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Synthesis of Daunorubicin Piperonal Derivatives by One-Step Reductive Amination

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Abstract—New *N*-derivatives of daunorubicin are obtained using aromatic aldehydes by one-step reductive amination. The obtained compounds are found to exhibit a high cytotoxic activity.

Keywords: anthracycline antibiotics, daunorubicin, reductive amination, aromatic aldehydes, anticancer agents

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Daunorubicin, like other anthracycline antibiotics, is widely applied in the modern chemotherapy of human cancer diseases, due to its properties, such as its therapeutic efficacy, availability, and relatively low price. However, this class of medicines possesses a number of serious drawbacks, such as a high level of cardiotoxicity, carcinogenicity, mutagenicity, and myelo- and immunodepressive effects, as well as the rapid growth of the resistance of cancer cells to medicines administered by the mechanism of multiple drug resistance [1]. To overcome these drawbacks, we used the chemical modification of daunorubicin, which involves both the anthraquinoid aglycone and the aminosugar (daunosamine) moiety [2]. At the present time, modification of the 3'-NH_2 group is considered to be the least labor-consuming and most effective approach, partially confirming the presumptive action mechanism of anthracyclines, according to which it is the daunosamine moiety that binds to the purine bases of DNA upon intercalation [3]. At the present time, a simple and quite efficient method has been developed for functionalization of the 3'-NH_2 group through its one-step reductive amination using aromatic aldehydes and sodium cyanoborohydride [5]. Earlier, we implemented this approach to modify daunorubicin with different substituted aromatic aldehydes. A series of conjugates containing both donor and acceptor substituents at the benzyl fragment were obtained [6]. The reductive amination was found to be quite efficient also in the synthesis of hybrid molecules containing metallocene fragments [7]. However, biotesting all the daunorubicin derivatives that we synthesized earlier showed that they are significantly inferior to the parent compound.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were recorded on a Bruker AMX-400 instrument in $CDCl_3$ using residual proton signals as the internal standard (^1H, $\delta = 7.28$ ppm). The chemical shift of $CDCl_3$ triplet in ^{13}C NMR spectra was taken equal to 76.91 ppm. ^{13}C NMR spectra were recorded in the JMODECHO mode; signals for carbon atoms with an odd and even number of protons have the opposite polarity. The course of the reactions was controlled by TLC on Alumina TLC plates w/UV254; the target products were purified by chromatography on a Macherey-Nagel silica gel (MNKieselgel 60, 70–230 mesh). Daunorubicin in the form of commercially available hydrochloride was purchased from China. Sodium cyanoborohydride $NaCNBH_3$ (95%) and aldehydes used were also commercially available; the latter were used as received. $NaCNBH_3$ was purified by recrystallization of its complex with dioxane as described in [8].

The elemental analysis of newly synthesized compounds was performed at the Laboratory of Microanalysis of Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences (INEOS RAS). The structure of the obtained compounds was studied using the equipment of the Center for Molecule Composition Studies of INEOS RAS. Biological tests for anticancer activity were carried out at the Institute of Physiologically Active Compounds of the Russian Academy of Sciences (Chernogolovka, Russia).

General Procedure for the Synthesis of Compounds 2 and 3

A mixture of the corresponding carboxaldehyde (8.0 mmol) and daunorubicin hydrochloride (225 mg, 0.4 mmol) in $CH_3CN–H_2O$ (3 : 1, 9 mL) was stirred

for 0.5 h at room temperature under darkroom conditions. $NaCNBH_3$ (76 mg, 1.2 mmol) was added and the reaction mixture was stirred for 0.5 h. H_2O (5 mL) was added and the mixture was extracted with $CHCl_3$ (3 × 10 mL). The organic layer was washed with H_2O (15 mL) and the aqueous layer was extracted with $CHCl_3$ (15 mL). The combined organic extracts were dried over Na_2SO_4 and filtered. The solvent was removed in a water-jet pump vacuum (10 mm Hg). The resulting residue was purified by chromatography on silica gel using a gradient $CHCl_3$–MeOH (100 : 1 → 1 : 1) mixture as the eluent.

Compound **2** was obtained as a dark-orange powder in yield of 0.04 g (32%). ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 14.00 (br s, 1H, $C^{16}OH$), 13.25 (br s, 1H, C^9OH), 8.06 (d, 1H, $^3J_{HH}$ = 7.6 Hz, C^1H), 7.81 (t, 1H, $^3J_{HH}$ = 8.0 Hz, C^2H), 7.41 (d, 1H, $^3J_{HH}$ = 8.0 Hz; C^3H); 6.78 (s, 1H, $C^{32}H$); 6.72 and 6.71 (d, 1H each, $^3J_{HH}$ = 8.0 Hz, $C^{28}H$, $C^{29}H$), 5.93 (s, 2H, $C^{33}H_2$), 5.54 (br s, 1H, $C^{21}H$), 5.32 (br s, 1H, $C^{14}H$), 4.71 (br s, 1H, $C^{23}H$), 4.11 (s, 3H, $C^{35}H_3$), 4.09 (q, 1H, $^3J_{HH}$ = 6.8 Hz, $C^{22}H$), 3.67 (br s, 1H, $C^{24}H$), 3.73 and 3.60 (d, 1H each, $^2J_{HH}$ = 12.4 Hz, $C^{26}H_2$), 3.25 and 2.99 (br d, 1H each, $^2J_{HH}$ = 18.8 Hz, $C^{11}H_2$), 2.45 (s, 3H, $C^{20}H_3$), 2.41–2.38 and 2.15–2.10 (m, 1H each, $C^{13}H_2$), 1.86–1.79 and 1.71–1.67 (m, 1H each, $C^{25}H_2$), 1.40 (d, 3H, $^3J_{HH}$ = 6.8 Hz, $C^{34}H_3$).^{13}C{1H} NMR, ($CDCl_3$, 100 MHz), δ: 211.71 (C^{19}), 186.64, 186.29 (C^{18}, C^7), 160.78 (C^4), 156.20, 155.55 (C^9, C^{16}), 147.54, 147.48 (C^{30}, C^{31}), 135.50 (C^2), 135.18 (C^6), 134.21, 134.07 (C^{10}, C^{15}), 133.41 (C^{27}), 120.96 (C^{28}), 120.55 (C^5), 119.55, 118.20 (C^1, C^3), 111.09, 110.94 (C^8, C^{17}), 108.36, 107.98 (C^{29}, C^{32}), 100.82 (C^{21}), 100.75 (C^{33}), 76.59 (C^{12}), 69.66, 66.70, 66.47 (C^{14}, C^{23}, C^{22}), 56.45 (C^{35}), 52.22 (C^{24}), 50.03 (C^{26}), 34.68, 33.05, 30.15 (C^{11}, C^{13}, C^{25}), 24.64 (C^{20}), 16.96 (C^{34}). Found, %: C, 58.34; H, 4.84; N, 1.97. Calculated for $C_{35}H_{35}NO_{12}$ $3H_2O$, %: C, 58.74; H, 5.77; N, 1.96.

Compound **3** was obtained as a dark-orange powder in yield of 0.05 g (34%). ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 14.00 (br s, 1H, $C^{16}OH$), 13.25 (br s, 1H, C^9OH), 8.06 (d, 1H, $^3J_{HH}$ = 8.0 Hz, C^1H), 7.81 (t, 1H, $^3J_{HH}$ = 8.0 Hz, C^2H), 7.41 (d, 1H, $^3J_{HH}$ = 8.0 Hz, C^3H), 6.37 (s, 1H, $C^{28}H$), 5.94 (s, 2H, $C^{33}H_2$), 5.54 (br s, 1H, $C^{21}H$), 5.33 (br s, 1H, $C^{14}H$), 4.73 (br s, 1H, $C^{23}H$), 4.11 (s, 3H, $C^{35}H_3$), 4.09 (q, 1H, $_3J_{HH}$ = 6.8 Hz, $C^{22}H$), 3.91 and 3.82 (s, 3H each, $C^{36}H_3$, $C^{37}H_3$), 3.73 (br s, 1H, $C^{24}H$), 3.72 and 3.61 (d, 1H each, $2J_{HH}$ = 12.0 Hz, $C^{26}H_2$), 3.25 and 3.00 (br d, 1H each, $^2J_{HH}$ = 16.0 Hz, $C^{11}H_2$), 2.45 (s, 3H, $C^{20}H_3$), 2.41–2.38 and 2.15–2.10 (m, 1H each, $C^{13}H_2$), 1.83–1.79 and 1.66–1.62 (m, 1H each, $C^{25}H_2$), 1.42 (d, 3H, $^3J_{HH}$ = 8.0 Hz, $C^{34}H_3$). ^{13}C{1H} NMR ($CDCl_3$, 100 MHz), δ: 211.73 (C^{19}), 187.01, 186.65 (C^{18}, C^7), 160.97 (C^4), 156.35, 155.85 (C^9, C^{16}), 138.66, 138.05 (C^{30}, C^{31}), 136.40, 136.19 (C^{29}, C^{32}), 135.59 (C^2), 135.49 (C^6), 134.36, 134.26 (C^{10}, C^{15}), 124.23 (C^{27}), 120.90 (C^5), 119.70, 118.28 (C^1, C^3), 111.35, 111.19 (C^8, C^{17}), 108.52 (C^{28}), 101.51 (C^{33}), 100.83 (C^{21}), 76.78 (C^{12}), 69.65, 66.50 (C^{14}, C^{23}, C^{22}), 59.88 (C^{35}), 56.81, 56.58 (C^{36}, C^{37}), 51.72 (C^{24}), 45.27 (C^{26}), 34.84, 33.31, 30.19 (C^{11}, C^{13}, C^{25}), 24.66 (C^{20}), 17.04 (C^{34}). Found, %: C, 52.44; H, 5.91; N, 3.00. Calculated for $C_{37}H_{39}NO_{14} \cdot 7H_2O$, %: C, 52.42; H, 6.30; N, 1.65.

RESULTS AND DISCUSSION

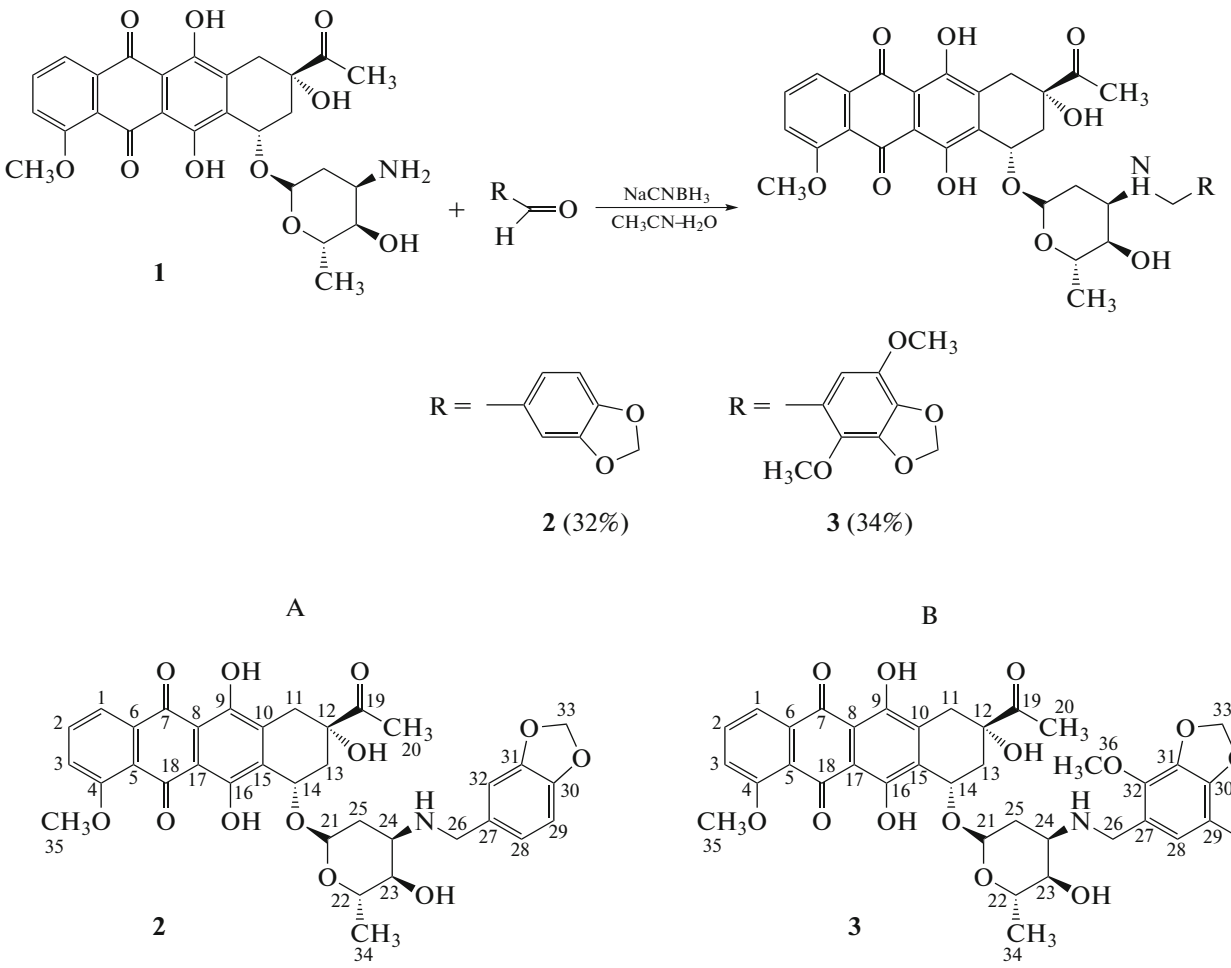
Compounds **2** and **3** (Fig. 1) were obtained in yields of 32–34% after chromatographic purification. Their structures were determined by spectral methods (^{1}H and ^{13}NMR spectroscopy) and the composition was confirmed by elemental analysis.

Although the yields of conjugates **2** and **3** were slightly lower than those of the earlier obtained daunorubicin derivatives [6], the test of their biological properties showed them to be superior in both the antiproliferative activity and the acute toxicity value to the parent compound, as can be seen from the data of Table 1 [9].

A wide spectrum of antiproliferative activity is a great advantage of compounds **2** and **3**. They are effective for the treatment of cancer diseases, including lung carcinoma, rhabdomyosarcoma, intestinal carcinoma, and breast adenocarcinoma. Further acute toxicity studies conducted according to the rapid method of Prozorovskii on white outbread mice with a weight of 22 to 24 g [10] showed that the acute toxicity (LD_{50})

Table 1. Cytotoxicity of obtained compounds

Compound	Inhibitory concentration IC_{50}, μmol/L				Acute toxicity LD_{50}, mg/kg
	A549 (non-small-cell lung cancer)	RD (rhabdomyosarcoma)	HCT116 (intestine carcinoma)	MCF7 (breast adenocarcinoma)	
Daunorubicin	0.34 ± 0.02	1.24 ± 0.00	0.12 ± 0.00	0.40 ± 0.02	1.8
2	0.22 ± 0.00	0.17 ± 0.01	0.10 ± 0.00	0.79 ± 0.02	108.0
3	1.57 ± 0.02	0.46 ± 0.00	0.19 ± 0.00	0.24 ± 0.02	108.0



Scheme 1. (A) is (8S-*cis*)-8-acetyl-10-[[3-(benzo[d][1,3]dioxol-5-ylmethylamino)-2,3,6-trideoxy-α-L-lyxohexopyranosyl]oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione (**2**); (B) is (8S-*cis*)-8-acetyl-10-[[3-(4,7-dimethoxybenzo[d][1,3]dioxol-5-ylmethylamino)-2,3,6-trideoxy-α-L-lyxohexopyranosyl]oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione (**3**).

of derivatives **2** and **3** upon intraperitoneal administration is 108.0 (160 ± 73) mg/kg. These compounds relate to hazard category 3, moderately toxic substances according to the Sidorov classification [1]. According to the published data, LD_{50} of daunorubicin upon intraperitoneal administration to mice is 1.8 mg/kg [12], which allows us to qualify this substance as hazard category 2 with a high level of toxicity according to the Sidorov classification. A much lower (by a factor of 60) acute toxicity of compounds **2** and **3** compared to the starting antibiotic make them more effective as anticancer drugs.

Summing up all the obtained data taking into account the novelty, low acute toxicity values, and a wide spectrum of antiproliferative activity of the obtained compounds (which suggests their potential applicability for the treatment of oncological diseases associated with proliferation of tumor cells), we succeeded in patenting compounds **2** and **3** [9]. The mechanism of action of these compounds is assumed not to be different from that of daunorubicin and other anthracycline medicines [13].

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

There are no supplementary materials for this work.

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