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Original article

Simple and efficient synthesis of novel glycosyl thiourea derivatives as potential antitumor agents

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Abstract

The practical synthesis of pseudonucleosides incorporating thiourea derivative by coupling of monosaccharides (D-galactose, D-glucose and D-xylose) per-*O*-acetylated glycosyl isothiocyanates and different heterocyclic hydrazide derivatives is reported. The method involves the preparation of per-*O*-acetylated glycosyl isothiocyanates from per-*O*-acetylated sugars (two-step synthesis), which couple with heterocyclic hydrazides from amines to give thiourea-linked pseudonucleosides. All newly synthesized pseudonucleosides were assayed against human lung cancer-cell lines (PG) and human liver cancer-cell lines (BEL-7402) *in vitro*. The 2-(4-methoxybenzamide)-benzoimidazole-1-yl-acetyl pseudonucleosides showed moderate inhibition against these two cancer-cell lines with EC_{50} from 22.8 to 76.4 μM and from 54.9 to 82.4 μM, respectively. And the other compounds did not demonstrate any significant cytotoxicity even at concentrations up to 200 μM.

Keywords: Glycosyl bromides; Glycosyl isothiocyanates; Heterocyclic hydrazides; Thiourea-linked pseudonucleosides; Cytotoxicity

1. Introduction

Isothiocyanates are versatile synthetic intermediates in organic chemistry due to their availability and their tendency to undergo nucleophilic additions and cycloadditions [1–3]. Particularly in carbohydrate field, sugar isothiocyanates play a pivotal role in the preparation of a broad spectrum of carbohydrate derivatives, mostly having thiourea structure, for biological and pharmaceutical interests [4,5]. Numerous antiviral, antibacterial and antitumor agents have been prepared by reaction of glycosyl isothiocyanates with biologically active amines [4,6,7]. Recently, glycosyl isothiocyanates are being used to prepare thiourea-linked symmetrical and unsymmetrical carbohydrate mimics, such as pseudooligosaccharides [8,9], thioureylene-di-nucleosides [10] and other glycosyl thioureas [11], for molecular recognition studies.

At the same time, nucleosides are compounds of antiviral and antitumor interests [12,13], so they have become an important subject of research in the field of organic and pharmaceutical chemistry due to the successful treatment of many infectious diseases [14], in particular for the therapy of AIDS [15,16]. The synthesis of analogues of natural nucleoside and oligonucleosides has been a growing research topic over the last few years, and several pseudonucleosides with imidazolidine or thioimidazole groups [17] as well as thiourea-linked oligonucleoside analogues [10] changing negatively charged phosphodiester linkage by non-ionic isosteric spacers have been synthesized [18].

In view of the advantage conferred by glycosyl isothiocyanate synthesis that allows rapid, convenient access to a wide array of thioureido carbohydrates [19], together with the notable biological activities of nucleoside analogues, a great deal of work has focused on the development of novel thiourea-linked glycoconjugate or nucleoside [20]. However, there are little reports on the synthesis and bioactivity of thiourea-linked pseudonucleoside, in which the base and sugar moieties are bound by an acylthiosemicarbazide. In this paper, a very short and efficient synthetic route to novel acylthiosemicarbazide derivatives and their cytotoxicity effects are reported.

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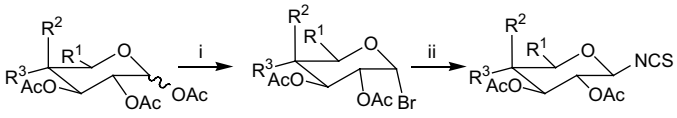
2. Chemistry

The synthetic strategy involved the coupling of the glycosyl isothiocyanates to the heterocyclic hydrazide to obtain acetylated *N*-neoglycoconjugates that could be subjected to hydrolysis to the deacetylated thiourea-linked pseudonucleoside. Accordingly, the first task was to prepare the glycosyl isothiocyanates (Scheme 1). The reaction of per-*O*-acetylated sugars (D-galactose **1**, D-glucose **2**, and D-xylose **3**) with brominating agent (added Br_2 in a suspension of P_4 in AcOH) [21] at room temperature led to the corresponding acetylated glycosyl bromides **4**–**6**, respectively. Treatment of **4**–**6** with thiocyanate in refluxing dry dimethylbenzene for 3.5 h gives the glycosyl isothiocyanates **7**–**9** [22] according to the classical Fischer's method [23], The hexopyranosyl isothiocyanates of D-galacto and D-gluco configuration **7** and **8** were obtained in about 8 h, while formation of the pentopyranosyl isothiocyanate **9** took about 8.5 h.

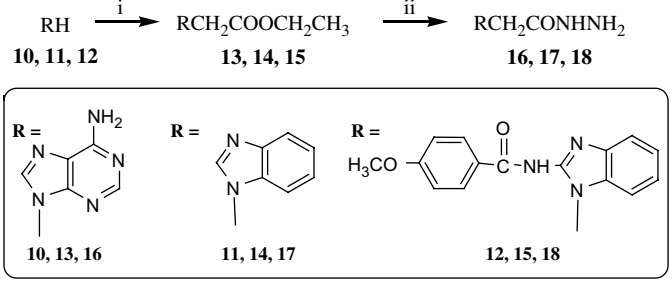
The heterocyclic hydrazides were prepared from amines **10**–**12** (Scheme 2). Adenine **10** was alkylated with ethyl chloroacetate in DMF by first generating the anion with sodium hydride. This procedure resulted in only one isolable compound which was recrystallized from methanol and identified as the expected light yellow solid adenine-9-yl-ethylacetate **13** [24] in a 70% yield. Then the reduction of **13** with hydrazine hydrate in methanol gave corresponding adenine hydrazide derivative **16** as white solid in good yield of 81%. By the same procedure, heterocyclic hydrazide derivatives **17** and **18** were also efficiently prepared from the corresponding heterocyclic compounds **11** and **12** within two steps in 52 and 47% yields, respectively.

The coupling of the glycosyl isothiocyanates to the heterocyclic hydrazide afforded acetylated *N*-neoglycoconjugates which finally led to the deacetylated thiourea-linked pseudonucleosides (*N*-glycoconjugates) (Scheme 3). Nucleophilic addition of the adenine-9-yl-acetyl hydrazide **16** to per-*O*-acetylated sugar isothiocyanates **7**–**9** in pyridine under Ar at room temperature afforded the corresponding per-*O*-protected adducts **19**–**21**, respectively. Then, these adducts **19**–**21** were subjected to sodium methoxide-catalyzed in methanol (pH = 8.0) deacetylation furnished the corresponding unprotected *N*-glycoconjugates **28**–**30**, respectively. Following the same protocols, the other deacetylated *N*-glycoconjugates **31**–**36** were prepared in two steps from the corresponding starting materials. All purification was achieved by column chromatography and possible recrystallization from methanol.



1, **4**, **7**: $R^1 = CH_2OAc$, $R^2 = OAc$, $R^3 = H$
2, **5**, **8**: $R^1 = CH_2OAc$, $R^2 = H$, $R^3 = OAc$
3, **6**, **9**: $R^1 = R^2 = H$, $R^3 = OAc$

Scheme 1. Reagents and conditions. (i) AcOH, P, dropwise Br_2, 30 min and (ii) $Pb(SCN)_2$, dry $(CH_3)_2C_6H_4$, 3.5–8.5 h.

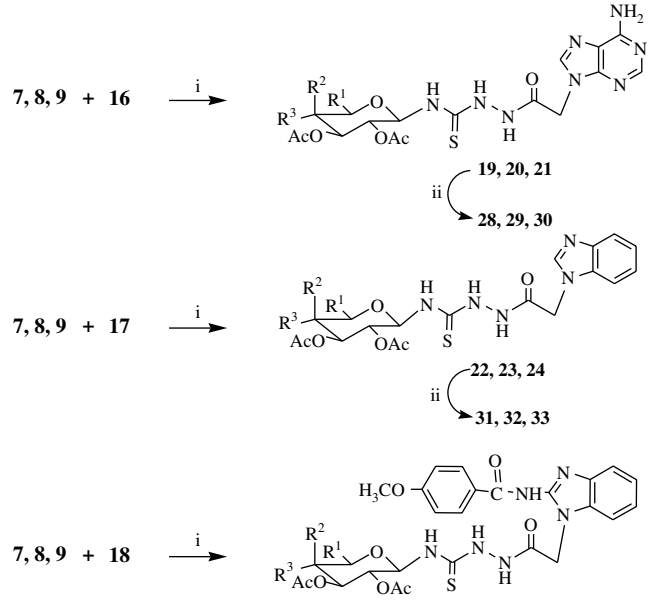


Scheme 2. Reagents and conditions. (i) NaH, $ClCH_2COOC_2H_5$, DMF and (ii) 80% $H_2NNH_2 \cdot H_2O$, MeOH.

It is interesting to note that the coupling between benzoimidazole-1-yl-acetyl hydrazide **17** with per-*O*-acetylated sugar isothiocyanates **7**–**9** gave the per-*O*-protected adducts **22**–**24** in good yield (see Table 1, entry 4–6, yields were more than 85%). On the other hand, the reaction of 2-(4-methoxybenzamide)-benzoimidazole-1-yl-acetyl hydrazide **18** was accomplished to afford the compounds **25**–**27** in 64–76% yield. These results can be explained that the weaker steric hindrance of the compound **17** makes its reaction smooth.

3. Biological activity

The newly synthesized compounds **28**–**36** were evaluated for their *in vitro* cytotoxicity by growth-inhibition studies



19, **22**, **25**: $R^1 = CH_2OAc$, $R^2 = OAc$, $R^3 = H$
20, **23**, **26**: $R^1 = CH_2OAc$, $R^2 = H$, $R^3 = OAc$
21, **24**, **27**: $R^1 = R^2 = H$, $R^3 = OAc$
28, **31**, **34**: $R^1 = CH_2OH$, $R^2 = OH$, $R^3 = H$
29, **32**, **35**: $R^1 = CH_2OH$, $R^2 = H$, $R^3 = OH$
30, **33**, **36**: $R^1 = R^2 = H$, $R^3 = OH$

Scheme 3. Reagents and conditions. (i) Ar, DMF, room temperature and (ii) MeOH, MeONa, pH = 8.0.

Table 1
Synthesis of the per-*O*-protected glycoconjugates **19**–**27**[a]

Entry	Sugar isothiocyanates	Hydrazide	Product	Yield[b] (%)
1	**7**	16	19	76
2	**8**	16	20	83
3	**9**	16	21	77
4	**7**	17	22	86
5	**8**	17	23	91
6	**9**	17	24	88
7	**7**	18	25	64
8	**8**	18	26	76
9	**9**	18	27	71

[a] All reactions conducted at room temperature; see Section 5 for details.
[b] Isolated yields based on sugar isothiocyanates after possible purification.

using two human cancer-cell lines: human liver (BEL-7402 cell) and human lung (PG cell).

As shown in Table 2, the total deacetylated 2-(4-methoxybenzamide)-benzoimidazole-1-yl-acetyl pseudonucleosides **34**–**36** exhibited inhibitory activity against human liver cancer-cell lines (BEL-7402) with EC_{50} from 22.8 to 76.4 μM and showed much less activity against human lung cancer-cell lines (PG) with EC_{50} from 54.9 to 82.4 μM. Additionally, only compound **29** in benzoimidazole-1-yl-acetyl glycoconjugates showed effect on human liver cancer-cells (BEL-7402) with EC_{50} of 86.2. However, the compounds **28** and **31** demonstrated inhibitory effects on human liver cancer-cell lines (BEL-7402) with EC_{50} of 94.5 and 82.2 μM, respectively. And the other compounds did not indicate any significant inhibition against these two human cancer-cell lines up to 200 μM.

4. Conclusions

In conclusion, a new type of sugar–heterocyclic compounds linked by thiourea derivative could be obtained from the readily available per-*O*-acetylated sugars under mild condition. To the best of our knowledge, this type of *N*-neoglycoconjugates is not known in the literature and some of them showed moderate inhibitory effects on cancer cells. This convenience of our strategy lies in the possibility of preparing a number of different analogues by the reaction of a simple glycosyl isothiocyanate with several heterocyclic hydrazides.

Table 2
Cytotoxicity of the final compounds **28**–**36** in two cancer-cell lines (PG, BEL-7402)

Compound	EC_{50}[a] (μM)	
	PG[b]	BEL-7402[c]
28	94.5	≥200
29	>100	86.2
30	>200	>100
31	82.2	>100
32	>100	>200
33	>200	>200
34	54.9	22.8
35	82.4	76.4
36	73.7	36.7
Zidovudine	0.58	4.62

[a] 50% Effective concentration.
[b] Human lung cells.
[c] Human liver cells.

5. Experimental

5.1. General procedures

Solvents were pretreated, when necessary, according to appropriate standard procedures before used. Column chromatography (CC) was performed on silica gel (200–300 mesh), and precoated silica gel plates (F_{254}: Qingdao Marine Chemical Co., Ltd., 20 × 10 cm, 0.5 mm thick) were used for TLC; the spots were examined with UV light, iodine vapor and sulfuric acid spray. And reaction mixtures were stirred magnetically. Melting points were determined on an RY-1 apparatus in capillaries and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a BRUCK 500 MHz spectrometer in DMSO-d_6 and chemical shifts are given in δ values (ppm) relative to tetramethylsilane as the internal standard. Splitting patterns are designated as follows: s = singlet, d = doublet, dd = double doublet, t = triplet, br s = broad singlet, and m = multiplet. UV spectra were arrived on a VARIAN spectrometer using a cell path length of 1 cm. Fourier transform infrared spectra were recorded using an Nicolet 501P FTIR spectrometer with KBr plates. Mass spectra were recorded on an ABI-API4000 mass spectrometer in *m*/*z*. Elemental analysis was reported with a Perkin Elmer 240C apparatus. Optical rotations were measured on a Perkin Elmer 241 polarimeter at 25°.

5.2. General procedure for synthesis of per-O-acetylated glycosyl isothiocyanates 7–9

To a suspension of P_4 (4.5 g, 3.63 mmol) in AcOH (60 mL) was added Br_2 (12 mL, 234.10 mmol) dropwise. The mixture was stirred for 30 min at room temperature, and then P_4 was filtrated. Per-*O*-acetylated sugar **1** (39 g, 100 mmol) was added and the mixture was stirred at the same temperature until TLC showed disappearance of **1**. The bulk of AcOH was removed under reduced pressure, and the residue was partitioned between saturated Na_2CO_3 solution and $CHCl_3$. The organic extracts were washed with water and brine, and dried (Na_2SO_4). The organic phase was concentrated under reduced pressure to afford **4** as a white solid. Without further purification, per-*O*-acetylated bromide **4** (7.9 g, 20 mmol) was added dropwise to a refluxed suspension of $Pb(SCN)_2$ (9.69 g, 30 mmol) in 50 mL anhydrous dimethylbenzene. The mixture refluxed for 3.5–8.5 h, then $Pb(SCN)_2$ was filtrated. The solvent was removed under reduced pressure and the residue was recrystalized to afford **7** as white solid from the mixture of toluene and petroleum ether. The corresponding compounds **8** and **9** were prepared by this method. Physical data of per-*O*-acetylated

glycosyl isothiocyanates **7–9** matched those described in the literature [21].

5.3. General procedure for synthesis of acetylhydrazine heterocycle compounds **16–18**

To a cooled suspension of adenine **10** (5.0 g, 37 mmol) in dry DMF (100 mL) was added NaH (6.1 g, 0.15 mol, 60% dispersion in oil, was washed with hexane) proportionally. The mixture was stirred for 2 h at room temperature. Subsequently ethyl chloroacetate (150 mL, 140 mmol) was added dropwise within 2 h at room temperature. After another 2 h, the solvent was removed by evaporate, *in vacuo*. The residue was dropped into water (250 mL) and stirring. The solids were filtered off and washed with water, followed by recrystallization from methanol to give **13** (5.56 g, 70%) as light yellow crystalline [25]. Without further purification, **13** (5.0 g, 23 mmol) was dissolved in 30 mL MeOH and the mixture was heated until becoming homogeneous solution. Then 80% hydrazine hydrate (2.8 mL, 48 mmol) was added and the mixture was continuously stirred at the same temperature. The mixture was stirred for an additional 8 h after white solid appeared. The resulting aqueous suspension was filtered under reduced pressure, and residue was washed with anhydrous EtOH to neutral. Then residue was dried *in vacuo* to afford **16** (3.60 g, 81%) as white solid. The corresponding compounds **17** and **18** were prepared by this method. Physical data of acetylhydrazine heterocycle compounds **16–18** matched those described in the literature [24].

5.4. General procedure for synthesis of per-O-acetylated thiourea-linked pseudonucleosides **19–27**

A stirred suspension of **16** (248 mg, 1.2 mmol) in anhydrous DMF (15 mL) was heated to 140 °C, the mixture continuously reacted for additional 5 min under the same conditions after solids were dissolved completely. Then the mixture was cooled slowly to 70 °C and was kept as a clear solution. Glycosyl isothiocyanate **7** (373 mg, 1 mmol) in 5 mL dry DMF was added dropwise. The reaction mixture was cool to room temperature and stirred vigorously under Ar for 8 h. After removal of DMF *in vacuo*, the resulting brown residue was diluted with anhydrous MeOH. Silica gel was added, and the mixture was evaporated to dryness. The dry powder was applied to silica gel CC (EtOAc/MeOH) to afford **19** (441 mg, 76%) as a light yellow powder. Mp: 168–169 °C; IR (KBr, ν_{max}): 3433 (NH), 1749, 1643 (C=O), 1369 (Me), 1231 (gly-H), 1081 (C=S) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 9.75 (1H, s, NH), 9.75 (1H, s, NH), 8.73 (1H, s, NH), 8.23 (1H, s, adenine ring-H), 8.14 (1H, s, adenine ring-H), 7.26 (2H, s, NH_2), 4.04–5.32 (7H, m, gly-H, CH_2), 4.30 (2H, s, $COCH_2$),1.91–2.16 (12H, m, CH_3CO). Anal. Calcd for $C_{22}H_{28}N_8O_{10}S$: C, 44.29; H, 4.73; N, 18.78. Found: C, 44.18; H, 4.69; N, 18.28. ESI-MS: (MH^+) 597.6.

5.4.1. Compound **20**

Light yellow solid, 495 mg, yield 83%; mp: 171–173 °C; IR (KBr, ν_{max}): 3340 (NH), 1643, 1574 (C=O), 1369 (Me), 1232 (gly-H), 1041 (C=S) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 9.81 (1H, s, NH), 8.90 (1H, s, NH), 8.68 (1H, s, NH), 8.25 (1H, s, adenine ring-H), 8.07 (1H, s, adenine ring-H), 7.27 (2H, s, NH_2), 3.97–5.91 (7H, m, gly-H, CH_2), 4.29 (2H, s, $COCH_2$), 1.75–2.03 (12H, m, CH_3CO). Anal. Calcd for $C_{22}H_{28}N_8O_{10}S$: C, 44.29; H, 4.73; N, 18.78. Found: C, 44.24; H, 4.66; N, 18.54. ESI-MS: (MH^+) 597.6.

5.4.2. Compound **21**

White solid 404 mg, yield 77%; mp: 183–184 °C; ^1H NMR (500 MHz, DMSO-d_6): δ 9.83 (1H, s, NH), 8.53 (1H, s, NH), 7.95 (1H, s, NH), 8.14 (1H, s, adenine ring-H), 8.04 (1H, s, adenine ring-H), 3.50–5.75 (7H, m, gly-H), 7.26 (2H, s, NH_2), 4.24 (2H, s, $COCH_2$), 1.86–2.00 (9H, m, CH_3CO). Anal. Calcd for $C_{19}H_{24}N_8O_8S$: C, 43.51; H, 4.61; N, 21.36. Found: C, 43.57; H, 4.65; N, 31.44. ESI-MS: (MH^+) 525.5.

5.4.3. Compound **22**

White solid 499 mg, yield 86%; ^1H NMR (500 MHz, DMSO-d_6): δ 10.25 (1 H, br s, NH), 9.84 (1H, br s, NH), 8.68 (1H, br s, NH), 7.16–8.19 (5H, m, benzimidazole ring-H), 3.90–5.88 (9H, m, gly-H and $COCH_2$), 1.85–2.01 (12H, 3s, CH_3CO). Anal. Calcd for $C_{24}H_{29}N_5O_{10}S$: C, 49.74; H, 5.04; N, 12.08. Found: C, 49.86; H, 5.08; N, 12.01. ESI-MS: (MH^+) 580.6.

5.4.4. Compound **23**

Light yellow solid 527 mg, yield 91%; ^1H NMR (500 MHz, DMSO-d_6): δ 10.22 (1H, br s, NH), 9.85 (1H, br s, NH), 8.54 (1H, br s, NH), 7.20–8.15 (5H, m, benzimidazole ring-H), 3.94–5.32 (9H, m, gly-H and $COCH_2$), 1.80–2.09 (12H, 3s, CH_3CO). Anal. Calcd for $C_{24}H_{29}N_5O_{10}S$: C, 49.74; H, 5.04; N, 12.08. Found: C, 49.67; H, 5.06; N, 12.14. ESI-MS: (MH^+) 580.6.

5.4.5. Compound **24**

White solid 447 mg, yield 88%; ^1H NMR (500 MHz, DMSO-d_6): δ 10.21 (1H, br s, NH), 9.83 (1H, br s, NH), 8.72 (1H, br s, NH), 7.15–8.16 (5H, m, benzimidazole ring-H), 3.98–5.87 (8H, m, gly-H and $COCH_2$), 1.83–2.15 (9H, 3s, CH_3CO). Anal. Calcd for $C_{21}H_{25}N_5O_8S$: C, 49.70; H, 4.97; N, 13.80. Found: C, 49.62; H, 5.03; N, 13.71. ESI-MS: (MH^+) 508.5.

5.4.6. Compound **25**

White solid 466 mg, yield 64%; ^1H NMR (500 MHz, DMSO-d_6): δ 12.72 (1H, br s, NH), 10.37 (1H, br s, NH), 9.98 (1H, br s, NH), 8.64 (1H, br s, NH), 7.01–8.28 (8H, m, benzene ring-H), 3.95–5.95 (9H, m, gly-H and $COCH_2$), 3.84 (3H, s, O–CH_3), 1.79–2.01(12H, 3s, CH_3CO). Anal. Calcd for $C_{32}H_{36}N_6O_{12}S$: C, 52.74; H, 4.98; N, 11.53. Found: C, 52.68; H, 5.03; N, 11.59. ESI-MS: (MH^+) 729.7.

5.4.7. Compound ***26***

White solid 554 mg, yield 76%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.71 (1H, br s, NH), 10.38 (1H, br s, NH), 9.98 (1H, br s, NH), 8.45 (1H, br s, NH), 7.16–8.23 (8H, m, benzene ring-H), 3.92–5.72 (9H, m, gly-H and $COCH_2$), 3.85 (3H, s, O–CH_3), 1.78–2.01(12H, 3s, CH_3CO). Anal. Calcd for $C_{32}H_{36}N_6O_{12}S$: C, 52.74; H, 4.98; N, 11.53. Found: C, 52.71; H, 4.94; N, 11.48. ESI-MS: (MH^+) 729.7.

5.4.8. Compound ***27***

White solid 466 mg, yield 71%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.70 (1H, br s, NH), 10.29 (1H, br s, NH), 9.95 (1H, br s, NH), 8.68 (1H, br s, NH), 7.15–8.16 (8H, m, benzene ring-H), 3.95–5.85 (8H, m, gly-H and $COCH_2$), 3.85 (3H, s, O–CH_3), 1.76–2.15 (9H, 3s, CH_3CO). Anal. Calcd for $C_{29}H_{32}N_6O_{10}S$: C, 53.04; H, 4.91; N, 12.80. Found: C, 52.97; H, 4.96; N, 12.74. ESI-MS: (MH^+) 657.7.

5.5. General procedure for synthesis of deacetylated thiourea-linked pseudonucleosides ***28–36***

A solution of per-*O*-acetylated thiourea-linked carbodydrate **19** (596 mg, 1 mmol) in 15 mL of anhydrous MeOH was treated with sodium methanol (dissolved in methanol) to pH = 8.0. The mixture was stirred at room temperature for 10–15 min until compound **19** disappeared monitored by TLC. The reaction solution was neutralized with cation-exchange resin and white floccule formed. The resulting suspension was filtered under reduced pressure, and residue was dried *in vacuo* to afford **28** (377 mg, 88%) as white powder. IR (KBr, ν_{max}): 3325 (OH), 1649 (C=O), 1255 (gly-H), 1082 (C=S) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.69 (1H, s, NH), 8.26 (1H, s, NH), 8.14 (1H, s, NH), 8.20 (1H, s, adenine ring-H), 8.04 (1H, s, adenine ring-H), 7.25 (s, 2H, NH_2), 5.59–3.39 (11H, m, gly-H, overlapped with H of OH), 3.53 (2H, m, $COCH_2$). Anal. Calcd for $C_{14}H_{20}N_8O_6S$: C, 39.25; H, 4.71; N, 26.15. Found: C, 39.02; H, 4.69; N, 25.99. ESI-MS: (MH^+) 429.4.

5.5.1. Compound ***29***

White powder 390 mg, yield 91%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.71 (1H, br s, NH), 8.35 (1H, br s, NH), 8.10 (1H, br s, NH), 8.15 (1H, s, adenine ring-H), 8.09 (1H, s, adenine ring-H), 7.28 (2H, s, NH_2), 5.31–4.95 (11H, m, gly-H overlapped with H of H–O), 3.13–3.69 (2H, m, $COCH_2$). Anal. Calcd for $C_{14}H_{20}N_8O_6S$: C, 39.25; H, 4.71; N, 26.15. Found: C, 39.02; H, 4.69; N, 25.99. ESI-MS: (MH^+) 429.4.

5.5.2. Compound ***30***

White powder 347 mg, yield 87%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.41 (1H, br s, NH), 9.75 (1H, br s, NH), 8.27 (1H, br s, NH), 7.71 (s, 2H, NH_2), 8.13 (1H, s, adenine ring-H), 7.97 (1H, s, adenine ring-H), 3.07–5.23 (9 H, m, gly-H overlapped with H of H–O), 3.60 (2H, m, $COCH_2$). Anal. Calcd for $C_{13}H_{18}N_8O_5S$: C, 39.19; H, 4.55; N, 12.80. Found: C, 39.26; H, 4.51; N, 12.87. ESI-MS: (MH^+) 399.4.

5.5.3. Compound ***31***

White powder 382 mg, yield 93%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.37 (1H, br s, NH), 9.71 (1H, br s, NH), 8.68 (1H, br s, NH), 7.20–8.49 (5H, m, benzimidazole ring-H), 3.15–5.65 (13H, m, gly-H and $COCH_2$ overlapped with H of H–O). Anal. Calcd for $C_{16}H_{21}N_5O_6S$: C, 46.71; H, 5.14; N, 17.02. Found: C, 46.67; H, 5.08; N, 17.10. ESI-MS: (MH^+) 412.4.

5.5.4. Compound ***32***

White powder 370 mg, yield 90%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.41 (1H, br s, NH), 9.90 (1H, br s, NH), 8.58 (1H, br s, NH), 7.28–8.48 (5H, m, benzimidazole ring-H), 3.30–5.78 (13H, m, gly-H and $COCH_2$ overlapped with H of H–O). Anal. Calcd for $C_{16}H_{21}N_5O_6S$: C, 46.71; H, 5.14; N, 17.02. Found: C, 46.75; H, 5.11; N, 17.09. ESI-MS: (MH^+) 412.4.

5.5.5. Compound ***33***

White powder 332 mg, yield 87%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.33 (1H, br s, NH), 9.45 (1H, br s, NH), 8.47 (1H, br s, NH), 7.18–8.39 (5H, m, benzimidazole ring-H), 3.12–5.28 (11H, m, gly-H and $COCH_2$ overlapped with H of H–O). Anal. Calcd for $C_{15}H_{19}N_5O_5S$: C, 47.24; H, 5.02; N, 18.36. Found: C, 47.31; H, 4.97; N, 18.31. ESI-MS: (MH^+) 382.4.

5.5.6. Compound ***34***

White powder 510 mg, yield 91%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.24 (1H, br s, NH), 10.18 (1H, br s, NH), 9.86 (1H, br s, NH), 8.73 (1H, br s, NH), 7.02–8.22 (8H, m, benzene ring-H), 3.20–5.78 (13H, m, gly-H and $COCH_2$ overlapped with H of H–O), 3.78 (3H, s, O–CH_3). Anal. Calcd for $C_{24}H_{28}N_6O_8S$: C, 51.42; H, 5.03; N, 14.99. Found: C, 51.47; H, 4.96; N, 15.04. ESI-MS: (MH^+) 561.6.

5.5.7. Compound ***35***

White powder 533 mg, yield 95%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.72 (1H, br s, NH), 10.35 (1H, br s, NH), 9.78 (1H, br s, NH), 9.14 (1H, br s, NH), 7.20–8.29 (8H, m, benzene ring-H), 3.25–5.32 (13H, m, gly-H and $COCH_2$ overlapped with H of H–O), 3.85 (3H, s, O–CH_3). Anal. Calcd for $C_{24}H_{28}N_6O_8S$: C, 51.42; H, 5.03; N, 14.99. Found: C, 51.37; H, 4.96; N, 14.92. ESI-MS: (MH^+) 561.6.

5.5.8. Compound ***36***

White powder 472 mg, yield 89%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.72 (1H, br s, NH), 10.30 (1H, br s, NH), 9.75 (1H, br s, NH), 9.09 (1H, br s, NH), 7.28–8.47 (8H, m, benzene ring-H), 3.32–5.38 (11H, m, gly-H and $COCH_2$ overlapped with H of H–O), 3.86 (3H, s, O–CH_3). Anal. Calcd for $C_{23}H_{26}N_6O_7S$: C, 52.07; H, 4.94; N, 15.84. Found: C, 51.98; H, 4.96; N, 15.90. ESI-MS: (MH^+) 531.6.

5.6. Cytotoxicity assays

Growth inhibition of the synthetic compounds to various tumor cells was determined by MTT assays [26]. Briefly, tumor

cells ($3-5 \times 10^4$ cells mL^{-1}) were inoculated in 96-well culture plates (100 μL/well). After 24 h culture, 50 μL of culture medium containing synthetic compound of various concentrations was added to the wells, and BCS-1640 medium in control cells, then the cells were incubated for 48 h. The absorbance of each well was measured using a microculture plate reader at 490 nm.

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