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Chiral δ-iodo-γ-lactones derived from cuminaldehyde, 2,5-dimethylbenzaldehyde and piperonal: chemoenzymatic synthesis and antiproliferative activity

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The paper dedicated to Prof. Czesław Wawrzeńczyk on the occasion of 50th anniversary of his scientific work

ABSTRACT

Six enantiomeric pairs of β-aryl-δ-iodo-γ-lactones **8a–c**, **9a–c** derived from cuminaldehyde, 2,5-dimethylbenzaldehyde and piperonal were synthesized with high enantiomeric purities (ee 93–99%) from enantiomerically enriched allyl alcohols **3a–c**. The key step in the synthesis of lactones **8a** and **9a** was the kinetic resolution of racemic (*E*)-4-(4′-isopropylphenyl)but-3-en-2-ol **3a** by a lipase-catalysed transesterification. Among the five tested enzymes, the most effective and enantioselective was lipase B from *Candida antarctica* and after 2 h (−)-(*S*)-alcohol **3a** and (+)-(*R*)-propionate **5** were obtained with ee's ⩾99%. The transfer of chirality from alcohols (*S*)-**3a–c** and (*R*)-**3a–c** to γ,δ-unsaturated esters (*S*)-**6a–c** and (*R*)-**6a–c** via a stereoselective Johnson–Claisen rearrangement followed by hydrolysis and iodolactonization afforded the final lactones **8a–c** and **9a–c**. The configurations of their stereogenic centres were assigned based on crystallographic analysis and/or the iodolactonization mechanism. In 42 of 48 tests, the synthesized lactones showed antiproliferative activity against four selected cancer lines (Jurkat, D17, GL-1, CLBL-1). The *trans*-stereoisomers were more active than the *cis*-stereoisomers and the highest activity was found for lactone (−)-*trans*-(4*S*,5*R*,6*S*)-**9c** with a 1,3-benzodioxole substituent and both enantiomers of the *trans*-lactone with a 2,5-dimethylphenyl substituent: (+)-**9b** and (−)-**9b**. Among the *trans*-lactones, those with a (4*S*,5*R*,6*S*)-configuration exhibited higher activity than their enantiomers and the most significant difference was observed for the enantiomers of the *trans*-lactone with a 1,3-benzodioxole substituent **9c** (IC_{50} = 5.29 and 5.08 vs 36.47 and 33.77 for Jurkat and GL-1 cancer lines respectively).

1. Introduction

Many natural and synthetic compounds possessing both a lactone function and an aromatic ring in their structures exhibit interesting biological activities, for example, antifungal,[1–5] antiparasite,[6–8] anti-inflammatory,[9] antiplatelet,[10] anticonvulsant,[11,12] insecticidal[13] or antifeedant.[14,15] The most characteristic activity for this class of compounds is their cytotoxicity against different cancer cell lines. These properties have been proved for compounds of natural origin e.g. camptothecin,[16] *trans*-α,β-dibenzyl-γ-butyrolactone lignans,[17–20] eupomatilones[21] and styryl lactones.[22] A variety of methods have also been elaborated to synthesize lactones with antitumour activity bearing an aromatic ring, including analogues of homocamptothecin,[23] paraconic acid,[24,25] isochaihulactone[26] and (*S*)-goniothalamin,[27] synthetic styryl lactones,[28] α-hydroxylated lactone lignans[29] as well as a series of 4-methylideneisoxazolidin-5-ones,[30] γ-aryl-α-alkylidene-γ-lactones,[31] β-aryl-δ-methyl-α-methylene-δ-lactones[32] or β-aryl-δ-iodo-γ-lactones.[33]

In our previous paper we reported on a convenient synthesis of racemic antiproliferative iodolactones from simple aromatic aldehydes.[34] The most promising activity towards Jurkat (human leukaemia) and D17 (canine osteosarcoma) was observed for the

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cis-δ-iodo-γ-lactone with a *p*-isopropylphenyl ring **8a** synthesized from cuminaldehyde **1**. Taking into account that the spatial structure of the compound influences its biological activity, we herein report on the chemoenzymatic synthesis of the enantiomers of *cis*- and *trans*-β-(4-isopropylphenyl)-δ-iodo-γ-lactones. We also decided to expand our studies by investigating analogues with more sterically hindered aromatic substituents, derived from 2,5-dimethylbenzaldehyde and piperonal. The former represents lactones with a disubstituted phenyl ring, whereas lactones derived from piperonal contain a 1,3-benzodioxole ring; the structural element which is characteristic for many natural and synthetic compounds that exhibit biological activities including anticancer properties.[19,26,35,36] By preparing both enantiomeric forms of the target lactones, we were able to compare their antiproliferative activity towards selected cancer lines.

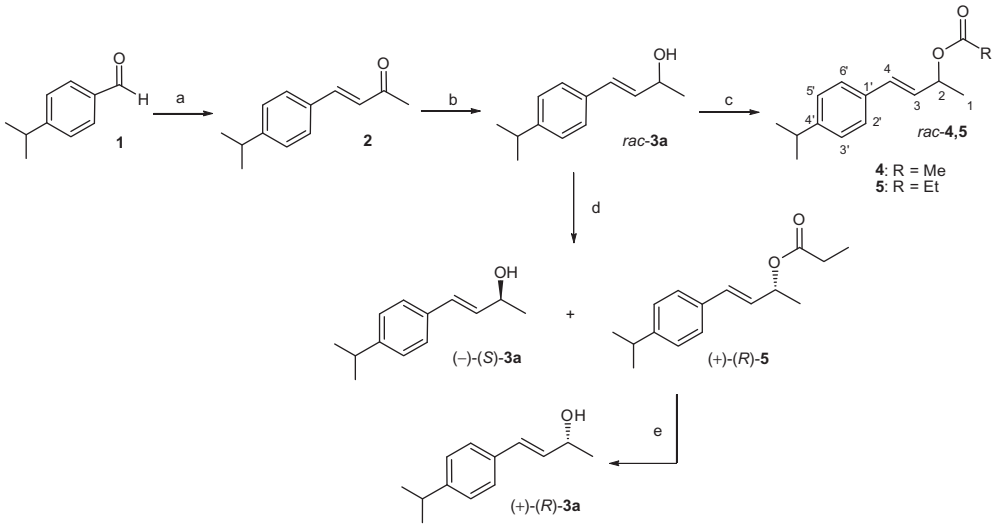
Lipases have been commonly used as enantioselective catalysts in the kinetic or dynamic kinetic resolution of racemic mixtures[37–41] and have found wide application in the asymmetric synthesis of chiral pharmaceuticals, agrochemicals, flavours and cosmetics.[42–45] The crucial biocatalytic step of our synthesis is the kinetic resolution of racemic 4-arylbut-3-en-2-ols, precursors of the corresponding lactones **8a**–**c** and **9a**–**c**. In our previous studies starting from corresponding aromatic aldehydes we synthesized (*E*)-4-(2′,5′-dimethylphenyl)but-3-en-2-ol **3b** and (*E*)-4-(benzo[*d*][1′,3′]-diox-5′-yl)but-3-en-2-ol **3c** and developed efficient methods of their lipase-catalysed transesterification.[46] As a result, we obtained the desired alcohols in both enantiomeric forms. Consequently, the aim of current studies was also the selection of lipases and establishing the conditions for the enantioselective transesterification of cuminaldehyde-derived (*E*)-4-(4′-isopropylphenyl)but-3-en-2-ol **3a**, the precursor of δ-iodo-γ-lactones **8a** and **9a**.

2. Results and discussion

Our approach to obtain pure enantiomers of iodolactones **8a**–**c** and **9a**–**c** was based on the enzymatic production of both enantiomers of their precursors, allyl alcohols **3a**–**c**. The biocatalytic method involved in the synthetic route was an enantioselective kinetic resolution of alcohols **3a**–**c** through lipase-catalysed transesterification. The conditions for the resolution of (*E*)-4-(2′,5′-dimethylphenyl)but-3-en-2-ol **3b** and (*E*)-4-(benzo[*d*][1′,3′]-diox-5′-yl)but-3-en-2-ol **3c** were established previously and enantiomerically pure or enriched alcohols were obtained (ee = 93 to over 99%).[46] In the present case, the first step was the screening of enzymes for the transesterification of (*E*)-4-(4′-isopropylphenyl)but-3-en-2-ol **3a**. Racemic alcohol **3a** was obtained via a two-step procedure starting from cuminaldehyde **1** by the Claisen–Schmidt condensation with acetone, followed by the reduction of the carbonyl group in the thus formed α,β-unsaturated ketone **2** using sodium borohydride.[34] Racemic esters **4** and **5**, standards in chiral GC analysis, were obtained in the reaction of *rac*-**3a** with the corresponding acyl chlorides (Scheme 1).

The selection of the enzymes was carried out at room temperature on an analytical scale, using vinyl propionate as the acyl donor and diisopropyl ether as the solvent. The products of the reaction were unreacted (−)-alcohol **3a** and (+)-propionate **5**. Five commercially available lipase preparations were screened for their catalytic activity and enantioselectivity. Lipozyme® (lipase from *Mucor miehei*), Lipozyme TL IM (*Thermomyces lanuginosus* lipase) and CAL-B (lipase B from *Candida antarctica*) were used in immobilized form, whereas CCL (lipase from *Candida cylindracea*) and Amano Lipase PS (lipase from *Burkholderia cepacia*) were applied in a free form. The results of the screening procedure are presented in Table 1.

The highest efficiency and enantioselectivity (E >1000) was observed for CAL-B; after 2 h reaction, the conversion of substrate reached 50% and both alcohol **3a** and ester **5** were obtained with excellent enantiomeric excesses (99% and >99%, respectively). Lipozyme TL IM catalysed the transesterification of alcohol **3a** at the same rate as CAL-B, but after 2 h, the ee of the alcohol was only 80%. Excellent resolution (99% ee of alcohol **3a** and ester **5**) was achieved after 4 h at 56% conversion. The enantioselectivity of the reaction was also very high (E >900). A comparable enantiomeric ratio was determined for this process with Amano Lipase PS but a significantly lower reaction rate was observed. In this case, the conversion exceeded 50% only after 24 h. The enantiomeric excess of unreacted alcohol **3a** gradually increased from 23% after 2 h up to 53% after 6 h, while the enantiomeric purity of propionate **5** remained stable (ee 99%). After extending the reaction time to 24 h, alcohol **3a** was formed with 97% ee. An increase in the enantiomeric excess of the unreacted alcohol, was observed for Lipozyme®-catalysed transesterification. Finally, after 48 h of the process at the 59% conversion, almost pure (+)-propionate **5** (ee 99%) and enantiomerically enriched (−)-alcohol **3a** (ee 94%) were



Scheme 1. Reagents and conditions: (a) acetone, NaOH, rt, 24 h; (b) $NaBH_4$, MeOH, 0 °C to rt, 3 h; (c) MeC(O)Cl or EtC(O)Cl, Et_2O, pyridine, 0 °C to rt, 24 h; (d) CAL-B, vinyl propionate, DIPE, rt, 2 h; (e) NaOH, reflux, 3 h.

Table 1
Results of enzymatic transesterification of racemic alcohol **3a** with vinyl propionate

Enzyme	Time (h)	Conversion[a] (mol %)	ee of (−)-(*S*)-alcohol **3a** (%)	ee of (+)-(*R*)-propionate **5** (%)	*E*[b]
Lipozyme®	2	24	33	99	
	4	42	62	99	
	6	48	79	99	
	24	53	84	99	
	48	59	94	99	>700
Lipozyme TL IM	2	50	80	99	
	4	56	99	99	>900
CCL	2	21	12	57	
	4	22	13	57	
	6	29	13	52	
	24	48	15	44	
	48	56	15	39	3
CAL-B	2	50	99	>99	>1000
Amano Lipase PS	2	22	23	99	
	4	44	39	99	
	6	47	53	99	
	24	53	97	99	>800

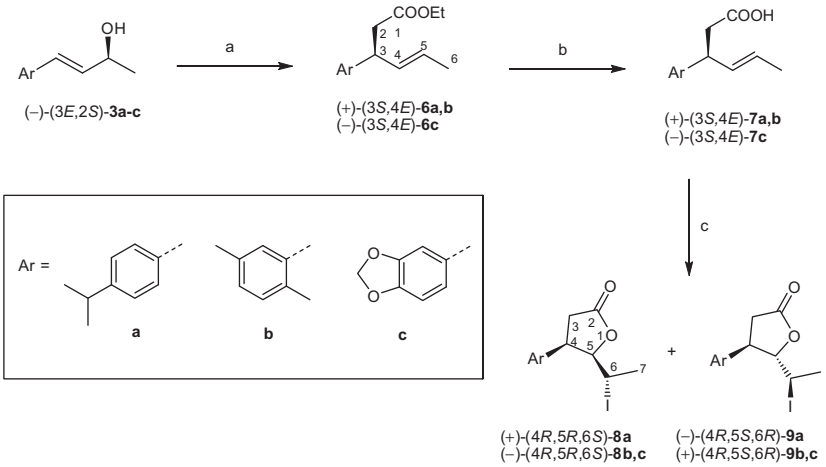
[a] According to chiral GC analysis.
[b] The enantiomeric ratio calculated at the highest conversion rate according to the following equation: $E = \ln[(1 - ee_s)/(1 + (ee_s/ee_p)]/\ln[(1 + ee_s)/(1 + (ee_s/ee_p)]$; ee_s-enantiomeric excess of unreacted alcohol, ee_p = enantiomeric excess of propionate.

obtained. The lowest reaction rate and poor enantioselectivity (E = 3) were seen for CCL. In this reaction, a conversion close to 50% was achieved after 24 h but the ee of alcohol **3a** was very low (15%) while the ee of propionate **5** decreased from 57% to 44%. A longer reaction time (48 h) was also not enough to achieve a satisfactory enantiomeric purity of the products.

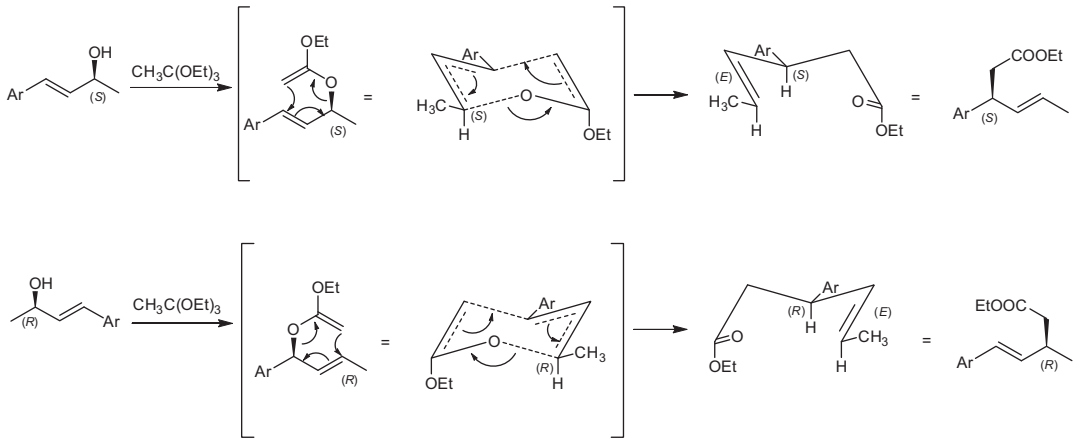
In order to make a choice of enzyme for the preparative-scale resolution, we were guided by such criteria as high enantiomeric excesses of the products and short reaction times. These were fulfilled to the greatest extent by CAL-B. The preparative-scale reaction was carried out starting with 5 g of alcohol **3a** under the same experimental conditions as described for the screening procedure. After 2 h the products were separated and purified to give (−)-alcohol **3a** (38% yield) and (+)-propionate **5** (43% yield) with ee 99% and >99%, respectively. Subsequently, (+)-propionate **5** was hydrolysed under alkaline conditions (NaOH) to afford (+)-alcohol **3a** in 74% yield and with >99% ee (Scheme 1).

The enantiomers of alcohol **3a** have not been obtained previously. At this stage, we could only presume their configurations based on the Kazlauskas′ rule[47] and the structural similarity of 4-(4′-isopropylphenyl)but-3-en-2-ol **3a** to a series of alcohols with 4-aryl-but-3-en-2-ol system resolved by CAL-B-mediated transesterification.[46,48] Under the same conditions as applied here for the resolution of alcohol **3a**, CAL-B preferentially catalysed esterification of the (+)-(*R*)-enantiomers, leaving the (−)-(*S*)-enantiomers unreacted. A negative specific rotation was determined for the unreacted enantiomer of **3a** after transesterification catalysed by CAL-B, which indicated an (*S*)-configuration and, consequently, an (*R*)-configuration for both (+)-propionate **5** and alcohol (+)-**3a** obtained as the product of its hydrolysis. Analogous reasoning was demonstrated previously in the assignment of the configuration for the CAL-B-produced enantiomers of allyl alcohol with 2,5-dimethylphenyl ring **3b**.[46]

Enantiomeric allyl alcohols (−)-(*S*)-**3a** and (+)-(*R*)-**3a** as well their analogues were obtained according to the literature;[46] (−)-(*S*)-**3b** (ee = 99%), (+)-(*R*)-**3b** (ee = 98%) and (−)-(*S*)-**3c** (ee = 99%), (+)-(*R*)-**3c** (ee = 93%), were used as the starting materials in the three-step synthesis of both enantiomers of *cis*-δ-iodo-γ-lactones **8a**–**c** and *trans*-δ-iodo-γ-lactones **9a**–**c**, respectively. The synthetic pathway for (−)-(*S*)-enantiomers of starting alcohols **3a**–**c**, is depicted in Scheme 2. The key step of this synthesis was the stereoselective Johnson–Claisen rearrangement of allyl alcohols **3a**–**c**.[49]



Scheme 2. Reagents and conditions: (a) $CH_3C(OEt)_3$, propionic acid, 138 °C; (b) (1) NaOH, reflux, 3 h; (2) HCl; (c) $NaHCO_3$, Et_2O, rt, 1 h, then I_2, KI, rt, 24 h.



Scheme 3. Transfer of chirality in the Johnson–Claisen rearrangement from enantiomeric allyl alcohols **3a**–**c** to the corresponding γ,δ-unsaturated esters **6a**–**c**.

Due to the high stereoselectivity of this reaction, the configuration at the C-2 position of the starting alcohol is transferred to the benzylic C-3 position of the forming γ,δ-unsaturated ester with a retention of the *E*-configuration of the double bond. For the alcohols with an *E*-configuration of the double bond, the configuration of stereogenic centre remains the same as that assigned for the starting alcohol due to the energetically favoured conformation of the chair-like transition state (Scheme 3). The result of the rearrangement of (*E*,*S*)-alcohols **3a**–**c** is therefore (*E*,*S*)-esters **6a**–**c** whereas the transformation of (*E*,*R*)-enantiomers of alcohols **3a**–**c** affords the corresponding (*E*,*R*)-esters **6a**–**c**. Chiral GC analysis of esters **6a**–**c** showed exactly the same enantiomeric compositions as those determined for the corresponding starting alcohols **3a**–**c**.

Enantiomeric γ,δ-unsaturated ethyl esters **6a**–**c** were hydrolysed under alkaline conditions to the corresponding enantiomeric acids **7a**–**c**. In the final step, acids **7a**–**c** were independently subjected to the iodolactonization using iodine and potassium iodide in the biphasic $Et_2O/NaHCO_3$ system to afford mixtures from which only products of 5-*exo*-cyclization (δ-iodo-γ-lactones) were isolated by silica gel column chromatography. As a result, starting from three pairs of enantiomeric acids, (+)-**7a**–**c** and (−)-**7a**–**c**, three enantiomeric pairs of *cis* δ-iodo-γ-lactones (+)-**8a**–**c** (−)-**8a**–**c** and three enantiomeric pairs of *trans* δ-iodo-γ-lactones (+)-**9a**–**c** and (−)-**9a**–**c** were obtained. Their structures were fully established by spectroscopic analysis. Furthermore, in the case of the crystalline enantiomers of five *cis*-isomers, X-ray analysis was carried out and the crystal structures were obtained. The anomalous dispersion for the iodine atom was sufficient to determine the absolute configurations of all of the stereogenic centres for these enantiomers. A configuration of (4*R*,5*R*,6*S*) was assigned for *cis*-lactones (+)-**8a** and (−)-**8c**, which were synthesized from (*S*)-alcohols **3a** and **3c**, respectively, and the opposite configuration was proved for enantiomers (−)-**8a** and (+)-**8c** obtained from (*R*)-alcohols **3a** and **3c** (Figs. 1 and 2). In the case of *cis*-lactone with a 2′,5′-dimethylphenyl ring, only X-ray analysis for the (−)-**8b** enantiomer obtained from (*S*)-alcohol **3b** was successful and confirmed its absolute configuration as (4*R*,5*R*,6*S*) (Fig. 3). Consequently, its enantiomer synthesized from (*R*)-alcohol **3b** possess a (4*S*,5*S*,6*R*)-configuration.

These findings were also essential to confirm the configurations of the enantiomeric esters **6a**–**c** formed during the Johnson–Claisen rearrangement. It is crucial that the relative configuration at C-4 of the lactone ring is retained during the iodolactonization of the corresponding acid. The (4*R*)-configuration of lactones **8a**–**c** must be the consequence of lactonization of (*S*)-acid **7** (Scheme 4) and analogously the (4*S*)-configuration of their enantiomers results from the (*R*)-configuration of acids **7a**–**c**. It should be noted that this inconsistency is the result of different priorities of substituents at C-4 after the formation of the lactone ring, and not an inversion of configuration during the reaction. The configurations of enantiomeric acids **7a**–**c** correspond with the configurations of their corresponding enantiomeric γ,δ-unsaturated ethyl esters **6a**–**c**. Taking into consideration the stereochemical outcome of Johnson–Claisen rearrangement depicted in Scheme 3, we are able to confirm the

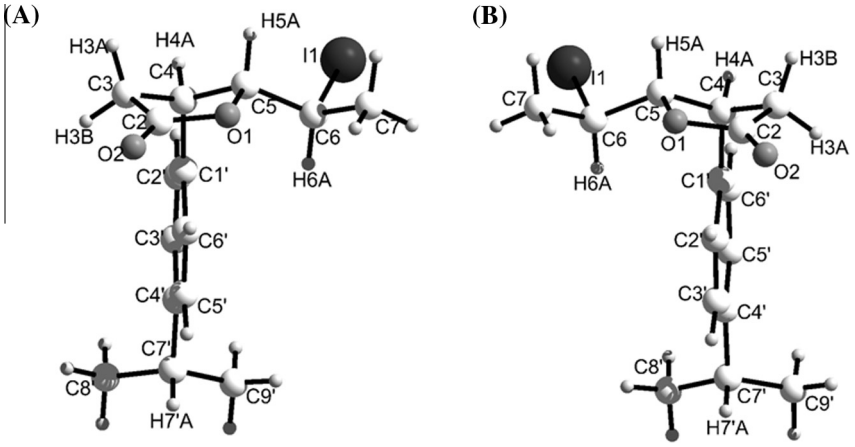


Figure 1. Crystal structures of the two enantiomeric *cis*-δ-iodo-γ-lactones derived from cuminaldehyde: (+)-(4*R*,5*R*,6*S*)-**8a** (A) and (−)-(4*S*,5*S*,6*R*)-**8a** (B) with crystallographic numbering.

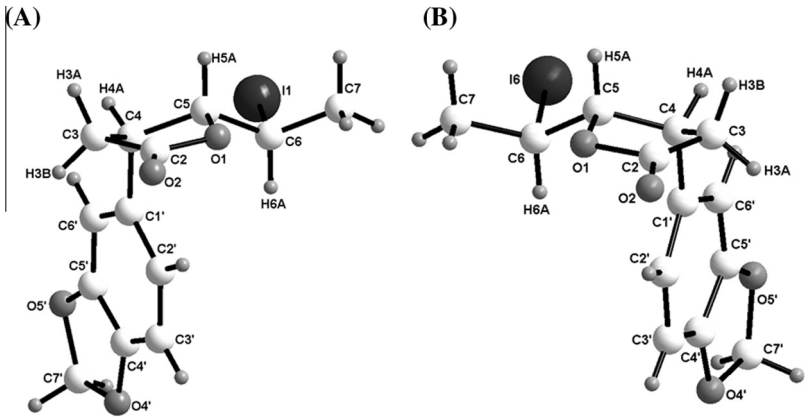


Figure 2. Crystal structures of the two enantiomeric *cis*-δ-iodo-γ-lactones derived from piperonal: (−)-(4*R*,5*R*,6*S*)-**8c** (A) and (+)-(4*S*,5*S*,6*R*)-**8c** (B) with crystallographic numbering.

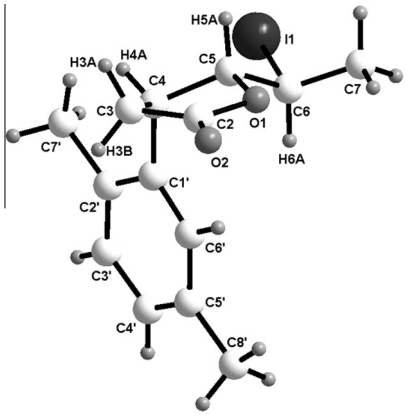
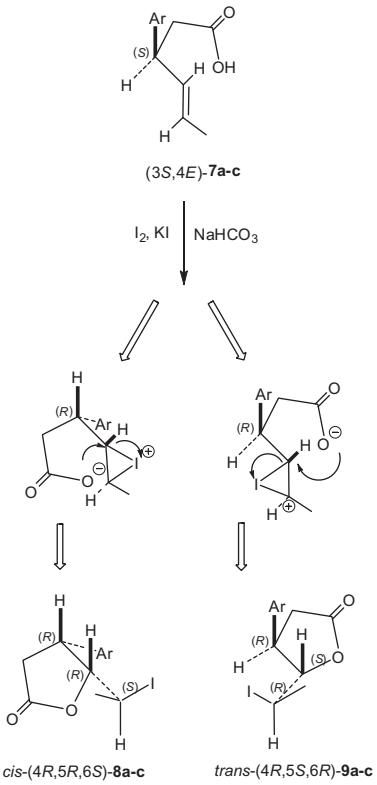


Figure 3. Crystal structures of *cis*-δ-iodo-γ-lactone derived from 2,5-dimethylbenzaldehyde: (−)-(4*R*,5*R*,6*S*)-**8b** with crystallographic numbering.

configurations of the starting enantiomeric allyl alcohols **3a** and **3b**, formerly presumed according to the Kazlauskas' rule.

For non-crystalline enantiomeric *trans*-δ-iodo-γ-lactones **9a–c**, the configuration at C-4 remained the same as for their corresponding *cis*-isomers. The configurations at C-5 and C-6 were assigned by taking into consideration an antiperiplanar orientation of the C–O and C–I bonds as a consequence of the mechanism of the iodolactonization in which the C-5 carbon is attacked by the carboxylate ion from the opposite side of the iodonium ion. This reaction mechanism, illustrated for the lactonization of (*S*)-acids and explaining the configurations of both *cis* and *trans* isomer, is shown in Scheme 4. The accuracy of the stereochemical assignment of the reaction was evidenced by the crystallographic analysis on the *cis*-isomers (Figs. 1 and 2). Based on the aforementioned mechanism, the configuration of *trans*-δ-iodo-γ-lactones **9a–c** derived from (*S*)-alcohols **3a–c** was assigned as (4*R*,5*S*,6*R*). Analogous proceeding allowed us to determine the configuration of their enantiomers as (4*S*,5*R*,6*S*).

Synthesized enantiomeric pairs of lactones: (+)-**8a–c**, (−)-**8a–c** and (−)-**9a–c**, (+)-**9a–c** were subjected to in vitro cytotoxic assay against four selected cancer cell lines: Jurkat (human T-cell leukaemia), D17 (canine osteosarcoma), GL-1 (canine B-cell leukaemia) and CLBL-1 (canine B-cell lymphoma cell line). Carboplatin was used as the reference drug. After treatment of the cell lines with various concentrations (0.05–50 μg/mL) of tested compounds, the metabolically active cells were measured by MTT assay. The effect of studied compounds on the cell viability is shown in Table 2 as IC_{50} values.



Scheme 4. Mechanism of iodolactonization of acids (*S*)-**6a–c** showing the configurations of stereogenic centres of forming diastereomeric γ-lactones.

In 42 tests, the studied lactones exhibited considerable concentration-dependent inhibitory effects on the proliferation of the examined cell lines. In eight tests the values of IC_{50} did not exceed 10 and in five cases the IC_{50} values were even lower than those determined for carboplatin. In six cases the activity of studied lactones was very low (IC_{50} >50). The highest antiproliferative effect towards all cancer lines was observed for lactone (−)-*trans*-(4*S*,5*R*,6*S*)-**9c** (entry 12) and both enantiomers of lactone *trans*-**9b** (entries 7 and 8) whereas the least active was the (−)-(4*R*,5*R*,6*S*)-enantiomer of *cis*-lactone **8c** (entry 9). Considering the effect of the spatial structure on the antiproliferative activity, it can be seen that *trans*-isomers were more active than *cis*, which was particularly noticeable for lactones with either a 2,5-dimethylphenyl ring (entries 5–8)

Table 2
The antiproliferative activity of synthesized iodolactones against the selected cancer cell lines

Entry	Compound	IC_{50} (μg/mL)[a]			
		Jurkat	D17	GL-1	CLBL-1
1	(+)-*cis*-(4*R*,5*R*,6*S*)-**8a**	38.68 ± 3.69[b]	>50	24.26 ± 6.56	28.15 ± 2.68
2	(−)-*cis*-(4*S*,5*S*,6*R*)-**8a**	17.89 ± 5.90	45.46 ± 3.01	20.88 ± 5.37	27.03 ± 8.29
3	(−)-*trans*-(4*R*,5*S*,6*R*)-**9a**	38.93 ± 8.65	44.40 ± 0.50	20.28 ± 6.04	19.15 ± 3.05
4	(+)-*trans*-(4*S*,5*R*,6*S*)-**9a**	34.75 ± 3.30	37.54 ± 4.40	14.48 ± 4.44	26.43 ± 4.27
5	(−)-*cis*-(4*R*,5*R*,6*S*)-**8b**	29.40 ± 1.66	19.39 ± 2.60	25.65 ± 4.3	8.07 ± 1.21
6	(+)-*cis*-(4*S*,5*S*,6*R*)-**8b**	33.84 ± 6.85	26.57 ± 3.54	20.48 ± 3.95	8.01 ± 0.96
7	(+)-*trans*-(4*R*,5*S*,6*R*)-**9b**	14.30 ± 3.72	14.81 ± 3.39	14.24 ± 6.06	7.10 ± 0.65
8	(−)-*trans*-(4*S*,5*R*,6*S*)-**9b**	16.16 ± 4.73	16.99 ± 4.88	16.30 ± 4.69	4.76 ± 0.52
9	(−)-*cis*-(4*R*,5*R*,6*S*)-**8c**	>50	>50	>50	24.72 ± 2.45
10	(+)-*cis*-(4*S*,5*S*,6*R*)-**8c**	>50	>50	29.85 ± 5.21	9.58 ± 1.13
11	(+)-*trans*-(4*R*,5*S*,6*R*)-**9c**	36.47 ± 5.43	36.84 ± 2.51	33.77 ± 2.14	13.46 ± 2.09
12	(−)-*trans*-(4*S*,5*R*,6*S*)-**9c**	5.29 ± 0.31	16.65 ± 2.56	5.08 ± 0.41	9.10 ± 0,96
13	Carboplatin	21.37 ± 4.00	13.10 ± 4.3	7.06 ± 1.15	n.i.[c]

[a] IC_{50}—anticancer drug concentration inhibiting cell viability by 50%.
[b] Results obtained from more than 3 independent experiments (four wells each) and expressed as mean value ± SD.
[c] n.i.—not investigated.

or a 1,3-benzodioxole ring (entries 9–12). For *trans*-lactones derived from 2,5-dimethylbenzaldehyde, the activities of both enantiomeric forms were comparable (entries 7 and 8). In the group of lactones derived from cuminaldehyde and piperonal, in almost all cases the *trans*-enantiomers with a (4*S*,5*R*,6*S*)-configuration exhibited higher activity than their antipodes. A particularly large difference between the activity of two enantiomers was found for *trans*-lactone with a 1,3-benzodioxole substituent against Jurkat and GL-1 line; in this case IC_{50} values determined for (−)-(4*S*,5*R*,6*S*)-**9c** (entry 12) were over 7-fold lower than those calculated for (+)-(4*R*,5*S*,6*R*)-**9c** (entry 11). In the case of *cis*-isomers, the observed relationship was not so clear and depended on an aryl substituent and tested cancer line. For the *cis*-lactone with a 4-isopropylphenyl substituent, IC_{50} values were generally lower for the (−)-(4*S*,5*S*,6*R*)-**8a** enantiomer (entry 2) and the same tendency was observed for the lactone with a 1,3-benzodioxole substituent (entry 10) towards GL-1 and CLBL-1. However, both enantiomers of this lactone were not active against Jurkat and D17. Comparing the activity of *cis*-enantiomers lactone with a 2,5-dimethylphenyl system, (−)-**8b** with a (4*R*,5*R*,6*S*)-configuration (entry 5) was slightly more active towards Jurkat and D17, but less active towards GL-1 and the activity of both enantiomers towards CLBL-1 was comparable.

3. Conclusion

In conclusion, six pairs of enantiomeric β-aryl-δ-iodo-γ-lactones have been obtained with ee = 93–99% using a combined chemoenzymatic route starting from enantiomerically enriched allyl alcohols **3a–c**. Lipase from *C. antarctica* was the most efficient and enantioselective biocatalyst in the process of transesterification of racemic (*E*)-4-isopropylphenyl-but-3-en-2-ol **3a** and showed a preference towards the (*R*)-enantiomer to produce (*R*)-propionate and (*S*)-alcohol with excellent enantiomeric purities (ee >99%). The integration of this biocatalytic step with the transfer of chirality by a Johnson–Claisen rearrangement and stereoselective iodolactonization may be involved to produce a variety of chiral lactones with the defined configurations of all stereogenic centres, which in turn allows us to find relationships between the stereochemical structure and the biological activity of a particular isomer. The results of cytotoxic assays showed the high antiproliferative activity of most synthesized lactones and indicated the *trans*-stereoisomers were the more active. The relationship between the configuration of stereogenic centres and the activity was often influenced by the cell line type and the aryl substituent.

4. Experimental

4.1. General

Synthesized compounds were purified by silica gel column chromatography (Kieselgel 60, 230–400 mesh, Merck) using various mixtures of hexane and acetone as the eluents. Analytical Thin Layer Chromatography (TLC) was carried out on silica gel coated aluminium plates (DC-Alufolien Kieselgel 60 F_{254}, Merck) with mixture of hexane/acetone (4:1, v/v) as the developing system. Compounds were visualized by spraying the plates with solution of 1% $Ce(SO_4)_2$ and 2% $H_3[P(Mo_3O_{10})_4]$ in 10% H_2SO_4.

Gas chromatography (GC) analysis was performed on Agilent Technologies 6890N instrument equipped with autosampler, split injection (50:1), FID detector and hydrogen as a carrier gas. The progress of reactions was monitored using DB-17 column [(50%-phenyl)-methylpolysiloxane, 30 m × 0.25 mm × 0.25 μm]. Products of iodolactonization **8a–c** and **9a–c** were analysed using the following temperature programme: injector 280 °C, detector (FID) 280 °C, initial column temperature: 220 °C (1 min), 220–260 °C (3 °C min^{-1}), 260–300 °C (30 °C min^{-1}), final column temperature 300 °C (2 min). The conditions for the analysis of alcohol **3a**, acetate **4**, propionate **5**, esters **6a–c** and acids **7a–c** were as follows: injector 280 °C, detector (FID) 280 °C, initial column temperature: 100 °C (1 min), 100–200 °C (20 °C min^{-1}), 200–300 °C (30 °C min^{-1}), final column temperature 300 °C (1 min). Chiral gas chromatography (CGC) was carried out using Varian CP Chirasil-DEX CB column (25 m × 0.25 mm × 0.25 μm) for acetate **4**, propionate **5**, esters **6a–c**, acids **7a–c**, iodolactones **8a–c** and **9b**,**c** and CycloSil-B column (30% heptakis(2,3-di-*O*-methyl-6-*O*-*t*-butyldimethylsilyl)-β-cyclodextrin in DB-1701, 30 m × 0.25 mm × 0.25 μm) for iodolactones **9a**. The following temperature programmes were applied: injector 280 °C, detector 250 °C; for enantiomers of acetate **4** and propionate **5**: initial column temperature 80 °C, 80–200 °C (5 °C min^{-1}), final column temperature 200 °C (1 min); for enantiomers of esters **6a–c**, acids **7a–c**, iodolactones **8b**,**c** and iodolactones **9b**,**c**: initial column temperature 50 °C, 80–200 °C (0.5 °C min^{-1}), final column temperature 200 °C (1 min); for enantiomers of iodolactones **8a**: initial column temperature 80 °C, 80–200 °C (0.5 °C min^{-1}), final column temperature 250 °C (1 min); for enantiomers of iodolactones **9a**: initial column temperature 80 °C, 80–250 °C (2 °C min^{-1}), final column temperature 250 °C (1 min). Due to the inseparability of its enantiomers, alcohol **3a** was analysed after derivatization into the corresponding acetate by treatment with acetyl chloride.

Nuclear magnetic resonance spectroscopy (^1H NMR, ^{13}C NMR, HMQC) was carried out on a Bruker Avance II 600 MHz

spectrometer for $CDCl_3$ solutions. Residual solvent signals (δ_H = 7.26, δ_C = 77.0) were used as references for chemical shifts. Infrared spectroscopy was conducted using Mattson IR 300 Thermo Nicolet spectrophotometer using KBr pellets or in liquid films. High resolution mass spectra (HRMS) were recorded using electron spray ionization (ESI) technique on spectrometer Waters ESI-Q-TOF Premier XE. Melting points (uncorrected) were measured with a Boetius apparatus. Refractive indexes were measured on Carl Zeiss Jena refractometer. Specific optical rotations were measured for samples dissolved in methylene chloride (concentration denoted in g/100 mL) on a Jasco P-2000-Na digital polarimeter with intelligent Remote Module (iRM) controller.

X-Ray data were collected on a Xcalibur Ruby diffractometer (Mo-Kα radiation; λ = 0.71073 Å). X-Ray data were collected at 100 K using an Oxford Cryosystem device. Data reduction and analysis were carried out with the CrysAlis 'RED' or CrysAlisPro programme (Oxford Diffraction, Wrocław (Poland), 2001, 2003). Analytical numeric absorption correction using a multifaceted crystal model based on expressions derived by Clark and Reid was applied.[50] Space groups were determined, based on systematic absences and intensity statistics. The structures were solved by direct methods using the SHELXS programme and refined using all F^2 data, as implemented by the SHELXL97 programme.[51] Non-hydrogen atoms were refined with anisotropic displacement parameters. All H atoms were placed at calculated positions. Before the last cycle of refinement all H atoms were fixed and were allowed to ride on their parent atoms. The absolute configurations were confirmed by anomalous dispersion effects in diffraction measurements on the crystal. Crystal data for (+)-**8a**, (−)-**8a**, (−)-**8b**, (−)-**8c**, (+)-**8c** reported herein, have been deposited with the Cambridge Crystallographic Data Centre (CCDC) as supplementary publication numbers 1408082, 1408083, 1446200, 1446201 and 1446202, respectively. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB12 1EZ, UK (fax + 44-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

4.2. Chemicals and enzymes

Cuminaldehyde **1** (purity 98%), acetyl chloride (purity ⩾ 99%), propionyl chloride (purity 98%), triethyl orthoacetate (purity 97%), vinyl propionate (purity 98%), diisopropyl ether (DIPE, purity ⩾ 98.5%) and diatomaceous earth (Celite 560) were purchased from Sigma–Aldrich (USA). Analytical grade chemicals: propionic acid, sodium hydrogen carbonate, sodium thiosulfate, sodium hydroxide, potassium iodide, iodine, anhydrous magnesium sulfate, organic solvents, sodium chloride and hydrochloric acid (35–37%) were purchased from P.P.H. Stanlab (Poland), Chempur (Poland) and POCH (Poland). Lipozyme® immobilized from *Mucor miehei* (30 U/g), lipase acrylic resin from *Candida antarctica* (CAL-B, >5000 U/g), lipase from *Candida cylindracea* (CCL, 5.18 U/mg) and Amano Lipase PS from *Burkholderia cepacia* (⩾30,000 U/g) were purchased from Sigma-Aldrich (USA). Immobilized silica-granulated *Thermomyces lanuginosus* lipase preparation (Lipozyme TL IM, 250 U/g) was purchased from Novozymes (Denmark).

Both enantiomers of (*E*)-4-(2′,5′-dimethylphenyl)but-3-en-2-ol [(−)-(*S*)-**3b**, ee = 98% and (+)-(*R*)-**3b**, ee = 98%] and (*E*)-4-(benzo[*d*][1′,3′]-diox-5′-yl)but-3-en-2-ol [(−)-(*S*)-**3c**, ee = 99% and (+)-(*R*)-**3c**, ee = 93%] were obtained previously by kinetic resolution of their racemic forms.[46]

4.3. Synthesis of racemic (*E*)-4-(4′-isopropylphenyl)but-3-en-2-ol *rac*-3a

Racemic alcohol **3a** was synthesized via a two-step synthesis from cuminaldehyde **1** (Scheme 1). The detailed procedures, yields of reactions, physical and spectroscopic data of the intermediate ketone **2** and alcohol **3a** were published in our previous paper.[34]

4.4. General procedure for the synthesis of racemic esters 4,5

A solution of *rac*-**3a** (2 mmol) in 10 mL of dry diethyl ether and 1 mL of dry pyridine was placed on a magnetic stirrer in an ice bath. The mixture was stirred for 0.5 h after which 12 mmol of the appropriate acyl chloride (acetyl chloride or propionyl chloride) were added dropwise. The reaction was continued at room temperature. When the substrate was fully esterified (TLC, 24 h), 5 mL of 0.1 M HCl were added and the products were extracted with diethyl ether (3 × 50 mL). Organic layers were pooled, washed with saturated $NaHCO_3$, brine (until neutral) and dried over anhydrous $MgSO_4$. After filtration the solvent was evaporated in vacuo and crude ester was subjected to the column chromatography (hexane/acetone, 15:1) to afford pure product **4**, **5**.

4.4.1. (*E*)-4-(4′-Isopropylphenyl)but-3-en-2-yl acetate *rac*-4

Obtained as the product of reaction of *rac*-**3a** (0.4 g, 2 mmol) with acetyl chloride. Yield 90% (0.43 g); pale-brown liquid; n_D^{20} = 1.5138; $t_R(S)$ = 18.9 min, t_R (*R*) = 19.1 min; IR (film, cm^{-1}): 1738 (s), 1654 (w), 1513 (m), 1240 (s), 1041 (m), 968 (m), 831 (m); ^1H NMR (600 MHz, $CDCl_3$): δ 1.24 (d, *J* = 6.9 Hz, 6H, $(CH_3)_2CH$–), 1.40 (d, *J* = 6.6 Hz, 3H, CH_3-1), 2.07 (s, 3H, $CH_3C(O)$–), 2.89 (septet, *J* = 6.9 Hz, 1H, $(CH_3)_2CH$–), 5.52 (m, 1H, H-2), 6.15 (dd, *J* = 15.9, 6.9 Hz, 1H, H-3), 6.59 (d, *J* = 15.9 Hz, 1H, H-4), 7.18 (m, 2H, H-3′, H-5′), 7.32 (m, 2H, H-2′, H-6′); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.4 (C-1), 21.4 ($CH_3C(O)$–), 23.9 ($(CH_3)_2CH$–), 33.8 ($(CH_3)_2CH$), 71.1 (C-2), 126.5, 126.6 (C-2′, C-3′, C-5′, C-6′), 127.9 (C-3), 131.5 (C-4), 133.9 (C-1′), 148.8 (C-4′), 170.3 ($CH_3C(O)$–). HRMS: calcd for $C_{15}H_{20}O_2$ $[M+Na]^+$: 255.1361, found 255.1367.

4.4.2. (*E*)-4-(4′-Isopropylphenyl)but-3-en-2-yl propionate *rac*-5

Obtained as the product of reaction of *rac*-**3a** (0.6 g, 3 mmol) with propionyl chloride. Yield 91% (0.71 g); pale-brown liquid; n_D^{20} = 1.5079; $t_R(S)$ = 20.4 min, t_R (*R*) = 20.5 min; IR (film, cm^{-1}): 1735 (s), 1654 (w), 1513 (m), 1187 (s), 1040 (m), 968 (m), 812 (m); ^1H NMR (300 MHz, $CDCl_3$): δ 1.15 (t, *J* = 7.8 Hz, 3H, $CH_3CH_2C(O)$–), 1.24 (d, *J* = 6.9 Hz, 6H, $(CH_3)_2CH$–), 1.40 (d, *J* = 6.6 Hz, 3H, CH_3-1), 2.34 (q, *J* = 7.8 Hz, 2H, $CH_3CH_2C(O)$–), 2.89 (septet, *J* = 6.9 Hz, 1H, $(CH_3)_2CH$–), 5.53 (m, 1H, H-2), 6.14 (dd, *J* = 15.9, 6.9 Hz, 1H, H-3), 6.58 (d, *J* = 15.9 Hz, 1H, H-4), 7.18 (m, 2H, H-3′, H-5′), 7.32 (m, 2H, H-2′, H-6′); ^{13}C NMR (150 MHz, $CDCl_3$): δ 9.1 ($CH_3CH_2C(O)$–), 20.4 (C-1), 23.9 ($(CH_3)_2CH$–), 27.9 ($CH_3CH_2C(O)$–), 33.8 ($(CH_3)_2CH$–), 70.9 (C-2), 126.5, 126.6 (C-2′, C-3′, C-5′, C-6′), 128.0 (C-3), 131.4 (C-4), 134.0 (C-1′), 148.8 (C-4′), 173.7 ($CH_3CH_2C(O)$–). HRMS: calcd for $C_{16}H_{22}O_2$ $[M+Na]^+$: 269.1518, found 269.1530.

4.5. Enzymatic transesterification of (*E*)-4-(4′-isopropylphenyl)but-3-en-2-ol *rac*-3a

4.5.1. Analytical scale

Racemic alcohol **3a** (100 mg) was dissolved in 3 mL of diisopropyl ether (DIPE), after which 50 mg of lipase and 1 mL of vinyl propionate were added. The mixture was stirred in a 10 mL vial on a magnetic stirrer at room temperature. Samples of the reaction mixtures (0.3 mL) were withdrawn at various time intervals and filtered through diatomaceous earth. Before chiral GC analysis, samples were derivatized as follows: 0.5 mL of dried pyridine, 0.3 mL of acetyl chloride and 1 mL were added to the filtered samples and the mixture was stirred for 0.5 h at room temperature. After dilution with 1 mL of 1 M HCl and 1 mL of diethyl ether, the organic layer was separated, washed with saturated $NaHCO_3$, brine and dried over anhydrous $MgSO_4$. Extracts were filtrated

and solvent was completely evaporated in vacuo. The residues were dissolved in 1 mL of hexane, transferred to the vials and analysed by GC.

4.5.2. Preparative scale

Lipase B from *C. antartica* (50% w/w of alcohol) was added to the solution of racemic alcohol **3a** (5 g, 26 mmol) and vinyl propionate (15 mmol) in 50 mL of DIPE placed in the 250 mL round-bottom flask. The reaction was carried out on a magnetic stirrer at room temperature. After 2 h the enzyme was removed by filtration and the organic solvent was evaporated in vacuo. Enantiomerically enriched products were separated by column chromatography (hexane/acetone, 10:1).

4.5.2.1. (−)-(2*S*,3*E*)-4-(4′-Isopropylphenyl)but-3-en-2-ol (−)-3a. Yield 38% (1.92 g); brown liquid; ee = 99% (determined after derivatization into acetate); $[\alpha]_D^{20} = -24.0$ (*c* 5.4, CH_2Cl_2); physical and spectroscopic data identical to those reported earlier for *rac*-**3a**.[34]

4.5.2.2. (+)-(2*R*, 3*E*)-4-(4′-Isopropylphenyl)but-3-en-2-yl propionate (+)-5. Yield 43% (2.8 g); pale-brown liquid; ee >99%; t_R = 20.5 min; $[\alpha]_D^{20} = +109.8$ (*c* 1.6, CH_2Cl_2); physical and spectroscopic data in accordance with those reported herein for *rac*-**5**.

4.6. Hydrolysis of (+)-(2*R*,3*E*)-4-(4′-isopropylphenyl)but-3-en-2-yl propionate (+)-5

Ester (+)-**5** (11 mmol) was heated at reflux in 30 mL of 2.5% ethanolic solution of NaOH. When the substrate was hydrolysed completely (3 h, TLC), ethanol was evaporated in vacuo and the residue was diluted with water. The product was extracted with methylene chloride (3 × 40 mL). Organic fractions were combined, washed with brine until neutral and dried over anhydrous $MgSO_4$. Evaporation of solvent in vacuo yielded pure alcohol (+)-**3a**.

4.6.1. (+)-(2*R*,3*E*)-4-(4′-Isopropylphenyl)but-3-en-2-ol (+)-3a

Yield 74% (1.61 g); brown liquid; ee >99% (determined after derivatization into acetate); $[\alpha]_D^{20} = +24.1$ (*c* 2.4, CH_2Cl_2, ee >99%); physical and spectroscopic data identical to those reported earlier for *rac*-**3a**.[34]

4.7. General procedure for the synthesis of γ,δ-unsaturated esters 6a–c

A solution of allyl alcohol **3** (10 mmol) in 30 mL of triethyl orthoacetate (164 mmol) with a drop of propionic acid was heated at 138 °C with continuous removal of ethanol by distillation. When the substrate reacted completely (24 h, TLC, GC), the mixture was purified by column chromatography (hexane/acetone, 40:1) to obtain pure ester **6**.

4.7.1. (+)-(3*S*,4*E*)-3-(4′-Isopropylphenyl)hex-4-enoic acid ethyl ester (+)-6a

Obtained from alcohol (−)-**3a** (1.9 g, 10 mmol); yield 85% (2.2 g); yellow liquid; ee = 99%; t_R = 158.9; $[\alpha]_D^{20} = +8.3$ (*c* 4.0, CH_2Cl_2); physical and spectroscopic data identical to those reported previously for *rac*-**6a**.[34]

4.7.2. (−)-(3*R*,4*E*)-3-(4′-Isopropylphenyl)hex-4-enoic acid ethyl ester (−)-6a

Obtained from alcohol (+)-**3a** (1.6 g, 8 mmol); yield 77% (1.68 g); yellow liquid; ee >99%; t_R = 158.4 min; $[\alpha]_D^{20} = -8.3$ (*c* 2.3, CH_2Cl_2); physical and spectroscopic data identical to those reported previously for *rac*-**6a**.[34]

4.7.3. (+)-(3*S*,4*E*)-3-(2′,5′-Dimethylphenyl)hex-4-enoic acid ethyl ester (+)-6b

Obtained from alcohol (−)-**3b** (1.7 g, 9 mmol). Yield 81% (1.92 g); light brown liquid; $n_D^{20} = 1.5160$; ee = 99%; t_R = 149.7 - min.; $[\alpha]_D^{20} = +22.1$ (*c* 1.2, CH_2Cl_2); IR (film, cm^{-1}): 1736 (s), 1502 (m), 1161 (s), 1037 (m), 968 (m), 809 (m); ^1H NMR (600 MHz, $CDCl_3$): δ 1.19 (t, *J* = 7.2 Hz, 3H, $-OCH_2CH_3$), 1.65 (dd, *J* = 6.0, 1.2 Hz, 3H, CH_3-6), 2.30, 2.33 (two s, 6H, 2 × CH_3-Ph), 2.66 (dd, *J* = 15.0, 7.2 Hz, 1H, one of CH_2-2), 2.71 (dd, *J* = 15.0, 8.4 Hz, 1H, one of CH_2-2), 4.03 (m, 1H, H-3), 4.09 (q, *J* = 7.2 Hz, 2H, $-OCH_2CH_3$), 5.45 (dqd, *J* = 15.6, 6.0, 0.6 Hz, 1H, H-5), 5.53 (ddq, *J* = 15.6, 7.2, 1.2 Hz, 1H, H-4), 6.92 (d, *J* = 7.8 Hz, H-4′), 6.96 (s, 1H, H-6′), 7.03 (d, *J* = 7.8 Hz, 1H, H-3′); ^{13}C NMR (150 MHz, $CDCl_3$): δ 14.1 ($-OCH_2CH_3$), 17.9 (C-6), 19.0, 21.1 (2 × CH_3-Ph), 40.2 (C-3), 40.3 (C-2), 60.2 ($-OCH_2CH_3$), 125.4 (C-5), 126.8 (C-6′), 126.9 (C-4′), 130.3 (C-3′), 132.5 (C-2′), 132.8 (C-4), 135.4 (C-5′), 141.1 (C-1′), 172.1 (C-1); HRMS: calcd for $C_{16}H_{22}O_2$ $[M+H]^+$: 247.1698, found 247.1701.

4.7.4. (−)-(3*R*,4*E*)-3-(2′,5′-Dimethylphenyl)hex-4-enoic acid ethyl ester (−)-6b

Obtained from alcohol (+)-**3b** (1.6 g, 9 mmol). Yield 90% (2.01 g); light brown liquid; ee = 98%; t_R = 149.0 min.; $[\alpha]_D^{20} = -22.3$ (*c* 1.4, CH_2Cl_2); physical and spectroscopic data identical to those reported herein for (+)-**6b**.

4.7.5. (−)-(3*S*,4*E*)-3-(Benzo[*d*][1′,3′]-dioxol-5′-yl)hex-4-enoic acid ethyl ester (−)-6c

Obtained from alcohol (−)-**3c** (1.8 g, 9 mmol). Yield 88% (2.16 g); light brown liquid; ee = 99%; t_R = 197.0 min.; $[\alpha]_D^{20} = -2.5$ (*c* 0.7, CH_2Cl_2); physical and spectroscopic data identical to those reported for *rac*-**6c** in our previous paper.[15]

4.7.6. (+)-(3*R*,4*E*)-3-(Benzo[*d*][1′,3′]-dioxol-5′-yl)hex-4-enoic acid ethyl ester (+)-6c

Obtained from alcohol (+)-**3c** (1.6 g, 8 mmol). Yield 82% (1.79 g); light brown liquid; ee = 93%; t_R = 196.6 min.; $[\alpha]_D^{20} = +2.1$ (*c* 0.8, CH_2Cl_2); physical and spectroscopic data identical to those reported for *rac*-**6c**.[15]

4.8. General protocol for the synthesis of γ,δ-unsaturated acids 7a–c

Ester **6** (7 mmol) was heated at reflux in a 2.5% ethanolic solution of NaOH (30 mL). When the reaction was finished (3 h, TLC, GC), ethanol was evaporated in vacuo, and the residue was diluted with water and washed by diethyl ether (20 mL) to remove organic impurities. The water layer was acidified with 1 M HCl and the product was extracted with diethyl ether (3 × 20 mL). The combined ether fractions were washed with brine until neutral and dried over anhydrous $MgSO_4$. After evaporation of the solvent in vacuo, pure acid **7** was obtained.

4.8.1. (+)-(3*S*,4*E*)-3-(4′-Isopropylphenyl)hex-4-enoic acid (+)-7a

Obtained from ester (+)-**6a** (2 g, 7 mmol); yield 86% (1.51 g); brown liquid; ee = 99%; t_R = 218.9 min; $[\alpha]_D^{20} = +7.8$ (*c* 1.7, CH_2Cl_2); physical and spectroscopic data consistent with those reported previously for *rac*-**7a**.[34]

4.8.2. (−)-(3*R*,4*E*)-3-(4′-Isopropylphenyl)hex-4-enoic acid (−)-7a

Obtained from ester (−)-**6a** (1.66 g, 6 mmol); yield 90% (1.34 g); brown liquid; ee >99%; t_R = 219.4 min; $[\alpha]_D^{20} = -7.9$ (*c* 1.9, CH_2Cl_2); physical and spectroscopic data in accordance with those reported previously for *rac*-**7a**.[34]

4.8.3. (+)-(3*S*,4*E*)-3-(2′,5′-Dimethylphenyl)hex-4-enoic acid (+)-7b

Obtained from ester (+)-**6b** (1.8 g, 7 mmol); yield 76% (1.21 g); brown, oily liquid; ee = 99%; t_R = 189.3 min; $[\alpha]_D^{20}$ = +23.0 (*c* 1.5, CH_2Cl_2); IR (film, cm^{-1}): 3100–2600 (s,b), 1708 (s), 1502 (s), 1440 (m), 967 (m), 810 (s); ^1H NMR (600 MHz, $CDCl_3$) δ 1.65 (d, *J* = 6.0 Hz, 3H, CH_3-6), 2.31, 2.32 (two s, 6H, 2 × CH_3-Ph), 2.71 (dd, *J* = 15.6, 7.2 Hz, 1H, one of CH_2-2), 2.75 (dd, *J* = 15.6, 9.0 Hz, 1H, one of CH_2-2), 4.02 (m, 1H, H-3), 5.46 (dq, *J* = 15.0, 6.0 Hz, 1H, H-5), 5.53 (dd, *J* = 15.0, 6.6 Hz, 1H, H-4), 6.93 (d, *J* = 7.2 Hz, 1H, H-4′), 6.95 (s, 1H, H-6′), 7.04 (d, *J* = 7.2 Hz, 1H, H-3′); ^{13}C NMR (150 MHz, $CDCl_3$) δ 17.9 (C-6), 19.0 (2 × CH_3-Ph), 39.8 (C-3), 39.9 (C-2), 125.6 (C-5), 126.8 (C-6′), 127.0 (C-4′), 130.4 (C-3′), 132.5 (C-4), 132.6 (C-2′), 135.6 (C-5′), 140.9 (C-1′), 178.2 (C-1); HRMS: calcd for $C_{14}H_{18}O_2$ $[M-H]^-$: 217.1228, found 217.1220.

4.8.4. (−)-(3*S*,4*E*)-3-(2′,5′-Dimethylphenyl)hex-4-enoic acid (−)-7b

Obtained from ester (−)-**6b** (1.9 g, 8 mmol); yield 77% (1.3 g); brown, oily liquid, ee = 98%; t_R = 188.7 min; $[\alpha]_D^{20}$ = −22.9 (*c* 1.5, CH_2Cl_2); spectroscopic data identical to those reported herein for (+)-**7a**.

4.8.5. (−)-(3*S*,4*E*)-3-(Benzo[*d*][1′,3′]-dioxol-5′-yl)hex-4-enoic acid (−)-7c

Obtained from ester (−)-**6c** (2 g, 7 mmol); yield 90% (1.6 g); dense, brown liquid; ee = 99%; t_R = 234.1; $[\alpha]_D^{20}$ = −7.4 (*c* 0.1, CH_2Cl_2); spectroscopic data consistent with those reported previously for *rac*-**7a**.[15]

4.8.6. (+)-(3*S*,4*E*)-3-(Benzo[*d*][1′,3′]-dioxol-5′-yl)hex-4-enoic acid (+)-7c

Obtained from ester (+)-**6c** (1.6 g, 6 mmol); yield 92% (1.31 g); brown, oily liquid; ee = 93%, t_R = 234.9 min; $[\alpha]_D^{20}$ = +7.0 (*c* 0.1, CH_2Cl_2); spectroscopic data identical to those reported for *rac*-**7a**.[15]

4.9. General procedure for the synthesis of iodolactones 8a–c and 9a–c

A solution of acid **7** (6 mmol) in 20 mL of diethyl ether was stirred with 20 mL of saturated solution of $NaHCO_3$ for 1 h at room temperature. Afterwards a solution of I_2 (12 mmol) and KI (59 mmol) in water (5 mL) was added dropwise and the mixture was stirred until the substrate reacted completely (24 h, TLC, GC). The mixture was washed with saturated $Na_2S_2O_3$ and the organic fraction was separated and dried over anhydrous $MgSO_4$. After evaporation of diethyl ether in vacuo the products were separated by column chromatography (hexane/acetone, 20:1).

4.9.1. Data of iodolactones obtained from acid (+)-7a (1.4 g, 6 mmol)

4.9.1.1. (+)-*cis*-(4*R*,5*R*,6*S*)-5-(1-Iodoethyl)-4-(4′-isopropylphenyl)-dihydrofuran-2-one (+)-8a. Yield 28% (0.60 g); colourless crystals (crystallization from mixture hexane/acetone 20:1); mp 63–65 °C; ee = 99%; t_R = 166.0 min; $[\alpha]_D^{20}$ = +1.9 (*c* 0.8, CH_2Cl_2); spectroscopic data in accordance with those reported for *rac*-**8a**.[34] Crystal data for (+)-**8a**: $C_{15}H_{19}IO_2$, *M* = 358.20, orthorhombic, $P2_12_12_1$, *a* = 8.090(2), *b* = 9.405(2), *c* = 19.074(3) Å, *V* = 1451.3 (5) Å^3, *Z* = 4, D_c = 1.639 Mg m^{-3}, *T* = 100(2) K, *R* = 0.044, *wR* = 0.097 (5163 reflections with $I > 2\sigma(I)$) for 163 variables. CCDC 1408082.

4.9.1.2. (−)-*trans*-(4*R*,5*S*,6*R*)-5-(1-Iodoethyl)-4-(4′-isopropylphenyl)dihydrofuran-2-one (−)-9a. Yield 20% (0.43 g); brown, dense liquid; ee = 99%; t_R = 70.1 min; $[\alpha]_D^{20}$ = −8.5 (*c* 1.7, CH_2Cl_2); spectroscopic data identical to those reported for *rac*-**9a**.[34]

4.9.2. Data of iodolactones obtained from acid (−)-7a (1.25 g, 5 mmol)

4.9.2.1. (−)-*cis*-(4*S*,5*S*,6*R*)-5-(1-Iodoethyl)-4-(4′-isopropylphenyl)dihydrofuran-2-one (−)-8a. Yield 33% (0.63 g); colourless crystals; (crystallization from mixture hexane/acetone 20:1); mp 68–70 °C; ee >99%; t_R = 163.4 min; $[\alpha]_D^{20}$ = −1.9 (*c* 1.0, CH_2Cl_2); spectroscopic data consistent with those reported for *rac*-**8a**.[34] Crystal data for (−)-**8a**: $C_{15}H_{19}IO_2$, *M* = 358.20, orthorhombic, $P2_12_12_1$, *a* = 8.093(2), *b* = 9.432(2), *c* = 19.072(3) Å, *V* = 1455.8(5) Å^3, *Z* = 4, D_c = 1.634 Mg m^{-3}, *T* = 100(2) K, *R* = 0.026, *wR* = 0.047 (11086 reflections with $I > 2\sigma(I)$) for 163 variables. CCDC 1408083.

4.9.2.2. (+)-*trans*-(4*S*,5*R*,6*S*)-5-(1-Iodoethyl)-4-(4′-isopropylphenyl)dihydrofuran-2-one (+)-9a. Yield 19% (0.41 g); brown, dense liquid; ee >99%; t_R = 70.3 min; $[\alpha]_D^{20}$ = +8.6 (*c* 0.6, CH_2Cl_2); spectroscopic data identical to those reported for *rac*-**9a**.[34]

4.9.3. Data of iodolactones obtained from acid (+)-7b (1.1 g, 5 mmol)

4.9.3.1. (−)-*cis*-(4*R*,5*R*,6*S*)-5-(1-Iodoethyl)-4-(2′,5′-dimethylphenyl)dihydrofuran-2-one (−)-8b. Yield 39% (0.67 g); colourless crystals (crystallization from mixture hexane/acetone 5:1); mp = 104–108 °C, ee >99%; t_R = 205.1 min; $[\alpha]_D^{20}$ = −31.1 (*c* 0.8, CH_2Cl_2); IR (film, cm^{-1}): 1783 (s), 1506 (m), 1169 (s), 1028 (m), 982 (m), 835 (s); ^1H NMR (600 MHz, $CDCl_3$) δ 2.05 (d, *J* = 6.6 Hz, 3H, CH_3-7), 2.29 (s, 3H, CH_3-5′), 2.40 (s, 3H, CH_3-2′), 2.59 (d, *J* = 17.4 Hz, 1H, one of CH_2-3), 3.10 (dd, *J* = 17.4, 9.0 Hz, 1H, one of CH_2-3), 3.91 (dq, *J* = 10.8, 6.6 Hz, 1H, H-6), 4.11 (m, 1H, H-4), 4.87 (dd, *J* = 10.8, 6.6 Hz, 1H, H-5), 6.88 (s, 1H, H-6′), 7.00 (d, *J* = 7.8 Hz, 1H, H-4′), 7.06 (d, *J* = 7.8 Hz, 1H, H-3′); ^{13}C NMR (150 MHz, $CDCl_3$) δ 20.2 (CH_3-2′), 21.2 (CH_3-5′), 22.9 (C-6), 25.7 (C-7), 38.9 (C-3), 39.6 (C-4), 88.2 (C-5), 126.3 (C-6′), 128.5 (C-4′), 131.0 (C-3′), 133.1 (C-2′), 136.1, 136.2 (C-1′, C-5′), 176.5 (C-2); HRMS: calcd for $C_{14}H_{17}IO_2$ $[M+Na]^+$: 367.0171, found 367.0179. Crystal data for (−)-**8b**: $C_{14}H_{17}IO_2$, *M* = 344.17, triclinic, *P*1, *a* = 8.437(3), *b* = 9.510(3), *c* = 9.517(3) Å, α = 97.86(2), β = 93.39(3), γ = 114.76(3)°, *V* = 681.1(4) Å^3, *Z* = 2, D_c = 1.678 Mg m^{-3}, *T* = 100 (2) K, *R* = 0.070, *wR* = 0.157 (3297 reflections with $I > 2\sigma(I)$) for 308 variables. CCDC 1446200.

4.9.3.2. (+)-*trans*-(4*R*,5*S*,6*R*)-5-(1-Iodoethyl)-4-(2′,5′-dimethylphenyl)dihydrofuran-2-one (+)-9b. Yield 20% (0.35 g); dense, brown liquid; ee = 99%; t_R = 220.1 min; $[\alpha]_D^{20}$ = +14.6 (*c* 0.3, CH_2Cl_2); IR (film, cm^{-1}): 1785 (s), 1505 (m), 1188 (s), 1150 (s), 998 (m), 812 (m); ^1H NMR (600 MHz, $CDCl_3$) δ 1.83 (d, *J* = 6.6 Hz, 3H, CH_3-7), 2.32 (s, 3H, CH_3-5′), 2.37 (s, 3H, CH_3-2′), 2.52 (dd, *J* = 18.6, 7.2 Hz, 1H, one of CH_2-3), 3.12 (dd, *J* = 18.6, 10.2 Hz, 1H, one of CH_2-3), 3.82 (ddd, *J* = 10.2, 7.2, 4.8 Hz, 1H, H-4), 4.35–4.40 (two m, 2H, H-5, H-6), 6.99 (d, *J* = 7.8 Hz, 1H, H-4′), 7.02 (s, 1H, H-6′), 7.07 (d, *J* = 7.8 Hz, 1H, H-3′); ^{13}C NMR (151 MHz, $CDCl_3$) δ 19.6 (CH_3-2′), 21.1 (CH_3-5′), 23.6 (C-7), 28.8 (C-6), 38.0 (C-3), 41.1 (C-4), 89.3 (C-5), 126.5 (C-6′), 128.2 (C-4′), 130.9 (C-3′), 132.0 (C-2′), 136.8 (C-5′), 139.5 (C-1′), 174.9 (C-2); HRMS: calcd for $C_{14}H_{17}IO_2$ $[M+Na]^+$: 367.0171, found 367.0172.

4.9.4. Data of iodolactones obtained from acid (−)-7b (1.2 g, 5 mmol)

4.9.4.1. (+)-*cis*-(4*S*,5*S*,6*R*)-5-(1-Iodoethyl)-4-(2′,5′-dimethylphenyl)dihydrofuran-2-one (+)-8b. Yield 40% (0.75 g); colourless crystals (crystallization from mixture hexane/acetone 5:1); mp 108–112 °C; ee >99%; t_R = 202.2 min; $[\alpha]_D^{20}$ = +31.1 (*c* 0.6, CH_2Cl_2); spectroscopic data identical to those reported herein for (−)-**8b**.

4.9.4.2. (−)-*trans*-(4*S*,5*R*,6*S*)-5-(1-Iodoethyl)-4-(2′,5′-dimethylphenyl)dihydrofuran-2-one (−)-9b. Yield 21% (0.39 g); brown, dense liquid; ee = 98%; t_R = 218.6 min; $[\alpha]_D^{20}$ = −14.4 (*c* 0.3, CH_2Cl_2); spectroscopic data identical to those reported herein for (+)-**9b**.

4.9.5. Data of iodolactones obtained from acid (−)-7c (1.5 g, 6 mmol)

4.9.5.1. (−)-*cis*-(4*R*,5*R*,6*S*)-5-(1-Iodoethyl)-4-(benzo[*d*][1′,3′]-dioxol-5′-yl)dihydrofuran-2-one (−)-8c. Yield 40% (0.92 g); yellow crystals; mp 148−151 °C; (crystallization from mixture hexane/acetone 20:1); ee >99%, t_R = 249.1 min; $[\alpha]_D^{20}$ = −4.8 (*c* 0.1, CH_2Cl_2); spectroscopic data in accordance with those reported for *rac*-**8c**.[15] Crystal data for (−)-**8c**: $C_{13}H_{13}IO_4$, *M* = 360.13, triclinic, *P*1, *a* = 6.794(2), *b* = 8.701(2), *c* = 11.499(3) Å, *α* = 98.03(2), *β* = 107.18(3), *γ* = 90.08(2)°, *V* = 642.4(3) Å^3, *Z* = 2, D_c = 1.862 Mg m^{-3}, *T* = 100.01(10) K, *R* = 0.026, *wR* = 0.047 (4666 reflections with $I > 2\sigma(I)$) for 325 variables. CCDC 1446201.

4.9.5.2. (+)-*trans*-(4*R*,5*S*,6*R*)-5-(1-Iodoethyl)-4-(benzo[*d*][1′,3′]-dioxol-5′-yl)dihydrofuran-2-one (+)-9c. Yield 24% (0.55 g); dense, brown liquid; ee >99%, t_R = 263.3 min; $[\alpha]_D^{20}$ = +7.4 (*c* 0.2, CH_2Cl_2); spectroscopic data identical to those reported for *rac*-**9c**.[15]

4.9.6. Data of iodolactones obtained from acid (+)-7c (1.2 g, 5 mmol)

4.9.6.1. (+)-*cis*-(4*S*,5*S*,6*R*)-5-(1-Iodoethyl)-4-(benzo[*d*][1′,3′]-dioxol-5′-yl)dihydrofuran-2-one (+)-8c. Yield 34% (0.62 g); colourless crystals; (crystallization from mixture hexane/acetone 20:1); mp 143−146 °C; ee >99%; t_R = 247.3 min; $[\alpha]_D^{20}$ = +4.8 (*c* 0.3, CH_2Cl_2); spectroscopic data consistent with those reported herein for (−)-**8c**. Crystal data for (+)-**8c**: $C_{13}H_{13}IO_4$, *M* = 360.13, triclinic, *P*1, *a* = 6.825(2), *b* = 8.736(2), *c* = 11.533(3) Å, *α* = 97.64 (2), *β* = 106.97(3), *γ* = 91.64(3)°, *V* = 650.2(3) Å^3, *Z* = 2, D_c = 1.839 Mg m^{-3}, *T* = 100.00(10) K, *R* = 0.058, *wR* = 0.136 (3278 reflections with $I > 2\sigma(I)$) for 316 variables. CCDC 1446202.

4.9.6.2. (−)-*trans*-(4*S*,5*R*,6*S*)-5-(1-Iodoethyl)-4-(benzo[*d*][1′,3′]-dioxol-5′-yl)dihydrofuran-2-one (−)-9c. Yield 21% (0.38 g); dense, brown liquid; ee = 93%; t_R = 263.9 min; $[\alpha]_D^{20}$ = −7.1 (*c* 0.1, CH_2Cl_2); spectroscopic data in accordance with those reported herein for (+)-**9c**.

4.10. Cell lines and assay for antiproliferative activity

The human Jurkat cell line (T-cell leukaemia) was obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and kindly provided from the collection of the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland. D17 cell line (canine osteosarcoma) was obtained from American Type Culture Collection (ATCC), Rockville, MD, USA. The canine cell line GL-1 (B-cell leukaemia) was obtained from Yasuhito Fujino and Hajime Tsujimoto from the University of Tokyo, Department of Veterinary Internal Medicine, Tokyo, Japan[52] and the CLBL-1 (B-cell lymphoma cell line) was obtained from Barbara C. Rütgen, Institute of Immunology, Department of Pathobiology of the University of Veterinary Medicine, Vienna, Austria.[53] All cell lines were maintained in RPMI 1640 culture medium (Institute of Immunology and Experimental Therapy, Wroclaw, Poland) supplemented with 2 mM L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin and 10% foetal bovine serum (FBS) (Sigma–Aldrich, Steinheim, Germany) for GL-1, D17 and Jurkat cells and 20% FBS for CLBL-1 cells. The culture was maintained in a CO_2 incubator at 37 °C in a humidified atmosphere. Cells were cultured in 75 mL cell culture flask (Corning, NY, USA) and subcultivated every other day to keep at a optimal density (50–70% of confluence).

The initial solutions of the tested compounds were freshly prepared for each experiment by dissolving each compound in 1 mL of DMSO (POCH, Gliwice, Poland). For poorly soluble compounds ultrasound treatment was applied to achieve their complete solubility. The culture media was used as a solvent for obtaining further solutions. To determine cell viability, 1×10^4 cells/well were seeded in 96-well-plates (NUNC, Roskilde, Denmark). The examined substances were prepared within a concentration range of 0.05–50 μg/mL in culture medium (DMSO or ethanol concentration was less than 1% in each dilution). The cells were incubated in medium alone or medium containing either the vehicle control (DMSO) or increasing concentrations of the tested substances for 72 h. After the respective incubation time, 20 μL of MTT solution (5 mg/mL) was added to every well for a further 4 h, then 80 μL of lysis buffer (225 mL of DMF, 67.5 g of SDS, 275 mL of distillated water) were added. The optical density of culture wells was measured after 24 h using a spectrophotometric microplate reader (Elx800, BioTek, Winooski, USA) at a reference wavelength of 570 nm. The optical density of formed formazan in control (untreated) cells was taken as 100%. Viability of test samples was determined as: % Viability = (average OD for test group/average OD for control group) × 100. The results of cytotoxic activity for the tested compounds are expressed as IC_{50} values (Table 2).

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