World Journal of Research and Review (WJRR)
ISSN:2455-3956, Volume-3, Issue-4, October 2016 Pages 50-62

Synthesis, Toxicological, Anticoagulant Activity and Quantumchemical Study of Bis-coumarins and Other Coumarin Derivatives

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***Abstract*— Fifteen 4-hydroxycoumarin derivatives were synthesized. Seven of them are described for the first time. By means of X-ray crystal structure analysis of 4-hydroxy-3-[(2-oxo-2H-chromen-3-yl)-(2-ethoxycarbonylphen yl) methyl]-chromen-2-one (3) and 3-[2,6-dichlorophenyl) (ethoxy) methyl]-4- hydroxy- 2H-chromen-2-one (6) were confirmed the structure of these compounds previously by us. A comparative pharmacological study of the anticoagulant effect with respect to warfarin showed that the synthesized compounds have different anticoagulant activities. The QSTR model derived on the set of seventeen 4-hydroxycoumarin derivatives indicates that low i.p. toxicity could be achieved for a compound with large surface, unbranched molecule, with electronegative neighbour substituents and missing phydroxylgroup in the aldehyde fragment of the molecule.**

***Index Terms*—4-Hydroxycoumarin derivatives, Biscoumarins, Benzopyranobenzopyranes, Toxicity, Blood clotting time, QSTR model.**

I. INTRODUCTION

4-Hydroxycoumarin derivatives are of interest because of their anticoagulant [1-3], spasmolytic [4,5] and rodenticidal [6-9] effects. Some coumarin derivatives are known for their antifungal and anti-HIV activities too [10, 11]. They are also extensively used as analytical reagents [12-14]. The most widely used anticoagulant in the USA and Canada is the racemic warfarin sodium. The enzyme target of coumarins is vitamin K 2,3-epoxide reductase in the liver microsomes. Recently, an 18-kDa protein has been identified in the endoplasmic reticulum, the quantity of which increases in the presence of coumarin anticoagulants. This protein inhibits the activity of 2,3-epoxide reductase in a dose-dependent manner [15]. However, the drugs of this group exhibit some side effects including the warfarin-related purple toes syndrome. By synthesis of different arylidenebis4-hydroxycoumarins and benzopyranobenzopyranes, it is possible to obtain compounds with biological activity comparable to that of warfarin, but with lower toxicity and fewer side effects. In this paper we describe the synthesis of 4-hydroxycoumarin derivatives and studied their toxicity, anticoagulant activity and the quantitative structure - toxicity relationships (QSTR) after i.p. administration.

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II. RESULTS AND DISCUSSION

A. Chemistry

Different substituted aromatic aldehydes were condensed with 4-hydroxycoumarin in ethanol, glacial acetic acid at a molar ratio 1:2. The products were biscoumarins, benzopyranobenzopyrans and monocoumarin derivatives. The structure of these compounds was confirmed by mass spectral, IR and X-ray structure analyses. The process lasted for 16 h (synthesis of biscoumarin derivatives in ethanol) and the product was 4-hydroxy-3-[(2-oxo-2H-chromen-3-yl)-(4-carboxyphenyl)m ethyl]-chromen-2-one - (carboxylic group in the p-position in aromatic nucleus of the aldehyde) (MS: 456) (1). 4-Hydroxy-3-[(2-oxo-2H-chromen-3-yl)-(2-ethoxycarbonylp henyl) methyl]-chromen-2-one – the process lasted 144 h (ethoxycarbonyl group is in the oposition in aromatic nucleus of the aldehyde) (MS: 484) (3). 3-[2,6-Dichlorophenyl)(ethoxy)methyl]-4-hydroxy-2H-chro men-2-one – the process lasted 13 h (chlorine atoms are in the o,o' or 2,6-dichlorobenzaldehyde) (MS: 365) (6). The other 12 compounds were synthesized in glacial acetic accarboxyphenyl)methyl]-chromen-2-one – the process lasted 6 h (carboxylic group is in m-position in aromatic nucleus of the aldehyde (MS: 456) (2); 4-hydroxy-3-[(2-oxo-2H-chromen-3-yl)-(4-hydroxyphenyl) methyl]-chromen-2-one) – the process lasted 7 h (hydroxyl group is in the pposition in the aromatic nucleus of the aldehyde (MS: 428) (4). 4-Hydroxy-3-[(2-oxo-2H-chromen-3-yl)-(3-bromo-4-hydrox yphenyl)methyl]-chromen-2-one – the process lasted 9 h (atom of bromine and hydroxyl group are in m- and p-position in the aromatic nucleus of the aldehyde). (MS: 507) (5). 3-[6-Oxo-(6H,7H)-benzopyrano[4,3-b]benzopyran-8,10-dim ethoxy-7-yl]-4-hydroxy-2H-chromen-2-one – the process lasted 16 h (two methoxy groups are in o- and p-positions and hydroxyl group is in o-position in the aromatic nucleus of the aldehyde). (MS: 470) (7). 4-Hydroxy-3-[(2-oxo-2H-chromen-3-yl)-(3-methoxy-4-hydr oxyphenyl)-methyl]-chromen-2-one – the process lasted 11 h

(methoxy group is in m-position and hydroxyl group is in p-position in the aromatic nucleus of the aldehyde). (MS: 458) (8). 3-[6-Oxo-(6H,7H)-benzopyrano[4,3-b]benzopyran-8-bromo-10-benzoylox-7-yl]-4-hydroxy-2H-chromen-2-one – the process lasted 21 h (benzoyloxy group is in p-position, bromine atom - in o-position and hydroxyl group - in o-position in the aromatic nucleus of the aldehyde). (MS: 609) (9). 3-[6-Oxo-(6H,7H)-benzopyrano[4,3-b]benzopyran-8-bromo-11-acetoxy-7-yl]-4-hydroxy-2H-chromen-2-one – the process lasted 17 h (acetoxy group is in m-position, bromine atom - in o-position and hydroxyl group - in o-position in the aromatic nucleus of the aldehyde). (MS: 547) (10). 4-Hydroxy-3-[(2-oxo-2H-chromen-3-yl)-(2,3,6-tribromo-5-methoxy-4-hydroxyphenyl)-methyl]-chromen-2-one – the process lasted 21 h (three atoms of bromine are in o-, o'- and m-positions, methoxy- group - in m'-position and hydroxyl group - in p-position in the aromatic nucleus of the aldehyde). (MS: 695) (11). 3-[6-Oxo-(6H,7H)-benzopyrano[4,3-b]benzopyran-8-nitro-1 1-methoxy-7-yl]-4-hydroxy-2H-chromen-2-one – the process lasted 16 h (methoxy group is in m-position, nitro group - in o'-position and hydroxyl group - in o-position in the aromatic nucleus of the aldehyde). (MS: 485) (12). 3-[6-Oxo-(6H,7H)-benzopyrano[4,3-b]benzopyran-10-hydro xy-7-yl]-4-hydroxy-2H-chromen-2-one – the process lasted 13 h (two hydroxyl groups are in o- and p-positions in the aromatic nucleus of the aldehyde). (MS: 426) (13). 3-[6-Oxo-(6H,7H)-benzopyrano[4,3-b]benzopyran-9-bromo-7-yl]-4-hydroxy-2H-chromen-2-one – the process lasted 13 h (bromine atom is in m-position and hydroxyl group - in oposition in the aromatic nucleus of the aldehyde). (MS: 426) (14).
4-Hydroxy-3-[(2-oxo-2H-chromen-3-yl)-(2-benzensulfonyl-3-methoxy-6-nitrophenyl)methyl]-chromen-2-one – the process lasted 32 h (benzensulfonyl group is in o-position, methoxy group - in m-position and nitro group - in o'-position in the aromatic nucleus of the aldehyde). (MS: 643) (15). (Table I)

B. Pharmacology – acute toxicity and anticoagulant activity

Acute per oral and intra peritoneal toxicity as well as the in vivo effects of the compounds on blood clotting time were studied in mice. Warfarin was used as a reference compound. The studies were approved by the Institutional Animal Care Committee at the Faculty of Pharmacy, Medical University of Sofia, Bulgaria.
The most toxic compounds after intraperitoneal administration were **8**, **2**, **9**, **13**, **14**, **15**, **5**, **4**, **11** with LD_{50} values ranging between 200 and 330 mg kg-1. Compounds **12** and **1** were less toxic with LD_{50} values ranging between 715.2 and 622,7 mg.kg-^{1}.
After oral administration the most toxic compound was **4** with LD_{50} value of 250 mg.kg-^{1}.
Less toxic were **1** and **14** (> 2000 mg.kg-^{1}) and **7** and **12** (> 1500 mg.kg-^{1}). Compound **4** showed an index of absorption twice higher than that of warfarin. Compounds **6**, **12** and warfarin had a comparable index of absorption. All other compounds had an index of absorption lower than that of warfarin (Table II).

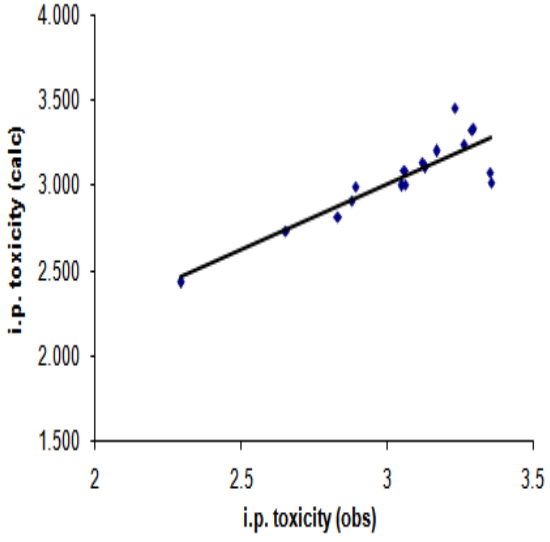
After oral administration of 1/10 of LD_{50} compounds **5, 6, 8** a statistically significantly increase blood clotting time compared to the controls was observed (Table III). These compounds were tested additionally for a dose-dependent effect after 4 days of oral administration using 1/20 of LD_{50} (Table IV). The other compounds did not affect significantly blood clotting time.

C. Quantitative structure - toxicity relationships (QSTR)

The i.p. toxicity was presented as $\log(1/LC_{50})$, where LC_{50} (mol.kg-^{1}) is the concentration causing death to 50 % of the tested population. The higher the $\log(1/LC_{50})$ value, the more toxic the compound is. The values of i.p. toxicity ranged from 2.29 to 3.41. The molecular structures of biscoumarines were described by 119 descriptors and indicator variables as described in the Experimental section. A selection procedure by genetic algorithm (GA) was applied to select the most predictive descriptors followed by a stepwise linear regression. Several models were derived and compared in terms of r2 and q2. The best performing one is given below:

log(1/*LC*)=0.203cOH0.014Surface+1.671xc3+0.249SaasC +3.951
n = 17, r2 =0.772, SEE = 0.154, F = 10.13, q2 = 0.504, p-value = 0.000802

No intercorrelation between the descriptors in the model was observed (R<0.7). The plot calculated i.p. toxicity versus observed i.p. toxicity is given in Fig. 1.
Figure 1
Calculated vs observed i.p. toxicity of the studied compounds



In Table V are given the descriptors of the 17 biscoumarins considered in the model and the outliers. The values of predicted and calculated toxicity ($\log(1/LC_{50})$ also are presented. The indicator variable **cOH** accounts for the presence of p- hydroxyl group in the aldehyde part of biscoumarines. It has a positive contribution to the toxicity, i.e. the substitution of p-hydroxyl group in the aldehyde part of biscoumarins increase the i.p. toxicity of the compound.

The descriptor **Surface** has a negative contribution to the i.p. toxicity. Hence, compounds with higher value for that descriptor would be less toxic. The values of Surface in the set vary between 269.4 and 430.5. The compound **2** has the lowest i.p.toxicity and one of the highest values of Surface (415.6). The topological state descriptor **xc3** is the third order cluster chi connectivity index. It is defined for a single branch point ("Y" type) and encodes the number and branching environments of such points. The main characteristic of the cluster type index is that all bonds are connected to the common central atom (star type structure). Its values vary between 1.56 for 2,6-dichlorobenzaldehyde(19) and 3.18 for 2-sulfophenyl-3-methoxy-6-nitrobenzaldehyde (22). Descriptor xc3 is an indicator of the degree of third order branching, and thus implicates the effect of substitution in a molecule. The positive coefficient of xc3 in the QSTR regression model means that increasing the branching of the molecule contributes to high i.p. toxicity.

The electrotopological state descriptor **SaasC** is the atom type state index for aromatic carbon with one sigma bond. It is calculated as the sum of the E-state values for all aromatic carbons with one sigma bond that are present in the molecule and reflects the electron accessibility of the substituted carbon atom. The values of SaacS in the training set vary between -0.138 for 3,4-dihydroxybenzaldehyde (7) and 2.82 for 2-hydroxy-4,6-dimethoxybenzaldehyde (8). The value of SaasC decreases as the electron accessibility of the substituted carbon atom decreases. The positive coefficient of SaasC in the QSTR regression model means that the lower is the value of the descriptor, the lower is i.p toxicity. Negative value of SaasC could be achieved for biscoumarines substituted at neighboring positions with strong electronegative substituents, thus limiting the electron accessibility of the substituted carbon atom as is the case of compound 7.

The application of the QSTR regression model for calculation of i.p. toxicity of the 5 outliers in the initial set of 22 biscoumarines showed good prediction ability for two of the outliers – co ability for two of the outliers – compounds 18 and 19.

III. Experimental Procedures

A. *Chemistry*

Melting points were measured on Boetius hot plate microscope (Germany) and are uncorrected. IR spectra (nujol) were recorded on an IR-spectrometer FTIR-8101M Shimadzu.

^{1}NMR spectra were recorded at ambient temperature on a Bruker 250 WM (250 MHz) spectrometer in [d_6]-acetone, $CDCl_3$. Chemical shifts are given in ppm (δ) relative to TMS used as an internal standard. Mass spectra were recorded on a Jeol JMS D 300 double focusing mass spectrometer coupled to a JMA 2000 data system. The compounds were introduced by direct inlet probe, heated from 50 ^oC to 400 ^oC at a rate of 100 ^oC min^{-1}. The ionization current was 300 mA, the accelerating voltage 3 kV and the chamber temperature 150 ^oC. TLC was performed on precoated plates Kieselgel 60 F254 (Merck, Germany) with layer thickness of 0.25 mm and UV detection (254 nm). Yields of TLC-homogeneous isolated products are presented. Results of elemental analyses were within ± 0.4 % of the theoretical values.

B. *General Procedure*

4-Hydroxycoumarin and the respective aromatic aldehyde at a molar ratio 2:1 in glacial acetic acid were mixed under stirring and heated at reflux until the appearance of an insoluble product. After cooling the product was filtered and was recrystallized. The following 8 3,3'-arylidenebis-(4-hydroxy-2H-1-benzopyran-2-ones), 6 benzopyrano-coumarins and a 2,6-dichlorophenyl-ethoxymethyl-4-hydroxycoumarin derivatives were synthesized according to this procedure.

(1.) 4-Hydroxy-3-[(2-oxo-2H-chromen-3-yl)-(4-carboxyphenyl)methyl]-chromen-2-one)

4-Hydroxycoumarin, 4-formylbenzoic acid, 16 h, ethanol, 72%, 280-282 °C.
[10, 16, 17]

(2.) 4-Hydroxy-3-[(2-oxo-2H-chromen-3-yl)-(3-carboxyphenyl)methyl]-chromen-2-one)

4-Hydroxycoumarin (3.24 g, 0.02 mol) and 3-formylbenzoic acid (1.50 g, 0.01 mol) in gl. acetic acid were refluxed and stirred for 6 h until white crystals appeared. After cooling the crude product was filteret off and recrystallized from ethanol. Yield 3.06 g (67 %),m. p. 228-231 ^oC (lit. m. p. 228-230 ^oC). Anal. Calc. for $C_{26}H_{16}O_8$, (456): C = 68.42; H = 3.51 %. Found C = 68.61, H = 3.39 %**.** IR (KBr) 3229, 1770, 1682, 1633, 1572, 1280, 754. 1H-NMR (DMSO-d6): 4.6-5.4 (s, 3H), 6.9-7.1 (s, 1H), 7.2-8.2 (m, 12H). FABMS m/z 456 (MH+).

(3) 4-Hydroxy-3-[(2-oxo-2H-chromen-3-yl)-(2-ethoxycarbonylphenyl)methyl]-chromen-2-one

4-Hydroxycoumarin, 2-formylbenzoic acid, 144 h, ethanol (6 h, gl. acetic acid), recrystallized from acetonitril, 63 %, 228-231 ^oC [17, 18].

(4.) 4-Hydroxy-3-[(2-oxo-2H-chromen-3-yl)-(4-hydroxyphenyl)methyl]-chromen-2-one)

4-Hydroxycoumarin, 4-hydroxybenzaldehide, 7 h, acetic acid, recrystallized from ethanol, 69 %, 212-214 ^oC [19 - 29].

(5.)4-Hydroxy-3-[(2-oxo-2H-chromen-3-yl)-(3-bromo-4-hydroxyphenyl)methyl]-chromen-2-one)

4-Hydroxycoumarin (3.24 g, 0.02 mol) and 3-bromo-4-hydroxybenzaldehide (2.01 g, 0.01 mol) were dissolved in gl. acetic acid. The solution was heated under reflux for 9 h until an insoluble solid appeared. After cooling, the crude product was filtered off and recrystallized from ethanol. Yield 4.05 g (80 %), m.p. 182.3-184 ^oC. Anal. calc. for $C_{25}H_{15}BrO_7$ (507): C 59.17; H 2.96; Br 15.78 %. Found: C 58.83; H 3.13; Br 15.45 %. IR (KBr): 2982, 2831, 2667,

2601, 2317, 2162, 1980, 1734, 1610, 1590, 1567, 1501, 1482, 1433, 1380, 1288, 1240, 1224, 1175, 1042, 978, 930, 881, 773, 738.

(6.)
3-[2,6-Dichlorophenyl)(ethoxy)methyl]-4-hydroxy-2H-chromen-2-one

4-Hydroxycoumarin, 2,6-dichlorobenzaldehyde, 13 h, ethanol, 78 %, 132-133 ^{o}C [30].

(7.)
3-[6-oxo-(6H,7H)-benzopyrano[4,3-b]benzopyran-8,10-dimethoxy-7-yl]-4-hydroxy-2H-chromen-2-one

4-Hydroxycoumarin,4,6-dimethoxy-2-hydroxybenzaldehyde, 16 h, gl. acetic acid, 62 %, 245.2-247 ^{o}C [31, 32].

(8.)
4-Hydroxy-3-[(2-oxo-2H-chromen-3-yl)-(3-methoxy-4-hydroxyphenyl)-methyl]-chromen-2-one)

4-Hydroxycoumarin,3-methoxy-4-hydroxybenzaldehyde 11 h, gl. acetic acid (recrystallized from ethanol), 70 %, 215-216.4 ^{o}C [33 - 36].

(9.)
3-[6-oxo-(6H,7H)-benzopyrano[4,3-b]benzopyran-8-bromo-10-benzoylox-7-yl]-4-hydroxy-2H-chromen-2-one

4-Hydroxycoumarin (3.24 g, 0.02 mol) and 4-benzoyloxy-6-bromo-2-hydroxybenzaldrhyde (3.21 g, 0.01 mol) were dissolved in gl. acetic acid. The solution was heated under reflux for 21 h until an insoluble solid appeared. After cooling, the crude product was filtered off and recrystallized from ethanol. Yield: 4.5 g (74 %), m. p. 252-254.3 ^{o}C. Anal. calc. for $C_{32}H_{17}BrO_8$ (609): C 63.05, H 2.79, Br 13.14 %. Found: C 63.34, H 3.01, Br 13.48 %. IR (KBr) cm^{-1}: 3062, 2931, 2560, 1695, 1626, 1600, 1564, 1511, 1452, 1394, 1302, 1240, 1196, 1082, 1063, 1031, 948, 899, 831, 745, 699, 644.

(10.)
3-[6-oxo-(6H,7H)-benzopyrano[4,3-b]benzopyran-8-bromo-11-acetoxy-7-yl]-4-hydroxy-2H-chromen-2-one

4-Hydroxycoumarin (3.24 g, 0.02 mol) and 3-acetoxy-6-bromo-2- hydroxybenzaldehyde (2.59 g, 0.01 mol) were dissolved in gl. acetic acid. The solution washeated under reflux for 17 h until an insoluble solid appeared. After cooling, the crude product was filtered off and recrystallized from ethanol. Yield: 4.2 g (77 %), m. p. 218-220.5 ^{o}C. Anal. calc. for $C_{27}H_{15}BrO_8$ (547): C 59.23, H 2.74, Br 14.62. Found: C 59.48, H 2.93, Br 14.29 %. IR (KBr) cm^{-1}: 1771, 1705, 1647, 1609, 1566, 1481, 1463, 1396, 1313, 1202, 1173, 1103, 1067, 948, 870, 790, 750, 700, 691,673.

(11.)
4-Hydroxy-3-[(2-oxo-2H-chromen-3-yl)-(2,3,6-tribromo-5-methoxy-4- hydroxyphenyl)-methyl]-chromen-2-one)

4-Hydroxycoumarin (3.24 g, 0.02 mol) and 2,3,6-tribromo-5-methoxy-4-hydroxybenzaldehyde (3.89 g, 0.01 mol) were dissolved in gl. acetic acid. The solution was heated under reflux for 21 h until an insoluble solid appeared. After cooling, the crude product was filtered off and recrystallized from ethanol. Yield: 4.12 g (59.3 %), m. p. 231-233 ^{o}C. Anal. calc. for $C_{26}H_{15}Br_3O_8$ (695): C 44.89, H 2.16, Br 34.53 %. Found: C 45.18, H 2.43, Br 34.17 %. IR (KBr) cm^{-1}: 3421, 2941, 1652, 1620, 1604, 1567, 1503, 1448, 1358, 1280, 1187, 1168, 1141, 1098, 910, 867, 759, 673, 648, 609.

(12.)
3-[6-oxo-(6H,7H)-benzopyrano[4,3-b]benzopyran-8-nitro-11-methoxy-7-yl]-4-hydroxy-2H-chromen-2-one

4-Hydroxycoumarin (3.24 g, 0.02 mol) and 3-methoxy-6-nitro-2-hydroxybenzaldehide (1.97 g, 0.01 mol) were dissolved in gl. acetic acid. The solution was heated under reflux for 16 h until an insoluble solid appeared. After cooling, the crude product was filtered off and recrystallized from ethanol. Yield: 3.76 g (77.5 %), m.p. 217-219 oC. Anal. calc. For $C_{26}H_{15}NO_9$ (485): C 64.33, H 3.09, N 2.89 %. Found: C 64.09, H 3.32, N 2.78 %. IR (KBr) cm-^{1}: 2945, 2707, 2568, 1695, 1662, 1608, 1541, 1508, 1462, 1308, 1276, 1242, 1195, 1101, 1072, 948, 832, 746, 614.

(13.)
3-[6-oxo-(6H,7H)-benzopyrano[4,3-b]benzopyran-10-hydroxy-7-yl]-4-hydroxy-2H-chromen-2-one

4-Hydroxycoumarin, 2,4-dihydroxybenzaldehyde, 13 h, gl. acetic acid (recrystallized from ethanol), 73 %, 267-269.4 ^{o}C [20, 21].

(14.)
3-[6-oxo-(6H,7H)-benzopyrano[4,3-b]benzopyran-9-bromo-7-yl]-4-hydroxy-2H-chromen-2-one

4-Hydroxycoumarin, 5-bromo-2-hydroxybenzaldehyde, 13 h, gl. acetic acid (recrystallized from ethanol), 73 %, 307-310 ^{o}C (decompn) [31, 37-39].

(15.)
4-Hydroxy-3-[(2-oxo-2H-chromen-3-yl)-(2-benzensulfonyl-3-methoxy-6- nitrophenyl)methyl]-chromen-2-one)

4-Hydroxycoumarin (3.24 g, 0.02 mol) and 2-benzensulfonyl-3-methoxy-6-nitrobenzaldehyde (3.37 g, 0.01 mol) were dissolved in gl. acetic acid. The solution was heated under reflux for 32 h until an insoluble solid appeared. After cooling, the crude product was filtered off and recrystallized from ethanol. Yield: 5.16 g (80 %), m. p.152.5-154.5 ^{o}C. Anal. calc. for $C_{32}H_{21}NO_{12}S$ (643): C 59.72, H 3.27, N 2.18, S 4.98 %. Found: C 59.48, H 3.42, N 2.33, S 5.17 %. IR (KBr) cm^{-1}: 2945, 1709, 1647, 1578, 1445, 1371, 1287, 1223, 1193, 1088, 1065, 1035, 993, 952, 898, 821, 765, 736, 660, 623, 607.

C. Pharmacology

Animals: Male albino mice line H, 20-25 g b.w. were used for acute intra peritoneal and oral toxicity and anticoagulation in vivo studies. The animals were housed according GLP instruction of animal care, water and food

being supplied temperature 22°C; humidity 30 %; lighting schedule 12 h light/dark cycle. Prior to administration the animals fasted for one day. The compounds were suspended in water using Tween 80 and saline. Acute per oral and intra peritoneal toxicity studies were performed according to OECD Guideline 425 "Up and Down procedure" (FDA, 2001) [40]. The index of absorption (IA) in % was calculated (100 x i.p. LD50/p.o. LD50) using the data from acute toxicity studies. The compounds were administered for 4 days at a dose equivalent to 1/10 – 1/20 of LD50 and blood clotting time was assessed 24 h thereafter according to the method of Morawitz [41, 42].

IV. QSTR STUDIES

Experimental Dataset

The set of 22 biscoumarines was used to derive a model for prediction of i.p. toxicity. Thestructures of the biscoumarines were built in HyperChem 7.5 (Hypercube Ltd.) software. Their geometries were optimized by MM+ force field.

Molecular descriptors

The chemical structure of the studied compounds was described by 99 molecular descriptors computed using the software package MDL QSAR version 2.2 (MDL Information Systems, Inc., San Leandro, USA), as well as 26 indicator variables describing the presence of specific substitutes in aldehyde part of biscoumarines. The molecular descriptors were grouped into five types: molecular connectivity χ (chi) indices [43] which represent molecular structure by encoding significant topological features of whole molecule; k shape indices - a family of graph-based structure descriptors that represent shape [44] ; electrotopological state (E- state) indices, which represent the electron density at each atom and their ability to participate in intermolecular interactions [45]; molecular properties - weight, log P, log D, number of rings, number of hydrogen bond donors and acceptors, etc.; and 3D molecular properties such as polarizability, surface area, volume, etc.

Variable selection

A genetic algorithm (GA) [46] and stepwise regression, as implemented in the MDL QSARpackage, were used as variable selection procedures. GA allows one to select a subset of the most significant predictors using two evolutionary operations: random mutation and genetic recombination (crossover). The parameters of the GA used in this study were initial population with size of 32, uniform crossover and one-point mutation. Adjusted R squared scoring function was used as a fitness function. The GA regression equations were generated on the basis of the selected variables by ordinary multiple linear regression (MLR). The stepwise regression was used in a forward mode with default value for F-to-enter (4.00) and F-to-remove (3.99). The final descriptor set was checked for intercorrelation.

Dataset optimization

PCA (Principle Analysis of Components) as implemented in MDL-QSAR package was applied on the matrix of descriptors in order to identify outliers in the dataset. The outliers were identified using the score plot and the Hotelling's T2 statistics with significance level 0.1. The components which are outside the Hotelling's tolerance ellipse are regarded asoutliers. After removing of 5 outliers, a set of 17 biscoumarines and 119 descriptors was formed to create a regression model for i.p. toxicity prediction.

Models assessment

Final models were assessed by explained variance (r2), standard error of estimate (SEE), and
cross-validated r2 (q2) according to the following equations:

$$r^2 = 1 - \frac{\sum_{i=1}^{n} (\log(1/LD_{50})_{obs,i} - \log(1/LD_{50})_{calc,i}}{\sum_{i=1}^{n} (\log(1/LD_{50})_{obs,i} - \log(1/LD_{50})_{obs,mean}},$$

$$SEE = \sqrt{\frac{\sum_{i=1}^{n} (\log(1/LD_{50})_{obs,i} - \log(1/LD_{50})_{calc,i}}{n-d-1}},$$

$$q^2 = 1 - \frac{\sum_{i=1}^{n} (\log(1/LD_{50})_{obs,i} - \log(1/LD_{50})_{pred,i}}{\sum_{i=1}^{n} (\log(1/LD_{50})_{obs,i} - \log(1/LD_{50})_{obs,mean}},$$

where $\log(1/LC_{50})$obs,i is the observed $\log(1/LC_{50})$ of the i -th compound, $\log(1/LC_{50})$calc,i is the calculated by the model $\log(1/LC_{50})$, $\log(1/LC_{50})$pred,i is the predicted by the model $\log(1/LC_{50})$, *n* - the number of compounds in the dataset, *d* - the number of molecular descriptors in the model. Fisher statistics (F) for models also was calculated. The models were validated by leave-one-out cross-validation (LOO-CV), i.e. one compound is excluded from the training subset, the model is derived based on the remaining *n-1* compounds and used to predict the $\log(1/LC_{50})$pred,i of the excluded *i* -th compound.

V. CONCLUSIONS

Our studies indicate that some of the original compounds (1 and 12) are less toxic than the standard warfarin. The blood clotting time was prolonged only by compounds 5, 6, 8 in a dose dependent manner but less than the standard warfarin.
The QSTR model derived on the set of seventeen 4-hydroxycoumarin derivatives indicates that low i.p. toxicity could be attained for a compound with a large surface, unbranched molecule, with an electronegative neighbour substituents and missing p-hydroxyl group in the aldehyde fragment of the molecule.

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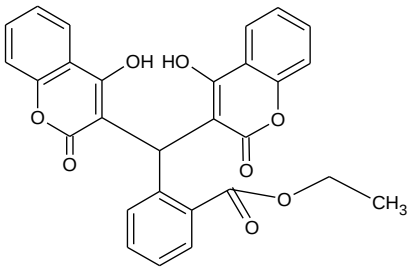
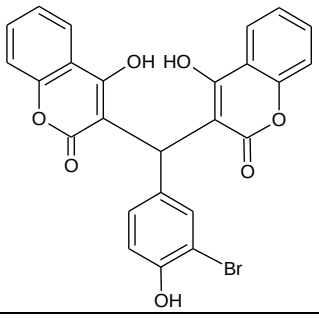
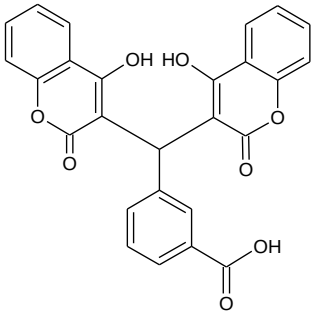
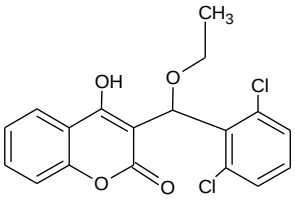
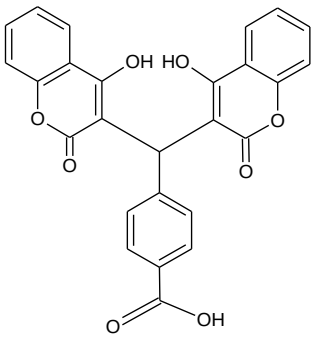
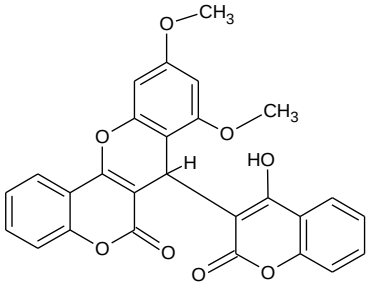
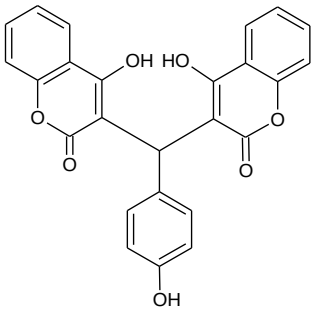
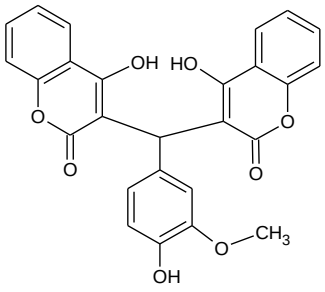
APPENDIX
Table I Synthesized compounds

No	Compound	No	Compound
1.		5.	
2.		6.	
3.		7.	
4.		8.	

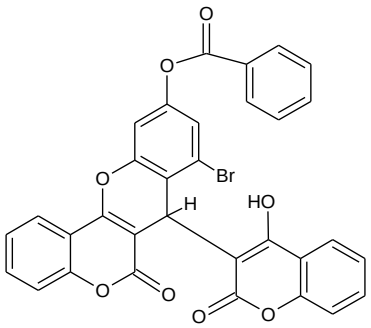
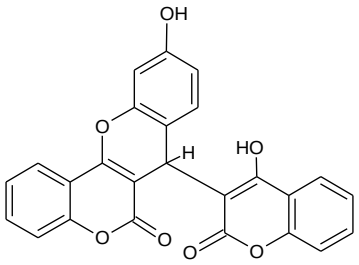
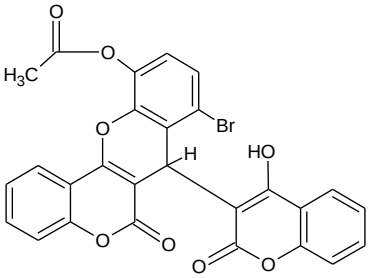
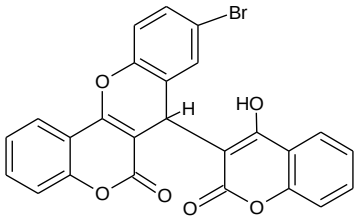
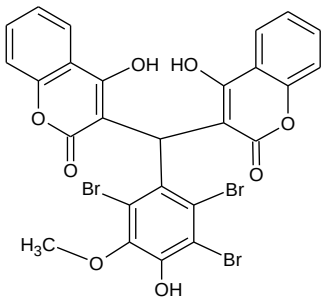
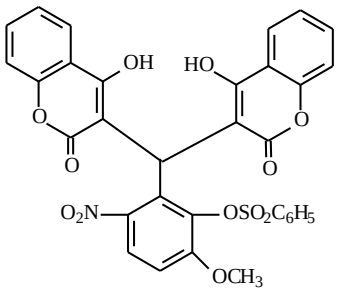
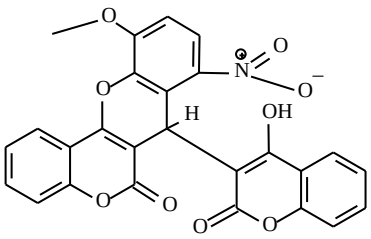
9.		13.	
10.		14.	
11.		15.	
12.			

Table II Acute toxicity (LD_{50} in mg kg^{-1}) in male mice after intra peritoneal (i.p.) and per oral (p.o.) administration and index of absorption (IA) in %.

Compound	i.p. LD_{50} (mg kg^{-1})	p.o. LD_{50} (mg kg^{-1})	IA (%)
1	622.7 (501.2 -:- 743.4)	> 2000	< 31.1
2	230.8 (184.8 -:- 277.4)	≈ 1000 (900 -:- 1100)	≈ 23.1
3	408.4 (320.1 -:- 492.3)	> 1000	< 40.8
4	289.6 (231.6 -:- 334.9)	≈ 250 (200 -:- 800)	100
5	276.6 (222.4 -:- 330.1)	> 1000	< 27.7
6	404.1 (322.1 -:- 486.3)	750 (600 -:- 900)	≈ 53.9
7	407.5 (327.5 -:- 487.5)	> 1500	< 27.2
8	202.7 (162.7 -:- 243.4)	≈ 750	≈ 27.0
9	248.5 (198.8 -:- 298.2)	> 1000	< 24.9
10	407.5 (325.5 -:- 481.0)	> 1000	< 40.8
11	323.4 (258.2 -:- 388.1)	> 1000	< 32.3
12	715.2 (572.2 -:- 858.2)	> 1500	< 47.7
13	250.0 (200.0 -:- 300.0)	> 1000	< 25.0
14	≈ 250.0	> 2000	< 12.5
15	≈ 250.0	> 1000	< 25.0
Warfarin	363.7 (288.5-:-442.4)	762(612.1-:-901.3)	47

LD_{50} values are expressed as mean and confidence interval given in parenthesis. The index of absorption (IA) was calculated as the ratio of LD_{50} i.p. to LD_{50} p.o. x 100.

Table III. The effects of compounds on blood clotting time after p.o. administration of 1/10 of LD_{50}

Compound	Dose (mg/kg^{-1}b.w.)	Clotting time (s)	Clotting time (%)	Statistically significant differences
Control group	_	86	100	
1	200	87	101.2	
2	100	108	125.6	
3	100	73	84.9	
4	25	89	103.5	
5	100	154	179	p<0,05
6	75	164	190.7	p<0,05
7	150	107	124,4	
8	75	181	210,5	p<0,05
Control group	_	167	100	
9	100	189	113.2	
10	100	103	61.7	
11	100	153	91,6	
12	150	154	92,2	
13	100	72	43,1	
14	200	52	31,1	
15	100	85	50,9	
Control group		172.38	100	
Warfarin	76	457,0	265.5	p<0,05

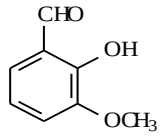
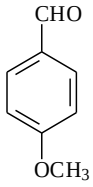
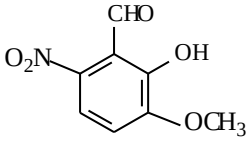
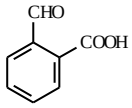
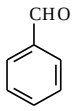
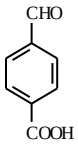
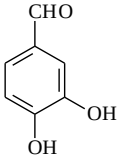
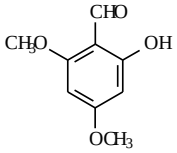
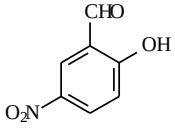
* - Statistically significant differences p<0,05 in comparison with control group

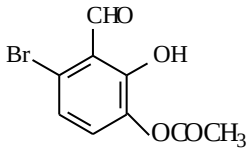
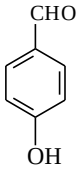
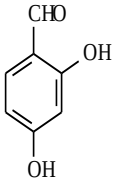
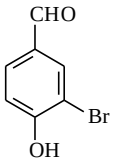
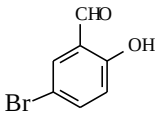
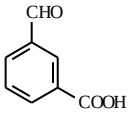
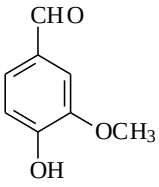
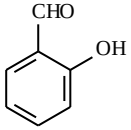
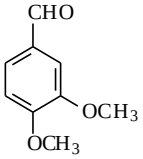
Table IV. The effects of compounds (5, 6, 8) on blood clotting time after p.o. administration of 1/20 part of LD_{50}

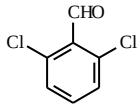
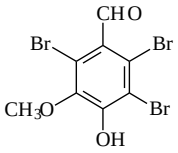
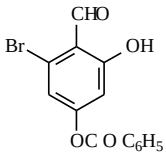
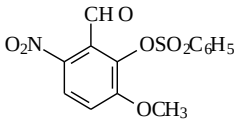
Compound	Dose (mg/kg^{-1}) b.w.	Clotting time (s)	Clotting time (%)	Statistically significant differences
Control group	-	157.5	100	
5	50	170	107,9	
Control group		173	100	
6	37,5	168	97,1	
8	37,5	116	67	
Control group		172,38	100	
Warfarin	73,5	656,7	380,9	$p<0,05$

* - Statistically significant differences $p<0,05$ in comparison with control group

Table V. Structure, observed and calculated toxicity (log(1/LC_{50})) and molecular descriptors of the studied compounds

№	Molecules	Log(1/LC_{50})	Log(1/LC_{50})(calc)	Residual	xc3	SaasC	Surface	cOH
1	2-hydroxy-3-methoxybenzaldehyde 	2.292	2.434	-0.142	2.178	2.737	415.602	0
2	4-methoxybenzaldehyde 	2.653	2.736	-0.083	2.166	2.012	379.841	0
3	2-hydroxy-3-methoxy-6-nitrobenzaldehyde 	2.832	2.818	0.014	2.622	0.386	399.404	0
4	2-formylbenzoic_acid 	2.878	2.915	-0.037	2.303	0.896	363.643	0
5	Benzaldehyde 	2.892	2.995	-0.103	1.962	1.592	329.697	0
6	4-formylbenzoic_acid 	3.048	3.001	0.047	2.462	0.989	378.053	0
7	3,4-dihydroxybenzaldehyde 	3.059	3.089	-0.030	2.433	-0.138	362.856	1
8	2-hydroxy-4,6-dimethoxybenzaldehyde 	3.062	3.012	0.050	2.387	2.821	400.843	0
9	2-hydroxy-5-nitrobenzaldehyde 	3.119	3.140	-0.021	2.542	1.229	381.865	0

10	4-bromo-3-formyl-2-hydroxyphenylacetate 	3.128	3.110	0.018	2.797	2.139	430.515	0
11	4-hydroxybenzaldehyde 	3.17	3.203	-0.033	2.250	1.234	357.347	1
12	2,4-dihydroxybenzaldehyde 	3.232	3.456	-0.224	2.330	2.010	362.525	1
13	3-bromo-4-hydroxybenzaldehyde 	3.263	3.248	0.015	2.433	1.421	379.146	1
14	5-bromo-2-hydroxybenzaldehyde 	3.292	3.325	-0.033	2.330	3.308	380.378	0
15	3-formylbenzoic acid 	3.296	3.339	-0.043	2.462	0.720	349.214	0
16	4-hydroxy-3-methoxybenzaldehyde 	3.354	3.082	0.272	2.364	0.975	374.846	1
17	2-hydroxybenzaldehyde 	3.356	3.024	0.332	2.042	2.672	356.242	0
18	3,4-dimethoxybenzaldehyde 	2.593	2.664	0.071	2.812	1.048	419.051	0

19	2,6-dichlorobenzaldehyde 	2.956	2.865	-0.091	1.561	1.811	269.406	0
20	2,3,6-tribromo-4-hydroxy-5-methoxybenzaldehyde 	3.332	2.867	-0.465	2.812	1.048	419.051	1
21	3-bromo-4-formyl-5-hydroxyphenylbenzoate 	3.39	4.028	0.638	2.859	2.671	356.242	0
22	3-Methoxy-6-nitro-2-(phenylsulfonyloxy)benzaldehyde 	3.411	1.297	-2.114	3.171	-2.135	502.603	0