Babeş-Bolyai University
Faculty of de Chemistry and Chemical Engineering
Department of Biochemstry and Biochemical Engineering

Chiral heterocyclic alcohols: chemoenzimatic synthesis and metabolic effects

Ph.D. Thesis Abstract

Scientific advisor
Prof. Dr. Florin Dan Irimie

Ph.D. student
Maria Trif

Cluj-Napoca
2012

Babeş-Bolyai University
Faculty of de Chemistry and Chemical Engineering
Department of Biochemstry and Biochemical Engineering

Maria TRIF

Chiral heterocyclic alcohols: chemoenzimatic synthesis and metabolic effects

Ph.D. Thesis Abstract

Jury

President: Conf. Dr. Cornelia Majdik – Dean of the Faculty of Chemistry and Chemical Engineering, Babeş-Bolyai University, Cluj-Napoca

Scientific advisor: Prof. Dr. Florin Dan Irimie – Faculty of Chemistry and Chemical Engineering, Babeş-Bolyai University, Cluj-Napoca

Reviewers:

Conf. Dr. Adela Pintea - Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca

Prof. Univ. Dr. Valentin Zaharia - Faculty of Pharmacy, University of Medicine and Pharmacy "Iuliu Haţieganu", Cluj-Napoca

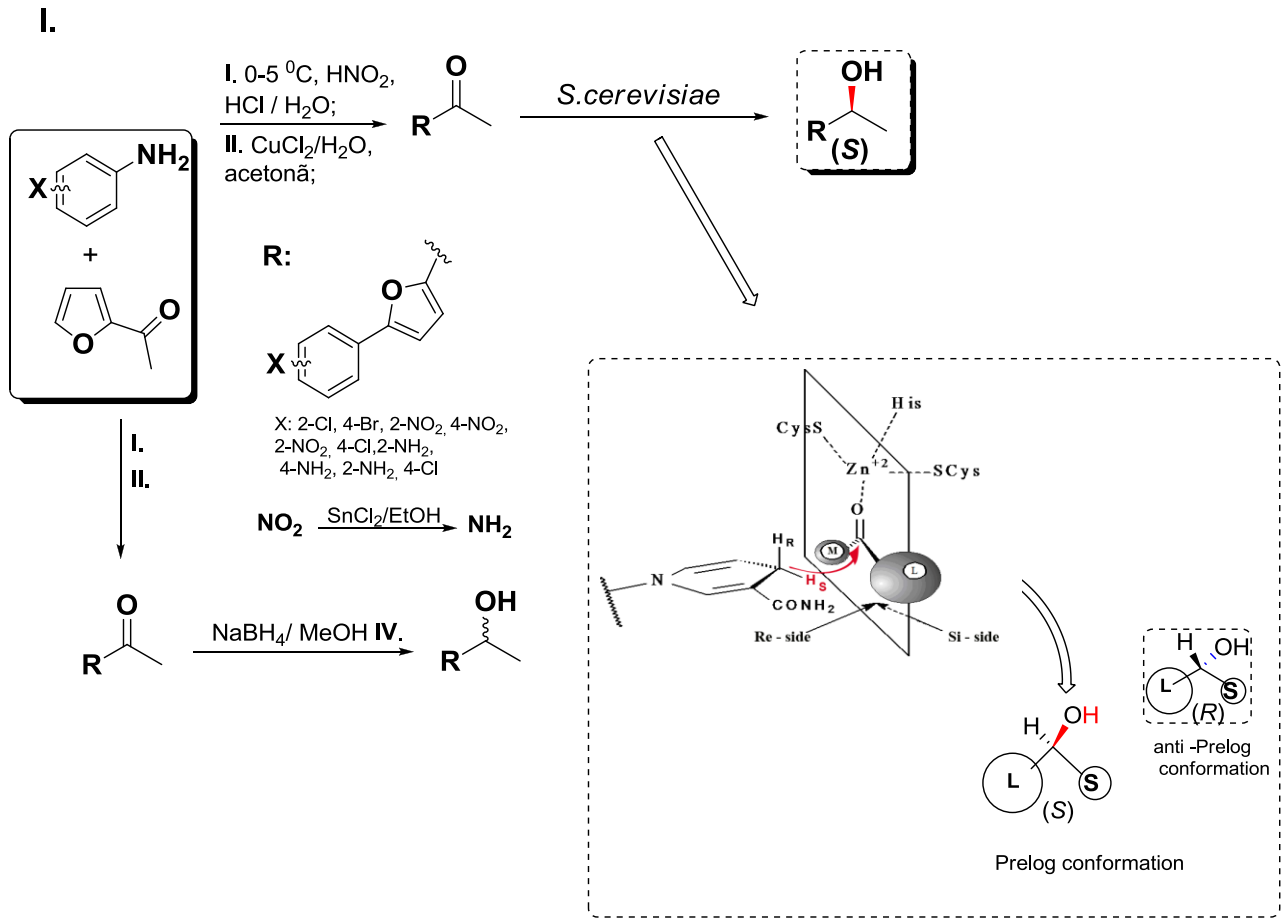
Prof. Univ. Dr. Ioan Grosu - Faculty of Chemistry and Chemical Engineering, Babeş-Bolyai University, Cluj-Napoca

Defense : 2 March 2012

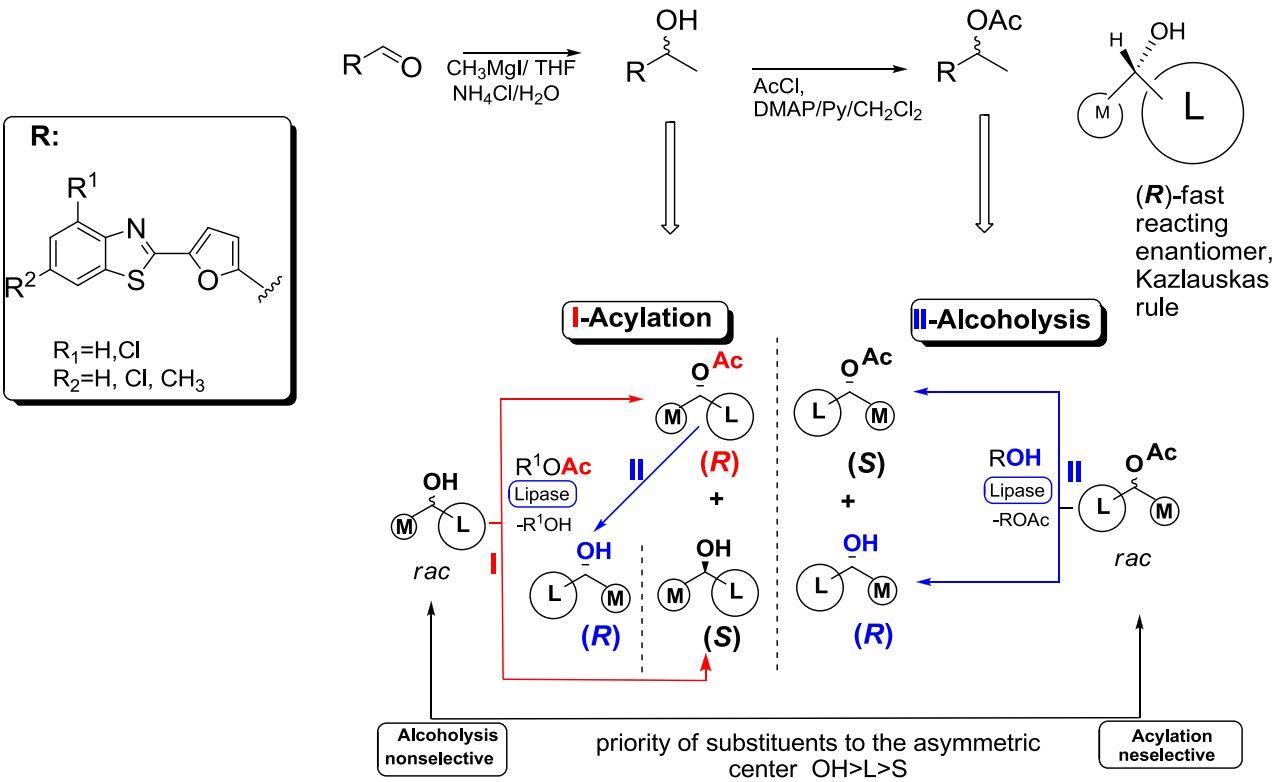
Content

Keywords: biocatalysis, baker's yeast, phenylfuran-2-yl ethanols, heterocyclic alcohols, enzymatic acylation , enzymatic methanolysis, selectivity, lipase, enantioselective synthesis.

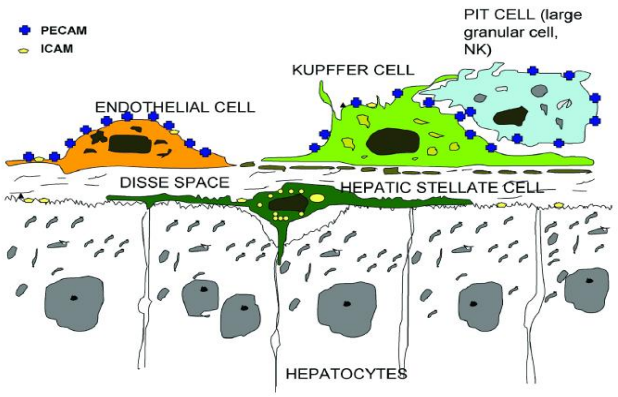
Graphical abstract:



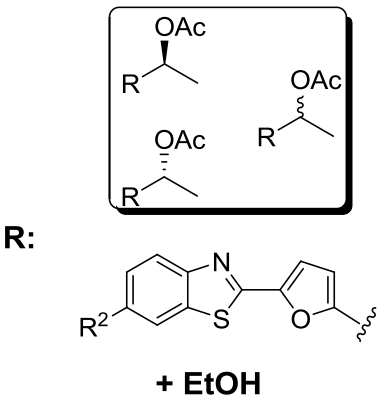
II.



III.



(Ramadori et al. 2008)[1]



1. Introduction

Biocatalysis is a valuable and attractive application for developing new synthesis methods, for a wide range of products in fields such as pharmaceutical and agrochemical industry, fine chemistry, forensic science, microbiology or genetic engineering. Pharmaceutical companies are increasingly using cellular and enzymatic bio-catalysis (with microorganisms or purified enzymes, respectively) as a source of enantio- and diastereopure products, that can be obtained by selective synthesis. In this respect, new microorganisms and/or their enzymes are subject to intensive screening.

There is a growing interest in the pharmaceutical field to obtain pure optically active compounds and precursors for drugs, showing high selectivity, high specificity, and reduced side effects. At the same time, synthesis strategies with minimal negative impact on the environment ("green processes"), high productivity, high degree use of raw material and minimum energy consumption are being sought. Today about 10% of all methods for drug synthesis involve the use of biocatalysts and more than half of all bioactive substances have at least one chiral center.

Biocatalysis may be a real alternative for achieving high chemo-, regio- and/or stereoselectivity under mild conditions (pH, temperature, pressure) and without the use of toxic or hazardous reagents in case of difficult chemical reactions. Also, biocatalysts tend to show better selectivity characteristics as compared to other catalysts. Integrating bioinformatics in genomics and exploiting biodiversity facilitates the discovery of new biocatalysts (microorganisms and / or enzymes); in addition, genetic engineering of existing enzymes and optimization of working conditions may allow adaptation to the requirements of the pursued aim.

A large number of studies prove the applicability of enzyme- or whole cell system-mediated biocatalysis in the synthesis of optically pure secondary heterocyclic alcohols. Both the heterocycles within and entire heterocyclic secondary alcohol structures can be precursors in the synthesis of chiral drugs with complex structures and diverse biological activity. Our interest was directed towards lipase-mediated kinetic resolution of racemic alcohols and enantioselective yeast-mediated biocatalytic reduction of prochirale ketones, in a synthetic strategy that combined biosynthesis steps with a chemocatalytic ones. These methods have proven potential for developing sustainable and cost-effective technologies, respecting the principles of green chemistry.

2. Theroretical part (*literature data*)

3. Research objectives

During the last decades, the development of new methods for stereoselective synthesis of enantiomerically enriched heterocyclic alcohols has attracted strong interest. Due to their biological and pharmacological properties, such molecules are found as active components in many drugs (Chapter 2.2). Biocatalysts are particularly suitable for the synthesis of enantiopure compounds, because of their many specific advantages. Moreover, biocatalytic processes proved to be more environmentally friendly, safer and less polluting. Biocatalysis as a strategy for chiral compound synthesis is no longer restricted to laboratory use, but has evolved to be employed on a large scale in the pharmaceutical and fine chemistry industry.

A first aim of this research was the synthesis of chiral alcohols with phenyl-furan and furyl-benzothiazole structures and of their acetates. We further aimed to assess the biological effects of the methyl-substituted acetate of furyl-benzothiazole. We have chosen these compounds because both the heterocycles within their structures and the secondary alcohols as a whole can be precursors in the synthesis of chiral drugs with complex structures and diverse biological activities. Compounds containing reactive functional groups, which can attach to other structural components of the final drug molecules, allowing easy conversion of intermediates in other compounds with high enantiopurity, meet the necessary criteria to be used as building blocks in pharmaceutical industry. Due to their structure, phenyl-furans and furyl-benzothiazols meet these criteria, acting as donor/acceptor of hydrogen bonds and also provide positive ions. Substitution of the heterocyclic ring with an aromatic or heteroaromatic one can further increased pharmacophoric diversity.

Our research work focused on three distinct directions:

1. Stereoselective synthesis of new differently substituted (hetero)aryl (*S*)-1-(5-phenylfuran-2-yl) ethanols (*S*)-**2a-e**, of both enantiomers of 1-(5-(benzo[d]thiazole-2-yl)furan-2-yl) ethanols (*R*)-(*S*)-**3-a-d** and their corresponding enantiopure acetates (*R*)-(*S*)-**4-a-d**. Two different biocatalytic methods were selected for their synthesis:

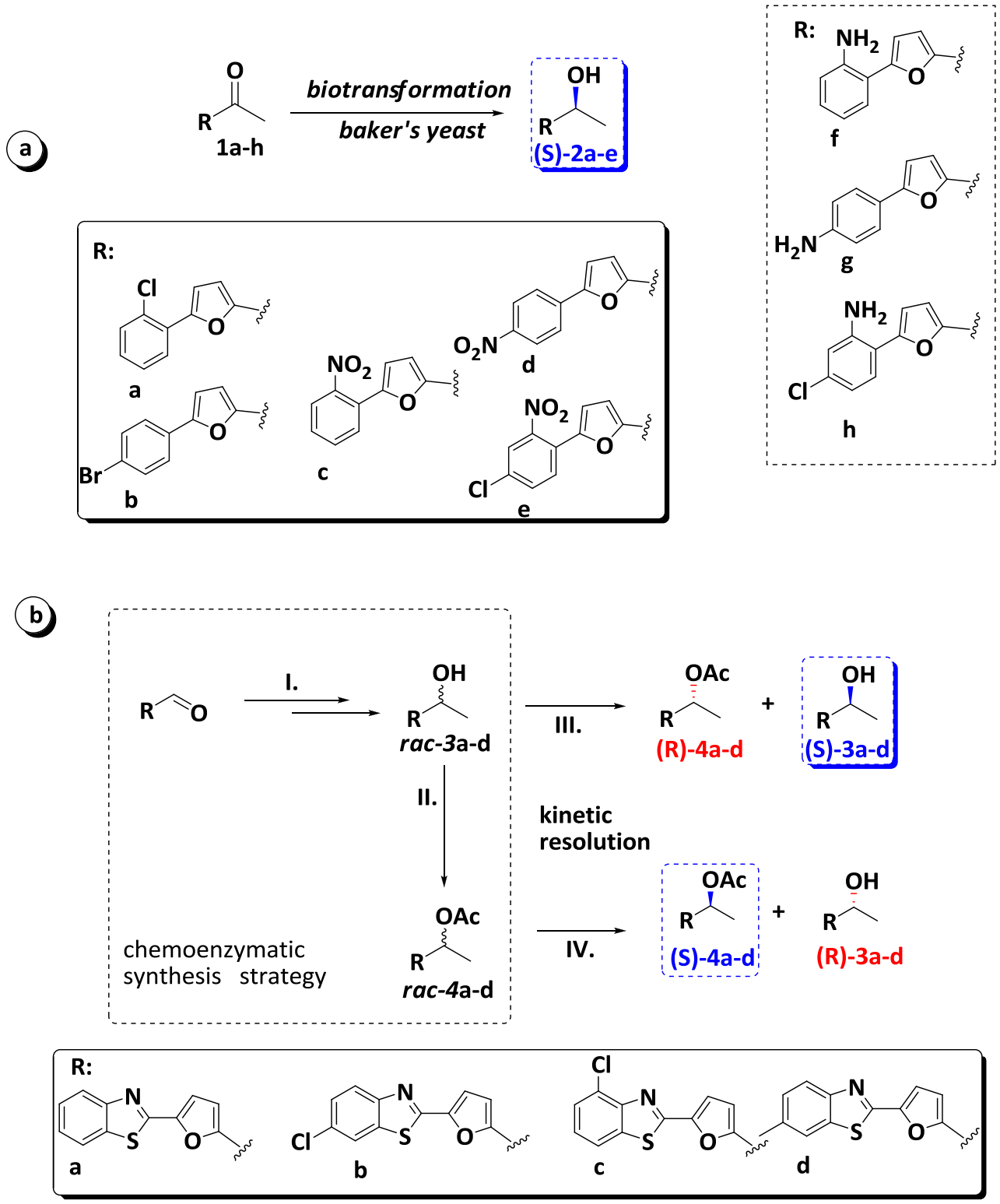
a. enantiotop-selective yeast-mediated bioreduction of prochiral 1-(5-phenylfuran-2-yl) ketones **1a-h** (Figure 1, a. This is one of the most practical, cheap, efficient and environmentally friendly methods, as described in Section 2.3.1. Although the yeast contains both (R)- and (S)-specific oxidoreductases, which may lead to decreased enantioselectivity of the obtained products, several procedures for enhancing stereoselectivity have been reported, as well as high values of enantiomeric excesses for products obtained from similar substrates. The above, together with the

favourable results obtained with the stereoselective synthesis of differently substituted phenylfuran-2-yl-ethan-1,2-diols, justify the selection of yeast as biocatalyst for phenylfuran alcohol synthesis.

b. stereoselective synthesis of racemic differently substituted 1-(5-(benzo[*d*]thiazole-2-yl)furan-2-yl) ethanols *rac*-**3a-d** and their acetates *rac*-**4a-d** by lipase-mediated kinetic resolution, using both enzymatic selective acylation of racemic ethanols (Scheme 1b, route III) and selective enzymatic alcoholysis of the corresponding racemic acetate (Scheme 1b, route IV). Lipase-mediated kinetic resolution of racemic alcohols proved to be an effective synthesis method for various optically pure secondary alcohols (Chapter 2.3.2). Making use of the lipase's characteristic to maintain its enantiomeric preference in both alcoholysis and acylation, and given the high selectivity of the enzyme in the synthesis of cyanohydrin acetates starting from furylbenzothiazoles, we chose to optimize the lipase-mediated kinetic resolution for the synthesis of optically pure furyl-benzothiazole alcohols and of their acetates.

2. Optimization of activity and stereoselectivity of the used biocatalysts in order to increase yield and enantiomeric excess, where required by less than optimal results.

3. Investigation of a few metabolic effects of 1-(5-(6-methylbenzo[d]thiazole-2-yl)furan-2-yl)ethyl acetate, comparatively for the racemic form and for each of its enantiomers. The acylated form was chosen for biological experiments considering its higher hydrophobicity, which allows a more facile penetration of cell membranes.



Scheme 1.

a. Synthesis of novel (*S*)-phenilfurane ethanols (*S*)-**2a-e**, by biotransformations with baker's yeast

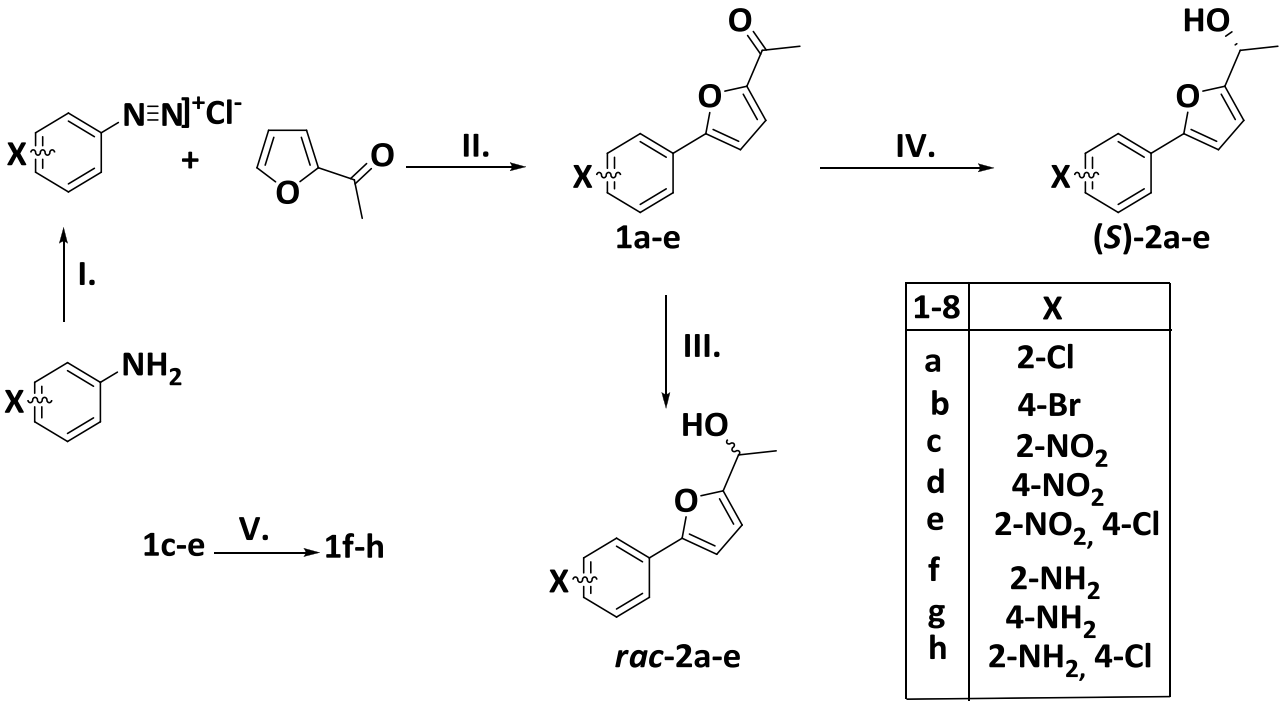
b. Synthesis of both enantiomers (*S*)- şi (*R*)- furylbenzthiazole-2-yl-ethanols **3a-d** and furylbenzthiazole-2-yl-acetates **4a-d** by lipase mediated kinetic resolution**.**

4. Results and discussion

4.1.Stereoselective bioreduction of 1-(5-phenylfuran--2-yl)ethanones mediated by baker's yeast

4.1.1. Synthesis of 1-(5-phenylfuran-2-yl)ethanones 1a-e

The prochiral heteroaryl-ethanones **1a-e** used as substrates were prepared by the Meerwein method[2] from the corresponding diazonium salts of various anilines and 2-acetyl-furan. Further by the chemical reduction of ketones **1a-e**, the racemic phenylfuran-2-yl-ethanols *rac*-**2a-e** were obtained (Scheme 2a).



1-8	X
a	2-Cl
b	4-Br
c	2-NO_2
d	4-NO_2
e	2-NO_2, 4-Cl
f	2-NH_2
g	4-NH_2
h	2-NH_2, 4-Cl

I. 0-5 0C, HNO2, HCl / H2O; II. CuCl2/H2O, acetone; III. NaBH4/ MeOH IV. baker's yeast.

Scheme 2. chemical synthesis of phenylfuran-2-yl-compounds and the baker's yeast mediated reduction of the ketones.

4.1.2. Analytical scale enzimatic biotransformation mediated by baker's yeast

Due to the high instability of the phenylfuran-2-yl-ethanols *rac*-**2a-e** during their isolation and purification processes, the use of even weak acidic media had to be avoided. The chromatographic enantiomeric separation of *rac*-**2a-e** was further developed, which allowed us to investigate the stereochemical outcome of the baker's yeast mediated bioreductions (Table 3).

First the analytical scale baker's yeast mediated transformation of **1a-e** was performed under fermenting (with sucrose) and non-fermenting (without sucrose) conditions (Table 1). In all cases the bioreduction in fermentative conditions underwent with higher stereoselectivity and conversion compared to the one performed in non-fermentative conditions.

Table 1. Fermenting and non-fermenting biotransformation of **1a-e**

Entry	Substrate	Product	Fermenting system		Nonfermenting system	
			ee (%)	Yield (%)	ee (%)	Yield (%)
1	**1a**	**(*S*)- 2a**	64	70	52	49
2	**1b**	**(*S*)- 2b**	-	-	-	-
3	**1c**	**(*S*)- 2c**	41	85	-	-
4	**1d**	**(*S*)- 2d**	98	80	80	57
5	**1e**	**(*S*)- 2e**	99	87	27	61

As an example, while the fermenting bioreduction of **1c** underwent with a relatively high yield, the non-fermenting bioreduction of the same substrate failed (Table 1, entry 3) as well as the biotransformation of **1b** in both conditions, recovering almost quantitatively the starting materials. This result is in good accordance with our earlier observation that both (5-(4-bromo-phenyl)furan-2-yl)-α-hydroxyethanone and (5-(4-bromophenyl)-furan-2-yl)-α-acetoxy-methylethanone are inadequate substrates for the baker's yeast enzymatic equipment which could be explained with the high sterical demand of 1-(5-(4-bromophenyl)furan-2-yl)ethanone, preventing the good interaction of these compounds with the catalytic site of cellular oxidoreductases.

The high enantiomeric excesses for (*S*)-**2d** and (*S*)-**2e** (Table 1, entries 4 and 5), are also in accordance with the previously obtained good results for the synthesis of the corresponding 1,2-ethanediols by baker's yeast mediated biotransformation. The difference between the ee values of the products obtained by bioreduction of **1c** and **1d** (Table 1, entries 3 and 4) proved that the *para* substituted substrates are preferred by baker's yeast enzymes to the *ortho* substituted ones.

Beside position, the electronic effects of the substituents could also influence the yields and the enantiomeric composition of the obtained compounds. By the selective reduction with $SnCl_2$ of **1c-e** the corresponding 1-(amino-5-phenyl-furan-2-yl)ethanones were synthesized. Unfortunately the bioreductions of these derivatives failed: as in case of **1b**, no trace of product could be found in the reaction mixture. The absence of the cellular transformation of the amino ketones suggested a structural incompatibility between these substances and the enzymatic equipment of the baker's yeast cells, which could be explained by the different electronic distribution in the amino-ketones compared to that of nitro-ketones.

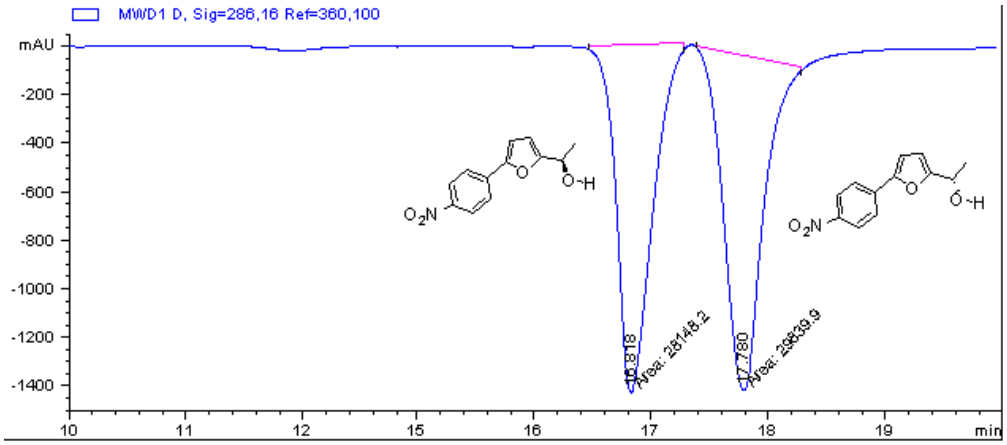
The characterization of new molecules produces was done by NMR Spectroscopy and MS Spectrometry. High performance liquid chromatography (HPLC) with chiral columns were employed for direct separation of enantiomers and quantification of enantiomeric excesses. In Figure 1 show, HPLC elution diagram for analysis of racemic *rac*-**2d** and *rac*-**2e** and product isolated from the baker's yeast mediated biotransformation of ketone.

Excepting the highly stereoselective bioreduction of **1d,e** (Table 1, entry 4,5), the biotransformation of the other substrates **1a,c** yielded the corresponding (*S*)-phenylfuran-2-yl-ethanols (*S*)-**2a,c** with poor enantiomeric excess (ee). These unsatisfactory results determined us to perform the fermenting baker's yeast reductions of **1a,c** in the presence of various additives which could enhance the stereoselectivity of the enzymatic reductions. These compounds are acting as specific inhibitors of (*S*)- or (*R*)-selective oxidoreductases. In most of the cases their presence in the reaction mixture caused the variation (increase or decrease) of ee of the produced (*S*)-alcohols. The influence of the used additives upon the stereoselectivity of the bioreduction of **1a,c** differed for each substrate (Table 2).

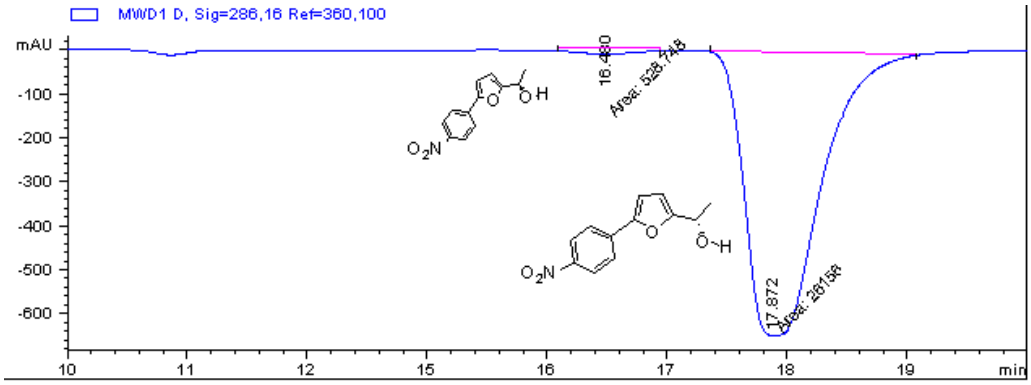
Table 2. The influence of various additives on the stereoselectivity of bioreduction of ketones **1a,c**

Entry	Additives	ee (%)		Yield (%)		Time (h)
		(*S*)-**2a**	(*S*)-**2c**	(*S*)-**2a**	(*S*)-**2c**	
1	Fermenting	64	41	70	85	48
2	Non-fermenting	52	-	49	-	48
3	*n*-hexane	46	58	40	79	48
4	L -cysteine	53	56	45	70	48
5	$MgCl_2$	35	54	43	73	48
6	$MnCl_2$	41	49	51	81	48

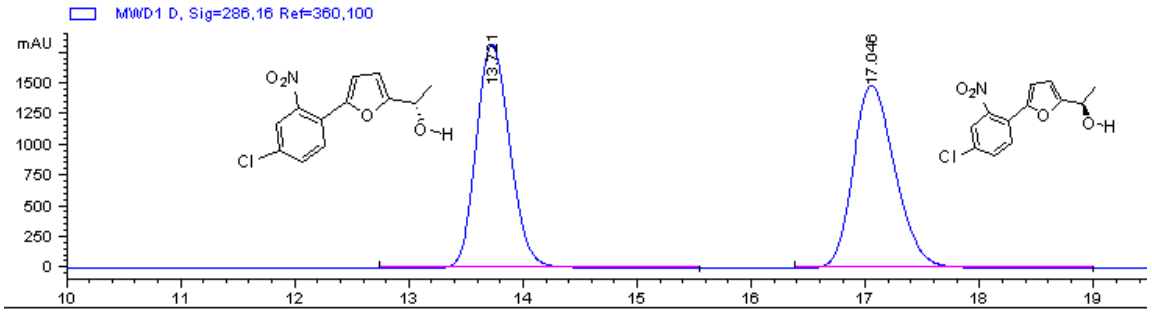
None of the additives improved the enantiomeric excess of the produced (*S*)-phenylfuran-2-yl-ethanol (*S*)-**2a** the best result remaining that obtained without additives in fermenting conditions (Table 2, entry 1). The bioreduction of **1c** in presence of 0.1% *n*-hexane showed the highest selectivity, however a slight decrease of the yield was observed (Table 2, entry 3). As it was expected, the use of phenacyl-chloride, known as an inhibitor of (*S*)-selective yeast oxidoreductases[3], impared the cellular reduction of **1a,c**. Surprisingly, in presence of allyl alcohol and ethyl bromoacetate, as inhibitors of (*R*)-selective yeast oxidoreductases,[4] the bioreduction of **1a,c** failed.



a.



b.



c.

d.

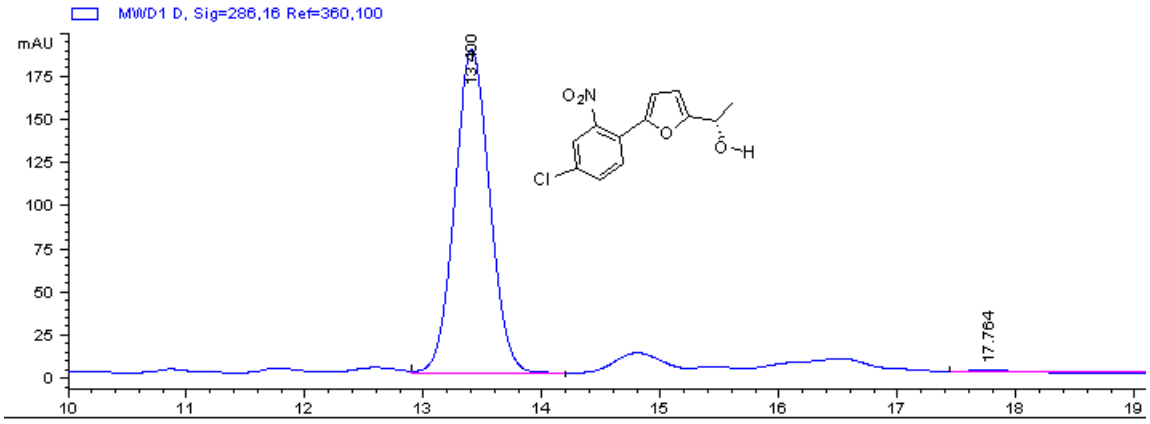


Figure 1. Elution diagram of the racemic 1-(5-(4-nitrophenyl)furan-2-yl)ethanol **a.** ***rac*-2d** and **b.** product of bioreduction of ketone **1d**. **c**. elution diagram of the racemic 1-(5-(4-chloro-2-nitrophenyl)furan-2-yl)ethanol *rac*-**2 e** and **d**. product isolated from the baker's yeast mediated biotransformation of ketone **1e**.

Table 3. Retention times of the enantiomers of *rac*-**2a-e**

Compound	t_R (min)	Compound	t_R (min)
(*S*)-**2a**	20.8	(*R*)-**2a**	22.3
(*S*)-**2b**	8.4	(*R*)-**2b**	9.7
(*S*)-**2c**	14.1	(*R*)-**2c**	15.9
(*S*)-**2d**	16.8	(*R*)-**2d**	17.7
(*S*)-**2e**	13.7	(*R*)-**2e**	17.1

4.1.3. Preparative scale synthesis of (S)-heteroarylethanols 2a,c-e with baker's yeast

Using the optimal conditions determined for the analytical scale reactions, the preparative scale bioreductions of **1a,c-e** was further performed. In the scale up procedure no significant changes in yield, stereoselectivity and reaction time were observed, as compared to those found for the analytical scale procedures (Table 4).

Table 4. Baker's yeast mediated preparative scale synthesis of (*S*)- phenylfuran-2-yl-ethanols **2a,c-e**

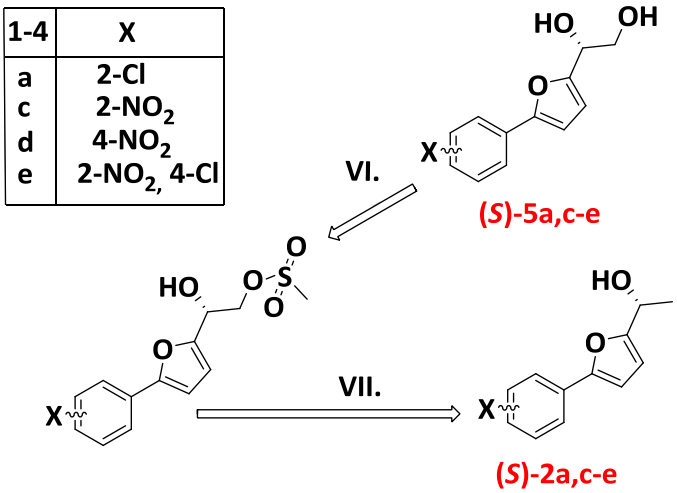
Entry	Substrate	Product	ee (%)	Yield (%)	[α_D^{25}]
1	**1a**	**(*S*)- 2a**[a]	64	65	- 6.2
2	**1c**	**(*S*)- 2c**[b]	58	81	- 28
3	**1d**	**(*S*)- 2d**[a]	98	76	+ 13
4	**1e**	**(*S*)- 2e**[a]	99	80	- 24

[a]fermenting system, 48 h

[b] fermenting system with *n*-hexane 0.1% as additive, 48 h

4.1.3. The absolute configuration of the synthesized heteroaryl-ethanediols.

To determine the absolute configuration of the novel heteroarylethanols an already known retrosynthetic pathway was employed. Thus the known optically active diols[5] (*S*)-**3a,c-e** were selectively mesylated at the primary hydroxyl group, followed by the subsequent reduction with $LiAlH_4$ of the mesylated intermediates, yielding the corresponding optically active heteroarylethanols (*S*)-**2a,c-e** (Scheme 3). The absolute configuration was established comparing the sign of the specific rotations and of the retention times of the heteroarylethanols obtained by the two different methods.



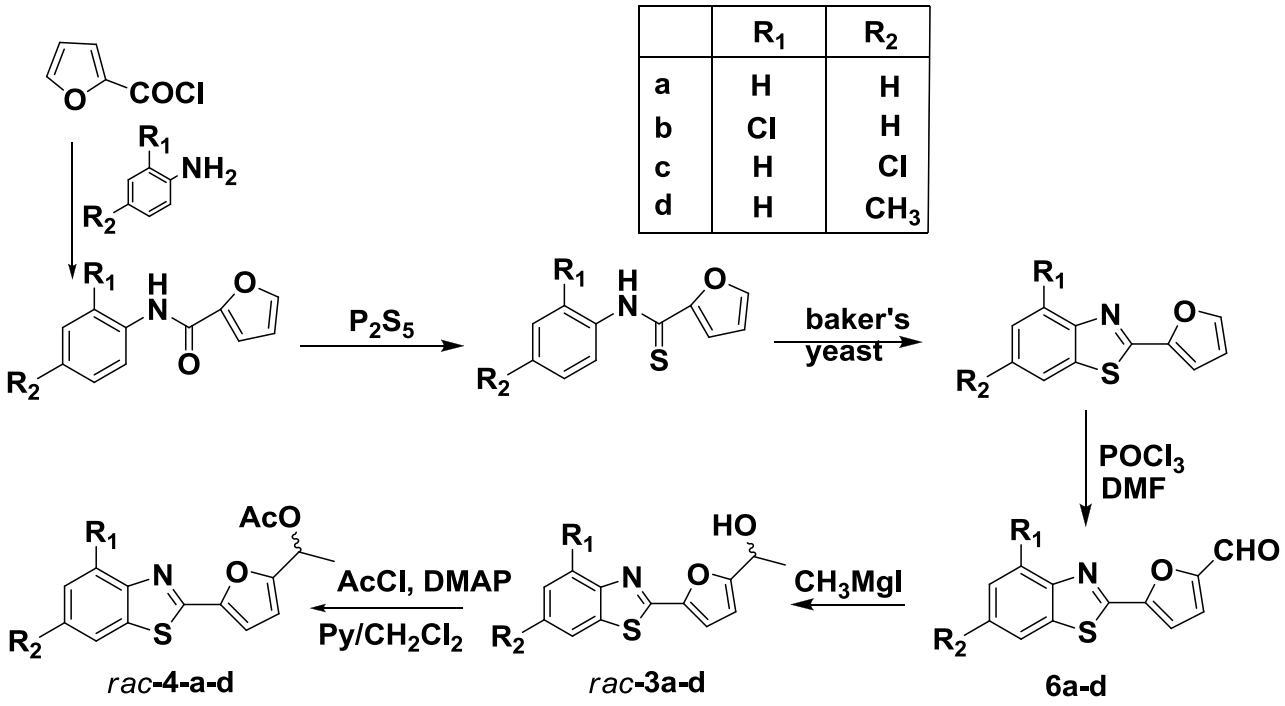
VI. CH_3SO_2Cl, Et_3N/THF, -20^{0}C; VII. $LiAlH_4$/THF

Schema 3. Retrosynthetic pathway for the absolute configuration of produced enantiomerically enriched heteroaryl-ethanols (*S*)-**2a,c-e**.

4.2. CaL-B a highly selective biocatalyst for the kinetic resolution of furylbenzthiazole-2-yl-ethanols and acetates

4.2.1. Chemoenyimatic synthesis of racemic alcohols *rac*-3a-d and their acetates *rac*-4a-d

First the synthesis of racemic 1-(5-(benzo[d]thiazol-2-yl)furan-2-yl)ethanols *rac*-**3a-d** from the corresponding heteroaryl aldehydes **6a-d** by a Grignard reaction was performed. The corresponding acetates *rac*-**4a-d** were obtained by the chemical acylation of the heteroarylethanols *rac*-**3a-d** (Scheme 4).

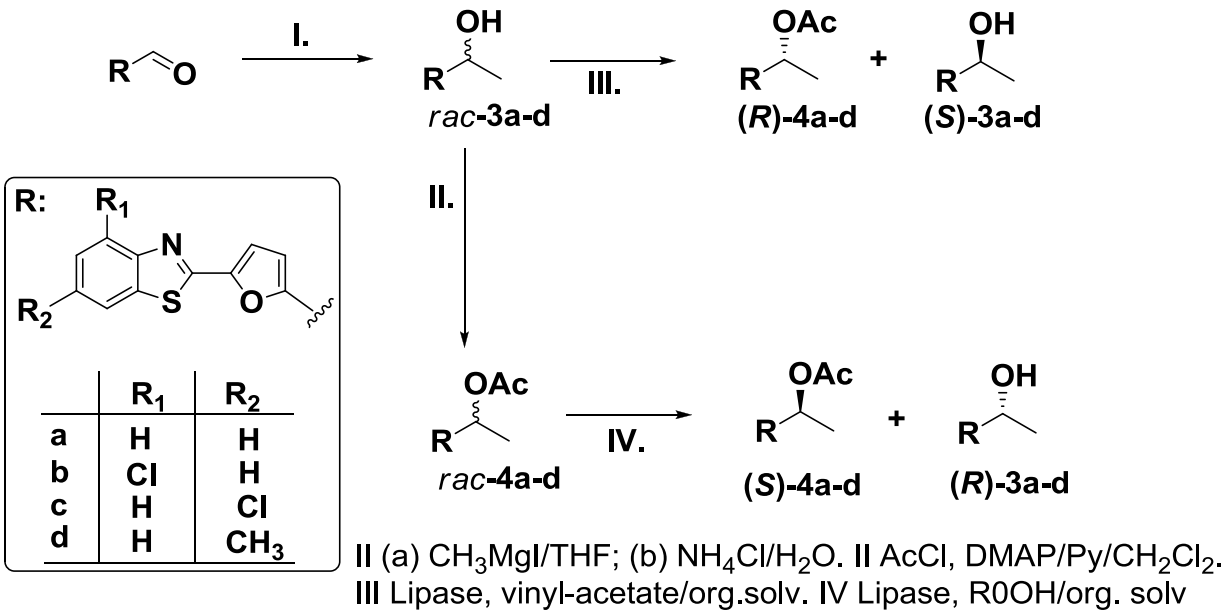


Scheme 4. The synthesis of 1-(5-(benzo[d]thiazol-2-yl)furan-2-yl) derivatives.

4.2.2. Lipase mediated kinetic resolutions of *rac*-3a-d şi *rac*-4a-d

To investigate the stereoselectivity of the enzymatic kinetic resolution and the activity of the enzymes, the chromatographic enantiomeric separation of the racemates *rac*-**3,4a-d** was first established. In order to obtain highly enantiomerically enriched resolution products, using racemic 1-(5-(benzo[d]thiazol-2-yl)furan-2-yl)ethanol *rac*-**3a** as a model compound. Potentially useful lipases were screened in various organic solvents for enantiomer selective acylation of racemic

alcohol *rac*-**3a**. with vinyl acetate (5 equiv), and for alcoholysis with methanol, ethanol, propanol and butanol (8 equiv) of racemic acetate *rac*-**4a**. (Scheme 5).



Scheme 5. Preparation and enzymatic kinetic resolution of racemic 1-(5-(benzo[d]thiazol-2-yl)furan-3-yl)ethanols *rac*-**3a-d** and their acetates *rac*-**4a-d**.

Due to the poor solubility of *rac*-**3a** in neat vinyl acetate screening with several lipases for the enzymatic acylation of the heteroaylethanol was performed in acetonitrile, in which the solubility of the substrates was highest. It was found that Novozyme 435 (lipase B from *Candida antarctica*, CaL-B) was the most active and selective enzyme for this purpose (Table 5, entry 5). Interestingly, lipase A from *C. antarctica*, (CaL-A) previously found to be the most efficient for the kinetic resolution and dynamic kinetic resolution of furylbenzthiazole-based cyanohydrins[6], showed lower activity (Table 5, entry 1) than lipase B from *C. antarctica* for the enzymatic acylation of *rac*-**3a**. Further, using the most efficient enzyme, solvent effects on the enzymatic acylation was tested. Due to the low solubility of the substrate, only a small number of organic solvents proved to be useful for the CaL-B-mediated acylation.

Table 5. The influence of the nature of the enzyme on the enzymatic acylation of *rac*-**3a** with vinyl acetate (8 equiv) in acetonitrile

Entry	Enzyme	Time (h)	c (%)	ee_P (%)	ee_S (%)	E
1	**CaL-A**	14	34	> 99.5	52.3	»200
2	**LPS**	14	15	> 99.5	18	> 200
3	**LF**	14	0	-	-	-
4	**LAK**	3	26	98	34.4	138
5	**CaL-B**	3	50	> 99.5	98	»200
6	**CCL**	14	9	> 99.5	10	> 200
7	**CRL**	14	8	98	9	108
8	**PPL**	14	0	-	-	-

A strong solvent influence upon the reaction rate was observed, while the selectivities were high in most cases. In accordance with the values shown in Table 6, acetonitrile (Table 6, entry 1) proved to be the most appropriate solvent (high ee and highest reaction rate value) for the enzymatic acylation of *rac*-**3a**. Performing the same screening procedure for the rest of the substrates *rac*-**3b-d**, the optimal method was the same as found for *rac*-**3a**.

Table 6. The influence of the nature of the solvent on the CaL-B-mediated acylation of *rac*-**3a** with vinyl acetate (8 equiv) in different solvents after 3 h

Entry	Solvent	c (%)	ee_P (%)	ee_S (%)	E
1	**Acetonitrile**	50	> 99.5	98	»200
2	**MTBE**	49	> 99.5	95	»200
3	**Toluene**	47	> 99.5	90	»200
4	**1,4-Dioxane**	16.4	> 99.5	19.6	> 200
5	**Cloroform**	38.4	> 99.5	62.3	»200

To prepare the opposite enantiomers of the resolution products, the (*R*)-1-heteroarylethanols (*R*)- **3a-d** and the (*S*)-1-heteroarylethyl acetates (*S*)- **4a-d**, the analytical scale enzymatic alcoholysis of racemic 1-(5-(benzo[d]thiazol-2-yl)furan-2-yl)ethyl acetates *rac*-**4a-d** was investigated further. The screening process involved enzymatic alcoholysis with various amounts (2, 4, 6, 8, 10 equiv) of methanol, ethanol, propanol and butanol, in the same solvents used for the enzymatic acylation. Interestingly, only CaL-B was catalytically active. With all the other lipases showing activity for the enzymatic acylation (CaL-A, LPS, LAK, CCL and CRL), the alcoholysis failed. The nature and the

concentration of the nucleophile strongly influenced the selectivity of the CaL-B-catalyzed alcoholysis. The best results were obtained when 8 equiv of methanol was used in acetonitrile. In Table 7 some selected results from the screening procedure for the enzymatic methanolysis of *rac*-**4a** are shown. To verify the general validity of the optimal conditions for the enzymatic methanolysis of *rac*-**4a**, the same screening procedure was further extended to the other racemic acetates *rac*-**4a-d**. As was expected, the CaL-B-catalyzed methanolysis (8 equiv) in acetonitrile was found to be the most selective procedure. The reaction times, conversions and ee values for both analytical scale enzymatic acylation of *rac*-**3a-d** and methanolysis of *rac*-**4a-d** are presented in Table 8. In all cases at 50% conversion, high ee values were obtained, for both product and unreacted enantiomer of the substrate, showing high enantioselectivity and activity of CaL-B in acetonitrile towards all the investigated 1-(5-(benzo[d]thiazol-2-yl)furan-2-yl)-based derivatives.

Table 7. The influence of the nature of solvent upon the selectivity and velocity of CaL-B catalyzed methanolysis (8 equiv) after 2 h

Entry	Solvent	c (%)	ee_P (%)	ee_S (%)	E
1	**Acetonitrile**	50	> 99.5	99	»200
2	**MTBE**	40	> 99.5	66	»200
3	**Toluene**	15	> 99.5	17	> 200
4	**1,4- Dioxane**	14	> 99.5	16.6	> 200
5	**Cloroform**	18	> 99.5	22	> 200

Table 8. The optimal conditions for the analytical scale enzymatic kinetic resolution of racemic alcohols *rac* **3a-d** and acetates *rac* **4a-d** with CaL-B in acetonitrile

Entry	Substrate	Timp(h)	c (%)	ee_p (%)	ee_s (%)
1	*rac*-**3a**	4	50	> 99.5	> 99.5
2	*rac*-**3b**	8	50	> 99.5	98
3	*rac*-**3c**	14	50	> 99.5	> 99.5
4	*rac*-**3d**	12	50	> 99.5	> 99.5
5	*rac*-**4a**	2	50	> 99.5	> 99.5
6	*rac*-**4b**	12	50	> 99.5	98
7	*rac*-**4c**	8	50	98	98
8	*rac*-**4d**	8	50	> 99.5	> 99.5

4.2.3. Preparative scale synthesis of optically pure (*R*)- , (S)-3a-d and (*R*)- , (*S*)-4a-d

Based on the analytical scale optimal procedure, the preparative scale enzymatic resolutions were performed for both *rac*-1-heteroarylethanols *rac*-**3a-d** and the corresponding ethyl acetates *rac*-**4a-d**. All dilutions, substrate–biocatalyst ratio and reaction conditions were the same as in the case of the analytical scale reactions. The reactions were monitored by HPLC and TLC and were stopped at an approx. 50% conversion, removing the enzyme by filtration. Data on yield, enantiomeric excess and specific rotatory value of the obtained enantiomers are presented in Table 9.

To investigate the stereoselectivity and the conditions of KR for the lipase mediated acylation and alcoholysis of 1-(5-(benzo[d]thiazol-2-yl)furan-2-yl)ethanol ***rac*-3a-d** and 1-(5-(benzo[d]thiazol-2-yl)furan-2-yl)ethyl acetate the chromatographic separation of the enantiomers of the reaction counterparts *rac*-**3a**,**4a** *rac*-**3d, 4d** was first established (Figure 2a-d).

Tabele 9. Yield, ee and specific rotatory value for the products of CaL-B-mediated kinetic resolutions.

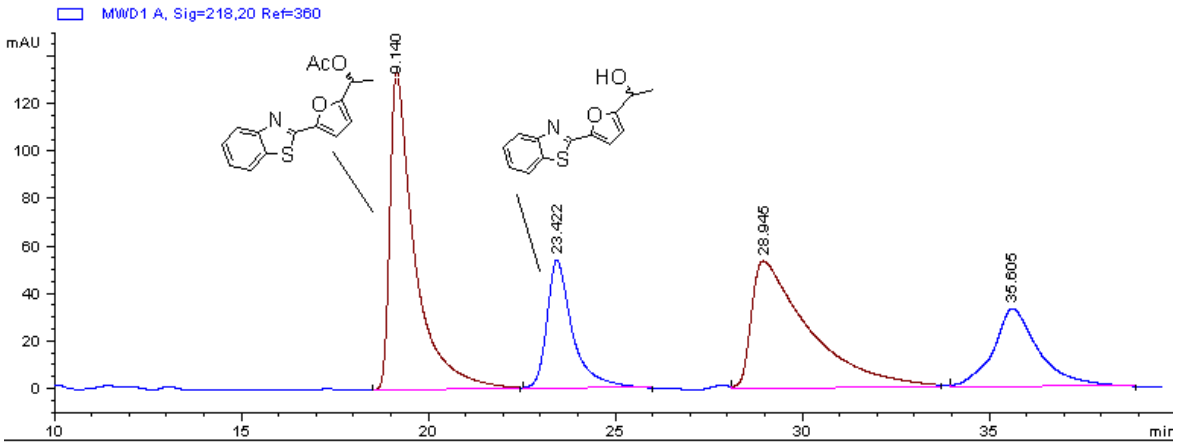
Entry	Product	c.(%)	ee (%)	$[\alpha]_D^{25}$*	Product	c(%)	ee (%)	$[\alpha]_D^{25}$**
1[a]	(*S*)-**3a**	49	> 99.5	-28.3	(*R*)-**4a**	49	> 99.5	+ 97
2[a]	(*S*)-**3b**	48	98	-40.3	(*R*)-**4b**	49	99	+ 92.9
3[a]	(*S*)-**3c**	49	99	-18.7	(*R*)-**4c**	49	99	+109.5
4[a]	(*S*)-**3d**	49	> 9.5	- 12.8	(*R*)-**4d**	47	> 99.5	+87.5
5[b]	(*R*)-**3a**	49	> 99.5	+ 31.1	(*S*)-**4a**	48	> 99.5	-107
6[b]	(*R*)-**3b**	47	98	+ 42.7	(*S*)-**4b**	49	99	-97
7[b]	(*R*)-**3c**	48	98	+ 19.8	(*S*)-**4c**	48	98	-103.4
8[b]	(*R*)-**3d**	50	> 99.5	+10.3	(*S*)-**4d**	37	99	-94.3

[a] Products obtained by enzymatic acylation.

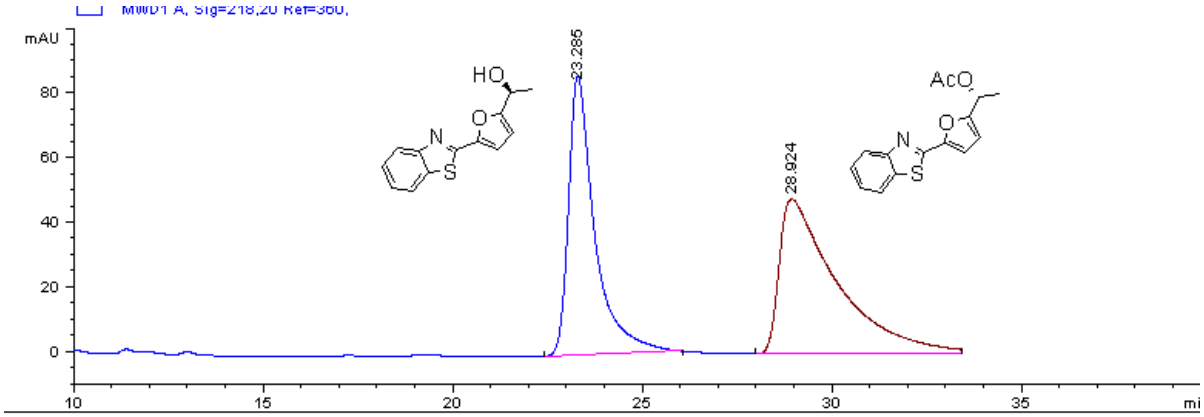
[b] Products obtained by enzymatic alcoholysis.

[c] c 0.5 mg/mL.

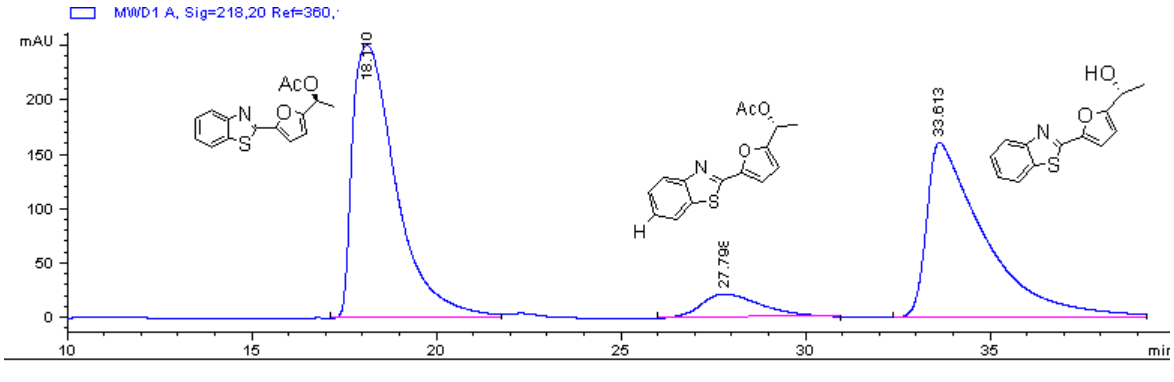
[d] c 0.25 mg/mL.


MWD1 A, Sig=218,20 Ref=360
mAU
AcO
HO
9.140
23.422
28.945
35.605
mir

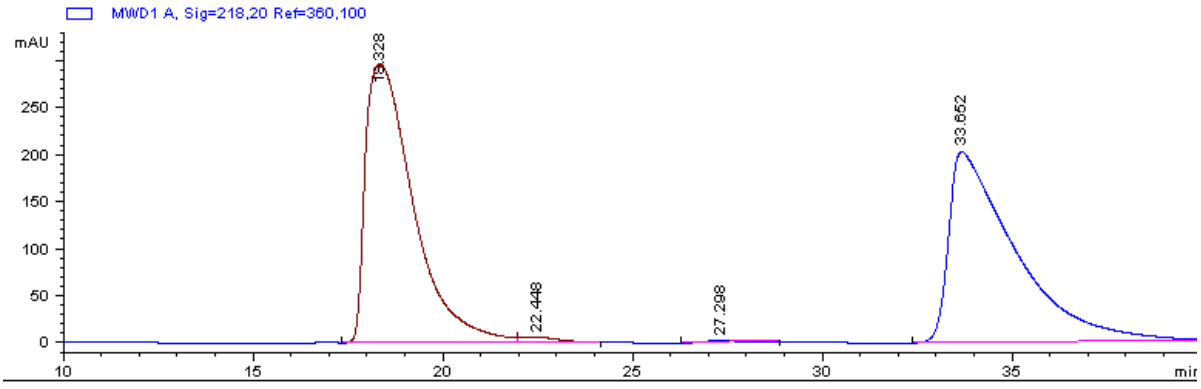
a.


mAU
HO
AcO
23.285
28.924
mi

b.


MWD1 A, Sig=218,20 Ref=360,
mAU
AcO
18.110
27.798
33.613
HO
mir

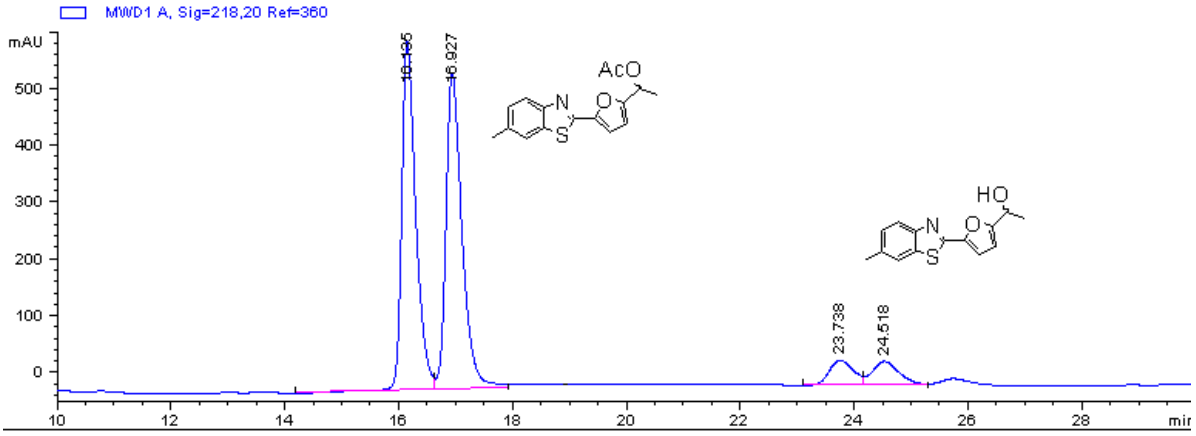
c.


MWD1 A, Sig=218,20 Ref=360,100
mAU
19.328
22.448
27.298
33.652
mir

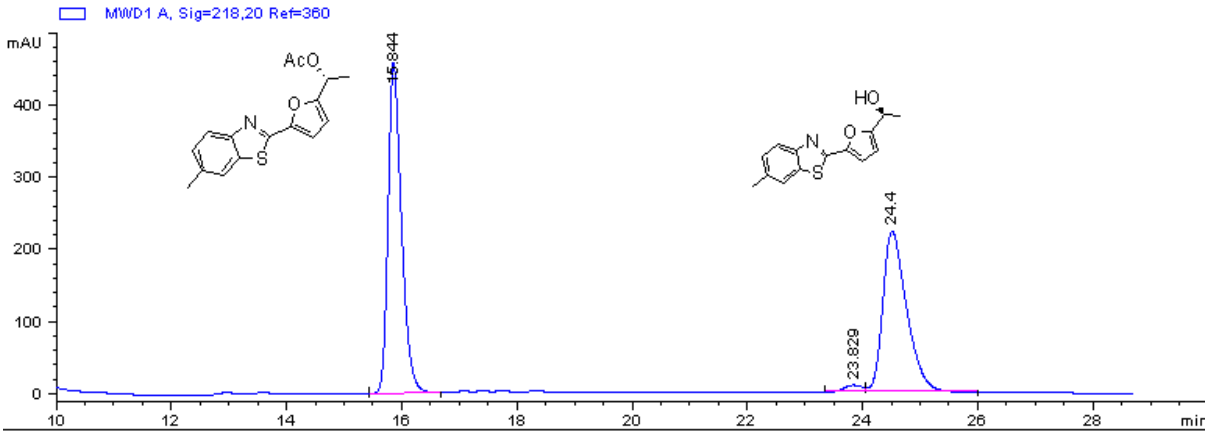
d.

Figure 2. a. Elution diagram of the mixture of the racemic of starting material *rac*-**3a** and racemic product *rac*-**4a** for the lipase catalysed KR. **b**. Elution diagram for the enzymatic KR of *rac*-**3a** after 2 hours, **c, d**. Elution diagram for the enzymatic KR of *rac*-**4a** after 4 hours and after 4 hours.

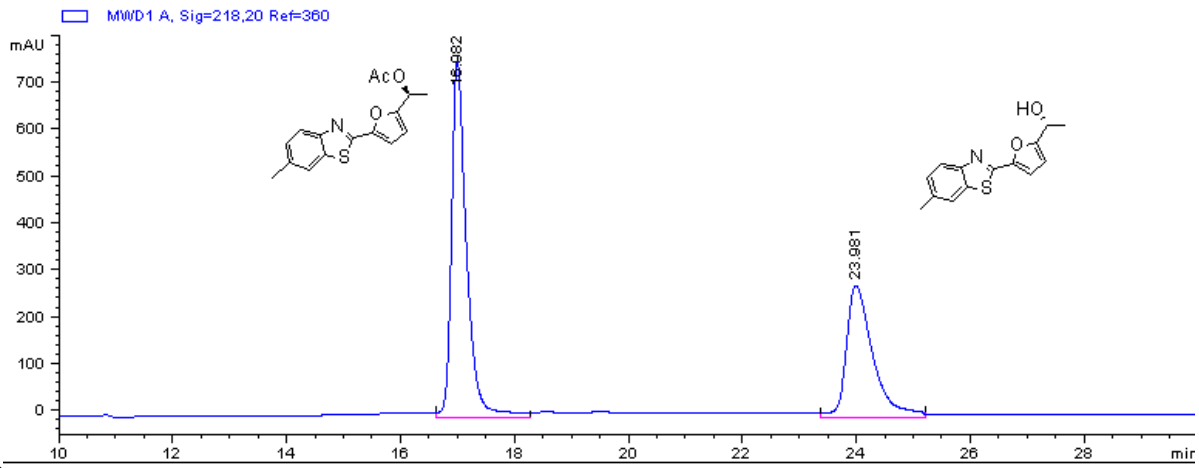
.



a.



b.



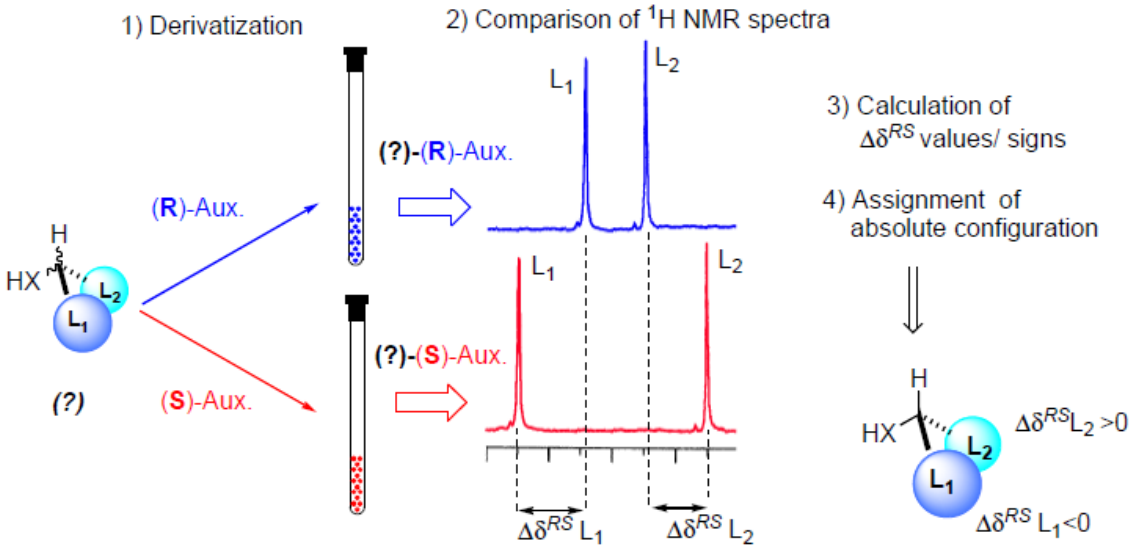
c.

Figure 2. a. Elution diagram of the mixture of the racemic of starting material *rac*-**3d** and racemic product *rac*-**4d** for the lipase catalysed KR. **b**. Elution diagram for the enzymatic KR of *rac*-**3d** after 2 hours, **c**. Elution diagram for the enzymatic KR of *rac*-**4d** after 5 hours.

4.2.4. The absolute configuration of the resolution products

According to Kazlauskas' empirical rule[7] for predicting which enantiomer reacts faster during the resolution of secondary chiral alcohols, the absolute configurations of the products obtained by the lipase-mediated kinetic resolution can be assigned[8]. The assignment of the absolute configuration of chiral secondary alcohols, by comparison of the ^{1}H-NMR spectra of the conjugates obtained by esterification enantiomers diastereomers (*R*) - and (*S*) - enantiomers of the alcohol with the two enantiomers of chiral auxiliary reagent (*R*) - and (*S*) – of MTPA (Scheme 6)[9].

However, a few exceptions to this rule have been reported so far[10]. Therefore, the absolute configurations of the novel enantiopure alcohols was determined by a detailed ^{1}H-NMR study in the case of the Mosher's derivative[11] of **3b**. Thus, the racemic and enantiomerically pure **3b** was esterified with (*R*)-MTPA-Cl and the resulting diastereomers were differentiated by their ^{1}H-NMR spectra.



Scheme 6. The assignment of the absolute configuration of chiral secondary by comparison of the ^{1}H-NMR[9].

The esterification of racemic *rac*-**3b** with the (*R*)-MTPA-Cl occured selectively affording the expected mixture of the corresponding Mosher esters, but in a 1.7:1 ratio (Figure. 4a) in agreement with the previous observation of Heathcock[12]. We noted that, depending on the sterical hindrance involving the OH group, the above unequal distribution is not always observed[13]. Next, previous structural data[14] suggested that the most plausible conformation of the (*S*)-MTPA fragment is that presented in (Figure 3). If so, in the case of (*S*)-MTPA-(*R*)-3b, there is a steric repulsion between the heteroaryl moiety of **3b** and the phenyl group of the MTPA counterpart. In contrast, in the case of (*S*)-MTPA-(*S*)-3b, the heteroaryl and phenyl groups are not proximal.

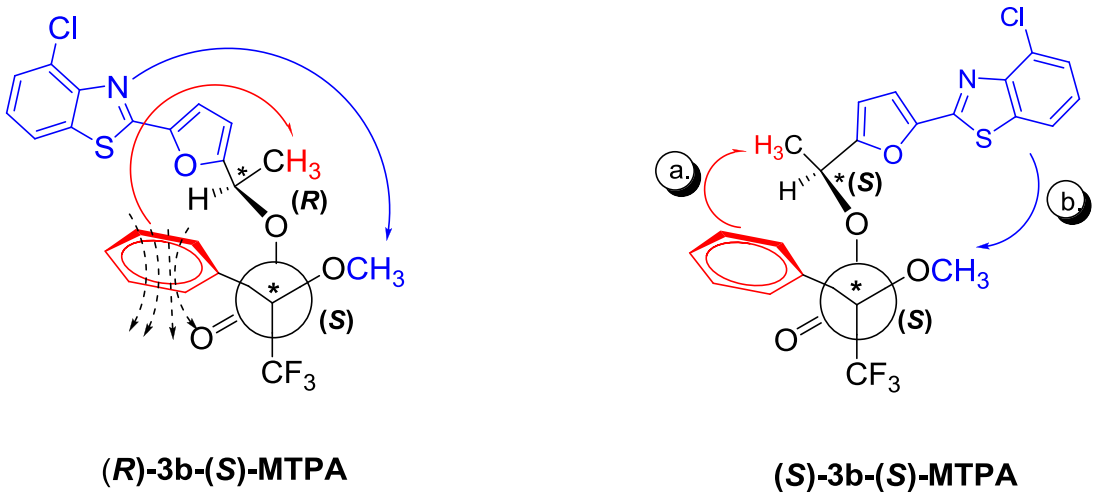
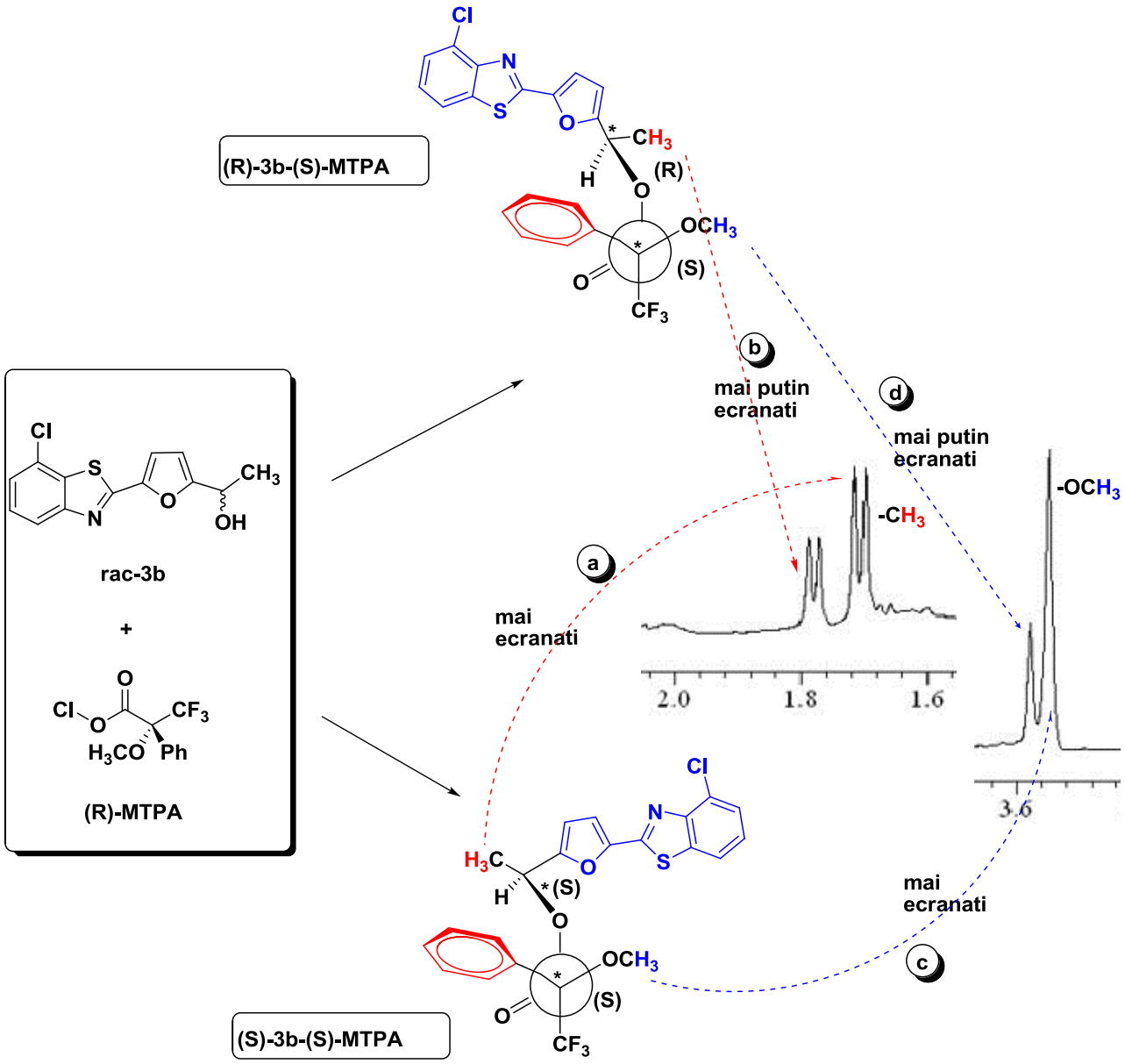


Figure 3. Role of the anisotropic group of the auxiliary and the importance of the conformation of the two resulting diastereomers (*R*)-**3b**-(*S*)-MTPA şi (*S*)-**3b**-(*S*)-MTPA.

Hence, based on the observed selectivity of the esterification, the ^{1}H-NMR signals of the two diastereomers, (*S*)-MTPA-(*R*)-3b and (*S*)-MTPA-(*S*)-3b, could be assigned unambiguously as follows: the signals with major intensity (δ = 1.70 ppm and δ = 3.54 ppm) belonged to the $-CH_3$ and $-OCH_3$ groups, respectively, in the (*S*)-MTPA-(*S*)-3b environment, while the minor signals at δ =1.78 ppm and δ = 3.58 ppm belonged to the (*S*)-MTPA-(*R*)-3b diastereomer (Scheme 7.). Supporting evidence is also provided by the phenyl group's diamagnetic effect on the proximal groups. Thus in the case of (*S*)-MTPA-(*S*)-3b the strong diamagnetic effect of the phenyl ring caused the proximal $-CH_3$ protons to resonate upfield (Scheme 6. a). Indeed, $-CH_3$ protons are found to be more shielded, δ $-CH_3$ = 1.70 ppm, in comparison with the $-CH_3$ protons of (*S*)-MTPA-(*R*)-3b (δ $-CH_3$ = 1.78 ppm) where the distance between the phenyl and the methyl groups is higher

(Scheme 7. b). The methoxy protons of the (*S*)-MTPA-(*S*)-3b were also found to be more shielded, δ -OCH_3 = 3.54 ppm, when compared with their analogues in (*S*)-MTPA-(*R*)-3b, δ -OCH_3 = 3.58 ppm. This is due to the diamagnetic effect of the proximal heteroaryl ring (Scheme 6. c,d). However, in this case the diamagnetic effect of the heteroaryl moiety is lower, as shown by the difference between the δ values as $\Delta\delta CH_3$ 0.08 ppm and $\Delta\delta OCH_3$ = 0.04 ppm, due to the larger distance between the -OCH_3 group and the heteroaryl moiety.



Scheme 7. Role of the diamagnetic effect and steric repulsion of the phenyl group and the heteroaryl grup on the protons -CH_3 respectively -OCH_3, importance of the conformation ester (*R*)-**3b**-(*S*)-MTPA and (*S*)-**3b**-(*S*)-MTPA.

The esterification of the enantiomerically pure alcohol remained untransformed during the CaL-B-mediated acylation reaction with (R)-MTPA chloride, producing the down fielded (*S*)-MTPA-(*S*)-3b diastereomer (Figure. 4b), thus confirming the -(*S*) absolute configuration predicted by the Kazlauskas empirical rule. The absolute configuration for the rest of the compounds was established by comparing their signs of the specific rotation with (+)-(*S*)-3b.

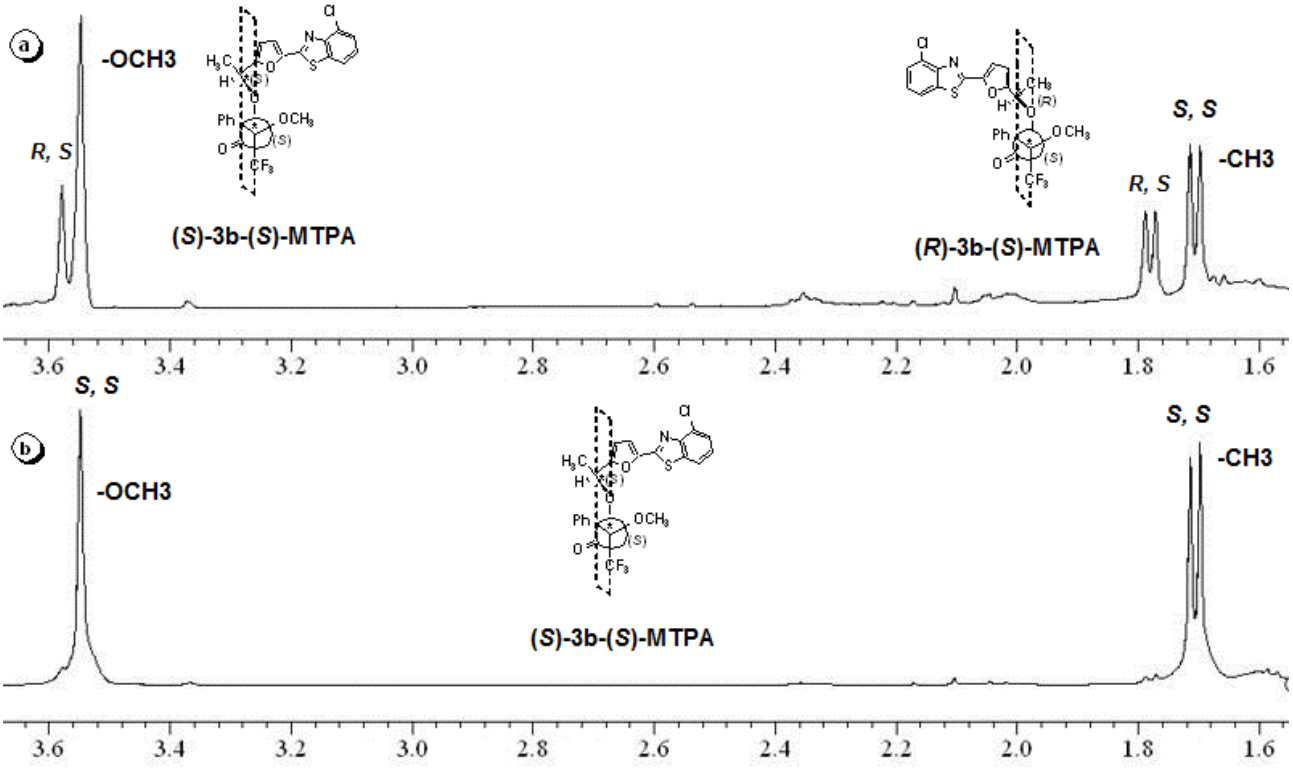


Figure 4. Signals of the -CH_3 protons and -OCH_3 protons from the ^{1}H RMN spectra of the (a). (*R*)-**3b**-(*S*)-MTPA (*S*)-**3b**-(*S*)-MTPA and (b). (*S*)-3b and of the (*S*)-MTPA-(*S*)-3b.

5. Conclusions

The baker's yeast mediated synthesis of novel phenylfuran-2-yl-ethanols was developed. The procedure yielded the enantiomerically enriched (*S*)-alcohols with moderate to high yield and ee. A strong substituent effect was observed and investigated in comparison with our previous results obtained in the baker's yeast synthesis of novel phenylfuran-2-yl-ethanediols.

An efficient enzymatic kinetic resolution of various furylbenzthiazole-based ethanols *rac*-**3a–d** and their acetates *rac*-**4a–d** has been achieved. Both processes, the enzymatic acylation and the enzymatic alcoholysis were efficient in acetonitrile using CaL-B as a catalyst, affording the highly enantiomerically enriched enantiomers of the target compounds.

The absolute configuration of the obtained products was determined by a detailed ^{1}H- NMR study of the Mosher's derivative of *rac*-**3b** and -(*S*)-**3b**.

7. Testing the biological activity of 1 - (5 - (6-methylbenzo [d] thiazole-2-yl) furan-2-yl) ethyl acetate *rac*-4d and (*S*) -, (*R*) - 4d

7.1. Material and methods

7.1.1. Biological material

Experimental animals were white Wistar rats, adult males, weighing 200 ± 20 g, maintained throughout the experiment in the zoobase of the Department of Experimental Biology, Faculty of Biology and Geology, under appropriate zoo-sanitary conditions: constant temperature and humidity, light/dark cycle 12/12 hours, Larsen diet (with or without added FBT), access to water, and ethanol solution respectively.

Animal handling was performed gently without causing distress or pain, and sacrifice by severing the carotid artery was done under anesthesia.

7.1.2 Experimental protocol

Our research was conducted on an experimental model *in vivo*. The animals received:

1. FBT, (*R*)- and (*S*)- enantiomers

Rats were divided into three groups:

- **M** (n = 10): control group that received the standard diet only and water *ad libitum*;
- **FBT-R** (n = 10): group that received the standard diet with added FBT (*R*)- enantiomer
- **FBT-S** (n = 10): as FBT-R, but with FBT (*S*)- enantiomer

The experiment has lasted for 15 days, after which the animals were sacrificed and blood was collected, together with samples of liver tissue for morphological and biochemical determinations.

2. in parallel ethanol and FBT.

Ethanol was administered daily for 30 days, as a 10% (v/v) solution in drinking water.

FBT administration (*R* and *S* enantiomers, the racemic respectively) was made by mixing the substance in the feed, in the following doses:

- 400 mg / kg of feed for each enantiomer;
- 800 mg / kg of feed for racemic.

Doses were calculated as to correspond to 20 mg/kg body weight/day for enantiomers and 40 mg/kg body weight/day racemic.

FBT was introduced in the diet of animals starting with the 16^{th} day of the experiment, for the next 15 days.

Rats were divided into five groups:

- **M** (n = 5): the control group, that received the standard diet and water *ad libitum*;
- **EtOH** (n = 5), group treated with ethanol 10% (v/v) in drinking water, and the standard diet without FBT;
- **(EtOH-*R*)** (n = 5), the group that received ethanol as the previous group, and the standard diet with added FBT, *R*-enantiomer
- **(EtOH-*S*)** (n = 5), as **EtOH-*R***, but with *S*-enantiomer
- **(EtOH-*Rac*)** (n = 5), the group that received ethanol and the racemic.

The experiment has lasted for 30 days, after which the animals were sacrificed, blood and samples of liver tissue were collected, for morphological and biochemical determinations.

7.2. Results and discussion

7.2.1. Effects of furil-benzothiazole administration (*R* and *S* enantiomers) at rats

7.2.1.1 Hematological parameters

The number of red blood cells (RBC), in the adult rat, range from 6 to 10x106 blood cells/mm^3. In our experiment, all the group mean values were within normal limits (Table 15). An increasing trend (14.4%) of RBC was recorded in the FBT-*R* group, becoming statistically significant under the influence of *S*-enantiomer (19.66%).

The hematocrit was within the physiological limits (from 35.5 to 43.6%) and showed no significant variations between groups.

It can be concluded that FBT, in the administered dose, had not negative effects on blood parameters characterizing RBC.

Table 15. Variation of some hematological parameters (RBCs) in rats treated with furylbenzothiazole.

Groups Parameters		M	FBT-R	FBT-S
Nr. RBC (nr. x 10^6/mm^3)	x±ES	7,12±0,39	8,17±0,47	8,52±0,46
	n	10	10	10
	p (M)		>0,05 NS	< 0,05*
Hemoglobin (g/dL)	x±ES	19,92±1,66	19,24±0,66	20,23±1,86
	n	10	10	10
	p (M)		>0,05 NS	>0,05 NS
Hematocrit (%)	x±ES	41,05±0,69	41,43±0,72	41,47±0,51
	n	10	10	10
	p (M)		>0,05 NS	>0,05 NS

x±ES = mean±standard error; n = no. Of animals/group; p (M) = statistical significance against the control group; the asterisks mark the statistical significance as follows :p < 0,05 -* ; p < 0,01 - ** ; p < 0,001 - ***.

In adult rats, the leukocytes number varies between 8 and $12x10^3$ white blood cells/mm^3 of blood. The values obtained in this experiment (Table 2, Fig. 2) fall between these limits, with a slight increase (+9.5%) in the group FBT-*R*, as compared to the control.

In FBT-*Rac* group, an increase of lymphocyte percentage, accompanied by a significant decrease in the percentage of circulating monocytes. This decrease is probably the result of fixing them in greater number in the sinusoids of liver tissue, in response to the additional metabolic burden represented by the added FBT.

7.2.1.2. Biochemical parameters

The blood sugar levels in adult rats ranges between 70 and 90 mg% [15]; the mean value of our control group falls between these limits (Table 3, Fig. 3). The *R*-enantiomer of FBT caused a decrease of blood glucose with 12,25%, uninsured statistically, while for *S*-enantiomer, the blood glucose reduction was significant, 26,39%. Hepatic glucose and liver glycogen concentrations were also reduced in both groups treated with FBT.

In one experiment on liver microsomes, Su et al. (2006)[16] have demonstrated the hypoglycaemic property of certain benzothiazole derivatives. Another *in vivo* study on obese rats demonstrated the effectiveness of benzothiazole compounds in reducing glucose and body weight[17].

Table 17. Variation of glycemia, glucose and glycogen concentrations in the liver tissue of rats treated with FBT

Groups Parameters		M	FBT-R	FBT-S
Glycemia (mg /dL)	x±ES	72,94±5,28	64,02±4,23	53,69±3,69
	n	10	10	10
	p (M)		>0,05 NS	<0,01**
Glucose (mg/g tissue)	x±ES	2,46±0,34	1,93±0,11	1,96±0,18
	n	8	10	8
	p (M)		>0,05 NS	> 0,05 NS
Glycogen (mg/g tissue)	x±ES	1,88±0,31	1,18±0,20	1,24±0,11
	n	10	10	10
	p (M)		< 0,05*	< 0,05*

Further research is necessary, to investigate the hypoglycaemiant capacity of *S*-enantiomer on an experimental model of streptozotocin-induced diabetes.

Recent data[18] (2011) indicate the ability of benzothiazole compounds to reduce triglyceride and cholesterol levels. In our experiment, only the *S*-enantiomer produced a statistically uninsured decrease, with 6% of total serum cholesterol, when the compound, non-optimized in terms of bioavailability, was administered in the food of normal animals (Table 4, Fig. 4). Under the same

conditions, there was a significant increase of liver cholesterol, with both enantiomers, but higher with FBT-*S*. Because only the total cholesterol was measured, we cannot provide information on the HDL and LDL fractions. However, one can suspect a low turnover of liver cholesterol.

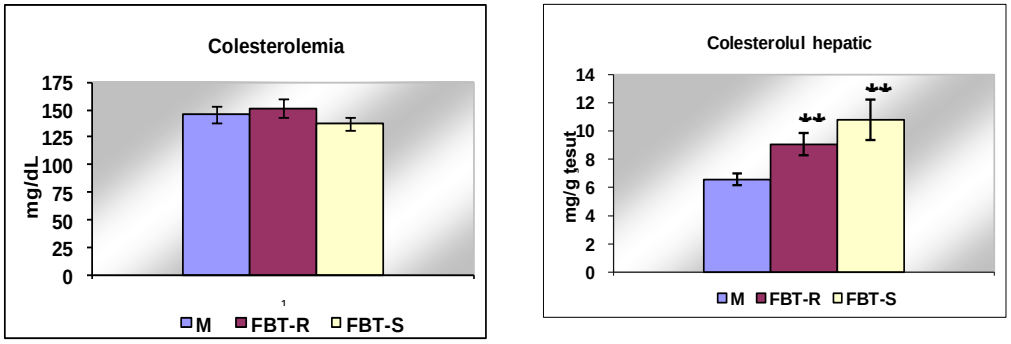


Fig. 13. Cholesterolemia and liver cholesterol concentration in rats treated with furylbenzothiazole.

Both FBT enantiomers slightly increased the total liver protein concentration; in the case of (*S*)- enantiomer, there was an increase of 10%. On the other hand, proteinemia dropped in both treated groups, significantly (+15%) for the *S*-enantiomer of FBT (Table 5 and Fig. 5). We assume that these results are due to a slight inhibition of protein export.

Table 19. Protein concentrations in the serum and liver of rats treated with furylbenzothiazole.

Groups Parameters		**M**	**FBT-R**	**FBT-S**
Proteine-mia (g/dL)	x±ES	5,14±0,29	4,68±0,32	4,36±0,24
	n	10	10	10
	p (M)		>0,05 NS	<0,05*
Liver proteins (mg/ g tissue)	x±ES	364,79±19,78	400,85±25,07	403,81±18,74
	n	10	10	10
	p (M)		>0,05 NS	>0,05 NS

7.1.2.2. Enzymatic parameters

Catalase. Given the intracellular localization of the enzyme, its activity in serum is a residual one, due to changes in plasma membrane permeability, and membrane destructions respectively. Therefore, a low presence of the enzyme in serum, as recorded in the FBT-*S* group (Table 9 and Fig. 9), is evidence of increased membrane stability.

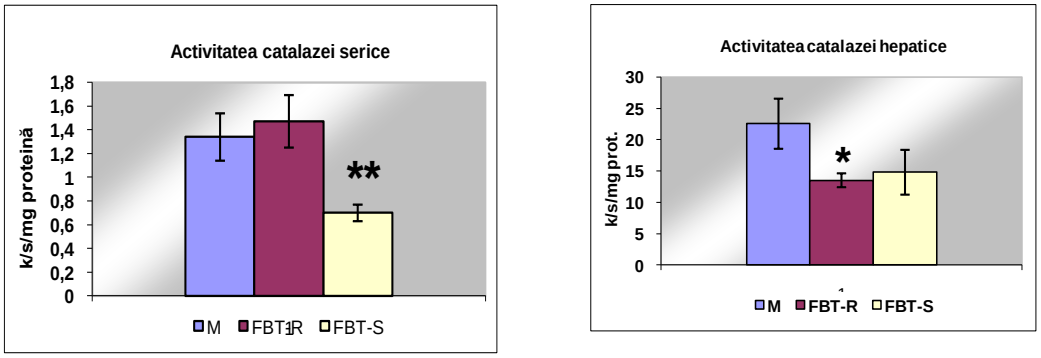


Fig. 18. Serum and liver catalase activity in rats treated with furylbenzothiazole.

CAT activity in the liver tissue has been reduced with 40% and 35% respectively in the presence of *R*- and *S*-enantiomers of the FBT, as compared to the average value of the control group. As the enzyme is induced proportional to the amount of substrate (H_2O_2) produced in the oxidative metabolism, we can assume that CAT synthesis is reduced as a result of low production of H_2O_2.

Superoxide dismutase. Since the reaction catalyzed by SOD provides substrate (H_2O_2) to catalase, proportionality is to be expected between the activities of the two enzymes. Consequently (Table 10 and Fig. 10), serum SOD activity was significantly reduced, with 45%, in FBT-*S* group, and hepatic SOD activity was also decreased in both groups treated with FBT, more in the case of FBT-*R*.

Table 24. Serum and liver superoxide dismutase activity in rats treated with furylbenzothiazole

Groups Parameters		**M**	**FBT-R**	**FBT-S**
Seric SOD (USOD/ml /min)	x±ES	0,86±0,08	0,82±0,14	0,48±0,09
	n	10	10	10
	p (M)		>0,05 NS	<0,01**
Liver SOD (USOD/mg protein/min)	x±ES	3,91±0,57	1,66±0,12	2,42±0,55
	n	10	10	10
	p (M)		<0,001***	<0,05*

In conclusion, this preliminary experiment, concerning the biological action of newly synthesized FBT on healthy mammals, revealed:

- An increase of the number of red blood cells;

- A decrease of the circulating monocytes, probably due to their transformation into fixed liver macrophages;

- A decrease of glycemia, and of liver glucose and glycogen, which makes the substance an interesting candidate for development of antidiabetic drugs;

- No harmful effects on protein turnover and the marker enzymes of membrane integrity;
- A low production of free radicals.

More significant effects were observed for *S*-enantiomer.

7.2.2. Effects of furil-benzothiazole (*R*- and *S*- enantiomers and racemic) administration at rats subchronic intoxicated with ethanol

As there are reports concerning furans antioxidant and cytoprotective actions in lipopolysaccharide poisoning, and anti-inflammatory properties of benzothiazole[18], we considered of interest to approach the effects of FBT on an experimental model of subchronic alcoholism.

7.2.2.1 Hematological parameters

The number of RBC significantly increased under the influence of ethanol, a phenomenon encountered in other similar experiments. This can be considered an attempt to adapt the body to an increased need for O_2. The two enantiomers and the racemic mixture have restored the RBC to values close to those of the control group (Fig. 17, Table16).

Table 25. Hematological parameters (RBCs) in rats intoxicated with ethanol and treated with furylbenzothiazole.

Groups Parameters		**M**	**EtOH**	**EtOH-R**	**EtOH-S**	**EtOH-Rac**
No. of RBC (no. x 10^6)	x±ES	9,53±0,50	12,24±0,98	9,32±0,37	9,66±0,82	9,80±0,32
	n	5	5	5	5	5
	p_1 (M)		< 0,05*	>0,05 NS	>0,05 NS	>0,05 NS
	p_2 (EtOH)			< 0,05*	< 0,05*	< 0,05*
Hemoglobin (g/dL)	x±ES	17,01±0,71	14,04±1,52	17,35±0,97	17,48±0,75	18,54±0,47
	n	5	5	5	5	5
	p_1 (M)		>0,05NS	>0,05 NS	>0,05 NS	>0,05NS
	p_2 (EtOH)			>0,05 NS	< 0,05*	< 0,05*
Hematocrit (%)	x±ES	44,98±0,36	45,62±1,23	46,01±0,97	45,94±1,00	45,84±0,42
	n	5	5	5	5	5
	p_1 (M)		>0,05 NS	>0,05 NS	>0,05 NS	>0,05 NS
	p_2 (EtOH)			>0,05 NS	>0,05 NS	>0,05 NS

x±ES = mean±standard error; n = no. Of animals/group; p_1 (M) = statistical significance against the control group; p_2 (EtOH) = statistical significance against the EtOH group;the asterisks mark the statistical significance as follows :$p < 0,05$ -* ; $p < 0,01$ - ** ; $p < 0,001$ - ***.

The two enantiomers of acetyl-furylbenzothiazole restored the hemoglobin concentration at almost identical values with the ones in the control group, while the racemic led to a slight increase of hemoglobin concentration, over the control group, but in physiological limits.

Leukocytes number has changed significantly as compared to the control group and the group with EtOH only, under the influence of (*S*)-enantiomer of the compound under study. These changes are not beneficial, meaning a depression of immune function. The (*S*)-enantiomer had a clear immunosuppressive effect that overlapped with EtOH action. Racemic mixture behaved differently from the two enantiomers, the leukocyte count in the EtOH-*Rac* being very close to the control group.

The two enantiomers present in racemic caused a potential decrease of inflammatory response (low neutrophil percentage), an increase of specific immune response (due to lymphocytes), and a monocyte proportion similar to the control group.

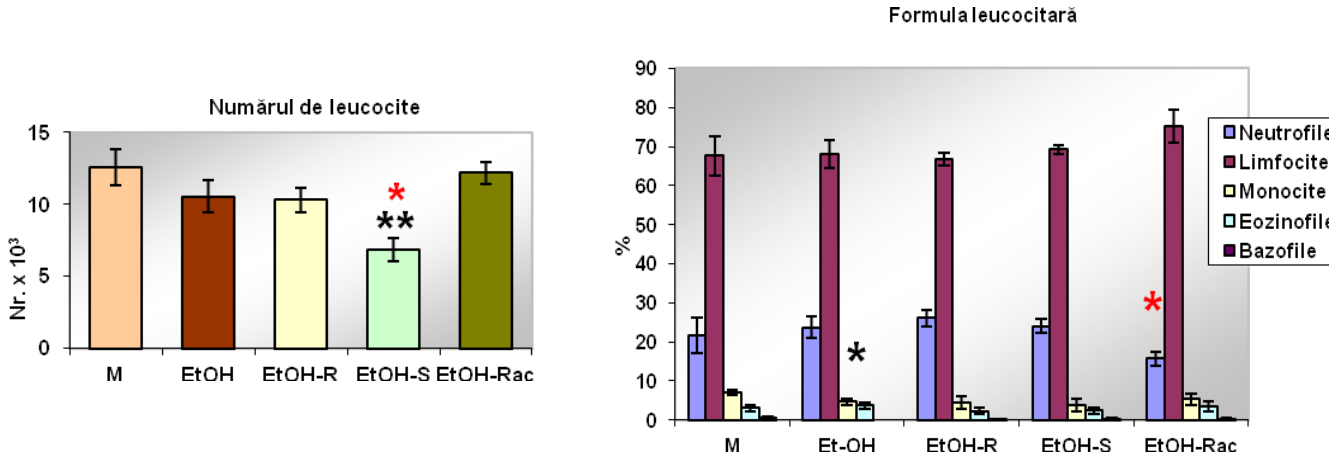


Fig. 21. Changes in hematological parameters (leukocytes, differential leukocyte count) in rats intoxicated with ethanol

7.2.2.2. Biochemical parameters

Blood sugar level did not undergo significant changes in any of the groups (table 18, fig. 19). Liver glucose significantly increased under influence of both EtOH and acetyl-furylbenzothiazole, as separate enantiomers and racemic mixture.

Table 27. Glycemia, liver glucose and glycogen concentrations in rats intoxicated with ethanol and treated with furylbenzothiazole.

Groups Parameters		**M**	**EtOH**	**EtOH-R**	**EtOH-S**	**EtOH-Rac**
Glycemia (mg /dL)	x±ES	206,72±20,11	214,53±18,11	232,85±22,24	249,66±31,50	245,92±26,27
	n	5	5	5	5	5
	p_1 (M)		>0,05 NS	>0,05 NS	>0,05 NS	>0,05 NS
	p_2 (EtOH)		>0,05 NS	>0,05 NS	>0,05 NS	>0,05 NS
Glucose (mg/g hepatic tissue)	x±ES	12,92±4,31	19,46±4,59	30,84±5,01	29,29±5,81	30,18±6,33
	n	5	5	5	5	5
	p_1 (M)		>0,05 NS	< 0,05*	< 0,05*	< 0,05*
	p_2 (EtOH)			>0,05NS	>0,05 NS	>0,05 NS
Glycogen (mg/g tissue)	x±ES	0,92±0,07	0,78±0,21	1,68±0,32	2,38±0,54	1,79±0,17
	n	5	5	5	5	5
	p_1 (M)		>0,05 NS	< 0,05*	< 0,05*	< 0,001***
	p_2 (EtOH)			< 0,05*	< 0,05*	< 0,01**

Cholesterol level underwent a distinct change in all treatmedgroups (Table 19, Fig. 20): if values in EtOH and EtOH-R groups were significantly lower than in the control group, groups EtOH-*S* and EtOH-*Rac* were characterized by significantly lower values than both control and EtOH group. It follows the hypocholesterolemiant properties of (*S*)- enantiomer and of racemic, as it has been reported earlier for other benzothiazole derivatives[18] .

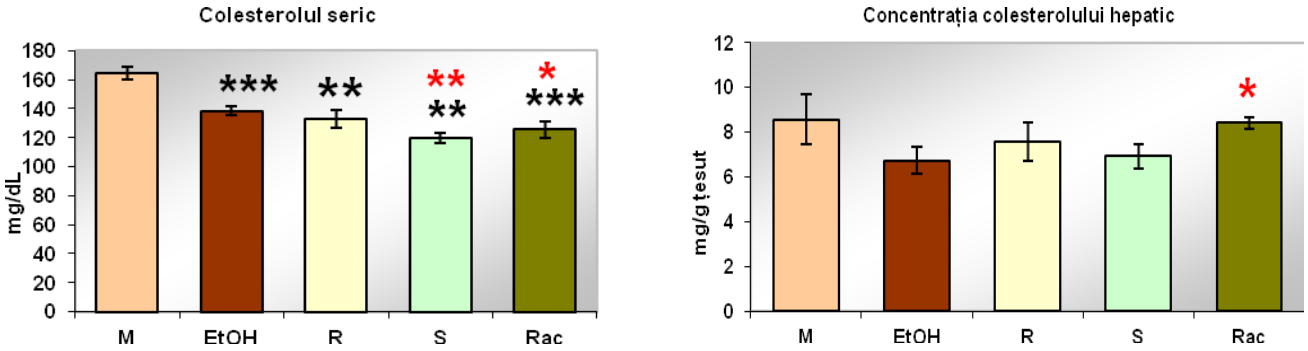


Fig. 23. Cholesterolemia and hepatic cholesterol concentration in rats intoxicated with ethanol and treated with furilbenzotiazol.

Proteinemia underwent significant changes only in the case of EtOH-*Rac* (Table 20, Fig. 21) where there was a marked an sharp increase due to increased hepatic protein synthesis. Acetyl-furylbenzothiazole, even administered under EtOH treatment, stimulated the hepatic protein synthesis (in a significant manner in (*S*)-enantiomer and racemic groups) and protein export from liver in the case of racemic (Table 20, Fig. 21).

Table 29. Proteinemia and liver protein content in rats intoxicated with ethanol and treated with furylbenzothiazole.

Groups Parameters		M	EtOH	EtOH-R	EtOH-S	EtOH-Rac
Proteine-mia (g/dL)	x±ES	6,89±0,07	7,46±0,92	7,40±0,91	7,44±1,10	9,21±1,30
	n	5	5	5	5	5
	p_1 (M)		>0,05 NS	>0,05 NS	>0,05 NS	>0,05 NS
	p_2 (EtOH)			>0,05 NS	>0,05 NS	>0,05 NS
Liver protein (mg/ g tissue)	x±ES	245,37±58,54	232,29±45,13	311,45±58,64	340,68±16,23	352,21±24,11
	n	5	5	5	5	5
	p_1 (M)		>0,05 NS	>0,05 NS	>0,05 NS	>0,05NS
	p_2 (EtOH)			>0,05 NS	< 0,05*	< 0,05*

7.2.2.3. Enzymatic parameters

Antioxidant enzymes. Our results on *catalase* activity in serum, although statistically uninsured due to the small number of animals used, demonstrates a strong hemolytic activity under the action of ethanol (Table 23, Fig.24); this effect was considerably reduced in the presence of the two enantiomers and especially of the racemic. These data correlate with hemoglobin concentration. Acetyl-furylbenzothiazole enantiomers stimulated catalase activity, bringing it to higher values as compared to the control group. In the EtOH-*Rac* group, this increase was even higher and statistically significant as compared with the EtOH group.

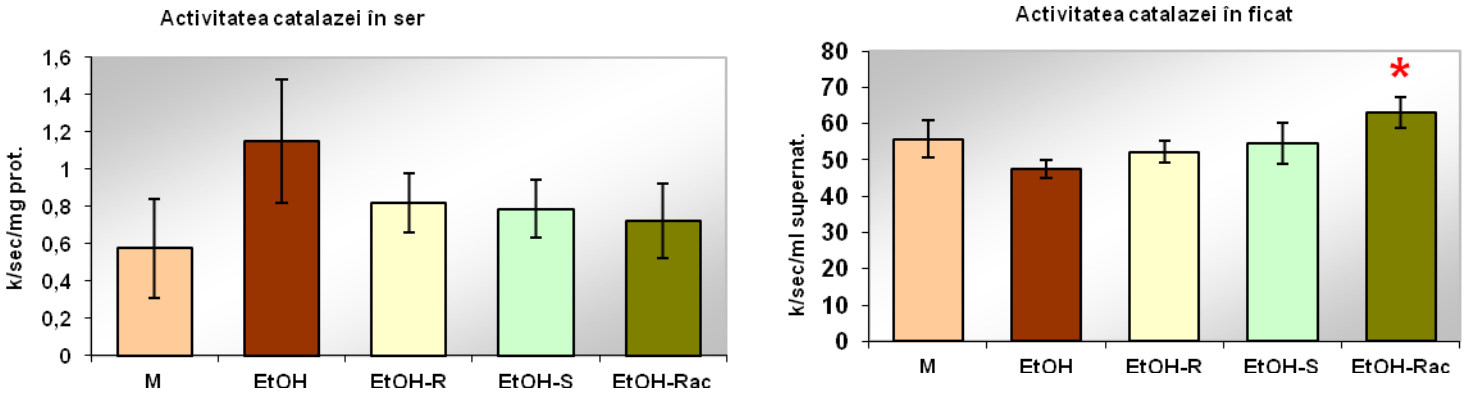


Fig. 28. Serum and liver catalase activities in rats intoxicated with ethanol and treated with furylbenzothiazole.

The inflammation produced by EtOH in subchronic treatment decreased tissue SOD activity in all four groups who have received EtOH. The enantiomers and the acetyl-furylbenzothiazole racemic did not counteract the effect of ethanol on this enzyme; (*R*)- and (*S*)- enantiomers had

further decreased the enzyme activity under ethanol, and their cumulative effect was greatest in the EtOH-*Rac* group (Table 25, Fig. 26).

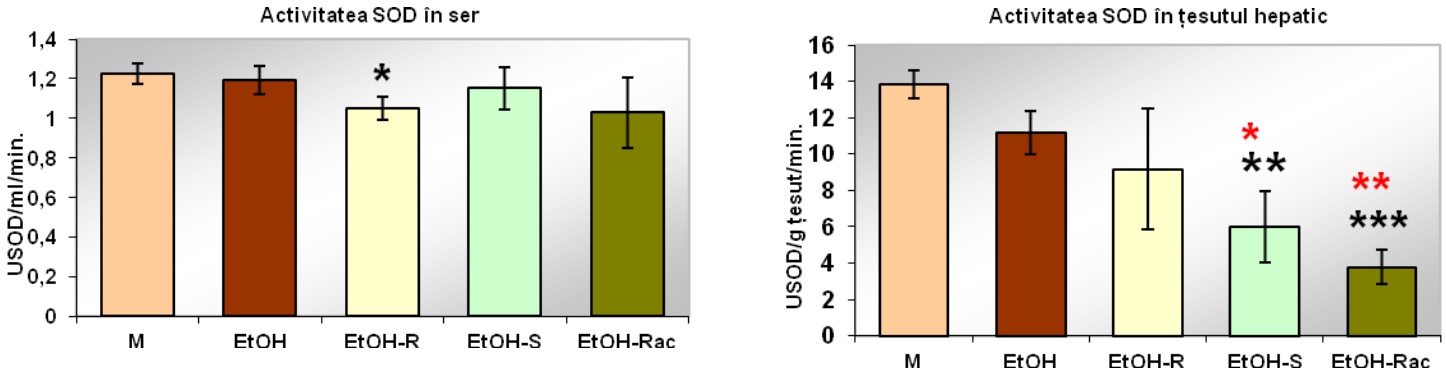


Figura 29. Serum and liver superoxide dismutase activity at rats intoxicated with ethanol and treated with furilbenzotiazol

7.2.2.4. Conclusions

1. The protective effect on blood parameters, exerted by the two FBT enantiomers and the racemic mixture, resulted in restoration of the control level of some parameters that were changed by alcohol intoxication: red blood cell count, hemoglobin concentration and hematocrit. However, the (*S*)-enantiomer maintained the depressed immune function induced by ethanol (low white blood cell count). The racemic reduced the inflammatory reaction by decreasing neutrophils and stimulated the specific immunity by increasing lymphocytes.

2. Concerning the carbohydrate metabolism, the three forms of FBT resulted in a load of hepatocytes with glycogen, and blood sugar and hepatic glucose increase. Both enantiomers and racemic mixture showed a cholesterol-lowering effect, most obvious for the (*S*)-enantiomer. Hepatic protein synthesis was stimulated by all three forms of the tested FBT, as compared with EtOH group, in a significant manner in cases of (*S*)-enantiomer and racemic.

3. The increased serum ALAT and LDH activity, under the influence of EtOH, was further amplified by the presence of FBT enantiomers and racemic, which may suggest a modification (at least early) of membrane permeability and structure. We expect electron microscopy data to bring further clarification of this issue. The antioxidant activity of the tested furylbenzothiazole compound, especially of the racemic mixture, resulted in a stimulation of hepatic catalase activity.

Bibliografie

[1] Ramandori, G., Moriconi, F., Malik, I., Dudas, J. *J Physiol Pharmacol*, **2008**, 107-117.

[2] Meerwein, H., Buchner, E., Emster, K. *J. Prakt. Chem.* **1939**, *152*, 237-266.

[3] a). Shi, Y. G., Fang, Y., Ren, Y. P., Guan, H. L., Zhang, J. Y. *J. Chem. Technol. Biotechnol.* **2009**, *84*, 681-688. (b). Cui, J. N., Ema, T., Sakai, T., Utaka, M. *Tetrahedron: Asymmetry*, **1998**, *9*, 2681-2692. (c). Forni, A., Moretti, I., Prati, F., Torre, G., *Tetrahedron*, **1994**, *50*, 11995-12000.

[4] a).Dao, D.H., Okamura, M., Akasaka, T., Kawai, Y., Hida, K., Ohno, A. *Tetrahedron: Asymmetry* **1998**, 9, 2725-2737. (b). Dao, D. H., Kawai, Y., Hida, K., Hornes, S., Nakamura, K., Ohno, A. *B. Chem. Soc. Jpn.* **1998**, *71*, 425-432.

[5] Bencze, L. Cs., Paizs, Cs., Toşa, M. I., Irimie, F. D. *Tetrahedron: Asymmetry*, **2011**, *22*, 675-683.

[6] (a) Paizs, Cs., Tosa, M., Majdik, C., Tähtinen, P., Irimie, F.D., Kanerva, L.T. *Tetrahedron: Asymmetry*, **2003**, *14*, 619-627; (b) Paizs, Cs., Toşa, M.I., Majdik, C., Mişca, R., Irimie, F.D. *Roum. Biotechnol. Lett*. **2001**, *6*, 325–331.

[9]Jing, Q., Kazlauskas, R. J. *Chirality*, **2008**, *20*, 724-735.

[10](a) Kazlauskas, R.J., Weissfloch A.N.E., Rappaport A.T., Cuccia L.A., *J. Org. Chem*., **1991**, *56*, 2656–2665. (b). Rotticci, D., Haeffner F., Orrenius C., Norin T., Hult K., *J. Mol. Catal. B: Enzymatic*. **1998**, *5*, 267–272. (c). Hwang, B.Y, Scheib, H., Pleiss, J., Kim, B.G,. Schmid, R.D. *J. Mol. Catal. B: Enzymatic*, **2000**, *10*, 223–231. (d). Schulz, T., Pleiss, J., Schmid, R.D. *Protein Sci*. **2000**, *9*, 1053–1062.

[9] (a). Seco, J. M., Quiñoá E., Riguera R., *Chem. Rev*. **2004**, *104*, 17-117. (b) Freire, F., Seco, J. M., Quiñoá, E., Riguera, R., *J. Org. Chem*. **2005**, *70*, 3778- 3790.

[12] Nagy, V., Tőke, E. R., Keong, L. C., Szatzker, G., Ibrahim, D., Omar, I. C., Szakács, G., Poppe, L. *J. Mol. Catal. B: Enzym*. **2006**, *39*, 141-148.

[11] Yang, Y., Kayser, M., Hooper, D. *Chirality*, **2005**, 131–134.

[14] Dutcher, J. S., Macmillan, J. G., Heathcock, C. H. *J. Org. Chem.* **1976**, *41*, 2663-2669.

[15](a). Baskar, B., Pandian, N. G., Priya, K., Chadha, A. *Tetrahedron Asymmetry*, **2004**, *15*, 3961-3966; (b). Chen, H., Nagabandi, S., Smith, S., Goodman, J. M., Plettner, E., *Tetrahedron Asymmetry*, **2009**, *20*, 449-456.

[16] (a). Dale, J. A., Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512-519; (b). Sullivan, G. R., Dale, J. A., Mosher, H. S. *J. Org. Chem.*, **1973**, *38*, 2143-2147; (c). Merckx, E. M., Vanhoeck, L., Lepoivre, J. A., Alderweireld, F. C., Van Der Veken, B. J., Tollenaere, J. P., Raymaekers, L. A. *Spectr: Int. J.* **1983**, *2*, 30; (d) Doesburg, H.M.; Petit, G. H.; Merckx, E. M. *Acta Crystallogr.* **1982**, *B38*, 1181-1185; (e) Oh, S. S.; Butler, W. H.; Koreeda, M. *J. Org. Chem*., **1989**, *54*, 4499-4503.

[15] Chiricuţă, I., Bologa, S., Rişcă, R., Ghergariu, S. *Enciclopedia Oncologică Inst. Oncologic Cluj-Napoca*, **1992**, 19.

[16] Su, X., Viker, N., Gansshapillai, D., Smith, A., Purohita, M., Reed, N.J., Potter, B.V.L. *Molec. Cell. Endocrinol*., **2006**, *248*, 214-217.

[17] Chi, B., Milne, J.C., Carney, D.P., Song, J., Choy, W., Lambert, P.D., Gagne, D.J., Hirsch, M., Cote, A., Davis, M., Lainez, E., Meade, N., Normington, K., Jirousek, M.R., Perni, R.B., *Bioorg. Med. Chem. Lett*., **2009**, *19*, 1416-1420.

[18] Yadav, P.S., Senthilkumar, G.P., *Int. J. Pharm. Sci. Drug Res*., **2011**, 3. 1-7.