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Spectroscopic Methods and Theoretical Studies of Bromoacetyl Substituted Derivatives of Bile Acids

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Abstract

The structure of seven bromoacetyl substituted derivatives of bile acids have been characterized by ^{1}H MMR, ^{13}C NMR, 2D NMR, FT-IR and mass spectrometry (ESI-MS) as well as PM5 semiempirical and B3LYP *ab initio* methods. Estimation of the pharmacotherapeutic potential has been accomplished for the synthesized compounds on the basis of Prediction of Activity Spectra for Substances (PASS).

Keywords: Bile acids, bromoacetyl substituted derivatives, Prediction of Activity Spectra for Substances, spectroscopic methods, PM5 and B3LYP calculations.

1. Introduction

Steroids are beside carbohydrates, amino acids, peptides and nucleobases a large class of natural compounds. Compounds of this type display a very important role in plant and animal organisms. Steroids are not only constituents of the cell membrane in eukaryotes (e.g., cholesterol, cholestanol, ergosterol), but they are also the main sex hormones in mammals (e.g., testosterone, estrogens, progesterone) and plants (e.g. brassinosteroids). Steroids play also important functions in the regulation of metabolism (e.g., bile acids and vitamin D).[1–4] Especially important compounds are bile acids (e.g., lithocholic, deoxycholic and cholic) and their derivatives. The cholic acid was first isolated from the bile of mammals in 1828 by L. Gmelin. The bile acids are produced from cholesterol in the liver and are stored in the gallbladder.[5–10] However, gallbladder contraction with feeding releases bile acids into the intestine. Additionally, the terminal carboxylic acid group in C(17) side chain may be conjugated with taurine or glycine.

The amphiphilic properties of bile acids together with their specific structure composed of a large, rigid and curved skeleton, as well as chirality and orientation of their chemically different polar hydroxy groups (3α; 3α,7α and 3α,7α,12α) toward the center of a concave face make them interesting starting materials for the synthesis of macrocyclic compounds as molecular dimers, molecular tweezers, cholaphanes or quasi podands.[11–18]

Moreover, the unique structural elements of bile acids are very important in the study of molecular recognition, host–guest chemistry and biomimetic chemistry. They also play a very significant role in supramolecular chemistry and as drugs in pharmacology. Bile acids themselves have been used as building blocks for the design and construction of new molecular receptors that are capable to recognize guest molecules of diverse chemical nature.[13–16] Bile acid dimers can be used for the synthesis of macrocyclic compounds as artificial receptors.[19–23] Some derivatives of bile acids are very good organogelators.[24–26] The above mentioned applications of bile acids make them very interesting and promising materials.

Halogenoacetyl (chloro- or bromo-) substituted derivatives of bile acids play an extremely useful role in various organic syntheses. Compounds of this type can be prepared in many different ways. Bile acids can react with halogenoacetic acid halides and potassium carbonate in chloroform or dichloromethane, with calcium or sodium hydride and tetrabutylammonium bromide (TEBA) in toluene, as well as pyridine or 4-(dimethylamino)pyridine (DMAP) in toluene.[27–35] These compounds were used in nucleophilic reactions with *N*-, *S*- or *O*-nucleophiles. In many cases bromo- or chloroacetyl substituted derivatives of bile acids react with pyrimidines (e.g. selective *N*-1-alkylation of uracil),[27] purines (e. g. selective *N*-9-alkylation of adenine),[28] thio analogs of pyrimidine bases (e. g.

selective *N*-1-alkylation of 2-thiouracil),[29] ammonium morpholinyl dithiocarbamate (selective *S*-alkylation),[30] *N*-1-alkylation of imidazole,[31] sodium azide[32–34] or tamoxifen (antagonist of the estrogen receptor in breast tissue).[35] In the case of sodium azide, the resulting products are used as substrates for click chemistry reactions. Singh *et al.* employed a series of chloro substituted derivatives of bile acids that were used in the synthesis of cationic bile acid-based facial amphiphiles featuring trimethyl ammonium head groups. The authors evaluated the role of these amphiphile compounds for cytotoxic activity against colon cancer cells.[36]

However, to the best of our knowledge, no work has been published on the spectroscopic (^{1}H MMR, ^{13}C NMR, 2D NMR, FT-IR), semiempirical (PM5) and *ab initio* (B3LYP) methods, as well as *in silico* (PASS) and mass spectrometry (ESI-MS) studies of bromoacetyl substituted bile acids.

2. Experimental Procedure

2. 1. Instrumentation

The NMR spectra were measured with a Spectrometer NMR Varian Mercury 300 MHz (Oxford, UK), operating at 300.07 and 75.4614 for ^{1}H and ^{13}C, respectively. Typical conditions for the proton spectra were: pulse width 32°, acquisition time 5 s, FT size 32 K and digital resolution 0.3 Hz per point, and for the carbon spectra pulse width 60°, FT size 60 K and digital resolution 0.6 Hz per point, the number of scans varied from 1200 to 10,000 per spectrum. The ^{13}C and ^{1}H chemical shifts were measured in $CDCl_3$ relative to TMS as the internal standard. The 2D ^{1}H-^{1}H (COSY) and Heteronuclear Multiple-Bond Connectivity (HMBC, HSQC) spectra were recorded on a Bruker Avance DRX spectrometer operating at 599.93 and 150.85 MHz for ^{1}H and ^{13}C, respectively. Infrared spectra were recorded as KBr pellets (1.5 mg/300 mg KBr) using a FT-IR Bruker IFS 66 spectrometer (Karlsruhe, Germany) at 295 K. The ESI (electron spray ionization) mass spectra were recorded on a Waters/Micromass (Manchester, UK) ZQ mass spectrometer equipped with a Harvard Apparatus (Saint Laurent, Canada) syringe pump. The sample solutions were prepared in methanol at the concentration of approximately 10^{-5} M. The standard ESI-MS mass spectra were recorded at the cone voltage 90 V.

2. 2. Computational Details

The PM5 semiempirical calculations were performed using the WinMopac 2003 program.[37–39] The calculations were performed using the GAUSSIAN 03 program package[40] at the B3LYP[41–43] levels of theory with the 6-31G(d,p) basis set.[44] The NMR isotropic shielding constants were calculated using the standard GIAO (Gauge-Independent Atomic Orbital) approach of Gaussian 03.[45,46]

Potential pharmacological activities of the compounds synthesized have been evaluated on the basis of computer-aided drug discovery approach with *in silico* Prediction of Activity Spectra for Substances (PASSs) program. It is based on a robust analysis of the structure–activity relationship in a heterogeneous training set currently including about 60,000 biologically active compounds from different chemical series with about 4,500 types of biological activities. Since only the structural formula of the chemical compound is necessary to obtain a PASS prediction, this approach can be used at the earliest stages of investigation. There are many examples of the successful use of the PASS approach leading to new pharmacological agents.[47–51] The PASS software is useful for the study of biological activity of secondary metabolites. We have selected the types of activities that were predicted for a potential compound with the highest probability (focal activities). If predicted activity (PA) > 0.7, the substance is very likely to exhibit the activity in experiment and the chance of the substance being the analogue of a known pharmaceutical agent is also high. And if 0.5 < PA < 0.7, the substance is unlikely to exhibit the activity in experiment, the probability is less, and the substance is unlike any known pharmaceutical agent.

2. 3. Synthesis

The methyl esters of bile acids (1 eq.) were dissolved in 6 mL anhydrous toluene. Then sodium hydride (3–5 eq.) and TEBA (0.1 eq.) were added and the reaction was carried out for 1 h at room temperature. Subsequently, bromoacetic acid bromide was added dropwise (1.1 eq. for each hydroxyl group at steroid skeleton) and the reaction mixture was kept at room temperature for 24 h. Then the excess of sodium hydride was filtered, and the filtrate was washed with $NaHCO_3$ (5%, 20 mL), brine (20 mL) and finally dried over Na_2CO_3. The solvent was evaporated under reduced pressure to give the crude product. Products were purified by chromatography on silica gel (Merck, type 60, 70–230 mesh).

Methyl 3α-bromoacetoxy-5β-cholan-24-oate (**4**) isolated yield 59%, mp 127–128 °C. Anal. Calcd for $C_{27}H_{43}BrO_4$: C 63.40, H 8.47. Found: C 63.58, H 8.30; ^{1}H NMR ($CDCl_3$): δ 4.83–4.75 (m, 1H, 3β-H), 3.80 (s, 2H, 3α-CH_2Br), 3.67 (s, 3H, OCH_3), 0.93 (s, 3H, CH_3-19), 0.91 (d, J = 6.4 Hz, 3H, CH_3-21), 0.65 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ 174.7 (C-24), 166.8 (3α-CO_2), 76.6 (C-3), 56.4, 55.9, 51.5 (C-25), 42.7, 41.9, 40.4, 40.1, 35.8, 35.3, 34.9, 34.6, 31.9, 31.0, 30.9, 28.2 (3α-CH_2Br), 26.9, 26.4, 26.4, 26.3, 24.2, 23.3 (C-19), 20.8, 18.3 (C-21), 12.0 (C-18). ESI-MS (MeOH): *m/z* (%) 533 (100) $[M+Na]^+$, 549 (25) $[M+K]^+$; FT-IR: ν 1730 (C=O), 1292 (C–O) cm^{-1}.

Methyl 3α-bromoacetoxy-12α-hydroxy-5β-cholan-24-oate (**5**), isolated yield 50%, oil. Anal. Calcd for $C_{27}H_{43}BrO_5$: C 61.47, H 8.22. Found: C 61.63, H 8.40; ^{1}H NMR ($CDCl_3$): δ 4.81–4.76 (m, 1H, 3β-H), 3.99 (bs,1H, 12β-H), 3.80 (s, 2H, 3α-CH_2Br), 3.67 (s, 3H, OCH_3), 0.97 (d, *J* = 6.4 Hz, 3H, CH_3-21), 0.93 (s, 3H, CH_3-19), 0.68 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ 174.6 (C-24), 166.8 (3α-CO_2), 76.5 (C-3), 73.1 (C-12), 51.5 (C-25), 48.25, 47.37, 46.47, 41.82, 35.94, 35.02, 34.73, 34.09, 33.65, 31.82, 31.03, 30.86, 28.7 (3α-CH_2Br), 27.40, 26.88, 26.40, 26.24, 25.96, 23.56, 23.1 (C-19), 17.3 (C-21), 12.7 (C-18); ESI-MS (MeOH): *m/z* (%) 551 (100) $[M+Na]^+$, 567 (30) $[M+K]^+$; FT-IR: ν 3543 (OH), 1734 (C=O), 1287 (C–O) cm^{-1}.

Methyl 3α,12α-dibromoacetoxy-5β-cholan-24-oate (**6**) isolated yield 70%, oil. Anal. Calcd for $C_{27}H_{43}Br_2O_6$: C 53.71, H 6.84. Found: C 53.49, H 6.52; ^{1}H NMR ($CDCl_3$): δ 5.16 (t, *J* = 2.8 Hz, 1H, 12β-H), 4.81–4.73 (m, 1H, 3β-H), 3.85 (s, 2H, 12α-CH_2Br), 3.79 (s, 2H, 3α-CH_2Br), 3.66 (s, 3H, OCH_3), 0.92 (s, 3H, CH_3-19), 0.83 (d, *J* = 6.3 Hz, 3H, CH_3-21), 0.75 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ 174.6 (C-24), 166.7 (3α-CO_2), 166.6 (12α-CO_2), 77.9 (C-12), 76.3 (C-3), 51.5 (C-25), 49.2, 47.3, 45.1, 41.7, 35.6, 34.8, 34.6, 34.3, 34.1, 31.8, 30.9, 30.8, 27.4 (3α-CH_2Br), 26.8 (12α-CH_2Br), 26.4, 26.2, 25.9, 25.9, 25.3, 23.4, 22.9 (C-19), 17.4 (C-21), 12.3 (C-18); ESI-MS (MeOH): *m/z* (%) 671 (100) $[M+Na]^+$, 687 (43) $[M+K]^+$; FT-IR: ν 1733 (C=O), 1286 (C–O) cm^{-1}.

Methyl 3α-bromoacetoxy-7α,12α-dihydroxy-5β-cholan-24-oate (**7**), isolated yield 57%, oil. Anal. Calcd for $C_{27}H_{43}BrO_6$: C 59.66, H 7.97. Found: C 59.34, H 7.82; ^{1}H NMR ($CDCl_3$): δ 4.69–4.61 (m, 1H, 3β-H), 4.00 (bs, 1H, 12β-H), 3.98-3.86 (m, 1H, 7β-H), 3.79 (s, 2H, 3α-CH_2Br), 3.67 (s, 3H, OCH_3), 0.99 (d, *J* = 6.3 Hz, 3H, CH_3-21), 0.91 (s, 3H, CH_3-19), 0.80 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ 174.7 (C-24), 166.8 (3α-CO_2), 76.5 (C-3), 72.8 (C-12), 68.2 (C-7), 51.5 (C-25), 47.2, 46.5, 42.0, 41.1, 39.5, 35.1, 34.8, 34.7, 34.6, 34.2, 31.9, 31.0, 30.8, 29.4, 28.4 (3α-CH_2Br), 27.40, 26.7, 26.5, 26.4, 23.1, 22.7 (C-19), 17.3 (C-21), 14.12, 12.6 (C-18); ESI-MS (MeOH): *m/z* (%) 567 (100) $[M+Na]^+$, 583 (55) $[M+K]^+$; 579 (100) $[M+Cl]^-$, 623 (35) $[M+Br]^-$; FT-IR: ν 3433 (OH), 1734 (C=O), 1286 (C–O) cm^{-1}.

Methyl 3α,12α-dibromoacetoxy-7α-hydroxy-5β-cholan-24-oate (**8**) isolated yield 23%, oil. Anal. Calcd for $C_{27}H_{43}Br_2O_7$: C 52.42, H 6.67. Found: C 52.74, H 6.43; ^{1}H NMR ($CDCl_3$): δ 5.17 (t, *J* = 2.0 Hz, 1H, 12β-H), 4.67–4.59 (m, 1H, 3β-H), 3.91-3.88 (m, 1H, 7β-H), 3.86 (s, 2H, 12α-CH_2Br), 3.79 (s, 2H, 3α-CH_2Br), 3.66 (s, 3H, OCH_3), 0.91 (s, 3H, CH_3-19), 0.85 (d, *J* = 6.4 Hz, 3H, CH_3-21), 0.77 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ 174.6 (C-24), 166.7 (3α-CO_2), 166.8 (12α-CO_2), 77.5 (C-12), 76.3 (C-3), 67.9 (C-7), 51.5 (C-25), 47.1, 45.1, 43.2, 41.0, 39.6, 34.8, 34.6, 34.6, 34.3, 30.9, 30.7, 27.5 (3α-CH_2Br), 27.3, 26.5 (12α-CH_2Br), 26.3, 25.9, 25.1, 22.9 (C-19), 22.3, 17.4 (C-21), 12.2 (C-18); ESI-MS (MeOH): *m/z* (%) 687 (100) $[M+Na]^+$; FT-IR: ν 3541 (OH), 1731 (C=O), 1286 (C–O) cm^{-1}.

Methyl 3α,7α-dibromoacetoxy-12α-hydroxy-5β-cholan-24-oate (**9**), isolated yield 24%, oil. Anal. Calcd for $C_{27}H_{43}Br_2O_7$: C 52.42, H 6.67. Found: C 52.59, H 6.58; ^{1}H NMR ($CDCl_3$): δ 4.99–4.96 (m, 1H, 7β-H), 4.70–4.63 (m, 1H, 3β-H), 4.01 (bs, 1H, 12β-H), 3.83 (s, 2H, 7α-CH_2Br), 3.80 (s, 2H, 3α-CH_2Br), 3.66 (s, 3H, OCH_3), 0.98 (d, *J* = 6.2 Hz, 3H, CH_3-21), 0.94 (s, 3H, CH_3-19), 0.69 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ 174.6 (C-24), 166.8 (3α-CO_2), 166.6 (7α-CO_2), 76.1 (C-3), 73.3 (C-7), 72.6 (C-12), 51.5 (C-25), 47.2, 46.6, 41.9, 40.7, 38.2, 34.9, 34.6, 34.4, 34.3, 31.1, 31.0, 30.8, 29.7, 28.5, 28.1, 27.2 (3α-CH_2Br), 26.3 (7α-CH_2Br), 26.3, 22.9 (C-19), 22.4, 17.3 (C-21), 12.5 (C-18); ESI-MS (MeOH): *m/z* (%) 687 (100) $[M+Na]^+$; 183 (100) $[NaBr+Br]^-$; 699 (13) $[M+Cl]^-$; 743 (75) $[M+Br]^-$; FT-IR: ν 3530 (OH), 1733 (C=O), 1287 (C–O) cm^{-1}.

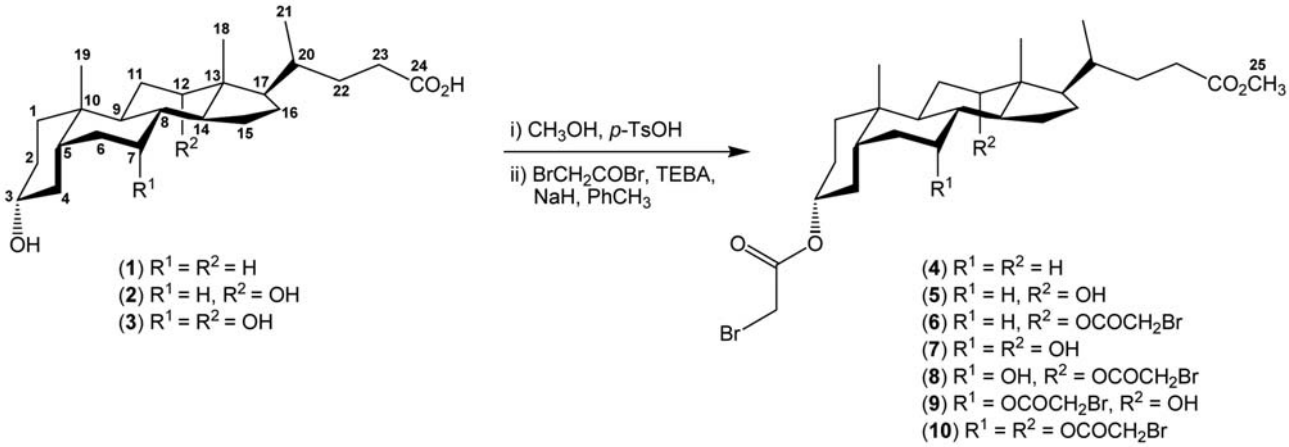
Methyl 3α,7α,12α-tribromoacetoxy-5β-cholan-24-oate (**10**), isolated yield 54%, oil. Anal. Calcd for $C_{27}H_{43}Br_3O_8$: C 47.41, H 5.78. Found: C 47.68, H 5.58; ^{1}H NMR ($CDCl_3$): δ 5.17 (t, *J* = 2.9 Hz, 1H, 12β-H), 5.02–5.00 (m, 1H, 7β-H), 4.68–4.60 (m, 1H, 3β-H), 3.89 (s, 2H, 12α-CH_2Br), 3.85 (s, 2H, 7α-CH_2Br), 3.79 (s, 2H, 3α-CH_2Br), 3.66 (s, 3H, OCH_3), 0.94 (s, 3H, CH_3-19), 0.84 (d, *J* = 6.4 Hz, 3H, CH_3-21), 0.76 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ 174.4 (C-24), 166.7 (3α-CO_2), 166.5 (12α-CO_2), 166.3 (7α-CO_2), 77.2 (C-12), 75.8 (C-3), 73.1 (C-7), 51.5 (C-25), 47.2, 45.1, 42.7, 40.6, 37.9, 34.7, 34.5, 34.3, 34.3, 31.1, 30.9, 30.7, 29.7, 28.4, 27.2, 26.3, 26.4 (3α-CH_2Br), 26.2 (7α-CH_2Br), 26.1 (12α-CH_2Br), 24.9, 22.9 (C-19), 22.2, 17.5 (C-21), 11.9 (C-18); ESI-MS (MeOH): *m/z* (%) 808 (100) $[M+Na]^+$; FT-IR: ν 1732 (C=O), 1286 (C–O) cm^{-1}.

3. Results and Discussion

3. 1. Synthesis

Bromoacetyl substituted derivatives of lithocholic, deoxycholic and cholic acid were obtained by reaction of methyl esters of bile acids with bromoacetic acid bromide in toluene with TEBA and sodium hydride to give compounds **4**–**10**.[33] The syntheses of compounds **4**–**10** are depicted in Scheme 1.

In the chemical literature there is no report on the compounds **6**–**10**. Some data about compounds **4** and **5**, which were synthesized with potassium carbonate in chloroform, are given by Chattopadhyay.[27] Products were obtained with very good yields, however no information about purification method is available. Among the com-



Scheme 1. Synthesis of bromoacetyl substituted derivatives of bile acids **4**–**10**.

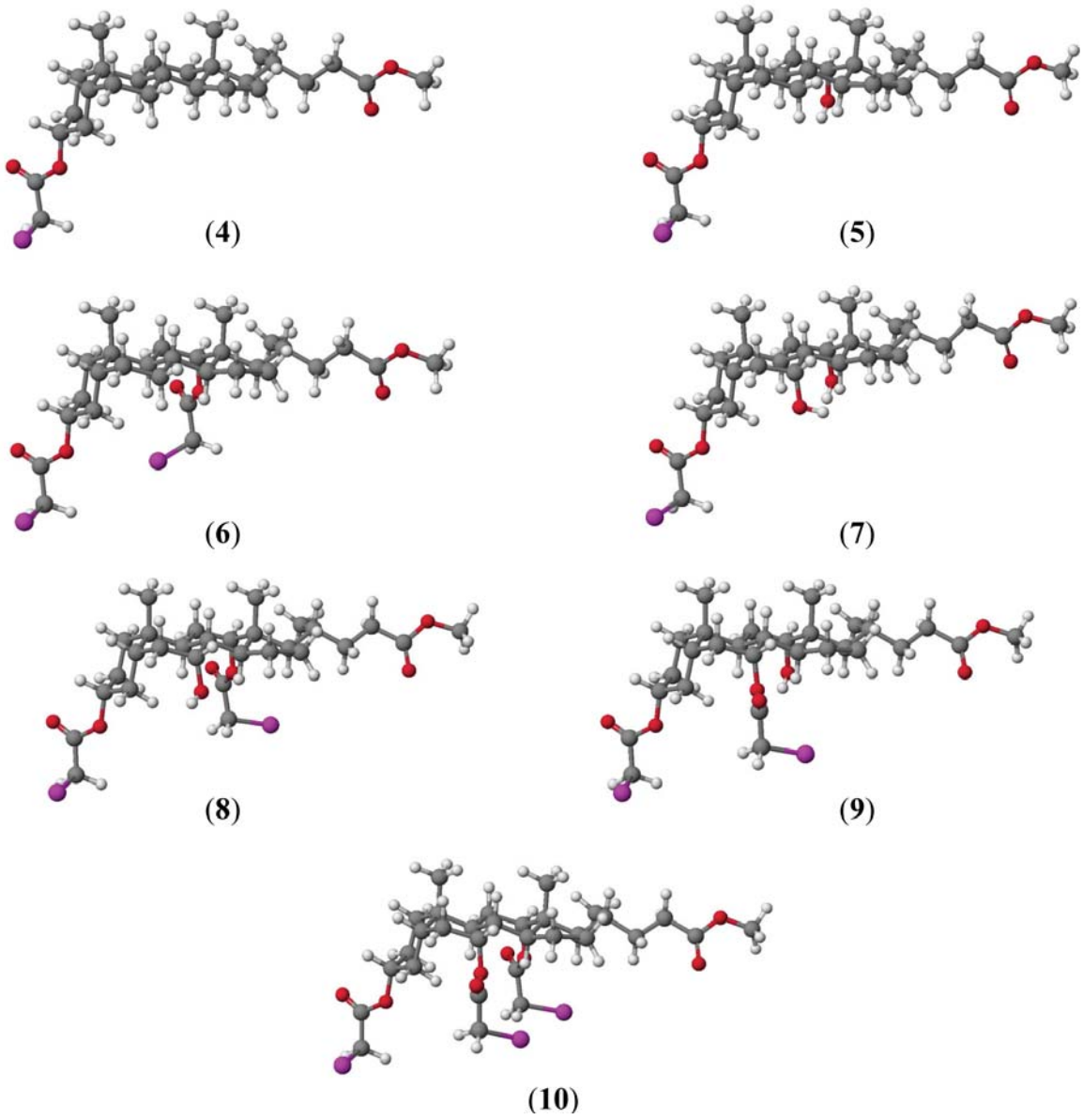


Figure 1. Molecular models of compounds **4**–**10** calculated by PM5 method.

pounds prepared only methyl 3α-bromoacetoxy-5β-cholan-24-oate (**4**) was a solid, the rest of the conjugates were oils. We are the first to offer a full spectroscopic characterization of bromoacetyl substituted derivatives of lithocholic, deoxycholic and cholic acid.

3. 2. PM5 and B3LYP Calculations

The PM5 semiempirical calculations were performed using the WinMopac 2003 program. The final heat of formation (HOF) for the methyl esters of bile acids **1**–**3** and its bromoacetyl substituted derivatives **4**–**10** is presented in Table 1. The molecular models of compounds **4**–**10** are shown in Figure 1.

The lowest HOF value is observed for methyl 3α,7α,12α-tribromoacetoxy-5β-cholan-24-oate (**10**). This fact can be explained by the greater stability by ester groups in isolated molecule. The HOF of methyl 3α,12α-dibromoacetoxy-7α-hydroxy-5β-cholan-24-oate (**8**) and methyl 3α,7α-dibromoacetoxy-12α-hydroxy-5β-cholan-24-oate (**9**) are comparable. The increase of the number of free hydroxyl groups causes the increase of the HOF values. It can be also caused by difficulty to form intramolecular hydrogen bonds. The spatial arrangement and interaction of the conjugate **8** is shown in Figure 2. The final heat of formation is –1941.6084 kcal/mol and the distances between the carbonyl group of 3α-CO_2 and 12α-CO_2 are 8.03 Å and 7.45 Å, respectively. In turn, the distance between the C(24)O_2 groups is 7.68 Å. Compensation charges occur only through intermolecular electrostatic interaction. This is a very good confirmation of the conclusion that interactions reduce HOF.

Table 1. Heat of formation (HOF) [kcal/mol] of compounds **4**–**10**.

Compound	HOF	ΔHOF	HOF of five molecules
1	–226.5896	–	–
2	–268.6761	–	–
3	–309.3719	–	–
4	–266.3263	–39.7367	–1348.8740
5	–308.5947	–39.9186	–1558.2560
6	–345.9485	–77.2724	–1744.5095
7	–348.0126	–38.6407	–1756.1661
8	–386.0620	–76.6901	–1941.6084
9	–388.6102	–79.2383	–1957.0453
10	–425.4859	–116.1140	–2108.7200

$\Delta HOF = HOF_{(\text{compounds } \mathbf{4–10})} - HOF_{(\text{methyl ester of bile acids } \mathbf{1–3})}$

3. 3. The Predicted Biological Activity

The biological activity spectra were predicted for all synthesized compounds using PASS. We selected the types of activity that were predicted for a potential compound with the highest probability (focal activities, Table 2). According to these data the most frequently predicted types of biological activity, e.g. inhibitors of acylcarnitine hydrolase (PA > 95%), alkenylglycerophosphocholine hydrolase (PA > 90%), alkylacetylglycerophosphatase (PA > 90%), D-lactaldehyde dehydrogenase, glucan endo-1,3-β-D-glucosidase, glyceryl-ether monooxygenase as well as choleretic, cholesterol antagonist, CYP3A substrate and CYP3A4 substrate.

3. 4. ^{1}H and ^{13}C NMR Spectra

The structures of all synthesized compounds were determined from their ^{1}H and ^{13}C, as well as two-dimen-

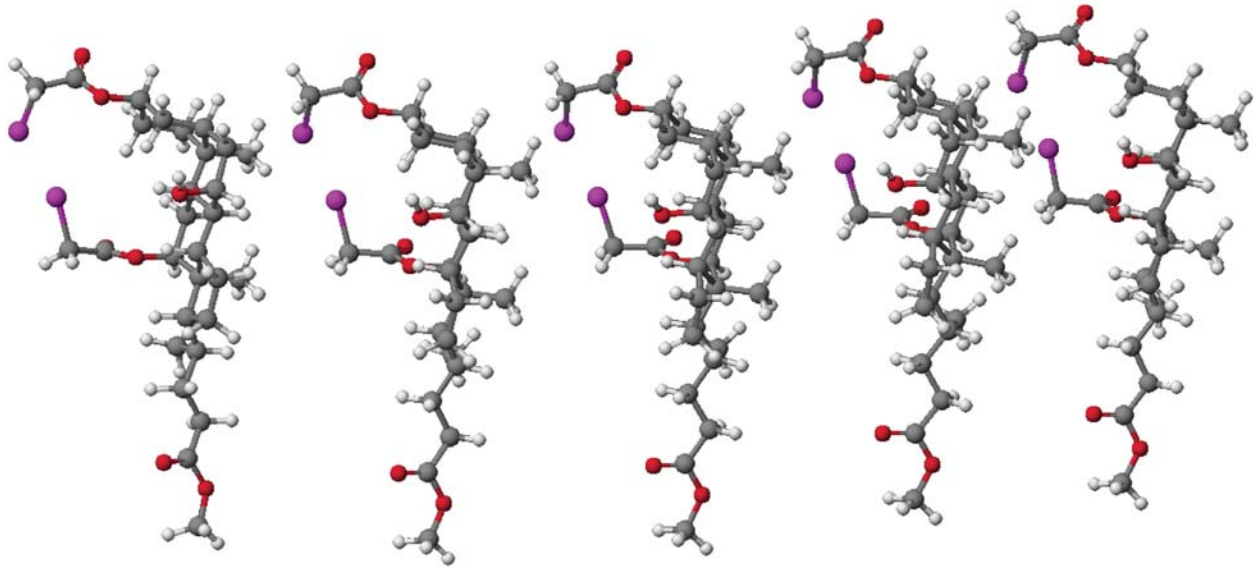


Figure 2. Molecular models of compound **8** calculated by PM5 method.

Table 2. Probability »to be Active« (PA) values for predicted biological activity of compounds **4**–**10**.

Focal predicted activity (PA > 80%)	Compounds						
	4	**5**	**6**	**7**	**8**	**9**	**10**
Acylcarnitine hydrolase inhibitor	96	98	97	99	98	98	97
Adenomatous polyposis treatment	81	–	–	82	82	–	–
Alkenylglycerophosphocholine hydrolase inhibitor	94	97	95	96	94	96	92
Alkenylglycerophosphoethanolamine hydrolase inhibitor	81	89	82	85	81	85	–
Alkylacetylglycerophosphatase inhibitor	94	97	95	95	94	95	92
Choleretic	–	86	–	90	87	86	–
Cholesterol antagonist	89	85	81	88	88	82	–
CYP3A substrate	–	82	–	83	–	83	–
CYP3A4 substrate	81	84	–	85	82	85	–
Dextranase inhibitor	88	–	89	91	88	91	83
D-lactaldehyde dehydrogenase inhibitor	–	–	–	86	81	81	–
Glucan endo-1,3-β-D-glucosidase inhibitor	87	95	89	91	88	91	83
Glyceryl-ether monooxygenase inhibitor	–	86	84	89	88	88	86
Peptidoglycan glycosyltransferase inhibitor	83	91	85	87	84	87	–
Plasmanylethanolamine desaturase inhibitor	–	–	–	84	81	81	–
Protein-disulfide reductase (glutathione) inhibitor	–	88	81	81	–	81	–
Vitamin-K-epoxide reductase inhibitor	–	81	–	87	83	83	–

sional NMR spectra (COSY, HSQC, HMBC). The assignments of proton and carbon-13 chemical shifts for **4**–**9** are listed in Table 3 and are based on 2D ^{1}H-^{1}H, ^{1}H-^{13}C NMR experiments. Additionally the ^{1}H-^{13}C HSQC spectrum shows correlations over three bonds between the C3β-H, C7β-H, C12β-H protons and C=O carbon atoms in **10**.[52] The HSQC spectrum of **10** is shown in Figure 3. Cross-peaks between H(21)–C(18), H(11)–C(12), H(18)–C(12) confirmed its structure.

The ^{1}H and ^{13}C NMR spectra were measured in chloroform (Table 3). The ^{1}H NMR spectra of compounds **4**–**10** show characteristic multiplets in the range

Table 3. The most characteristic chemical shifts (ppm) in ^{1}H and ^{13}C NMR spectra of compounds **4**–**9**.

No of atom	4	5	6	7	8	9
^{1}H NMR						
18	0.65	0.68	0.75	0.80	0.77	0.69
19	0.93	0.93	0.92	0.91	0.91	0.94
21	0.91	0.97	0.83	0.99	0.85	0.98
25	3.67	3.67	3.66	3.67	3.66	3.66
3β-H	4.83–4.75	4.81–4.76	4.81–4.73	4.69–4.61	4.67–4.59	4.70–4.63
7β-H	–	–	–	3.98–3.86	3.91–3.88	4.99–4.96
12β-H	–	3.99	5.16	4.00	5.17	4.01
3α-CH_2Br	3.80	3.80	3.79	3.79	3.79	3.80
7α-CH_2Br	–	–	–	–	–	3.83
12α-CH_2Br	–	–	3.85	–	3.86	–
^{13}C NMR						
18	12.0	12.7	12.3	12.6	12.2	12.5
19	23.3	23.1	22.9	22.7	22.9	22.9
21	18.3	17.3	17.4	17.3	17.4	17.3
24	174.7	174.6	174.6	174.7	174.6	174.6
25	51.5	51.5	51.5	51.5	51.5	51.5
3β-H	76.6	76.5	76.3	76.5	76.3	76.1
7β-H	–	–	–	68.2	67.9	73.3
12β-H	–	73.1	77.9	72.8	77.5	72.6
3α-CO_2	166.8	166.8	166.7	166.8	166.7	166.8
7α-CO_2	–	–	–	–	–	166.6
12α-CO_2	–	–	166.6	–	166.8	–
3α-CH_2Br	28.2	28.7	27.4	28.4	27.5	27.2
7α-CH_2Br	–	–	–	–	–	26.3
12α-CH_2Br	–	–	26.8	–	v26.5	–

4.83–4.59 ppm assigned to the C3β-H protons (in axial positions) of the steroid skeleton.

In the spectra of compounds **5**, **7** and **9** where unsubstituted hydroxy group in position C(12) is present, characteristic broad singlets in the range 4.01–3.99 ppm are observed which are due to the C12β-H protons (in equatorial positions). However, in the case of the bromoacetates (compounds **6**, **8** and **10**) protons C12β-H appear as triplets in the range of 5.17–5.16 ppm.

^{1}H NMR spectra of derivatives of cholic acid **7**, **8** and **9**, **10** show characteristic multiplets in the ranges of 3.98–3.86 and 5.02–4.96 ppm assigned to the C(7β)-H protons (in equatorial positions) of the steroid skeleton, respectively. The position of the signals at lower chemical shift values is related to the presence of the unsubstituted hydroxyl group in **7** and **8**, while the higher chemical shift values correspond to the $OOCCH_2Br$ group in **9** and **10** (Figure 4).

Two hydrogen singlets in the range of 0.80–0.65 and 0.94–0.91, as well as characteristic doublets at 0.99–0.83 ppm are assigned to CH_3-18, CH_3-19, and CH_3-21, respectively.

The characteristic singlets of CO_2CH_3 protons in the range 3.67–3.66 ppm for all discussed compounds were observed in ^{1}H NMR spectra.

The ^{1}H NMR spectra of compounds **4–10** show characteristic singlets in the range 3.80–3.79 ppm for the protons of the 3α-$OCOCH_2Br$ group, whereas for compounds **9** and **10** characteristic doublets at 3.83 and 3.85 ppm for the protons of the 7α-$OCOCH_2Br$ group are observed. However, protons of 12α-$OCOCH_2Br$ group for the compound **6** appear as a doublet and for compounds **8** and **10** as a singlet at 3.85, 3.86 and 3.89 ppm, respectively. The characteristic protons shifts for compounds **4–10** are collected in Table 3.

The ^{13}C NMR spectra of compounds **4–10** show characteristic signals at 12.7–11.9, 23.3–22.7 and 18.3–17.3 ppm which are assigned to CH_3-18, CH_3-19 and CH_3-21, respectively. The carbon atoms of the CO_2CH_3 group are observed in the range 174.4–174.7 ppm and at 51.5 ppm and are assigned to CO_2 and CH_3, respectively. On the other hand, carbon atoms of bromoacetoxy groups in positions 3α, 7α or 12α resonate in the range of 166.8–166.3 ppm. Unusually, a relationship was observed between the signals of carbon atoms C(3) and C(12) of the steroid skeleton. The carbon atoms of the C(12) steroid skeleton gave signals in the range of 73.1–72.6 and 77.9–77.2 ppm assigned to C(12)OH and $C(12)OCOCH_2Br$, respectively. However, carbons of the C(7) gave signals in the range 68.2–67.9 and 73.1–73.3

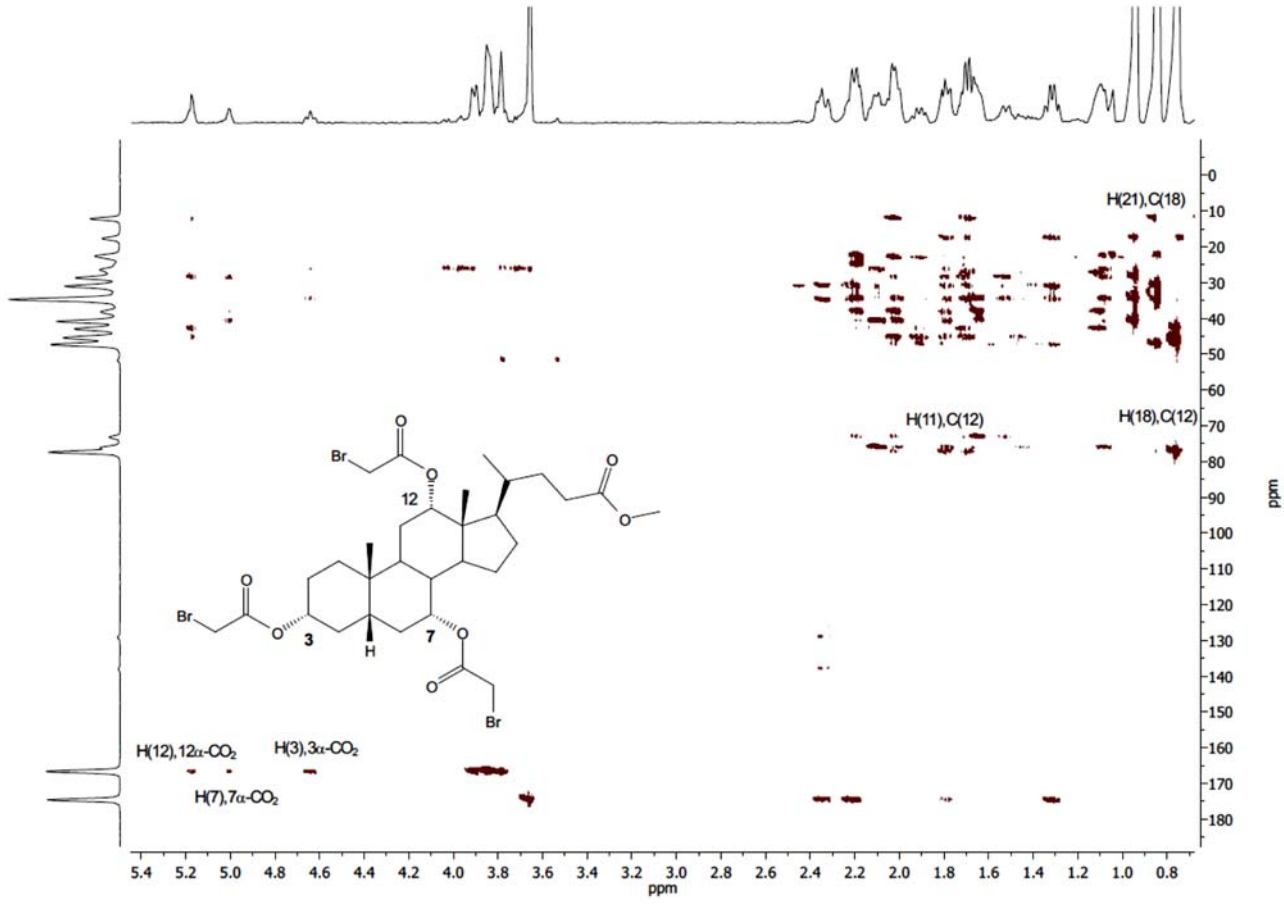


Figure 3. HSQC NMR spectrum for methyl 3α,7α,12α-tribromoacetoxy-5β-cholan-24-oate (**10**).

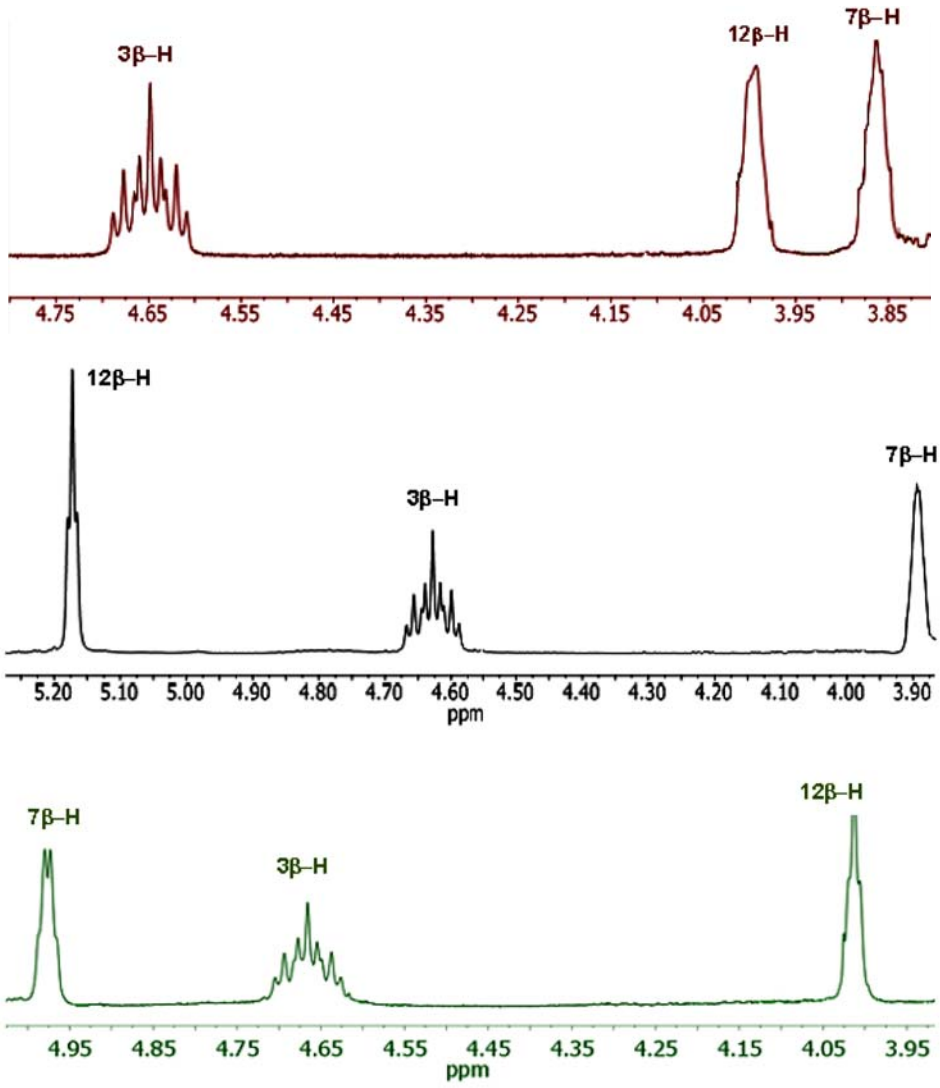


Figure 4. ^{1}H NMR spectra in the 5.20–3.80 ppm region showing the most characteristic signals of compounds **7** (red), **8** (black) and **9** (green).

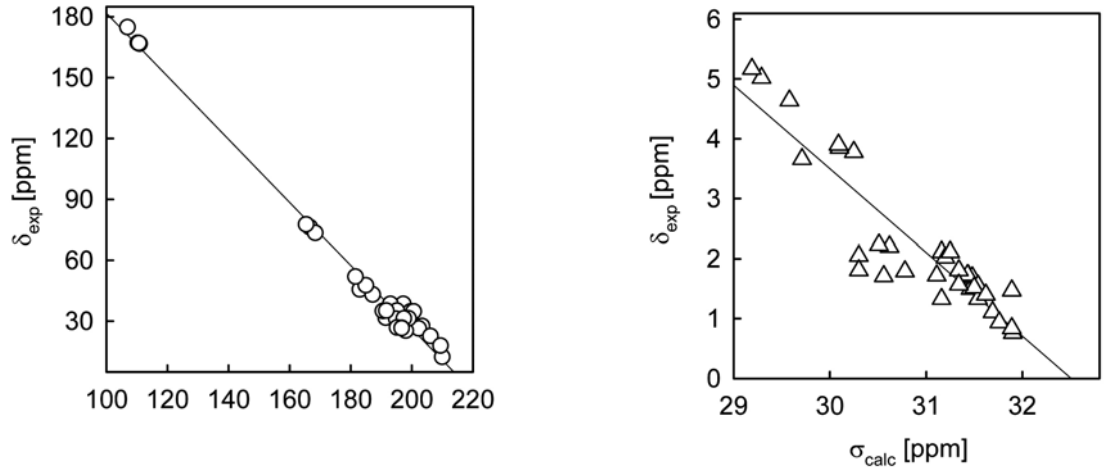


Figure 5. Plots of the experimental chemical shifts (δ) vs. the magnetic isotropic shielding constants (σ) from the GIAO/B3LYP/6-31G(d,p) approach calculated for **10** in $CDCl_3$; $\delta_{exp} = a + b\ \sigma_{calc}$; (a) carbon-13, (b) proton.

ppm assigned to C(7)OH and C(7)OCOCH$_2$Br, respectively. The presence of bromoacetoxy group at C(7) shift the signals of carbon atoms C(3) to lower chemical shift in comparison to bromoacetoxy group at C(12). The diagnostic signal for BrCH$_2$ groups is observed at 28.7–26.1 ppm.

The relation between the experimental ^{13}C and ^{1}H chemical shifts (δ_{exp}) and the Gauge Including Atomic Orbitals (GIAO) magnetic isotropic shielding constants (σ_{calc}), which are widely used,[45,46,53–55] are usually linear and described by the equation $\delta_{exp} = a + b\,\sigma_{calc}$. The slope and intercept of the least-square correlation lines are used to scale the GIAO isotropic absolute shielding constants σ, and to predict chemical shifts in CDCl$_3$ for **10** (Figure 5, Table 4).

As can be seen from Figure 5 the agreement between the experimental and the calculated data for protons is worse than for carbons-13.[55] The protons are located on the periphery of the molecule, thus their interactions with solvent molecules are much stronger than the interactions of the more hidden carbon atoms. The differences between the calculated and experimental shifts for protons are probably due to the fact that the shifts are calculated for single molecules in a gas phase.

3. 5. FT-IR Spectra

The FT-IR spectra of the representative conjugates **7**, **9** and **10** are shown in Figure 6. The most characteristic in the FT-IR spectra are the bands at 3543 cm^{-1} (**5**), 3433

Table 4. Chemical shifts (δ, ppm) in CDCl$_3$ calculating GIAO nuclear magnetic shielding tensors (σ_{calc}) for **10**. The predicted GIAO chemical shifts were computed from the linear equation $\delta_{exp.} = a + b\cdot\sigma_{calc}$ with *a* and *b* determined from the fit of the experimental data.

carbon-13	$\delta_{exp.}$	δ_{calc}	σ_{calc}	proton	$\delta_{exp.}$	δ_{calc}	σ_{calc}
C(1)	38.00	30.24	197.52	H(1)	1.72	1.49	31.44
C(2)	27.15	20.66	203.68	H(1)	1.11	1.14	31.69
C(3)	75.83	77.78	166.92	H(2)	1.50	1.46	31.46
C(4)	34.30	26.49	199.93	H(2)	1.47	0.86	31.89
C(5)	31.14	39.19	191.76	H(3)	4.64	4.09	29.58
C(6)	34.32	24.94	200.93	H(4)	1.69	1.43	31.48
C(7)	73.05	75.00	168.71	H(4)	2.11	1.88	31.16
C(8)	38.00	36.81	193.29	H(5)	1.33	1.35	31.54
C(9)	34.71	33.44	195.46	H(6)	1.72	1.50	31.43
C(10)	34.50	40.60	190.85	H(6)	2.02	1.83	31.20
C(11)	25.91	22.36	202.59	H(7)	5.02	4.49	29.29
C(12)	77.22	79.62	165.74	H(8)	1.73	1.50	31.43
C(13)	45.11	52.36	183.28	H(9)	2.11	1.76	31.25
C(14)	42.70	45.82	187.49	H(11)	1.55	1.35	31.54
C(15)	24.87	28.98	198.33	H(11)	1.81	1.63	31.34
C(16)	30.67	33.83	195.21	H(12)	5.17	4.63	29.19
C(17)	47.18	49.29	185.26	H(14)	2.05	3.08	30.30
C(18)	11.92	10.39	210.29	H(15)	1.52	1.41	31.50
C(19)	22.86	16.39	206.43	H(15)	1.57	1.63	31.34
C(20)	34.71	38.86	191.97	H(16)	1.79	2.41	30.78
C(21)	17.46	11.26	209.73	H(16)	1.81	3.08	30.30
C(22)	30.88	27.56	199.24	H(17)	1.71	2.72	30.56
C(23)	30.88	29.92	197.72	H(18)	0.76	0.85	31.90
C(24)	174.40	170.54	107.23	H(19)	0.94	1.04	31.76
CH$_3$O	51.48	54.54	181.88	H(20)	1.40	1.24	31.62
3α-CO$_2$	166.70	165.25	110.63	H(21)	0.84	0.86	31.89
7α-CO$_2$	166.25	164.34	111.22	H(22)	1.72	1.95	31.11
12α-CO$_2$	166.51	164.79	110.93	H(22)	1.33	1.88	31.16
3α-CH$_2$	26.36	33.41	195.48	H(23)	2.20	2.63	30.62
7α-CH$_2$	26.19	31.06	196.99	H(23)	2.23	2.79	30.51
12α-CH$_2$	26.06	31.00	197.03	CH$_3$O	3.66	3.90	29.71
				3α-CH$_2$	3.79	3.15	30.25
				7α-CH$_2$	3.85	3.36	30.10
				12α-CH$_2$	3.89	3.37	30.09
a^a		337.1619				45.3617	
b^b		–1.5539				–1.3954	
r^{2c}		0.9895				0.8322	

[a] intercept; [b] slope; [c] correlation coefficient

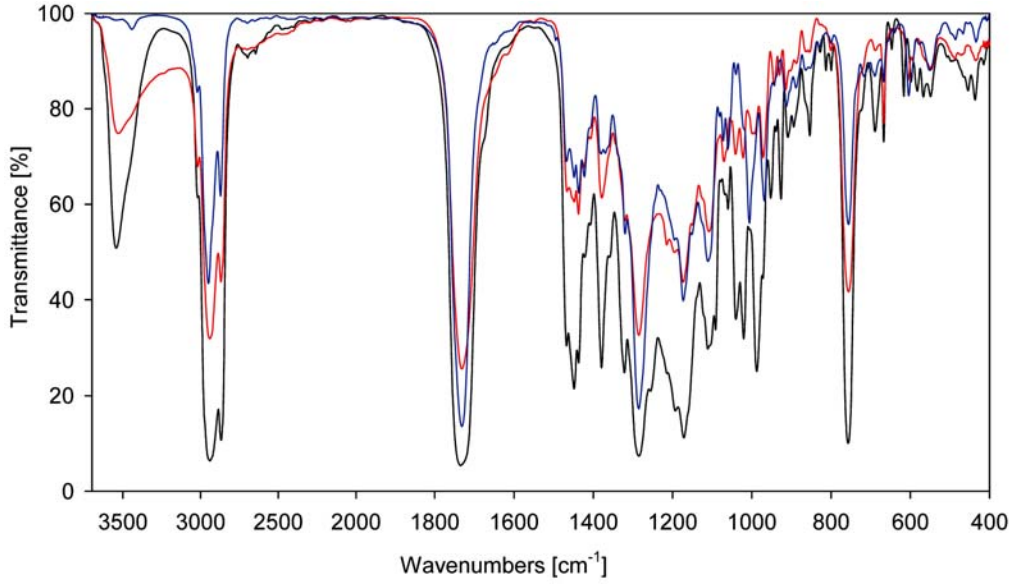


Figure 6. FT-IR spectra (film) of compounds **7** (black), **9** (red) and **10** (blue) in the 3600–400 cm^{-1} region.

cm^{-1} (**7**), 3541 cm^{-1} (**8**) and 3530 cm^{-1} (**9**) assigned to the stretching vibrations ν(OH) of the O(7)H, O(12)H or O(7)H and O(12)H. The FT-IR spectra of all compounds revealed two strong characteristic signals in the regions 1734–1730 cm^{-1} and 1287–1284 cm^{-1}, assigned to ν(C=O) and ν(C–O), respectively. On the other hand, the presence of chloroacetoxy groups in disubstituted derivatives of methyl ester of deoxycholic acid at position C(3) and C(12) or trisubstituted derivatives of methyl ester of cholic acid at position C(3), C(7) and C(12) slightly shift the carbonyl groups ν(C=O) to higher wavenumbers, 1737–1731 cm^{-1}, which is due to the inductive influence of chlorine.[33]

Furthermore, the comparison of carbonyl groups ν(C=O) bands for methyl and ethyl derivatives show that carbonyl band of ethyl esters are shifted by about 20 cm^{-1} to higher wavenumbers.[56]

3. 5. ESI-MS Spectra

The ESI-MS spectra were recorded in methanol. In Figure 7 we present the ESI-MS spectra of compounds **7** and **9**.

In all cases, the molecular ion is present as a $[M+Na]^+$. Synthesized compounds showed a higher affinity for sodium cation than potassium ion. In all spectra the molecular ion peak is 100% relative abundance, furthermore the elimination of $BrCH_2CO_2H$ is observed from which the fragmented ions $[M - BrCH_2CO_2H+Na]^+$ come. Because these ions are seen in all the discussed compounds, it can be concluded that the neutral molecule of $BrCH_2CO_2H$ is eliminated from the C(3) position of the steroid skeleton. Furthermore, we observed for compounds **4**–**7** in the ESI mass spectra ions $[M+K]^+$. These ions have a relative abundances of 30–35%, only for compound **7** it amounts to 55%. In this case, there was no elimination of the $BrCH_2CO_2H$ molecule. For compounds **4**, **5** and **7**–**9** ions $[M+Br]^-$ (100%) in the negative ion mode were observed. In the spectra of the compounds **7** and **9** in the negative ion mode the presence of ion $[M+Cl]^-$ was also observed. For 3α-bromoacetoxy-7α,12α-dihydroxy-5β-cholan-24-oate (**7**) the relative abundance of this ion was 100% (Figure 7).

4. Conclusions

Seven bromoacetyl substituted derivatives **4**–**10** of lithocholic, deoxycholic and cholic acid were obtained by the reaction of methyl esters of bile acids with bromoacetic acid bromide in toluene with TEBA and sodium hydride.

The structures of all synthesized compounds **4**–**10** were determined from their ^1H and ^{13}C NMR, 2D NMR (COSY, HSQC, HMBC), FT-IR as well as ESI-MS spectra. Moreover, PM5 calculations were performed on all compounds. Additionally, analyses of the biological prediction activity spectra for bromoacetyl substituted derivatives of bile acids prepared herein are examples of *in silico* studies of chemical compounds.

Linear correlations between the experimental ^1H and ^{13}C chemical shifts and the computed screening constants confirm the optimized geometry. Estimation of the pharmacotherapeutic potential has been accomplished for the synthesized compounds on the basis of Prediction of Activity Spectra for Substances (PASS). The obtained compounds may find applications as subtrates in organic synthesis.

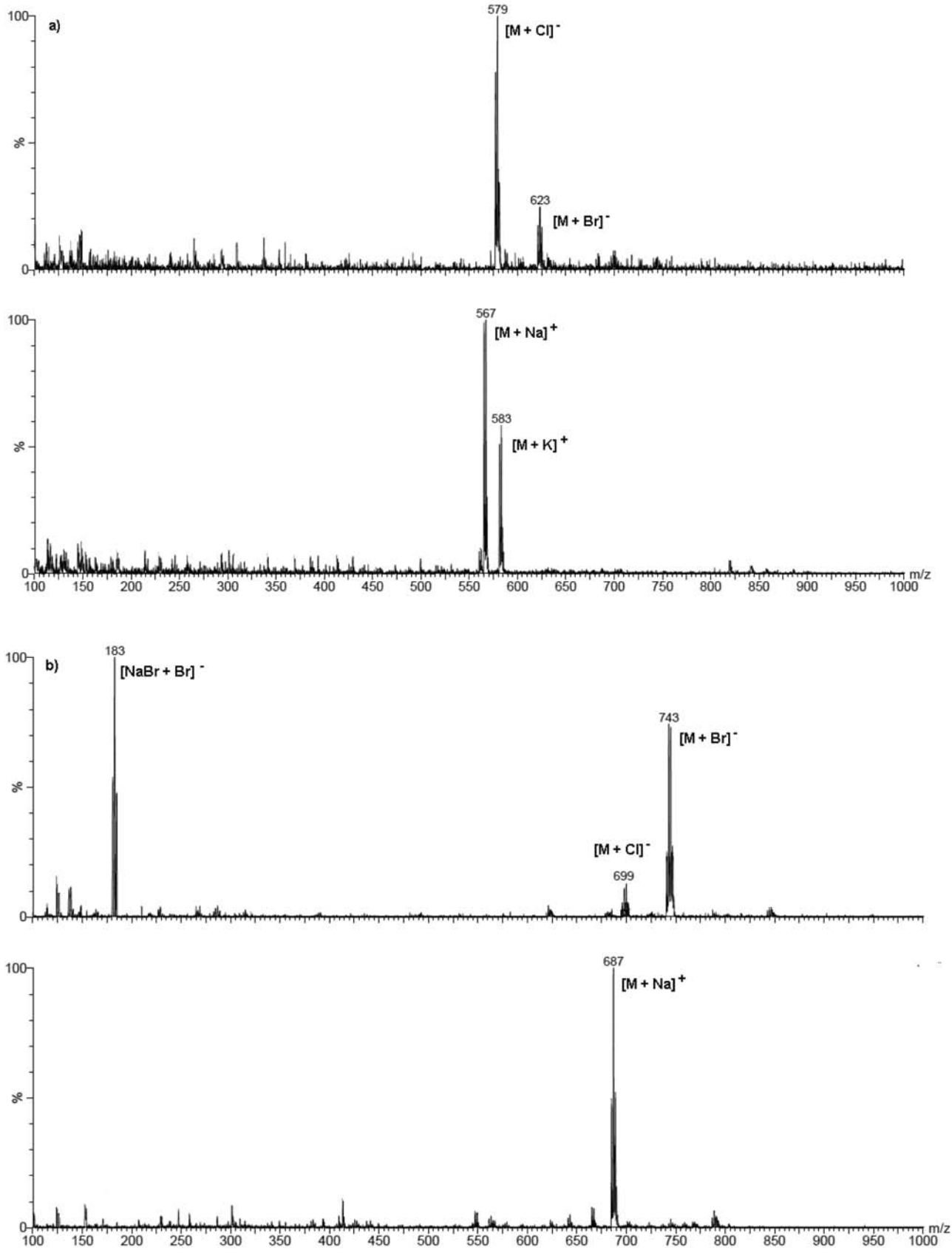


Figure 7. ESI-MS spectra in the negative and positive ion mode of compounds **7** (a) and **9** (b).

5. Acknowledgements

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6. References

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Povzetek

Z uporabo ^{1}H MMR, ^{13}C NMR, 2D NMR, FT-IR in masne spektrometrije (ESI-MS) smo določili strukturo sedmih bromoacetil substituiranih derivatov žolčnih kislin. Ob tem smo uporabili tudi PM5 semiempirične izračune in B3LYP *ab initio* metode. Oceno morebitnega farmakoterapevtskega potenciala sintetiziranih spojin smo izvedli s pomočjo programa »napovedovanje aktivnostnega spektra spojin« (*Prediction of Activity Spectra for Substances* oz. PASS).