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SYNTHESIS AND CYTOTOXIC ACTIVITY OF A SERIES OF BILE ACID DERIVATIVES

*The new conjugates of selected bile acids (hyocholic (**2**), deoxycholic (**3**), hyodeoxycholic (**4**) and 12-ketocholic (**5**) acids) with ethyl 11-aminoundecanoate **7**, **8**, **11**, and **13** were synthesized. The conjugation reaction was carried out in ethyl acetate in the presence of N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) and triethylamine. Under the same experimental conditions, the conjugation reaction involving ethyl 6-aminohexanoate resulted in formation of a conjugate **9** only in the case of deoxycholic acid (**3**) in addition to the unexpected ethyl ester **10**. In the case of the other bile acids (cholic (**1**), hyodeoxycholic (**4**) and 12-ketocholic (**5**) acids) only an unexpected ester formation took place giving esters **6**, **12**, and **14**. Cytotoxic activity against four tumor cell lines (human breast adenocarcinoma ER-, MDA-MB-231; breast adenocarcinoma ER+, MCF-7; cervix epiteloid carcinoma, HeLa S-3; and prostate cancer, PC-3) was evaluated. Conjugate **8** showed strong activity against HeLa S-3 and conjugate **11** for PC-3. Ethyl ester of 12-ketocholic acid **14** showed very strong antiproliferative activity against MCF-7 and HeLa S-3.*

Studies of bile acids, their physiology and metabolism, their role in carcinogenesis and other major human diseases, have recently experienced a significant progress [1,2]. They have emerged as key regulators of their own metabolism and of lipid and carbohydrate metabolism, and have an important role as promoters of esophageal and colon cancers, cholangiocarcinoma, as well as new implications in breast cancer development and metastasis. In the past several years, it has been reported that synthetic bile acid derivatives induced apoptosis in several human cancer cells [2], including heaptocellular carcinoma cells [3,4], breast carcinoma cells [5,6], leukemic T cells [7], prostate cancer cells [8], colon cancer cells [9], osteosarcoma cells [10], and cervical carcinoma cells [11]. Among the synthetic bile acid derivatives tested, a synthetic derivatives of ursodeoxycholic acid (glycine methyl ester conjugate, HS-1030, and L-phenylalanine benzyl ester conjugate, HS-1183) and those of chenodeoxycholic acid (L-phenyl alanine benzyl ester conjugate, HS-1199 and β-alanine benzyl ester conjugate, HS-1200) showed the activity in inducing apoptosis. The IC_{50} values of these derivatives range from 25 to 50 μM for SiHa human cervical carcinoma cells for compounds HS-1200, HS-1199 and HS--1183; for MDA-MB-231 IC_{50} values is from 30 to 45 μM for HS-1200, HS-1199 and HS-1183; for MCF-7 they are from 30 to 150 μM for HS-1199, HS-1200 and HS-1183 [6,11].

The synthesis and growth inhibition studies against the MCF-7 human breast cancer and SKOV-3 ovarian carcinoma cell lines of derivatives of lithocholic acid and cholic acid in which quinoline-3-carboxylate and acridine-9-carboxylate are substituted at 3 and/or the 24 position are reported [12], along with weak activity against the MCF-7 and SKOV-3 lines was exhibited.

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In view of all the above, the aim of this work was to investigate the possibility of the synthesis of some new conjugates of selected bile acids (cholic, hyocholic, deoxycholic, hyodeoxycholic and 12-ketocholic acids) with natural amino acids of the different carbon chain length and examine their effect on the mechanism of formation of the conjugates of 11-aminoundecanoic and 6-aminoundecanoic acid. The syntheses were performed by the method for preparing conjugates of natural bile acids using *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) as a coupling reagent [13]. A series of new conjugates and unexpectedly obtained ethyl esters of bile acids, as well as the starting substances, were tested as potential cytotoxic agents against human tumor cells MDA-MB-231, MCF-7, HeLa S-3, PC-3, and against healthy MRC-5 cells.

EXPERIMENTAL

General procedure

IR spectra were recorded on a NEXUS 670 SP-IR spectrometer (wavenumbers in cm^{-1}). NMR spectra were taken on a Bruker AC 250E spectrometer operating at 250 MHz (^1H) and 62.5 MHz (^{13}C) and are reported in ppm (δ-scale) downfield from the tetramethylsilane internal standard; coupling constants (*J*) are given in Hz. Mass spectra were recorded on a Finnigan MAT 8230 instrument, using chemical ionization (isobutane) technique. All the reagents used were of analytical grade. All solutions were dried over anhydrous Na_2SO_4.

General procedure for preparation of compounds 6–14

A suspension of bile acid (**1**–**5**, 1 mmol), ethyl 6--aminohexanoate or ethyl 11-aminoundecanoate (1.4 mmol), *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ, 1.4 mmol), and triethylamine (0,2 ml) in ethyl acetate (14 ml) was refluxed for 6 h. The reaction mixture was cooled to room temperature and washed with solution of NaOH (0.5 M, 3×10 ml) and HCl (0.5 M,

3×10 ml) and water (2×10 ml). After drying and evaporating to 2 ml, *n*-hexane (2 ml) was added and the crude product left at 5 °C for 12 h. The yellow oil was purified by column chromatography on silica gel (40 g, CH_2Cl_2–acetone 4:1).

Ethyl 3α,7α,12α-trihydroxy-5β-cholanate (**6**). White amorphous solid (26%). IR spectrum: 3400, 2950, 1750, 1390, 1100, 1050. ^{1}H-NMR spectrum ($CDCl_3$): 0.64 (*s*, 3H, H-18); 0.85 (*s*, 3H, H-19); 0.95 (*d*, 3H, *J* = 6.2 Hz, H-21); 1.22 (*t*, 3H, *J* = 7.2 Hz, CH_3 from Et); 3.42 (*m*, 1H, H-3); 3.80 (*m*, 1H, H-7); 3.93 (*m*, 1H, H-12); 4.08 (*q*, 2H, *J* = 7.2 Hz, CH_2 from Et). ^{13}C-NMR spectrum ($CDCl_3$): 12.40 (C-18); 14.20 (CH_3 from Et); 17.26 (C--21); 22.39; 23.17 (C-19); 26.25; 27.46; 28.08; 29.21; 30.27; 30.87; 31.33; 34.62; 34.72; 35.24; 39.43; 41.45; 41.53; 46.36; 46.95; 53.81; 60.13 (CH_2 from Et); 68.34 (C-7); 71.83 (C-3); 73.03 (C-12); 174.36 (C=O). Mass spectrum (*m*/*z*): 400 (M^+ - $2H_2O$).

Ethyl 11-(3α,6α,7α-trihydroxy-5β-cholanamido)-undecanoate (**7**). Pale-yellow oil (27%). IR spectrum: 3350, 2950, 2870, 1720, 1650, 1380, 1050. ^{1}H-NMR spectrum ($CDCl_3$): 0.58 (*s*, 3H, H-18'); 0.84 (*s*, 3H, H-19'); 0.87 (*d*, 3H, *J* = 6.2 Hz, H-21'); 1.22 (*t*, 3H, *J* = = 7.2 Hz, CH_3 from Et); 2.22 (*t*, 2H, *J* = 7.4 Hz H-2); 3.13 (*m*, 2H, H-11); 3.55 (*m*, 1H, H-3'); 3.75 (*m*, 2H, H-6' and H-7'); 4.05 (*q*, 2H, *J* = 7.2 Hz, CH_2 from Et); 6.33 (br *s*, 1H, NH). ^{13}C-NMR spectrum ($CDCl_3$): 11.67 (C--18'); 14.11 (CH_3 from Et); 18.31 (C-21'); 23.05 (C-19'); 23.43; 24.81; 26.85; 28.18; 28.97; 29.11; 29.19; 29.25; 29.36; 29.48; 31.97; 32.52; 33.37; 34.22; 35.40; 35.82; 38.46; 39.43; 42.51; 47.85; 50.03; 55.72; 60.05 (CH_2 from Et); 69.60 (C-6'); 71.69 (C-7'); 77.21 (C-3'); 173.27 (C=O from amide); 173.90 (C=O from ester). Mass spectrum (*m*/*z*): 621 (M^+ + 1).

Ethyl 11-(3α,12α-dihydroxy-5β-cholanamido)undecanoate (**8**). Colorless oil (19%). IR spectrum: 3350, 2920, 1720, 1650. ^{1}H-NMR spectrum ($CDCl_3$): 0.65 (*s*, 3H, H-18'); 0.88 (*s*, 3H, H-19'); 0.95 (*d*, 3H, *J* = 6.2 Hz, H-21'); 1.23 (*t*, 3H, *J* = 7.2 Hz, CH_3 from Et); 2.26 (*t*, 2H, *J* = 7.4 Hz, H-2); 3.19 (*m*, 2H, H-11); 3.57 (*m*, 1H, H-3'); 3.95 (*m*, 1H, H-12'); 4.10 (*q*, 2H, *J* = 7.2, CH_2 from Et); 5.77 (br *s*, 1H, NH). ^{13}C-NMR spectrum ($CDCl_3$): 12.66 (C-18'); 14.19 (CH_3 from Et); 17.35 (C--21'); 23.08 (C-19'); 23.63; 24.89; 26.09; 26.85; 27.11; 27.47; 28.52; 29.04; 29.15; 29.20; 29.28; 29.38; 29.58; 30.38; 31.35; 33.41; 33.54; 34.07; 34.30; 35.20; 35.95; 36.32; 39.46; 42.03; 46.43; 46.98; 48.09; 60.10 (CH_2 from Et); 71.64 (C-12'); 73.06 (C-3'); 173.57 (C=O from amide); 173.87 (C=O from ester). Mass spectrum (*m*/*z*): 605 (M^+ + 1).

Ethyl 6-(3α,12α-dihydroxy-5β-cholanamido)hexanoate (**9**). White amorphous solid (10%). IR spectrum: 3350, 2920, 1720, 1650, 1560. ^{1}H-NMR spectrum ($CDCl_3$): 0.67 (*s*, 3H, H-18'); 0.90 (*s*, 3H, H-19'); 0.97 (*d*, 3H, *J* = 6.2 Hz, H-21'); 1.25 (*t*, 3H, *J* = 7.2 Hz, CH_3 from Et); 2.28 (*t*, 2H, *J* = 7.4 Hz, H-2); 3.24 (*m*, 2H, H--6); 3.60 (*m*, 1H, H-3'); 3.97 (*m*, 1H, H-12'); 4.12 (*q*, 2H, *J* = 7.2 Hz, CH_2 from Et); 5.64 (br *s*, 1H, NH). ^{13}C--NMR spectrum ($CDCl_3$): 12.74 (C-18'); 14.23 (CH_3 from Et); 17.43 (C-21'); 23.12 (C-19'); 23.63; 24.44; 26.11; 26.33; 27.11; 27.46; 28.64; 29.22; 30.44; 31.71; 33.41; 33.64; 34.10; 35.17; 35.20; 36.01; 36.39; 39.25; 42.06; 46.48; 47.12; 48.25; 60.27 (CH_2 from Et); 71.79 (C-12'); 73.15 (C-3'); 173.66 (C=O). Mass spectrum (*m*/*z*): 533 (M^+).

Ethyl 3α,12α-dihydroxy-5β-cholanate (**10**). White amorphous solid (19%). IR spectrum: 3400, 2950, 1750, 1390, 1100, 1050. ^{1}H-NMR spectrum ($CDCl_3$): 0.67 (*s*, 3H, H-18); 0.90 (*s*, 3H, H-19); 0.96 (*d*, 3H, *J* = 6.2 Hz, H-21); 1.25 (*t*, 3H, *J* = 7.2 Hz, CH_3 from Et); 3.60 (*m*, 1H, H-3); 3.96 (*m*, 1H, H-12); 4.10 (*q*, 2H, *J* = 7.2 Hz, CH_2 from Et). ^{13}C-NMR spectrum ($CDCl_3$): 12.67 (C--18); 14.21 (CH_3 from Et); 17.24 (C-21); 23.08 (C-19); 23.63; 26.08; 27.08; 27.44; 28.50; 30.28; 30.84; 31.34; 33.56; 34.07; 35.11; 35.19; 35.96; 36.27; 42.02; 46.42; 47.24; 48.17; 60.18 (CH_2 from Et); 71.56 (C-12); 72.98 (C-3); 174.32 (C=O). Mass spectrum (*m*/*z*): 403 (M^+ - H_2O).

Ethyl 11-(3α,6α-dihydroxy-5β-cholanamido)undecanoate (**11**). Colorless oil (14%). IR spectrum: 3350, 2950, 2880, 1720, 1650, 1380, 1100, 1050. ^{1}H-NMR spectrum ($CDCl_3$): 0.60 (*s*, 3H, H-18'); 0.87 (*s*, 3H, H-19'); 1.00 (*d*, 3H, *J* = 6.2 Hz, H-21'); 1.24 (*t*, 3H, *J* = 7.2 Hz, CH_3 from Et); 2.25 (*t*, 2H, *J* = 7.4 Hz, H-2); 3.18 (*m*, 2H, H-11); 3.58 (*m*, 1H, H-3'); 4.04 (*m*, 1H, H-6'); 4.10 (*q*, 2H, *J* = 7.2 Hz, CH_2 from Et); 5.75 (br *s*, 1H, NH). ^{13}C-NMR spectrum ($CDCl_3$): 11.98 (C-18'); 14.19 (CH_3 from Et); 18.32 (C-21'); 20.71; 23.46 (C-19'); 24.89; 26.85; 29.04; 29.16; 29.20; 29.28; 29.39; 29.58; 30.09; 34.32; 34.79; 34.86; 35.47; 35.56; 35.88; 39.49; 39.81; 39.95; 42.79; 48.38; 56.00; 56.15; 60.13 (CH_2 from Et); 67.91 (C-6'); 71.44 (C-3'); 173.59 (C=O from amide); 173.90 (C=O from ester). Mass spectrum (*m*/*z*): 605 (M^+ + + 1).

Ethyl 3α,6α-dihydroxy-5β-cholanate (**12**). White amorphous solid (17%). IR spectrum: 3350, 2950, 1720, 1390, 1050. ^{1}H NMR spectrum ($CDCl_3$): 0.63 (s, 3H, H--18); 0.89 (*s*, 3H, H-19); 0.92 (*d*, 3H, *J* = 6.2 Hz, H-21); 1.24 (*t*, 3H, *J* = 7.2 Hz, CH_3 from Et); 3.60 (*m*, 1H, H--3); 4.06 (*m*, 1H, H-6); 4.12 (*q*, 2H, *J* = 7.2 Hz, CH_2 from Et). ^{13}C-NMR spectrum ($CDCl_3$): 11.97 (C-18); 14.21 (CH_3 from Et); 18.21 (C-21); 20.70; 23.46 (C-19); 24.15; 28.07; 29.16; 30.14; 30.90; 31.28; 34.79; 34.91; 35.29; 35.53; 35.90; 39.78; 39.90; 42.80; 48.36; 55.89; 56.11; 60.17 (CH_2 from Et); 68.00 (C-6); 71.51 (C-3); 174.31 (C=O). Mass spectrum (*m*/*z*): 385 (M^+ - $2H_2O$).

Ethyl 11-(3α,7α-dihydroxy-12-oxo-5β-cholanamido)undecanoate (**13**). Yellow oil (72%). IR spectrum: 3350, 2920, 2870, 1720, 1650. ^{1}H-NMR spectrum ($CDCl_3$): 0.79 (*d*, 3H, *J* = 6.2 Hz, H-21'); 0.94 (*s*, 3H, H-18'); 0.96 (*s*, 3H, H-19'); 1.20 (*t*, 3H, *J* = 7.2 Hz, CH_3 from Et); 2.23 (*t*, 2H, *J* = 7.4 Hz, H-2); 3.15 (*m*, 2H, H--11); 3.36 (*m*, 1H, H-3'); 3.87 (*m*, 1H, H-7'); 4.06 (*q*, 2H, *J* = 7.2 Hz, CH_2 from Et); 6.03 (br *s*, 1H, NH). ^{13}C-

-NMR spectrum ($CDCl_3$): 11.43 (C-18'); 14.12 (CH_3 from Et); 18.60 (C-21'); 22.10; 23.67 (C-19'); 24.83; 26.81; 27.47; 28.97; 29.10; 29.16; 29.23; 29.34; 29.50; 30.28; 31.24; 33.44; 34.24; 34.75; 35.31; 35.49; 35.70; 36.94; 39.24; 39.40; 41.07; 46.09; 53.31; 56.90; 60.05 (CH_2 from Et); 67.53 (C-7'); 71.40 (C-3'); 173.61 (C=O from amide); 173.82 (C=O from ester); 215.19 (C-12). Mass spectrum (*m*/*z*): 619 ($M^+ + 1$).

Ethyl 3α,7α-dihydroxy-12-oxo-5β-cholanate (**14**). White amorphous solid (18%). IR spectrum: 3400, 2950, 1720, 1050. ^{1}H-NMR spectrum ($CDCl_3$): 0.82 (*d*, 3H, *J* = 6.2 Hz, H-21); 0.96 (*s*, 3H, H-18); 0.98 (*s*, 3H, H--19); 1.22 (*t*, 3H, *J* = 7.2 Hz, CH_3 from Et); 3.40 (*m*, 1H, H-3); 3.90 (*m*, 1H, H-7); 4.08 (*q*, 2H, *J* = 7.2 Hz, CH_2 from Et). ^{13}C-NMR spectrum ($CDCl_3$): 11.63 (C-18); 14.18 (CH_3 from Et); 18.50 (C-21); 22.12; 23.73 (C-19); 26.13; 27.46; 30.43; 31.49; 34.83; 30.87; 35.30; 35.52; 35.74; 36.89; 37.64; 39.25; 41.03; 46.30; 53.26; 56.92 (CH_2 from Et); 58.83; 60.13 (C-7); 67.77 (C-3); 174.25 (C=O); 214.85 (C-12). Mass spectrum (*m*/*z*): 435 (M^+).

Cytotoxic activity

Cell lines. Four human tumor cell lines and one human non-tumor cell line were used in the study: human breast adenocarcinoma ER-, MDA-MB-231; human breast adenocarcinoma ER+, MCF-7; cervix epiteloid carcinoma, HeLa S-3; prostate cancer PC-3, and normal fetal lung fibroblasts, MRC-5.

The cells were grown in Dulbecco's modified Eagle's medium (DMEM) with 4.5% of glucose. Media were supplemented with 10% of fetal calf serum (FCS, NIVNS) and antibiotics: 100 IU/ml of penicillin and 100 μg/ml of streptomycin (ICN Galenika). All cell lines were cultured in flasks (Costar, 25 cm^2) at 37 °C in the 100% humidity atmosphere and 5% of CO_2. Only viable cells were used in the assay. Viability was determined by dye exclusion assay with Trypan Blue.

Cytotoxicity assay. Cytotoxicity was evaluated by the colorimetric sulforhodamine B (SRB) assay according to Skehan *et al*. [14]. Briefly, single cell suspension was plated into 96-well microtitre plates (Costar, flat bottom): 5×10^3 cells per 180 ml of medium. Plates were pre-incubated for 24 h at 37 °C and in 5% of CO_2. Tested substances at concentrations ranging from 10^{-8} to 10^{-4} M were added to all wells except for the controls. After the incubation (48 h/37 °C/5% CO_2) SRB assay was carried out as follows: 50 μl of 80% trichloroacetic acid (TCA) was added to all wells; 1 h later the plates were washed with distilled water, and 75 μl of 0.4% SRB was added to all wells; 30 min later the plates were washed with citric acid (1%) and dried at room temperature. Finally, 200 μl of 10 mM Tris (pH 10.5) were added to all wells. Absorbance (*A*) was measured on the microplate reader (Multiscan MCC340, Labsystems) at 540/690 nm. The wells without cells, containing complete medium only, served as blank.

Cytotoxicity was calculated according to the formula:

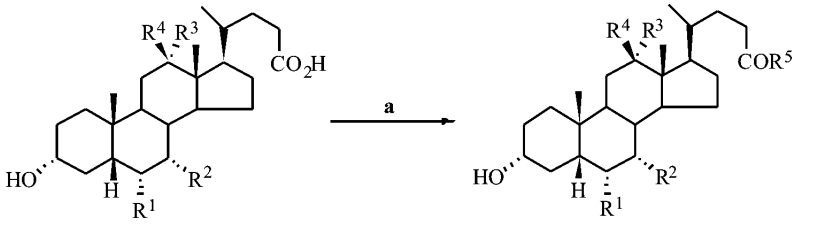
$$(1-A_{test}/A_{control})\times100$$

and expressed as a percentage of cytotoxicity (*CI*, %).

*Data analysis***.** Two independent experiments were performed in quadruplicate for each concentration of the compound. IC_{50} value defines the dose of compound that inhibits cell growth by 50%. The IC_{50} of compounds was determined by median effect analysis.

RESULTS AND DISCUSSION

The new conjugates and ethyl esters of bile acids **6**–**14** (Scheme 1) were synthesized starting from cholic (**1**), hyocholic (**2**), deoxycholic (**3**), hyodeoxycholic (**4**),



1, R^1=R^4=H; R^2=R^3=OH
2, R^1=R^2=OH; R^3=R^4=H
3, R^1=R^2=R^4=H; R^3=OH
4, R^1=OH; R^2=R^3=R^4=H
5, R^1=H; R^2=OH; R^3,R^4=O

6, R^1- R^4 = as **1**, R^5=OEt
7, R^1 - R^4 as **2**, R^5=NH$(CH_2)_{10}CO_2$Et
8, R^1 - R^4 as **3**, R^5=NH$(CH_2)_{10}CO_2$Et
9, R^1 - R^4 as **3**, R^5=NH$(CH_2)_5CO_2$Et
10, R^1 - R^4 as **3**, R^5=OEt
11, R^1 - R^4 as **4**, R^5=NH$(CH_2)_{10}CO_2$Et
12, R^1 - R^4 as **4**, R^5=OEt
13, R^1 - R^4 as **5**, R^5=NH$(CH_2)_{10}CO_2$Et
14, R^1 - R^4 as **5**, R^5=OEt

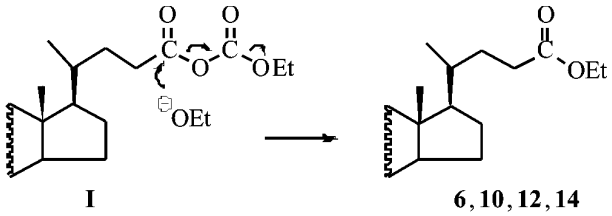
*Scheme 1. Synthesis of bile acid derivatives **6**–**14**.*
*Šema 1. Sinteza derivata žučnih kiselina **6**–**14**.*

and 12-ketocholic (**5**) acids. The reaction of conjugation of deoxycholic acid (**3**) was carried out under similar reaction conditions as the preparation of conjugates with natural amino acids [13].

Namely, the reaction of deoxycholic acid with ethyl 6-aminohexanoate was performed in the presence of EEDQ and triethylamine, in ethyl acetate, at the boiling temperature of the reaction mixture during 6 hours. This resulted in a mixture of the conjugate **9** and the unexpected ester **10**. We say "unexpected" because the application of the same reaction conditions in the synthesis of bile acid conjugates and ethyl glycinate yielded no bile acid esters [13]. On the other hand, it has been reported that bile acids could be esterified by conventional methods, with the corresponding alcohols in the presence of catalytic amounts of acids [15], as well as by modern methods that involve high-intensity ultrasound (HIU) and microwave (MW) irradiation [16,17]. Also, in our previous paper [18] we described a selective one-pot esterification and selective 3α-acetylation of cholic and deoxycholic acids with ethyl, propyl, and butyl acetate in the presence of a catalytic amount of 4-toluenesulfonic acid. In contrast to deoxycholic acid (**3**), which in the reaction with ethyl 6-aminohexanoate gave two products, cholic (**1**), hyodeoxycholic (**4**) and 12-ketocholic (**5**) acids gave under the same reaction conditions only the corresponding ethyl esters. Formation of the mentioned ethyl esters **6**, **10**, **12** and **14**, described in the present work, represents an unusual way of esterification of bile acids.

The reaction of deoxycholic acid and ethyl 11-aminoundecanoate was carried out by applying the same conditions. In this case only the amide, *i.e*. conjugate **8**, was formed. Amides were also formed with hyocholic (**2**), hyodeoxycholic (**4**), and 12-ketocholic (**5**) acids as only products, *i.e*. the conjugates **7**, **11**, and **13** respectively. The reaction yields ranged from 10 (conjugate **9**) to 27% (conjugate **7**), except for the conjugate of 12-ketocholic acid (**12**), which was obtained in a yield of 72%. The relatively low yields of the obtained conjugates are in concordance with the similar results obtained by Iida *et al*. [19] in the synthesis of tauroconjugates of selected bile acids.

As can be seen, bile acids and ethyl 6-aminohexanoate, in the presence of EEDQ, yielded esters, whereas the reaction involving ethyl 11-aminoundecanoate gave the expected conjugates. The obtained results may be explained in the following way. First, the intramolecular cyclization of ethyl 6-aminohexanoate under the given basic conditions gives a seven-membered lactam. This yields the loss of the nucleophilic amino group and formation of the ethoxide anion which attacks the mixed carbonic anhydride **I**, formed as the intermediate in the reaction of the bile acid and EEDQ (Scheme 2), which is in accordance with the mechanism proposed by Belleau and Malek [20].



Scheme 2. Proposed mechanism for ethyl esters formation in the reaction of the bile acids and EEDQ.
Šema 2. Predpostavljeni mehanizam dobijanja etil-estara u reakciji žučnih kiselina i EEDQ.

In the case of ethyl 11-aminoundecanoate, the formation of a 12-membered lactam ring is not suitable, so that the 11-amino group is available for the attack of the intermediate **I** to form the corresponding conjugate.

The synthesized compounds **6**–**8** and **10**–**14**, as well as starting bile acids **1**–**5** were evaluated for their cytotoxic activity against MDA-MB-231, MCF-7, HeLa S-3, PC-3, and MRC-5. *In vitro*, cytotoxicity was evaluated after 48-h cell treatment by the SRB assay [14]. Doxorubicin served as reference compound. The IC_{50} values in comparison with that for doxorubicin are shown in Table 1.

Of all the synthesized compounds the most active was ethyl ester 12-ketocholic acid **14**, which showed a potent activity against the MCF-7 (IC_{50} = 1.94 μM) and HeLa S-3 (IC_{50} = 4.19 μM) cells. From the ester group, ethyl ester of deoxycholic acid **10** showed also a rather strong activity against the HeLa S-3 (IC_{50} = 13.43 μM).

As far as the conjugates are concerned, a strong cytotoxicity showed the conjugate of deoxycholic acid **8** against HeLa S-3 (IC_{50} = 10.04 μM), conjugate of hyodeoxycholic acid **11** against PC-3 cells (IC_{50} = 9.83 μM), and conjugate of 12-ketocholic acid **13** against MCF-7 cells (IC_{50} = 12.18 μM). The conjugate of hyocholic acid **7** showed a satisfactory activity against HeLa S-3 cells (IC_{50} = 17.50 μM). Compared to doxorubicin, the conjugate **11** showed almost ten times higher activity against the PC-3 calls, whereas it was not toxic against MRC-5 cells.

Of all the starting bile acids the highest and very potent antiproliferative activity showed deoxycholic acid (**3**) against PC-3 cells (IC_{50} = 1.94 μM). A strong cytotoxicity showed also 12-ketocholic acid (**5**) against MDA-MB-231 (IC_{50} = 10.64 μM) and hyodeoxycholic acid (**4**) against HeLa S-3 (IC_{50} = 9.21 μM). None of these acids exhibited activity against healthy MRC-5 cells.

The conjugates **7** and **13** showed an increase in cytotoxicity compared to the starting 12-ketocholic (**5**) and hyocholic (**2**) acids, which were inactive against MCF-7. The esters **12** and **14** also showed an increased cytotoxicity against MCF-7 compared to the starting 12-ketocholic and hyodeoxycholic (**4**) acids, which were inactive. In the case of HeLa S-3, compared to the non-toxic 12-ketocholic and hyocholic acids, a great increase of cytotoxicity showed the conjugate **7** and ester **14**. The ester **14** showed a significantly increased cytotoxicity

*Table 1. In vitro cytotoxycity of compounds **1–8** and **10–14** against MDA-MB-231, MCF-7, HeLa S-3, PC-3, and MRC-5 cell lines*
*Tabela 1. In vitro citotokstičnost jedinjenja **1–8** i **10–14** prema MDA-MB-231, MCF-7, HeLa S-3, PC-3 i MRC-5 ćelijskim linijama*

Compound	IC_{50} / μM				
	Cell lines				
	MDA-MB-231	MCF-7	HeLa S-3	PC-3	MRC-5
Cholic acid (**1**)	85.75	73.81	>100	58.55	>100
Hyocholic acid (**2**)	9.83	>100	>100	>100	>100
Deoxycholic acid (**3**)	33.14	>100	14.02	1.94	>100
Hyodeoxycholic acid (**4**)	>100	>100	9.21	12.08	>100
12-Ketocholic acid (**5**)	10.64	>100	>100	>100	>100
Ethyl 3α,7α,12α-trihydroxy-5β-cholanate (**6**)	> 100	84.21	51.11	> 100	30.68
Ethyl 11-(3α,6α,7α-trihydroxy-5β-cholanamido)undecanoate (**7**)	42.01	58.55	17.50	85.75	73.81
Ethyl 11-(3α,12α-dihydroxy-5β-cholanamido)undecanoate (**8**)	> 100	> 100	10.04	61.32	>100
Ethyl 3α,12α-dihydroxy-5β-cholanate (**10**)	27.52	61.32	13.43	> 100	>100
Ethyl 11-(3α,6α-dihydroxy-5β-cholanamido)undecanoate (**11**)	>100	>100	20.79	9.83	>100
Ethyl 3α,6α-dihydroxy-5β-cholanate (**12**)	>100	30.68	54.41	84.21	>100
Ethyl 11-(3α,7α-dihydroxy-12-oxo-5β-cholanamido)undecanoate (**13**)	11.43	12.18	> 100	> 100	>100
Ethyl 3α,7α-dihydroxy-12-oxo-5β-cholanate (**14**)	> 100	1.94	4.19	33.14	>100
Doxorubicin	0.12	0.75	1.17	95.61	0.12

against PC-3 cells compared to the inactive starting 12--ketocholic acid, whereas the conjugate **11** exhibited a small increase in cytotoxicity compared to the starting hyodeoxycholic acid.

CONCLUSION

In conclusion it can be said that the modification of the side chain in the molecules of the five selected bile acids **1**–**5** by the conjugation with ethyl ester of 11-aminoundecanoic acid significantly influenced cell proliferation: the obtained conjugates and ethyl esters showed enhanced antiproliferative activity compared with the starting bile acids. In this sense, the most effective were the ester **14** and amide **13**, which showed a potent activity against MCF-7 cells compared to the inactive starting bile acids. A similar behavior showed also compounds **14** and **7** against HeLa S-3 cells. These compounds (**7**, **13**, and **14**) could serve as the basis for potentially more active agents against some tumor cell lines.

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IZVOD

SINTEZA I CITOTOKSIČNA AKTIVNOST SERIJE NOVIH DERIVATA ŽUČNIH KISELINA

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(Naučni rad)

U ovom radu su sintetizovani novi konjugati **7**, **8**, **11** i **13** odabranih žučnih kiselina (hioholne (**2**), deoksiholne (**3**), hiodeoksiholne (**4**) i 12-ketoholne (**5**) kiseline) sa etil 11-amino-undekanoatom. Reakcija konjugacije je izvedena u etil-acetatu u prisustvu *N*-etoksikarbonil-2-etoksi-1,2-dihidrohinolina (EEDQ) i trietilamina. Pri pomenutim reakcionim uslovima žučnih kiselina sa etil 6-aminoheksanoatom formirao se konjugat **9** samo sa deoksiholnom kiselinom (**3**), ali je i neočekivano nastao i etil-estar deoksiholne kiseline **10**. Kod ostalih žučnih kiselina (holne (**1**), hiodeoksiholne (**4**) i 12-ketoholne (**5**) kiseline) nastali su samo neočekivani estri **6**, **12** i **14**. Sintetizovanim jedinjenjima je određena citotoksična aktivnost prema četiri linije humanih tumora (adenokarcinom dojke ER-, MDA-MB-231; adenokarcinom dojke ER+, MCF-7; karcinom grlića materice, HeLa S-3 i adenokarcinom prostate, PC-3). Konjugat **8** je pokazao snažnu aktivnost prema HeLa S-3, a konjugat **11** prema PC-3 ćelijama. Etil estar 12-ketoholne kiseline **14** pokazao je veoma snažnu citotoksičnu aktivnost prema MCF-7 i HeLa S-3 ćelijama.

Ključne reči: Žučne kiseline • Citotoksičnost • Etil 6-aminoheksanoat konjugati • Etil 11-aminoundekanoat konjugati

Key words: Bile acids • Cytotoxicity • Ethyl 6-aminohexanoate conjugates • Ethyl 11-aminoundecanoate conjugates