hz, 1H), 6.27 (d, J = 10.0, 1H), 4.50 (s, 2H). ^{13}CNMR (DMSO-*d6*, 125 MHz) δ 165.30, 151.81, 130.61, 117.74. ESI-MS calculated for $C_8H_9N_6OS$ $(M+H)^+$ 237.06, found 237.3.

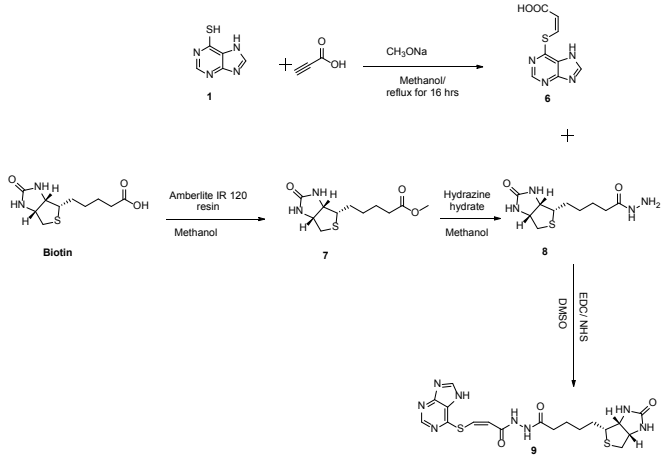
Synthesis of 6-MP-CNW-biotin (Compound 12)

200 mL containing 0.74W/V% of compound 10 suspension was used. The flocculation of compound 10 was done by using 6 mL of saturated sodium chloride solution. Compound 10 was then obtained by centrifugation and washed (by redispersion using sonication, followed by centrifugation) 3 times using 100 mL of acetone, followed by 2 times with 100 mL of DMF. Then Compound 10 was dispersed in 200 mL of DMF and sonicated for 5 min. The concentration of compound 10 was determined by the gravimetrical method and it was found to be 0.65W/V% containing about 1.5 mmole of COOH (the COOH concentration was determined by conductimetric titration). 144 mg of EDC-HCl (0.74 mmole) and 86 mg of NHS (0.74 mmole) were added for the activation of half of COOH of compound 10. After stirring the suspension for 2 hours at R.T., 194 mg (0.75 mmole) of compound 8 was added to the suspension with continuous stirring for 48 hours at room temp. The reaction mixture was then transferred to be dialyzed with dimethylformamide for 24 hours (three times) using a dialysis membrane 8-14 kDa.

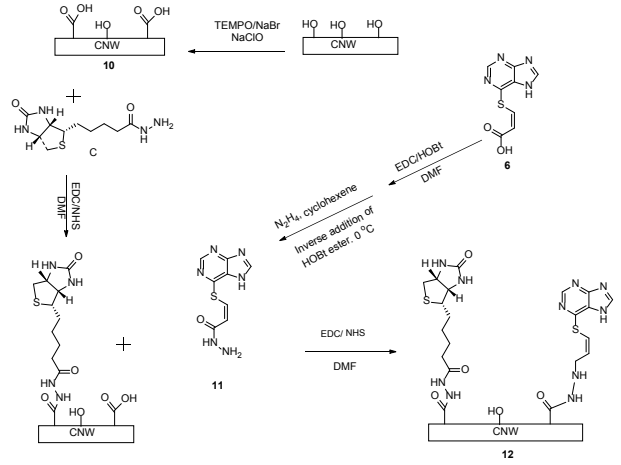
At the end of dialysis, the reaction mixture was poured again into a round flask. 230 mg of EDC-HCl (1.2 mmole) and 138 mg of NHS (1.2 mmole) were added for activating the unreacted COOH groups of compound 10. After stirring the suspension for 2 hours at RT, 284 mg (1.2 mmole) of compound 11 was added, and the mixture was stirred for a further 48 hours at RT. The reaction mixture was then transferred to be dialyzed with dimethylformamide for 24 hours (3 times) and then dialyzed with deionized water for 48 hours.

In vitro Drug Release Study

The release of 6 MP was studied by a dialysis tube technique using two different media, FBS with 100 µM GSH and acetate buffer with pH 5.8 and GSH millimolar concentration (10 mM).



Scheme 1: Synthesis schematics of compound 9



Scheme 2: Synthesis schematics of compound 12.

In brief, compound 12 (25 mg) was transferred to a dialysis tube containing 10 mL of the dialysis medium then dialyzed against 50 mL of this dialysis medium. At the desired time intervals of 1, 2, 4, 6, 8, 12, or 24 hours, 1-mL of released medium was taken out and replaced with an equal volume of fresh medium. The concentration of the drug was determined using a UV spectrophotometer at 320 nm.

In vitro Cytotoxicity Assay

The MTT assay was used to evaluate the *in vitro* cytotoxicity of compound 9 and compound 12. Three cell lines were used, two cell lines that overexpress biotin receptor, human hepatic carcinoma (HepG2) and human breast cancer cell (MCF-7). The third cell line rarely expresses the biotin receptor which is Chinese hamster ovarian (CHO). The results of the assay were shown as a mean of three independent experiments and IC_{50s} were calculated to show the effectiveness of the tested compounds on growth inhibition of the three used cell lines.

RESULTS AND DISCUSSION

Chemical Synthesis of Compounds 6-9, and 11

Compound 6 was prepared by the reaction of 6 mercaptopurine with propiolic acid by Michael reaction.[22] The IR spectrum shows the appearance of a band at 1703 cm^{-1} due to the stretching of carbonyl of carboxylic acid. ^{1}HNMR shows the appearance of vinylic protons signals at 8.77 and 6.29 p.p.m., and carboxylic acid proton signal at 12.77 p.p.m. ^{13}CNMR of compound 6 shows the appearance of a new signal related to COOH carbon at 167.80 p.p.m.

Compound **7** was prepared by Fischer esterification of biotin with methanol, as acid catalysis Amberlite IR120 resign was used.[23] The IR spectrum shows shifting of C=O absorption from 1685 cm^{-1} to 1743 cm^{-1} due to the conversion of carboxylic acid to ester. ^{1}HNMR of compound **7** shows the disappearance of the signal of the carboxylic acid proton (COOH) at 12.00 p.p.m. and the appearance of the signal of methoxy protons ($COOCH_3$) at 3.58 p.p.m. as a singlet. ^{13}CNMR of compound **7** shows the appearance of a new signal related to $COOCH_3$ carbon at 51.65 p.p.m., also a change in the value of C=O at the site of reaction from174.92 to 173.77 p.p.m. due to the conversion of carboxylic acid to ester.

Compound 8 was prepared by the hydrazinolysis of compound 7 using an excess of hydrazine.[24] The IR spectrum shows shifting of C=O absorption from 1743 cm^{-1} to 1689 cm^{-1} due to the conversion of ester to hydrazide. ^{1}HNMR shows the disappearance of the signal of methoxy protons ($COOCH_3$) at 3.58 p.p.m. and the appearance of two new signals related to $CONHNH_2$ protons at 8.92 p.p.m. and 4.14 ppm, respectively. ^{13}CNMR of compound 8 shows the disappearance of $COOCH_3$ carbon at 51.65 ppm, also a change in the value of C=O at the site of reaction from 173.77 to 172.04 ppm, due to the conversion of ester to hydrazide.

Compound 9 was obtained by the coupling of compound 6 with compound 8 using EDC-HCl as a dehydrating agent.[25] The IR spectrum shows broadband at 3400–3250 cm^{-1} due to the stretching of secondary amines and amides, and a band at 1687 cm^{-1} for the stretching of carbonyl of hydrazide. ^{1}HNMR shows the disappearance $CONHNH_2$ signal at 4.14 p.p.m. and the appearance of a new signal at 9.98 p.p.m. ^{13}CNMR of the compound 9 shows a change in the value of C=O at the site of reaction from 167.80 to 164.61 p.p.m. due to the conversion of carboxylic acid to hydrazide.

Compound 11 was obtained as hydrazide of compound 6, the process involves the activation of the carboxylic acid by EDC-HCl to get HOBt esters. Then by the hydrazinolysis of these esters to get compound 11.[26] The IR spectrum shows a shifting of C=O absorption from 1703 cm^{-1} to 1674 cm^{-1} due to the conversion of carboxylic acid to hydrazide. ^{1}HNMR shows the disappearance of the signal of the carboxylic acid proton (COOH) at 12.77 p.p.m. and the appearance of new signals related to $CONHNH_2$ protons at 9.45 p.p.m. and 4.50 p.p.m. respectively both as a singlet. ^{13}CNMR of compound 11 shows a change in the chemical shift of C=O at the site of reaction from 167.80 to 165.30 p.p.m. due to the conversion of carboxylic acid to hydrazide.

The Biotin and 6-MP Content

The contents of biotin and 6 mercaptopurine in compound 12 were determined and the results are shown in Table 1.

Particle Size and Zeta Potential

Quantitative measurements of particle size and zeta potential showed that CNW and the conjugates of CNW have a particle size in the range of 68 to 98 nm with negatively charged surfaces, and the results are shown in Table 1.

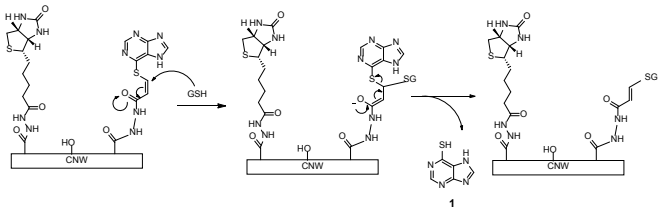
In vitro Release Study

6 mercaptopurine release from compound 12 is based on Michael addition-elimination reaction (Scheme 3).[22] Since the α-β unsaturated bond is sensitive to the GSH (thiol group), the GSH will attack the β carbon of the unsaturated bond leading to GS-conjugate formation, and this will be followed by 6 MP release.

The accumulative 6 mercaptopurine release from compound 12 is 78% in the acetate buffer with pH 5.8 and glutathione millimolar concentration (10 mM), while the cumulative release in PBS with 100 μM glutathione is only 11.4%, as shown in Figure 2. The obtained results suggested that 6 mercaptopurine release is accelerated by the elevated

Table 1: Characterization data of CNW and the conjugates of CNW

Sample	*Particle size*	*PDI*	*Zeta potential*	*Biotin%*	*6 MP %*
CNW	68.12 ± 4.19	0.38 ± 0.12	-34.33 ± 1.59	0	0
Compound 10	81.23 ± 0.3.53	0.46 ± 0.05	-65.22 ± 2.40	0	0
Compound 12	98.63 ± 5.41	0.35 ± 0.11	-42.04 ± 0.91	8.67	4.3



Scheme 3: Drug release mechanism.

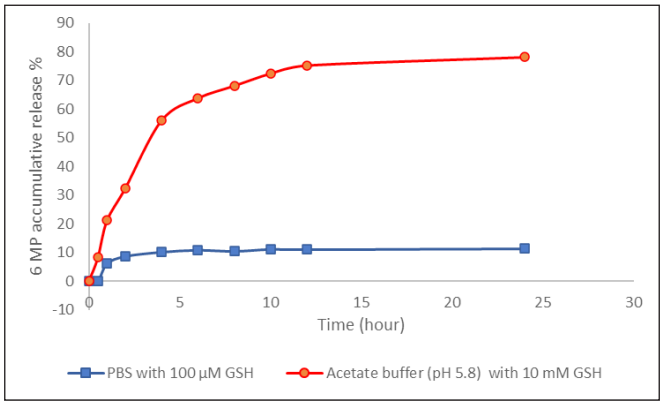


Figure 2: The release of 6 mercaptopurine from compound 12 at different conditions

glutathione concentrations in the cellular environment (especially cancer cells). In contrast, a minimum amount of 6 mercaptopurine is released at the low glutathione concentrations in the extracellular environment.

In vitro Cytotoxicity Assay

In the current study, three types of cell lines with various levels of biotin receptors expression were used for the cytotoxicity assay of compounds 9 and 12. HepG2 and MCF-7 cells are characterized by biotin receptor overexpression, while CHO is a biotin negative cell line.[20] Figures 3a, b, and c show the viability of the cells after the incubation for 72 hours. The IC_{50s} were measured to show the growth inhibitory effect of compounds 9 and 12 on the three cell lines (Figure 3d). The IC_{50} of compound 9 is less than half that of six mercaptopurine on HepG2 and MCF-7 (cell lines with biotin receptor overexpression), and, and about 3 times higher than 6 mercaptopurine on CHO (the biotin negative cell line). Compound 12 showed activity comparable to that of six mercaptopurine on HepG2 and MCF-7. Against CHO, compound 12 showed a much lower inhibitory effect and IC_{50} is more than 8 times higher than that of 6 mercaptopurine. The obtained results suggested that the cytotoxicity and the uptake of compounds 9 and 12 are higher for biotin positive cells than for biotin negative cells and they can effectively reduce six mercaptopurine side effects by the selective killing of biotin-positive cancer cells.

CONCLUSIONS

Two 6 mercaptopurine prodrugs were prepared using the biotin as a guiding moiety and glutathione sensitive α, β unsaturated linker. The chemical synthesis was monitored by FT-IR, ^{1}H and ^{13}C NMR, and MS. The evaluation of the nanoparticles was done by the DLS. The *in vitro* release study showed that

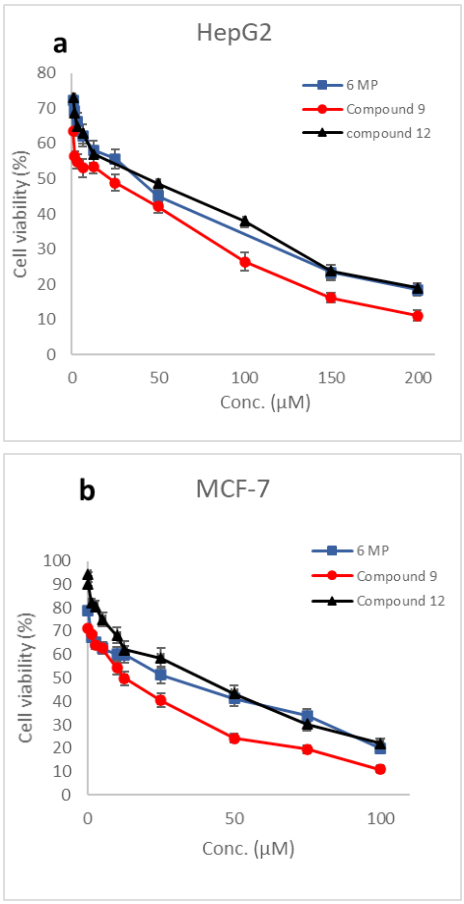


Figure 3: Cytotoxicity of 6 mercaptopurine, compound 9, and compound 12 against (a) HepG2, (b)MCF-7, and (c) CHO cell lines after 72 h incubation (d) IC_{50}s of 6 mercaptopurine, compound 9, and compound 12 in HepG2, MCF-7, and CHO cell lines.

glutathione can promote the release of 6 mercaptopurine at the intracellular millimolar concentration compared with glutathione micromolar concentration. MTT assay showed that these prodrugs have had a higher inhibitory effect on cell lines with biotin overexpression (HepG2 and MCF-7) and a low inhibitory effect on biotin negative cells (CHO). These results suggested that biotin-guided, glutathione-sensitive prodrugs had a potential for cancer targeting.

REFERENCES:

1. van der Merwe E, Huang D, Peterson D, Kilian G, Milne PJ, Van de Venter M, et al. The synthesis and anticancer activity of selected diketopiperazines. Peptides. 2008;29(8):1305-1311.
2. Pérez-Herrero E, Fernández-Medarde A. Advanced targeted therapies in cancer: Drug nanocarriers, the future of chemotherapy. Eur J Pharm Biopharm. 2015;93(1):52-79.
3. Al-Amily DH, Mohammed MH. Design, synthesis, and docking study of acyl thiourea derivatives as possible histone deacetylase inhibitors with a novel zinc binding group. Sci Pharm. 2019;87(4):1-15.
4. Yang JJ, Landier W, Yang W, Liu C, Hageman L, Cheng C, et al. Inherited NUDT15 variant is a genetic determinant of mercaptopurine intolerance in children with acute lymphoblastic leukemia. J Clin Oncol. 2015;33(11):1235-1242.

5. Newton DW, Ratanamaneichatara S, Murray WJ. Dissociation, solubility and lipophilicity of azathioprine. Int J Pharm. 1982;11(3):209-213.
6. Zacchigna M, Cateni F, Di Luca G, Drioli S. A simple method for the preparation of PEG-6-mercaptopurine for oral administration. Bioorganic Med Chem Lett. 2007;17(23):6607-6609.
7. Kumar GP, Sanganal JS, Phani AR, Manohara C, Tripathi SM, Raghavendra HL, et al. Anti-cancerous efficacy and pharmacokinetics of 6-mercaptopurine loaded chitosan nanoparticles. Pharmacol Res. 2015;100:47-57.
8. Al-Darraji AS, Mohamed MH. Synthesis and preliminary anticancer evaluation of 6-mercaptopurine-methotrexate conjugate as possible mutual prodrug. Iraqi J Pharm Sci. 2019;28(1):114-124.
9. Hwang Y, Elfarra AA. Cysteine S-conjugates may act as kidney-selective prodrugs: formation of 6-mercaptopurine by the renal metabolism of S-(6-purinyl)-L-cysteine. J Pharmacol Exp Ther. 1989;251(2):448-454.
10. Miron T, Arditti F, Konstantinovski L, Rabinkov A, Mirelman D, Berrebi A, et al. Novel derivatives of 6-mercaptopurine: Synthesis, characterization and antiproliferative activities of S-allylthio-mercaptopurines. Eur J Med Chem. 2009;44(2):554--550.
11. Mohammed MH, Taher MA. Synthesis of New Two Derivatives of 6-Mercaptopurine (6MP) 6-[5-Pyridine-4-yl- 1, 2, 3, 4-oxazolezole-2-yl)dithiol]-9H-purine (38) And 9H-purine-6-yl-benyldithiocarbamate (45) With Cytotoxicity Results From The National Cancer Institute's Anticancer Drug. Int J Pharm Sci Res. 2012;3(08):2613-2622.
12. Wei X, Liao J, Davoudi Z, Zheng H, Chen J, Li D, et al. Folate receptor-targeted and gsh-responsive carboxymethyl chitosan nanoparticles containing covalently entrapped 6-mercaptopurine for enhanced intracellular drug delivery in leukemia. Mar Drugs. 2018;16(11):439.
13. Klemm D, Schumann D, Udhardt U, Marsch S. Bacterial synthesized cellulose - Artificial blood vessels for microsurgery. Vol. 26, Progress in Polymer Science (Oxford). Elsevier Ltd; 2001. p. 1561-1603.
14. Owens DE, Peppas NA. Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. Int J Pharm. 2006;307(1):93-102.
15. Eyley S, Thielemans W. Surface modification of cellulose nanocrystals. Nanoscale. 2014;6(14):7764-7779.
16. Wu G, Fang Y-Z, Yang S, Lupton JR, Turner ND. Glutathione Metabolism and Its. J Nutr. 2004;134(3):489-492.
17. Espinosa-Diez C, Miguel V, Mennerich D, Kietzmann T, Sánchez-Pérez P, Cadenas S, et al. Antioxidant responses and cellular adjustments to oxidative stress. Redox Biol. 2015;6:183-197.
18. Ruzza P, Rosato A, Nassi A, Rondina M, Zorzin M, Rossi CR, et al. Synthesis and preliminary in vitro biological evaluation of 4-[(4-hydroxyphenyl)sulfanyl]but-3-en-2-one, a 4-mercaptophenol derivative designed as a novel bifunctional antimelanoma agent. J Med Chem. 2009;52(15):4973-4976.
19. Saito G, Swanson JA, Lee K-D. Drug delivery strategy utilizing conjugation via reversible disulfide linkages: role and site of cellular reducing activities. Adv Drug Deliv Rev. 2003;55(2): 199-215.
20. Ren WX, Han J, Uhm S, Jang YJ, Kang C, Kim JH, et al. Recent development of biotin conjugation in biological imaging, sensing, and target delivery. Chem Commun. 2015;51(52):10403-10418.
21. Luo Z, Hu Y, Cai K, Ding X, Zhang Q, Li M, et al. Intracellular redox-activated anticancer drug delivery by functionalized hollow mesoporous silica nanoreservoirs with tumor specificity. Biomaterials. 2014;35(27):7951-7952.
22. Shi L, Ding K, Sun X, Zhang L, Zeng T, Yin Y, et al. Preparation, characterization, and in vitro drug release behavior of glutathione-sensitive long-circulation micelles based on polyethylene glycol prodrug. J Biomater Sci Polym Ed. 2016;27(6):472-489.
23. Zör Kİ, Amberl O. Deactivation of Amberlite Ir-120 Used in the Esterification of Acetic Acid With Isobutanol. 2006;19(2);47-58.
24. Murar CE, Thuaud F, Bode JW. KAHA ligations that form aspartyl aldehyde residues as synthetic handles for protein modification and purification. J Am Chem Soc. 2014;136(52):18140-18148.
25. Gong XY, Yin YH, Huang ZJ, Lu B, Xu PH, Zheng H, et al. Preparation, characterization and in vitro release study of a glutathione-dependent polymeric prodrug Cis-3-(9H-purin-6-ylthio)-acrylic acid-graft-carboxymethyl chitosan. Int J Pharm. 2012;436(1–2):240-247.
26. Zhang X, Breslav M, Grimm J, Guan K, Huang A, Liu F, et al. A new procedure for preparation of carboxylic acid hydrazides. J Org Chem. 2002;67(26):9471-9474.