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Design, synthesis, and antitumor activity of bile acid–polyamine–nucleoside conjugates

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Abstract—A series of bile acid–polyamine amides conjugated with 3′-azido-3′-deoxythymidine (AZT) as potential antitumor prodrugs in the form of phosphoramidates were synthesized in good yields and their antitumor activities were assayed against two human cancer cells in vitro: cervix cancer HeLa cells and renal cancer 7860 cells. The improved antitumor activity probably derived from the enhanced delivery efficiency of AZT due to bile acid–polyamine conjugates.

3′-Azido-3′-deoxythymidine (AZT, Zidovudine, Fig. 1), clinically approved by FDA to treat human HIV infection, is a typical member of the nucleoside analogues. It is designed as a current or potential antiviral drug competitively inhibiting reverse transcriptase (RT), one of the critical enzymes in the viral replicative cycle.[1] However, AZT was initially tested as an anticancer agent.[2] Combined with some other antitumor agents,[2] such as 5-fluorouracil,[3,4] cisplatin,[5] and paclitaxel,[6] AZT can be used in treatment of some cancers. Recently, AZT has also been found to be effective in inducing apoptosis and inhibiting cell growth of parathyroid cancer cells.[7]

On the other hand, the clinical limitations related with AZT therapy (i.e., bone marrow suppression, myopathy, low brain uptake, and a short half-life in plasma) have prompted the development of strategies for designing of AZT prodrugs, especially various phosphate esters.[8] Until recent years, the prodrugs of AZT targeted in antitumor activity have been developed, mostly represented by phosphoramidate monoesters and ether phospholipids of AZT (Fig. 1).[9,10] However, improvements are still needed in designing potential antitumor prodrugs of AZT since current prodrugs have led to some defections, such as low oral bioavailability, instability in gastric fluid, and decreased selective index.[9–11]

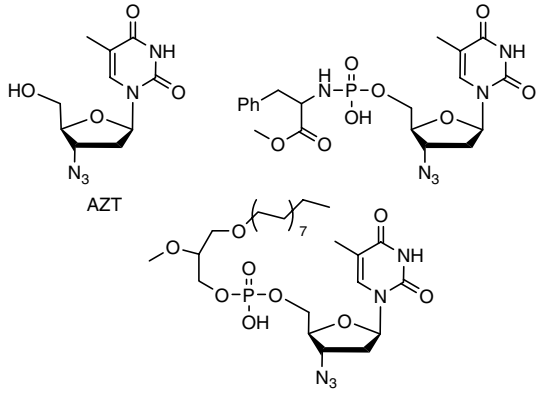

Figure 1. AZT and its antitumor prodrugs.

Bile acids, the acidic sterols commonly existing in bile, physiologically undergo the enterohepatic circulation, depending on several Na^+-dependent bile acid transport systems in different organs.[12,13] The organotropism revealed by bile acids in the enterohepatic circulation probably makes bile acid–drug conjugates very useful in: (a) specifically delivering the drug to the liver and the intestine while reducing undesired side-effects in

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other organs or tissues; and (b) improving the intestinal absorption of poorly absorbable drugs through the bile acid transport systems used as shuttling systems for drugs.[14] Moreover, owing to their own facially amphiphilic structures, it is possible to utilize bile acids as building blocks for other more complex amphiphilic molecules.[15] Actually, these aforementioned physiological and structural properties of bile acids resulted in their wide applications in pharmacology, supramolecules, functional polymer, etc.[16,17]

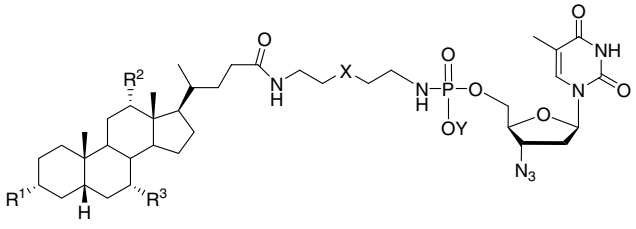
Besides, the natural polyamines–putrescine, spermidine, and spermine, essential for cell growth and viability, have been numerously linked with the apoptotic pathways exploited for tumor therapy.[18] For a long time, it has also been recognized that the polyamine metabolism and polyamine transport system are significant potential targets and tools to suppress proliferative processes in cancers.[19]

Particularly, a great number of bile acid–polyamine conjugates with various impressive biological activities due to their facial amphiphilicity have been designed and studied.[20] A series of works of Regen's group revealed that bile acid–polyamine conjugates could assist various bioactive molecules (such as peptides and oligonucleotides) to cross the phospholipid bilayers owing to the facial amphiphilicity of the conjugates.[21] Furthermore, the polycationic state of polyamines under normal physiological conditions could form ionic interaction between polyamines and long anionic sites of macromolecules, such as phosphate backbone of DNA and negative face of cell membrane.[19] This kind of interaction enables polyamines and their conjugates to be utilized in oriented delivery (i.e., bile acid–polyamine conjugates used in nonviral gene delivery techniques).[22] These facts definitely indicate that amphiphilic bile acid–polyamine conjugates should be considered to enhance the efficiency of drug delivery and improve drugs' bioactivity.

Based on the above viewpoints and continuous research work on the steroidal phosphate conjugates in our laboratory,[23–25] a series of novel bile acid–AZT phosphoramidate conjugates, deriving from deoxycholic acid, cholic acid, and dehydrocholic acid, respectively, were designed and synthesized (Fig. 2). This design concerns not only the potential improved drug delivery and oral absorption given by bile acid–polyamine conjugates,[13] but also the fact that the release of AZT–monophosphate from its phosphoramidate monoesters could prolong the elimination of AZT in the animal pharmacokinetic model.[11]

The target molecules were synthesized according to the general synthetic procedure as shown in Figure 3. H-phosphates of AZT[24] reacted with polyamines by the Atherton–Todd reaction to provide polyamine phosphoramidates.[26] With activated bile acid, the phosphoramidates were then easily converted to the target products.[27] This protocol was proven to be facile, efficient, and in high overall yield (about 70% in average). The structures of all bile acid–polyamine–AZT conjugates have been confirmed by the NMR and ESI–HRMS.[28]

The bile acid–polyamine–AZT conjugates were tested in preliminary assays against two human cancer cell lines in vitro by the standard MTT method: cervix cancer HeLa and renal cancer 7860 (Table 1). The results showed that only the conjugates deriving from dipropylenetriamine and stearyl alcohol (**1c**, **2c**, **3c**) possessed



	R^1	R^2	R^3	X	Y-OH
1a	OH	OH	H	NH	$CH_3(CH_2)_{17}OH$
1b	OH	OH	H	NH	AZT
1c	OH	OH	H	CH_2NHCH_2	$CH_3(CH_2)_{17}OH$
1d	OH	OH	H	CH_2NHCH_2	AZT
2a	OH	OH	OH	NH	$CH_3(CH_2)_{17}OH$
2b	OH	OH	OH	NH	AZT
2c	OH	OH	OH	CH_2NHCH_2	$CH_3(CH_2)_{17}OH$
2d	OH	OH	OH	CH_2NHCH_2	AZT
3a	=O	=O	=O	NH	$CH_3(CH_2)_{17}OH$
3b	=O	=O	=O	NH	AZT
3c	=O	=O	=O	CH_2NHCH_2	$CH_3(CH_2)_{17}OH$
3d	=O	=O	=O	CH_2NHCH_2	AZT

Figure 2. Bile acid–polyamine–AZT conjugates.

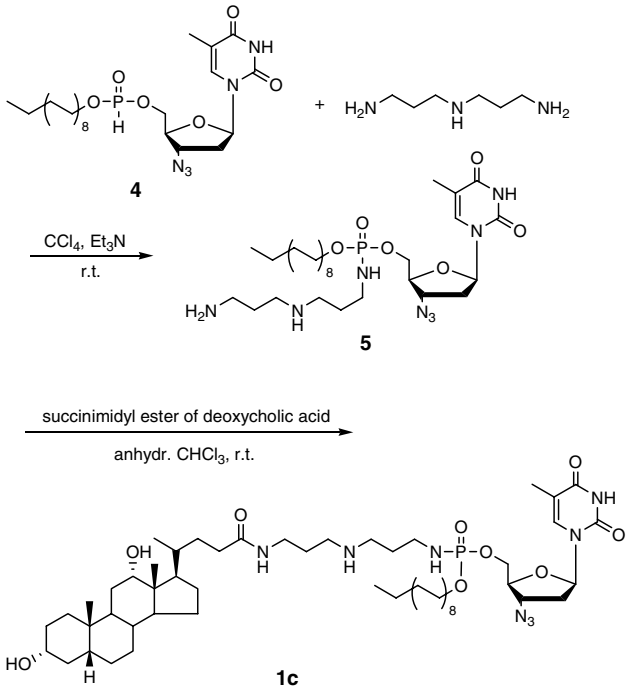


Figure 3. Typical synthetic procedure of bile acid conjugates.

Table 1. In vitro cytotoxic activity against cervix cancer HeLa and renal cancer 7860

Compound	IC_{50}[a] (μM)	
	HeLa	7860
AZT	>100[b]	>100
1a	58 (±4.9)	>100
1b	>100	>100
1c	6.8 (±0.2)	89 (±33)
1d	>100	>100
2a	59 (±2.6)	>100
2b	>100	>100
2c	7.2 (±0.2)	94 (±22)
2d	>100	>100
3a	42 (±2.4)	>100
3b	>100	>100
3c	7.1 (±0.1)	91 (±16)
3d	>100	>100
4	86 (±12)	>100

[a] IC_{50} values are defined as the minimal drug concentration necessary to inhibit the growth of 50% of the cells cultivated in 48 h and are means of three separate experiments, standard deviation is given in parentheses. IC_{50} values are given only if they are less than 100 μM, which is the maximum concentration tested.

[b] It has been reported that AZT itself inhibited about 50% HeLa cells at the concentration of at least 125 μM in 48 h.[29]

notable antitumor bioactivity against HeLa cell line and fairly against renal cancer 7860 cell line, comparing with AZT which had low inhibition against both cancer cell lines (the cell viability of HeLa and 7860 is 70 ± 3% and 75 ± 2% in 100 μM AZT, respectively). The conjugation between bile acid–polyamine amide and AZT definitely improved the antitumor activity of AZT and its H-phosphate **4**, however, the compounds of dipropylenetriamine replaced by diethylenetriamine decreased the IC_{50} values of conjugates. Through the comparison between monoesters and diesters of AZT, the aliphatic long chain component was also evidently important, possibly due to its lipophilicity.

In order to confirm the respective contributions of AZT component and the reminder structures in the above bile acid conjugates to their bioactivity, three bile acid–polyamine–thymidine conjugates **6**–**8** (Fig. 4) were synthesized according to the similar general method demonstrated in Figure 3.

Conjugates **6**–**8** possessed low cytotoxic activity against both HeLa and 7860 (the lowest cell viability of HeLa

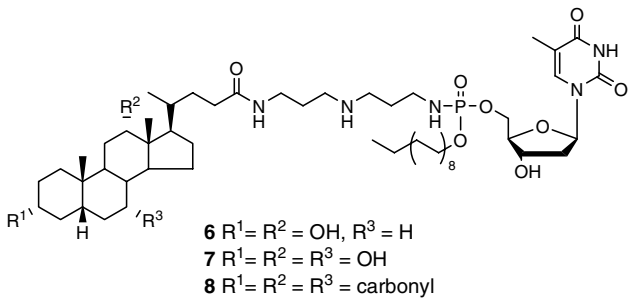


Figure 4. Bile acid–polyamine–thymidine conjugates.

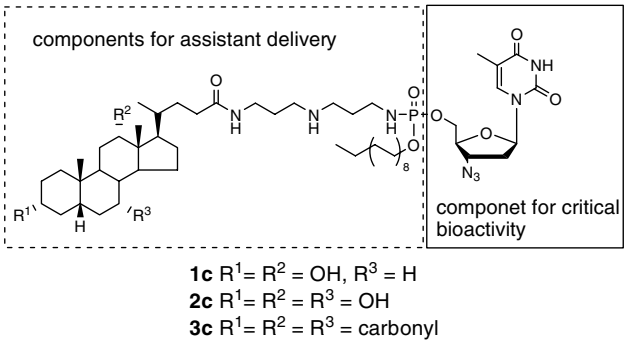


Figure 5. The functions of components in conjugates **1c**, **2c**, and **3c**.

and 7860 is 92 ± 5% and 95 ± 4% in 100 μM, respectively) in bioassays. Combined with the bioassay results of the conjugates **1c**, **2c**, and **3c**, this result further indicates that AZT moiety plays a key role in the bioactivity of its conjugates and the components other than AZT in the conjugates are significant in assisting AZT to be delivered into tumor cells (Fig. 5).

In summary, a series of novel bile acid–polyamine–AZT phosphoramidate conjugates have been designed and synthesized in a facile and efficient methodology. Among them, **1c**, **2c**, and **3c** considerably suppressed the growth of cervix cancer HeLa cells in bioassays. The above results provide a useful method to enhance the therapeutic efficacy of drugs by covalent linkage of drugs to amphiphilic bile acid–polyamine conjugates and greatly encourage us to further investigate on the detailed delivery procedure of this strategy.

Acknowledgments

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28. Details of the typical synthetic procedure and spectrum data of the representative conjugate **1c** are displayed as follows.

    Preparation of polyamine phosphoramidate of AZT **5**: The H-phosphate of AZT **4** was prepared according to the previous work in our lab (Ref. 24). A solution of **4** (195 mg, 0.33 mmol) in CH_2Cl_2 (3 ml) was dropwise added to a stirred CH_2Cl_2 solution (5 ml) of the mixture of dipropylenetriamine (88 mg, 0.67 mmol), triethylamine (0.1 ml), and CCl_4 (0.1 ml) in 5 min, and stirred at room temperature for another 15 min. After filtration, the solvent was removed under reduced pressure. The crude product was purified by the silica-gel column chromatography (1:7:13, 30% NH_4OH:CH_3OH:CH_2Cl_2) to give **5** (220 mg, 92%) as colorless oil. ^{1}HNMR (300 MHz, CD_3OD) δ 7.51 (d, 1H, J = 2.4 Hz), 6.16 (q, 1H, J = 6.5 Hz), 4.44–4.39 (m, 1H), 4.20–4.14 (m, 2H), 4.04 (t, 2H, J = 6.5 Hz), 4.01–3.96 (m, 1H), 2.99–2.91 (m, 2H), 2.78 (t, 2H, J = 7.2 Hz), 2.68–2.62 (m, 4H), 2.41 (dd, 2H, J = 6.5, 6.2 Hz), 1.90 (s, 3H), 1.72–1.68 (m, 6H), 1.41–1.18 (m, 30H), 0.87 (t, 3H); ^{13}CNMR (75 MHz, CD_3OD) δ 164.7, 150.5, 135.7, 110.2, 84.6, 81.9, 66.2, 64.5 (1C), 59.9, 46.4, 45.9, 38.5, 38.4, 36.2, 31.2, 30.2 (2C), 29.6, 29.5–28.5 (12C), 24.8, 21.9, 12.8, 11.1; ^{31}PNMR (121 MHz, CD_3OD) δ 11.03. HRMS–ESI (*m*/*z*): calcd for $[C_{34}H_{65}N_8O_6P+H]^+$ 713.4834, found 713.4823.

    Preparation of deoxycholic acid–AZT conjugate **1c**: To the stirred solution of **5** (58 mg, 0.081 mmol) in anhydrous $CHCl_3$ (4 ml) was added a solution of the succinimidyl ester of deoxycholic acid (40 mg, 0.082 mmol) in anhydrous $CHCl_3$ (4 ml) under argon atmosphere over a 5-min period. After stirring under argon atmosphere at room temperature for another 24 h, the solvent was evaporated. The crude product was purified by the silica-gel column chromatography (1:7:35, 30% NH_4OH:CH_3OH:CH_2Cl_2) to give **1c** (74 mg, 84%) as colorless solid. ^{1}HNMR (300 MHz, $CDCl_3$) δ 7.41 (s, 1H), 7.20 (m, 1 NH), 6.11 (q, 1H, J = 6.5 Hz), 4.43 (m, 1H), 5.10–4.10 (br, 2 NH), 4.21 (m, 2NH), 4.03 (m, 1H), 4.00–3.98 (m, 2H, J = 6.5 Hz), 3.95 (s, 1H), 3.66-3.49 (m, 1H), 3.51 (br, 1NH), 3.31 (m, 2H), 3.04–3.02 (m, 2H), 2.85–2.80 (m, 4H), 2.44–2.42 (m, 2H, J = 6.7 Hz), 2.14 (m, 2H), 1.92 (s, 3H), 1.90–1.00 (m, 60H), 0.98 (d, 3H, J = 5.5 Hz), 0.88 (s, 3H), 0.86 (t, 3H, J = 6.9 Hz), 0.65 (s, 3H); ^{13}CNMR (75 MHz, CD_3OD/$CDCl_3$) δ 174.5, 164.0, 150.4, 135.5, 110.6, 84.3, 82.0, 71.9, 70.9, 67.2, 65.2, 60.4, 47.6, 46.3, 46.1, 46.0, 45.9, 41.7, 41.4, 36.5, 36.0, 35.7 (2C), 35.1 (2C), 34.6, 34.4(2C), 32.0, 31.9, 31.4, 30.1, 29.2-28.4 (13C), 28.0, 27.3, 26.0, 25.2, 22.9, 22.3, 22.2, 17.0, 13.9, 12.4, 12.2; ^{31}PNMR (121 MHz, $CDCl_3$) δ 10.43. HRMS–ESI (*m*/*z*): calcd for $[C_{58}H_{103}N_8O_9P+H]^+$ 1087.7658, found 1087.7652.
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