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Antiproliferative Activity of β-Hydroxy-β-Arylalkanoic Acids

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Abstract: Article describes the synthesis of fifteen β-hydroxy-β-arylalkanoic acids by Reformatsky reaction using the 1-ethoxyethyl-2-bromoalkanoates, aromatic or cycloalkyl ketones or aromatic aldehydes. The short survey of previously reported synthetic procedures for title compounds, is given. The majority of obtained compounds exert antiproliferative activity *in vitro* toward human: HeLa, Fem-X cells, K562, and LS174 cells, having IC_{50} values from 62.20 to 205 μM. The most active compound is 3-OH-2,2-di-Me-3-(4-biphenylyl)-butanoic acid, having the IC_{50} value 62.20 μM toward HeLa cells. Seven examined compounds did not affect proliferation of healthy human blood peripheral mononuclear cells (PBMC and PBMC+ PHA), IC_{50} > 300 μM. The preliminary QSAR results show that estimated lipophilicity of compounds influences their antiproliferative activity in the first place. The ability of dehydration, and the spatial arrangement of hydrophobic portion, HBD and HBA in molecules are has almost equal importance as lipophilicity.

Keywords: β-hydroxy-β-arylalkanoic acids, Reformatsky reaction, antiproliferative activity, human dedifferentiate cells, QSAR

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1. Introduction

Cyclooxygenases (COX) or prostaglandin endoperoxide synthases (PGHS) are the key enzymes in the synthesis of prostaglandins from their precursor, arachidonic acid.

Arachidonic acid is cleaved from cell membrane phospholipids by phospholipase A2. The COX-1 or COX-2 converts arachidonic acid in unstable endoperoxides PGG2 and PGH2 that are subsequently metabolized by synthases to primary prostaglandins PGD2, PGE2, PGF2a, TXA2 (Tromboxane A2) and PGI2 (Prostacycline). Prostaglandins are the lipid mediators made by most cells in the body, except for red blood cells, and they are released by almost any type of chemical or mechanical stimulus [1]. The two definitely known isoforms, COX-1 and COX-2, show distinct expressions patterns and distinct biological activities.

The COX-1 is built in many different cells to create prostaglandins used for basic "housekeeping" messages throughout the body. COX-1 is a constitutively expressed protein that is responsible for the physiological production of prostaglandins [2]. The COX-2 is built only in special cells and is used for signaling pain and inflammation. This isoform is also called inducible isoform of enzime COX [3]. In inflammatory process COX-2 is overexpressed. The COX-1 variant protein, named COX-3, is sensitive to inhibition with paracetamol.

Main therapeutic actions of NSAID-s (nonsteroidal anti-inflammatory drugs) are analgesic, antipyretic and anti-inflammatory. Recently was reported that some of NSAIDs (Diclofenac, Naproxen, Etodolac, …) were investigated as antiproliferative agents [4].

We have synthesized fifteen β-hydroxy-β-arylalkanoic acids that are structurally similar to COX inhibitors: *p*-isobutylphenylacetic acid (Ibufenak) [5], 4-biphenylacetic acid (Felbinac) [6], α-(4-isobutylphenyl)propanoic acid (Brufen) [7], α-(6-methoxy-2-naphtyl)propanoic acid (Naproxen) [8]. Introducing the oxo-functionality in position four of 4-biphenylylbutanoic acid increases their anti-inflammatory activity (Fenbufen) [9]. Our compounds belong to the class of aryl- and cycloalkylpropanoic acids, structurally similar with commercially available NSAID-s. Their anti-proliferative activity toward malignant cell lines was evaluated in this work. With the aim to determine the undesirable cytotoxic effect of investigated compounds on immune competent cells the normal peripheral blood mononuclear cells were used as target cells, too.

There are several pathways in direct synthesis of β-hydroxy acids. The careful control of the conditions used for the hydrolysis can suppress the dehydration, and good yield of some hydroxy acids have been reported [10]. In accordance with the literature, β-hydroxy acids could be produced through Reformatsky reaction by using *tert*-butyl [10], trimethylsilyl [11], or tetrahydropyranyl [12] esters as starting materials, which provide mild acidic conditions. This approach is commonly known as modified Reformatsky reaction for direct synthesis of β-hydroxy acids. Another method for direct synthesis β-hydroxy acids can be used when α-bromo acid is not accessible, and utilizes the dianion of carboxylic acids obtained by the reaction between acids and extremely strong bases as Li-diisopropylamide, instead of Reformatsky reagent [13]. In this reaction carboxylic proton and α-hydrogen are both substituted by lithium. The class of substituted α-phenyl-β-hydroxypropanoic acids was obtained in this way.

It has been shown that the carboxyl group of α-bromo acids can be protected by allyl-zincbromide. The obtained bromozinc salt undergoes the Reformatsky reaction [14]. This method is applicable to α-

bromo acids having a bromine atom on secondary or tertiary carbon. In case of bromoacetic acid this method is ineffective.

A method for direct preparation of β-hydroxy acids using corresponding halomagnesium salts of α-bromo acids instead of α-bromo esters, was described [15]. Ethyl-vinyl esters can be used instead of 2-tetrahydropyranyl esters as intermediates in Reformatsky reaction [16,17].

Readily accessible α-bromo ester-acetals, obtained by the action of aliphatic vinyl ethers on α-bromo acids, can be used in the Reformatsky reaction for direct preparation of β-hydroxy acids [16,17]. The authors who reported these syntheses did not report full physicochemical characterization of obtained compounds.

In our work we have synthesized these compounds using previously described methods [16,17]. All compounds were characterized by their melting points, MS, IR, ^{1}H, and ^{13}C NMR spectra, and by elemental analysis, as well.

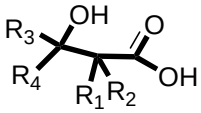
2. Results and Discussion

2.1. Antiproliferative activity

In our previous communications the antiproliferative activity of β-hydroxy-β-arylalkanoic acids was reported [20-21]. Antiproliferative activity of studied compounds (**1-15)** was assessed using Kenacid Blue R dye binding method, as described in literature [19]. Concentrations of examined compounds (**1-15)** that induced the 50% decrease of survival (S) of HeLa, Fem-X, K562, and LS174 cells (IC_{50}), obtained from graph (S%)=*f*(c) are given in *Table 1*. IC_{50} Of known cytostatic drug, *cis*-diammindichloroplatinum (*cis*-DDP) was used as positive control. Seven examined compounds **1**,**3**,**4**,**7,10-12** did not affect proliferation of healthy human blood peripheral mononuclear cells (PBMC and PBMC + PHA) $IC_{50} > 300$ μM.

All the studied compounds, except compound **15**, affected the survival of HeLa cells, while compound **3** affected the survival of all four examined cell lines. The most active compound toward HeLa cells is compound **1**, while the least active one is compound **14**. *Figure 1* shows light microscopy of four target cell lines after treatment for 72 h with medium alone (control), or with 150 μM of compound **1**.

Table 1. Structures and IC_{50} values of studied β-hydroxy-β-arylalkanoic acids toward HeLa, Fem-X, K562, LS174. *cis*-Diammindichloro platinum (cisplatin, *cis*-DDP) was used as a positive control.



					IC_{50} (μM)			
№	R_1-	R_2-	R_3-	R_4-	HeLa	Fem-X	K562	LS174
1	Me-	Me-	Me-	Ph-Ph-	62.20	205	141	154
2	Me-	Me-	Ph-	Ph-	80.56	/	/	/
3	Me-	H-	H-	2-Cl-Ph-	97.20	89	92	93
4	H-	H-	Me-	Ph-Ph-	98.70	>200	>200	>200
5	Me-	Me-	fluorenyl-		99.07	/	/	/
6	Me-	H-	Ph-	Ph-	99.41	/	/	/
7	Me-	Me-	H-	4-Cl-Ph-	106.30	>200	>200	>200
8	H-	H-	H-	2-Cl-Ph-	117.80	>200	>200	>200
9	Me-	Me-	Me-	4-MeO-Ph-	125.80	136	>200	>200
10	Me-	Me-	cyclohexyl-		128.40	>200	>200	>200
11	Me-	Me-	Me-	Ph-	133.17	/	/	/
12	H-	H-	Me-	4-*i*-Bu-Ph-	151.40	>200	>200	>200
13	Me-	Me-	H-	Ph-	164.88	/	/	/
14	H-	H-	Ph-	Ph-	182.55	/	/	/
15	H-	Et-	cyclohexyl-		>200	>200	/	/
Cis-DDP	/				4.31	4.70	5.77	/

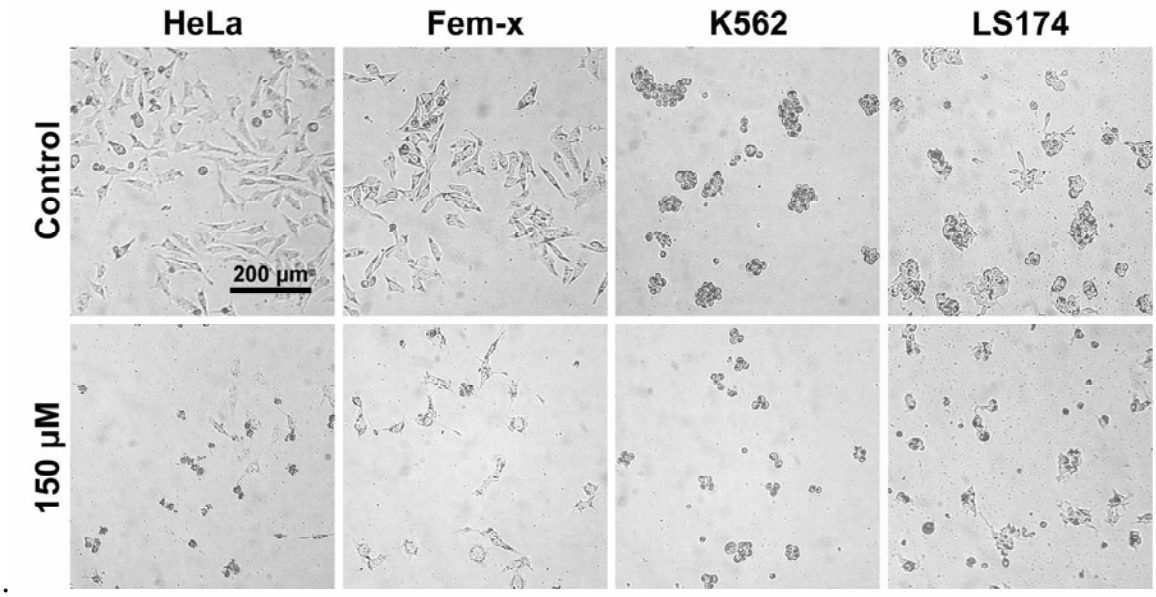


Figure 1. Light microscopy of four studied cell lines after treatment for 72 h with medium alone (control, upper row), or with 150 μM of compound **1** (bottom row). Cells were observed using an inverted microscope and photographed by a digital camera (Olympus C-4040 Zoom).

2.2. QSAR

The QSAR study was performed using estimated lipophilicity (log*P*) and indicator variable ***I***, which cumulatively represents the mutual arrangement of hydrogen bond donors (HBD), hydrogen bond acceptors (HBA) and hydrophobic area within molecules. A QSAR study of antiproliferative activity toward HeLa cells, against which compounds **1**-**14** are active, results with two-parameter correlation:

$$\log(1/IC_{50}) = +\ 0.167\ (\pm 0.043)\ \log P - 0.154\ (\pm 0.051)\ \boldsymbol{I} - 2.527\ (\pm 0.12)$$
$$(n = 14;\ r = 0.941;\ s = 0.046;\ F = 42.467;\ Q^2 = 0.810;\ s_{PRESS} = 0.059) \quad (1)$$

Log*P* = log (K_{OW}) values were estimated by the Crippen fragmentation method [22]. Indicator variable, ***I***, bearing value 1 for compounds having H on α carbon, and implicitly are prone for the dehydration, *i.e.*, loosing hydroxyl functionality (HBD) in position near the aromatic moiety. Indicator variable bearing value –1 was ascribed to compounds which have *ortho* substituent(s) on the aryl (or cycloalkyl) moiety, that stericaly hinders their coplanarity with C-OH bond. In this way it accounts for changed electronic environment of oxygen atom - influencing the HBD ability of β-OH group. Inclusion of indicator variable ***I*** is a reminiscent of classical Free-Wilson approach [23], that imply factorization of differences on distinct position of molecules in separate groups, and posterior multiple regression analysis of obtained matrix. Two distinct structural features were described by one indicator variable in order to obtain statistics of higher quality.

Table 2. Descriptors used in QSAR, obtained and from Equation 1 predicted log(1/(IC_{50})) values for HeLa cells.

№	log*P*	I	Obtained log(1/(IC_{50}))	Predicted log(1/(IC_{50}))
1	4.16	0	–1.794	–1.833
2	3.88	0	–1.906	–1.880
3	2.12	–1	–1.988	–2.020
4	2.89	0	–1.994	–2.045
5	3.53	0	–1.996	–1.938
6	3.18	0	–1.997	–1.997
7	2.83	0	–2.027	–2.055
8	1.56	–1	–2.071	–2.113
9	2.36	0	–2.100	–2.133
10	1.99	–1	–2.109	–2.041
11	2.49	0	–2.124	–2.112
12	2.87	1	–2.180	–2.202
13	2.27	0	–2.217	–2.148
14	2.61	1	–2.261	–2.246

By using only log*P* values, the good correlation could be obtained for compounds **1**, **2**, **5**, **12** and **14** (r = 0.997), while something inferior one (r = 0.858) by additional inclusion of **4**, **6**, **7**, **9**, **11** and **13**.

After the energy minimization using molecular mechanics (MM+ force field), the similarity search was performed using VegaZZ 2.0.5 software [24]. Superimposition of molecular structure details (phenyl rings linked [25] to C-OH (*β*-OH group), C-OH and carboxyl carbonyl) of compounds **2** – **14** upon the structure of the most active one (**1**), and posterior correlation of root mean square deviation (RMSD) of considered atoms positions with activity and indicator variable ***I*** (***I*** as defined above), gives low intercorrelation coefficients: activity/RMSD, $r = 0.61$ while for ***I***/RMSD, $r = 0.69$. This shows that assumptions used for introduction of ***I*** (as defined above) are well chosen.

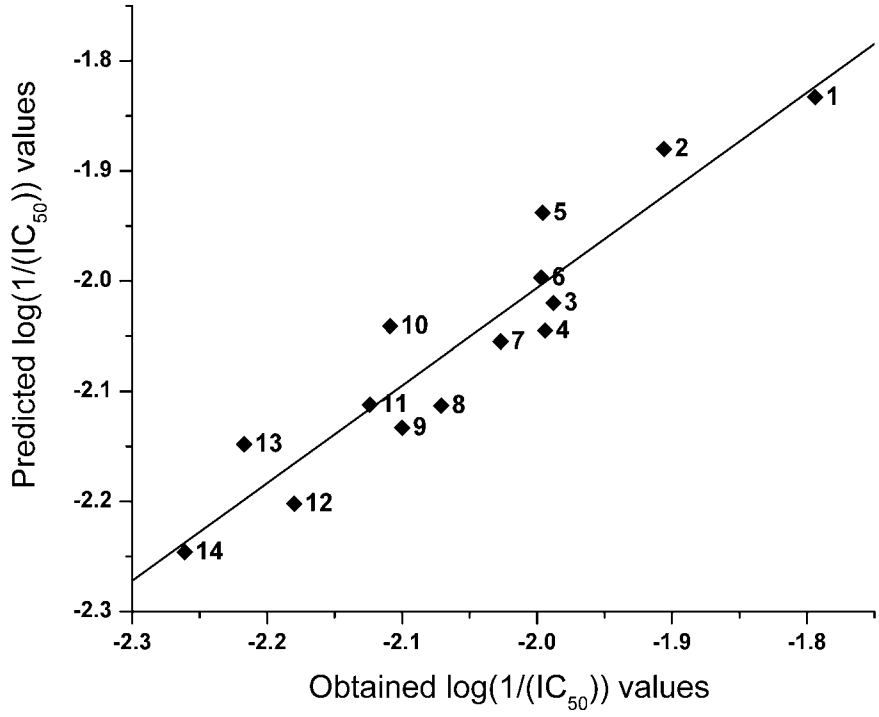


Figure 2. Correlation of predicted (from Eq. 1) and experimentally obtained log(1/(IC_{50}) values for HeLa cells (n = 14; $r = 0.941$)

Values of used descriptors, obtained, and from the *Equation 1* predicted log(1/(IC_{50})), are given in *Table 2*. The relationship between obtained *vs.* predicted log(1/(IC_{50})) in graphical form are given in the *Figure 2*. The higher weight descriptor in correlation is the estimated log*P*, while indicator variable that describes the stereoelectronic demands for the activity has a comparable weight.

2.3. Conclusion

The majority of synthesized *β*-hydroxy-*β*-arylalkanoic acids exert antiproliferative activity *in vitro* toward HeLa, Fem-X, K562 and LS174 having IC_{50} values from 62.20 to 205 μM. The most active compound is 3-hydroxy-2,2-dimethyl-3-(4-biphenylyl)butanoic acid. Seven examined compounds did not affect proliferation of healthy human blood peripheral proliferation of healthy human blood peripheral mononuclear cells (PBMC and PBMC+PHA), $IC_{50} > 300$ μM.

The presence of COX in HeLa cells is documented [26]. It could explain the higher sensitivity of HeLa cell line to our compounds.

A QSAR study of antiproliferative activity toward HeLa cells, against which compounds **1-14** are active indicate that estimated lipophilicity of compounds influences their antiproliferative activity in the first place, and indicate that spatial arrangement of HBD and HBA (exerted by indicator variable) has a comparable importance.

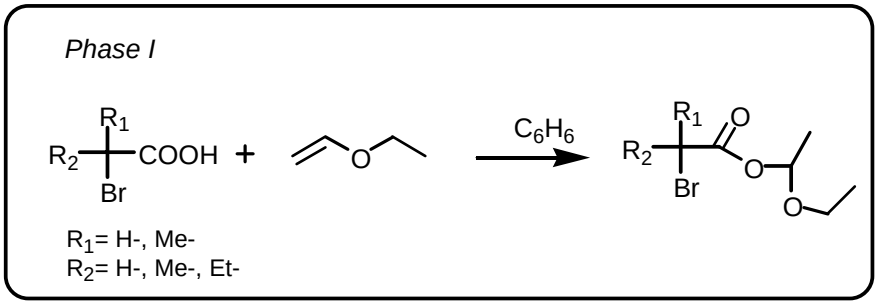
3. Experimental

Aromatic ketones, aromatic aldehydes, α-bromo acids and ethyl-vinyl ether were reagent grade and were purchased from Sigma-Aldrich or Fluka. Zinc powder was activated by treatment with conc. hydrochloric acid, subsequently washed with distilled water, ethanol, acetone and ether, dried for 2 hours at 120 ºC and finally dried in the presence of $CaCl_2$ under the reduced pressure. Tetrahydrofuran was purified by treatment with $LiAlH_4$ (LAH), distilled in the presence of the excess of LAH and used immediately after the distillation. For the synthesis of α-bromo esters-acetals, dry, thiophene-free, benzene was used. All other solvents were *p.a*. grade.

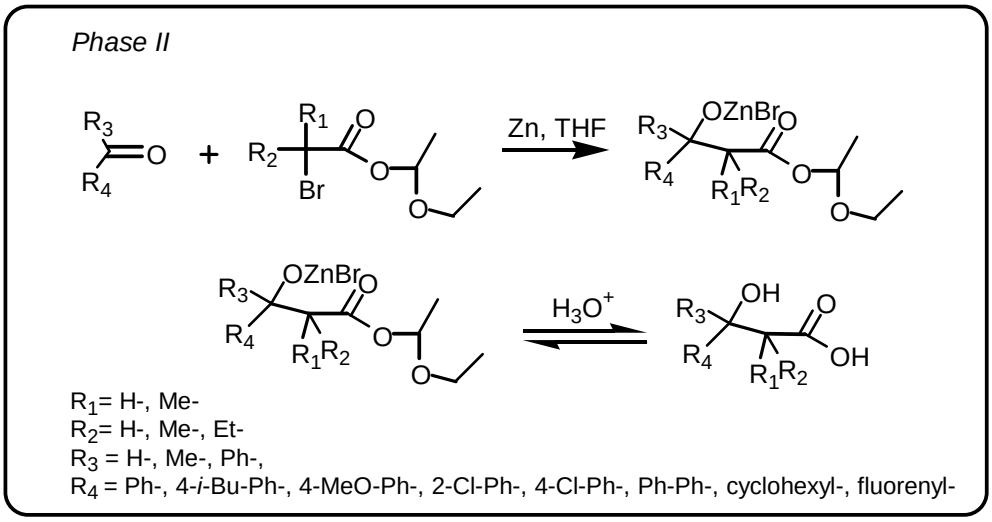
IR Spectra were taken in KBR pellet on Perkin-Elmer 1725 spectrophotometer. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded at 200/50 MHz with tetramethylsilane (TMS) as internal standard on a Varian "Gemini 200" spectrometer in $CDCl_3$. The mass spectra were taken on a Finnigan-MAT 8230 BE MS, employing both chemical ionization (i-C_4H_{10}) (CI), and electron impact (70 eV) (EI). The elemental analyses were done on Elemetar-Vario EL III equipment. Melting points were determined in open capillary tubes on Büchi apparatus and are uncorrected.

3.1. Syntheses

Compounds **1-15** were synthesized by two-step reaction. In the first phase (*Scheme 1*) the intermediate, α-bromo alkanoic 1-ethoxyethyl esters (α-bromo esters-acetals, **PI-1** to **PI-4**), were synthesized.



Scheme 1. Synthesis of α-bromo esters-acetals (Phase I)



Scheme 2. Synthesis of β-hydroxy-β-arylalkanoic acids (Phase II)

The second phase was the modified Reformatsky reaction - reaction between previously synthesized intermediates and suitable aldehydes or ketones in presence of Zn in tetrahydrofuran (THF) at –5 °C during the first few hours, followed by subsequent rising to the room temperature (*Scheme 2*). The β-hydroxy-β-aryl, or β-cycloalkylalkanoic acids (**1-15**) were obtained (Table 1).

3.1.1 Synthesis of α-bromo esters-acetals (Scheme 1, Phase I)**.**

In a 100 ml two-necked round bottom flask equipped with $CaCl_2$ tube, argon inlet and magnetic stirrer, the α-bromo acid (0.03 mol), ethyl-vinyl ether (3.37 g, 5.00 ml, ~ 0.05 mol), 4-6 ml dry benzene were placed, and stirred at room temperature for 2 hours. After the evaporation of benzene and excess of ethyl-vinyl ether, the residual mixture was distilled under the reduced pressure. In this way obtained esters were analyzed immediately by instrumental methods. By the longer staying at room temperature, or even on the lower temperatures (~4 °C), obtained esters become yellow, probably due to decomposition. The boiling points of synthesized compounds were determined during distillation. The compounds were characterized by elemental analyses, MS, IR, ^{1}H, and ^{13}C NMR spectroscopy. The bromoethyl-vinyl ester of bromoethanoic acid, synthesis of which is previously reported [18] was characterized only by spectral data. These intermediates are labeled as **PI-1** to **PI**-4, being result of Phase I synthesis.

According to procedure *3.1.1*, the following intermediate compounds were synthesized:

PI-1. 1-Ethoxyethyl-2-bromopropanoate: $C_7H_{13}O_3Br$; M_w = 225.02; **Boiling point:** 87 ^{0}C, (3 mm Hg); **IR (Film)**: $\bar{\nu}_{max}$ (cm^{-1}) 2984, 2935, 1738 ($\bar{\nu}$ >C=O), 1139 cm^{-1}($\bar{\nu}$ -C-O-C-); **^{1}H NMR ($CDCl_3$) (δ)**: 1.22 (*t*, *J* 7.1 Hz, 3H); [1.23 (*t*, *J* 7.1 Hz, 3H)]; 1.43 (*d*, *J*5.2 Hz, 3H); [1.44 (*d*, *J* 5.2 Hz, 3H)]; 1.82 (d, *J* 6.9 Hz, 3H); 3.5-3.89 (*m*, 4H); 4.38 (*q*, *J* 7.00 Hz; 2H); 5.97 (*q*, *J* 5.2 Hz; 2H); **^{13}C NMR ($CDCl_3$) (δ)**: δ 14.78 (O-CH-$\underline{C}H_3$); 20.23 [20.45] (CH_2-$\underline{C}H_3$); 21.16 [21.33] (Br-CH-$\underline{C}H_3$); 40.02 [40.31] (Br-$\underline{C}$H-$\underline{C}H_3$); 64.37 [64.76] (O-$\underline{C}H_2$-); 97.66 [97.96] (O-$\underline{C}$H-O); 169.71 169.76 ($\underline{C}$=O$_{ester}$); **MS (CI):** 225 M^+, 223, 207 (M-18), 52 (M-73), 144 (M-80); **Yield (%):** 68; **Elemental analysis (%):** Calc. C, 37.35 H, 5.82; Found C, 37.16 H, 5.83.

PI-2. 1-Ethoxyethyl-2-bromobuthanoate: $C_8H_{15}O_3Br$; M_w = 239.11**; Boiling point:** 60 ^{0}C (1 mm Hg); **IR (Film)**: $\bar{\nu}_{max}$ (cm^{-1}) 2978, 2939, 1737 ($\bar{\nu}$, >C=O), 1137 ($\bar{\nu}$ -C-O-C-); **^{1}H NMR ($CDCl_3$) (δ)**: 1.04 (*t, J* 7.4 Hz, 6H); 1.21 (*t, J* 7.1 Hz, 6H); 1.45 (*d, J* 5.0 Hz, 6H)]; 1.97-2.21 (*m*, 4H); 3.51-3.85 (*m*, 4H); 4.16 (*t, J* 7.0 Hz, 1H); [4.17 (*t, J* 7.0 Hz, 1H)]; 5.98 (*q, J* 5.4 Hz, 2H); **^{13}C NMR ($CDCl_3$) (δ)**: 11.51 (Br-CH-CH_2-$\underline{C}H_3$); 14.68 (-O-CH_2-$\underline{C}H_3$); 20.18 [20.36] ($\underline{C}H_3$CH(OEt)-O); 27.82 [27.93] (Br-CH-$\underline{C}H_2$-CH_3); 47.47 [47.67] (Br-$\underline{C}$H-); 64.60 (-O-$\underline{C}H_2$-CH_3); 97.66 [97.72] ($CH_3\underline{C}$H(OEt)-O); 169.02 (169.14) ($C=O_{ester}$).; **MS (CI):** 239 (M^+), 241, 167 (M-73).; **Yield (%):** 81; **Elemental analysis (%):** Calc. C, 40.18; H ,6.32; Found C, 40.47; H, 6.50.

The compounds **1** and **2** are the distereomeric mixtures. In proton NMR spectra, the shift values attributed to the minor diasteromer are given in square brackets.

PI-3. **1-Ethoxyethyl-2-bromo-2-methylpropanoate:** $C_8H_{15}O_3Br$; Mw = 239.11; **Boiling point:** 94 ^{0}C, 20 mm Hg; **IR (Film)**: $\bar{\nu}_{max}$ (cm^{-1}) 2982, 2932, 1732 ($\bar{\nu}$, >C=O), 1140 ($\bar{\nu}$ -C-O-C-); **^{1}H NMR ($CDCl_3$) (δ)**: 1.23 (*t, J* 7.0 Hz, 3 H); 1.45 (*d, J* 5.4 Hz; 3 H); 1.94 (*s*, 6 H); 3.51-3.88 (*m*; 2 H); 5.97 (*q, J* 5.2 Hz, 1H); **^{13}C NMR ($CDCl_3$) (δ)**: 14.86 (-O-CH_2-$\underline{C}H_3$); 20.29 ($\underline{C}H_3$CH(OEt)-O); 30.37 (Br-C($\underline{C}H_3$)$_2$-); 55.86 (Br-$\underline{C}$(CH_3)$_2$-); 64.76 (-O-$\underline{C}H_2$-CH_3); 98.12 ($CH_3\underline{C}$H(OEt)-O); 171.16 ($C=O_{ester}$).; **MS (CI):** 240 $(M+H)^+$, 169 (M-73); **Yield (%):** 54; **Elemental analysis (%):** Calc.: C, 40.18; H, 6.32. Found: C, 40.14; H, 6.57.

PI-4. **1-Ethoxyethyl-2-bromoethanoate:** $C_6H_{11}O_3Br$; M_w = 211.05; **Boiling point:** 85 ^{0}C (3 mm Hg); **IR (Film)**: $\bar{\nu}_{max}$ (cm^{-1}) 2983, 2937, 1734 ($\bar{\nu}$, >C=O), 1285 ($\bar{\nu}$, >C-O), 1129 ($\bar{\nu}$ -C-O-C-); **^{1}H NMR ($CDCl_3$) (δ)**: 1.22 (*t, J* 7 Hz, 3 H); 1.44 (*d, J* 5 Hz, 3 H); 3.5-3.91 (*m*, 2 H); 3,85 (*d, J* 1.6 Hz, 2H); 5.97 (*q, J* 5.2 Hz, 1 H); **^{13}C NMR ($CDCl_3$) (δ)**: 14.86 (-O-CH_2-$\underline{C}H_3$); 20.57 ($\underline{C}H_3$CH(OEt)-O); 26.06 (Br-$\underline{C}H_2$-); 64.98 (-O-$\underline{C}H_2$-CH_3); 98.43 ($CH_3\underline{C}$H(OEt)-O); 166.97 ($\underline{C}=O_{ester}$); **MS (EI):** 168 (M-45), 121 (M-89), 82 (Br); **Yield (%):** 71.

3.1.2. Synthesis of *β*-hydroxy-*β*-arylalkanoic acids (Scheme 2, Phase II)

In 100 ml three-necked, round-bottom flask, equipped with $CaCl_2$ tube, argon inlet and magnetic stirrer, the Zn (0.02 mol, 1.30 g), aldehyde or ketone (0.01 mol), 40 ml of dried THF, small amounts of $HgCl_2$ and I_2 are placed. Previously prepared esters (0.02 mol) were added from dropping funnel during 30 min under the argon atmosphere. The reaction mixtures were cooled in ice bath and constantly stirred magnetically, until whole amount of Zn was disappeared (10 - 48 h usually) [27]. The THF was removed under reduced pressure followed by addition of 30 ml of benzene and 10 ml of cold 3M HCl. This reaction mixture was cooled at 0 °C in ice bath, and stirred for ~3 hours. The organic layers were taken. Obtained aqueous solutions were additionally extracted with benzene. The combined organic extracts were treated with 10% aqueous $KHCO_3$ until pH ~8 was reached (hydroxy acids were converted in potassium salts). Alkaline aqueous solutions were extracted by small amount of ether to remove unreacted aldehyde or ketone. This solution was cooled at 0 °C and cold 10% HCl was carefully added to pH~2.5, yielding *β*-hydroxy acids, mostly as oil at first, turning into crystals by prolonged keeping on the low temperatures. In this way obtained acids were recrystallized from benzene or *i*-PrOH.

1. 3-Hydroxy-2,2-dimethyl-3-(4-biphenylyl)butanoic acid: $C_{18}H_{20}O_3$; M_w = 284.35**; Melting point:** 142 ^{0}C; **IR (KBr)**: $\bar{\nu}_{max}$ (cm^{-1}) 3513 ($\bar{\nu}$,-CH-OH), 3034 ($\bar{\nu}$,-C(O)-$\underline{O}$H), 2977, 1688 ($\bar{\nu}$,

>C=O), 1238 ($\bar{\nu}$, -C-O); **^{1}H NMR ($CDCl_3$) (δ)**: 1.17 (*s*, 3 H); 1.20 (*s*, 3 H); 1.69 (*s*, 3 H); 7.31-7.63 (*m*, 9 H); **^{13}C NMR ($CDCl_3$) (δ)**: 21.65 (-C($\underline{C}H_3$)$_2$-); 25.10 (-C(OH)$\underline{C}H_3$-); 50.05 (-$\underline{C}$(CH$_3$)$_2$-); 77.36 (-$\underline{C}$(OH)CH$_3$-); 126.07 (*o*-Ph); 126.98 (*o*'-Ph); 127.31 (*p*'-Ph); 127.63 (*m*-Ph); 128.74 (*m*'-Ph); 139.94 (p_{ipso}-Ph); 140.47 (C_{ipso}'-Ph); 141.63 (C_{ipso}-Ph); 182.91 (COOH); **MS (CI):** 285 $(M+H)^+$, 267 (M-17), 197 (M-87); **Yield (%):** 32; **Elemental analysis (%):** Calc.: C, 76.03; H, 7.09. Found: C, 75.57; H, 7.28.

2. 3-Hydroxy-2,2-dimethyl-3,3-diphenylpropanoic acid; $C_{17}H_{18}O_3$; M_w = 270.32; **Melting point:** 162 ^{0}C; **IR (KBr**): $\bar{\nu}_{max}$ (cm^{-1}) 3570 ($\bar{\nu}$,-CH-$\underline{OH}$), 3520 ($\bar{\nu}$,-C(O)-$\underline{OH}$), 1678 ($\bar{\nu}$, >C=O), 1160 ($\bar{\nu}$, -C-O); **^{1}H NMR ($CDCl_3$) (δ)**: 1.35 (*s*, 6H); 7.13-7.47 (*m*, 10 H, after the subtraction of $CHCl_3$ signal at 7.263 δ); **^{13}C NMR ($CDCl_3$) (δ)**: 23.93 (-C($\underline{C}H_3$)$_2$-); 48.71 (-$\underline{C}$(CH$_3$)$_2$-); 82.44 ($\underline{C}$-OH); 127.25 (*o*-Ph); 127.39 (*p*-Ph); 128.63 (*m*-Ph); 144.62 (C_{ipso}-Ph); 185.24 (COOH); **MS (CI):** 253 (M-17), 183 (M-87); **Yield (%):** 40; **Elemental analysis (%):** Calcd.**:** C, 75.53; H, 6,71. Found: C, 75.22; H, 6,74.

3. 3-Hydroxy-2-methyl-3-(2-chlorophenyl)propanoic acid; $C_{10}H_{11}ClO_3$; M_w = 214.65; **Melting point:** 84 ^{0}C; **IR (KBr):** $\bar{\nu}_{max}$ (cm^{-1}) 3325, 3420 ($\bar{\nu}$,-CH-$\underline{OH}$), 3057, 2992 ($\bar{\nu}$,-C(O)-$\underline{OH}$), 1707 ($\bar{\nu}$, >C=O), 1232 ($\bar{\nu}$, -$\underline{C}$-O-); **^{1}H NMR ($CDCl_3$) (δ)**: 1.09 (*d*, *J* 7.2 Hz, 3 H, after the subtraction of $CHCl_3$ signal at 7.263 δ); 1.16 (*d*, *J* 7.2 Hz, 3 H); 2.88-3.11 (*m*, 1 H); 5.31(*d*, *J* 8 Hz, 1 H); 5.62 (*d*, *J* 2.2 Hz, 1 H); 7.18-7.63 (*m*, 4 H); **^{13}C NMR ($CDCl_3$) (δ)**: 9.14 (14.15) (-CH($\underline{C}H_3$)-); 42.55 (46.45) (-$\underline{C}$H(CH$_3$)-); 69.68 (71.99) (-$\underline{C}$H(OH)-); 126.74 (127.34) (*m*-Ph); 127.91 (128.23) (*m*-Ph); 128.73 (*p*-Ph); 129.16 (129.44) (*o*-Ph); 129.52 (131.29) (C-Cl); 138.14 (C_{ipso}-Ph); 181.15 (181.66) (COOH); (**MS (EI):** 214 (M^+),142 (M-73), 77; **Yield (%):** 25; **Elemental analysis (%):** Calcd.**:** C, 55.96; H, 5.17. Found: C, 55.41; H, 5.02.

4. 3-Hydroxy-3-(4-biphenylyl)butanoic acid; $C_{16}H_{16}O_3$; Mw = 256.30; **Melting point:** 136 ^{o}C; **IR (KBr):** $\bar{\nu}_{max}$ (cm^{-1}) 3521 ($\bar{\nu}$,-CH-$\underline{OH}$), 2977 ($\bar{\nu}$,-C(O)-$\underline{OH}$), 1689 ($\bar{\nu}$, >C=O), 1238 ($\bar{\nu}$, -$\underline{C}$-O-); **^{1}H NMR ($CDCl_3$) (δ)**: 1.58 (*s*, 1 H); 2.96 (*dd*, J_1 J_2 16.4 Hz; 2 H); 5.63 (*s*, OH); 7.30-7.60 (*m*, 9 H); **^{13}C NMR ($CDCl_3$) (δ)**: 30.57 (CH_3); 47.92 (-CH_2-); 72.70 (C-OH); 124.85 (*o*-Ph); 127.10 (*o*'-Ph); 127.14 (*o*- and *p*'-Ph); 127.32 (*m*'-Ph); 128.78 (*m*-Ph); 139.98 (p_{ipso}-Ph); 140.58 (C_{ipso}'-Ph); 145.40 (C_{ipso}-Ph); **MS (EI):** 256 (M^+), 238 (M-18), 197 (M-59), 153; **Yield (%):** 27; **Elemental analysis (%):** Calcd.: C, 74.98; H, 6.29. Found: C, 74.70; H, 6.48.

5. 2-[9-(9-Hydroxyfluorenyl)]-2-methylpropanoic acid; $C_{17}H_{16}O_3$; M_w = 268.31; **Melting point:** 138 ^{o}C; **IR (KBr):** $\bar{\nu}_{max}$ (cm^{-1}) 3387 ($\bar{\nu}$, -CH-$\underline{OH}$), 2984 ($\bar{\nu}$,-C(O)-$\underline{OH}$), 1725 ($\bar{\nu}$, >C=O), 1156 ($\bar{\nu}$, -C-O); **^{1}H NMR ($CDCl_3$) (δ)**: 1.07 (*s*, 6H); 7.19-7.61 (*m*, 8 H); **^{13}C NMR (**$CDCl_3$**) (δ)**: 20.98 (-C($\underline{C}H_3$)$_2$-); 48.72 (-$\underline{C}$(CH$_3$)$_2$-); 85.39 (C-OH); 119.97 (*m*-Ph); 124.14 (*m*-Ph); 127.80 (*p*-Ph); 129.45 (*o*-Ph); 140.43 (C_{ipso}-Ar); 145.69 (C_{ipso}); 180.65 (COOH); (**MS (CI):** 268 (M^+), 251 (M-17), 223 (M-45), 181 (M-87); **Yield (%):** 20; **Elemental analysis (%):** Calcd.: C, 76.10; H, 6.01. Found: C, 76.19; H, 6.19.

6. 3-Hydroxy-2-methyl-3,3-diphenylpropanoic acid; $C_{16}H_{16}O_3$; M_w = 256.30; **Melting point:** 180 ^{o}C; **IR (KBr):** $\bar{\nu}_{max}$ (cm^{-1}) 3525 ($\bar{\nu}$, -CH-$\underline{OH}$), 2991 ($\bar{\nu}$, -C(O)-$\underline{OH}$), 1677 ($\bar{\nu}$, >C=O), 1225 ($\bar{\nu}$, -C-O); **^{1}H NMR ($CDCl_3$) (δ)** : 1.19 (*d*, *J* 7.2 Hz, 3 H); 3.65 (*q*, *J* 7.2 Hz, 1 H); 7.13-7.56 (*m*, 10 H); **^{13}C NMR ($CDCl_3$) (δ)** 13.04 (CH_3); 46.41 (-$\underline{C}$H(CH$_3$)-); 77.91 (C-OH); 125.30 (*o*- and *o*'-Ph); 126.71 (*m*-Ph); 127.12 (*m*'-Ph); 128.18 (*p*- and *p*'-Ph); 128.47 (*o*- and *o*'-Ph); 143.80 (C_{ipso}); 147.15 (C_{ipso}');

182.04 (COOH); **MS (CI):**. 257 $(M+H)^+$, 239 (M-17), 183 (M-73); **Yield (%):** 35; **Elemental analysis (%):** Calcd.: C, 74.98; H, 6.29. Found: C, 75.08; H, 6.11.

7. 3-Hydroxy-2,2-dimethyl-3-(4-chlorophenyl)propanoic acid; $C_{11}H_{13}ClO_3$; M_w = 228.67; **Melting point:** 142 ^oC; **IR (KBr):** $\bar{\nu}_{max}$ (cm^{-1}) 3501 ($\bar{\nu}$, -CH-OH), 2989 ($\bar{\nu}$, -C(O)-OH), 1683 ($\bar{\nu}$, >C=O), 1167 ($\bar{\nu}$, -C-O); **^{1}H NMR ($CDCl_3$) (δ)** : 1.13 (*s*, 3 H); 1.15 (*s*, 3 H); 4.94 (*s*, 3 H); 7.25-7.36 (*m*, 4 H); **^{13}C NMR ($CDCl_3$) (δ)** 18.34 ($\underline{C}H_3$); 23.05 ($\underline{C}H_3$); 47.50 (-$\underline{C}(CH_3)_2$-); 77.64 (-$\underline{C}$H(OH)-); 128.07 (*m*-Ph); 129.07 (*o*-Ph); 137.96 (C_{ipso}); 183.08 (COOH); **MS (CI)** 229 $(M+H)^+$, 211 (M-18), 141; **Yield (%):** 40; **Elemental analysis (%):** Calcd.: C, 57.78; H, 5.73. Found: C, 57.73; H, 5.75.

8. 3-Hydroxy-3-(2-chlorophenyl)propanoic acid; $C_9H_9ClO_3$; M_w = 200.62; **Melting point:** 92 ^oC; **IR (KBr):** $\bar{\nu}_{max}$ (cm^{-1}) 3476, 3311 ($\bar{\nu}$, -C(O)-OH), 1709 ($\bar{\nu}$, >C=O), 1164 ($\bar{\nu}$, -C-O); **^{1}H NMR ($CDCl_3$) (δ)**: 2.71 (*dd*, *J* 9.8 Hz, *J* 16.8 Hz, 1H); 2.97 (*dd*, *J* 2.6 Hz, *J* 16.8 Hz, 1H); 5.53 (*dd*, *J* 2.2 Hz, *J* 8 Hz, 1H); 6.51 (*s*, OH) 7.19-7.64 (*m*, 4 H); **^{13}C NMR ($CDCl_3$) (δ)**: 41.29 (-CH_2-); 66.91 (-$\underline{C}$H(OH)-); 126.96 (*m*-Ph); 127.32 (*p*-Ph); 128.98 (*m*-Ph); 129.49 (*o*-Ph); 131.38 (C-Cl); 139.45 (C_{ipso}); 177.57 (COOH); **MS (CI)** 201 (M^+), 183 (M-18), 141 (M-59); **Yield (%):** 20; **Elemental analysis (%):** Calcd.: C, 53.88; H, 4.52. Found: C, 53.65; H, 4.63.

9. 3-Hydroxy-2,2-dimethyl-(4-methoxyphenyl)butanoic acid; $C_{13}H_{18}O_4$; M_w = 238.28; **Melting point:** 120 ^oC; **IR (KBr):** $\bar{\nu}_{max}$ (cm^{-1}) 3420 ($\bar{\nu}$, -C(O)-OH), 2996 ($\bar{\nu}$, -C(O)-OH), 1728 ($\bar{\nu}$, >C=O),1251 ($\bar{\nu}$, -C-O); **^{1}H NMR ($CDCl_3$) (δ)**: 1.12 (*s*, 3 H); 1.15 (*s*, 3 H); 1.64 (*s*, 3 H); 3.79 (*s*, 3 H); 6.81-7.37 (*m*, 4 H); **^{13}C NMR ($CDCl_3$) (δ)**: 21.56 (-C($\underline{C}H_3$)$_2$-); 25.13 (-C(OH)$\underline{C}H_3$-); 50.11 (-$\underline{C}(CH_3)_2$-); 55.13 ($\underline{C}H_3$-O-Ar); 77.18 (-$\underline{C}$(OH)CH_3-); 112.70 (*m*-Ar); 128.32 (*o*-Ar); 134.72 (C-OCH_3); 158.59 (C_{ipso}); 182.67 (COOH); **MS (CI)** 239 $(M+H)^+$, 221 (M-18), 151 (M-87); **Yield (%):** 45; **Elemental analysis (%):** Calcd.: C, 65.53; H, 7.61. Found: C, 65.76; H, 7.94.

10. 2-Hethyl-2-(1-(1-hydroxycyclohexyl))propanoic acid; $C_{10}H_{18}O_3$; M_w = 186.25; **Melting point:** 89 ^oC; **IR (KBr)**: $\bar{\nu}_{max}$ (cm^{-1}) 3497 ($\bar{\nu}$, -CH-OH), 3411 ($\bar{\nu}$, -C(O)-OH), 2970 ($\bar{\nu}$, -C(O)-OH), 1686 ($\bar{\nu}$, >C=O), 1144 ($\bar{\nu}$, -C-O); **^{1}H NMR ($CDCl_3$) (δ)** : 1.25 (*s*, 6 H); 1.21-1.69 (*m*, 10 H); **^{13}C NMR ($CDCl_3$) (δ)**: 20.74 (-C($\underline{C}H_3$)$_2$-); 21.41 (2'(4')-CH_2-); 25.53 (4'-CH_2-); 31.30 (2'(6')-CH_2-); 50.11 (-$\underline{C}(CH_3)_2$-); 74.63 (C-OH); 183.09 (COOH); **MS (CI)** 187 $(M+H)^+$, 169 (M-17); **Yield (%):** 28; **Elemental analysis (%):** Calcd.**: C,** 64.49; H, 9.74. Found: C, 64.20; H, 10.02.

11. 3-Hydroxy-2,2-dimethyl-3-phenylbutanoic acid; $C_{12}H_{16}O_3$; M_w = 208.26; **Melting point:** 89 ^oC; **IR (KBr)**: $\bar{\nu}_{max}$ (cm^{-1}) 3230 ($\bar{\nu}$, -CH-OH), 3205, ($\bar{\nu}$, -C(O)-OH), 2989 ($\bar{\nu}$, -C(O)-OH), 1723 ($\bar{\nu}$, >C=O), 1159 ($\bar{\nu}$, -C-O); **^{1}H NMR ($CDCl_3$) (δ)**: 1.13 (*s*, 3 H); 1.16 (*s*, 3 H); 1.66 (*s*, 3 H); 7.25-7.46 (*m*, 5 H); **^{13}C NMR (DMSO) (δ)** 21.54 (-C($\underline{C}H_3$)$_2$-); 21.59 (-C($\underline{C}H_3$)$_2$-); 25.00 (-C(OH)$\underline{C}H_3$-); 49.98 (-$\underline{C}(CH_3)_2$-); 77.38 (-$\underline{C}$(OH)CH_3-); 127.12 (*o*-Ph); 127.23 (*p*-Ph); 127.43 (*m*-Ph); 142.52 (C_{ipso}-Ph); 182.98 (COOH); **MS (CI)** 208 (M^+), 191 (M-17), 121 (M-87); **Yield (%):** 33; **Elemental analysis (%)**: Calcd.: C, 69.21; H, 7.77. Found: C, 68.97; H, 7.74.

12. 3-Hydroxy-3(4-isobutylphenyl)butanoic acid;$C_{14}H_{20}O_3$; M_w = 236.31; **Melting point**: 90 ^oC; **IR (KBr):** $\bar{\nu}_{max}$ (cm^{-1}) 3515, 3400 ($\bar{\nu}$, -C(O)-OH), 2957 ($\bar{\nu}$, -C(O)-OH), 1683 ($\bar{\nu}$, >C=O), 1244 ($\bar{\nu}$, -C-O); **^{1}H NMR ($CDCl_3$) (δ)**: 0.87 (*d*, *J* 6.6 Hz, 3H); 0.90 (*d*, *J* 6.6 Hz, 3H); 1.55 (*s*, 3H); 1.85 (*h*, 1 H); 2.45 (*d*, *J* 7.2 Hz, 2 H); 2.91 *(dd*, *J* 16 Hz, *J* 39.2 Hz, 2 H); 5.76 (*b*, OH); 7.10 (*d*, *J* 8 Hz) 7.30 (*d*, *J* 8 Hz); **^{13}C NMR ($CDCl_3$) (δ)**: 22.34 (-CH($\underline{C}H_3$)$_2$); 30.10 (-$\underline{C}$H(CH_3)$_2$); 30.39 (CH_3-$\underline{C}$(OH)-); 44.88 (Ar-$\underline{C}H_2$-); 46.08 (-$\underline{C}H_2$-COOH); 72.74 (CH_3-$\underline{C}$(OH)-); 124.06 (*o*-Ph); 129.09 (*m*-Ph); 140.51 (C_{ipso}-*i*-Bu);

143.57 (C_{ipso}); 176.98 (COOH); **MS (CI)** 236 (M+), 218 (M-18), 177 (M-59); **Yield (%):** 42; **Elemental analysis (%):** Calcd.: C, 70.11; H, 8.53; Found: C, 70.08; H, 8.58. (with 0.2 mol H_2O; in ^{1}H NMR spectra, presence of water is observed)

13. **3-Hydroxy-2,2-dimethyl-3-phenylpropanoic acid;** $C_{11}H_{14}O_3$; M_w = 194.23**; Melting point:** 132 ^{0}C; **IR (KBr):** $\bar{\nu}_{max}$ (cm^{-1}) 3439, 3380 ($\bar{\nu}$, -OH), 2984 ($\bar{\nu}$, -C(O)-OH), 1707 ($\bar{\nu}$, >C=O), 1134 ($\bar{\nu}$, -C-O); **^{1}H NMR ($CDCl_3$) (δ)** 1.17 (*s*, 6 H); 4.96 (*s*, 1 H); 7.32-7.36 (*m*, 5 H); **^{13}C NMR ($CDCl_3$) (δ)** 18.50 (-C(CH$_3$)$_2$-); 23.29 (-C(CH$_3$)$_2$-); 47.49 (-C(CH$_3$)$_2$-); 78.47 (HO-CH(Ph)-); 127.72 (*o*-Ph); 127.96 (*m*-Ph); 128.07 (*p*-Ph); 139.47 (C_{ipso}-Ph); 182.80 (COOH); **MS (CI)**. 194 (M^+), 176 (M-18), 107 (M-87); **Yield (%):** 25; **Elemental analysis (%)**: Calcd.: C, 68.02; H, 7.26; Found: C, 67.71; H, 7.55.

14. 3-Hydroxy-3,3-diphenylpropanoic acid; $C_{15}H_{14}O_3$; Mw = 242.09; **Melting point:** 217 ^{0}C; **IR (KBr):** $\bar{\nu}_{max}$ (cm^{-1}) 3478 ($\bar{\nu}$, -OH), 2908 ($\bar{\nu}$, -C(O)-OH), 1688 ($\bar{\nu}$, >C=O), 1232 ($\bar{\nu}$, -C-O); **^{1}H NMR (DMSO) (δ)** 3.28 (*s*, 2 H); 7.12-7.48 (*m*, 10 H); **^{13}C NMR (DMSO) (δ)** 45.33 (-CH$_2$-COOH); 75.71 (HO-C(Ph)$_2$-); 125.69 (*o*-Ph); 126.60 (*p*-Ph); 128.07 (*m*-Ph); 147.54 (C_{ipso}-Ph); 173.35 (COOH); **MS (CI)**. 242 (M^+) 183 (M-59); **Yield (%):** 40; **Elemental analysis (%):** Calcd.**: C,** 74.36; H**,**. 5.82; Found: C, 74.22; H, 6.01.

15. 2-(1'-(1'-Hydroxycyclohexyl))butanoic acid; $C_{10}H_{18}O_3$; M_w = 186.25; **Melting point:** 86 ^{0}C; **IR (KBr):** $\bar{\nu}_{max}$ (cm^{-1}) 3384 ($\bar{\nu}$,-OH), 2936 ($\bar{\nu}$, -C(O)-OH), 1710 ($\bar{\nu}$, >C=O), 1193 ($\bar{\nu}$, -C-O); **^{1}HNMR ($CDCl_3$) (δ)** 0.97 (*t, J* 7.3 Hz. 3H); 1.22-1.81 (*m*, 12H); 2.38 (*t, J* 7.5 Hz, 3H); **^{13}C NMR ($CDCl_3$) (δ)** 12.32 (-CH$_2$-CH$_3$); 19.41(-CH$_2$-CH$_3$) ; 21.59 (3'(5')-CH$_2$); 21.79 (5'(3')-CH$_2$); 25.51 (4'-CH$_2$); 34.19 (2'(6')-CH$_2$); 37.11 (6'(2')-CH$_2$); 56.48 (-CH(Et)COOH); 71.96 (1'-COH); 181.00 (COOH); (**MS (CI)** 186 (M+), 169 (M-17), 100 (M-87); **Yield (%):** 40; **Elemental analysis (%)**: Calcd.: C, 64.49; H, 9.74; Found: C, 64.23; H, 9.86.

3.2. Cell culture

Human: colon carcinoma LS174, cervix carcinoma HeLa, and melanoma Fem-X cells, were cultured as monolayers in the nutrient medium. Human myelogenous leukemia K562 cells were maintained as suspension culture. Nutrient medium was RPMI 1640 medium, supplemented with L-glutamine (3 mM), streptomycin (100 μg/ml), and penicillin (100 UI/mL), 10% heat inactivated (56 ^{0}C) fetal bovine serum (FBS) and 25 mM Hepes, and was adjusted to pH 7.2 by bicarbonate solution. The cells were grown at 37 ^{o}C in 5% CO_2 and humidified air atmosphere.

3.2.1. Investigated compounds

Stock solutions of 15 acids, in concentrations of 20 mM in DMSO, were diluted in nutrient medium to get final concentration in the range from 0-300 μM.

3.2.2. Preparation of peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMC) were separated from whole heparinized blood of two healthy volunteers by Lymphoprep^{TM} (Oslo, Norway) gradient centrifugation. Cells, washed three times

with Haemaccel (aqueous solution supplemented with 145 mM Na^+, 5.1 mM K^+, 6.2 mM Ca^+, 145 mM Cl^-, and 35 g/L gelatin polymers, pH=7.4) were counted and resuspended in nutrient medium.

3.2.3. Treatment of LS174, HeLa, Fem-x and K562 cells

Neoplastic LS174 cells were seeded (6,000 cells per well), while HeLa and Fem-X cells were seeded at density of 2,000 cells per well, into 96-well microtiter plates, and 20 h later, after the cell adherence, five different, double diluted, concentrations of investigated compounds were added to the wells. Only to 3,000 per well of K562 cells, investigated compounds were added 2h after the cell seeding.

3.2.4. Treatment of PBMC

PBMC Were seeded (150,000 cells per well), into nutrient medium without, or enriched with (5μg/ml) phytohaemaglutinin (PHA) (Welcome) in 96-well microtiter plates. Two hours later, investigated extracts were added to the wells, in triplicate, to five final concentrations, except to the control wells where only a nutrient medium was added to the cells. Nutrient medium with corresponding concentrations of compounds, but void of cells was used as the blank.

3.2.5. Determination of cell survival

Cell survival was determined indirectly by measuring total protein by the Kenacid Blue R (KBR) dye binding method [19]. Briefly, after 72 h of continuous agent's action, medium was discarded and target cells were washed twice with warm (37 ^oC) phosphate buffered saline (PBS). PBMC were always centrifuged 10 min at 2.000 rpm and supernatant was aspirated, leaving a small amount of medium, just to not disturb cells in the pellet. Then target cells were fixed for 20 min with 150 μl of a mixture of methanol and acetic acid (3:1) and stained 2-3 h with 0.04% Coomassie Brilliant Blue R-250 in 25% ethanol and 12% glacial acetic acid, washed, and bound dye was dissolved in desorbing solution (1M potassium acetate, 70% ethanol). Absorbance (A) at 570 nm was measured 2h later. To get cell survival (%), A of a sample with cells grown in the presence of various concentrations of the investigated agent was divided with control optical density (the A of control cells grown only in nutrient medium), and multiplied by 100. It was implied that A of the blank was always subtracted from A of the concentration of the corresponding sample with target cells. IC_{50} concentration was defined as the concentration of an agent inhibiting cell survival by 50%, compared with a vehicle-treated control.

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24. For superimposition of the molecules the arbitrary chosen, but *same* stereochemistry (*S* or *S,R*) of enantiomers or diastereomers are used. For the superimposition of cycloalkyl-substituted compound **10** the C2 and C6 of cyclohexyl ring were superimposed on the corresponding carbons of phenyl ring of the **1**. For the 3,3-diphenyl compound, one of phenyl rings is arbitrarily chosen.
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