LATVIJAS UNIVERSITĀTE
ĶĪMIJAS FAKULTĀTE

ZIGMĀRS ANDŽĀNS

OPTISKI AKTĪVU 1,4-DIHIDROPIRIDĪN-6-SULFANILALKILKARBONSKĀBJU ESTERU SINTĒZE UN ĪPAŠĪBAS

PROMOCIJAS DARBS

Darba vadītāji: Dr.ķīm. Aivars Krauze
Dr.h.ķīm., prof. Andris Zicmanis

RĪGA 2014

Promocijas darbs izstrādāts Latvijas Organiskās sintēzes institūtā laika posmā no 2009. gada līdz 2014.gadam. Darbs veikts ar Eiropas Sociālā fonda projekta ”Atbalsts doktora studijām Latvijas Universitātē” Nr.2009/0138/ 1DP/1.1.2.1.2./ 09/IPIA/ VIAA/004 finansiālu atbalstu.

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2) Dr.ķīm., prof. Ērika Bizdēna, Rīgas Tehniskā universitāte;

3) Dr.hab.ķīm., Grigorijs Veinbergs, Latvijas Organiskās sintēzes institūts.

Promocijas darba aizstāvēšana notiks Latvijas Universitātes Ķīmijas nozares promocijas padomes atklātā sēdē 2014. gada 8. maijā plkst. 17.00 Latvijas Universitātes Ķīmijas fakultātē Kr. Valdemāra ielā 48.

Ar promocijas darbu un tā kopsavilkumu var iepazīties Latvijas Universitātes bibliotēkā Rīgā, Kalpaka bulvārī 4.

LU Ķīmijas zinātņu nozares promocijas

padomes priekšsēdētājs _____/ A. Zicmanis/
(paraksts)

promocijas padomes sekretāre _____/ V. Rudoviča/
(paraksts)

SATURS

APZĪMĒJUMU SARAKSTS

1,4-DHP – 1,4-dihidropiridīns;

^{13}C-KMR – oglekļa kodolu magnētiskā rezonanse;

^{1}H-KMR – ūdeņraža kodolu magnētiskā rezonanse;

AEŠH – augsti efektīva šķidruma hromatogrāfija;

CAL-B – Candida antarctica lipase B;

c – konversija;

CAL-B – lipāze *Candida antarctica* B;

de – diastereomērais pārākums;

DMSO – dimetilsulfoksīds;

$CHCl_3$ – hloroforms;

H_2O – ūdens;

ep – enantiomērais pārākums;

EtOH – etanols;

IPA – izopropanols;

IPE – diizopropilēteris;

IS – infrasarkanā spektroskopija;

Pi – piperidīns;

PSH – plānslāņa hromatogrāfija;

ŠH-MS – šķidruma hromatogrāfija/masspektrometrija;

st. – stunda;

MeOH – metanols;

DHM – dihlormetāns;

K_2CO_3 – kālija karbonāts;

NaOH –nātrija hidroksīds;

HCl – sālskābe;

KCl – kālija hlorīds;

mg- miligrams;

g – grams;

M – molārs;

ml – mililitrs;

T – laiks;

t – triplets;

kv. – kvartets;

m – multiplets;

MeCN – acetonitrls;

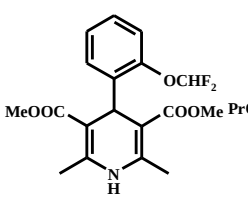
k.t. – kušanas temperatūra;

THF – tetrahidrofurāns.

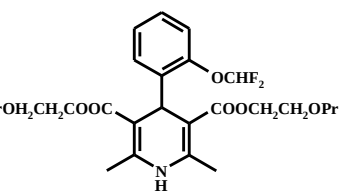
IEVADS

Mūsdienu organiskās un medicīnas ķīmijas aktuāls jautājums ir jaunu regio-, stereo- un enantioselektīvu metožu izstrāde bioloģiski nozīmīgu heterociklu sintēzei. Jaunajām metodēm jābūt vienkāršām, ērtām, ar viegli pieejamiem reaģentiem, videi draudzīgām un ar pēc iespējas zemākām produktu izmaksām. Gandrīz obligāta prasība ir farmaceitiski nozīmīgo produktu, kas satur hirālo centru, sadalīšana pretējos enantiomēros. Optiski aktīvus savienojumus var iegūt, izmantojot gan hirālus katalizatorus vai hirālas palīggrupas, gan arī - enantioselektīvus enzīmus. Pielietojot hirālos katalizatorus, metodes ieguvums ir spēja sadalīt enantiomēros plašu klāstu substrātu, augsta katalītiskā aktivitāte, mīnuss – ilgstošs process un iespējama vides piesārņošana ar smagajiem metāliem. Enzimātiskās katalīzes metodes plusi ir augsta selektivitāte, maigi reakcijas apstākļi un augsta katalītiskā aktivitāte, bet tās mīnusi ir limitēts substrātu daudzums (jāsatur enzimātiski labila grupa) un pagaidām vairumā gadījumu iegūst tikai vienu enantiomēru, jo sākotnējā struktūra satur prohirālo centru. Lai 1,4-dihidropiridīnus (DHP) sadalītu enantiomēros, pielietojot enzimātisko metodi, tiem jāsatur aktivējošās grupas. Hidrolāzes grupas enzīmi ir izmantojami enzimātiskajās reakcijās, ja DHP satur estera, glikozidāzes, ētera, peptīda, halogenīda vai amīda grupu.

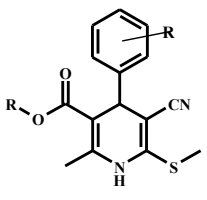
Tēmas aktualitāte. Ir zināms, ka farmaceitiski nozīmīgu vielu enantiomēriem novēro īpašību atšķirības: viens optiskais izomērs var būt daudzkārt aktīvāks par otru optisko izomēru, tiem var novērot pat pretēju bioloģisko aktivitāti, kā arī iespējama atšķirīga toksicitāte. Ja abiem enantiomēriem ir atšķirīgas farmakoloģiskās īpašības, tad racemāts uzskatāms par vielu maisījumu ar 50% tīrību. Šādas atšķirības novēro amlodipīna un felodipīna enantiomēriem. Abos gadījumos (*S*)-enantiomēri ir aktīvāki par (*R*)-enantiomēriem un, lietojot aktīvāko izomēru, pacientiem nenovēro tik daudz blaknes kā tad, ja pielietots racemāts.



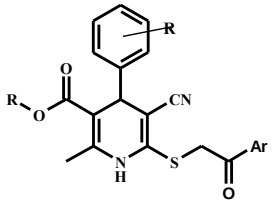
Foridons



Cerebrokrasts



Kardiovaskulārās īp.



MDR modulators

Latvijas Organiskās sintēzes institūta Membrānaktīvo savienojumu un *β*–diketonu laboratorijā ir radīti preparāti foridons (antihipertensīva aktivitāte) [1] un cerebrokrasts (uzlabo un veicina atmiņas procesus) [2, 3]. Tie ir simetriski 1,4-dihidropiridīni.

Vairāki nozīmīgi ārstniecības līdzekļi ir radīti asimetrisku 1,4-dihidropiridīnu rindās: bez amlodipīna un felodipīna vēl klevidipīns, lekanidipīns, nikardipīns, nimodipīns u.c.

Farmakoloģiski nozīmīgi ir arī 6-alkilsulfanil-1,4-dihidropiridīni. Tiem piemīt mazāk izteikts Ca antagonisms un tā kā tie kopumā noraksturoti kā maztoksiskāki savienojumi, tad tiek pētītas šo savienojumu pielietošanas iespējas ļaundabīgo audzēju ārstēšanā. Aktuāla problēma ir zāļu multirezistence (MDR – *multidrug resistance*), un tas ir galvenais šķērslis ķīmijterapijas lietošanai vēža ārstēšanā [4]. Galvenā multirezistences pazīme ir pārmērīga P-glikoproteīna (P-gp) un citu transporta proteīnu ekspresija, kas izsauc aktīvās zāļu vielas uzkrāšanās samazinājumu šūnā [5, 6]. P-gp inhibējošas īpašības piemīt dažādām vielu grupām, tai skaitā, Ca^{2+} kanālu blokatoriem, piemēram, verapamilam, daudziem 1,4-dihidropiridīniem (1,4-DHP) [7, 8]. Pamatšķērslis šo savienojumu izmantošanā MDR novēršanai ir to kardiotoksiskums. Tā kā pasaulē joprojām nav šīs indikācijas preparātu, tad ir aktuāli izstrādāt maztoksiskus multirezistences modulatorus.

Arī 6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes esteru individuālajiem enantiomēriem sagaidāmas atšķirīgas farmakoloģiskās īpašības. Ja izstrādājot potencialo kardiovaskulāro preparātu, uzdevums ir atrast aktīvāko enantiomēru, tad konstruējot multirezistences modulatorus uz DHP bāzes, pielietojumu varētu atrast pretējais mazāk aktīvais enantomērs. Mūsu piedāvātie pretējo enantiomēru iegūšanas ceļi ir izaicinoši, jo hirālais centrs no reakcijas centra atrodas 6 saišu attālumā, un tas ir pilnīgi oriģināls pētījums. Tā kā ieplānoto reakciju rezultātā ģenerēti optiski aktīvs reaģētspējīgs 1,4-dihidropiridīn-6-tiolāts, tad metode varētu pavērt iespējas ērti iegūt plašu klāstu enantiotīrus (enantiobagātinātus) sēru saturošus 1,4-DHP.

Darba mērķis - izstrādāt metodes S saturošu 1,4-dihidropiridīnkarbonskābju esteru enantiosadalīšanai un rast risinājumus praktiski nozīmīgo kardiovaskulāro aktivitāti uzrādošo 6-metilsulfanil-1,4-dihidropiridīnu iegūšanai individuālu enantiomēru veidā. Mērķa sasniegšanai tika izvirzīti vairāki uzdevumi:

- sintezēt 6-alkilsulfanil-1,4-DHP, kuri 3.vietā satur enzimātiski hidrolizēties spējīgas estera grupas ($COOCH_2COOR$ un $COOCH_2OCOR$), kā arī 6-alkilsulfanil-1,4-DHP-3-COOH vai 1,4-DHP-6-SCH_2COOH un veikt enantiosadalīšanu ar enzīmiem vai komerciāli pieejamiem optiski aktīviem amīniem.

- sintezēt 1,4-DHP-SCH_2OCOR un 1,4-DHP-SCH_2CH_2COOR un izvērtēt, vai līdz šim nepētītās merkaptometiloksikarbonil- un merkaptopropionskābes esteru grupas uzrāda pietiekoši augstu hidrolītisko aktivitāti, kas ļautu veikt farmaceitiski perspektīvo 1,4-dihidropiridīnu atvasinājumu enantioselektīvo enzimātisko hidrolīzi.

- izvērtēt vai hirālajam centram 1,4-DHP-S$(CH_2)_n$COOR tipa savienojumos atrodoties 5 - 9 saišu attālumā no reakcijas centra, iespējama optiski aktīvo savienojumu rašanās un dot hipotētisku skaidrojumu.

- izstrādāt preparatīvu metodi kā veikt enantiotīru (enantiobagātinātu) 1,4-DHP-SCH_2OCOR (1,4-DHP-SCH_2CH_2COOR) hidrolīzi, deformilēšanu (deakrilāciju) un alkilēšanu, lai iegūtu optiski aktīvus mērķa produktus.

- iegūt optiski aktīvus savienojumus, veicot 1,4-dihidropiridīn-6-merkaptoetiķskābes esteru aminolīzi enzīmu klātbūtnē.

Darba praktiskā nozīme

- Sagaidāms, ka jaunsintezēto enantiotīro savienojumu kardiovaskulāro un multirezisrences modulējošo īpašību izpēte ļaus radīt izejas platformu, lai radītu potenciālos preparātus.

- Izstrādātas divas jaunas metodes kardiovaskulāro aktivitāti uzrādošo 5-ciāno-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes esteru iegūšanai individuālu enantiomēru veidā.

Viena metode ietver 1,4-dihidropiridīn-6-merkaptopropionskābes esteru sintēzi, to enantioselektīvo hidrolīzi enzīma klātbūtnē, deakrilāciju un alkilēšanu.

Otra metode ietver 6-alkilkarboksimetilsulfanil-1,4-dihidropiridīnu sintēzi, to enzimātisko hidrolīzi, 6-alkilkarboksimetilgrupas nošķelšanu un alkilēšanu.

Šīm metodēm sagaidāma liela praktiska nozīme, jo tiek ģenerēts hirāls, ļoti reaģētspējīgs tiolāts, kurš savukārt reaģējot ar elektrofīlajiem reaģentiem, paver iespējas ērti iegūt plašu klāstu enantiobagātinātus (ideāli enantiotīrus) sēru saturošus 1,4-DHP.

Promocijas darba rezultātu aprobācija

Zinātniskās publikācijas:

1. A. Krauze, L. Sile, L. Chernova, Z.Andzans, G. Duburs. Synthesis of 6-alkoxycarbonylmethylsulfanyl-5-cyano-2-methyl-4-phenyl-1,4-dihydropyridine-3-carboxylates, Heterocyclic Communications, 15(4), 2009, 297-301.
2. L. Beķere, A. Krauze, I. Šestakova, I. Domračeva, Z. Andžāns, G. Duburs. Synthesis and properties of methyl 6-alkylsulfanyl-4-(2-chlorophenyl)-1,4-dihydropyridine-3-carboxylates, Latvijas ķīmijas žurnāls, 2, 2010, 146-151.
3. A.Krauze, Z.; Andžāns, G.Duburs. Synthesis and properties of partially hydrogenated ethyl ([3,4']bipyridin-6'-ylsulfanyl) acetates, Latvijas ķīmijas žurnāls, 1, 2010, 66-71.
4. Z.Andzans, A.Krauze, L.Bekere, S.Grinberga, I.Adlere, G.Duburs. Synthesis and hydrolysis of ethoxycarbonylmethyl and cyanoethyl 5-cyano-6-methylsulfanyl-1,4-dihydropyridine-3-carboxylates, Heterocyclic Letters, 1(3), 2011, 197-204.
5. Z. Andzans, A. Krauze, I. Adlere, L. Krasnova, G. Duburs. Synthesis and enantioselective lipase-catalyzed kinetic resolution of methyl 6-(methoxycarbonyl-methylsulfanyl)-1,4-dihydropyridine-3-carboxylates, Chemistry of Heterocyclic Compounds, 3, 2013, 454-460.
6. A. Krauze, Z. Andzans, L. Krasnova, S. Germane, I. Domracheva, I. Adlere, S. Grinberga, G. Duburs. Synthesis of 5-alkoxycarbonyl-6-alkylsulfanyl-4-(2-difluoromethoxyphenyl)-1,4-dihydropyridines and related derivatives as analogues of cognition enhancer cerebrocrast, Chemistry of Heterocyclic Compounds, 4, 2013, 607-614.
7. Z.Andzans, I.Adlere, A. Versilovskis, L. Krasnova, S. Grinberga, G. Duburs, A. Krauze. Effective method of lipase-catalyzed enantioresolution of 6-alkylsulfanyl-1,4-dihydropyridines, Heterocycles, 89(1), 2014, 43-58.

Konferenču tēzes:

1. Andzans Z. Hydrolysis of ethoxycarbonylmethyl-6-methylsulfanyl-1,4-dihydropyridine-3-carboxylate. Latvijas Ķīmijas žurnāls Rīga, Latvija, 2009 372, P. Walden 6th Symposium on Organic Synthesis, Rīga.

2. Z. Andžāns, I. Adlere, L. Beķere, A. Krauze, G. Duburs. Reactivity of 6-ethoxycarbonylalkylsulfanyl-1,4-dihydropyridine-3-carbonitriles with nucleophiles. Abstracts book of 6th International Conference on Organic Synthesis, 2010, 50, Rīga, Latvija.

3. Andzans Z. 1,4-Dihidropiridīn-6-sulfanilalkilkarbonskābju esteru sintēze un to enzimātiskā hidrolīze. RTU 51. starptautiskā zinātniskā konference, Rīga, Latvija, 2010, mutiskais ziņojums.

4. Andzans Z. Enantioselective enzymatic hydrolysis of 1,4-dihydropyridin-6-ylsulfanyl alkanoic acid methyl esters. Latvijas Ķīmijas žurnāls Rīga, Latvija, 2012, 52, P. Walden 7th Symposium on Organic Synthesis, Rīga, Latvija, 2011.

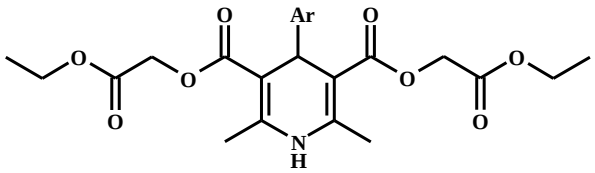
5. Z. Andzans, I. Adlere, G. Duburs, A.Krauze. Lipase-catalyzed enantioresolution of 6-methoxycarbonylethylsulfanyl-1,4-dihydropyridines. Abstracts book of 7th International Conference on Organic Synthesis, 2012, 48, Tallinn, Estonia.

6. Z. Andzans, I.Adlere. „XVth Conference on Heterocycles in Bio-organic Chemistry – 2013" ar stenda referātu "Enantioseparation of 1,4-dihydropyridine-6-mercaptoethanol"

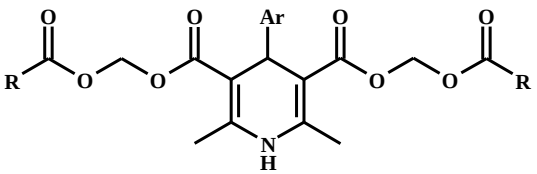
1. LITERATŪRAS APSKATS

Literatūrā ir aprakstīta simetrisku 1,4-dihidropiridīn-3,5-dikarbonskābju dietoksikarbonilmetilesteru **1** sintēze un to ķīmiskā un enzimātiskā hidrolīze.

Enantiotīri 1,4-DHP iegūti pielietojot gan ķīmiskās, gan ķīmiski enzimātiskās metodes. Enzīmi sekmīgi pielietoti tādos gadījumos, kad DHP gredzena 3. un 5. vietās ievestas enzimātiski labilas grupas - dubultesteru (sav. **1**) vai apgriezto esteru grupas (sav. **2**) [9, 10].



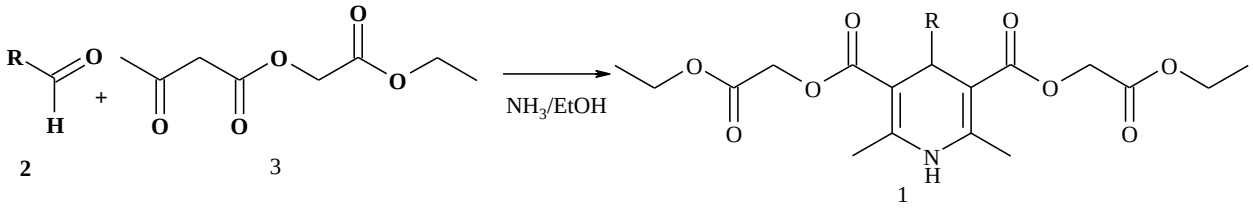
1



2

1.1. 1,4-Dihidropiridīn-3,5-dietoksikarbonilmetilesteru iegūšana

Galvenā 1,4-dihidropiridīn-3,5-dietoksikarbonilmetilesteru **1** iegūšanas metode ir Hanča sintēze. Divu ekvivalentu etoksikarbonilmetilacetoacetāta **3** reakcijā ar vienu ekvivalentu aromātisko aldehīdu amonjaka klātbūtnē ilgstoši vārot etanola šķīdumā, veidojas 1,4-DHP-3,5-dikarbonskābes dikarboksimetilesteri **1** ar 42-67% iznākumiem [9].



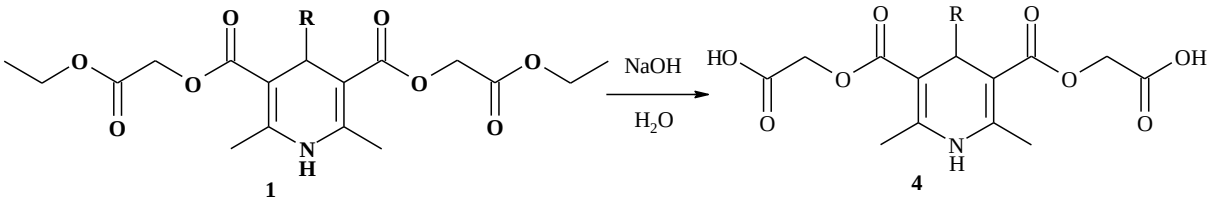
2 **3** **1**

a) R = 4-Cl-C_6H_4; b) R = 2-$OCHF_2$-C_6H_4; c) R = 2-Cl-C_6H_4;
d) R = C_6H_5; e) R = 3-NO_2-C_6H_4

Iznākumus izdodas paaugstināt, ja reakcijas maisījumam pievieno pakāpeniski amonjaku. Ja viss nepieciešamais amonjaka daudzums tiek pievienots jau reakcijas sākumā, tad laika gaitā tas iztvaiko, kā rezultātā nenotiek 1,4-dihidropiridīna cikla saslēgšanās [9].

1.2. 1,4-Dihidropiridīn-3,5-dietoksikarbonilmetilesteru hidrolīze

4-Aril-2,6-dimetil-1,4-dihidropiridīn-3,5-dietoksikarbonilmetilesteru ķīmiskā hidrolīze. Esteru **1** hidrolīze norit nātrija vai kālija hidroksīdu ūdens šķīdumos istabas temperatūrā un tās rezultātā iegūst 2,6-dimetil-1,4-dihidropiridīn-3,5-dikarbonskābes esterus **4**, pie tam hidrolīze notiek pie „galējām" esteru grupām abās pusēs. Hidrolīze nenotiek pie „iekšējām" esteru grupām stērisko un elektronisko faktoru dēļ [9].

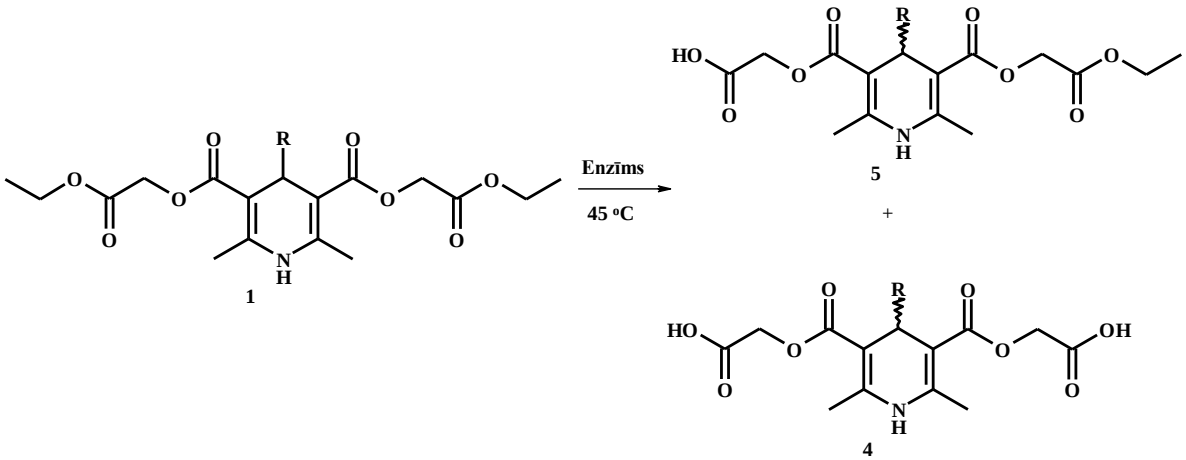


1 **4**

a) R = 4-Cl-C_6H_4; b) R = 2-$OCHF_2$-C_6H_4; c) R = 2-Cl-C_6H_4;
d) R = C_6H_5; e) R = 3-NO_2-C_6H_4

4-Aril-2,6-dimetil-1,4-dihidropiridīn-3,5-dietoksikarbonilmetilesteru enzimātiskā hidrolīze. Plaši pētīta 2,6-dimetil-1,4-dihidropiridīn-3,5-dietoksikarbonilmetilesteru **1** enzimātiskā hidrolīze. Piemeklējot attiecīgos enzīmus un reakcijas apstākļus, ir iespējams 1,4-DHP **1** sadalīt enantiomēros.

Simetrisku 1,4-dihidropiridīn-3,5-dietoksikarbonilmetilesteru **1** gadījumā, svarīgi ir piemeklēt tādus reakcijas apstākļus, kur enzīms hidrolizē vienu estera grupu, iegūstot enantiotīru 1,4-DHP **5**.



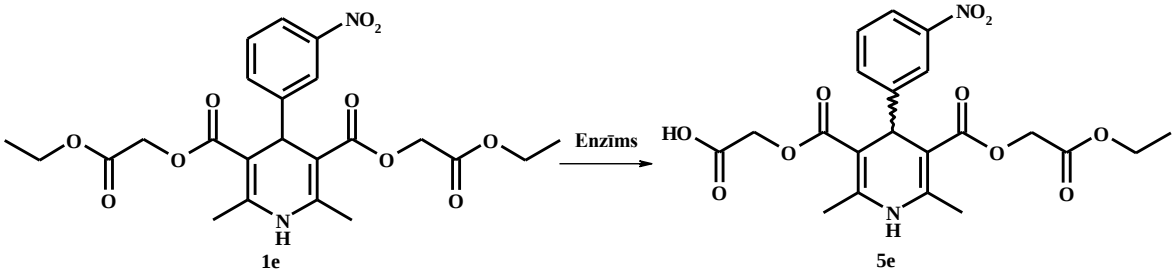
1 **5** **4**

a) R = 4-Cl-C_6H_4; b) R = 2-$OCHF_2$-C_6H_4; c) R = 2-Cl-C_6H_4;
d) R = C_6H_5; e) R = 3-NO_2-C_6H_4

Esteru grupu enzimātiskajai hidrolīzei 1,4-dihidropiridīn-3,5-dietoksikarbonilmetilesteriem ir izmantotas hidrolāzes: Proteāze P6 (*Aspergillus melleus*), Acilāze 30 000 (*Aspergillus sp.*), *Candida antarctica* lipāze B (CAL-B), lipāze AH (*Pseudomonas sp.*) un lipāze PS (*Pseudomonas cepacia*).

Proteāzes P6 un Acilāzes 30 000 gadījumā dietoksikarbonilmetilesterus **1** hidrolizē fosfāta buferšķīdumā ar pH = 7,5, tam pievieno 15% acetonitrila un uztur 45 ^{o}C temperatūru. Abi enzīmi šādos apstākļos hidrolizē estera grupu no abām pusēm, veidojot diskābi **4**, kas ir racemisks savienojums [9].

Lipāzes AH (*Pseudomonas sp.*) gadījumā, mainot šķīdinātājus, mainās enzīma stereoselektivitāte. Savienojuma **1e** hidrolīze ar lipāzi diizopropilēterī (IPE), kas piesātināts ar ūdeni, reakcijas laiks ir 1 st., iznākums ir 83% un enantioselektivitāte ir 68%. Reakciju veicot 20 ^{o}C temperatūrā cikloheksānā, kas satur ūdeni, reakcijas laiks ir 17 st., ķīmiskais iznākums ir 57%, savukārt enantioselektivitāte palielinās līdz 91% [10].



1e 5e

Enzimātiskā hidrolīze ar lipāzi PS (*Pseudomonas cepacia*) notiek līdzīgi kā ar lipāzi AH. Veicot enzimātisko hidrolīzi ar lipāzi PS, ir pētīta divu šķīdinātāju ietekme uz stereoselektivitāti, reakcijas laiku un iznākumu. Enzimātiskā hidrolīze veikta savienojumam **1e** IPE un cikloheksānā ūdens klātbūtnē 20 ^{o}C temperatūrā. Izmantojot kā šķīdinātāju IPE, reakcijas laiks ir 24 st., iznākums 86% un enantioselektivitāte 99%. Ja tiek lietots cikloheksāns, tad reakcijas laiks ir 72 st., iznākums tikai 31% un enantioselektivitāte 92% [10].

Kā viens no labākajiem enzīmiem literatūrā ir minēts *Candida antarctica* lipāze B (CAL-B, Novozym 435®). CAL-B ir ļoti efektīvs katalizators dažādu substrātu enantioselektīvām transformācijām. Esteru **1** hidrolīzes procesā rodas gan vēlamais produkts **5**, gan arī diskābes **4**. Hidrolīzes ierobežojošais faktors ir substrātu **1a-e** šķīdība ūdens saturošos šķīdumos. Sajaucot fosfāta buferšķīdumu (pH = 7,5) ar acetonitrilu un uzturot maisījumam 45 ^{o}C temperatūru, paaugstinās substrāta **1** šķīdība, bet tik un tā sākumā reakcijas maisījums ir heterogēns. Ir grūti savstarpēji salīdzināt enzimātiskās reakcijas ar dažādiem

substrātiem, ja pētāmais objekts šķīst reakcijas gaitā, kā tas ir ar **1a,c** savienojumiem. Enzīma stereoselektivitāte ievērojami izmainās, ja izmanto dažādus šķīdinātājus. Izmantojot enzīmu CAL-B un mainot organiskos šķīdinātājus, iegūti atšķirīgi rezultāti (skatīt 1.1. tabulu).

1.1. tabula

Enzīma CAL-B katalizēta hidrolīze savienojumiem 1a-e dažādos šķīdinātājos 45 °C

Nr.	Substrāts	Reakcijas laiks, st.	Enantiomērais pārākums (ep) skābei **5**, %
1.	1a	19[1]	93[1]
2.	1a	22[2]	55[2]
3.	1b	48[1]	79[1]
4.	1b	282[3]	97[3]
5.	1c	20[1]	77[1]
6.	1c	168[3]	67[3]
7.	1d	96[1]	68[1]
8.	1d	48[4]	69[4]
9.	1e	48[1]	72[1]
10.	1e	138[5]	93[5]

1 – acetonitrila fosfāta buferšķīdums

2 – terc-butilspirta fosfāta buferšķīdums

3 – diizopropilētera šķīdums ūdenī

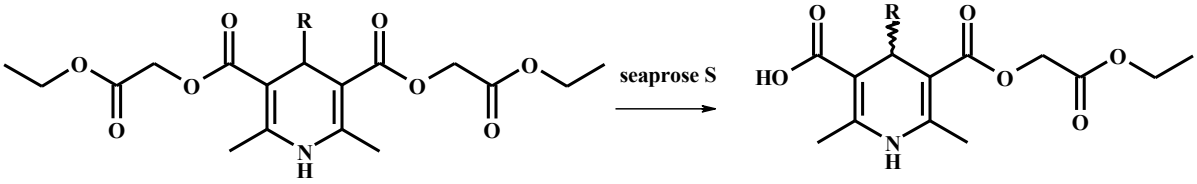
4 – acetona fosfāta buferšķīdums

5 – 20% DMSO fosfāta buferšķīdums

Standartā tiek izmantots acetonitrila modificētais buferšķīdums. Laba enantioselektivitāte iegūta savienojumam **5b** (97%), kad reakcija veikta IPE, kas satur ūdeni, bet savienojumam **5c** tikai 67%. Reakciju veicot 20% dimetilsulfoksīda (DMSO), fosfāta buferšķīdumā ar pH = 7,5 savienojuma **5e** enantioselektivitāte ir 93%. Eksperimentos ar acetona fosfāta buferšķīdumiem iegūti **5d** ar 69% enantiomēro pārākumu. Ar terc-butilspirta fosfāta buferšķīdumiem iegūti sliktāki rezultāti nekā ar acetonitrila modificēto buferšķīdumu, savienojums **5a** iegūts ar 55% enantiomēro pārākumu [9].

CAL-B katalizētās reakcijās produktus **7** iegūst ar labu enantioselektivitāti un īsākā laikā, salīdzinoši ar pārējiem enzīmiem. Ķīmiskie iznākumi savienojumiem **5a-e** ir 29-87%, reakcijas laiki 18-96 st. un enantioselektivitāte 68-93%, par šķīdinātāju izmantojot acetonitrilu [9].

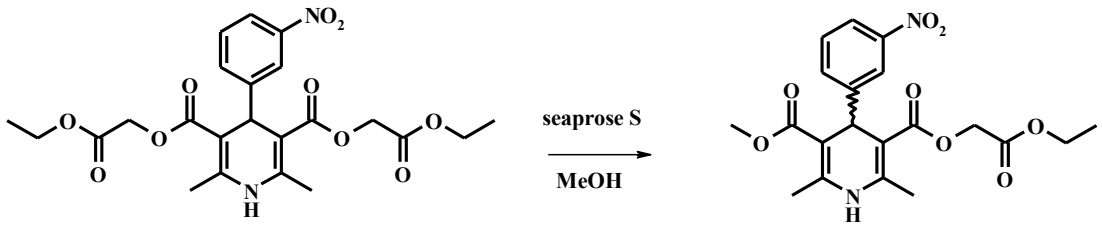
Ja enzimātiskajai hidrolīzei izmanto enzīmu seaprose S (*Aspergillus melleus*), tad hidrolīze notiek tikai pie vienas no esteru grupām, kas atrodas pie piridīna gredzena, iegūstot attiecīgo karbonskābi **6**. Ar seaprose S neizdodas iegūt ne dubultesteru monoskābes **5**, ne diskābes **4**. Reakcija tiek veikta 2% DMSO, fosfāta buferšķīdumā ar pH = 7,5, istabas temperatūrā. Reakcijas laiks ir 72 st., savienojumu **6** ķīmiskie iznākums ir ~ 60% un enantioselektivitāte ir virs 99% [11, 12].



1 6

a) R = 4-Cl-C_6H_4; b) R = 2-$OCHF_2$-C_6H_4; c) R = 2-Cl-C_6H_4;
d) R = C_6H_5; e) R = 3-NO_2-C_6H_4

Seaprose S katalizē arī 1,4-DHP etoksikarbonilmetilesteru pāresterifikāciju, ja šķīdumam pievieno 5% metanola un maisījumu silda 30 ^oC temperatūrā. Rodas metilesteris **7** ar 52% ķīmisko iznākumu un 99% enantiomēro pārākumu. Diemžēl publikācijas autori – japāņi nav uzdevuši ^1H-KMR un IS spektru datus [13].



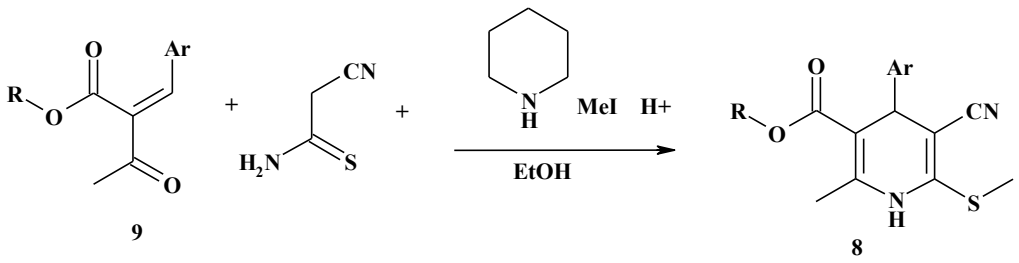
1e 7

1.3. 5-Ciāno-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes esteru iegūšana

Mums vairāk interesē sēru saturoši 1,4-DHP. Pašreiz literatūrā ir atrodami dati par 6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes etil- vai metilesteru sintēzi. Šāda tipa savienojumus var iegūt gan pakāpeniskā sintēzē, gan daudzkomponentu viena reaktora sintēzē.

Četru komponentu viena reaktora metode, par izejvielu izmantojot 2-(arilmetilēn)acetetiķskābes etilesterus. Literatūrā [14, 15] 5-ciāno-4-(2-hlorfenil)-2-metil-

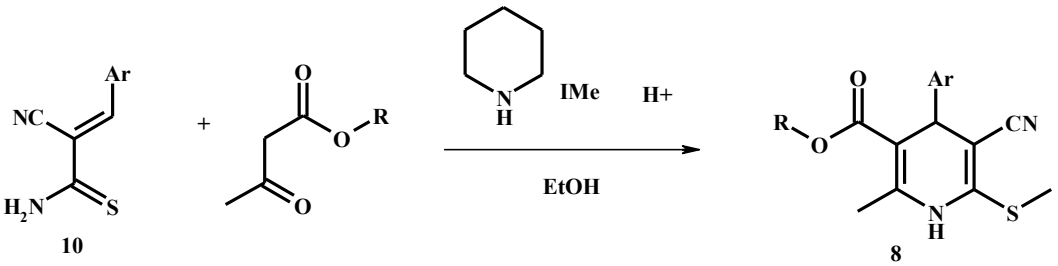
6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes etilesteris **8** iegūts pēc trīs metodēm, kā viena no tām ir četru komponentu viena reaktora metode. Kā izejas savienojumi izmantoti 2-(arilmetilēn)acetetiķskābes esteris **9** un 2-ciānotioacetamīds. Reakcija noris istabas temperatūrā. 6-Metilsulfanil-1,4-dihidropiridīnu **8a** iegūst ar 80% iznākumu, savienojumu **8b** ar 86%, savienojumu **8c** ar 80% un savienojumu **8d** ar 90% iznākumu.



9 8

a) R = Et, Ar = Ph; b) R = Et, Ar = 2-Cl-C_6H_4; c) R = Me, Ar = Ph; d) R = Me, Ar = 2-Cl-C_6H_4

Četru komponentu viena reaktora metode, kur kā izejvielas izmanto 3-arilmetilēn-2-ciānotioakrilamīdus. Izmantojot par pamatizejvielām 3-arilmetilēn-2-ciānotioakrilamīdu **10** un acetetiķskābes etilesteri un pielietojot četru komponentu viena reaktora metodi, iegūst 5-ciāno-4-(2-hlorfenil)-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes etilesteri **8**. Arī šajā gadījumā reakcija noris istabas temperatūrā. 6-Metilsulfanil-1,4-dihidropiridīnu **8a** iegūst ar 91% iznākumu, savienojumu **8b** ar 86%, , savienojumu **8c** ar 87% un savienojumu **8b** ar 89% iznākumu [14, 15].

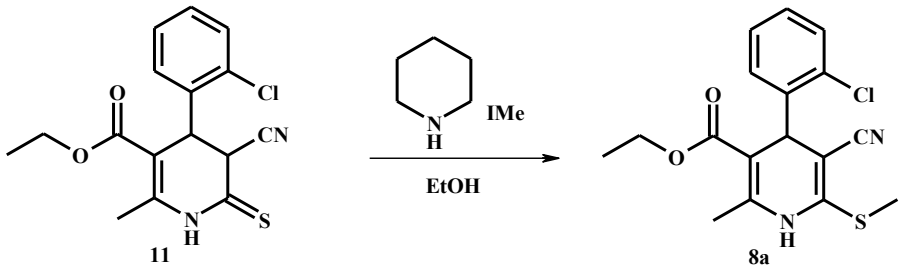


10 8

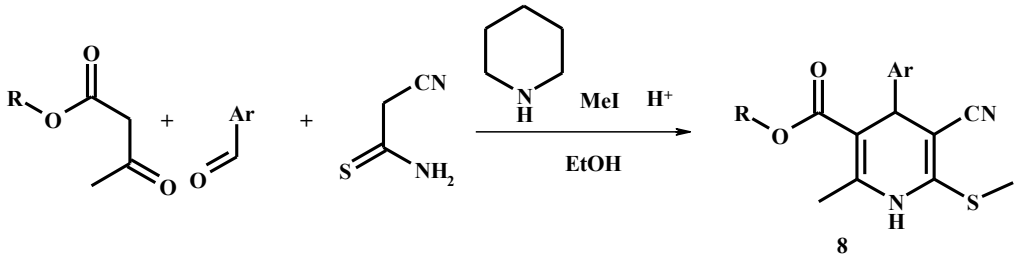
a) R = Et, Ar = Ph; b) R = Et, Ar = 2-Cl-C_6H_4; c) R = Me, Ar = Ph; d) R = Me, Ar = 2-Cl-C_6H_4

5-Ciāno-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes etilestera iegūšana, izmantojot 1,4-dihidropiridīn-2-(3H)-tionu. 6-Metilsulfanil-1,4-dihidropiridīnu

8a var iegūt arī no iepriekš uzkonstruēta tiona. 1,4-Dihidropiridīn-2-(3H)-tiona **11** alkilēšanas reakcijā ar metiljodīdu piperidīna klātbūtnē etanola šķīdumā 6-metilsulfanil-1,4-dihidropiridīns **8a** izkristalizējas ar 76% iznākumu [14, 15].



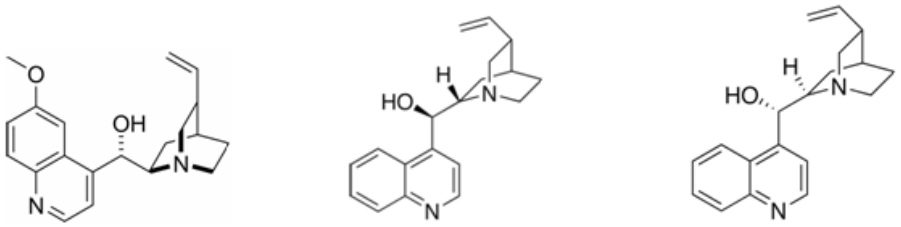
Piecu komponentu viena reaktora metode. Labākā metode 6-metilsulfanil-1,4-dihidropiridīnu **8** iegūšanai ir piecu komponentu viena reaktora metode, kur neizdala starpproduktus. Zinot pareizo reaģentu pievienošanas secību, savienojumus **8** var iegūt ar augstiem iznākumiem. Aromātiskā aldehīda, acetetiķskābes estera, 2-ciānotioacetamīda, piperidīna un metiljodīda reakcijā ar sintēzes summāro iznākumu 96% iegūst savienojumu **8a**, ar 78% iznākumu – savienojumu **8b**, ar 81% iznākumu – savienojumu **8c** un ar 88% iznākumu – savienojumu **8d** [14, 15].



a) R = Et, Ar = Ph; b) R = Et, Ar = 2-Cl-C_6H_4; c) R = Me, Ar = Ph; d) R = Me, Ar = 2-Cl-C_6H_4

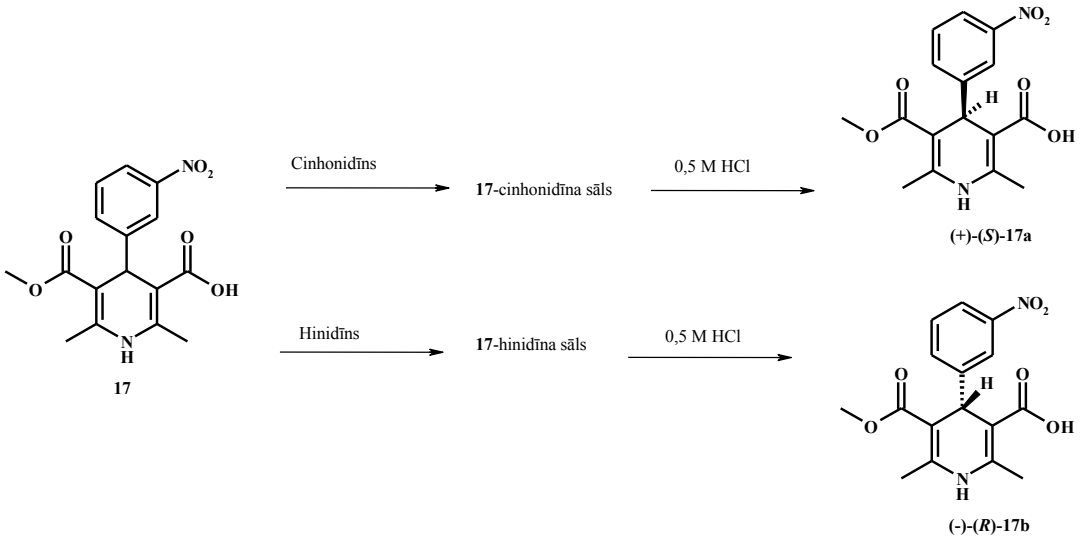
1.4. Racemisku 1,4-DHP-karbonskābju sadalīšana enantiomēros ar hirāliem amīniem

Literatūras avotos atspoguļoti pētījumi, kad 1,4-DHP-karbonskābes tiek sadalītas enantiomēros, pielietojot hinīna alkaloīdus: cinhonidīnu un cinhonīnu (pseidoenantiomēru pārus) un hinidīnu. Tie ir komerciāli pieejami, salīdzinoši lēti optiski aktīvi amīni:



Hinidīns **Cinhonidīns** **Cinhonīns**

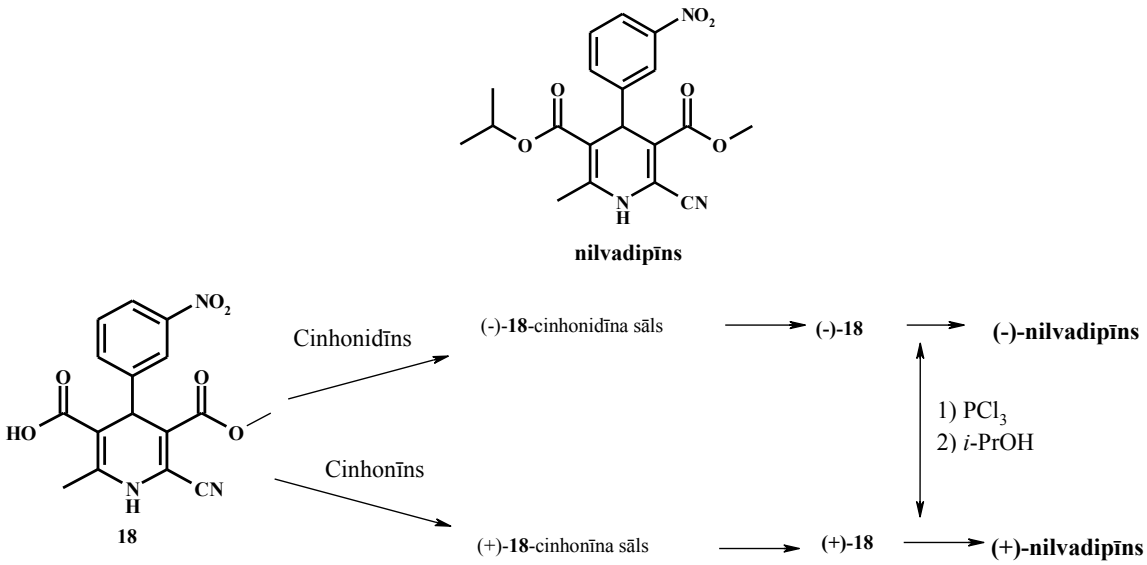
Racemiskās karbonskābes **17** sadalīšana enantiomēros veikta, kristalizējot skābes attiecīgos diastereomēros amīnu sāļus. Kā piemērotākā šķīdinātāju sistēma piemeklēta DMF-ūdens maisījums attiecībā 8 pret 5. Tālāk apstrādājot sāļus ar skābi, izolē enantiotīrās skābes **17a** (84%, ep = 99,5%) un **17b** (93%, ep = 99,5%) (skat. 1.1. att.). Cinhonidīnu un hinidīnu reģenerē atpakaļ no reakcijas filtrāta, bet tie zaudē aktivitāti. Svarīga ir šķīdinātāja ietekme, etanolā un acetonā kristalizācijas iznākumi un skābju enantiotīrība nav augsta (ep = 63-72%). DMF paaugstina enantiotīrību, savukārt ūdens pievienošana – ķīmiskos iznākumus [16].



1.1. att. **1,4-DHP-karbonskābes sadalīšana enantiomēros pēc frakcionētās kristalizācijas ar optiski aktīvajiem amīniem – cinhonidīnu un hinidīnu**

Ziņots par 1,4-DHP-karbonskābes sadalīšanu, kur hinidīna vietā par sadalīšanas aģentu lieto cinhonīnu. Sadalīšanas procesā 1,4-DHP bija nepieciešams ieviest NH-aizsarggrupu. 1-Etoksimetil-aizsarggrupu noņem, apstrādājot ar 1M HCl, un iegūst skābes **17a** un **17b** ar augstu enantiotīrību (> 99%) [17].

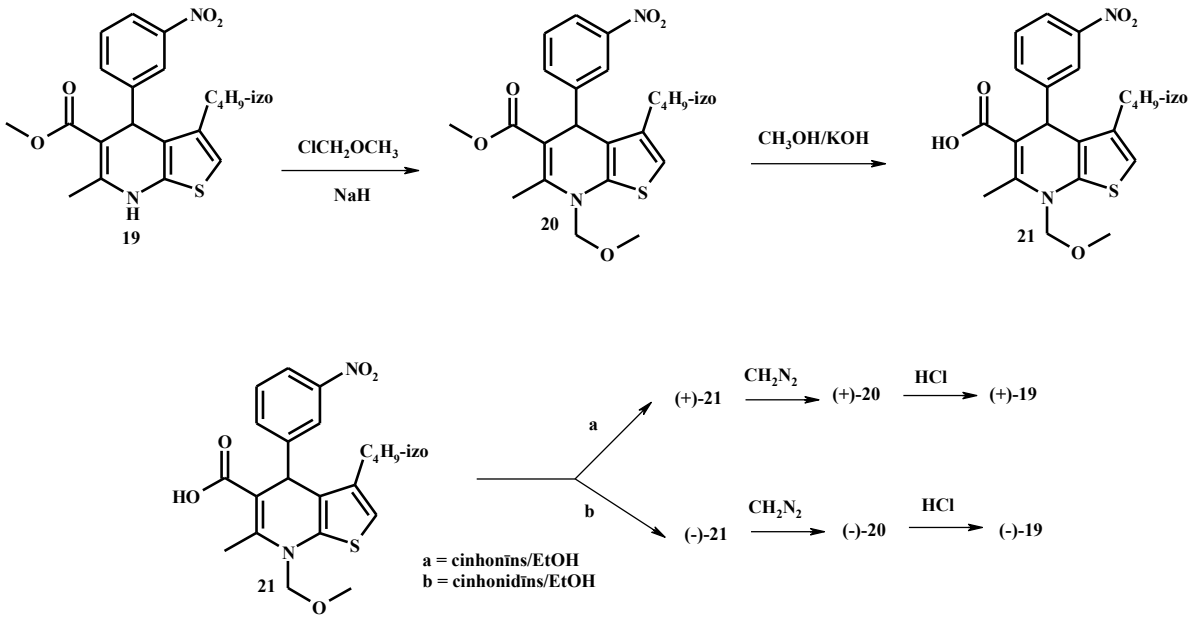
Līdzīgi kā sadalīta skābe **17**, strukturāli nedaudz atšķirīgu nesimetrisku 1,4-DHP-karbonskābi **18** sadalīja enantiomēros ar cinhonidīnu un cinhonīnu. Sadalīšanas shēma attēlota 1.2. attēlā. Skābes attiecīgais izopropilesteris ir antihipertensīvs preparāts nilvadipīns:



1.2. att. **Nilvadipīna pretējo enantiomēru iegūšana, sadalot 1,4-DHP-karbonskābi enantiomēros ar sadalīšanas aģentiem – cinhonidīnu un cinhonīnu**

Analogi arī šajā gadījumā diastereomērie sāļi kristalizēti frakcionēti. Sākumā uz racemisko skābi **18** iedarbojas ar cinhonidīnu, vārot metanola šķīdumā 15 minūtes, kristalizējot diastereomēru **(-)-18**-sāli no metanola, un tā iznākums ir tikai 30%. Šī sāls suspensiju etilacetātā apstrādā ar 2M HCl ūdens šķīdumu, un organisko slāni apstrādājot, iegūst enantiotīro skābi **(-)-18**. Uz atlikušo filtrātu iedarbojas ar otru sadalīšanas aģentu – cinhonīnu, analogi tīro **(+)-18**-sāli iegūst tikai ar 25% iznākumu. Lai no enantiotīrajām skābēm **(-)-18** un **(+)-18** iegūtu aktīvā preparāta atsevišķos enantiomērus, skābes ar fosfora trihlorīdu pārvērš skābes hlorīdos un tad ar izopropanolu esterificē tos, iegūstot (-)-nilvadipīnu un (+)-nilvadipīnu ar 98% un 95% iznākumiem attiecīgi [18].

Optiskajos izomēros sadalīts arī 4,7-dihidrotieno[2,3-*b*]piridīns **19** (1.3. att.). Savienojums **19** pārvērsts tā 7-metoksimetil-aizvietotā atvasinājumā **20**, tādējādi mazinot β-aminovinilkarbonilgrupas konjugācijas elektronisko efektu. Savienojumu **20** pēc tam hidrolizē, iegūstot skābi **21**, kuru sadala enantiomēros kā aprakstīts augstāk minētajos piemēros. Enantiotīrās skābes tad alkilē ar diazometānu un NH-aizsarggrupu noņem ar HCl šķīdumu, produktu absolūtās konfigurācijas noteiktas ar rentgenstruktūranalīzi [19]:



1.3. att. **4,7-Dihidrotiēno[2,3-*b*]piridīna sadalīšana enantimēros**

Visos līdz šim minētajos gadījumos tika pētīta 1,4-dihidropiridīnkarbonskābju sadalīšana enantiomēros, un karboksi-(skābes)-grupas oglekļa atoms šajās molekulās tieši piesaistīts 1,4-DHP gredzenam un attālums no reakcijas centra līdz hirālajam centram ir 2-3 saites. Taču šāda diatereomēru kristalizācijas metode enantiotīru karbonskābju iegūšanā varētu būt veiksmīgs risinājums arī tādu 1,4-DHP sadalīšanā enantiomēros, kur skābes grupa nav tieši piesaistīta 1,4-DHP gredzenam. Mūsu pētījuma objekti ir arī sēra saturošas 1,4-DHP-karbonskābes, kur skābes grupa neatrodas pie 1,4-DHP gredzena. Patentā [20] atspoguļota racemisku sēra atomu saturošu karbonskābju **22** sadalīšana optiskajos izomēros.

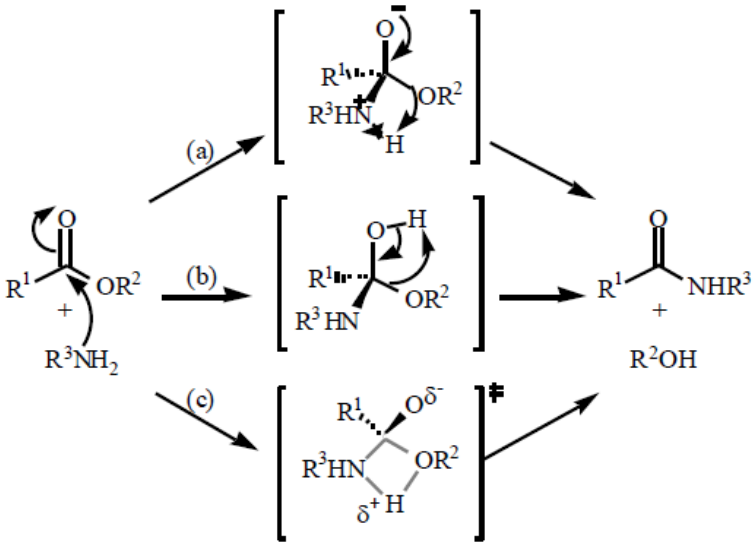
Sadalīšanas procedūras apstākļi tiek variēti. Par šķīdinātājiem lieto acetonu, hloroformu, benzolu, izopropilēteri u.c. Tiek pievienoti tādi hirālie amīni kā brucīns, hinīns, strihnīns, hinidīns, cinhonidīns, cinhonīns, (*S*)-(–)- vai (*R*)-(–)-α-feniletilamīns u.c. Diastereomērie sāļi frakcionēti kristalizēti un pārvērsti par attiecīgajiem skābes **22** pretējiem enantiomēriem. Tā piemēram, (+/–)-*S*-benzoil-2-merkaptopropānskābi vāra etanolā, pievieno cinhonidīnu un šķīdumu dzesē diennakti. Kristalizētais cinhonidīna sāls apstrādāts un iegūta (–)-*S*-benzoil-2-merkaptopropānskābe ar 35% iznākumu. Citu minēto piemēru iznākumi svārstās no 21% līdz 62%.

22

1.5. Enzīmu katalizēta 1,4-DHP esteru aminolīze

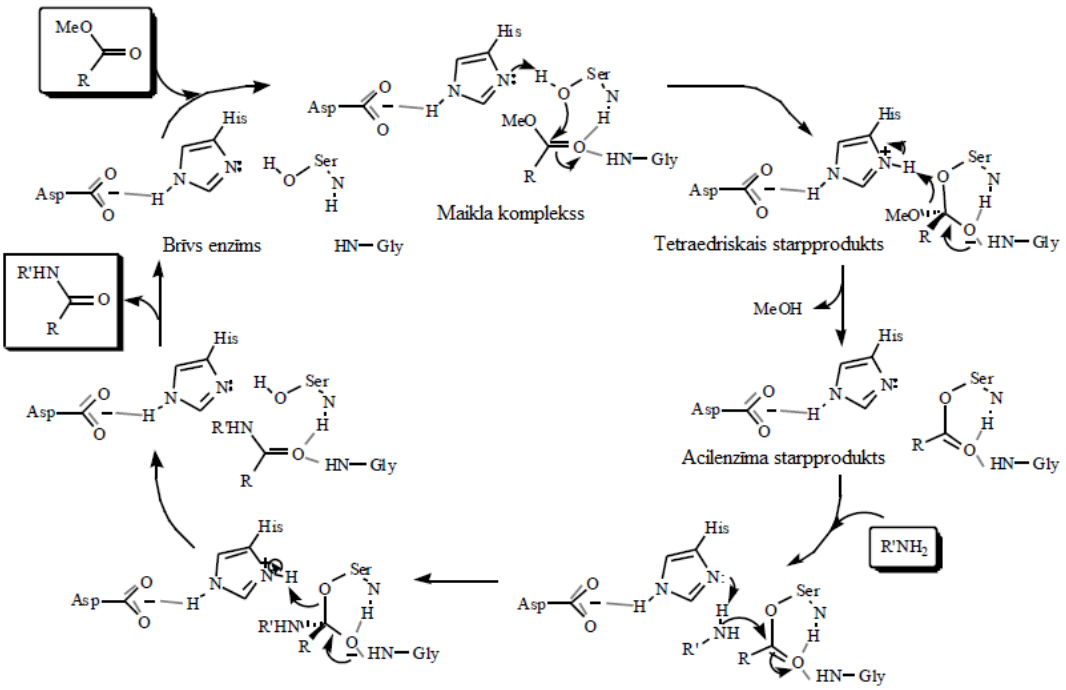
Plaši pētīta literatūrā un mūsu laboratorijā ir esteru sadalīšana enantiomēros, pielietojot enzimātisko hidrolīzi. Alternatīvs variants hidrolīzei savienojumu sadalīšanai eantiomēros, literatūrā ir aprakstītas dažādu substrātu enzīmu katalizētas aminolīzes reakcijas [21-26].

Aminolīze ir klasisks piemērs karbonilgrupas mijiedarbībai ar nukleofīliem, kā arī plaši pielietota reakcija peptīdu saišu veidošanai. Literatūrā ir daudzi kinētiskie un teorētiskie pētījumi par šo reakciju [27]. Balstoties uz šiem pētījumiem var izdalīt trīs iespējamos reakcijas norises mehānismus: (a) pakāpeniskais mehānisms caur cviterjona starpproduktu, (b) pakāpeniskais ceļš caur neitrāliem starpproduktiem un (c) saskaņotais ceļš (1.4. att.).



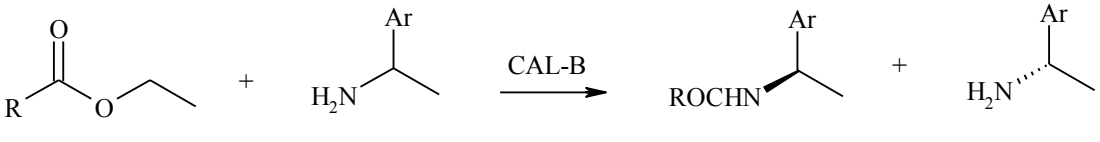
1.4. att. **Aminolīzes reakcijas mehānisms**.

Hidrolāžu vispārpieņemtais darbības mehānisms ir redzams 1.5. attēlā [28]. Katalītiski aktīvo vietu enzīmā veido katalītiskā triāde (serīns, aspargīnskābe un histidīns). Serīna atlikumam pievienojas estera acilgrupa, izveidojot acilenzīma aktivētu starpproduktu. Šis acilenzīma starpprodukts reaģē ar nukleofīlu (amīnu), kā rezultātā iegūst gala produkta amīdu un atbrīvojas enzīms brīvā formā, kas var atkal iesaistītites katalītiskajā ciklā. Aspartāta sānu ķēde aktivē histidīna atlikumu, kas ir atbildīgs par protonu pārnešanu, katalīzes procesā. Vēl viens svarīgs faktors ir oksianjona caurums, ko veido dažādi aminoskābju atlikumi, kas stabilizē negatīvi lādēto skābekli gan pārejas stāvoklī, gan tetraedriskajā starpstāvokļa veidošanās procesā. Sarežģītā enzīma struktūra uzrāda ļoti lielu substrāta/enzīmu mijiedarbības specifiskumu, kas var novest pie augstas regio vai stereoselektivitātes. Šī iemesla dēļ biotransformācija mūsdienās ir lielisks risinājums organiskajā ķīmijā.

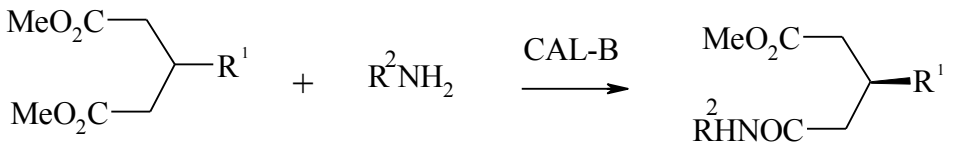


1.5. att. **Serīna hidrolāzes katalizēts aminolīzes reakcijas mehānisms**.

Laba metode racemisku esteru sadalīšanai enantiomēros ir transformēt tos par amīdiem, izmantojot lipāzes katalizētu aminolīzi. Par substrātiem ir iespējams izmantot ne tikai esterus, bet arī skābes. Augstus enantiomēros iznākumus iegūst, izmantojot tādus šķīdinātājus kā metilizobutilketons, tercbutanols, heksāns, diizopropilēteris, heptāns un metiltercbutilēteris [29-34]. Literatūrā pieminēts, ka enantioselektīvās aminolīzes reakcijās tiek izmantots plašs klāsts enzīmu, bet kā viens no effektīgākajiem enzīmiem tiek aprakstīts - Candida antarctica lipāze B. Ar tās palīdzību tiek iegūti augsti enantiomērie iznākumi 80-99% [21, 22 un 35].



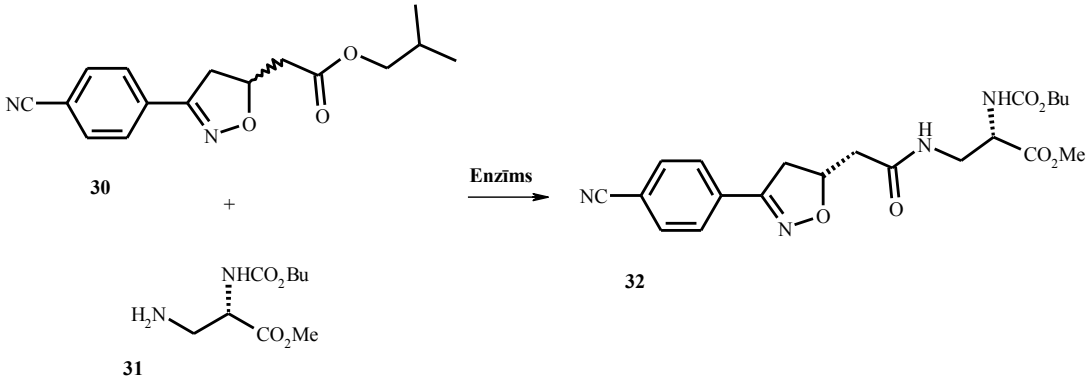
Autoriem izdodas iegūt amīdus **25** ar >98% enantiomēro pārākumu, veicot aminolīzi istabas temperatūrā, pielietojot enzīmu CAL un kā šķīdinātāju izoproponolu [31]. Arī neizreaģējušajam amīnam ir sasniegs >91% enantiomērais pārākums.



27 **28** **29**

Savienojumus **29** autori iegūst ar ļoti augstiem enantiomērajiem pārākumiem >99%, reakcijas veicot dioksānā, 30^{o}C temperatūrā [29].

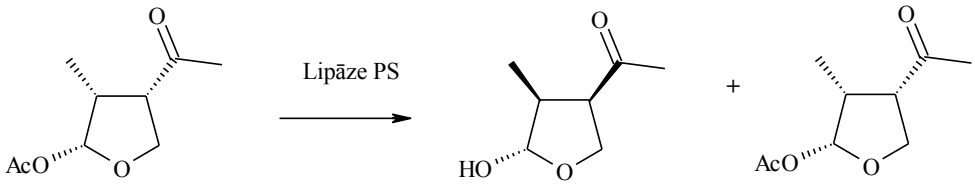
Literatūrā ir aprakstīti pētījumi par enzīmu pielietotām aminolīzes reakcijām, kur tiek pētīti diasteriomēri **32**. Enzimātiskās aminolīzes reakcijas norit IPE, MTBE un heptāna šķīdinātājos, 24-40 ^{o}C temperatūras amplitūdās, kā rezultātā iegūti savienojumi **32** ar > 80% de.



Augstākais rezultāts, veicot aminolīzi ar enzīmu CAL-B metiltercbutilēterī 24^{o}C temperatūrā, iegūts mērķsavienojumus **(R,S)-32** ar 92% de [35].

1.6. Attālināta hirālā centra ietekme uz enantioselektivitāti

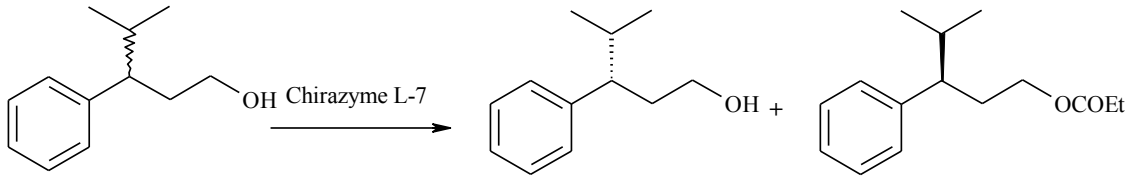
Vairumā gadījumu enzimātiskās hidrolīzes, esterifikācijas, acilēšanas u.c. reakciju substrātu hirālais un reakcijas centrs atrodas tikai vienas vai divu ķīmisko saišu attālumā. Ir minēti piemēri, kad šis attālums ir trīs vai vairāk saites [36]. Lipāzes PS (*Pseudomonas cepacia*) katalizēta Botriodiplodīna acetāta **33** deacilēšanas reakcijā iegūts Botriodiplodīns **34** ar augstu enantioselektivitāti (e.p. >80%) [37]:



33 **(+)-34** **(+)-33**

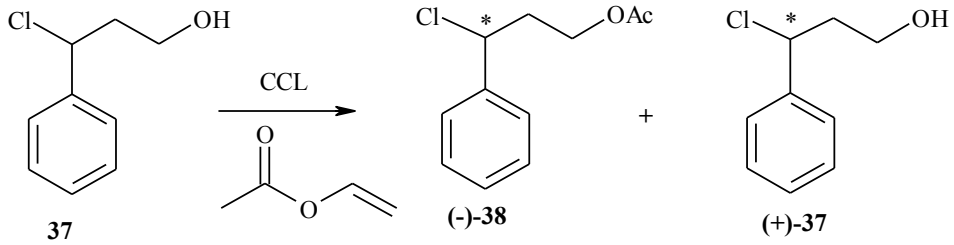
Pētīta γ-aizvietotu pirmējo spirtu enantioselektīva acilēšana lipāžu Chirazyme® L-6, L-7 un L-10 promotētās reakcijās. To enantioselektivitāte (E) sasniedz 11,6 vienības.

4,4-Dimetil-3-fenil-1-pentanola **35** gadījumā, veicot enantiosadalīšanu izopropilēterī/vinilpropionātā, variējot temperatūru, parādīts ka visaugstāko enantiomēro pārākumu izdodas sasniegt 0 °C temperatūrā. Acilētais produkts **36** iegūts ar 60%, bet neizreaģējošais spirts **(S)-35** ar 66% enantiomēro pārakumu. Citu γ-aizvietotu pirmējo spirtu kā substrātu gadījumā (variēti visdažādākie reakcijas apstākļi) enantiomērais pārākums ir robežās no 2% līdz 41% [38].



35 **(S)-35** **(R)-36**

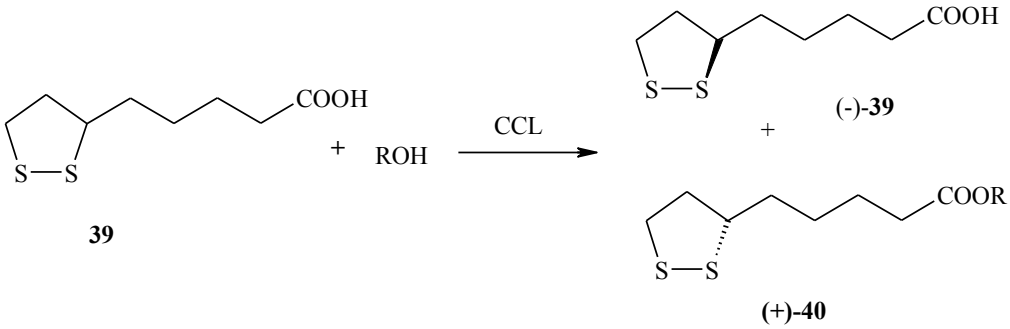
Tāpat enantiosadalīšana veikta lipāzes katalizētās reakcijās, esterificējot 3-aril-3-hlorpropanolus **37** ar vinilacetātu. Augstākais enantiomērais pārākums spirtam **(+)-37** un esterim **(-)-38** (ep. 29% un 31%) sasniegts lipāzes CCL (*Candida rugosa*) promotētā reakcijā cikloheksānā istabas temperatūrā. Tikai pēc otrreizējas spirta **(+)-37** lipāzes katalizētas esterifikācijas izdodas sasniegt 78% enantiomēro pārakumu. [36]:



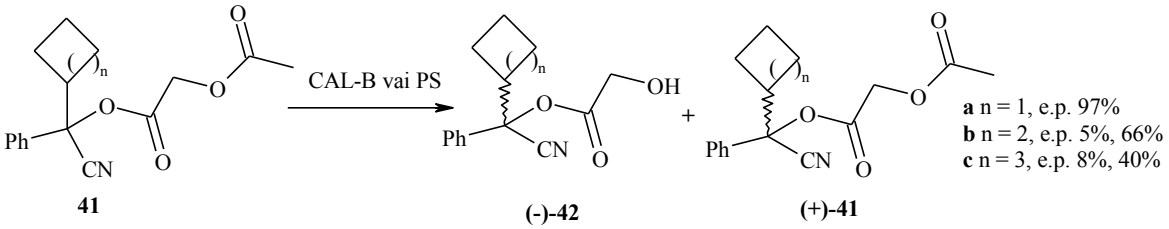
37 **(-)-38** **(+)-37**

Iepriekšminēto trīs piemēru gadījumā substrātu hirālā un reakcijas centra attālums ir trīs ķīmiskās saites.

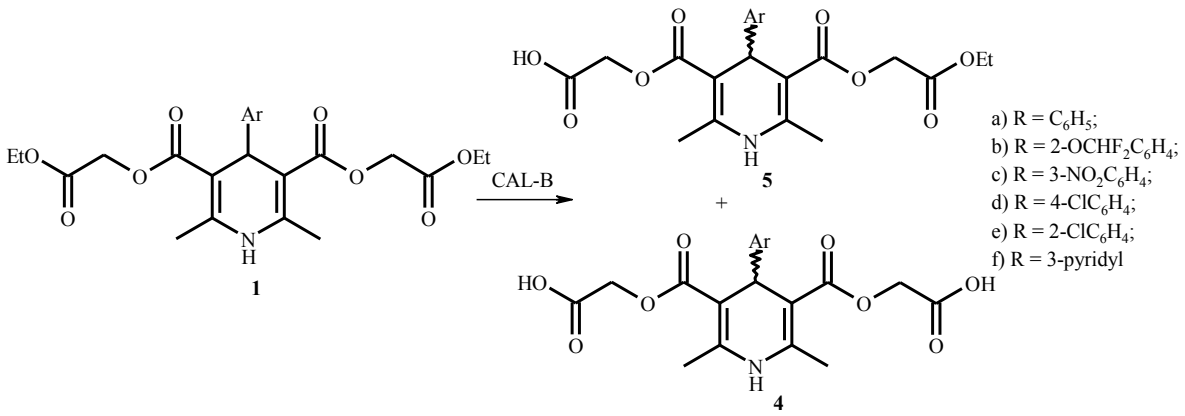
Lipāzes CCL katalizētās 5-(1,2-ditiolān-3-il)pentānskābes (**39)** esterifikācijas reakcijās ar alifātiskiem spirtiem (skābes **39** hirālais centrs ir attālināts no reaģējošās karbonilgrupas par 5 ķīmiskajām saitēm) produktus **40** iegūst ar 12% līdz 75% augstu enantiomēro pārākumu [39]:



Enzimātiski hidrolizējot esterus **41** lipāzes CAL-B vai PS promotētās reakcijās, sasniegta skābes **42a** 99% enantiotīrība. Atrasti optimālie apstākļi: fosfāta buferšķīduma un dioksāna attiecība 1 pret 2 un 30 °C temperatūra. Šai gadījumā reakcijas centrs no hirālā centra atrodas 5 saišu attālumā. Autori parādījuši, ka enantioselektivitāti būtiski ietekmē cikloalkilaizvietotājs. Interesanti, ka atkarībā no pielietotās lipāzes izdodas iegūt dažādas konfigurācijas produktus. Tāpat novēro produktu konfigurācijas atšķiras atkarībā no pielietotās lipāzes. Enzīmu enantioselektivitāte atkarīga no cikloalkilaizvietotāja. Autori to skaidro ar substrāta spēju saistīties ar enzīma aktīvā centra hidrofobisko vietu.



1,4-DHP-3,5-dietoksikarbonilmetilesteru **1** gadījumā CAL-B promotētās reakcijās, atkarībā no reakcijas apstākļiem un substrāta **1** aromātiskā aizvietotāja DHP gredzena 4. vietā, 1,4-DHP **5** iegūti ar 55-99% enantiomēro pārākumu. Augstākais rezultāts sasniegts, ja Ar aivietotājs ir 2-$OCHF_2C_6H_4$. Enantioselektivitāti ietekmē arī reakcijas vide – šķīdinātāji un temperatūra [9]. 1,4-DHP esteru **1** molekulās transformējamā grupa atrodas 5 saišu attālumā no hirālā centra:



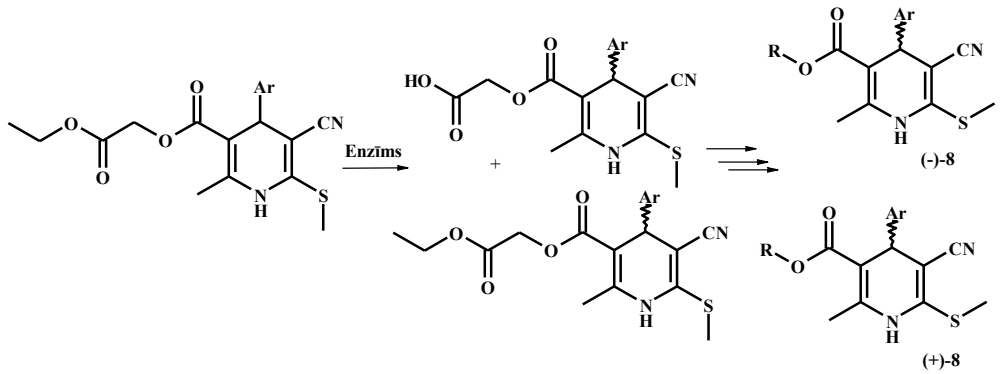
Literatūrā ir ļoti maz pieejama informācija par lipāžu katalizēto reakciju substrātu hirālā centra attālumu no reakcijas centra un to ietekmi uz enantioselektivitāti. Minētie saišu attālumi ir viens no faktoriem, kas ietekmē enantioselektivitāti. Literatūras dati liecina, ka būtiski ir arī reakciju apstākļi un individuāla substrāta mijiedarbība ar atsevišķiem enzīmiem.

2. REZULTĀTI UN TO IZVĒRTĒJUMS

1,4-Dihidropiridīnu sadalīšana optiskajos izomēros ir svarīgs uzdevums, jo bieži vien šo savienojumu enantiomēriem piemīt dažāda bioloģiskā aktivitāte, novēro farmakoloģisko īpašību atšķirības: viens optiskais izomērs ir daudzkārt aktīvāks par otru optisko izomēru, tiem ir pretēja bioloģiskā aktivitāte vai arī viens izomērs ir toksiskāks par otru izomēru.

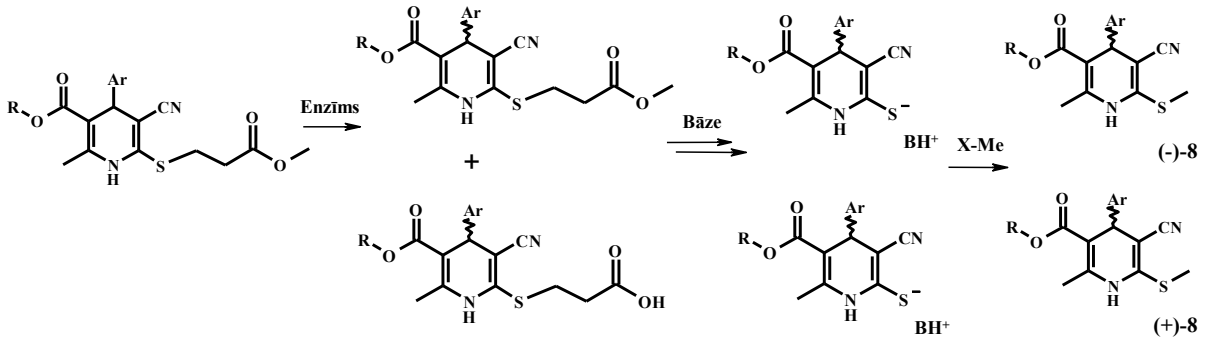
Darba mērķis ir izstrādāt metodes S saturošu 1,4-dihidropiridīnkarbonskābju esteru enantiosadalīšanai, kā arī rast risinājumus praktiski nozīmīgo kardiovaskulāro aktivitāti uzrādošo 5-ciāno-4-aril-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes esteru **8** [14] sadalīšanai enantiomēros. Lai iegūtu abus optiski aktīvos 6-metilsulfanil-1,4-dihidropiridīnu **8** enantiomērus, teorētiski iespējami vairāki risinājumi:

1. iespējamais ceļš (2.1. attēls) ir tādu sēru saturošu 1,4-dihidropiridīn-3-karbonskābju atvasinājumu sintēze, kuri saturētu enzimātiski labilo etoksikarbonilmetilestera grupu, to enantioselektīvā hidrolīze enzīmu klātbūtnē, attālinātās karbonskābes dekarboksilācija un pie cikla tuvākās karbonskābes alkilēšana. Šī metode darbojas sēru nesaturošo simetrisko DHP gadījumā. Nav sistemātiski pētīta attālinātās karbonskābes dekarboksilācija.



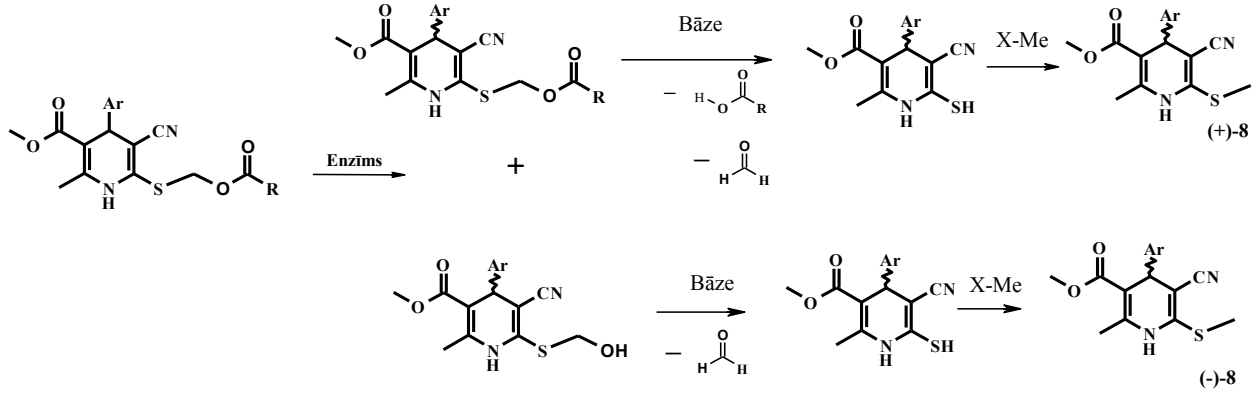
2.1. att. **Shēma mērķprodukta iegūšanai**

2. iespējamais ceļš (2.2. attēls) ir 1,4-dihidropiridīn-6-merkaptopropionskābes esteru sintēze, enantioselektīvā hidrolīze enzīmu klātbūtnē, deakrilācija un tiolāta alkilēšana. Šis ceļš ir izaicinošs, jo hirālais centrs no reakcijas centra atrodas 6 saišu attālumā un tas ir pilnīgi oriģināls pētījums.



2.2. att. **Shēma mērķprodukta iegūšanai**

3. iespējamais ceļš (2.3. attēls) ir 6-alkilkarboksimetilsulfanil-1,4-dihidropiridīnu sintēze, to enzimātiskā hidrolīze, formaldehīda izšķelšana vai 6-alkilkarboksimetilgrupas nošķelšana (hidrolīze un deformilēšana) un tiolāta alkilēšana. Arī šis ceļš ir izaicinošs, jo hirālais centrs no reakcijas centra atrodas 6 saišu attālumā un literatūrā nav datu par alkilkarboksimetilsulfanilgrupas enantioselektīvo hidrolīzi enzīmu klātbūtnē.



2.3. att. **Shēma mērķprodukta iegūšanai**

2.1. 6-Alkilsulfanil-1,4-dihidropiridīn-3-karbonskābju etoksikarbonilmetilesteru sintēze un enzimātiskā hidrolīze kinētiskās kontroles apstākļos

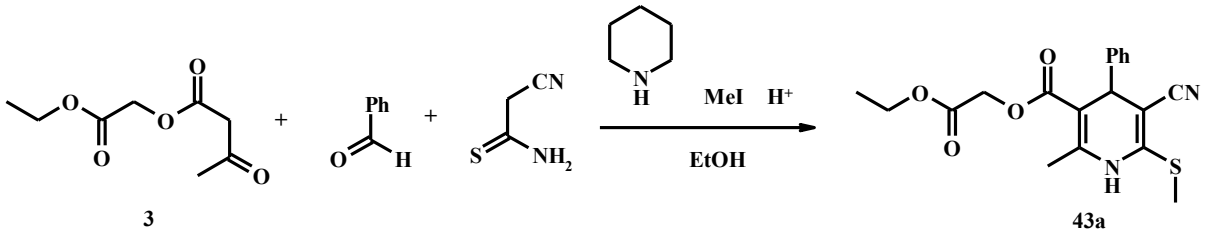
Darba mērķis ir veikt tādu sēru saturošu 1,4-dihidropiridīnu sintēzi, kuri saturētu enzimātiski labilās grupas. Literatūras analīze rāda, ka 3-etoksikarbonilmetilestera grupa 1,4-dihidropiridīnos dažādu enzīmu klātbūtnē hidrolizējas gan līdz 3-karboksimetilgrupai [9] („ārējā" estera grupa), gan līdz 3-karboksigrupai [11, 12] („iekšējā" estera grupa). Sintezēti 6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes etoksikarbonilmetilesteri, variējot aizvietotājus 4. vietā. Lai izpētītu esteru hidrolītiskās īpašības, veikta arī to ķīmiskā hidrolīze,

kas ļauj iegūt attiecīgās karbonskābes, kas kalpo kā modeļvielas (hirālās augsti efektīvās hromatogrāfijas enantioizšķiršanas metodes izstrāde) enzimātiskās enantiosadalīšanas izpētei.

2.1.1. 5-Ciāno-4-fenil-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes etoksikarbonilmetilestera sintēze

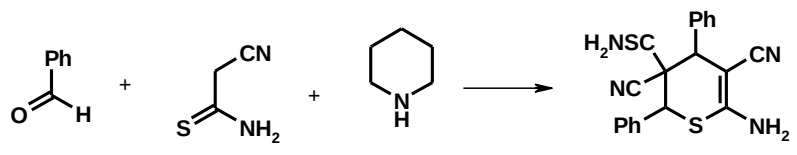
Literatūras analīze ļauj secināt, ka 6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes etoksikarbonilmetilestera iegūšanai visefektīvākā varētu būt piecu komponentu viena reaktora metode [14].

Piecu komponentu viena reaktora sintēze. 6-Metilsulfanil-1,4-dihidropiridīn-3-karbonskābes etoksikarbonilmetilesteris **43a** iegūts pielietojot piecu komponentu viena reaktora metodi. Svarīga ir komponentu pievienošanas secība: 1,3-dikarbonilsavienojums **3,** benzaldehīds, ciānotioacetamīds, piperidīns un tad metiljodīds. 6-Metilsulfanil-1,4-dihidropiridīns **43a** veidojas ar augstu iznākumu (pēc šķidrumu hromatogrāfijas/masspektrometrijas datiem), bet tā labās šķīdības dēļ spirtos un citos šķīdinātājos, produkts no metanola kristalizējas tikai ar 34% iznākumu. Izolēšanai neizmantojām kolonnas hromatogrāfiju, jo reakcijas gaitā rodas merkaptāni, kam ir ļoti nepatīkama smaka.



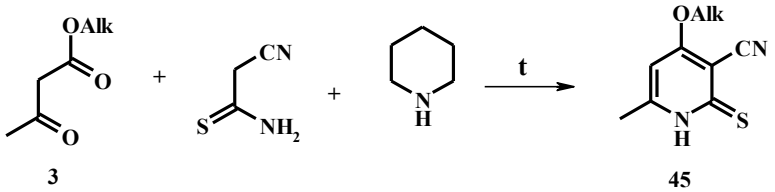
3 **43a**

Svarīga ir ne tikai reaģentu secība, bet arī katalizatora pievienošana un reakcijas temperatūra. Ja vispirms kolbā ievieto benzaldehīdu, ciānotioacetamīdu un piperidīnu, tad rodas 6-amino-3,5-diciāno-2,4-difenil-3,4-dihidro-2H-tiopirān-3-karbotioskābes amīds **44** [41].



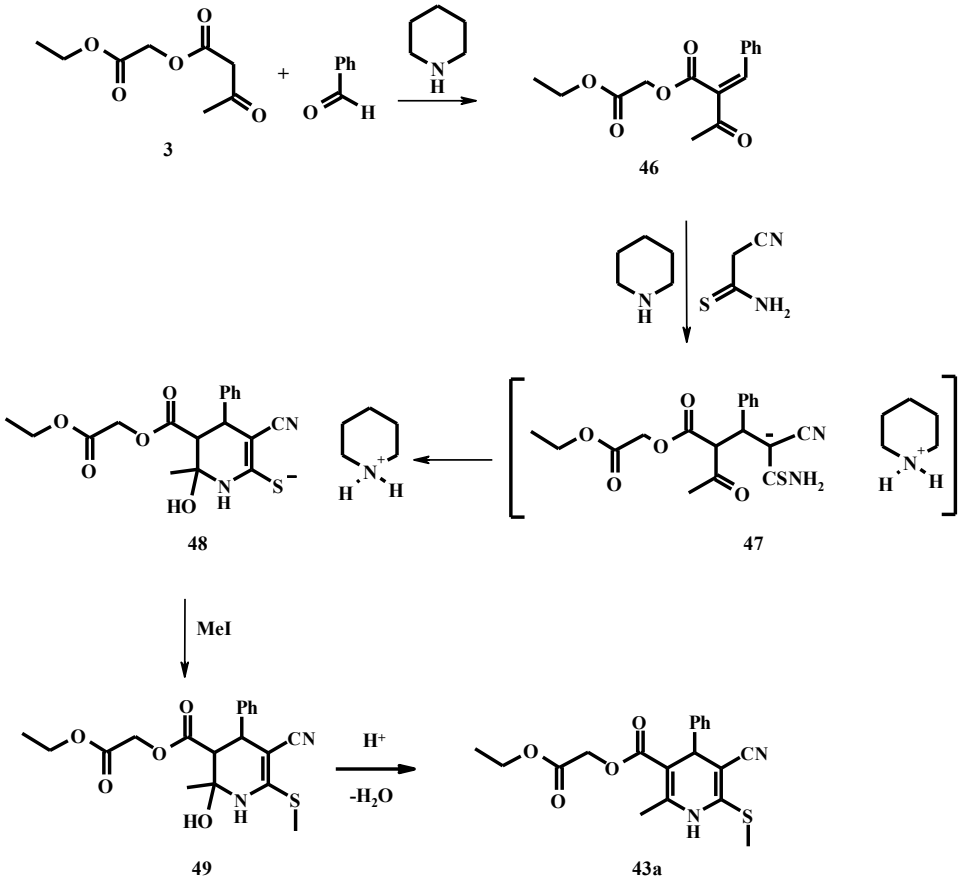
44

Nevēlami blakusprodukti veidojas arī tad, ja ne tikai samaina izejvielu pievienošanas kārtību, bet arī, ja izmaina reakcijas apstākļus. Ir zināms, ka dikarbonilsavienojumam **3** reaģējot ar ciānotioacetamīdu piperidīna klātbūtnē paaugstinātā temperatūrā veidojas tikai 4-alkoksi-3-ciāno-6-metil-2-tiokso-1,2-dihidropiridīns **45** [42].



3 **45**

5-Ciāno-4-fenil-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes etoksikarbonilmetilestera sintēzes mehānisms. Aromātiskajam aldehīdam reaģējot ar 1,3-dikarbonilsavienojumu **3** katalītiska piperidīna klātbūtnē veidojas Knēvenāgela reakcijas produkts – 3-okso-2-(1-fenilmetilidēn)butānskābes etoksikarbonilmetilesteris **46**. Tam reaģējot ar 2-ciānotioacetamīdu un ekvimolāru piperidīna daudzumu, veidojas pievienošanās produkts – Maikla reakcijas adukts **47**, kas varētu eksistēt sāls veidā. Pēdējais bāzes klātbūtnē ciklizējas, izveidojot piperidīnija 2-hidroksi-tetrahidropiridīn-6-tiolātu **48**. Tiolātam **48** pievienojot metiljodīdu un īslaicīgi uzvārot līdz šķīdinātāja viršanas temperatūrai, veidojas 2-hidroksi-6-metilsulfanil-1,4,5,6-tetrahidropiridīns **49**. Siltam reakcijas maisījumam pievienojot skābi, izšķeļas ūdens molekula un veidojas 1,4-dihidropiridīns **43a.**

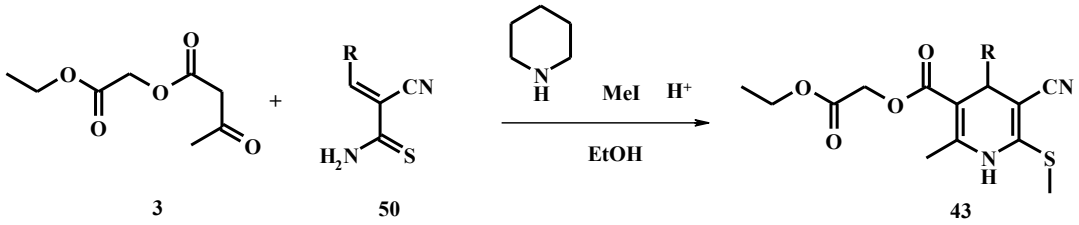


2.1.2. 5-Ciāno-4-((2-hlorfenil)- un (2,3-dihlorfenil)-)-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes etoksikarbonilmetilesteru sintēze

Tā kā, pielietojot 5-komponentu viena reaktora metodi 4-fenil-6-metilsulfanil-1,4-dihidropiridīns **43a**, sakarā ar izdalīšanas neērtībām (merkaptāni, sarežģīts vielu maisījums) kristalizējas tikai ar 34% iznākumu, tad 1,4-DHP **43b,c** sintezēti pielietojot literatūras apskatā minēto četru komponentu (tioakrilamīds, 1,3-dikarbonilsavienojums, piperidīns un metiljodīds) viena reaktora metodi [13], tādējādi padarot reakcijas maisījumu vienkāršāku.

2-Ciāno-3-(2-hlorfenil)tioakrilamīda **50b** reakcijā ar acetetiķskābes etoksikarbonilmetilesteri **3** ekvimolāra piperidīna daudzuma klātbūtnē un tālāk veicot secīgi alkilēšanas reakciju ar metiljodīdu un reakcijas maisījumu paskābinot, iegūst 6-metilsulfanil-1,4-dihidropiridīnu **43b** ar 53% iznākumu. Savienojuma **43b** zemo iznākumu var izskaidrot ar tā nestabilitāti šķīdumā. Ar AEŠH metodi noskaidrojās, ka gaismas ietekmē jau pusstundas laikā pieaug pašreiz nenoskaidrotu piemaisījumu daudzums par 10%. Līdzīgi iegūts

6-metilsulfanil-1,4-dihidropiridīns **43c** ar 75% iznākumu, kā pamatizejvielu izmantojot 2-ciāno-3-(2,3-dihlorfenil)tioakrilamīds **50c.**

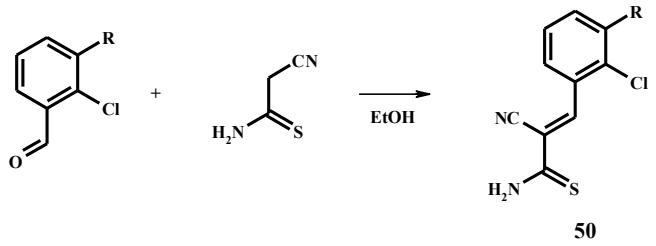


3 50 43

b) R = 2-ClC_6H_4; c) R = 2,3-$Cl_2C_6H_3$

Tā kā 3-(hlorfenil)-2-ciānotioakrilamīdi **50** nav komerciāli pieejami, tad tie iegūti aromātiskā aldehīda un ciānotioacetamīda kondensācijas reakcijā, reakcijas maisījumu īslaicīgi sildot līdz etanola viršanas temperatūrai.

3-(2-Hlorfenil)-2-ciānotioakrilamīds **50b** izdalīts ar 68% iznākumu un 2-ciāno-3-(2,3-dihlorfenil)tioakrilamīds **50c** ar 95% iznākumu.

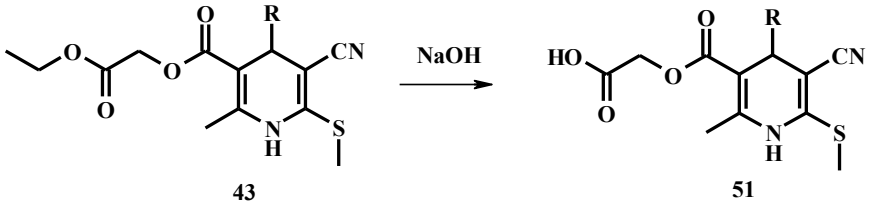


50

b) R = H; c) R = Cl

2.1.3. 4-Aril-5-ciāno-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes etoksikarbonilmetilesteru hidrolīze

6-Metilsulfanil-1,4-dihidropiridīnu **43** ķīmiskā hidrolīze, veikta ar mērķi iegūt modeļvielas enzimātiskajām reakcijām.



43 51

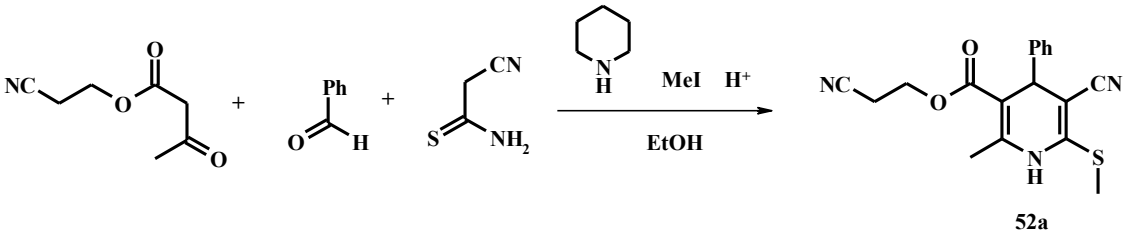
a) R = C_6H_5; b) R = 2-ClC_6H_4; c) R = 2,3-$Cl_2C_6H_3$

5-Ciāno-4-fenil-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes karboksimetilesteris **51a** ar 82%, 4-(2-hlorfenil)-1,4-dihidropiridīn-3-karbonskābes karboksimetilesteris **51b** ar 63% un 4-(2,3-dihlorfenil)-1,4-dihidropiridīn-3-karbonskābes karboksimetilesteris **51c** ar 90% iznākumu iegūti iedarbojoties uz dubultesteriem **43** ar nātrija hidroksīda ūdens šķīdumā, maisot 8h istabas temperatūrā. Skābi **51a** izdevās sakristalizēt no etanola. Savukārt skābes **51b,c** nešķīst dihlormetānā, līdz ar to tās sekmīgi izolētas no reakcijas maisījuma nofiltrējot. Tātad ķīmiski iegūtas 1,4-DHP-3-karboksimetilkarbonskābes **51**, kas norāda uz to, ka dotajos apstākļos hidrolizējas „ārējā", nevis „iekšējā" estera grupa, kā tas uz simetriskiem 1,4-DHP aprakstīts darbos [11, 12].

2.1.4. 4-Aril-5-ciāno-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes 2-ciānoetilesteru sintēze

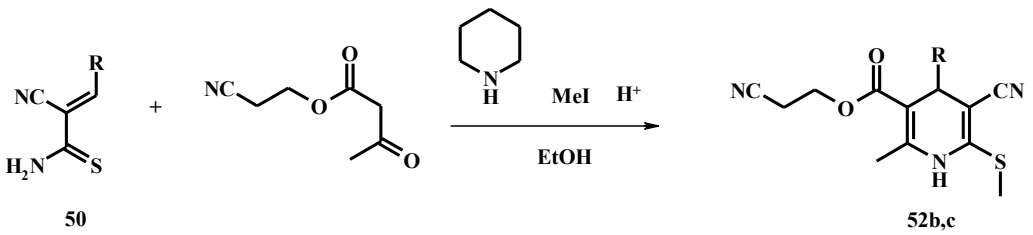
Literatūrā nav aprakstīta sēru saturošu 1,4-dihidropiridīn-3-karbonskābju iegūšana, bet ir atrodami dati, kur 1,4-dihidropiridīn-3-karbonskābes var iegūt hidrolizējot simetriskus 1,4-dihidropiridīn-3,5-dikarbonskābes 2-bisciānoetilesterus [11].

4-Fenil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes 2-ciānoetilesteris **43a** ar 43% iznākumu iegūts līdzīgi kā dubultesteris **43a**, pielietojot piecu komponentu viena reaktora metodi. Arī šajā gadījumā problēmas sagādā produkta izolēšana. Iegūtie rezultāti ir pirmie eksperimenti, ja savienojumi **52** aktualizēsies, tad tiks piemeklēti piemērotāki apstākļi, lai mērķa produktu izolētu ar augstāku iznākumu.



52a

4-(2-Hlorfenil)- un 4-(2,3-dihlorfenil)-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes 2-ciānoetilesteri **52b,c** iegūti pielietojot 4-komponentu viena reaktora metodi, kā izejas savienojumus izmantojot iepriekš pagatavotos 2-ciāno-3-(2-hlorfenil)- un 3-(2,3-dihlorfenil)tioakrilamīdus **50**. Savienojums **52b** izolēts ar 32 % iznākumu un savienojums **52c** izolēts ar 80 % iznākumu.

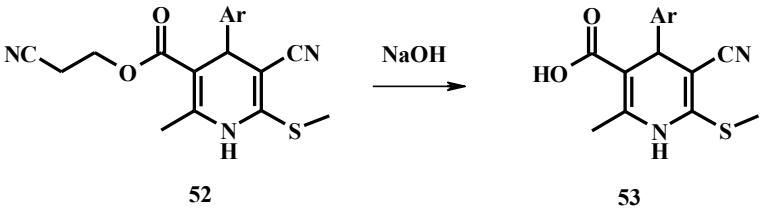


b) R = 2-ClC_6H_4; c) R = 2,3-$Cl_2C_6H_3$

Ciānoestera **52b** zemais iznākums izskaidrojams ar tā nestabilitāti šķīdumos, ko pierāda AEŠH analīze.

2.1.5. 4-Aril-5-ciāno-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes 2-ciānoetilesteru hidrolīze

6-Metilsulfanil-1,4-dihidropiridīna **52** ķīmiskā hidrolīze veikta ar mērķi iegūt otru svarīgu modeļvielu enzimātisko reakciju izpētei.



a) R = C_6H_5 b) R = 2-ClC_6H_4; c) R = 2,3-$Cl_2C_6H_3$

Esterus **52** hidrolīzē ar nātrija hidroksīda ūdens šķīdumu, maisot 8 stundas istabas temperatūrā. 4-Aril-1,4-dihidropiridīn-3-karbonskābes **53a-c** iegūtas ar attiecīgi 78%, 75% un 79% iznākumu, sakristalizējot no etanola.

2.1.6. 6-Metilsulfanil-1,4-dihidropiridīn-3-karbonskābes etoksikarbonilmetilesteru un 2-ciānoetilesteru un to hidrolīzes produktu struktūru pierādīšana

Sintezēto savienojumu **43**, **51-53** struktūras pierādītas ar infrasarkanajiem spektriem (IS), protonu kodolmagnētiskās rezonanses (^{1}H-KMR) spektriem un elementanalīzes datiem. Savienojumu **43**, **51-53** IS spektros novēro karbonilgrupu (C=O) valences svārstības pie 1631-1776 cm^{-1}, ciāno grupu (C≡N) svārstības pie 2193-2205 cm^{-1}, NH grupu svārstības pie

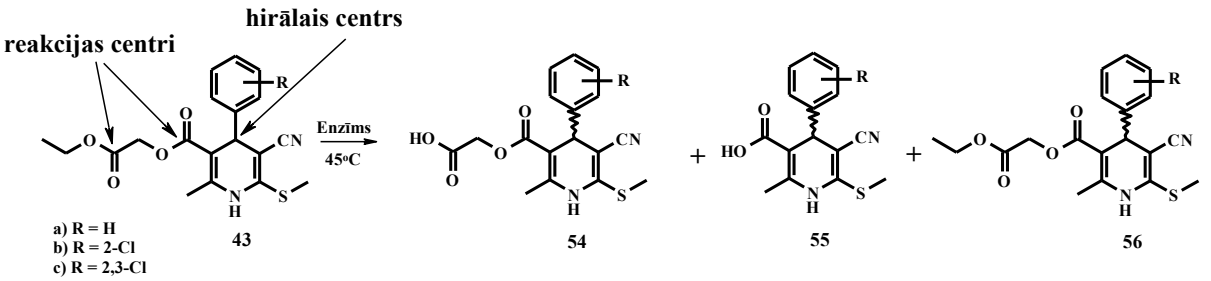
3066-3336 cm^{-1}. Karbonskābēm **51** un **53** IS spektros novēro OH grupu valences svārstības pie 3321-3625 cm^{-1}.

^{1}H-KMR spektros 6-metilsulfanil-1,4-dihidropiridīniem **43**, **51-53** raksturīgākais ir 4-H signāls, kas pierāda, ka savienojumiem ir 1,4-dihidrostruktūra. Ja piridīna gredzena 4. vietā ir neaizvietots benzola gredzens, tad ^{1}H-KMR spektros 4-H signāls ir pie 4,47-4,75 m.d. Savukārt 4-(2-hlorfenil)- un 4-(2,3-dihlorfenil)-1,4-dihidropiridīnu gadījumā 4-H signāls spektros ir nobīdīts uz vājākiem laukiem un ir robežās no 5,05-5,40 m.d.

Ar ^{1}H-KMR spektriem tiek pierādīts, ka savienojumi **43** ir dubultesteri, par to liecina 3-COO$\underline{CH_2}$ grupas signāls AB kvarteta veidā. Savienojumam **43a** ^{1}H-KMR spektros 3-$COOCH_2$ signāls ir pie 4,54 m.d. Savukārt 4-(2-hlorfenil)- un 4-(2,3-dihlorfenil)-1,4-dihidropiridīnu gadījumā 3-$COOCH_2$ signāls spektros ir robežās no 4,44-4,93 m.d. Ar ^{1}H-KMR spektriem tiek pierādīts, ka karbonskābēm **51** ir saglabājies 3-COO$\underline{CH_2}$ fragments, kas redzams, kā AB kvartets. ^{1}H-KMR spektros 3-$COOCH_2$ signālus novēro pie 4,27-4,93 m.d. Tātad, iegūti 1,4-dihidropiridīn-3-karbonskābes etoksikarbonilmetilesteri **43** un 2-ciānoetilesteri **52**. Tiem kā reakciju substrātiem sagaidāma atšķirīga hidrolītiskā aktivitāte. „Ārējās" karbonskābes **51** un vai „iekšējās" karbonskābes **53** kalpos kā modeļvielas. Pirms uzsākt enzīmu katalizētās enantiosektīvās hidrolīzes izpēti bija svarīgi izstrādāt AEŠH analīzes metodes, kas ļauj, pielietojot parastās kolonas, kontrolēt „ārējās" vai „iekšējās" karbonskābes veidošanos un, savukārt, pielietojot hirālās kolonas, gan neizreaģējošo esteru, gan dažādo karbonskābju enantiosadalīšanas pakāpi.

2.1.7. 4-Aril-5-ciāno-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes etoksikarbonilmetilesteru enzimātiskā hidrolīze

Kā minēts ievadā optiski aktīvus savienojumus var iegūt, izmantojot kā promotorus enantioselektīvus enzīmus. Ir zināms, ka lipāzes sekmē skābju esteru grupu enantioselektīvo hidrolīzi līdz karbonskābei. Plaši pētīta enzimātiskā hidrolīze tādiem 1,4-DHP, kuri 3. un 5. vietās satur modificētas, hidrolizēties spējīgas estera grupas. Uzsākot mūsu pētījumus, literatūrā nebija datu par sēru saturošu 1,4-DHP enzimātisko hidrolīzi. Tāpat nav sistemātisku pētījumu par to, cik tālu reakcijas centrs var atrasties no hirālā centra, lai notikt apmierinoša enantiosadalīšanās.



1,4-Dihidropiridīn-3-karbonskābes etoksikarbonilmetilestera **43** gadījumā reakcijas centrs ir attālināts no hirālā centra par 2 vai 5 saitēm. Veicot estera **43** enzimātisko hidrolīzi kinētiskās kontroles apstākļos, t.i., apstādinot reakciju „pusceļā", varējām sagaidīt karbonskābju **53** un **55** un neizreaģējošā estera **56** kā enantiotīru vai enantiobagātinātu produktu veidošanos.

2.1.8. Hirālās AEŠH metodes izstrāde 4-aril-1,4-dihidropiridīn-3-karbonskābes etoksikarbonilmetilesteru enantioselektīvās hidrolīzes izpētei.

Sākumā racemiskiem dubultesteriem un attiecīgajām skābēm tika piemeklēta optimālā mobīlā fāze uz hirālās kolonnas Whelk 01, lai varētu noteikt enantiomēro pārākumu.

Lai varētu raksturot joslu atdalīšanu vienu no otras, izmanto joslu izšķiršanas raksturlielumu R_S. Divu blakus esošu joslu izšķiršanu R_s definē kā attiecību attālumam starp to maksimumiem, jeb izdalīšanas laiku starpību Δt_R un vidējo aritmētisko no divu joslu pusplatumiem (w_1 un w_2) (skatīt 2.4. attēlu). Labs joslu sadalījums ir tad, ja R_S>1,2.

$$R_S = \frac{t_{R_2} - t_{R_1}}{0{,}5(w_2 + w_1)}$$

2.4. att. **Joslu izšķiršanas raksturlieluma aprēķināšanas formula**

Pamatojoties uz iepriekšēju pieredzi par līdzīgiem savienojumiem tika izmēģinātas 3 sistēmas (skatīt 2.1. tabulu).

2.1. tabula

Sākotnēji AEŠH metodei izmantotās mobīlās fāzes un to sadalītspēja

Nr.	Sistēma	Substrāts	Joslu izšķirtspēja R_S
1	Heksāns 46 Dihlormetāns 46 Etanols 8 Amonija acetāts 0,01 M	**43c**	0,4
2	Heksāns 80 Izopropanols 20 Amonija acetāts 0,01 M	**43c**	0,2
3	Heksāns 70 Etanols 30 Amonija acetāts 0,01 M	**51c**	0,2

Diemžēl šajās sistēmās nevarēja sadalīt nevienu no substrātiem ar pietiekami lielu sadalījuma koeficentu. Tāpēc tās tika veikti eksperimenti līdz tika atrastas optimālākās sistēmas (skatīt 2.2. tabulu).

2.2. tabula

AEŠH metodei izmantotās mobīlās fāzes un to sadalītspēja

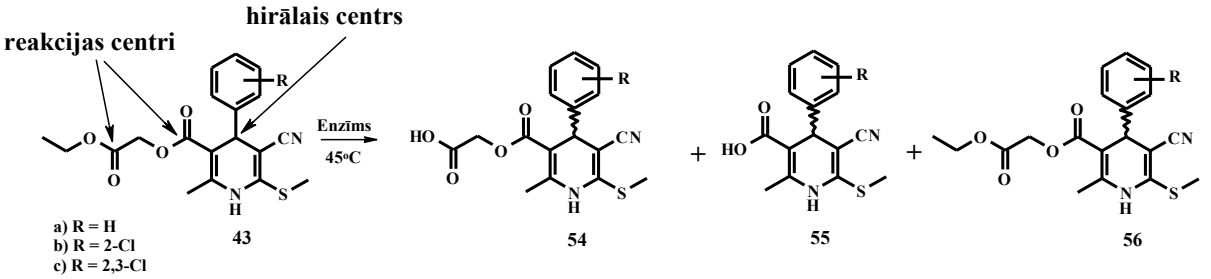
Nr.	Sistēma	Substrāts	Joslu izšķirtspēja R_S
4	Heksāns 80 Dihlormetāns 10 Izopropanols 10 Etiķskābe 0,1 %	**43a** **43b** **40c**	1,04 1,13 1,20
5	Heksāns 70 Dihlormetāns 15 Izopropanols 15 Etiķskābe 0,1 %	**43a** **43b** **43c**	1,02 1,11 1,18
6	Heksāns 40 Dihlormetāns 40 Etanols 20 + Amonija acetāts 0,01 M	**51a** **51b** **51c**	1,00 1,15 1,21

2.2. Tabulas dati ļauj secināt, ka dotajos apstākļos labs racemātu sadalījums enantiomēros ir savienojumiem **43b,c** un **51b,c**. Diemžēl savienojumiem **43a** un **51a** netika atrasti pietiekoši labi apstākļi pilnīgai enantiomērās tīrības noteikšanai.

2.1.9. 4-Aril-1,4-dihidropiridīn-3-karbonskābes etoksikarbonilmetilesteru enzimātiskās hidrolīzes optimālo apstākļu piemeklēšana

Enzīmu promotētās kinētiskās enantiosadalīšanas reakcijās tiek iegūti pretējie optiskie izomēri (kā enantiobagātināti savienojumi). Enzimātisko hidrolīzi kontrolēti veic līdz brīdim, kad ir izreaģējusi puse no estera, jo ideālā gadījumā enzīms selektīvi hidrolizē tikai vienu enantiomēru. Šī metode ir efektīva, jo tiek iegūti abi enantiobagātināti enantiomēri, viens ir reakcija produkts – karbonskābe, bet otrs - neizreaģējušais esteris.

Veikti dubultesteru **43** hidrolīzes eksperimenti enzīmu klātbūtnē. Tika pārbaudītas lipāzes: Amano Lipase G, A, M, AK, PS, Novozym 435®, *Candida rugosa*. Amano lipāzes nehidrolizē dubultesterus, bet lipāzes Novozym 435® un *Candida rugosa* uzrāda hidrolītisko aktivitāti (skatīt 2.3. tabulu).



2.3. tabula

Dubultesteru 43 enzimātiskās hidrolīzes rezultāti

Substrāts **43**	Enzīms	Temp., °C	Laiks, st.	Skābe **54**		Skābe **55**		Neizreaģējušais esteris **56**	
				ķīm. izn., %	ep, %	ķīm. izn., %	ep, %	ķīm. izn., %	ep, %
43a	Novozym 435®	45	24	33	10	15	2	46	11
43a	*Candida rugosa*	45	110	45	6	-	-	48	5
43b	Novozym 435®	45	34	31	9	13	2	45	10
43c	Novozym 435®	45	48	30	11	15	3	46	12

Enantiomērā tīrība noteikta, pielietojot AEŠH metodi un izmantojot hirālo kolonnu (R,R)-Whelk O1, kur kā stacionārā fāze izmantota ar silikagelu saistīts 1-(3,5-dinitrobenzamido)-1,2,3,4-tetrahidrofenantrēns un mobīlā fāze heksāns/DHM/izopropanols+0,01% ledus etiķskābes attiecībā 80/10/10 (skatīt 2.3. tabulu). Diemžēl pagaidām dotajos apstākļos (acetonitrils/fosfāta buferis pH = 7,5 attiecībā 15/85) enzimātiskā hidrolīze norit ar neapmierinošu enantioselektivitāti.

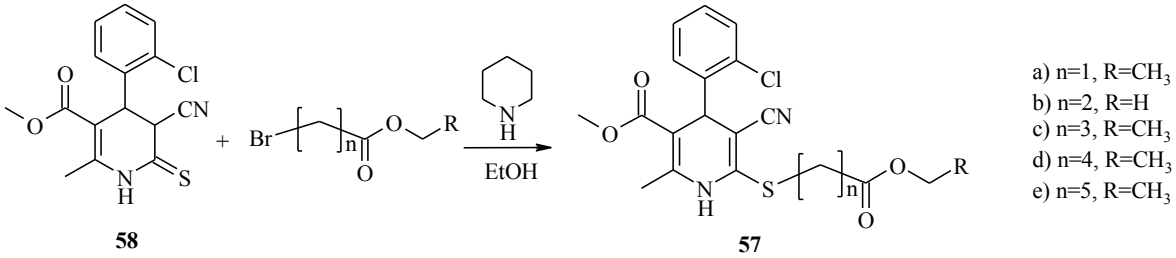
Tā kā darba mērķis ir izstrādāt metodi kardiovaskulāro aktivitāti uzrādošo 5-ciāno-4-aril-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes esteru [13] sadalīšanai enantiomēros, bet enzimātiski labilo etoksikarbonilmetilestera grupu saturošie 1,4-dihidropiridīn-3-karbonskābes atvasinājumi hidrolizējas ar neapmierinošu enantioselektivitāti, tad netika pētīta nedaudz (ep<12%) enatiobagātināto savienojumu **54** un **56** dekarboksilācija un alkilēšana, bet tika meklēts principiāli jauns alternatīvs risinājums.

2.2. 1,4-DHP-6-merkaptoalkilkarbonskābes esteru sintēze un enzimātiskā hidrolīze kinētiskās kontroles apstākļos

2.2.1. 1,4-DHP-6-merkaptoalkilkarbonskābes esteru sintēze

Ar mērķi palielināt 6-alkilsulfanil-1,4-DHP lipofilitāti, kā arī izpētīt enzimātisko hidrolītisko aktivitāti savienojumiem, kur reakcijas centrs no hirālā centra atrodas līdz pat 9 saišu attālumā, sintezēti oriģināli 1,4-DHP-6-merkaptoalkilkarbonskābes esteri **57.**

Tā kā ieplānota vielu sērijas sintēze, tad izdevīgi ir vispirms iegūt 6-tiokso-1,4,5,6-tetrahidropiridīnu **58** un tad to alkilēt ar atbilstošo ω-bromalkilkarbonskābes esteri.

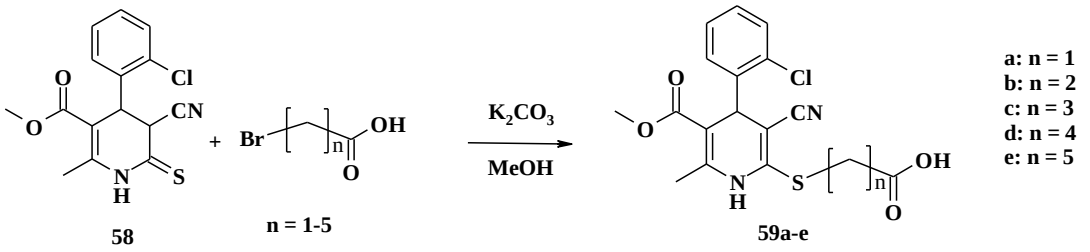


5-Ciāno-4-(2-hlorfenil)-2-metil-6-etoksikarbonilmetilsulfanil-1,4-dihidropiridīn-3-karbonskābes metilesteris 57a ar 85% iznākumu iegūts alkilējot 6-tiokso-1,4,5,6-tetrahidropiridīnu **58** ar brometiķskābes etilesteri. 1,4-DHP-6-merkaptoetiķskābes etilesteris **57a** kristalizējas no reakcijas maisījuma kā tīrs produkts. Savienojumu **57a** var iegūt arī pielietojot piecu komponentu viena reaktora metodi, bet tad to izolēšanai un attīrīšanai nepieciešams pielietot kolonnas hromatogrāfiju.

5-Ciāno-4-(2-hlorfenil)-2-metil-6-(2-metoksikarboniletilsulfanil)-1,4-dihidropiridīn-3-karbonskābes metilesteris **57b** ar 86% iznākumu iegūts alkilējot 5-ciāno-4-(2-hlorfenil)-2-metil-6-tiokso-1,2,3,4-dihidropiridīn-3-karbonskābes metilesteri **58** ar ω-bromalkilkarbonskābes esteri. Līdzīgi iegūti savienojumi **57c** ar 86%, **57d** ar 71% un **57e** ar 73% augstiem ķīmiskajiem iznākumiem.

2.2.2. 1,4-DHP-6-merkaptoalkilkarbonskābju sintēze

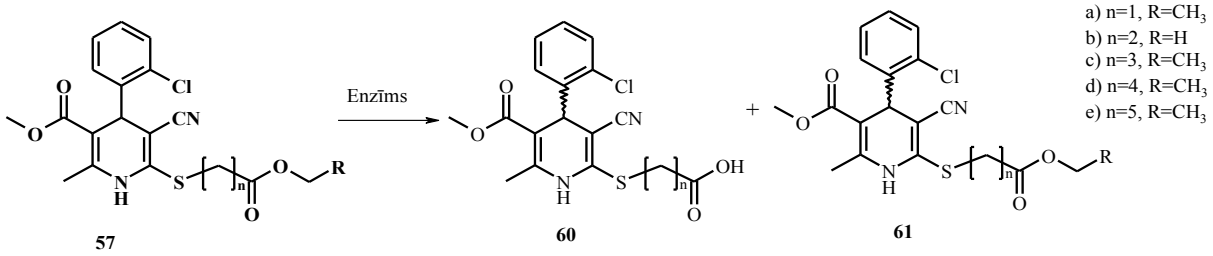
Attiecīgās 1,4-DHP-6-merkaptoalkilkarbonskābes **59** sintezētas ar mērķi iegūt modeļvielas enzimātiskajām reakcijām, lai piemeklētu apstākļus reakciju kinētiskai kontrolei un enantiomērās tīrības noteikšanai.



Attiecīgās skābes **59a-e** ar 76-87% iznākumiem iegūtas 6-tiokso-tetrahidropiridīnus **58** alkilējot ar ω-bromalkilkarbonskābēm ekvimolāra kālija karbonāta klātbūtnē metanola šķīdumā. Šajā gadījumā 1,4-DHP-2-tiolāta ģenerēšanai jāizmanto vājāka bāze, kā arī reakcijas temperatūra nedrīkst būt augstāka par ~ 50 ^{o}C. Neievērojot šos nosacījumus, notiek skābju **59a-e** esterifikācija un veidojas esteri **57a-e**.

2.2.3. 1,4-DHP-6-merkaptoalkilkarbonskābes esteru enzimātiskā hidrolīze kinētiskās kontroles apstākļos

Lai veiktu 1,4-DHP-6-merkaptoalkilkarbonskābes esteru **57** enzimātisko hidrolīzi kinētiskās kontroles apstākļos un izstrādātu metodi sadalīšanai enantiomēros, vispirms bija jānovērtē 3-COOMe un 6-merkaptoalkilkarbonskābes esteru hidrolītiskā aktivitāte. Literatūrā nav datu par šādu savienojumu sadalīšanu enantiomēros enzīmu katalizētās reakcijās. Nav arī datu par sistemātiskiem pētījumiem dažādu savienojumu klasēs, lai noskaidrotu kāds būtu optimālākais reakcijas centra attālums no hirālā centra.



Pēc analoģijas ar iepriekšējiem pētījumiem 1,4-DHP-3,5-dikarbonskābju esteru gadījumā, 6-alkoksikarbonilalkilsulfanil-1,4-DHP savienojumu **57** hidrolīzei izvēlējāmies populārākās lipāzes – *Candida antarctica* lipāzes B imobilizēto formu Novozym 435®. Substrāts **57** papildus tika pārbaudīts uz šādiem enzīmiem: *Candida cyclindracea, Pencillium camemberti, Candida rugosa type VII, Amano acylase*, Amano lipase PS, M, G. No 8 enzīmiem hidrolitisko aktivitāti uzrādīja tikai 2: Novozym 435® un *Amano acylase*.

Rezultāti parādīja, ka izvēlētās lipāzes 3-metoksikarbonilgrupu nehidrolizē, jo tā atrodas konjugācijā ar β-aminovinilgrupu un ir dezaktivēta. Savukārt, 6-alkoksikarbonilalkilsulfanilgrupa uzrādīja augstu hidrolītisko aktivitāti. Tika veikta enzimātiskā hidrolīze un iegūti labi rezultāti (skatīt 2.4. tabulu).

2.4. tabula

1**,4-DHP-6-merkaptoalkilkarbonskābes esteru 57 enzimātiskās hidrolīzes rezultāti**

Substrāts 57	**Lipāze**	**T, °C**	**DHM/ IPE**	**Reakc. laiks, st.**	**Ķīm. izn. skābei 60, %**	**Ķīm. izn. neizreaģē-jušajam esterim 61, %**	**Enantiotīrība neizreaģē-jušajam esterim 61, %**
a, n=1	Novozym 435®	25	1/20	45	49	45	37
b, n=2	Novozym 435®	25	1/20	190	46	47	68
c, n=3	Novozym 435®	25	1/20	20	48	44	11
d, n=4	Novozym 435®	25	1/20	20	49	44	5
e, n=5	Novozym 435®	25	1/20	17	48	47	15
a, n=1	Amano acylase	25	1/20	310	49	43	19
b, n=2	Amano acylase	25	1/20	310	44	45	21
c, n=3	Amano acylase	25	1/20	310	35	60	10
d, n=4	Amano acylase	25	1/20	310	22	76	12
e, n=5	Amano acylase	25	1/20	190	30	69	2

Balstoties uz literatūru par šķīdinātāja ietekmi uz enantioselektivitāti, tika samazināta dihlormetāna un IPE attiecība no 1/20 līdz 1/30 [43]. No 2.5. tabulas datiem varam secināt, ka tas būtiski uzlabo enantioselektivitāti 6-alkoksikarbonilalkilsulfanil-1,4-DHP **57a,b** gadījumā.

2.5. tabula

1,4-DHP-6-merkaptoalkilkarbonskābes esteru 57 enzimātiskās hidrolīzes rezultāti

Substrāts 57	Lipāze	T, °C	DHM/ IPE	Reakc. laiks, st.	Ķīm. izn. skābei 60, %	Ķīm. izn. neizreaģē-jušajam esterim 61, %	Enantiotīrība neizreaģē-jušajam esterim 61, %
a, n=1	Novozym 435®	25	1/30	45	49	45	50
b, n=2	Novozym 435®	25	1/30	165	47	45	80
c, n=3	Novozym 435®	25	1/30	20	46	43	6
d, n=4	Novozym 435®	25	1/30	20	49	45	5
e, n=5	Novozym 435®	25	1/30	15	46	48	20
a, n=1	Amano acylase	25	1/30	310	48	49	20
b, n=2	Amano acylase	25	1/30	310	49	47	89
c, n=3	Amano acylase	25	1/30	310	40	58	13
d, n=4	Amano acylase	25	1/30	310	34	62	10
e, n=5	Amano acylase	25	1/30	190	43	55	5

No iepriekšējas pieredzes ar līdzīgiem savienojumiem ir zināms, ka temperatūras paaugstināšana reizēm gan paātrina enzimātisko hidrolīzi, gan paaugstina enantioselektivitāti. Analizējot 2.6. tabulas datus, var secināt, ka savienojuma **57b** gadījumā salīdzinot ar pirmo eksperimentu enantioselektivitāte, izmantojot Novozym 435® ir uzlabota par 24%, kā arī novērojama stereoselektīva reakcija pat tad, ja hirālais un reakcijas centrs atrodas 9 saišu attālumā, savienojumam **57e**. Savukārt enzīms Amano acylase 45 °C temperatūrā hidrolītisko aktivitāti uzrāda tikai **57d** un **57e** gadījumā, bet reakcija nav stereoselektīva.

2.6. tabula

1,4-DHP-6-merkaptoalkilkarbonskābes esteru 54 enzimātiskās hidrolīzes rezultāti

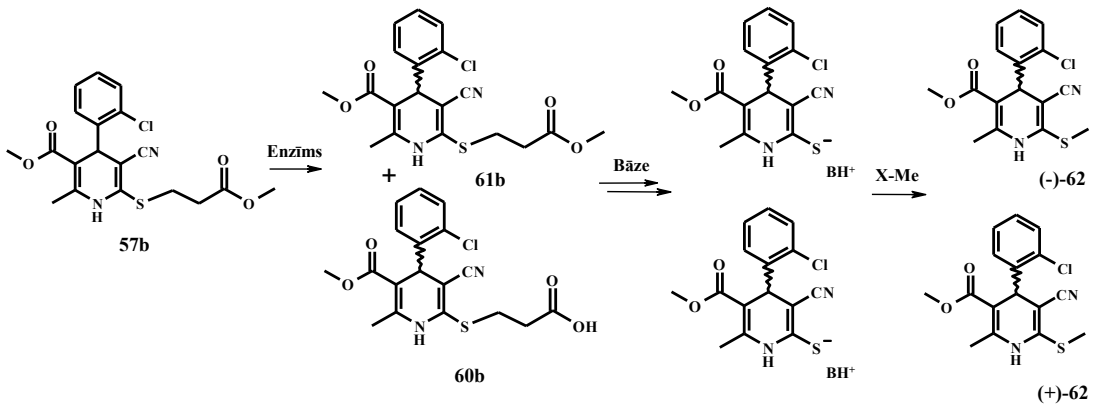
Substrāts 57	Lipāze	T, °C	DHM/ IPE	Reakc. laiks, st.	Ķīm. izn. skābei 60, %	Ķīm. izn. neizreaģē-jušajam esterim 61, %	Enantiotīrība neizreaģē-jušajam esterim 61, %
a)	**b)**	**c)**	**d)**	**e)**	**f)**	**g)**	**h)**
a, n=1	Novozym 435®	45	1/30	25	48	45	45
b, n=2	Novozym 435®	45	1/30	19	48	43	92
c, n=3	Novozym 435®	45	1/30	3	48	41	12

a)	b)	c)	d)	e)	f)	g)	h)
d, n=4	Novozym 435®	45	1/30	2	45	49	10
e, n=5	Novozym 435®	45	1/30	1	49	47	25
a, n=1	Amano acylase	45	1/30	400	-	100	-
b, n=2	Amano acylase	45	1/30	400	-	100	-
c, n=3	Amano acylase	45	1/30	400	-	100	-
d, n=4	Amano acylase	45	1/30	400	8	90	2
e, n=5	Amano acylase	45	1/30	400	26	72	3

Enantiomērā tīrība noteikta, pielietojot AEŠH metodi un izmantojot hirālo kolonnu (R,R)-Whelk O1, kur kā stacionārā fāze izmantota ar silikagelu saistīts 1-(3,5-dinitrobenzamido)-1,2,3,4-tetrahidrofenantrēns un mobīlā fāze heksāns/DHM/izopropanols + 0,05M amonija acetāts 65/30/5.

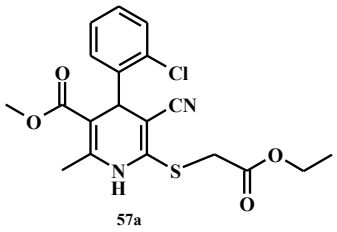
2.2.4. 6-Metilsulfanil-1,4-DHP kā enantiosadalītu produktu iegūšana, veicot 6-metoksikarboniletilsulfanil-1,4-DHP sintēzi, enzimātisko hidrolīzi kinētiskās kontroles apstākļos, deakrilēšanu un alkilēšanu

Kā jau pieminēts rezultātu un to izvērtējuma ievaddaļā 2. iespējamais ceļš ir 1,4-dihidropiridīn-6-merkaptopropionskābes esteru sintēze, enantioselektīvā hidrolīze enzīmu klātbūtnē, deakrilācija un alkilēšana. Šis ceļš ir izaicinošs, jo literatūrā nav datu par merkaptopropionskābes un citu merkaptoalkilkarbonskābju esteru enantioselektīvo hidrolīzi enzīmu klātbūtnē, piedevām hirālais centrs no reakcijas centra atrodas 6 saišu attālumā, bet 6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābju etoksikarbonilmetilesteru **43** gadījumā, kad hidrolīze noritēja ar neapmierinošu enantioselektivitāti, hirālais centrs no reakcijas centra atrodās 2 vai 5 saišu attālumā.



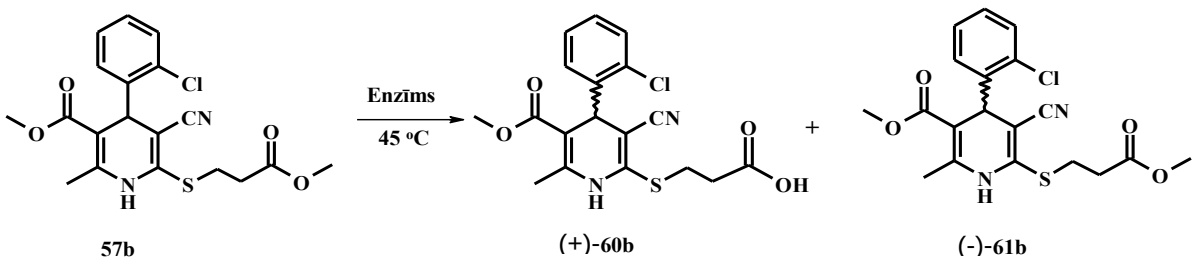
2.2. att. **Shēma mērķprodukta iegūšanai**

Risinot šo oriģinālo pieeju, veiksmes gadījumā varētu atrisināt vēl otru uzdevumu – sadalīt enantiomēros 5-ciāno-6-etoksikarbonilmetilsulfanil-4-(2-hlorfenil)-2-metil-1,4-DHP-3-karbonskābes metilesteri **57a**, kam nesen atrasta izteikta ar amlodipīnu salīdzinoša asinsspiedienu pazeminošā aktivitāte. Uz šūnu kultūrām **57a** dažos testos pārspēj amlodipīnu un, piedevām, tas izdevīgi atšķiras ar to, ka ir 6 reizes mazāk kaitīgs (LD_{50} ir attiecīgi 2197 un 368 mg/kg).



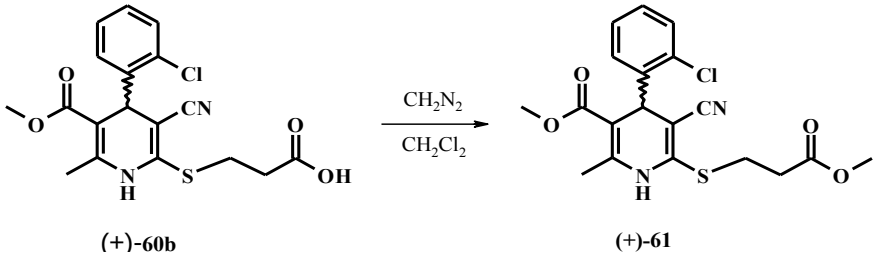
2.2.5. (-)-6-(2-metoksikarboniletilsulfanil)- un (+)-6-(2-karboksietilsulfanil)- 2-metil-1,4-dihidropiridīn-3-karbonskābes metilesteru iegūšana

Izmantojot labākos rezultātus no 2.6. tabulas, (-)-5-ciāno-4-(2-hlorfenil)-6-(2-metoksikarboniletilsulfanil)-2-metil-1,4-dihidropiridīn-3-karbonskābes metilesteris **61b** preparatīvi tiek iegūts enzimātiski hidrolizējot racemātu **57b**. Savienojumu **61b** atdala no **60b** pielietojot kolonnu hromatogrāfiju. (-)-5-Ciāno-4-(2-hlorfenil)-6-(2-metoksikarboniletilsulfanil)-2-metil-1,4-dihidropiridīn-3-karbonskābes metilesteris **61b** iegūts ar 92% enantiomēro tīrību.



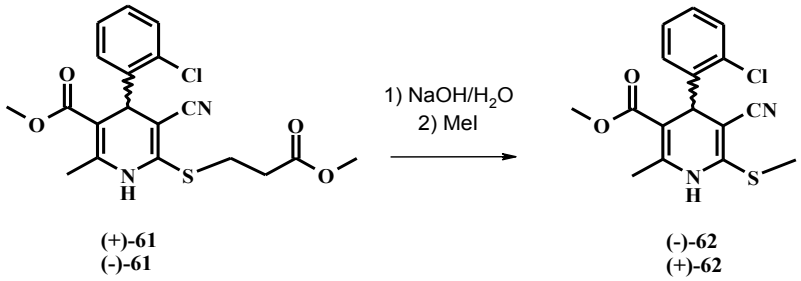
2.2.6. (+)-5-Ciāno-4-(2-hlorfenil)-6-(2-metoksikarboniletilsulfanil)-2-metil-1,4-dihidropiridīn-3-karbonskābes metilestera iegūšana

(+)-5-Ciāno-4-(2-hlorfenil)-6-(2-metoksikarboniletilsulfanil)-2-metil-1,4-dihidropiridīn-3-karbonskābes metilesteris (+)-**61** iegūts 6-(2-karboksietilsulfanil)-1,4-DHP **60b** alkilēšanas reakcijā ar diazometānu. Reakcija noris ar 95% ķīmisko iznākumu un 92% enantiomēro tīrību.



2.2.7. (-) un (+) 5-Ciāno-4-(2-hlorfenil)-6-metilsulfanil-2-metil-1,4-dihidropiridīn-3-karbonskābes metilestera iegūšana

Mērķa produkti (-)- un (+)-5-ciāno-4-(2-hlorfenil)-6-metilsulfanil-2-metil-1,4-dihidropiridīn-3-karbonskābes metilestera enantiomēri **62** iegūti veicot enantiobagātināto esteru (-)- un (+)-**61** deakrilāciju (iedarbojoties ar NaOH šķīdumu) un alkilējot ar metiljodīdu. Reakcijas noris ar augstiem iznākumiem attiecīgi (-)-**62** (94%) un (+)-**62** (92%), kā arī saglabājot izejvielu enantiotīrību 92%.

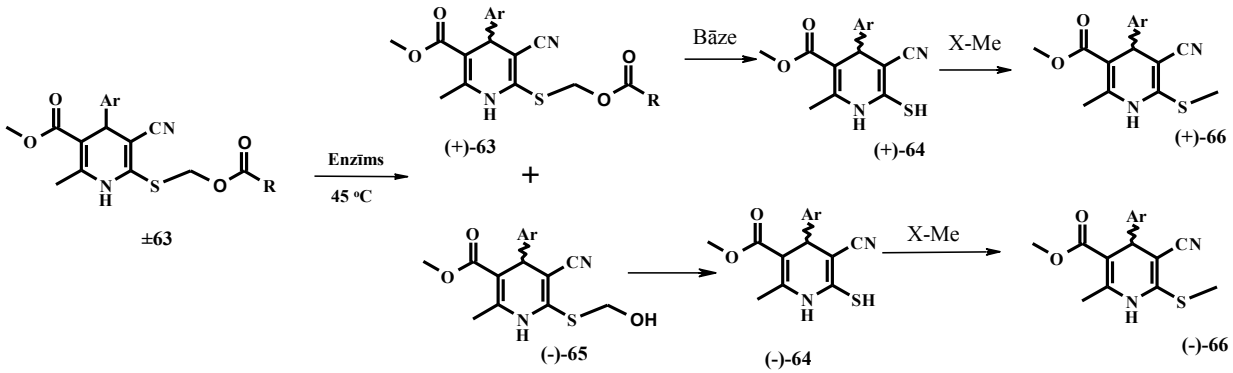


(+)-61
(-)-61

(-)-62
(+)-62

2.3. 6-Metilsulfanil-1,4-DHP kā enantiosadalītu produktu iegūšana, veicot 6-alkilkarboksimetilsulfanil-1,4-DHP sintēzi, enzimātisko hidrolīzi kinētiskās kontroles apstākļos, aizsarggrupas nošķelšana un alkilēšanu

Kā jau iepriekš pieminēts 3. iespējamais ceļš 6-metilsulfanil-1,4-DHP **(+)-66** un **(-)-66** kā enantiosadalītu produktu iegūšanai ir 6-alkilkarboksimetilsulfanil-1,4-dihidropiridīnu sintēze, to enzimātiskā hidrolīze, 6-alkilkarboksimetilgrupas nošķelšana un alkilēšana. Šis ceļš ir izaicinošs, jo hirālais centrs no reakcijas centra atrodas 6 saišu attālumā un literatūrā nav datu par alkilkarboksimetilsulfanilgrupas enantioselektīvo hidrolīzi enzīmu klātbūtnē. Literatūrā ir zināms, ka α-acetoksimetilsulfīdi viegli ķīmiski hidrolizējas ūdens klātūtnē, veidojot tiolu, formaldehīdu un etiķskābi. Mēs plānojam ievest 6-alkilkarboksimetilgrupu 1,4-DHP ar domu, ka šī grupa ir hidrolizēties spējīga un viegli nošķeļama.



±63

(+)-63

(+)-64

(+)-66

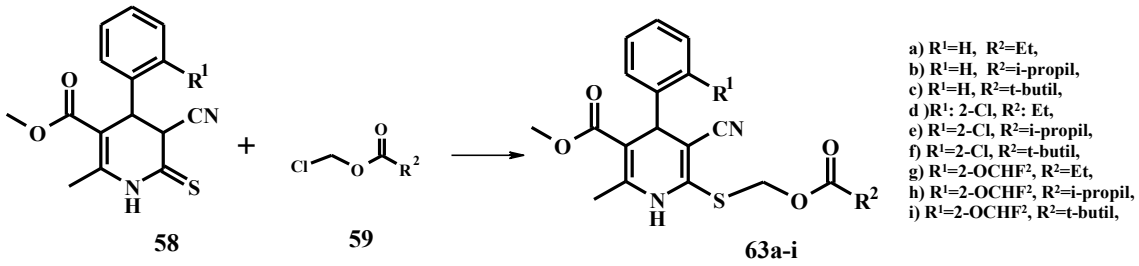
(-)-65

(-)-64

(-)-66

Oriģināli 6-alkilkarboksimetilsulfanil-1,4-DHP **63** sintezēti alkilējot 1,4-DHP-6-tionus **58** ar attiecīgās karbonskābes hlormetilesteri **59**. Lai noskaidrotu labilāko grupu enzimātiskās hidrolīzes reakcijai, tika variēts alkilaizvietotājs R, respektīvi, tika iegūti karbonskābes etil, *i*-propil un *t*-butilatvasinājumi **63**. Mēs sagaidījām, ka lineārā etilatvasinājuma gadījumā reakcija notiks vieglāk, bet sazaroto *i*-propil- un *t*-butilatvasinājumu gadījumā enantioselektīvāk. Ar mērķi iegūt savienojumus, kuriem sagaidāma kardiovaskulārā aktivitāte,

variēts 4.vietas R^1 aizvietotājs. 4-(2-ClC_6H_4)-grupu saturošiem kā amlodipīna analogiem bija sagaidāma antihipertensīvā aktivitāte, bet 4-(2-$F_2CHOC_6H_4$)-grupu saturošiem kā cerebrokrasta analogiem sagaidāma smadzeņu darbību veicinošā aktivitāte. Lai novērtētu arilgrupas ortoaizvietotāja ietekmi uz substrāta enzimātiskās hidrolīzes enantioselektivitāti, kā atskaites savienojums pētīts 4-Ph saturošs 1,4-DHP.



58 **59** **63a-i**

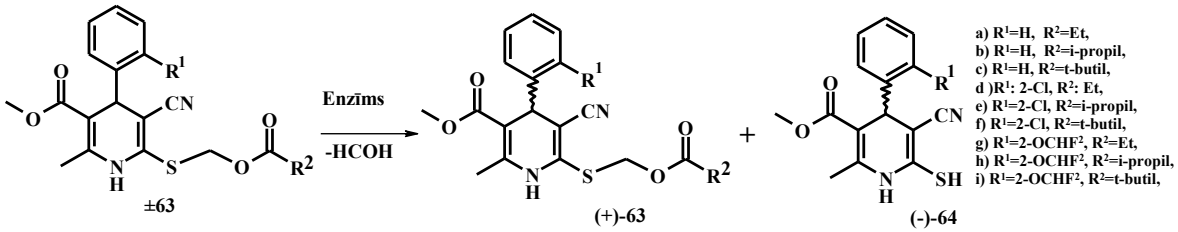
6-Izobutiriloksimetilsulfanil-1,4-DHP **63b**, **63e** un **63h** kristalizējas no reakcijas maisījuma kā tīri produkti ar 65-85% iznākumiem, savukārt etil- un *t*-butilatvasinājumi **63a**, **63c**, **63d**, **63f**, **63g** un **63i** (80-87%) tika attīrīti pielietojot kolonnas hromatogrāfiju.

Savienojumu **63** IS spektros novēro raksturīgo $\nu_{C\equiv N}$ joslas absorbcijas maksimumu pie 2190-2198 cm^{-1}. ^{1}H-KMR spektros 6-alkilkarboksimetilsulfanil-1,4-DHP **63** raksturīgākie ir 4-H un NH signāli singleta veidā, kas apstiprina hidrēto struktūru. 4-Fenil-1,4-DHP **63a-c** gadījumā 4-H signāls ir pie 4,61-4,63 m.d., bet 4-(2-ClC_6H_4)- un 4-(2-$F_2CHOC_6H_4$)-grupu saturošiem savienojumiem, tas ir nobīdīts uz vājākiem laukiem līdz 5,01-5,24 m.d. Savukārt NH signāls ir pie 7,27-7,75 m.d. Par to, ka tions **58** ir noalkilējies, liecina metilsulfanilgrupas 6-SCH_2OCO protonu signāli pie 5,29-5,34 m.d. (J=11,7 Hz). AB tipa kvartets norāda uz metilēngrupas protonu neekvivalentumu, jo savienojuma molekulā ir asimetriskais centrs pie 4-C atoma. Salīdzinot SCH_2OCO ar 6-SCH_2COO (3,50 m.d. un J=16,4 Hz) metilēngrupas signāli jūtami nobīdīti uz vājākiem laukiem O atoma ietekmes dēļ. 6-Terc-butilkarboksimetilsulfanil-1,4-DHP **63c**, **63f** un **63i** gadījumā nenovēro SCH_2OCO signālu sašķelšanos AB kvarteta veidā, tas ir redzams singleta veidā. To var skaidrot ar to, ka terc-butilkarboksimetilsulfanilgrupa nespēj telpiski pielocīties pietiekami tuvu pie 1,4-DHP gredzena 4-C atoma, lai varētu notikt protonu sadarbība, kas izraisītu AB kvarteta signālu veidošanos.

2.3.1. 1,4-DHP-6-alkilkarboksimetilsulfanil-3-karbonskābes esteru enzimātiskā hidrolīze kinētiskās kontroles apstākļos

Kā norādīts literatūras apskatā (1.2. nod.), 3,5-dialkanilkarboksimetoksikarbonil-1,4-DHP enzimātiski hidrolizējas ar >90% enantiomērajiem pārākumiem, izmantojot enzīmus: *Candida antarctica* lipāze B (CAL-B, Novozym 435®), lipāzi PS (*Pseudomonas*

cepacia), proteāze P6 (*Aspergillus melleus*), Acilāze 30 000 (*Aspergillus sp*.) un lipāze AH (*Pseudomonas sp*.). Reakcijas apstākļi, kuros tika sasniegta augsta enantioselektivitāte: fosfāta buferšķīdums ar pH = 7,5, tam pievienots 15% acetonitrila un 45 ^{o}C temperatūrā, diizopropilēteris (IPE), kas piesātināts ar ūdeni, 45 ^{o}C temperatūrā, cikloheksāns, kas satur ūdeni, 20 ^{o}C temperatūra un 20% dimetilsulfoksīda fosfāta buferšķīdumā ar pH = 7,5, 20 ^{o}C temperatūrā [9].



Substrāti **63** tika pārbaudīti uz šādiem enzīmiem: *Candida antarctica* lipāzes B imobilizēto formu Novozym 435®, *Candida cyclindracea*, *Pencillium camemberti*, *Candida rugosa type VII*, *Amano acylase*, Amano lipase PS, M, G. No 8 enzīmiem hidrolītisko aktivitāti uzrādīja tikai Novozym 435®.

Rezultāti parādīja, ka izvēlētā lipāze mums izdevīgi nehidrolizē 3-metoksikarbonilgrupu, jo tā atrodas konjugācijā ar β-aminovinilgrupu un ir dezaktivēta. Savukārt, 6-alkilkarboksimetilsulfanilgrupa uzrādīja augstu hidrolītisko aktivitāti. Lipāze Novozym 435® katalizē 6-alkilkarboksimetilsulfanil-1,4-DHP **63** hidrolīzi, kur aizvietotājs R^2 bija etil un *t*-butil, bet diemžēl reakcijas nenorit enantioselektīvi. Enantiomērais pārākums šajos gadījumos bija <5%. Savukārt savienojumiem **63b**, **63e** un **63h**, kas satur 6-izopropilkarboksimetilsulfanilgrupu enzīma Novozym 435® promotētās hidrolīzes rezultātā enantiomērais pārākums bija virs 80%. Turpmāk padziļināti tika pētīti 6-izopropilkarboksimetilsulfanilgrupu saturoši 1,4-DHP, mainot šķīdinātāju sistēmas un reakcijas temperatūras (skatīt 2.7. tab.). Pielietojot literatūrā sekmīgi izmantoto šķīdinātāju sistēmu MeCN/Buferis [9] enzimātiskās reakcijas norit, bet nav enantioselektīvas. Mūsu gadījumā 6-alkilkarboksimetilsulfanil-1,4-DHP **63** viegli hidrolizējas buferšķīdumā arī bez enzīma, kas arī ir iemesls tam, ka reakcija nav enantioselektīva.

2.7. tabula

1,4-DHP-6-alkilkarboksimetilsulfanil-3-karbonskābes esteru **63** enzimātiskās hidrolīzes rezultāti

Substrāts 63	**Enzīms**	**T, °C**	**Šķīd. Sist.**	**Reakc. laiks, st.**	**Enantiomērais pārākums (+)-63, %**
a)	**b)**	**c)**	**d)**	**e)**	**f)**
63a	Novozym 435®	25	MeCN/Buf.	240	racemāts
63e	Novozym 435®	25	MeCN/Buf.	240	racemāts
63h	Novozym 435®	25	MeCN/Buf.	240	racemāts
63a	Novozym 435®	25	DMSO/IPE/H_2O 1/5/0,03	168	80
63e	Novozym 435®	25	DMSO/IPE/H_2O 1/5/0,03	168	80
63h	Novozym 435®	25	DMSO/IPE/H_2O 1/5/0,03	168	81
63a	Novozym 435®	35	DMSO/IPE/H_2O 1/5/0,03	96	80
63e	Novozym 435®	35	DMSO/IPE/H_2O 1/5/0,03	96	80
63h	Novozym 435®	35	DMSO/IPE/H_2O 1/5/0,03	96	82
63a	Novozym 435®	45	DMSO/IPE/H_2O 1/5/0,03	48	81
63e	Novozym 435®	45	DMSO/IPE/H_2O 1/5/0,03	48	81
63h	Novozym 435®	45	DMSO/IPE/H_2O 1/5/0,03	48	84
63a	Novozym 435®	25	DHM/IPE/H_2O 1/20/0,03	288	80
63e	Novozym 435®	25	DHM/IPE/H_2O 1/34/0,03	240	84
63h	Novozym 435®	25	DHM/IPE/H_2O 1/200/0,03	240	85
63a	Novozym 435®	35	DHM/IPE/H_2O 1/20/0,03	72	85

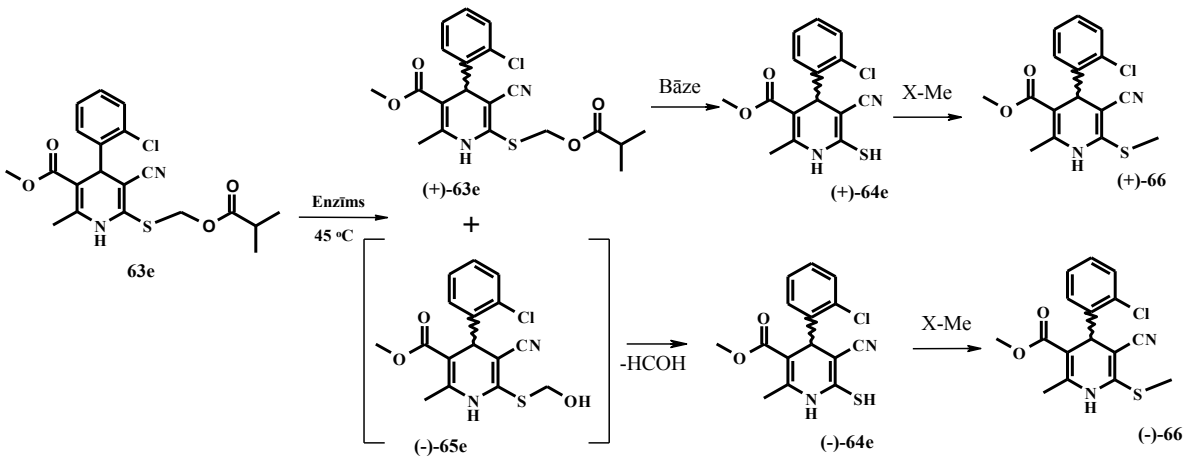
a)	b)	c)	d)	e)	f)
63e	Novozym 435®	35	DHM/IPE/H_2O 1/34/0,03	48	90
63h	Novozym 435®	35	DHM/IPE/H_2O 1/200/0,03	48	92
63a	Novozym 435®	45	DHM/IPE/H_2O 1/20/0,03	48	90
63e	Novozym 435®	45	DHM/IPE/H_2O 1/34/0,03	36	96
63h	Novozym 435®	45	DHM/IPE/H_2O 1/200/0,03	36	96

Lipāze Novozym 435® katalizē substrātu **63b**, **63e** un **63h** hidrolīzi šķīdinātāju sistēmā DMSO/IPE/H_2O, kuras rezultātā iegūst produktus **(+)-63b**, **(+)-63e** un **(+)-63h** ar enantiomēro pārākumu virs 80%. Paaugstinot reakcijas temperatūru no 25^{o}C līdz 45^{o}C, izdodas samazināt reakcijas laiku 3,5 reizes, bet enantioselektivitāte neuzlabojas. DHM/IPE/H_2O maisījums tika atrasts kā labākā šķīdinātāju sistēma. Veicot substrātu **63e** un **63h** Novozym 435® promotēto enzimātisko hidrolīzi 36 st. 45^{o}C temperatūrā, izdodas sasniegt >96% enantiomēro pārākumu.

Karbonskābju etil, *i*-propil un *t*-butilatvasinājumu **63** enzimātiskās hidrolīzes enantioselektivitāti būtiski ietekmēja alkilaizvietotājs. Savukārt variējot 4.vietas aizvietotājus: 4-Ph-, 4-(2-ClC_6H_4)- un 4-(2-F_2CHOC_6H_4)-, 1,4-DHP enzimātiskās hidrolīzes enantioselektivitātes izmaiņas ir nelielas.

2.3.2. (+)- un (-)- 5-Ciāno-4-(2-hlorfenil)-6-metilsulfanil-2-metil-1,4-dihidropiridīn-3-karbonskābes metilestera iegūšana

(+)-5-Ciāno-4-(2-hlorfenil)-6-metilsulfanil-2-metil-1,4-dihidropiridīn-3-karbonskābes metilesterus **(+)-66** un **(-)-66** preparatīvā daudzumā iegūst, veicot 6-izopropilkarboksimetilsulfanil-1,4-DHP **63e** enzimātisko hidrolīzi kinētiski kontrolētos apstākļos, izmantojot optimālos apstākļus (skat. 2.7. tabulu). Reakcija tika veikta 36 st. laikā, 45 ^{o}C temperatūrā, dihlormetāna/diizopropilētera/ūdens (1/34/0,03) šķīdinātāju maisījumā.



Kad fiksēta ~ 50 %, (±)-**63e** transformācija par (+)-**63e** (ķīmiski neizreaģējošais, bet enantiotransformējies produkts), reakciju apstādina. 5-Ciāno-4-(2-hlorfenil)-6-izobutiriloksimetilsulfanil-2-metil-1,4-dihidropiridīn-3-karbonskābes metilesteris (+)-**63e** iegūts ar 98% enantiomēro tīrību. Otra racemiskā estera (±)-**63e** daļa transformējas par 6-tiokso-1,4,5,6-tetrahidropiridīnu **(-)-64e**. Diemžēl, tiona hromatogrāfiskās nestabilitātes dēļ (tion-entiol tautomerizācija) neizdodas precīzi fiksēt 50% reakcijas norisi. Tiona **(-)-64e** veidošanos skaidrojam ar hidrolīzes rezultātā radušos 6-hidroksimetilsulfanil-1,4-DHP **(-)-65e** spontāno deformilēšanos. Iedarbojoties ar sārmu uz **(+)-63e,** iegūst tionu **(+)-64e**. Mērķa produkti 5-ciāno-4-(2-hlorfenil)-6-metilsulfanil-2-metil-1,4-dihidropiridīn-3-karbonskābes metilestera enantiomēri **(+)-66** un **(-)-66** iegūti veicot enantiobagātināto tionu **(+)-64e** un **(-)-64e** alkilēšanu ar metiljodīdu. Reakcija noris ar augstiem ķīmiskajiem iznākumiem 91% un 93%, bet šo transformāciju rezultātā samazinās enantiomērā tīrība. Hidrolīzes rezultātā atbrīvojas karbonskābe un formaldehīds, kuri hidrēto piridīn-2-tionu **(-)-64e** un daļēji nooksidē, tā samazinot ķīmisko un optisko iznākumu. Enantiomērais pārākums **(+)-66** gadījumā ir 80%, bet **(-)-66** gadījumā 50%. Enantiomērais pārākums noteikts, pielietojot AEŠH metodi, hirālo kolonu Lux Cellulose-2 (mobilā fāze: izopropilspirts/ heksāns, 1:1).

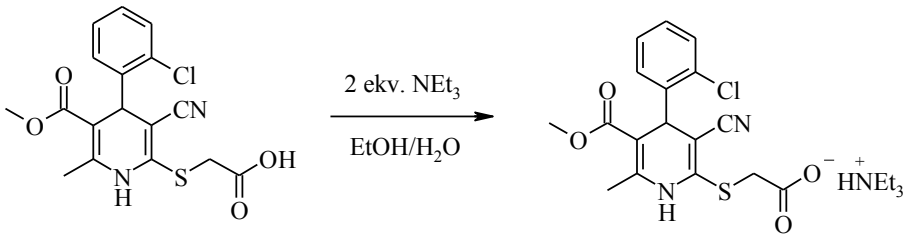
2.4. 4-(2-Hlorfenil)-6-(2-karboksimetilsulfanil)-1,4-dihidropiridīn-3-karbonskābes metilestera enantiosadalīšana, pielietojot hirālus amīnus

Daudzu hirālu ķīmisku savienojumu, dabisku vielu un farmaceitisko preparātu racionāla sintēze pieprasa efektīvas metodes šo savienojumu iegūšanai tīru enantiomēru veidā. Optiskā sadalīšana, veidojot diastereomērus sāļus, arvien ir plaši izmantota metode racemisku savienojumu sadalīšanai enantiomēros ķīmiskajā industrijā. Iedarbojoties uz racemiskām skābēm ar tādiem sadalīšanas aģentiem kā hirālas bāzes (amīni), iespējams iegūt skābes

atsevišķus enantiomērus. Darba ietvaros sintezēti arī 1,4-DHP-merkaptokarbonskābes atvasinājumi. Par turpmāko mērķi tika izvirzīts veikt eksperimentu sēriju, piemeklēt optimālos apstākļus un iegūt potenciāli aktīvus savienojumus – sēra saturošus 1,4-DHP – jau enantiotīrā (enantiobagātinātā veidā).

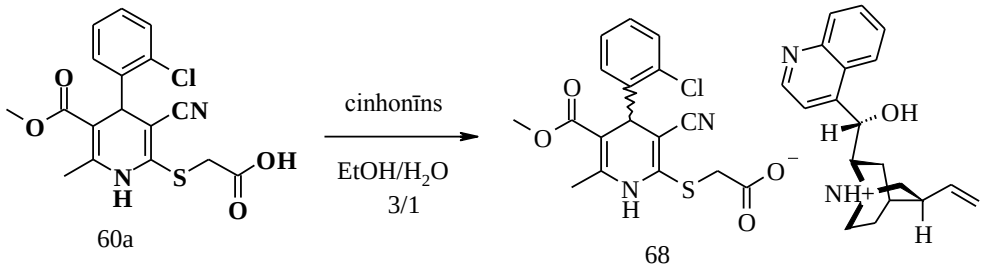
Literatūras apskata 1.4. apakšnodaļā galvenokārt minēti gadījumi, kad tika pētīta racemisku 1,4-dihidropiridīn-karbonskābju sadalīšana enantiomēros un karboksi-(skābes)-grupas oglekļa atoms šajās molekulās tieši piesaistīts 1,4-DHP gredzenam. Taču šāda diastereomēru kristalizācijas metode enantiotīru karbonskābju iegūšanā varētu būt veiksmīgs risinājums arī tādu 1,4-DHP sadalīšanā enantiomēros, kur skābes grupa nav tieši piesaistīta 1,4-DHP gredzenam.

Lai novērtētu 6-(2-karboksimetilsulfanil)-1,4-dihidropiridīna **60a** spēju veidot sāļus ar trešējiem amīniem, sākumā tika piemeklēti atbilstošie apstākļi tā reakcijai ar trietilamīnu. Tā kā substrāts (skābe) nešķīst nepolāros šķīdinātājos, izmantoti polāri šķīdinātāji un 2 ekvivalentu daudzums trietilamīna. 4-(2-Hlorfenil)-1,4-DHP-6-merkaptoetiķskābes trietilamonija sāls **67** iegūts etanola/ūdens (3/1) šķīdumā istabas temperatūrā pēc 24 stundām ar 90% iznākumu:



60a **67**

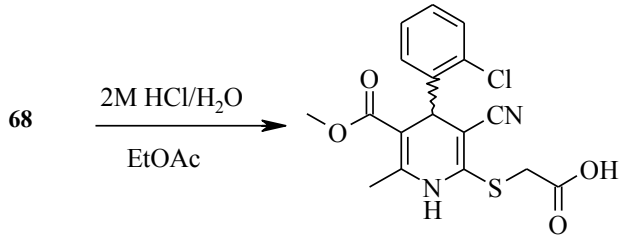
Racemiskas 4-(2-hlorfenil)-1,4-DHP-6-merkaptoetiķskābes diastereomēro amīna sāļu kristalizācijas eksperimenti veikti, variējot reakcijas apstākļus. Par hirālajiem amīniem izmantoti cinhonidīns, cinhonīns, hinidīns. Izmantotie šķīdinātāji: DMF/H_2O, acetons, EtOH/H_2O, DHM, etilacetāts. Lielākajā daļā gadījumu, amīns nesaistījās ar skābi **60a**, bet gan kristalizējas atpakaļ no reakcijas vides. Tikai vienā gadījumā tika iegūts cinhonīna 4-(2-hlorfenil)-1,4-DHP-6-merkaptoacetāta sāls **68** (59% iznākums pret vienu enantiomēru), tā ^{1}H-KMR spektrā redzami gan attiecīgās skābes **60a** raksturīgie 4-H, SCH_2 signāli, gan cinhonīna signāli:



60a 68

Noteikta diastereomērā sāls **68** īpatnējā optiskā griešana $[\alpha]_D^{20}$ +87,8 (*c* 10,0, $CHCl_3$). Jāpiebilst, ka pašam cinhonidīnam tā ir liela – $[\alpha]_D^{20}$ +224 (*c* 0,5, EtOH), tāpēc pat nelieli amīna piemaisījumi sniegtu neprecīzus optiskās griešanas datus. Lai precīzāk raksturotu diastereoselektivitāti, nepieciešams noteikt diastereomēro pārākumu (de). Pielietojot AEŠH metodi ar hirālo kolonnu „Whelk O1 (*R,R*)" (mobīlā fāze – IPA(AmAc, 0,1M)/CH_2Cl_2/heksāns – 10/10/80; 1 ml/min) noteica sāls **68** diastereomēro pārākumu, kas bija 4% de (t_{r1}=10,1 min, t_{r2}=11,4 min). Diemžēl šādas kristalizācijas diastereoselektivitāte ir zema. To var skaidrot ar substrāta skābes **60a** asimetrisko molekulu, kas var būt kā iemesls diastereomēro kristālu nelabvēlīgam sapakojumam. Tomēr šajā gadījumā, kad kristāli veidojas, zemo selektivitāti var skaidrot ar to, ka diastereomēra **68** hirālie centri atrodas tālu viens no otra.

Lai iegūtu enantiobagātinātu 4-(2-hlorfenil)-1,4-DHP-6-merkaptoetiķskābi **69**, nepieciešams apstrādāt sāli **68** skābā vidē:



69

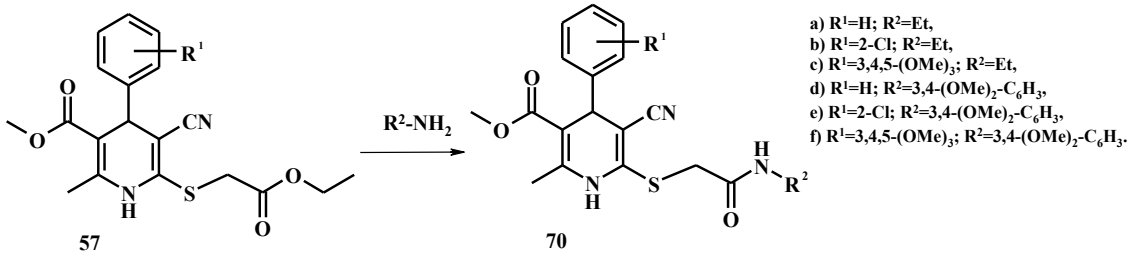
Iegūtās enantiobagātinātās skābes **69** (95% ķīm. iznākums) īpatnējā optiskā griešana $[\alpha]_D^{20}$ bija 0. Tas nozīmē, ka 1,4-dihidropiridīn-6-merkaptoetiķskābei **69** ir tieksme racemizēties skābā vidē. Šādu skābju sadalīšana ar diastereomērās kristalizāciju ir vēl neizpētīta metode un prasa plašākus pētījumus.

Veiksmīgs šādas problēmas risinājums varētu būt citu hirālu amīnu izmantošana, kā arī enantiosadalīšana pielietojot ķīmijenzimātiskas metodes.

2.5. 4-Aril-ciāno-6-alkilsulfanil-2-metil-1,4-dihidropiridīn-3-karbonskābes metilesteru ķīmiskā un enzimātiskā aminolīze.

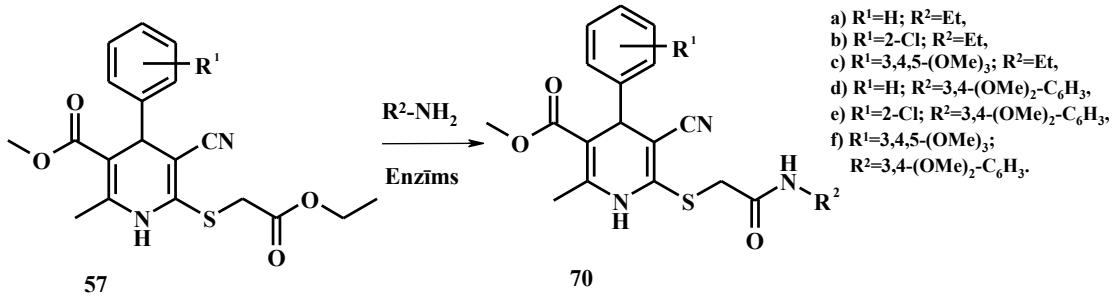
Alternatīvs variants 1,4-DHP-karbonskābju esteru enantiosadalīšanai, veicot enzimātisko hidrolīzi ir dažādu substrātu enzimātiskā aminolīze. Par substrātiem ir iespējams izmantot skābes un esterus. Augstus enantiomēros iznākumus iegūst izmantojot tādus šķīdinātājus kā metilizobutilketons, tercbutanols, heksāns, diizopropilēteris, heptāns un metiltercbutilēteris[21-26]. Kā pieminēts literatūras apskatā aprakstīts, augstus enantiomēros iznākumus 80-99% iegūst, pielietojot lipāzi - Candida antarctica lipāze B.

4-Aril-ciāno-6-alkilkarbomoilmetilsulfanil-2-metil-1,4-dihidropiridīn-3-karbonskābes metilesteru sintēzes shēma:



Savienojumi **70** tika sintezēti kā modeļvielas enzimātiskajām reakcijām, lai piemeklētu apstākļus enantiomērās tīrības noteikšanai. Tika uzsintezēti 1,4-DHP **70** ar 4. vietā dažādiem fenilgrupas aizvietotājiem un 6. vietā atšķirīgiem amīdiem. Izejvielu **57a-c** iegūšana ir iepriekš aprakstīta rezultātu izvērtējumā 2.2.1. nodaļā. Ķīmiskā aminolīze norit EtOH/H_2O šķīdumā istabas temperatūrā, maisot 3 stundas. Savienojumus **70a-c** izdodas iegūt ar 94-95% iznākumu sakristalizējot no reakcijas maisījuma. 1,4-DHP **57** reakcijā ar dimetoksianilīnu veidojas blakusprodukti, kas attīrīti ar kolonnas hromatogrāfiju un iegūti mērķaprodukti **70d-f** ar 81-87% iznākumu.

4-Aril-ciāno-6-alkilsulfanil-2-metil-1,4-dihidropiridīn-3-karbonskābes metilesteru enzimātiskā aminolīze tika veikta izmantojot enzīmus: Candida antarctica lipāze B, lipāze Candida rugosa un Amano Acylase.



Katalītisko aktivitāti uzrādīja viens enzīms - Candida antarctica lipāze B (imobilizētā forma Novozym 435®). Literatūrā aprakstīts, ka aminolīzes reakcijas tiek veiktas 25-40 ^{o}C temperatūrās. Līdz ar to mēs izvēlējāmies veikt enzimātiskās reakcijas 25 un 45 ^{o}C temperatūrās, lai izvērtētu to ietekmi uz substrātu reaģētspēju un produktu sterioselektivitāti. Analizējot mūsu rezultātus varam secināt, ka temperatūras izmaiņas būtiski neietekmē ne reaģētspēju, ne sterioselektivitāti. Vislielākās izmaiņas tika novērotas, pielietojot dažādas šķīdinātāju sistēmas. IPE/DHM un tīrā DHM aminolīzes enzimātiski katalizētās reakcijas nenotiek, pielietojot gan etilamīnu, gan dimetoksianilīnu. Racemiski produkti veidojas, izmantojot heksāna/DHM šķīdinātāju sistēmu, THF un dioksānu. Iegūtos rezultātus var aplūkot 2.8. tabulā. Diemžēl salīdzinot ar literatūras augstajiem enantiomērajiem pārākumiem (>90%), mums izdevās iegūt tikai racemiskus produktus. Tas skaidrojams ar to, ka atšķirībā no literatūrā aprakstītajiem savienojumiem, kur reakcijas un hirālā centra attālums ir 1-2 saites, mūsu gadījumā tās ir 5 saites.

2.8. tabula

1,4-DHP-6-alkanilsulfanil-3-karbonskābes esteru **54** enzimātiskās aminolīzes rezultāti

Substrāts	**T, ^{o}C**	**Enzīms**	**Šķīdinātājs**	**$EtNH_2$**	**3,4-dimetoksianilīns**
a)	**b)**	**c)**	**d)**	**e)**	**f)**
57a-c	25	Novozym 435®	IPE/DHM (15÷1)	**nenotiek**	**nenotiek**
57a-c	25	Novozym 435®	IPE/DHM (1÷1)	**nenotiek**	**nenotiek**
57a-c	25	Novozym 435®	DHM	**nenotiek**	**nenotiek**
57a-c	25	Novozym 435®	Heksāns/DHM (15÷1)	**racemāts**	**racemāts**
57a-c	25	Novozym 435®	Toluols	**racemāts**	**racemāts**
57a-c	25	Novozym 435®	Dioksāns	**nenotiek**	**racemāts**
57a-c	45	Novozym 435®	IPE/DHM (15÷1)	**nenotiek**	**nenotiek**
57a-c	45	Novozym 435®	IPE/DHM (1÷1)	**nenotiek**	**nenotiek**

a)	b)	c)	d)	e)	f)
57a-c	45	Novozym 435®	DHM	**nenotiek**	**nenotiek**
57a-c	45	Novozym 435®	Heksāns/DHM (15÷1)	**racemāts**	**racemāts**
57a-c	45	Novozym 435®	Toluols	**racemāts**	**racemāts**
57a-c	45	Novozym 435®	Dioksāns	**nenotiek**	**racemāts**

3. EKSPERIMENTĀLĀ DAĻA

Metodes un aparatūra

Reakcijas norise un sintezējamo savienojumu tīrības kontrole veikta ar plānslāņa hromatogrāfijas metodi, izmantojot „Silufol UV 254" plāksnītes. Hromatogrammas detektētas ar UV lampu „UVSL-58" („Ultra-Violet Product Inc.", ASV) pie viļņu garumiem: 254 un 366 nm. Par eluentu sistēmām izmantotas: hloroforms : heksāns : acetons – 2 : 1 : 1, heksāns : dihlormetāns : etanols – 16 : 5 : 1, heksāns : dihlormetāns : etanols – 5 : 5 : 1.

Kušanas temperatūras noteiktas ar automātisko kušanas aparātu "Optimelt". IS spektri uzņemti ar spektrometru „Shimadzu IR Prestige-21" paraugu suspensijām nujolā 400-4000 cm^{-1} diapazonā.

^{1}H-KMR spektri uzņemti ar aparātu „Bruker" pie 200 un 400 MHz DMSO-d_6 vai $CDCl_3$ šķīdumā, par iekšējo standartu izmantojot tetrametilsilānu. Elementu sastāva analīze izdarīta ar automātisko analizatoru „EA 1106" („Carlo Erba Instruments", Itālija).

^{1}H-KMR un IS spektri uzņemti OSI Fizikālorganiskās ķīmijas laboratorijā.

Dubultesteru enantiomērās tīrības noteikšanai izmantots šķidrumu hromatogrāfs „Shimadzu", augstspiediena sūknis LC-20AD, autosamplers SIL-20AC, diožu matricas detektors SPD-M20A, kolonnu termostats CTO-20AC.

Dubultesteru enzimātiskās reakcijas veiktas orbitālajā kratītājā ar termostatu „Max Q 4000".

Vielas:

- Etanols (F, R11, S16, S7);
- Benzaldehīds (Xn, R22, S24) ;
- 2-Hlorbenzaldehīds (C, R34, S26, S45);
- 2,3-dihlorbenzaldehīds (Xi, R36, R37, R38, S26, S37, S39);
- Dihlormetāns (Xn, R40, S23, S24, S25, S36/37);
- Piperidīns (F, T, R11, R22, R23/24, S16, S26, S27, S45);
- Metanols (F, T, R11, R23, R24, R25, R39, S7, S16, S36, S37);
- Ciānotioacetamīds (Xn, R21, S24);
- Metiljodīds (F, T, R11, R21, R23, R25, R37, R38, R40, S9, S16, S24, S36, S37, S38, S45);
- Nātrija hidroksīda ūdens šķīdums (C, R35, S26, S37, S39, S45).

Paskaidrojumi:

F – viegli uzliesmojošs;

Xn – kaitīgs;

T – tosisks;

Xi - kairinošs;

C – kodīgs;

R11 – viegli uzliesmojošs;

R20/21/22 – kaitīgs ieelpojot, kontaktā ar ādu un norijot;

R23/24/25 – toksisks ieelpojot, kontaktā ar ādu un norijot;

R23/25 – toksisks kontaktā ar ādu un norijot;

R24/25 – toksisks kontaktā ar ādu un norijot;

R26 – ļoti toksisks ieelpojot;

R34 – rada apdegumus;

R36/37/38/ - kairinošs acīm, elpošanas sistemai un ādai;

S7 – turēt noslēgtā traukā;

S9 – turēt noslēgtā traukā labi ventilējamās telpās;

S16 – sargāt no uguns – nesmēķēt;

S26 – ja nonāk kontaktā ar acīm, nekavējoties noskalot ar lielu ūdens daudzumu un meklēt medicīnisko palīdzību;

S28A – Ja nonāk saskarē ar ādu, nekavējoties skalot ar lielu ūdens daudzumu;

S36/37/39 – valkāt piemērotu aizsargapģērbu, cimdus un acu/sejas aizsargu;

S38 – nepietiekamas ventilācijas gadījumā valkāt piemērotus elpošanas aizsarglīdzekļus;

S45 – ja izmantojot rodas slikta pašsajūta, nekavējoties meklēt medicīnisko palīdzību (ja iespējams, uzrādīt etiķeti).

5-Ciāno-4-fenil-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes etoksikarbonilmetilesteris (43a)

0,94 g (5 mmol) acetetiķskābes etoksikarbonilmetilestera **3** un 0,53 g (5 mmol) benzaldehīda izšķīdina 20 ml etanola un pievieno 0,03 ml (0,3 mmol) piperidīna. Reakcijas maisījumu maisa istabas temperatūrā 5 min., tad pievieno 0,50 g (5 mmol) 2-ciānotioacetamīda un 0,53 ml (5,3 mmol) piperidīna un maisa istabas temperatūrā 25 min. Iegūtajam maisījumam pievieno 0,74 ml (5,3 mmol) metiljodīda un to vāra 5 min. Siltam reakcijas maisījumam pievieno 4 ml 3M HCl etanola šķīduma. Radušās nogulsnes filtrē, uz filtra tās mazgā ar 10 ml auksta metanola, 5 ml dest. ūdens. Iegūst 0,50 g (34%) bezkrāsainas kristāliskas vielas **43a** ar k.t. 129 - 130^{o}C.

IS spektrs (nujolā, ν): 1681, 1756 (C=O); 2200 (C≡N); 3066, 3270 (NH) cm^{-1}.

^{1}H–KMR spektrs ($CDCl_3$, 200 MHz, δ): 1,23 un 4,16 (5H, t un kv, J = 7,0 Hz, CH_2CH_3); 2,41 (3H, s, 2-CH_3); 2,48 (3H, s, SCH_3); 4,54 (2H, ABkv, J = 16.1 Hz, 3-$COOCH_2$); 4,75 (1H, s, 4-H); 6,09 (1H, s, NH); 7,20–7,38 (5H, kompl, C_6H_5) m.d.

Aprēķināts: $C_{19}H_{20}N_2O_4S$: C 61,27%; H 5,41%; N 7,52%; S 8,61%. Noteikts: C 60,75%; H 5,40%; N 7,46%; S 8,69%.

5-Ciāno-4-(2-hlorfenil)-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes etoksikarbonilmetilesteris (43b)

0,94 g (5 mmol) acetetiķskābes etoksikarbonilmetilestera **5** un 1,11 g (5 mmol) 3-(2-hlorfenil)-2-ciāno-tioakrilamīda **50b** izšķīdina 20,00 ml etanola un pievieno 0,53 ml (5,3 mmol) piperidīna. Reakcijas maisījumu maisa istabas temperatūrā 25 min. Iegūtajam šķīdumam pievieno 0,74 ml (5,3 mmol) metiljodīda un to vāra 5 min. Siltam reakcijas maisījumam pievieno 4 ml 3M HCl etanola šķīduma. Radušās nogulsnes filtrē, uz filtra tās mazgā ar 10 ml auksta metanola, 5 ml dest. ūdens. Iegūst 1,09 g (53%) bezkrāsainas kristāliskas vielas **43b** ar k.t. 144 – 145 ^oC.

IS spektrs (nujolā, ν): 1684, 1728 (C=O); 2199 (C≡N); 3254; 3336 (NH) cm^{-1}.

^{1}H – KMR spektrs ($CDCl_3$, 200 MHz, δ): 1,16 un 4,07 (5H, t un kv, J = 7.0 Hz, CH_2CH_3); 2,34 (3H, s, 2-CH_3); 2,40 (3H, s, SCH_3); 4,44 (2H, ABkv, J = 16.1 Hz, 3-$COOCH_2$); 5,29 (1H, s, 4-H); 6,20 (1H, s, NH); 7,02–7,30 (4H, kompl, C_6H_4Cl) m.d.

Aprēķināts: $C_{19}H_{19}ClN_2O_4S$: C 56,09%; H 4,71%; N 6,88%; S 7,88%. Noteikts: C 55,44%; H 4,83%; N 6,68%; S 7,87%.

5-Ciāno-4-(2,3-dihlorfenil)-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes etoksikarbonilmetilesteris (43c)

0,94 g (5 mmol) acetetiķskābes etoksikarbonilmetilestera **3** un 1,29 g (5 mmol) 2-ciāno-3-(2,3-dihlorfenil)tioakrilamīda **50c** izšķīdina 20 ml etanola un pievieno 0,53 ml (5,3 mmol) piperidīna. Reakcijas maisījumu maisa istabas temperatūrā 25 min. Iegūtajam šķīdumam pievieno 0,74 ml (5,3 mmol) metiljodīda un to vāra 5 min. Siltam reakcijas maisījumam pievieno 4 ml 3M HCl etanola šķīduma. Radušās nogulsnes filtrē, uz filtra tās mazgā ar 10 ml auksta metanola, 5 ml dest. ūdens. Iegūst 1,66 g (75%) bezkrāsainas kristāliskas vielas **43c** ar k.t. 140 – 141 ^oC.

IS spektrs (nujolā, ν): 1631, 1707 (C=O); 2204 (C≡N); 3323, (NH) cm^{-1}.

^{1}H – KMR spektrs ($CDCl_3$, 200 MHz, δ): 1,20 un 4,13 (5H, t un kv, J = 7.0 Hz, CH_2CH_3); 2,40 (3H, s, 2-CH_3); 2,47 (3H, s, SCH_3); 4,93 (2H, ABkv, J = 16.1 Hz, 3-$COOCH_2$); 5,40 (1H, s, 4-H); 6,19 (1H, s, NH); 7,16–7,37 (3H, kompl, $C_6H_3Cl_2$) m.d.

Aprēķināts: $C_{19}H_{18}Cl_2N_2O_4S$: C 51,71%; H 4,11%; N 6,35%; S 7,27%. Noteikts: C 51,52%; H 4,09%; N 6,31%; S 7,32%.

5-Ciāno-4-fenil-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes karboksimetilesteris (51a)

0,19 g (0,5 mmol) 5-ciāno-4-fenil-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes etoksikarbonilmetilestera **43a** izšķīdina 10 ml etanola, pievieno 0,16 ml (0,5 mmol) 3M NaOH ūdens šķīdumā un maisa istabas temperatūrā 8 st. Maisījumu apstradā ar 2M HCl ūdens šķīdumā līdz pH = 2-3. Nogulsnes filtrē, uz filtra tās mazgā ar 10 ml auksta metanola, 10 ml dest. ūdens. Iegūst 0,14 g (82%) bezkrāsainas kristāliskas vielas **51a** ar k.t. 229 - 231 ^{o}C.

IS spektrs (nujolā, ν): 1688, 1776 (C=O); 2201 (C≡N); 3189, 3273; 3607 (NH, OH) cm^{-1}.

^{1}H – KMR spektrs (DMSO-d_6, 200 MHz, δ): 2,41 (3H, s, 2-CH_3); 2,48 (3H, s, SCH_3); 4,59 (2H, ABkv, J = 15.6 Hz, 3-$COOCH_2$); 4,73 (1H, s, 4-H); 6,18 (1H, s, NH); 7,20–7,38 (5H, kompl, C_6H_5) m.d.

Aprēķināts: $C_{17}H_{16}N_2O_4S$: C 59,29%; H 4,68%; N 8,13%; S 9,31%. Noteikts: C 59,70%; H 4,18%; N 8,63%; S 9,73%.

5-Ciāno-4-(2-hlorfenil)-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes karboksimetilesteris (51b)

0,20 g (0,5 mmol) 5-ciāno-4-(2-hlorfenil)-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes etoksikarbonilmetilestera **43b** izšķīdina 10 ml etanola, pievieno 0,16 ml (0,5 mmol) 3M NaOH ūdens šķīdumā un maisa istabas temperatūrā 8 st. Maisījumu apstradā ar 2M HCl ūdens šķīdumā līdz pH = 2-3. Produktu nevar izolēt no etanola un metanola, tāpēc maisījumu ierotē sausu, mazgā ar 10 ml dest. ūdens un dekantē. Produktu kristalizē no 10 ml dihlormetāna. Noglusnes nofiltrē. Iegūst 0,12 g (63%) bezkrāsainas kristāliskas vielas **51b** ar k.t. 119 - 121 ^{o}C.

IS spektrs (nujolā, ν): 1644, 1717 (C=O); 2193 (C≡N); 3325, 3625 (NH, OH) cm^{-1}.

^{1}H – KMR spektrs (DMSO-d_6, 200 MHz, δ): 2,27 (3H, s, 2-CH_3); 2,40 (3H, s, SCH_3); 4,33 (2H, ABkv, J = 15.6 Hz, 3-$COOCH_2$); 5,05 (1H, s, 4-H); 7,06–7,36 (4H, kompl, C_6H_4Cl); 9,50 (1H, s, NH) m.d.

Aprēķināts: $C_{17}H_{15}ClN_2O_4S$: C 53,90%; H 3,99%; N 7,39%; S 8,46%. Noteikts: C 51,22%; H 3,83%; N 6,91%; S 7,90%.

5-Ciāno-4-(2,3-dihlorfenil)-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes karboksimetilesteris (51c)

0,22 g (0,5 mmol) 5-ciāno-4-(2,3-dihlorfenil)-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes etoksikarbonilmetilestera **43c** izšķīdina 10 ml etanola, pievieno 0,16 ml (0,5 mmol) 3M NaOH ūdens šķīdumā un maisa istabas temperatūrā 8 st. Maisījumu apstradā ar 2M HCl ūdens šķīdumā līdz pH = 2-3. Produktu daļēji var izolēt no etanola, lai paaugstinātu iznākumu filtrātu ierotē sausu, mazgā ar 10 ml dest. ūdens un dekantē. Produktu kristalizē no 5 ml dihlormetāna. Nogulsnes nofiltrē. Iegūst 0,19 g (90%) bezkrāsainas kristāliskas vielas **51c** ar k.t. 170 - 171 ^oC.

IS spektrs (nujolā, ν): 1632, 1723 (C=O); 2212 (C≡N); 3252 (NH) cm^{-1}.

^1H – KMR spektrs (DMSO-d_6, 200 MHz, δ): 2,35 (3H, s, 2-CH_3); 2,48 (3H, s, SCH_3); 4,27 (2H, ABkv, J = 15.6 Hz, 3-$COOCH_2$); 5,17 (1H, s, 4-H); 7,20–7,73 (3H, kompl, $C_6H_3Cl_2$); 9,60 (1H, s, NH) m.d.

Aprēķināts: $C_{17}H_{14}Cl_2N_2O_4S$: C 49,41%; H 3,41%; N 6,78%; S 7,76%. Noteikts: C 48,49%; H 3,31%; N 6,65%; S 7,69%.

5-Ciāno-4-fenil-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes 2-ciānoetilesteris (52a)

0,78 g (5 mmol) 3-oksobutānskābes-2-ciānoetilestera un 0,53 g (5 mmol) benzaldehīda izšķīdina 20 ml etanola un pievieno 0,03 ml (0,3 mmol) piperidīna. Reakcijas maisījumu maisa istabas temperatūrā 5 min., tad pievieno 0,5 g (5 mmol) 2-ciānotioacetamīda un 0,53 ml (5,3 mmol) piperidīna un maisa istabas temperatūrā 25 min. Iegūtajam maisījumam pievieno 0,74 ml (5,3 mmol) metiljodīda un to vāra 5 min. Siltam reakcijas maisījumam pievieno 4 ml 3M HCl etanola šķīduma. Radušās nogulsnes filtrē, uz filtra tās mazgā ar 10 ml auksta metanola, 5 ml dest. ūdens. Iegūst 0,73 g (43%) bezkrāsainas kristāliskas vielas **52a** ar k.t. 90 - 93 ^oC.

IS spektrs (nujolā, ν): 1695 (C=O); 2196, 2265 (C≡N); 3310 (NH) cm^{-1}.

^1H – KMR spektrs ($CDCl_3$, 200 MHz, δ): 2,41 (3H, s, 2-CH_3); 2,48 (3H, s, SCH_3); 2,53 (2H, t un t, CN$\underline{CH_2}CH_2O$); 4,21 (2H, t un t, $CNCH_2\underline{CH_2}O$); 4,47 (1H, s, 4-H); 6,10 (1H, s, NH); 7,20–7,40 (5H, kompl, C_6H_5) m.d.

Aprēķināts: $C_{18}H_{17}N_3O_2S$: C 63,69%; H 5,05%; N 12,38%; S 9,45%. Noteikts: C 63,88%; H 5,01%; N 12,31%; S 9,40%.

5-Ciāno-4-(2-hlorfenil)-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes 2-ciānoetilesteris (52b)

0,47 g (3 mmol) 3-oksobutānskābes-2-ciānoetilestera un 0,67 g (3 mmol) 2-ciāno-3-(2-hlorfenil)tioakrilamīdu **50b** izšķīdina 20 ml etanola un pievieno 0,33 ml (3,3 mmol) piperidīna. Reakcijas maisījumu maisa istabas temperatūrā 5 min., tad pievieno 3 ml 3M HCl/etanola šķīduma un turpina maisīt vēl 1 st. Iegūtajam maisījumam pievieno 0,33 ml (3,3 mmol) piperidīna un 0,74 ml (3,3 mmol) metiljodīda un to vāra 5 min. Radušās nogulsnes filtrē, uz filtra tās mazgā ar 10 ml auksta metanola, 5 ml dest. ūdens. Iegūst 0,35 g (32%) bezkrāsainas kristāliskas vielas **52b** ar k.t. 150 - 153 ^{o}C.

IS spektrs (nujolā, ν): 1654 (C=O); 2199, 2250 (C≡N); 3299 (NH) cm^{-1}.

^{1}H – KMR spektrs ($CDCl_3$, 200 MHz, δ): 2,35 (3H, s, 2-CH_3); 2,41 (3H, s, SCH_3); 2,48 (2H, t, $CNCH_2\underline{CH_2}O$); 4,11 (2H, t, $CN\underline{CH_2}CH_2O$); 5,23 (1H, s, 4-H); 6,08 (1H, s, NH); 7,10–7,34 (4H, kompl, C_6H_4Cl) m.d.

Aprēķināts: $C_{18}H_{16}ClN_3O_2S$: C 57,83%; H 4,31%; N 11,24%; S 8,58%. Noteikts: C 54,59%; H 4,63%; N 10,74%; S 7,71%.

5-Ciāno-4-(2,3-dihlorfenil)-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes 2-ciānoetilesteris (52c)

0,47 g (3 mmol) 3-oksobutānskābes-2-ciānoetilestera un 0,78 g (3 mmol) 2-ciāno-3-(2,3-dihlorfenil)tioakrilamīdu **50c** izšķīdina 20 ml etanola un pievieno 0,33 ml (3,3 mmol) piperidīna. Reakcijas maisījumu maisa istabas temperatūrā 5 min., tad pievieno 3 ml 3M HCl/etanola šķīduma un turpina maisīt vēl 1 st. Iegūtajam maisījumam pievieno 0,33 ml (3,3 mmol) piperidīna un 0,74 ml (3,3 mmol) metiljodīda un to vāra 5 min. Radušās nogulsnes filtrē, uz filtra tās mazgā ar 10 ml auksta metanola, 5 ml dest. ūdens. Iegūst 0,98 g (80%) bezkrāsainas kristāliskas vielas **52c** ar k.t. 163 - 165 ^{o}C.

IS spektrs (nujolā, ν): 1675 (C=O); 2197, 2260 (C≡N); 3316 (NH) cm^{-1}.

^{1}H – KMR spektrs ($CDCl_3$, 200 MHz, δ): 2,35 (3H, s, 2-CH_3); 2,42 (3H, s, SCH_3); 2,50 (2H, t, $CNCH_2\underline{CH_2}O$); 4,11 (2H, t, $CN\underline{CH_2}CH_2O$); 5,29 (1H, s, 4-H); 6,10 (1H, s, NH); 7,11–7,34 (3H, kompl, $C_6H_3Cl_2$) m.d.

Aprēķināts: $C_{18}H_{15}Cl_2N_3O_2S$: C 52,95%; H 3,70%; N 10,29%; S 7,85%. Noteikts: C 52,30%; H 3,61%; N 10,05%; S 7,89%.

5-Ciāno-4-fenil-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābe (53a)

0,17 g (0,5 mmol) 5-Ciāno-4-fenil-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes 2-ciānoetilestera **52a** izšķīdina 10 ml etanola, pievieno 0,16 ml (0,5 mmol) 3M NaOH ūdens šķīduma un maisa istabas temperatūrā 8 st. Maisījumu apstrādā ar 2M HCl ūdens šķīdumu līdz pH = 2-3. Nogulsnes filtrē, uz filtra tās mazgā ar 10 ml auksta metanola, 10 ml dest. ūdens. Iegūst 0,11 g (78%) bezkrāsainas kristāliskas vielas **53a** ar k.t. 231 – 233 ^{o}C.

IS spektrs (nujolā, ν): 1702 (C=O); 2204 (C≡N); 3273 (NH), 3321 (OH) cm^{-1}.

^{1}H – KMR spektrs (DMSO-d$_6$, 200 MHz, δ): 2,31 (3H, s, 2-CH$_3$); 2,48 (3H, s, SCH$_3$); 4,46 (1H, s, 4-H); 7,10–7,4 (5H, kompl, C$_6$H$_5$); 9,33 (1H, s, NH); 11,90 (1H, pl.s, OH) m.d.

Aprēķināts: $C_{15}H_{14}N_2O_2S$: C 62,92%; H 4,93%; N 9,78%; S 11,20%. Noteikts: C 63,35%; H 5,12%; N 9,87%; S 11,32%.

5-Ciāno-4-(2-hlorfenil-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābe (53b)

0,19 g (0,5 mmol) 5-Ciāno-4-(2-hlorfenil-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes 2-ciānoetilestera **52b** izšķīdina 10 ml etanola, pievieno 0,16 ml (0,5 mmol) 3M NaOH ūdens šķīduma un maisa istabas temperatūrā 8 st. Maisījumu apstrādā ar 2M HCl ūdens šķīdumu līdz pH = 2-3. Nogulsnes filtrē, uz filtra tās mazgā ar 10 ml auksta metanola, 10 ml dest. ūdens. Iegūst 0,12 g (75%) bezkrāsainas kristāliskas vielas **53b** ar k.t. 250 - 253 ^{o}C.

IS spektrs (nujolā, ν): 1704 (C=O); 2200 (C≡N); 3273 (NH), 3328 (OH) cm^{-1}.

^{1}H – KMR spektrs (DMSO-d$_6$, 200 MHz, δ): 2,31 (3H, s, 2-CH$_3$); 2,47 (3H, s, SCH$_3$); 5,21 (1H, s, 4-H); 7,13–7,42 (4H, kompl, C$_6$H$_4$Cl); 9,33 (1H, s, NH); 11,81 (1H, pl.s, OH) m.d.

Aprēķināts: $C_{15}H_{13}ClN_2O_2S$: C 56,16%; H 4,08%; N 8,73%; S 10,00%. Noteikts: C 56,10%; H 4,05%; N 8,80%; S 10,14%.

5-Ciāno-4-(2,3-dihlorfenil-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābe (53c)

0,20 g (0,5 mmol) 5-Ciāno-4-(2,3-dihlorfenil-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes 2-ciānoetilestera **52c** izšķīdina 10 ml etanola, pievieno 0,16 ml (0,5 mmol) 3M NaOH ūdens šķīduma un maisa istabas temperatūrā 8 st. Maisījumu apstrādā ar 2M HCl ūdens šķīdumu līdz pH = 2-3. Nogulsnes filtrē, uz filtra tās mazgā ar 10 ml auksta metanola,

10 ml dest. ūdens. Iegūst 0,14 g (79%) bezkrāsainas kristāliskas vielas **53c** ar k.t. 256 - 258 ^{o}C.

IS spektrs (nujolā, ν): 1704 (C=O); 2209 (C≡N); 3270 (NH), 3322 (OH) cm^{-1}.

^{1}H – KMR spektrs (DMSO-d_6, 200 MHz, δ): 2,30 (3H, s, 2-CH_3); 2,43 (3H, s, SCH_3); 5,27 (1H, s, 4-H); 7,11–7,46 (3H, kompl, $C_6H_3Cl_2$); 9,30 (1H, s, NH); 11,77 (1H, pl.s, OH) m.d.

Aprēķināts: $C_{15}H_{12}Cl_2N_2O_2S$: C 50,72%; H 3,40%; N 7,89%; S 9,03%. Noteikts: C 50,79%; H 3,41%; N 7,80%; S 9,14%.

5-Ciāno-4-(2-hlorfenil)-2-metil-6-etoksikarbonilmetilsulfanil-1,4-dihidropiridīn-3-karbonskābes metilesteris (57a)

0,96 g (3 mmol) 5-ciāno-4-(2-hlorfenil)-2-metil-6-tiokso-1,2,3,4-dihidropiridīn-3-karbonskābes metilestera **58** izšķīdina 20 ml metanola, pievieno 0,33 ml (3,3 mmol) piperidīna. Reakcijas maisījumu maisa istabas temperatūrā 5 min. Iegūtajam šķīdumam pievieno 0,37 ml (3,3 mmol) brometiķskābes etilestera un to vāra 2 st. Nogulsnes filtrē, uz filtra tās mazgā ar 10 ml auksta metanola, 10 ml dest. ūdens. Iegūst 1,02 g (84%) bezkrāsainas kristāliskas vielas **57a** ar k.t. 174 – 176 ^{o}C.

IS (nujolā, ν): 1688 (C=O); 2194 (C≡N); 3290 (NH) cm^{-1}.

^{1}H KMR (400 MHz, $CDCl_3$, δ): 1,08 un 3,98 (5H, t un kv, J = 16.1 Hz, 3-$COOCH_2CH_3$); 2,37 (3H, s, 2-CH_3); 2,42 (3H, s, 6-SCH_3); 5,30 (1H, s, 4-H); 6,40 (1H, s, NH); 7,0- 7,4 (4H, m, C_6H_4Cl) m.d.

Aprēķināts: $C_{19}H_{19}ClN_2O_4S$: C 56,09%; H 4,71%; N 6,88%; S 7,88%. Noteikts: C 56,00%; H 4,69%; N 6,89%; S 7,80%.

5-Ciāno-4-(2-hlorfenil)-2-metil-6-(2-metoksikarboniletilsulfanil)-1,4-dihidropiridīn-3-karbonskābes metilesteris (57b)

0,96 g (3 mmol) 5-ciāno-4-(2-hlorfenil)-2-metil-6-tiokso-1,2,3,4-dihidropiridīn-3-karbonskābes metilestera **58** izšķīdina 20 ml metanola, pievieno 0,33 ml (3,3 mmol) piperidīna. Reakcijas maisījumu maisa istabas temperatūrā 5 min. Iegūtajam šķīdumam pievieno 0,40 ml (3,3 mmol) metil-3-brompropionāta un to vāra 2 st. Nogulsnes filtrē, uz filtra tās mazgā ar 10 ml auksta metanola, 10 ml dest. ūdens. Iegūst 1,05 g (86%) bezkrāsainas kristāliskas vielas **57b** ar k.t. 79 – 80 ^{o}C.

IS (nujolā, ν): 1716 (C=O); 2206 (C≡N); 3359 (NH) cm^{-1}.

^{1}H KMR (δ, m.d., $CDCl_3$): 2,45 (3H, s, 2-CH_3); 2,67-2,75 (2H, m, $SCH_2\underline{CH_2}$); 2,96-3,25 (2H, m, $S\underline{CH_2}CH_2$); 3,56 (3H, s, 3-$COOCH_3$); 3,79 (3H, s, 6-$SCH_2CH_2COO\underline{CH_3}$); 5,29 (1H, s, 4-H); 7,10–7,35 (4H, m, C_6H_4Cl); 8,15 (1H, s, NH).

Aprēķināts: $C_{19}H_{19}ClN_2O_4S$: C 56,09%; H 4,71%; N 6,88%; S 7,88%. Noteikts: C 56,02%; H 4,74%; N 6,92%; S 7,80%.

5-Ciāno-4-(2-hlorfenil)-2-metil-6-(3-etoksikarbonilpropilsulfanil)-1,4-dihidropiridīn-3-karbonskābes metilesteris (57c)

0,96 g (3 mmol) 5-ciāno-4-(2-hlorfenil)-2-metil-6-tiokso-1,2,3,4-dihidropiridīn-3-karbonskābes metilestera **58** izšķīdina 20 ml metanola, pievieno 0,33 ml (3,3 mmol) piperidīna. Reakcijas maisījumu maisa istabas temperatūrā 5 min. Iegūtajam šķīdumam pievieno 0,34 ml (3,3 mmol) etil-4-brombutirāta un to vāra 2 st. Iegūst 1,13 g (86%) eļļainas vielas **57c**.

^{1}H KMR (δ, m.d., $CDCl_3$): 1,25 un 4,15 (5H, t un kv, J = 7.0 Hz, 6-C_2H_5); 1,90-1,97 (2H, m, $SCH_2\underline{CH_2}CH_2$); 2,42 (3H, s, 2-$CH_3$); 2,45 (2H, t, $SCH_2CH_2\underline{CH_2}$); 2,82-3,01 (2H, m, $S\underline{CH_2}CH_2CH_2$); 3,54 (3H, s, 3-$OCH_3$); 5,28 (1H, s, 4-H); 6,95 (1H, s, NH), 7,11–7,34 (4H, m, C_6H_4Cl).

ŠH/MS (m/z, %): 321,01 (65); 365,66 (6); 401,87 (5); 433,25 (100).

5-Ciāno-4-(2-hlorfenil)-2-metil-6-(4-etoksikarbonilbutilsulfanil)-1,4-dihidropiridīn-3-karbonskābes metilesteris (57d)

0,96 g (3 mmol) 5-ciāno-4-(2-hlorfenil)-2-metil-6-tiokso-1,2,3,4-dihidropiridīn-3-karbonskābes metilestera **58** izšķīdina 20 ml metanola, pievieno 0,33 ml (3,3 mmol) piperidīna. Reakcijas maisījumu maisa istabas temperatūrā 5 min. Iegūtajam šķīdumam pievieno 0,40 ml (3,3 mmol) etil-5-bromvalerāta un to vāra 2 st. Nogulsnes filtrē, uz filtra tās mazgā ar 10 ml auksta metanola, 10 ml dest. ūdens. Iegūst 0,96 g (71%) bezkrāsainas kristāliskas vielas **57d** ar k.t. 73 – 74 ^{o}C.

IS (nujolā, ν): 1595, 1636, 1707, 1733 (CO); 2204 (CN); 3259 (NH) cm^{-1}.

^{1}H KMR (δ, m.d., $CDCl_3$): 1,24 un 4,13 (5H, t un kv, J = 7.0 Hz, 6- $SC_4H_8COO\underline{C_2H_5}$); 1,56-1,80 (4H, m, $SCH_2\underline{CH_2}\underline{CH_2}CH_2$); 2,30 (2H, t, 6-$SCH_2CH_2CH_2\underline{CH_2}COOC_2H_5$); 2,39 (3H, s, 2-$CH_3$); 2,73-3,01 (2H, m, $S\underline{CH_2}CH_2CH_2CH_2$); 3,55 (3H, s, 3-$COOCH_3$); 5,29 (1H, s, 4-H); 6,48 (1H, s, NH), 7,09–7,35 (4H, m, C_6H_4Cl).

Aprēķināts: $C_{22}H_{25}ClN_2O_4S$: C 58,86%; H 5,61%; N 6,24%. Noteikts: C 58,92%; H 5,54%; N 6,19.

5-Ciāno-4-(2-hlorfenil)-2-metil-6-(5-etoksikarbonilpentilsulfanil)-1,4-dihidropiridīn-3-karbonskābes metilesteris (57e)

0,96 g (3 mmol) 5-ciāno-4-(2-hlorfenil)-2-metil-6-tiokso-1,2,3,4-dihidropiridīn-3-karbonskābes metilestera **58** izšķīdina 20 ml metanola, pievieno 0,33 ml (3,3 mmol) piperidīna. Reakcijas maisījumu maisa istabas temperatūrā 5 min. Iegūtajam šķīdumam pievieno 0,54 ml (3,3 mmol) etil-6-bromheksāndionāta un to vāra 2 st. Nogulsnes filtrē, uz filtra tās mazgā ar 10 ml auksta metanola, 10 ml dest. ūdens. Iegūst 0,98 g (73%) bezkrāsainas kristāliskas vielas **57e** ar k.t. 94 – 95 ^{o}C.

IS (nujolā, ν): 1594, 1635, 1706, 1733 (CO); 2201 (CN); 3261 (NH) cm^{-1}.

^{1}H-KMR (δ, m.d., $CDCl_3$): 1,21 un 4,08 (5H, t un kv, *J* = 7.0 Hz, 6-$SC_5H_{10}COO\underline{C_2}\underline{H_5}$); 1,34-1,57 (6H, m, 6-$SCH_2\underline{CH_2}\underline{CH_2}\underline{CH_2}CH_2$), 2,23 (2H, t 6- $SCH_2CH_2CH_2CH_2\underline{CH_2}$), 2,34 (3H, s, 2-$CH_3$); 2,72-2,94 (2H, m, $S\underline{CH_2}CH_2CH_2CH_2CH_2$); 3,50 (3H, s, 3-$COOCH_3$); 5,25 (1H, s, 4-H); 6,29 (1H, s, NH), 7,07–7,30 (4H, m, C_6H_4Cl).

Aprēķināts: $C_{23}H_{27}ClN_2O_4S$: C 59,67%; H 5,88%; N 6,05%. Noteikts: C 59,73%; H 5,74%; N 6,02%.

5-Ciāno-4-(2-hlorfenil)-6-karboksimetilsulfanil-2-metil-1,4-dihidropiridīn-3-karbonskābes metilesteris (59a)

0,32 g (1 mmol) 5-ciāno-4-(2-hlorfenil)-2-metil-6-tiokso-1,2,3,4-dihidropiridīn-3-karbonskābes metilestera **58** šķīdina 5 ml metanola, pievieno 0,28 g (2 mmol) kālija karbonāta un silda 2 min 40-50 ^{o}C. Siltam reakcijas maisījumam pievieno 0,17 g (1,2 mmol) brometiķskābi un turpina sildīt 2 min. Tad reakcijas maisījumu maisa istabas temperatūrā 15 min. Filtrējot atdala kālija bromīdu un filtrātu neitralizē ar 2M HCl/H_2O līdz pH = 6. Nogulsnes (KCl) atdala filtrējot, filtrātam pievieno 10 ml H_2O un neitralizē ar 2M HCl/H_2O līdz pH = 2. Pievieno 10 ml metanola. Nogulsnes atdala filtrējot, uz filtra produktu mazgā ar aukstu metanolu un ūdeni. Iegūst 0,33 g (87%) savienojuma **59a** ar k.t. 165 - 167 ^{o}C.

IS (nujolā, ν): 1650, 1715 (C=O); 2200 (C≡N); 3195, 3260, 3450 (NH, OH) cm^{-1}.

^{1}H KMR (δ, m.d., DMSO-d_6): 2,30 (3H, s, 2-Me); 3,44 (3H, s, 3-$COOCH_3$); 3,84 (2H, ABkv, 6-$S\underline{CH_2}COOH$); 5,07 (1H, s, 4-H); 7,17-7,39 (4H, m, C_6H_4Cl); 9,59 (1H, s, NH).

Aprēķināts $C_{17}H_{15}ClN_2O_4S$: C 53,90; H 3,99; N 7,39. Atrasts: C 52,30; H 3,70; N 7,02.

5-Ciāno-4-(2-hlorfenil)-6-(2-karboksietilsulfanil)-2-metil-1,4-dihidropiridīn-3-karbonskābes metilesteris (59b)

0,32 g (1 mmol) 5-ciāno-4-(2-hlorfenil)-2-metil-6-tiokso-1,2,3,4-dihidropiridīn-3-karbonskābes metilestera **58** šķīdina 5 ml metanola, pievieno 0,28 g (2 mmol) kālija karbonāta un silda 2 min 40-50 ^{o}C. Siltam reakcijas maisījumam pievieno 0,18 g (1,2 mmol) 3-brompropionskābi un turpina sildīt 2 min. Tad reakcijas maisījumu maisa istabas temperatūrā 15 min. Filtrējot atdala kālija bromīdu un filtrātu neitralizē ar 2M HCl/H_2O līdz pH = 6. Nogulsnes (KCl) atdala filtrējot, filtrātam pievieno 10 ml H_2O un neitralizē ar 2M HCl/H_2O līdz pH = 2. Pievieno 10 ml metanola. Nogulsnes atdala filtrējot, uz filtra produktu mazgā ar aukstu metanolu un ūdeni. Iegūst 0,31 g (80%) savienojuma **59b** ar k.t. 177 – 180 ^{o}C.

IS (nujolā, ν): 1685, 1715 (C=O); 2216 (C≡N); 3175, 3225 (NH, OH) cm^{-1}.

^{1}H KMR (δ, m.d., DMSO-d_6): 2,30 (3H, s, 2-Me); 2,51-2,44 (2H, m, 6-$SCH_2\underline{CH_2}COOH$); 2,98-3,18 (2H, m, 6-$S\underline{CH_2}CH_2COOH$); 3,44 (3H, s, 3-$COOCH_3$); 5,07 (1H, s, 4-H); 7,17-7,39 (4H, m, C_6H_4Cl); 9,59 (1H, s, NH).

Aprēķināts $C_{18}H_{17}ClN_2O_4S$: C 55,03; H 4,36; N 7,13. Atrasts: C 54,48; H 4,32; N 6,90.

5-Ciāno-4-(2-hlorfenil)-6-(3-karboksipropilsulfanil)-2-metil-1,4-dihidropiridīn-3-karbonskābes metilesteris (59c)

0,32 g (1,0 mmol) 5-ciāno-4-(2-hlorfenil)-2-metil-6-tiokso-1,2,3,4-dihidropiridīn-3-karbonskābes metilestera **58** šķīdina 5 ml metanola, pievieno 0,28 g (2,0 mmol) kālija karbonāta un silda 2 min 40-50 ^{o}C. Siltam reakcijas maisījumam pievieno 0,20 g (1,2 mmol) 4-brombutānskābi un turpina sildīt 2 min. Tad reakcijas maisījumu maisa istabas temperatūrā 15 min. Filtrējot atdala kālija bromīdu un filtrātu neitralizē ar 2M HCl/H_2O līdz pH = 6. Nogulsnes (KCl) atdala filtrējot, filtrātam pievieno 10 ml H_2O un neitralizē ar 2M HCl/H_2O līdz pH = 2. Vielu ekstraģē no ūdens ar etilacetātu. Etilacetāta frakciju ietvaicē vakuumrotācijas iekārtā un iegūst 0,33 g (81%) eļļainas vielas **59c.**

^{1}H KMR (δ, m.d., DMSO-d_6): 1.92-1.99 (2H, t, 6-$SCH_2\underline{CH_2}CH_2COOH$); 2.23 (2H, t, 6-$SCH_2\underline{CH_2}CH_2COOH$); 2,30 (3H, s, 2-$CH_3$); 2,74-2,93 (2H, m, 6-$S\underline{CH_2}CH_2CH_2COOH$); 3,44 (3H, s, 3-$COOCH_3$); 5,07 (1H, s, 4-H); 7,17-7,39 (4H, m, C_6H_4Cl); 9,59 (1H, s, NH).

ŠH/MS (m/z, %): 270,96 (20); 302 (10); 323,00 (41); 429,65 (100).

5-Ciāno-4-(2-hlorfenil)-6-(4-karboksibutilsulfanil)-2-metil-1,4-dihidropiridīn-3-karbonskābes metilesteris (59d)

0,32 g (1,0 mmol) 5-ciāno-4-(2-hlorfenil)-2-metil-6-tiokso-1,2,3,4-dihidropiridīn-3-karbonskābes metilestera **58** šķīdina 5 ml metanola, pievieno 0,28 g (2,0 mmol) kālija karbonāta un silda 2 min 40-50 ^{o}C. Siltam reakcijas maisījumam pievieno 0,22 g (1,2 mmol) 5-brompentānskābi un turpina sildīt 2 min. Tad reakcijas maisījumu maisa istabas temperatūrā 15 min. Filtrējot atdala kālija bromīdu un filtrātu neitralizē ar 2M HCl/H_2O līdz pH = 6. Nogulsnes (KCl) atdala filtrējot, filtrātam pievieno 10 ml H_2O un neitralizē ar 2M HCl/H_2O līdz pH = 2. Vielu ekstraģē no ūdens ar etilacetātu. Etilacetāta frakciju ietvaicē vakuumrotācijas iekārtā un iegūst 0,36 g (86%) eļļainas vielas **59d.**

^{1}H KMR (δ, m.d., DMSO-d_6): 1,58-1,77 (4H, m, 6-$SCH_2\underline{CH_2}\underline{CH_2}CH_2COOH$); 2,33 (2H, t, 6-$SCH_2CH_2CH_2\underline{CH_2}COOH$); x2,37 (3H, s, 2-$CH_3$); 2,76-2,99 (2H, m, 6-$S\underline{CH_2}CH_2CH_2CH_2COOH$); 3,55 (3H, s, 3-$COOCH_3$); 5,29 (1H, s, 4-H); 6,22 (1H, s, NH); 7,17-7,39 (4H, m, C_6H_4Cl).

ŠH/MS (m/z, %): 209,06 (28); 291,10 (23); 419,22 (40); 443,21 (100).

5-Ciāno-4-(2-hlorfenil)-6-(5-karboksipentilsulfanil)-2-metil-1,4-dihidropiridīn-3-karbonskābes metilesteris (59e)

0,32 g (1,0 mmol) 5-ciāno-4-(2-hlorfenil)-2-metil-6-tiokso-1,2,3,4-dihidropiridīn-3-karbonskābes metilestera **58** šķīdina 5 ml metanola, pievieno 0,28 g (2,0 mmol) kālija karbonāta un silda 2 min 40-50 ^{o}C. Siltam reakcijas maisījumam pievieno 0,22 g (1,2 mmol) 6-bromheksānskābi un turpina sildīt 2 min. Tad reakcijas maisījumu maisa istabas temperatūrā 15 min. Filtrējot atdala kālija bromīdu un filtrātu neitralizē ar 2M HCl/H_2O līdz pH=6. Nogulsnes (KCl) atdala filtrējot, filtrātam pievieno 10 ml H_2O un neitralizē ar 2M HCl/H_2O līdz pH=2. Vielu ekstraģē no ūdens ar etilacetātu. Etilacetāta frakciju ietvaicē vakuumrotācijas iekārtā un iegūst 0,33 g (76%) eļļainas vielas **56e.**

^{1}H KMR (δ, m.d., DMSO-d_6): 1,39-1,60 (6H, m, 6-$SCH_2\underline{CH_2}\underline{CH_2}\underline{CH_2}CH_2COOH$); 2,37 (3H, s, 2-$CH_3$); 2,32 (2H, t, 6-$SCH_2CH_2CH_2\underline{CH_2}COOH$); 2,76-2,98 (2H, m, 6-$S\underline{CH_2}CH_2CH_2CH_2COOH$); 3,55 (3H, s, 3-$COOCH_3$); 5,29 (1H, s, 4-H); 6,22 (1H, s, NH); 7,12-7,34 (4H, m, C_6H_4Cl).

ŠH/MS (m/z, %): 207,86 (8); 259,36 (9); 319,10 (20); 433,21 (100).

4-Aril-5-ciāno-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes etoksikarbonilmetilestera (43a-c) enzimātiskā hidrolīze

Tika pārbaudītas lipāzes: *Amano Lipase G, A, M, AK, PS,* Novozym 435®, *Candida rugosa*.

0,01 g 4-aril-5-ciāno-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes etoksikarbonilmetilestera un 0,01 g enzīma šķīdina 3 ml acetonitrilā un 17 ml 20 mM kālija fosfāta buferšķīdumā (pH = 7,5). Reakcija noris 45 ^{o}C temperatūrā orbitālajā kratītājā (ātrums ir 200 rpm) ar termostatu. Reakcija tiek kontrolēta ar AEŠH metodi, izmantojot apgrieztās fāzes kolonnas: Altima C18, mobīlā fāze acetonitils/H_2O+0,01% ledus etiķskābes attiecībā 50/50 un hirālo kolonnu (R,R)-Whelk O1, kur kā stacionārā fāze izmantota ar silikagelu saistīts 1-(3,5-dinitrobenzamido)-1,2,3,4-tetrahidrofenantrēns un mobīlā fāze heksāns/CH_2Cl_2/izopropanols+0,01% ledus etiķskābes attiecībā 80/10/10.

5-Ciāno-4-(2-hlorfenil)-2-metil-6-alkilsulfanil-1,4-dihidropiridīn-3-karbonskābes metilesteru (57a-e) enzimātiskā hidrolīze

Tika pārbaudīti šādi enzīmi: *Candida cyclindracea, Pencillium camemberti, Candida rugosa type VII, Amano acylase,* Amano lipase PS, M, G.

0,01 g 5-ciāno-4-(2-hlorfenil)-2-metil-6-alkilsulfanil-1,4-dihidropiridīn-3-karbonskābes metilestera un 0,008 g enzīma šķīdina 1 ml dihlormetānā un 20 ml vai 30 ml ar ūdeni piesātinātā IPE. Reakcija noris 25 ^{o}C vai 45 ^{o}C temperatūrā orbitālajā kratītājā (ātrums ir 200 rpm) ar termostatu. Reakcija tiek kontrolēta ar AEŠH metodi, izmantojot hirālo kolonnu (R,R)-Whelk O1, kur kā stacionārā fāze izmantota ar silikagelu saistīts 1-(3,5-dinitrobenzamido)-1,2,3,4-tetrahidrofenantrēns un mobīlā fāze heksāns/CH_2Cl_2/izopropanols+0,05 M amonija acetāts attiecībā 60/35/5.

(-)-5-Ciāno-4-(2-hlorfenil)-2-metil-6-(2-metoksikarboniletilsulfanil)-1,4-dihidropiridīn-3-karbonskābes metilesteris ((-)-61b)

2,00 g 5-ciāno-4-(2-hlorfenil)-2-metil-6-(2-metoksikarboniletilsulfanil)-1,4-dihidropiridīn-3-karbonskābes metilestera **57b** un 1,50 g enzīma Novozym 435® šķīdina 20 ml dihlormetānā un 600 ml ar ūdeni piesātinātā IPE. Reakcija noris 45 ^{o}C temperatūrā orbitālajā kratītājā (ātrums ir 200 rpm) ar termostatu. Pēc 19 stundām nofiltrē enzīmu un reakcijas maisījumu ierotē. Produktu attīra ar kolonnas hromatogrāfiju uz silikagela eluentu sistēmā CH_2Cl_2/etilacetāts. Iegūst 0,95 g (48%) eļļainas vielas (-)-**61b**, ee = 92%, [α]=-134,92 (c=1, MeOH).

IS (nujolā, ν): 1716 (C=O); 2206 (C≡N); 3359 (NH) cm^{-1}.

^{1}H KMR (δ, m.d., $CDCl_3$): 2,45 (3H, s, 2-CH_3); 2,67-2,75 (2H, m, $SCH_2\underline{CH_2}$); 2,96-3,25 (2H, m, $S\underline{CH_2}CH_2$); 3,56 (3H, s, 3-$COOCH_3$); 3,79 (3H, s, 6-$SCH_2CH_2COO\underline{CH_3}$); 5,29 (1H, s, 4-H); 7,10–7,35 (4H, m, C_6H_4Cl); 8,15 (1H, s, NH).

ŠH/MS (m/z, %): 295 (90); 321 (50); 377 (60); 407 (100).

(+)-5-Ciāno-4-(2-hlorfenil)-2-metil-6-(2-karboksietilsulfanil)-1,4-dihidropiridīn-3-karbonskābes metilesteris ((+)-60b)

2,00 g 5-ciāno-4-(2-hlorfenil)-2-metil-6-(2-metoksikarboniletilsulfanil)-1,4-dihidropiridīn-3-karbonskābes metilestera **57b** un 1,5 g enzīma Novozym 435® šķīdina 20 ml dihlormetānā un 600 ml ar ūdeni piesātinātā IPE. Reakcija noris 45 ^oC temperatūrā orbitālajā kratītājā (ātrums ir 200 rpm) ar termostatu. Pēc 19 stundām nofiltrē enzīmu un reakcijas maisījumu ierotē. Produktu sakristalizē ar 5 ml dihlormetāna. Iegūst 0,90 g (45%) bezkrāsainas kristāliskas vielas (+)-**60b** ar k.t. 81 – 83 ^oC, [α]=+171,90 (c=1, MeOH).

IS (nujolā, ν): 1685, 1715 (C=O); 2216 (C≡N); 3175, 3225 (NH, OH) cm^{-1}.

^{1}H KMR (δ, m.d., DMSO-d_6): 2,30 (3H, s, 2-Me); 2,51-2,44 (2H, m, 6-$SCH_2\underline{CH_2}COOH$); 2,98-3,18 (2H, m, 6-$S\underline{CH_2}CH_2COOH$); 3,44 (3H, s, 3-$COOCH_3$); 5,07 (1H, s, 4-H); 7,17-7,39 (4H, m, C_6H_4Cl); 9,59 (1H, s, NH).

Aprēķināts $C_{18}H_{17}ClN_2O_4S$: C 55,03; H 4,36; N 7,13. Atrasts: C 54,90; H 4,37; N 6,90.

(+)-5-Ciāno-4-(2-hlorfenil)-2-metil-6-(2-metoksikarboniletilsulfanil)-1,4-dihidropiridīn-3-karbonskābes metilesteris ((+)-61)

1,00 g (2,55 mmol) (+)-5-Ciāno-4-(2-hlorfenil)-2-metil-6-(2-karboksietilsulfanil)-1,4-dihidropiridīn-3-karbonskābes metilesteri (+)-**60b** šķīdina 10 ml dihlormetānā, pievieno 10 ml diazometāna ētera šķīdumu un maisa istabas temperatūrā 5 min. Reakcijas maisījumu ietvaicē vakuumrotācijas iekārtā un iegūst 0,90 g (87%) eļļainas vielas **(+)-61**, ee = 92%, [α]=+133,92 (c=1, MeOH).

IS (nujolā, ν): 1716 (C=O); 2206 (C≡N); 3359 (NH) cm^{-1}.

^{1}H KMR (δ, m.d., $CDCl_3$): 2,45 (3H, s, 2-CH_3); 2,67-2,75 (2H, m, $SCH_2\underline{CH_2}$); 2,96-3,25 (2H, m, $S\underline{CH_2}CH_2$); 3,56 (3H, s, 3-$COOCH_3$); 3,79 (3H, s, 6-$SCH_2CH_2COO\underline{CH_3}$); 5,29 (1H, s, 4-H); 7,10–7,35 (4H, m, C_6H_4Cl); 8,15 (1H, s, NH).

ŠH/MS (m/z, %): 295 (90); 321 (50); 377 (60); 407 (100).

Aprēķināts: $C_{19}H_{19}ClN_2O_4S$: C 56,09%; H 4,71%; N 6,88%; S 7,88%. Noteikts: C 55,98%; H 4,77%; N 6,90%; S 7,84%.

(-)-5-Ciāno-4-(2-hlorfenil)-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes metilesteris ((-)-62)

1,00 g (2,5 mmol) (-)-5-Ciāno-4-(2-hlorfenil)-2-metil-6-(2-metoksikarboniletilsulfanil)-1,4-dihidropiridīn-3-karbonskābes metilesteris **(-)-61** šķīdina 15 ml metanolā, pievieno 0,85 ml 3N $NaOH/H_2O$ un maisa istabas temperatūrā 5 min. Tad pievieno 1,59 ml metiljodīda un maisa 30 ^oC temperatūrā. Reakcijas maisījumu ietvaicē vakuumrotācijas iekārtā un sakristalizē ar 40 ml ūdens. Iegūst 0,77 g (94%) bezkrāsainas kristaliskas vielas **(-)-62**, ar k.t. 70 - 74 ^oC, ee = 93%.

IS (nujolā, ν): 1695 (C=O); 2200 (C≡N); 3330 (NH) cm^{-1}.

^1H KMR (δ, m.d., $CDCl_3$): 2,32 (3H, s, SCH_3); 2,41 (3H, s, 2-CH_3); 3,50 (3H, s, 3-$COOCH_3$); 5,29 (1H, s, 4-H); 7,10–7,35 (4H, m, C_6H_4Cl); 8,15 (1H, s, NH).

ŠH/MS (m/z, %): 223 (50); 303 (70); 334 (80); 357 (100).

(+)-5-Ciāno-4-(2-hlorfenil)-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes metilesteris ((+)-62)

1,00 g (2,5 mmol) (+)-5-Ciāno-4-(2-hlorfenil)-2-metil-6-(2-metoksikarboniletilsulfanil)-1,4-dihidropiridīn-3-karbonskābes metilesteris **(+)-61** šķīdina 15 ml metanolā, pievieno 0,85 ml 3N $NaOH/H_2O$ un maisa istabas temperatūrā 5 min. Tad pievieno 1,59 ml metiljodīda un maisa 30 ^oC temperatūrā. Reakcijas maisījumu ietvaicē vakuumrotācijas iekārtā un kristalizē no 40 ml ūdens. Iegūst 0,80 g (94%) bezkrāsainas kristaliskas vielas **(+)-62**, ar k.t. 72 - 76 ^oC, ee = 92%.

IS (nujolā, ν): 1695 (C=O); 2200 (C≡N); 3330 (NH) cm^{-1}.

^1H KMR (δ, m.d., $CDCl_3$): 2,32 (3H, s, SCH_3); 2,41 (3H, s, 2-CH_3); 3,50 (3H, s, 3-$COOCH_3$); 5,29 (1H, s, 4-H); 7,10–7,35 (4H, m, C_6H_4Cl); 8,15 (1H, s, NH).

ŠH/MS (m/z, %): 223 (50); 303 (70); 334 (80); 357 (100).

5-Ciāno-4-fenil-2-metil-6-propioniloksimetilsulfanil-1,4-dihidropiridīn-3-karbonskābes metilesteris (63a)

0,95 g (3,3 mmol) 5-ciāno-4-fenil-2-metil-6-tiokso-1,2,3,4-tetrahidropiridīn-3-karbonskābes metilestera **58a** izšķīdina 20 ml metanola, pievieno 0,33 ml (3,3 mmol) piperidīna un 5 min maisa istabas temperatūrā. Iegūtajam šķīdumam pievieno 0,41 ml (3,3 mmol) propionskābes hlormetilestera, vāra 30 sekundes un turpina maisīt 1 st. istabas temperatūrā. Nogulsnes filtrē, uz filtra tās mazgā ar 5 ml auksta metanola un 10 ml dest. ūdens. Iegūst 0,91 g (84%) bezkrāsainas kristāliskas vielas **63a** ar k.t. 143-144 ^oC.

IS (nujolā, ν): 1698, 1701 (C=O); 2198 (C≡N); 3288 (NH) cm^{-1}.

^{1}H KMR (δ, m.d., $CDCl_3$): 1,15 un 2,37 (5H, t un kv, *J*=7,0 Hz, 6-OCO$\underline{CH_2CH_3}$); 2,39 (3H, s, 6-CH_3); 3,50 (3H, s, 3-$COOCH_3$); 4,61 (1H, s, 4-H); 5.29 (2H, ABkv, *J*=11.7 Hz, 6-SCH_2); 7,06-7,21 (5H, m, C_6H_5); 7,27 (1H, s, NH).

Aprēķināts: $C_{19}H_{20}N_2O_4S$: C 61,27%; H 5,41%; N 7,52%; S 8,61%. Atrasts: C 61,20%; H 5,22%; N 7,67%; S 8,69%.

5-Ciāno-4-fenil-2-metil-6-izobutiriloksimetilsulfanil-1,4-dihidropiridīn-3-karbonskābes metilesteris (63b)

0,95 g (3,3 mmol) 5-ciāno-4-fenil-2-metil-6-tiokso-1,2,3,4-tetrahidropiridīn-3-karbonskābes metilestera **58a** izšķīdina 20 ml metanola, pievieno 0,33 ml (3,3 mmol) piperidīna un maisa istabas temperatūrā 5 min. Iegūtajam šķīdumam pievieno 0,42 ml (3,3 mmol) izobutānskābes hlormetilestera, vāra 30 sekundes un turpina 1 st. maisīt istabas temperatūrā. Nogulsnes filtrē, uz filtra tās mazgā ar 5 ml auksta metanola un 10 ml dest. ūdens. Iegūst 1,02 g (80%) bezkrāsainas kristāliskas vielas **63b** ar k.t. 138 – 139 ^{o}C.

IS (nujolā, ν): 1704, 1712 (C=O); 2198 (C≡N); 3286 (NH) cm^{-1}.

^{1}H KMR (δ, m.d., $CDCl_3$): 1,15 (6H, d, 6-OCOCH($\underline{CH_3}$)$_2$); 2,39 (3H, s, 6-CH_3); 2,53-2,62 (1H, m, 6-OCO$\underline{CH}$(CH_3)$_2$); 3,50 (3H, s, 3-$COOCH_3$); 4,61 (1H, s, 4-H); 5.29 (2H, ABkv, *J*=11.7 Hz, 6-SCH_2); 7,06-7,21 (5H, m, C_6H_5); 7,27 (1H, s, NH).

Aprēķināts: $C_{20}H_{22}N_2O_4S$: C 62,16%; H 5,74%; N 7,25%; S 8,30%. Atrasts: C 62,00%; H 5,79%; N 7,35%; S 8,46%.

5-Ciāno-6-(2,2-dimetilpropioniloksimetilsulfanil-4-fenil-2-metil-1,4-dihidropiridīn-3-karbonskābes metilesteris (63c)

0,95 g (3,3 mmol) 5-ciāno-4-fenil-2-metil-6-tiokso-1,2,3,4-tetrahidropiridīn-3-karbonskābes metilestera **58a** izšķīdina 20 ml metanola, pievieno 0,33 ml (3,3 mmol) piperidīna un maisa istabas temperatūrā 5 min. Iegūtajam šķīdumam pievieno 0,43 ml (3,3 mmol) hlormetilestera pivalātu, vāra 30 sekundes un turpina maisīt 1 st. istabas temperatūrā. Nogulsnes filtrē, uz filtra tās mazgā ar 5 ml auksta metanola un 10 ml dest. ūdens. Iegūst 1,06 g (80%) bezkrāsainas kristāliskas vielas **63c** ar k.t. 125 – 126 ^{o}C.

IS (nujolā, ν): 1702, 1710 (C=O); 2198 (C≡N); 3290 (NH) cm^{-1}.

^{1}H KMR (δ, m.d., $CDCl_3$): 1,18 (9H, s, 6-OCOC($\underline{CH_3}$)$_3$); 2,39 (3H, s, 6-CH_3); 3,54 (3H, s, 3-$COOCH_3$); 4,63 (1H, s, 4-H); 5.29 (2H, s, 6-SCH_2); 7,14-7,26 (5H, m, C_6H_5); 7,71 (1H, s, NH).

Aprēķināts: $C_{21}H_{24}N_2O_4S$: C 62,98%; H 6,04%; N 6,99%; S 8,01%. Atrasts: C 62,84%; H 6,18%; N 7,23%; S 7,85%.

5-Ciāno-4-(2-hlorfenil)-2-metil-6-propioniloksimetilsulfanil-1,4-dihidropiridīn-3-karbonskābes metilesteris (63d)

1,06 g (3,3 mmol) 5-ciāno-4-(2-hlorfenil)-2-metil-6-tiokso-1,2,3,4-tetrahidropiridīn-3-karbonskābes metilestera **58d** izšķīdina 20 ml metanola, pievieno 0,33 ml (3,3 mmol) piperidīna un maisa istabas temperatūrā 5 min. Iegūtajam šķīdumam pievieno 0,41 ml (3,3 mmol) propionskābes hlormetilestera, vāra 30 sekundes un turpina maisīt 1 st. istabas temperatūrā. Nogulsnes filtrē, uz filtra tās mazgā ar 5 ml auksta metanola un 10 ml dest. ūdens. Iegūst 0,88 g (74%) bezkrāsainas kristāliskas vielas **63d** ar k.t. 194 – 195 ^oC.

IS (nujolā, ν): 1688, 1700 (C=O); 2194 (C≡N); 3290 (NH) cm^{-1}.

^1H KMR (δ, m.d., $CDCl_3$): 1,13 un 2,38 (5H, t un kv, *J*=7,4 Hz, 6-OCO$\underline{CH_2}\underline{CH_3}$); 2,39 (3H, s, 6-$CH_3$); 3,50 (3H, s, 3-$COOCH_3$); 5,24 (1H, s, 4-H); 5.29 (2H, ABkv, *J*=11.7 Hz, 6-SCH_2); 7,07-7,29 (4H, m, C_6H_4Cl); 7,68 (1H, s, NH).

Aprēķināts: $C_{19}H_{19}ClN_2O_4S$: C 56,09%; H 4,71%; N 6,88%; S 7,88%. Atrasts: C 56,01%; H 4,67%; N 6,94%; S 7,76%.

5-Ciāno-4-(2-hlorfenil)-2-metil-6-izobutiriloksimetilsulfanil-1,4-dihidropiridīn-3-karbonskābes metilesteris (63e)

1,06 g (3,3 mmol) 5-ciāno-4-(2-hlorfenil)-2-metil-6-tiokso-1,2,3,4-tetrahidropiridīn-3-karbonskābes metilestera **58d** izšķīdina 20 ml metanola, pievieno 0,33 ml (3,3 mmol) piperidīna un maisa istabas temperatūrā 5 min. Iegūtajam šķīdumam pievieno 0,42 ml (3,3 mmol) izobutānskābes hlormetilestera, vāra 30 sekundes un turpina maisīt 1 st. istabas temperatūrā. Nogulsnes filtrē, uz filtra tās mazgā ar 5 ml auksta metanola un 10 ml dest. ūdens. Iegūst 0,82 g (65%) bezkrāsainas kristāliskas vielas **63e** ar k.t. 174 – 176 ^oC.

IS (nujolā, ν): 1687, 1701 (C=O); 2191 (C≡N); 3285 (NH) cm^{-1}.

^1H KMR (δ, m.d., $CDCl_3$): 1,15 (6H, d, 6-OCOCH($\underline{CH_3}$)$_2$); 2,39 (3H, s, 6-CH_3); 2,53-2,62 (1H, m, 6-OCO$\underline{CH}$(CH_3)$_2$); 3,50 (3H, s, 3-$COOCH_3$); 5,22 (1H, s, 4-H); 5,29 (2H, ABkv, *J*=11,7 Hz, 6-SCH_2); 7,06-7,21 (4H, m, C_6H_4Cl); 7,27 (1H, s, NH).

Aprēķināts: $C_{20}H_{21}ClN_2O_4S$: C 57,07%; H 5,03%; N 6,66%; S 7,62%. Atrasts: C 56,92%; H 5,21%; N 6,78%; S 7,53%.

5-Ciāno-4-(2-hlorfenil)-2-metil-6-(2,2-dimetilpropioniloksimetilsulfanil-1,4-dihidropiridīn-3-karbonskābes metilesteris (63f)

1,06 g (3,3 mmol) 5-ciāno-4-(2-hlorfenil)-2-metil-6-tiokso-1,2,3,4-tetrahidropiridīn-3-karbonskābes metilestera **58d** izšķīdina 20 ml metanola, pievieno 0,33 ml (3,3 mmol) piperidīna un maisa istabas temperatūrā 5 min. Iegūtajam šķīdumam pievieno 0,43 ml (3,3 mmol) hlormetilestera pivalātu, vāra 30 sekundes un turpina maisīt 1 st. istabas temperatūrā. Nogulsnes filtrē, uz filtra tās mazgā ar 5 ml auksta metanola un 10 ml dest. ūdens. Iegūst 1,06 g (81%) bezkrāsainas kristāliskas vielas **63f** ar k.t. 152 – 154 ^{o}C.

IS (nujolā, ν): 1710, 1720 (C=O); 2205 (C≡N); 3208 (NH) cm^{-1}.

^{1}H KMR (δ, m.d., $CDCl_3$): 1,19 (9H, s, 6-OCOC$(CH_3)_3$); 2,39 (3H, s, 6-CH_3); 3,50 (3H, s, 3-COOCH_3); 5,24 (1H, s, 4-H); 5,30 (2H, s, 6-SCH_2); 7,06-7,21 (4H, m, C_6H_4Cl); 7,27 (1H, s, NH).

Aprēķināts: $C_{21}H_{23}ClN_2O_4S$: C 57,99%; H 5,33%; N 6,44%; S 7,37%. Atrasts: C 8,23%; H 5,21%; N 6,56%; S 7,50%.

5-Ciāno-4-(2-difluorometoksifenil)-2-metil-6-propioniloksimetilsulfanil-1,4-dihidropiridīn-3-karbonskābes metilesteri (63g)

1,16 g (3,3 mmol) 5-ciāno-4-(2-difluorometoksifenil)-2-metil-6-tiokso-1,2,3,4-tetrahidropiridīn-3-karbonskābes metilestera **58g** izšķīdina 20 ml metanola, pievieno 0,33 ml (3,3 mmol) piperidīna un maisa istabas temperatūrā 5 min. Iegūtajam šķīdumam pievieno 0,41 ml (3,3 mmol) propionskābes hlormetilestera, vāra 30 sekundes un turpina maisīt 1 st. istabas temperatūrā. Nogulsnes filtrē, uz filtra tās mazgā ar 5 ml auksta metanola un 10 ml dest. ūdens. Iegūst 1,14g (87%) bezkrāsainas kristāliskas vielas **63g** ar k.t. 127 – 128 ^{o}C.

IS (nujolā, ν): 1693, 1706 (C=O); 2212 (C≡N); 3284 (NH) cm^{-1}.

^{1}H KMR (δ, m.d., $CDCl_3$): 1,13 un 2,37 (5H, t un kv, *J*=7,4 Hz, 6-OCO$\underline{CH_2}\underline{CH_3}$); 2,36 (3H, s, 6-$CH_3$); 3,50 (3H, s, 3-COO$CH_3$); 5,01 (1H, s, 4-H); 5,30 (2H, ABkv, *J*=11,7 Hz, 6-SCH_2); 6,39 un 6,57 (1H, d un d, *J*=73,2 Hz, $C_6H_4O\underline{CH}F_2$); 7,02-7,20 (4H, m, $\underline{C_6H_4}OCHF_2$); 7,64 (1H, s, NH).

Aprēķināts: $C_{20}H_{20}F_2N_2O_5S$: C 54,79%; H 4,60%; N 6,39%; S 7,31%. Atrasts: C 54,89%; H 4,25%; N 6,47%; S 7,22%.

5-Ciāno-4-(2-difluorometoksifenil)-2-metil-6-izobutiriloksimetilsulfanil-1,4-dihidropiridīn-3-karbonskābes metilesteris (63h)

1,16 g (3,3 mmol) 5-ciāno-4-(2-difluorometoksifenil)-2-metil-6-tiokso-1,2,3,4-tetrahidropiridīn-3-karbonskābes metilestera **58g** izšķīdina 20 ml metanola, pievieno 0,33 ml (3,3 mmol) piperidīna un maisa istabas temperatūrā 5 min. Iegūtajam šķīdumam pievieno 0,42 ml (3,3 mmol) izobutānskābes hlormetilestera, vāra 30 sekundes un turpina maisīt 1 st. istabas temperatūrā. Nogulsnes filtrē, uz filtra tās mazgā ar 5 ml auksta metanola un 10 ml dest. ūdens. Iegūst 1,15g (85%) bezkrāsainas kristāliskas vielas **63h** ar k.t. 113 – 114 ^{o}C.

IS (nujolā, ν): 1678, 1725 (C=O); 2209 (C≡N); 3254 (NH) cm^{-1}.

^{1}H KMR (δ, m.d., $CDCl_3$): 1,19 (6H, d, 6-OCOCH($\underline{CH_3}$)$_2$); 2,40 (3H, s, 6-CH_3); 2,57-2,68 (1H, m, 6-OCO$\underline{CH}$(CH_3)$_2$); 3,55 (3H, s, 3-$COOCH_3$); 5,06 (1H, s, 4-H); 5,34 (2H, ABkv, *J*=11,7 Hz, 6-SCH_2); 6,40 un 6,61 (1H, d un d, *J*=73,2 Hz, $C_6H_4O\underline{CH}F_2$); 7,06-7,21 (4H, m, $\underline{C_6H_4}OCHF_2$); 7,75 (1H, s, NH).

Aprēķināts: $C_{21}H_{22}F_2N_2O_5S$: C 55,74%; H 4,90%; N 6,19%; S 7,09%. Atrasts: C 55,70%; H 4,78%; N 6,37%; S 7,21%.

5-Ciāno-4-(2-difluorometoksifenil)-2-metil-6-(2,2-dimetilpropioniloksimetil-sulfanil-1,4-dihidropiridīn-3-karbonskābes metilesteris (63i)

1,16 g (3,3 mmol) 5-ciāno-4-(2-difluorometoksifenil)-2-metil-6-tiokso-1,2,3,4-tetrahidropiridīn-3-karbonskābes metilestera **58g** izšķīdina 20 ml metanola, pievieno 0,33 ml (3,3 mmol) piperidīna un maisa istabas temperatūrā 5 min. Iegūtajam šķīdumam pievieno 0,43 ml (3,3 mmol) hlormetilestera pivalātu, vāra 30 sekundes un turpina maisīt 1 st. istabas temperatūrā. Nogulsnes filtrē, uz filtra tās mazgā ar 5 ml auksta metanola un 10 ml dest. ūdens. Iegūst 1,13g (81%) bezkrāsainas kristāliskas vielas **63i** ar k.t. 58 – 62 ^{o}C.

IS (nujolā, ν): 1685, 1740 (C=O); 2212 (C≡N); 3419 (NH) cm^{-1}.

^{1}H KMR (δ, m.d., $CDCl_3$): 1,19 (9H, d, 6-OCOC($\underline{CH_3}$)$_3$); 2,36 (3H, s, 6-CH_3); 3,50 (3H, s, 3-$COOCH_3$); 5,01 (1H, s, 4-H); 5,30 (2H, s, 6-SCH_2); 6,39 un 6,57 (1H, d un d, *J*=73,2 Hz, $C_6H_4O\underline{CH}F_2$); 7,02-7,20 (4H, m, $\underline{C_6H_4}OCHF_2$); 7,70 (1H, s, NH).

Aprēķināts: $C_{22}H_{24}F_2N_2O_5S$: C 56,64%; H 5,19%; N 6,00%; S 6,87%. Atrasts: C 56,55%; H 5,32%; N 6,12%; S 6,88%.

6-alkilkarboksimetilsulfanil-5-ciāno-2-metil-1,4-dihidropiridīn-3-karbonskābes metilesteru 63 enzimātiskā hidrolīze acetonitrila/bufera šķīdinātāju sistēmā

Tika pārbaudītas lipāzes: *Candida antarctica* lipāze B (CAL-B, Novozym 435®), lipāzi PS (*Pseudomonas cepacia*), proteāze P6 (*Aspergillus melleus*), Acilāze 30 000 (*Aspergillus sp*.) un lipāze AH (*Pseudomonas sp*.).

0,025 mmol 6-alkilkarboksimetilsulfanil-5-ciāno-2-metil-1,4-dihidropiridīn-3-karbonskābes metilestera **63** šķīdina 15% acetonitrila buferšķīdumā (20 mM kālija fosfāta buferšķīdums, pH = 7,5) un pievieno 0,01 g enzīma. Šķīdinātāja sistēmu pievieno tik daudz, lai substrāta koncentrācija šķīdumā būtu konstanta - 0,01 mol/L. Reakcija noris 25 ^{o}C temperatūrā orbitālajā kratītājā (ātrums ir 200 rpm) ar termostatu. Reakcija tiek kontrolēta ar AEŠH metodi, izmantojot apgrieztās fāzes kolonnu Altima C18, mobilā fāze - acetonitils/H_2O+0,01% ledus etiķskābes attiecībā 50:50 un hirālo kolonnu Lux Cellulose-2 mobilā fāze - izopropilspirts/heksāns, 1:1.

6-alkilkarboksimetilsulfanil-5-ciāno-2-metil-1,4-dihidropiridīn-3-karbonskābes metilesteru 63 enzimātiskā hidrolīze DMSO/IPE/H_2O šķīdinātāju sistēmā

Tika pārbaudītas lipāzes: *Candida antarctica* lipāze B (CAL-B, Novozym 435®), lipāzi PS (*Pseudomonas cepacia*), proteāze P6 (*Aspergillus melleus*), Acilāze 30 000 (*Aspergillus sp*.) un lipāze AH (*Pseudomonas sp*.).

0,025 mmol 6-alkilkarboksimetilsulfanil-5-ciāno-2-metil-1,4-dihidropiridīn-3-karbonskābes metilestera **63** šķīdina 20% dimetilsulfoksīda ar ūdeni piesātinātā diizopropilētera šķīdinātāju sistēmā un pievieno 0,01 g enzīma. Šķīdinātāja sistēmu pievieno tik daudz, lai substrāta koncentrācija šķīdumā būtu konstanta - 0,01 mol/L. Reakcija noris 25 vai 35, vai 45 ^{o}C temperatūrā orbitālajā kratītājā (ātrums ir 200 rpm) ar termostatu. Reakcija tiek kontrolēta ar AEŠH metodi, izmantojot apgrieztās fāzes kolonnu Altima C18, mobilā fāze - acetonitils/H_2O+0,01% ledus etiķskābes attiecībā 50:50 un hirālo kolonnu Lux Cellulose-2 mobilā fāze - izopropilspirts/heksāns, 1:1.

6-alkilkarboksimetilsulfanil-5-ciāno-2-metil-1,4-dihidropiridīn-3-karbonskābes metilesteru 63 enzimātiskā hidrolīze DCM/IPE/H_2O šķīdinātāju sistēmā

Tika pārbaudītas lipāzes: *Candida antarctica* lipāze B (CAL-B, Novozym 435®), lipāzi PS (*Pseudomonas cepacia*), proteāze P6 (*Aspergillus melleus*), Acilāze 30 000 (*Aspergillus sp*.) un lipāze AH (*Pseudomonas sp*.).

0,025 mmol 6-alkilkarboksimetilsulfanil-5-ciāno-2-metil-1,4-dihidropiridīn-3-karbonskābes metilestera **63** šķīdina dihlormetāna/diizopropilētera/ūdens šķīdinātāju sistēmā un pievieno 0,01 g enzīma. Šķīdinātāja sistēmu pievieno tik daudz, lai substrāta koncentrācija šķīdumā būtu konstanta - 0,01 mol/L. Reakcija noris 25 vai 35, vai 45 ^{o}C temperatūrā

orbitālajā kratītājā (ātrums ir 200 rpm) ar termostatu. Reakcija tiek kontrolēta ar AEŠH metodi, izmantojot apgrieztās fāzes kolonnu Altima C18, mobilā fāze - acetonitils/H_2O+0,01% ledus etiķskābes attiecībā 50:50 un hirālo kolonnu Lux Cellulose-2 mobilā fāze - izopropilspirts/heksāns, 1:1.

(+)-5-Ciāno-4-(2-hlorfenil)-2-metil-6-izobutiriloksimetilsulfanil-1,4-dihidropiridīn-3-karbonskābes metilesteris ((+)-63e)

2,00 g (4,75 mmol) 5-Ciāno-4-(2-hlorfenil)-2-metil-6-izobutiriloksimetilsulfanil-1,4-dihidropiridīn-3-karbonskābes metilestera **(±63e)** šķīdina 13,57 ml dihlormetānā un 461,38 ml ar ūdeni piesātinātā IPE un pievieno 1,90 g enzīma Novozym 435®. Reakcija noris 45 ^oC temperatūrā orbitālajā kratītājā (ātrums ir 200 rpm) ar termostatu. Pēc 36 stundām nofiltrē enzīmu un reakcijas maisījumu ierotē. Ķīmiski neizreaģējušo - enantiotransformējušo produktu attīra ar kolonnas hromatogrāfiju uz silikagela eluentu sistēmā CH_2Cl_2/etilacetāts (2:3). Iegūst 0,95 g (48%) eļļainas vielas **(+)-63e**, ep = 96%, [α]=+145,25 (c=1, MeOH).

^{1}H KMR (δ, m.d., $CDCl_3$): 1,15 (6H, d, 6-OCOCH(CH_3)$_2$); 2,39 (3H, s, 6-CH_3); 2,53-2,62 (1H, m, 6-OCOCH(CH_3)$_2$); 3,50 (3H, s, 3-COOCH_3); 5,22 (1H, s, 4-H); 5,29 (2H, ABkv, *J*=11,7 Hz, 6-SCH_2); 7,06-7,21 (4H, m, C_6H_4Cl); 7,27 (1H, s, NH).

Aprēķināts: $C_{20}H_{21}ClN_2O_4S$: C 57,07%; H 5,03%; N 6,66%; S 7,62%. Atrasts: C 57,21%; H 5,00%; N 6,52%; S 7,71%.

(-)-5-Ciāno-4-(2-hlorfenil)-2-metil-6-tiokso-1,2,3,4-tetrahidropiridīn-3-karbonskābes metilesteris ((-)-64)

2,00 g (4,75 mmol) 5-Ciāno-4-(2-hlorfenil)-2-metil-6-izobutiriloksimetilsulfanil-1,4-dihidropiridīn-3-karbonskābes metilestera **(±63e)** šķīdina 13,57 ml dihlormetānā un 461,38 ml ar ūdeni piesātinātā IPE un pievieno 1,90 g enzīma Novozym 435®. Reakcija noris 45 ^oC temperatūrā orbitālajā kratītājā (ātrums ir 200 rpm) ar termostatu. Pēc 36 stundām nofiltrē enzīmu un reakcijas maisījumu ierotē. Produktu attīra ar kolonnas hromatogrāfiju uz silikagela no sākuma eluentu sistēmā CH_2Cl_2/etilacetāts (2:3) nepolāros savienojumus, bet pēc tam tionu nomazgā ar MeOH. Iegūst 0,80 g (40%) eļļainas vielas **(-)-64e**.

^{1}H KMR (δ, m.d., $CDCl_3$): 2,50 un 2,60 (3H, s un s, cis- un trans-, 2-CH_3); 3,65 (3H, s, 3-COOCH_3); 4,90-5,20 (2H, m, 4-H un 5-H); 6,80-7,50 (4H, m, C_6H_4); 8,78 (1H, pl.s, NH).

(-)-5-Ciāno-4-(2-hlorfenil)-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes metilesteris (-)-66

1,00 g (1 mmol) tiona **(-)-64** šķīdina 15 ml metanolā, pievieno 0,1 ml piperidīna un 1,59 ml metiljodīda un maisa 30 ^{o}C temperatūrā. Reakcijas maisījumu ietvaicē vakuumrotācijas iekārtā un sakristalizē ar 40 ml ūdens. Iegūst 0,77 g (93%) bezkrāsainas kristaliskas vielas **(-)-66**, ar k.t. 70 - 74 ^{o}C, ep = 50%.

IS (nujolā, ν): 1695 (C=O); 2200 (C≡N); 3330 (NH) cm^{-1}.

^{1}H KMR (δ, m.d., $CDCl_3$): 2,32 (3H, s, SCH_3); 2,41 (3H, s, 2-CH_3); 3,50 (3H, s, 3-$COOCH_3$); 5,29 (1H, s, 4-H); 7,10–7,35 (4H, m, C_6H_4Cl); 8,15 (1H, s, NH).

ŠH/MS (m/z, %): 223 (50); 303 (70); 334 (80); 357 (100).

(+)-5-Ciāno-4-(2-hlorfenil)-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes metilesteris ((+)-66)

1,00 g (1 mmol) (+)-5-Ciāno-4-(2-hlorfenil)-2-metil-6-izobutiriloksimetilsulfanil-1,4-dihidropiridīn-3-karbonskābes metilestera **63e** šķīdina 15 ml metanolā, pievieno 0,34 ml 3N $NaOH/H_2O$ un maisa istabas temperatūrā 5 min. Tad pievieno 0.64 ml metiljodīda un maisa 30 ^{o}C temperatūrā. Reakcijas maisījumu ietvaicē vakuumrotācijas iekārtā un kristalizē no 40 ml ūdens. Iegūst 0,80 g (94%) bezkrāsainas kristaliskas vielas **(+)-66**, ar k.t. 72 - 76 ^{o}C, ep = 80%.

IS (nujolā, ν): 1695 (C=O); 2200 (C≡N); 3330 (NH) cm^{-1}.

^{1}H KMR (δ, m.d., $CDCl_3$): 2,32 (3H, s, SCH_3); 2,41 (3H, s, 2-CH_3); 3,50 (3H, s, 3-$COOCH_3$); 5,29 (1H, s, 4-H); 7,10–7,35 (4H, m, C_6H_4Cl); 8,15 (1H, s, NH).

ŠH/MS (m/z, %): 223 (50); 303 (70); 334 (80); 357 (100).

5-Ciāno-6-etilkarbamoilmetilsulfanil-4-fenil-2-metil-1,4-dihidropiridīn-3-karbonskābes metilesteris (70a)

0,37 g (1 mmol) 5-ciāno-6-etoksikarbonilmetilsulfanil-4-fenil-2-metil-1,4-dihidropiridīn-3-karbonskābes metilestera **57a** izšķīdina 10 ml etilamīnā un 3 st. maisa istabas temperatūrā. Nogulsnes filtrē, uz filtra tās mazgā ar 5 ml auksta etanola un 10 ml dest. ūdens. Iegūst 0.37g (95%) dzeltenīgas kristāliskas vielas **70a** ar k.t. 175-176 ^{o}C.

IS (nujolā, ν): 1686 (C=O); 2192 (C≡N); 3281, 3341 (NH) cm^{-1}.

^{1}H KMR (δ, m.d., $CDCl_3$): 0,98 un 3,06 (5H, t un kv., *J*=7,4 Hz CH_2CH_3); 2,28 (3H, s, 6-CH_3); 3,41 (3H, s, 3-$COOCH_3$); 3,67 (2H, ABkv, *J*=14,9 Hz, 6-SCH_2); 4,71 (1H, s, 4-H); 7,16-7,34 (5H, m, C_6H_5); 8,45 (1H, s, 1-NH); 10,45 (1H, s, 6-NH).

Aprēķināts: $C_{19}H_{21}N_3O_3S$: C 61,44%; H 5,70%; N 11,31%; S 8,63%. Atrasts: C 61,40%; H 5,52%; N 11,18%; S 8,87%.

5-Ciāno-6-etilkarbamoilmetilsulfanil-4-(2-hlorfenil)-2-metil-1,4-dihidropiridīn-3-karbonskābes metilesteris (70b)

0,37 g (1 mmol) 5-ciāno-6-etoksikarbonilmetilsulfanil-4-(2-hlorfenil)-2-metil-1,4-dihidropiridīn-3-karbonskābes metilestera **57b** izšķīdina 10 ml etilamīnā un 3 st. maisa istabas temperatūrā. Nogulsnes filtrē, uz filtra tās mazgā ar 5 ml auksta etanola un 10 ml dest. ūdens. Iegūst 0.37g (95%) bezkrāsainas kristāliskas vielas **70b** ar k.t. 196-197 ^oC.

IS (nujolā, ν): 1686 (C=O); 2188 (C≡N); 3142, 3259 (NH) cm^{-1}.

^1H KMR (δ, m.d., $CDCl_3$): 0,98 un 3,08 (5H, t un kv., *J*=7,4 Hz CH_2CH_3); 2,28 (3H, s, 6-CH_3); 3,40 (3H, s, 3-$COOCH_3$); 3,63 (2H, ABkv, *J*=14,9 Hz, 6-SCH_2); 5,04 (1H, s, 4-H); 7,16-7,35 (4H, m, C_6H_4); 8,45 (1H, s, 1-NH); 10,41 (1H, s, 6-NH).

Aprēķināts: $C_{19}H_{20}ClN_3O_3S$: C 56,22%; H 4,97%; N 10,35%; S 7,90%. Atrasts: C 56,14%; H 5,27%; N 10,47%; S 8,16%.

4-Aril-ciāno-6-alkilsulfanil-2-metil-1,4-dihidropiridīn-3-karbonskābes metilesteru enzimātiskā aminolīze

Tika pārbaudītas lipāzes: *Candida antarctica* lipāze B (CAL-B, Novozym 435®), Candida rugosa un Amano Acylase.

0,025 mmol 6-alkilkarboksimetilsulfanil-5-ciāno-2-metil-1,4-dihidropiridīn-3-karbonskābes metilestera **57** šķīdina 4 ml šķīdinātāju sistēmā un pievieno 0,01 g enzīma. Reakcija noris 25 vai 45 ^oC temperatūrā orbitālajā kratītājā (ātrums ir 200 rpm) ar termostatu. Reakcija tiek kontrolēta ar AEŠH metodi, izmantojot apgrieztās fāzes kolonnu Altima C18, mobilā fāze - acetonitils/H_2O+0,01% ledus etiķskābes attiecībā 50:50 un hirālo kolonnu Lux Cellulose-2 mobilā fāze - izopropilspirts/heksāns, 1:1.

Trietilamonija (5-ciāno-4-(2-hlorfenil)-2-metil-3-metoksikarbonil-1,4-dihidropiridīn-6-ilsulfanil)-acetāts (67)

4-(2-Hlorfenil)-6-(2-karboksimetilsulfanil)-1,4-dihidropiridīnu **60a** (0,19 g; 0,5 mmol) šķīdināja etanola-ūdens (3:1) maisījumā un pievienoja 0,07 ml (0,5 mmol) trietilamīna un maisīja istabas temperatūrā vienu stundu. Pēc tam pievienoja vēl 0,07 ml (0,5 mmol) trietilamīna (kopā 1 mmol) un maisīja 24 stundas. Maisījumu ietvaicēja vakuumā un kristālisko atlikumu pārkristalizēja no etanola. Ieguva 0,22 g (90%) bezkrāsainas kristāliskas vielas **67** ar k.t. 145-146 ^oC.

IS (nujolā, ν): 2192 (C≡N), 1676 (C=O) cm^{-1}.

^{1}H–KMR (400 MHz, DMSO-d_6, δ): 1.10-1.14 (t, 9H, HN($CH_2\underline{CH_3}$)), 2.27 (s, 3H, 2-Me), 2.95-3.07 (m, 6H, HN($\underline{CH_2}CH_3$)), 3.08-3.44 (ABkv, *J*=14.8 Hz, SCH_2COO^-), 3.40 (s, 3H, 3-COOMe), 5.02 (s, 1H, 4-H), 7.15-7.34 (m, 4H, C_6H_4) m.d.;

^{13}C–KMR (100 MHz, DMSO-d_6, δ): 9.2, 19.0, 38.6, 460, 51.2, 82.8, 99.5, 119.5, 128.5, 128.9, 129.5, 130.4, 131.0, 144.2, 147.9, 167.2, 172.1 m.d.

Aprēķināts: C, 57,67; H, 6,10; N, 8,77. $C_{23}H_{29}ClN_3O_4S$. Noteikts: C, 57,38; H, 6,16; N, 8,57.

SECINĀJUMI

1. Izstrādāta metode kardiovaskulāro aktivitāti uzrādošo 5-ciāno-4-aril-2-metil-6-metilsulfanil-1,4-dihidropiridīn-3-karbonskābes esteru iegūšanai individuālu enantiomēru (>95 % optiskā tīrība) veidā.
2. Atrastas principiāli jaunas aktivējošās grupas – merkapto-alkilkarbonskābes esteri, kas ļauj veikt farmaceitiski perspektīvo 1,4-dihidropiridīna atvasinājumu enantioselektīvu enzimātisko hidrolīzi. Stereoselektivitāte realizējas hirālajam centram atrodoties līdz pat 9 saišu attālumā no reakcijas centra, bet visaugstākais enantiomērais pārākums sasniegts, ja attālums ir 6 saites.
3. 4-(2-Hlorfenil)-1,4-dihidropiridīn-6-merkaptoetiķskābes enantiosadalīšana ar diastereomērās kristalizācijas metodi, pielietojot cinhonīnu kā sadalīšanas aģentu, norit ar zemu selektivitāti un prasa apstākļu optimizāciju.
4. 4-Aril-ciāno-6-alkilsulfanil-2-metil-1,4-dihidropiridīn-3-karbonskābes metilesteru enzimātiskajā aminolīzē, pielietojot lipāzi Novozym 435®, iegūti racemiski produkti.
5. Ar labiem iznākumiem iegūti S saturoši 1,4-DHP-3- un/vai 6- karbonskābju esteri kā substrāti enzimātiskai hidrolīzei. Šo savienojumu hidrolītiskās aktivitātes novērtējums rāda, ka no plašā pārbaudīto enzīmu klāsta visefektīvākās ir lipāzes Novozym 435® un *Amano acylase*.
6. 6-Metilsulfanil-1,4-dihidropiridīn-3-karbonskābes etoksikarbonilmetilesteru enzimātiskā hidrolīze (literatūrā aprakstītā sekmīga metode simetriskiem 1,4-DHP), pielietojot lipāzes Novozym 435® un *Candida rugosa* uzrādīja neapmierinošu enantioselektivitāti.
7. 1,4-dihidropiridīn-6-merkaptopropionskābes un 1,4-dihidropiridīn-6-merkaptometiloksikarbonskābes esteru enzimātiskā hidrolīze, pielietojot lipāzes Novozym 435® un *Candida rugosa*, norit ar teicamu enantioselektivitāti (ep. >95%).
8. Enantiobagātināto 1,4-dihidropiridīn-6-merkaptopropionskābes un 1,4-dihidropiridīn-6-merkaptometiloksikarbonskābes esteru deformilēšana (deakrilācija) un sekojošā alkilēšana dod mērķa produktus. Šīs metodes paver iespējas ērti iegūt plašu klāstu enantiobagātinātus sēru saturošus 1,4-DHP, jo satur enantiobagātinātus tiolātus, kas ir reaģētspējigi nukleofīli.

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Promocijas darbs „Optiski aktīvu 1,4-dihidropiridīn-6-sulfanilalkilkarbonskābju esteru sintēze un īpašības" izstrādāts LU Ķīmijas fakultātē.

Ar savu parakstu apliecinu, ka pētījums veikts patstāvīgi, izmantoti tikai tajā norādītie informācijas avoti un iesniegtā darba elektroniskā kopija atbilst izdrukai.

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Darbs iesniegts

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EFFECTIVE METHOD OF LIPASE-CATALYZED ENANTIORESOLUTION OF 6-ALKYLSULFANYL-1,4-DIHYDROPYRIDINES

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Abstract – A series of 6-alkylsulfanyl-1,4-dihydropyridines **(±)2** bearing a methoxycarbonylethyl group as a mild easily removable protecting group at S atom have been prepared by alkylation of 6-thioxo-1,4-dihydropyridines **1** with methyl bromopropionate. *Candida antarctica* lipase B (Novozym 435®, CAL-B) - and Amano Acylase (*Aspergillus mellus*)-catalyzed kinetic resolution has been investigated in water-saturated diisopropylether (IPE) at 25 and 45 °C. Further deacrylation and alkylation of enantioenriched 1,4-dihydropyridine-6-thiolates **(-)4** and **(+)4** gave rise to optically active 1,4-dihydropyridines **(-)2**, **(+)2**, **(-)5** and **(+)5** in 85-99% enantiomeric excess. The experiments present the 6-(methoxycarbonylethyl)sulfanyl group as an essentially new enzymatically labile (activating) group. The ester group being 6 bonds remote from the chiral center undergoes easy enzymatic hydrolysis and could be used for kinetic resolution of racemic 1,4-DHPs. This developed method offers access to mild optically active nucleophilic tiolates, which could be easily derivatized with electrophilic reagents.

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1. **INTRODUCTION**

1,4-Dihydropyridine (DHP) scaffold represents a heterocyclic unit of remarkable pharmacological efficiency.[1-4] DHP derivatives depending on their structure may bind to the L-, T- and N-types calcium, sodium, potassium or chloride channels and act as selective or multifunctional molecules for the various pharmacobiological activities such as bioprotective,[5] neurotropic,[6] membrane protecting,[7-9] radioprotecting,[10] antidiabetic,[11] gene-transfection,[12] and antibacterial.[13]

6-Alkylsulfanyl-1,4-DHPs display cardiovascular,[14-15] hepatoprotective,[16] antioxidant,[17] and antiradical[18] activities (in addition to the above mentioned activities), however, these compounds are still insufficiently studied.

Chirality plays an important role in the activity of 1,4-DHPs and both quantitative and qualitative differences between different stereoisomers (enantiomers) have been reported.[19,20] Pharmaceutical evaluations of chiral 1,4-DHPs revealed that their strereoisomers usually have different biological activities. Sometimes the undesired enantiomer caused serious side effects, while in other cases enantiomers were reported to have even the opposite action profile (calcium antagonist - calcium agonist; hypotensive activity - hypertensive activity).[21]

Chemoenzymatic methods for preparation of chiral drugs have a number of distinct advantages: they are simple, direct, efficient, mild, and cheap in case of multiple (repeated) use of the enzyme. The standard resolution technique, such as incorporation of an enzymatically labile group for the resolution of monocyclic 1,4-DHPs has been in use for the last decade. This approach has been pioneered by groups of Sih[22] and Achiwa,[23] applied to 6-derivatised 1,4-DHPs[24-26] and also used by our research group.[27-32]

It is worth mentioning that many activating groups applicable to enantioselective lipase-catalysed kinetic resolution of 1,4-dihydropyridine-3-carboxylates have been screened. An asymmetric 1,4-DHPs alkoxycarbonylmethoxycarbonyl (double esters),[27,28] alkylcarboxymethyloxycarbonyl (reverse esters) in position 5[25,30-33] and acetoxymethyl group in position 6[24,26,34,35] were the best characterised.

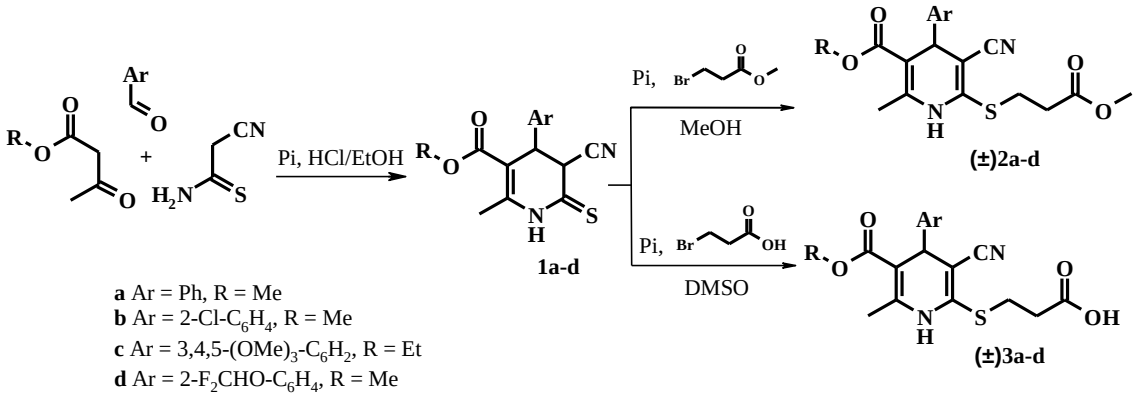
Esters are good substrates for the lipases and nowadays a lot of enzymes are commercially available. Lipases such as CAL-B and Amano acylase were evaluated for the resolution of esters and often possitive results are achieved.[33-37]

2. RESULTS AND DISCUSSION

The aim of our research was the synthesis of new 1,4-DHPs containing lipophilic methoxycarbonylethylsulfanyl group at position 6, which could act as mild and easily

removable protecting group at S atom. Though the ester group is 6 bonds remote from the chiral center, we expected that the enzymatic hydrolysis of this group could promote kinetic resolution of the target 1,4-DHPs. It is worth mentioning that our efforts to prepare optically active 6-alkylsulfanyl-1,4-DHPs by carrying out enzyme catalysed hydrolysis of so called activated double esters - ethoxycarbonylmethyl 6-methylsulfanyl-1,4-dihydropyridine-3-carboxylates were unsuccessful,[40] at the same time enantioresolution of 6-(methoxycarbonylmethyl)sulfanyl-1,4-dihydropyridines gave target products with up to 70% enantiomeric excess.[32]

The starting substrates 3-methoxycarbonyl-5-cyano-2-methyl-4-aryl-1,4-dihydropyridine-6-thiones **1** were prepared by a one-pot three-component condensation of alkyl acetoacetate, aromatic aldehyde and 2-cyanothioacetamide according to the synthesis protocol mentioned previously.[41]



Scheme 1

Alkylation of thiones **1** bearing several nucleophilic reaction centres (5-C, S, N) in basic (alkaline) medium under mild reaction conditions with methyl bromopropionate proceeds preferably at the sulphur atom giving rise to methyl 4-aryl-6-methoxycarbonylethylsulfanyl-1,4-dihydropyridine-3-carboxylates **(±)2** in 71-87% yields.

Carboxylic acids **(±)3**, as authentic samples for investigation of enzyme catalysed hydrolysis, were prepared by alkylation of thiones **1** with bromopropionic, acid in the presence of piperidine, in 64-85% yields.

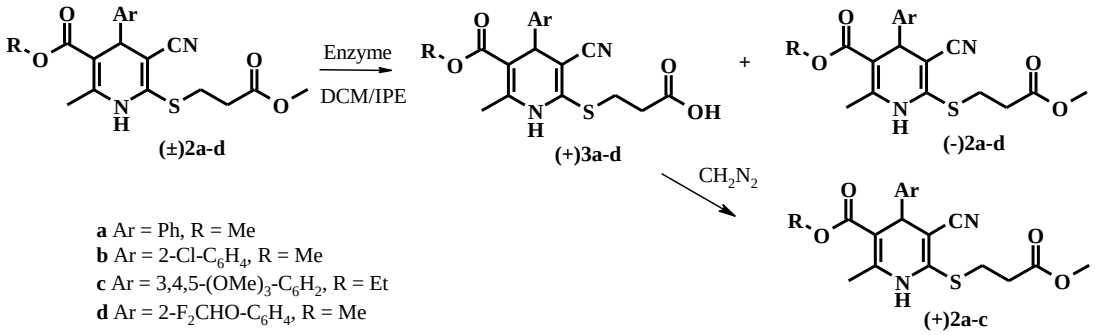
Enzymatic screening of the substrates **(±)2** was performed in water-saturated diisopropyl ether (IPE) (was found to be the best solvent) by using an Amano Acylase (*Aspergillus mellus*) and *Candida antarctica* lipase B (CAL-B, Novozym 435®). The reaction was monitored by HPLC and when ~50% of acid **(+)3** was formed the reaction was stopped, enzyme was filtered off, washed with IPE and the filtrate was evaporated. The mixture of acid **(+)3** and the remaining

ester (**-)2** was separated by column chromatography. The enantiomeric excess (ee) of the remaining esters **(-)2** were determined by HPLC using chiral column (Table 1).

Due to usage of IPE as a solvent, the concentration of substrate needs to be less than 0.01%, otherwise precipitation takes place. To increase the solubility of 6-methoxycarbonylethylsulfanyl-1,4-dihydropyridines **(±)2**, 0.5-10% of dichloromethane (DCM) was added to the reaction mixture. It increased the reaction rate, but enantioselectivity of the reaction decreased. When the ratio between DCM and IPE was 1:10, ee of ester **(-)2a** was 83%, but when the content of DCM was decreased to 5%, ee of **(-)2a** was increased to 95% (entry 5 and 6).

To hydrolyse 6-methoxycarbonylethylsulfanyl-1,4-DHPs **(±)2** an Amano Acylase was used. To our surprise, hydrolysis of DHPs **(±)2a** and **(±)2b** in water-saturated IPE did not take place at 45 ^{o}C (entry 14 and 15). In case of substrate **(±)2d** hydrolysis yielding racemic acid **(±)3d** and ester **(±)2d** (entry 17) was observed, but in case of DHP **(±)2c** just 20% ee of unreacted ester **(-)2c** was reached. By carrying out hydrolysis of the ester **(±)2b** with Amano Acylase at 25 ^{o}C 89% ee of **(-)2b** was reached. In case of DHPs **(-)2a**, **(-)2c** and **(-)2d** (entry 10, 12, 13) 38-52% ee was reached.

Table 1 shows that CAL-B gave better results and in case of 4-(3,4,5-trimethoxyphenyl)substituted 6-(2-methoxycarbonylethylsulfanyl)-1,4-DHP **(±)2c** enzyme catalysed hydrolysis carried out at 45 ^{o}C over 26 h provided 99% ee of **(-)2c**.



Scheme 2

Table 1 shows that in contrast to Amano Acylase, better results for CAL-B were reached by raising the temperature from 25 ^{o}C (ee 65-82%) to 45 ^{o}C (ee 86-99%).

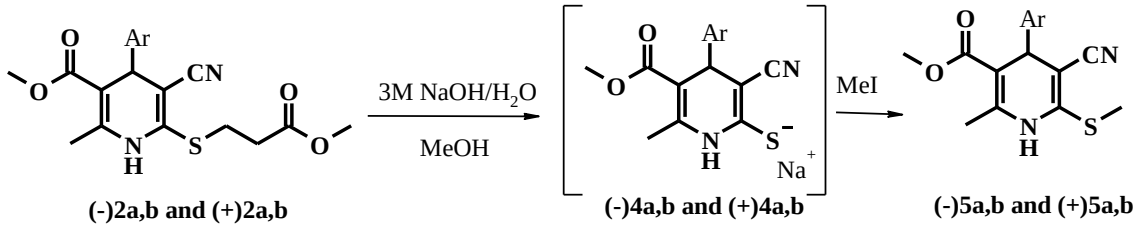
Table 1 Yields of enzymatic hydrolysis (reaction conditions)

Entry	Substrate	Enzyme	Ratio between DCM and	Temp., ^{o}C	T, h	Yield of product (-)2	Enantiomeric excess of (-)2, %	Yield of product (+)3

			IPE, v/v					
1	**(±)2a**	CAL-B	1:20	25	48	49	82	47
2	**(±)2b**	CAL-B	1:20	25	165	46	80	48
3	**(±)2c**	CAL-B	1:200	25	51	46	77	47
4	**(±)2d**	CAL-B	1:60	25	264	46	65	46
5	**(±)2a**	CAL-B	1:10	45	40	48	83	47
6	**(±)2a**	CAL-B	1:20	45	18	49	95	47
7	**(±)2b**	CAL-B	1:20	45	48	46	92	48
8	**(±)2c**	CAL-B	1:200	45	26	46	99	46
9	**(±)2d**	CAL-B	1:60	45	168	46	86	46
10	**(±)2a**	Amano Acylase	1:20	25	96	47	52	48
11	**(±)2b**	Amano Acylase	1:20	25	310	45	89	48
12	**(±)2c**	Amano Acylase	1:200	25	100	48	38	47
13	**(±)2d**	Amano Acylase	1:60	25	336	45	48	48
14	**(±)2a**	Amano Acylase	1:20	45	*	-	-	-
15	**(±)2b**	Amano Acylase	1:20	45	*	-	-	-
16	**(±)2c**	Amano Acylase	1:200	45	90	46	20	47
17	**(±)2d**	Amano Acylase	1:60	45	336	47	racemate	48

* Reaction doesn`t take place within 6 weeks

We did not succeed to find out an appropriate HPLC conditions for enantioseparation of carboxylic acids **(+)3** nor on Whelk O1, nor Lux Cellulose-2 chiral HPLC columns. To characterise the opposite enantiomer optically active acids **(+)3b** and **(+)3c** were methylated with diazomethane and enantiomeric excess of the corresponding methyl 6-methoxycarbonylethylsulfanyl-1,4-dihydropyridine-3-carboxylates **(+)2b** and **(+)2c** were determined (Table 2).



a) Ar = Ph; **b)** Ar = 2-Cl-C_6H_4

Scheme 3

Enantioenriched thiolates **(-)4** and **(+)4** were prepared by deacrylation of mercaptopropionates **(-)2** and **(+)2** with 3N NaOH water solution. Even after alkylation of **(-)4** and **(+)4** with methyl iodide enantioenriched methyl 6-methylsulfanyl-1,4-dihydropyridine-3-carboxylates **(-)5** and **(+)5** were still obtained (Table 2).

Table 2 Enantiomeric excess and optical yields of compounds **(-)2**, **(+)2**, **(+)3**, **(-)5** and **(+)5**

Entry	Compound	ee, %	$[\alpha]^{20}$, deg, (c=1, MeOH)
1	**(+)3a**	-	+168.6
2	**(+)3b**	-	+171.9
3	**(+)3c**	-	+182.1
4	**(-)2a**	95	-127.8
5	**(-)2b**	92	-134.9
6	**(-)2c**	99	-152.9
7	**(+)2a**	93	+120.3
8	**(+)2b**	92	+133.9
9	**(+)2c**	94	+134.9
10	**(-)5a**	93	-104.8
11	**(+)5a**	85	+92.6
12	**(-)5b**	92	-110.4
13	**(+)5b**	92	+80.5

3. **EXPERIMENTAL**

IR spectra were recorded on a Perkin-Elmer 580 B spectrometer in Nujol, or in thin layer. NMR spectra were recorded with a Varian 400-MR (400 and 100 MHz). Chemical shifts are reported in ppm relative to hexamethyldisiloxane (δ 0.055). Mass spectral data and chromatographic separation were obtained by using an Q-TOF mass spectrometer (Micromass) operating in the ESI positive or negative ion mode connected to an Acquity

UPLC system (Waters) with Acquity UPLC BEH C18 column (1.7 μm, 2.1×50 mm). A gradient elution with MeCN–HCOOH (0.1%) in water was used to separate analytes. The enantiomeric excesses were determinated by HPLC on a Lux Cellulose-2 column (4 μm, 4.6×150 mm), as a mobile phase a 0.1% acetic acid in 2-PrOH:hexane (50:50, v/v) was used, flow rate was 1 ml/min, UV detector was operated at 254 nm. Melting points were determined using OptiMelt (SRS Stanford Research Systems). Elemental analyses were performed by using an EA 1106 (Carlo Erba Instruments). Optical rotation values were measured with a Rudolph Research Analytical autopol VI automatic polarimeter. TLC was performed on 20×20 cm Silica gel TLC-PET F254 foils (Fluka) by using different elution solvent systems. All reagents were purchased from Aldrich, Acros, Fluka or Merck and used without further purification.

Preparation of compounds **1a**, **(±)2a** and **(±)3a** was described in[41], **1b** in[40], and **1d** in[32].

1.1. Methyl 5-cyano-2-methyl-6-thioxo-4-(3,4,5-trimetoxyphenyl)-1,4,5,6-tetrahydropyridine- 3-carboxylate (1c)

A mixture of 3,4,5-trimethoxybenzaldehyde (0.20 g, 1.0 mmol), methyl acetoacetate (0.12 g, 1.0 mmol) and piperidine (0.03 mL, 0.3 mmol) in EtOH (20 mL) was stirred for 5 min at room temperature. Then 2-cyanothioacetamide (0.1 g, 1.0 mmol) and piperidine (0.1 mL, 1.0 mmol) were added and reaction mixture was stirred for 30 min at room temperature. The resulting reaction mixture was acidified with 0.6 ml of 3M hydrochloric acid in ethanol. The precipitate was separated by filtration, washed with cold (-10 ^{o}C) MeOH (5 mL) and water (20 mL) to give 0.28 g (58%) of thione **1c** as yellow powder, mp 174-176 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1,03 (t, cis-, J = 7.0 Hz, 3H); 1,07 (t, trans-, J = 7.0 Hz, 3H); 2.45 (s, cis-, 3H); 2.53 (s, trans-, 3H); 3.31 (s, cis-, 3H); 3.37 (s, trans-, 3H); 3.55-3.69 (m, 9H); 3,99 (q, cis-, J = 7.0 Hz, 3H); 4,03 (q, trans-, J = 7.0 Hz, 3H); 4.16 (d, cis-, J = 6.3 Hz, 1H); 4.27 (d, cis-, J = 6.3 Hz, 1H); 4.53 (d, trans-, J = 2.7 Hz, 1H); 5.03 (d, trans-, J = 2.7 Hz, 1H); 6.39 and 6.46 (s and s, 2H); 12.10 (br s, cis-, 1H); 12.26 (br s, trans-, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 18.9, 41.6, 42.3, 46.1, 48.4, 56.2, 60.8, 104.1, 105.4, 115.6, 131.4, 133.4, 137.9, 142.9, 153.5, 165.3, 189.2, 190.8. IR (Nujol) 1684 (C=O); 2170 (C≡N); 3259 (NH) cm^{-1}. Anal. calcd. for $C_{24}H_{33}N_3O_5S$: C 61.61, H 6.99, N 8.83. Found C 61.55; H 7.08; N 8.77.

1.2. Methyl 5-cyano-6-(2-methoxycarbonylethylsulfanyl)-2-methyl-4-phenyl- 1,4-dihydropyridine-3-carboxylate ((±)2a)

A mixture of thione **1a** (0.29 g, 1.0 mmol) and piperidine (0.1 mL, 1.0 mmol) in MeOH (20 mL) was stirred for 10 min at room temperature. Then methyl bromopropionate (0.14 mL, 1.3 mmol) was added and the reaction mixture was stirred at 80 ^{o}C for 1 h. The precipitate was separated by filtration, washed with cold (-10 ^{o}C) MeOH (5 mL) and water (20 mL) to give 0.30 g (80%) of ester **(±)2a** as white powder, mp 109-110 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.43 (s, 3H); 2.60-2.76 (m, 2H); 2.97-3.20 (m, 2H); 3.58 (s, 3H); 3.76 (s, 3H); 4.64 (s, 1H); 7.17–7.29 (m, 4H); 7.99 (s, 1H). IR (Nujol) 1714, (C=O); 2198 (C≡N); 3309 (NH)

cm^{-1}. Mass-spectrum, m/z 373 (M^+), 342, 296, 287. Anal. calcd. for $C_{19}H_{20}N_2O_4S$: C 61.27; H 5.41; N 7.52. Found C 61.20; H 5.44; N 7.49.

1.3. Methyl 4-(2-chlorophenyl)-5-cyano-6-(2-methoxycarbonylethylsulfanyl)-2-methyl- 1,4-dihydropyridine-3-carboxylate ((±)2b)

Compound **(±)2b** was prepared in the same manner as **(±)2a** using thione **1b** instead of **1a**. Yield 0.29 g (71%) of ester **(±)2b** as white powder, mp 109-110 ^oC. ^1H NMR (400 MHz, $CDCl_3$): δ 2.45 (s, 3H); 2.67-2.75 (m, 2H); 2.96-3.25 (m, 2H); 3.56 (s, 3H); 3.79 (s, 3H); 5.29 (s, 1H); 7.10–7.35 (m, 4H); 8.15 (s, 1H). IR (Nujol) 1714, (C=O); 2198 (C≡N); 3309 (NH) cm^{-1}. Mass-spectrum, m/z 407 (M^+), 377, 343, 295, 289, 209. Anal. Calcd. For $C_{19}H_{19}ClN_2O_4S$: C 56.09; H 4.70; N 6.88. Found C 55.95; H 4.58; N 6.78.

1.4. Ethyl 5-cyano-6-(2-methoxycarbonylethylsulfanyl)-2-methyl-4-(3,4,5-trimethoxyphenyl)- 1,4-dihydropyridine-3-carboxylate ((±)2c)

Compound **(±)2c** was prepared in the same manner as **(±)2a** using thione **1c** instead of **1a**. Yield 0.38 g (79%) of ester **(±)2c** as white powder, mp 138-139 ^oC. ^1H NMR (400 MHz, $CDCl_3$): δ 1.11 (t, J = 7.0 Hz, 3H); 2.39 (s, 3H); 2.58-2.68 (m, 2H); 2.94-3.10 (m, 2H); 3.71 (s, 3H); 3.75 (s, 3H); 3.76 (s, 3H); 3.77 (s, 3H); 3.99 (q, J = 7.0 Hz, 3H); 4.58 (s, 1H); 6.39 (s, 2H); 7.88 (s, 1H); IR (Nujol) 1684, 1712 (C=O); 2198 (C≡N); 3240 (NH) cm^{-1}. Mass-spectrum, m/z (rel. int.) 499 ($M+Na^+$), 447, 432, 309, 277, 223. Anal. calcd. for $C_{23}H_{28}N_2O_7S$: C 57.97, H 5.92, N 5.88. Found C 57.89, H 5.91, N 5.81.

1.5. Methyl 5-cyano-4-(2-difluoromethoxyphenyl)-6-(2-methoxycarbonylethylsulfanyl)- 2-methyl-1,4-dihydropyridine-3-carboxylate ((±)2d)

Compound **(±)2d** was prepared in the same manner as **(±)2a** using thione **1d** instead of **1a**. Yield 0.38 g (87%) of ester **(±)2d** as white powder, mp 134-135 ^oC. ^1H NMR (400 MHz, $CDCl_3$): δ 2.42 (s, 3H); 2.67-2.74 (m, 2H); 2.96-3.24 (m, 2H); 3.55 (s, 3H); 3.78 (s, 3H); 5.06 (s, 1H); 6.54 (q, J = 73.2 Hz, 1H); 6.96–7.27 (m, 4H), 8.03 (s, 1H). IR (Nujol) 1710, (C=O); 2201 (C≡N); 3183 (NH) cm^{-1}. Mass-spectrum, m/z (rel. int.) 439 (M^+), 420, 399, 333, 296. Anal. calcd. for $C_{20}H_{20}F_2N_2O_5S$: C 54.79; H 4.60; N 6.39. Found C 54.79; H 4.47; N 6.26.

1.6. Methyl 6-carboxyethylsulfanyl-5-cyano-2-methyl-4-phenyl-1,4-dihydropyridine- 3-carboxylate ((±)3a)

A mixture of thione **1a** (0.29 g, 1.0 mmol) and piperidine (0.1 mL, 1.0 mmol) in DMSO (20 mL) was stirred for 10 min at room temperature. Then bromopropionic acid (0.15 g, 1.0 mmol) was added and the reaction mixture was stirred at 70 ^oC for 30 min. Resulting mixture was diluted with water (30 mL) and extracted with ethyl acetate (3x30 mL). The combined organic extracts were evaporated and crystallized from dichloromethane. The precipitate was separated by filtration, washed with cold (-10 ^oC) MeOH (5 mL) and water (20 mL) to give 0.31 g (85%) of acid **(±)3a** as white powder, mp 165-167 ^oC. ^1H NMR (400 MHz, DMSO): δ 2.30 (s, 3H), 2.40-2.50 (m, 2H), 2.97-3.17 (m, 2H), 3.48 (s, 3H), 4.46 (s, 1H), 7.10-7.30 (m, 3H), 10.33 (s, 1H) 12.42 (s, 1H). IR (Nujol) 1685 (C=O); 2216 (C≡N); 3175 and 3225 (NH and OH) cm^{-1}. Mass-spectrum, m/z (rel. int.) 359 (M^+), 327, 281, 209. Anal. calcd. for $C_{18}H_{18}N_2O_4S$: C 60.32 H, 5.06; N 7.82. Found C 60.25, H 5.17, N 7.88.

1.7. Methyl 6-carboxyethylsulfanyl-4-(2-chlorophenyl)-5-cyano-2-methyl-1,4-dihydro- pyridine-3-carboxylate ((±)3b)

Compound **(±)3b** was prepared in the same manner as **(±)3a** using thione **1b** instead of **1a**. Yield 0.29 g (69%) of acid **(±)3b** as white powder, mp 177-180 ^{o}C. ^{1}H NMR (400 MHz, DMSO): δ 2.30 (s, 3H); 2.51-2.44 (m, 2H); 2.98-3.18 (m, 2H); 3.42 (s, 3H); 5.07 (s, 1H); 7.21-7.37 (m, 4H); 9.54 (s, 1H); 12.42 (s, 1H); IR (Nujol) 1685, (C=O); 2216 (C≡N); 3175, 3225 (NH and OH) cm^{-1}. Mass-spectrum, m/z (rel. int.) 393 (M^{+}), 375, 281, 209. Anal. calcd. for $C_{18}H_{17}ClN_2O_4S$: C 55.03; H 4.36; N 7.13. Found C 54.48; H 4.32; N 6.90.

1.8. Ethyl 6-carboxyethylsulfanyl-5-cyano-2-methyl-4-(3,4,5-trimethoxyphenyl-1,4-dihydro- pyridine-3-carboxylate ((±)3c)

Compound **(±)3c** was prepared in the same manner as **(±)3a** using thione **1c** instead of **1a**. Yield 0.31 g (85%) of acid **(±)3c** as white powder, mp 105-107 ^{o}C. ^{1}H NMR (400 MHz, DMSO): δ 1,11 (t, J = 7.0 Hz, 3H); 2.30 (s, 3H); 2.40-2.50 (m, 2H); 2.97-3.17 (m, 2H); 3,99 (q, J = 7.0 Hz, 2H); 4.46 (s, 1H); 7.10-7.30 (m, 3H); 10.33 (s, 1H); 12.42 (s, 1H). IR (Nujol) 1685, (C=O); 2216 (C≡N); 3175, 3225 (NH and OH) cm^{-1}. Mass-spectrum, m/z (rel. int.) 449 (M^{+}), 418, 283, 263, 235. Anal. calcd. for $C_{18}H_{20}N_2O_5S$: C 57.43, H 5.36, N 7.44. Found C 57.32, H 5.42, N 7.53.

1.9. Methyl 6-carboxyethylsulfanyl-5-cyano-4-(2-difluoromethoxy-phenyl-2-methyl- 1,4-dihydropyridine-3-carboxylate ((±)3d)

Compound **(±)3d** was prepared in the same manner as **(±)3a** using thione **1d** instead of **1a**. Yield 0.27 g (64%) of acid **(±)3d** as white powder, mp 157-158 ^{o}C. ^{1}H NMR (400 MHz, DMSO): δ 2.26 (s, 3H), 2.43-2.48 (m, 2H), 2.95-3.17 (m, 2H), 3.40 (s, 3H), 4.87 (s, 1H), 7.17-7.39 (m, 3H), 9.49 (s, 1H) 12.37 (s, 1H). IR (Nujol) 1685, (C=O); 2216 (C≡N); 3175 and 3225 (NH and OH) cm^{-1}. Mass-spectrum, m/z (rel. int.) 425 (M^{+}), 405, 385, 333, 281. Anal. calcd. for $C_{19}H_{18}F_2N_2O_5S$: C 53.77, H 4.27, N 6.60. Found C 53.84, H 4.20, N 6.51.

1.10. General procedure of preparative *Candida antarctica* lipase B, Novozym 435 (CAL-B) catalysed hydrolysis of esters (±)2.

1 mmol of ester **(±)2** was dissolved in 2 mL of dichloromethane and appropriate amount of diisopropylether was added as mentioned in table 1 to give substrate concentration and ratio between dichloromethane and diisopropylether. Then 0.4 g of Novozym 435® (≥10,000 U/g) was added and the reaction mixture was placed in an incubator – shaker (45 ^{o}C) and stirred at 300 rpm. Every 10 - 20 min a 10-20 μL samples were taken with a syringe from the reaction mixture, transferred into the 1 mL vial containing75% of acetonitrile - water solution. Obtained solution was stirred for 15 sec and analysed by HPLC. Reaction was stopped when ~ 50% of acid **4** was formed. Blank reactions without enzyme showed no conversion of substrate. The enzyme was separated from the reaction mixture by filtration. Filtrate was evaporated to dryness under reduced pressure at 50 ^{o}C and the residue was purified by flash chromatography. Purified acids **(+)3** and esters **(+)2** were analysed by HPLC.

1.11. (+) Methyl 6-carboxyethylsulfanyl-5-cyano-2-methyl-4-phenyl-1,4-dihydro- pyridine-3-carboxylate ((+)3a)

0.17 g (47%) of acid **(+)3a** as white powder, mp 165-167 ^{o}C. ^{1}H NMR (400 MHz, DMSO): δ 2.30 (s, 3H), 2.40-2.50 (m, 2H), 2.97-3.17 (m, 2H), 3.48 (s, 3H), 4.46 (s, 1H), 7.10-7.30 (m, 3H), 10.33 (s, 1H) 12.42 (s, 1H). IR (Nujol) 1685, (C=O); 2216 (C≡N); 3175, 3225 (NH and OH) cm^{-1}. Mass-spectrum, m/z (rel. int.) 359 (M^{+}), 327, 281, 209. Anal. calcd.

for $C_{18}H_{18}N_2O_4S$: C 60.32 H, 5.06; N 7.82. Found C 60.22, H 5.19, N 7.88. $[\alpha]^{20}_D$ +168.6 (c 1.0, MeOH).

1.12. (+) Methyl 6-carboxyethylsulfanyl-4-(2-chlorophenyl)-5-cyano-2-methyl-1,4-dihydropyridine-3-carboxylate ((+)3b)

0.19 g (48%) of acid **(+)3b** as white powder, mp 177-180 ^oC. ^1H NMR (400 MHz, DMSO): δ 2.30 (s, 3H); 2.51-2.44 (m, 2H); 2.98-3.18 (m, 2H); 3.42 (s, 3H); 5.07 (s, 1H); 7.21–7.37 (m, 4H); 9.54 (s, 1H); 12.42 (s, 1H); IR (Nujol) 1685, (C=O); 2216 (C≡N); 3175, 3225 (NH and OH) cm^{-1}. Mass-spectrum, m/z (rel. int.) 393 (M^+), 375, 281, 209. Anal. Calcd. For $C_{18}H_{17}ClN_2O_4S$: C 55.03; H 4.36; N 7.13. Found C 54.86; H 4.25; N 6.88. $[\alpha]^{20}_D$ +171.9 (c 1.0, MeOH).

1.13. (+) Ethyl 6-carboxyethylsulfanyl-5-cyano-2-methyl-4-(3,4,5-trimethoxyphenyl- 1,4-dihydropyridine-3-carboxylate ((+)3c)

0.21 g (46%) of acid **(+)3c** as white powder, mp 105-107 ^oC. ^1H NMR (400 MHz, $CDCl_3$): δ 1.11 (t, J = 7.0 Hz, 3H); 2.39 (s, 3H); 2.58-2.68 (m, 2H); 2.94-3.10 (m, 2H); 3.71 (s, 3H); 3.75 (s, 3H); 3.76 (s, 3H); 3.77 (s, 3H); 3.99 (q, J = 7.0 Hz, 3H); 4.58 (s, 1H); 6.39 (s, 2H); 7.88 (s, 1H); IR (Nujol) 1684, 1712 (C=O); 2198 (C≡N); 3240 (NH) cm^{-1}. Mass-spectrum, m/z (rel. int.) 499 ($M+Na^+$), 447, 432, 309, 277, 223. Anal. Calcd. For $C_{18}H_{20}N_2O_5S$: C 57.43, H 5.36, N 7.44. Found C 57.32, H 5.42, N 7.53. $[\alpha]^{20}_D$ +182.1 (c 1.0, MeOH).

1.14. (-) Methyl 5-cyano-6-(2-methoxycarbonylethylsulfanyl)-2-methyl-4-phenyl- 1,4-dihydropyridine-3-carboxylate ((-)2a)

0.30 g (80%) of ester **(-)2a** as white powder, mp 109-110 ^oC. ^1H NMR (400 MHz, $CDCl_3$): δ 2.43 (s, 3H); 2.60-2.76 (m, 2H); 2.97-3.20 (m, 2H); 3.58 (s, 3H); 3.76 (s, 3H); 4.64 (s, 1H); 7.17–7.29 (m, 4H); 7.99 (s, 1H). IR (Nujol) 1714, (C=O); 2198 (C≡N); 3309 (NH) cm^{-1}. Mass-spectrum, m/z 373 (M^+), 342, 296, 287. Anal. Calcd. For $C_{19}H_{20}N_2O_4S$: C 61.27; H 5.41; N 7.52. Found C 61.20; H 5.44; N 7.49. $[\alpha]^{20}_D$ -127.8 (c 1.0, MeOH).

1.15. (-) Methyl 4-(2-chlorophenyl)-5-cyano-6-(2-methoxycarbonyl-ethylsulfanyl)- 2-methyl-1,4-dihydropyridine-3-carboxylate ((-)2b)

0.29 g (71%) of ester **(-)2b** as white powder, mp 109-110 ^oC. ^1H NMR (400 MHz, $CDCl_3$): δ 2.45 (s, 3H); 2.67-2.75 (m, 2H); 2.96-3.25 (m, 2H); 3.56 (s, 3H); 3.79 (s, 3H); 5.29 (s, 1H); 7.10-7.35 (m, 4H); 8.15 (s, 1H). IR (Nujol) 1714, (C=O); 2198 (C≡N); 3309 (NH) cm^{-1}. Mass-spectrum, m/z 407 (M^+), 377, 343, 295, 289, 209. Anal. calcd. for $C_{19}H_{19}ClN_2O_4S$: C 56.09; H 4.70; N 6.88. Found C 55.95; H 4.58; N 6.78. $[\alpha]^{20}_D$ -134.9 (c 1.0, MeOH).

1.16. (-) Ethyl 5-cyano-6-(2-methoxycarbonylethylsulfanyl)-2-methyl-4-(3,4,5-tri- methoxyphenyl)-1,4-dihydropyridine-3-carboxylate ((-)2c)

0.38 g (79%) of ester **(-)2c** as white powder, mp 138-139 ^oC. ^1H NMR (400 MHz, $CDCl_3$): δ 1.11 (t, J = 7.0 Hz, 3H); 2.39 (s, 3H); 2.58-2.68 (m, 2H); 2.94-3.10 (m, 2H); 3.71 (s, 3H); 3.75 (s, 3H); 3.76 (s, 3H); 3.77 (s, 3H); 3.99 (q, J = 7.0 Hz, 3H); 4.58 (s, 1H); 6.39 (s, 2H); 7.88 (s, 1H); IR (Nujol) 1684, 1712 (C=O); 2198 (C≡N); 3240 (NH) cm^{-1}. Mass-

spectrum, m/z (rel. int.) 499 ($M+Na^+$), 447, 432, 309, 277, 223. Anal. calcd. for $C_{23}H_{28}N_2O_7S$: C 57.97, H 5.92, N 5.88. Found C 57.89, H 5.91, N 5.81. $[\alpha]^{20}_D$ -152.9 (c 1.0, MeOH).

1.17. (+) Methyl 5-cyano-6-methoxycarbonylethylsulfanyl-2-methyl-4-phenyl-1,4-dihydropyridine-3-carboxylate ((+)2a)

A mixture of acid **(+)3a** (0.11 g, 0.3 mmol) and 0.5M diazomethane ether solution (1.0 mL) in dichloromethane (7 mL) was stirred for a 10 min at room temperature. After 10 min reaction mixture was evaporated to dryness under reduced pressure at 50°C to give 0.10 g (97%) of ester **(+)2a** as oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.43 (s, 3H); 2.60-2.76 (m, 2H); 2.97-3.20 (m, 2H); 3.58 (s, 3H); 3.76 (s, 3H); 4.64 (s, 1H); 7.17-7.29 (m, 4H); 7.99 (s, 1H). IR (Nujol) 1714, (C=O); 2198 (C≡N); 3309 (NH) cm^{-1}. Mass-spectrum, m/z 373 (M^+), 342, 296, 287. Anal. calcd. for $C_{19}H_{20}N_2O_4S$: C 61.27; H 5.41; N 7.52. Found C 61.18; H 5.47; N 7.43. $[\alpha]^{20}_D$ +120.3 (c 1.0, MeOH).

1.18. (+) Methyl 4-(2-chlorophenyl)-5-cyano-6-methoxycarbonylethyl-sulfanyl-2-methyl- 1,4-dihydropyridine-3-carboxylate (+)2b

Compound **(+)2b** was prepared in the same manner as **(+)2a**. Yield 0.10 g (97%) of ester **(+)2b** as oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.45 (s, 3H); 2.67-2.75 (m, 2H); 2.96-3.25 (m, 2H); 3.56 (s, 3H); 3.79 (s, 3H); 5.29 (s, 1H); 7.10–7.35 (m, 4H); 8.15 (s, 1H). IR (Nujol) 1714, (C=O); 2198 (C≡N); 3309 (NH) cm^{-1}. Mass-spectrum, m/z 407 (M^+), 377, 343, 295, 289, 209. $[\alpha]^{20}_D$ +133.9 (c 1.0, MeOH).

1.19. (+) Ethyl 5-cyano-6-methoxycarbonylethylsulfanyl-2-methyl-4-(3,4,5-trimethoxy- phenyl)-1,4-dihydropyridine-3-carboxylate (+)2c

Compound **(+)2c** was prepared in the same manner as **(+)2a**. Yield 0.10 g (95%) of ester **(+)2c** as oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.11 (t, J = 7.0 Hz, 3H); 2.39 (s, 3H); 2.58-2.68 (m, 2H); 2.94-3.10 (m, 2H); 3.71 (s, 3H); 3.75 (s, 3H); 3.76 (s, 3H); 3.77 (s, 3H); 3.99 (q, J = 7.0 Hz, 3H); 4.58 (s, 1H); 6.39 (s, 2H); 7.88 (s, 1H); IR (Nujol) 1684, 1712 (C=O); 2198 (C≡N); 3240 (NH) cm^{-1}. Mass-spectrum, m/z (rel. int.) 499 ($M+Na^+$), 447, 432, 309, 277, 223. $[\alpha]^{20}_D$ +134.9 (c 1.0, MeOH).

1.20. (-) Methyl 5-cyano-2-methyl-6-methylsulfanyl-4-phenyl-1,4-dihydro-pyridine-3-carboxylates ((-)5a)

A mixture of ester **(-)2a** (0.37g, 1.0 mmol) and 3N $NaOH/H_2O$ (0.34 mL, 1.0 mmol) in MeOH (20 mL) was stirred for 30 min at room temperature. Then iodomethane (0.08 mL, 1.3 mmol) was added and the reaction mixture was stirred at 40 °C for 1 h. The precipitate was separated by filtration, washed with cold (-10 °C) MeOH (5 mL) and water (20 mL) to give 0.29 g (95%) of ester **(-)5a** as white powder, mp 120-121 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.37 (s, 3H), 2.45 (s, 3H), 3.55 (s, 3H), 5.27 (s, 1H), 5.98 (s, 1H); 7.12-7.34 (m, 5H). IR (Nujol) 1685 (C=O); 2216 (C≡N); 3175 (NH) cm^{-1}. Mass-spectrum, m/z (rel. Int.) 301 (M^+), 269, 223. Anal. Calcd. For $C_{16}H_{16}N_2O_2S$: C 63.98, H 5.37, N 9.33. Found C 64.12, H 5.31, N 9.24.
$[\alpha]^{20}_D$ -104.8 (c 1.0, MeOH).

1.21. (-) Methyl 4-(2-chlorophenyl)-5-cyano-2-methyl-6-methylsulfanyl-1,4-dihydro- pyridine-3-carboxylates (-)5b

Compound **(-)5b** was prepared in the same manner as **(-)5a**. Yield 0.32 g (94%) of ester **(-)5b** as white powder, mp 120-121 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.37 (s, 3H); 2.45 (s, 3H); 3.55 (s, 3H); 5.27 (s, 1H); 5.98 (s, 1H); 7.12-7.34 (m, 4H). IR (Nujol) 1685 (C=O); 2216 (C≡N) 3225 (NH) cm^{-1}. Mass-spectrum, m/z (rel. int.) 335 (M^+), 303, 223. Anal. calcd. for $C_{16}H_{15}ClN_2O_2S$: C 57.40, H 4.52, N 8.37. Found C 57.29, H 4.63, N 8.38. $[\alpha]^{20}_D$ -110.4 (c 1.0, MeOH).

1.22. (+) Methyl 5-cyano-2-methyl-6-methylsulfanyl-4-phenyl-1,4-dihydro-pyridine-3-carboxylates ((+)5a)

Compound **(+)5a** was prepared in the same manner as **(-)5a**. Yield 0.29 g (96%) of ester **(+)5a** as white powder, mp 120-121 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.37 (s, 3H), 2.45 (s, 3H), 3.55 (s, 3H), 5.27 (s, 1H), 5.98 (s, 1H); 7.12-7.34 (m, 5H). IR (Nujol) 1685 (C=O); 2216 (C≡N); 3175 (NH) cm^{-1}. Mass-spectrum, m/z (rel. Int.) 301 (M^+), 269, 223. Anal. calcd. for $C_{16}H_{16}N_2O_2S$: C 63.98, H 5.37, N 9.33. Found C 64.20, H 5.30, N 9.19. $[\alpha]^{20}_D$ +92.6 (c 1.0, MeOH).

1.23. (+) Methyl 4-(2-chlorophenyl)-5-cyano-2-methyl-6-methylsulfanyl-1,4-dihydro- pyridine-3-carboxylates ((+5)b)

Compound **(+)5b** was prepared in the same manner as **(-)5a**. Yield 0.32 g (95%) of ester **(+)5b** as white powder, mp 120-121 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.37 (s, 3H); 2.45 (s, 3H); 3.55 (s, 3H); 5.27 (s, 1H); 5.98 (s, 1H); 7.12-7.34 (m, 4H). IR (Nujol) 1685 (C=O); 2216 (C≡N) 3225 (NH) cm^{-1}. Mass-spectrum, m/z (rel. int.) 335 (M^+), 303, 223. Anal. calcd. for $C_{16}H_{15}ClN_2O_2S$: C 57.40, H 4.52, N 8.37. Found C 57.29, H 4.63, N 8.38. $[\alpha]^{20}_D$ +80.5 (c 1.0, MeOH).

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ХИМИЯ ГЕТЕРОЦИКЛИЧЕСКИХ СОЕДИНЕНИЙ. — 2013. — № . 3 С. 454—460

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SYNTHESIS OF 3-ALKOXYCARBONYL-6-ALKYLSULFANYL-4-(2-DIFLUOROMETHOXYPHENYL)-1,4-DIHYDROPYRIDINES AND RELATED DERIVATIVES AS ANALOGUES OF COGNITION ENHANCER CEREBROCRAST

4-(2-Difluoromethoxyphenyl) substituted 3-alkoxycarbonyl-6-alkylsulfanyl-3-cyano-2-methyl-1,4-dihydropyridines **2**, related pyridines **2** and 4,7-dihydrothieno [2,3-b]pyridines **3** have been prepared and their memory improving activity by making use passive avoidance responses in acquisition test and calcium overload preventing activity in SH-SY5Y neuroblastoma cell line in presence of agonist carbachol were examined. 1,4-Dihydropyridines **2f**,**i** bearing propoxyethoxycarbonyl group in position 3 and possessing week influence on calcium overload in neuronal cells, showed high activity comparable with that of cerebrocrast.

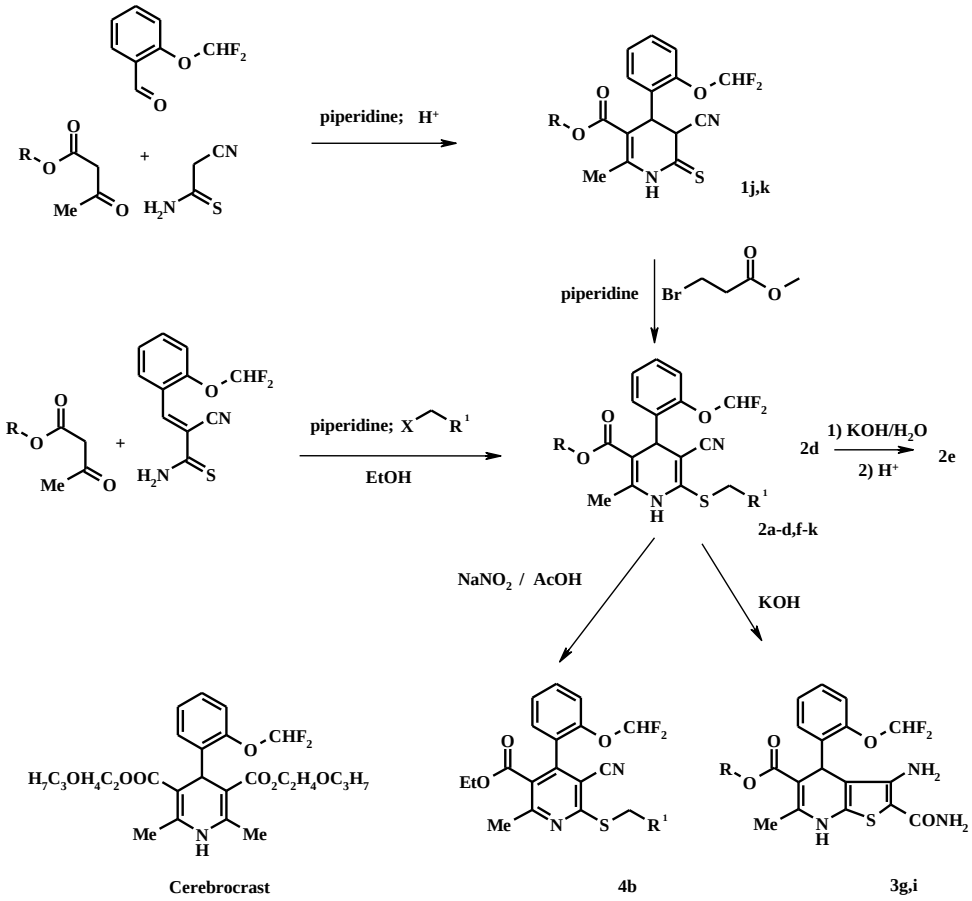
Keywords: 2-Alkylsulfanyl-1,4-dihydropyridines, thieno[2,3-b]pyridines, passive avoidance responses in acquisition test, calcium overload preventing activity.

1,4-Dihydropyridine (DHP) structure, when appropriately substituted, can exert potent and selective actions at a diverse set of membrane structures, including ion channels, G-protein coupled receptors and enzymes [1]. DHPs possess different - pleiotropic properties [2-4]. Thus, depending on the chemical structure peculiarities, 1,4-DHPs have many regulatory activities: neuro- and radioprotection, anti-mutagenic, anti-diabetic, anti-inflammatory, anti-ischaemic/anti-anginal and anti-hypertensive actions, as well as growth stimulating, life span prolongation and gene transfection properties [5]. Therefore, studies of specific properties of a group of DHP derivatives comprising minor changes of substituent structure could be useful for elucidation of biochemical interactions, which play a major role in the understanding of drug's structure-activity, drug's development and therapeutic success [6].

Several studies have shown that some 1,4-DHPs exert direct protective activity in experimental models of stroke [7] and neurodegenerative diseases [8]. As calcium overload has been linked to the apoptosis and death of the cell, it is suggested that the neuroprotective properties of 1,4-DHPs could be connected with their ability to prevent calcium overload in neurons [9]. 1,4-DHPs that simultaneously inhibit calcium influx in muscular cells as well as prevent calcium overload in neuronal cells are developed [10].

Decoration of 1,4-DHP ring with cyano and alkylsulfanyl substituents have resulted in compounds with cardiovascular [11,12], hepatoprotective [13], antioxidant (AOA) [14], and antiradical (ARA) [15] properties, however, calcium channel blocking activities of such type of compounds are less pronounced.

This study was performed to elaborate synthesis of novel asymmetric 6-alkylsulfanyl-1,4-DHPs containing structural fragments of cognition enhancer cerebrocrast [16-19], examine their memory improving activity by making use passive avoidance responses in acquisition test and calcium overload preventing activity in SH-SY5Y neuroblastoma cell line.



a) R = Me, R^1 = H; b) R = Et, R^1 = H; c) R = i-Pr, R^1 = H; d) R = $(CH_2)_2CN$, R^1 = H;
e) R = H, R^1 = H; f) R = $(CH_2)_2OC_3H_7$, R^1 = H; g) R = Et, R^1 = $CONH_2$; h) R = Et, R^1 = COOEt; i) R = $(CH_2)_2OC_3H_7$, R^1 = $CONH_2$; j) R = $(CH_2)_2OC_3H_7$, R^1 = CH_2COOMe; k) R = Me, R^1 = CH_2COOMe

6-Alkylsulfanyl substituted 5-cyano-4-(2-difluoromethoxyphenyl)-3-alkoxycarbonyl-2-methyl-1,4-DHPs **2a-d**,**f** were prepared in 62-82% yields by Michael reaction of alkyl acetoacetates with 2-cyano-3-(2-difluoromethoxyphenyl)thioacrylamide in presence of stoichiometric amount of piperidine as catalyst in ethanol with subsequent treatment with two fold excess of iodomethane.

By treatment of 1,4-DHP **2d** with KOH water solution hydrolysis took place and acid **2e** in 97% yield was formed.

To enhance solubility and lipophilicity of 1,4-DHPs **2** ester function (COOEt, CH_2COOMe groups) was introduced in 6-methylsulfanyl substituent, but ester group in position 3 (substituent R) was derivatized with propoxyethyl group. 1,4-DHPs **2g**,**i** containing amide function in 6-methylsulfanyl substituent and lipophilic $COO(CH_2)_2OC_3H_7$ group in position 3 were synthesized as well.

1,4-DHPs **2g-i** were prepared in 70-82% yields similarly to compounds **2a-f**, only by making use 1.05-1.10 fold excess of ethyl bromoacetate or iodoacetamide instead of iodomethane. 1,4-DHPs **2j**,**k** were prepared in 70-87% yields by alkylation of the corresponding thiones **1j**,**k** with methyl 3-bromopropionate which in turn were obtained by one pot four-component condensation of acetoacetate, aromatic aldehyde, 2-cyanoethanethioacetamide and stoichiometric amount of piperidine in 57-79% yields. Though the summary yields were intermediate (40-68%) the last pathway (obtaining

of intermediates **1**) has advantage because the target compounds **2j**,**k** crystallize as pure substances from reaction mixture, but in case of 5-component one-pot method separation with flash chromatography was necessary.

By treatment 2-carbamoylmethylsulfanyl-3-cyano-1,4-DHPs **2g** and **2i** with KOH in water - ethanol solution Thorpe's cyclization took place and 4,7-dihydrothieno[2,3-b]pyridines **3g** and **3i** in 59-73% yield were obtained.

Pyridines are the most possible metabolites of 1,4-DHPs *in vivo*. Compound **4b** was prepared in 29% yield by oxidation of **2b** with sodium nitrite in acetic acid.

The structures of synthesized compounds **2-4** were proved by spectroscopic and elemental analysis data. In the IR spectra of 1,4-DHPs **2a-k** the absorption band of cyano group were observed at 2188-2206 cm^{-1} (characteristic for β-aminovinylcarbonitriles) which disappeared after Thorpe's cyclization (in case of 4,7-dihydrothieno[2,3-b]pyridines **3g** and **3i**). In the ^{1}H NMR spectra characteristic singlet of 4-H proton at 4.93-5.22 ppm was observed for 1,4-DHPs **2a-k** and at 5.24-5.32 for 4,7-dihydrothieno[2,3-b]pyridines **3g** and **3i** confirming their partially structure.

TABLE 1. Influence of compounds **2**-**4** on Ca^{2+} accumulation in cell line SH-SY5Y in presence of agonist carbachol and on passive avoidance responses (PAR) in acquisition test in male ICR mice (18 – 24 g, t – 21°C, n = 6)

Com-pound	*R*	*R*[1]	*Log P**	*IC_{50}, μM SH-SY5Y*	*PAR test*	
					Dose, mg/kg	*Latency, Δ t, s*
Control saline						77.0 ± 22.3
Cereb-rocrast			5.08	> 100	0.05	158.3 ± 1.9**
2a	Me	H	3.41	n.e.		n.d.
2b	Et	H	3.78	n.e.	5.0	112.0 ± 9.4
2c	i-Pr	H	4.15	n.d.		n.d.
2d	C_2H_4CN	H	3.84	n.d.		n.d.
2e	H	H	2.79	n.d.		n.d.
2f	$C_2H_4OC_3H_7$	H	4.08	> 100	0.05	152.3 ± 3.6**
2g	Et	$CONH_2$	2.51	n.d.		n.d.
2h	Et	CO_2Et	4.02	n.e.	5.0	153.0 ± 9.5**
2i	$C_2H_4OC_3H_7$	$CONH_2$	2.81	n.e.	0.05	162.2 ± 4.3**
2j	$C_2H_4OC_3H_7$	CH_2CO_2Me	4.32	100		n.d.
2k	Me	CH_2CO_2Me	3.23	100		n.d.
3g	Et	$CONH_2$	3.15	n.d.		n.d.
3i	$C_2H_4OC_3H_7$	$CONH_2$	3.45	n.d.	5.0	101.3 ± 48.8
4b	Me	H	4.20	n.d.		n.d.

* calculated with program Molinspiration; ** $P < 0.05$ vs control; n.d. – not determined, n.e. – no effect

Cognitive enhancing effects of the studied substances **2** and **3** are shown in table 1. 1,4-DHPs **2f** and **2i** at the dose 0.05 mg/kg in memory test (PAR, acquisition) in mice showed activity which is comparable with that of cerebrocrast. 1,4-DHP **2h** lacking propoxyethyl ester group was active at the dose 5.0 mg/kg. So, prolongation of COOEt with $COO(CH_2)_2OC_3H_7$ group in position 3 and SMe with SCH_2COOEt group (compounds become more lipophilic), gave rise to the increase of activity. Thorpe's cyclization of active 2-carbamoylmethylsulfanyl-1,4-DHP **2i** to the corresponding 4,7-thieno[2,3-b]pyridine **3i** led to the significant lowering of activity.

As are seen from table, 1,4-DHPs 2 that are with cyano and alkylsulfanyl substituents similarly to symmetric 1,4-DHP – cerebrocrast and asymmetric 1,4-DHP-3,5-dicarboxylates [10] have weak influence on prevention of calcium overload in neuronal cells. 1,4-DHP 2i bearing propoxyethoxycarbonyl group in position 3 was the most potent cognition enhancer. These results together with the known data [16-19] allow to characterize 1,4-DHP-3-$COOC_2H_4OC_3H_7$ moiety as pharmacophore determining the memory improvement.

EXPERIMENTAL

All reagents were purchased from *Aldrich* or *Acros* and used without further purification. Melting points were determined on *OptiMelt MPA100* apparatus and are uncorrected. IR spectra have been recorded on a *„Shimadzu" IRPrestige-21* spectrometer (in nujol) and absorption band positions ν_{max} were expressed in cm^{-1}. ^1H NMR spectra were recorded on a *Varian Mercury-400* spectrometer (400 MHz). Chemical shifts are reported in ppm relative to HMDS. Elemental analyses were performed on an *EA 1106* (*Carlo Erba Instrument*). The course of the reactions and the individuality of substances were monitored by TLC on Kieselgel 60 F Merck plates with dichloromethane/hexane/methanol (5:5:1) as eluent. Synthesis of thione **1k** (yield 67%) is published in [20].

2-Propoxyethyl 5-cyano-4-(2-difluoromethoxyphenyl)-2-methyl-6-thioxo-1,4,5,6-tetrahydropyridine-3-carboxylate (1j).

A mixture of 2-difluoromethoxybenzaldehyde (0.69 g, 4.0 mmol), propoxyethyl acetoacetate (0.75 g, 4.0 mmol) and piperidine (0.04 ml, 0.4 mmol) in EtOH (20 ml) was stirred for 5 min at room temperature. Then 2-cyanoethanethioacetamide (0.4 g, 4.0 mmol) and piperidine (0.4 ml, 4.0 mmol) were added and the reaction mixture was stirred for 30 min. The resulting reaction mixture was acidified with 2.4 ml of 3M hydrochloric acid in ethanol. The precipitate was separated by filtration, washed with 5 ml of cold MeOH and 20 ml of water to give 0.97 g (57%) of thione **1j** as yellow powder, mp 90-91 ^oC. IR spectrum, ν, cm^{-1}: 3309 (N-H), 2198 (C≡N), 1714 (C=O) cm^{-1}. ^1H NMR spectrum ($CDCl_3$), *δ*, ppm (*J*, Hz): 0.78-0.89 (3H, m and m, cis- and trans-, 3-$OCH_2CH_2\underline{CH_3}$), 1.35-1.55 (2H, m and m, cis- and trans-, 3-$OCH_2\underline{CH_2}CH_3$), 2.51 and 2.59 (3H, s and s, cis- and trans-,
2-Me), 3.21-3.33 (2H, m and m, cis- and trans-, $COO\underline{CH_2}CH_2O$), 3.47-3.54 (2H, m and m, cis- and trans-, 3-$O\underline{CH_2}CH_2CH_3$), 4.15-4.24 (2H, m and m, cis- and trans-, $COOCH_2\underline{CH_2}O$), 4.17 and 4.86, 4.25and 5.08 (2H, d and d, *J* = 1.8, trans- H-5 and H-4, d and d, *J* = 7.5, cis-H-5 and H-4), 6.25 and 6.61 (1H, d and d, *J* = 74.0, cis- and *J* = 70.7, trans-, $OCHF_2$), 6.90-7.35 (4H, m, cis- and trans-, H Ar), 8.82 (1H, br.s, NH). Found, %: C 54.40, H 4.11, N 7.87, S 7.32. $C_{20}H_{22}F_2N_2O_4S$. Calculated, %: C 54.54, H 4.00, N 7.95, S 7.55.

Methyl 5-cyano-4-(2-difluoromethoxyphenyl)-2-methyl-6-methylsulfanyl-1,4-dihydropyridine-3-carboxylate (2a).

A mixture of methyl acetoacetate (0.58 g, 5 mmol), 2-cyano-3-(2-difluoro-methoxyphenyl)thioacrylamide (1.27 g, 5 mmol) and piperidine (0.55 ml, 5.5 mmol) in 10 ml of ethanol was heated to 40-50 ^oC, stirred 1 h at the ambient temperature. Then iodomethane (1.24 ml, 20 mmol) was added, the reaction mixture was refluxed for 15 min, cooled to 0 ^oC. The precipitated crude product was recrystallized from ethanol to give 1.30 g (71%) of **2a** as slightly yellow powder, mp 161-163 ^oC. IR spectrum, ν, cm^{-1}: 1672 (C=O), 2198 (C≡N), 3315 (N-H). ^1H NMR spectrum ($CDCl_3$) δ, ppm (*J*, Hz): 2.36 (3H, s, 6-CH_3), 2.47 (3H, s, SCH_3), 3.56 (3H, s, $COOCH_3$), 5.06 (1H, s, H-4), 6.00 (1H, br.s, NH), 6.54 (1H, q, *J* = 73.2, $OCHF_2$), 7.00-7.40 (4H, m, H Ar). Found, %: C 55.61, H 4.45, N 7.72, S 8.81. $C_{17}H_{16}F_2N_2O_3S$. Calculated, %: C 55.73, H 4.40, N 7.65, S 8.75.

Ethyl 5-cyano-4-(2-difluoromethoxyphenyl)-2-methyl-6-methylsulfanyl-1,4-dihydro-pyridine-3-carboxylate (2b).

Compound **2b** was prepared in the same manner as **2a** by making use ethyl acetoacetate instead of methyl acetoacetate. Yield 82%, mp 173-175 ^oC. IR spectrum, ν, cm^{-1}: 1693 (C=O), 2190 (C≡N), 3342 (N-H). ^1H NMR spectrum ($CDCl_3$) δ, ppm (*J*, Hz): 1.08 and 4.00 (5H, t and q, *J* = 7.0, $COOCH_2CH_3$), 2.34 (3H, s, 6-CH_3), 2.44 (3H, s, SCH_3), 5.07 (1H, s, H-4), 6.30 (1H, s, NH), 6.56 (1H, q, *J* = 73.2, $OCHF_2$), 7.00-7.30 (4H, m, H Ar). Found, %: C 56.81, H 4.57, N 7.52, S 8.39. $C_{18}H_{18}F_2N_2O_3S$. Calculated, %: C 56.83, H 4.77, N 7.36, S 8.43.

Isopropyl 5-cyano-4-(2-difluoromethoxyphenyl)-2-methyl-6-methylsulfanyl-1,4-dihydropyridine-3-carboxylate (2c).

Compound **2c** was prepared in the same manner as **2a** by making use isopropyl acetoacetate instead of methyl acetoacetate. Yield 62%, mp 114-116 ^oC. IR spectrum, ν, cm^{-1}: 1694 (C=O), 2194 (C≡N), 3364 (N-H). ^1H NMR spectrum ($CDCl_3$) δ, ppm (*J*, Hz): 0.90, 1.17 and 4.88 (7H, d and d, *J* = 6.2, and quintet, $COOCH(CH_3)_2$), 2.38 (3H, s, 6-CH_3), 2.47 (3H, s, SCH_3), 5.08 (1H, s, H-4), 6.08 (1H, s, NH), 6.57 (1H, q, *J* = 73.2, $OCHF_2$), 7.00-7.30 (4H, m, H Ar). Found, %: C 57.81, H 4.17, N 7.20, S 8.19. $C_{19}H_{20}F_2N_2O_3S$. Calculated, %: C 57.86, H 5.11, N 7.10, S 8.13.

Cyanoethyl 5-cyano-4-(2-difluoromethoxyphenyl)-2-methyl-6-methylsulfanyl-1,4-dihydropyridine-3-carboxylate (2d).

A mixture of cyanoethyl acetoacetate (0.8 g, 5 mmol), 2-cyano-3-(2-difluoromethoxyphenyl)thioacrylamide (1.27 g, 5 mmol) and piperidine (0.55 ml, 5.5 mmol) in 10 ml of ethanol was heated to 40-50 °C, stirred 1 h at the ambient temperature. Then iodomethane (1.24 ml, 20 ml) was added, the reaction mixture was refluxed for 15 min, chilled to 0 °C and poured in 50 ml of cold water. The precipitated crude product was recrystallized from ethanol to give 1.31 g (65%) of **2d** as slightly yellow powder, mp 134-135 °C. IR spectrum, ν, cm^{-1}: 1712 (C=O), 2207, 2266 (C≡N), 3288 (N-H). ^{1}H NMR spectrum ($CDCl_3$) δ, ppm (*J*, Hz): 2.37 (3H, s, 6-CH_3), 2.46 (3H, s, SCH_3), 2.53 and 4.17 (4H, t and t, *J* = 7.0, $COO(CH_2)_2$), 5.04 (1H, s, H-4), 6.18 (1H, br.s, NH), 6.58 (1H, q, *J* = 73.2, $OCHF_2$), 7.00-7.30 (4H, m, H Ar). Found, %: C 56.19, H 4.17, N 10.31, S 7.85 $C_{19}H_{17}F_2N_3O_3S$. Calculated, %: C 56.29, H 4.23, N 10.36, S 7.91.

5-Cyano-4-(2-difluoromethoxyphenyl)-2-methyl-6-methylsulfanyl-1,4-dihydropyridine-3-carboxylic acid (2e).

A mixture of 1,4-DHP **2d** (1.22 g, 3 mmol) and 1 ml 4 M KOH water solution in 4 ml of ethanol was stirred at 30 °C for 3 h. Then 1.3 ml of 3 M HCl ethanol solution were added and stirred at the ambient temperature for 1 h. The precipitate was separated by filtration and washed with 2 ml of ethanol and 10 ml of water to give 1.03 g (97%) of **2e** as colourless powder, mp 190-191 °C. IR spectrum, ν, cm^{-1}: 1680 (C=O), 2204 (C≡N), 3272, 3334 (N-H, O-H). ^{1}H NMR spectrum (DMSO-d_6) δ, ppm (*J*, Hz): 2.27 (3H, s, 6-CH_3), 2.40 (3H, s, SCH_3), 4.82 (1H, s, H-4), 7.08 (1H, q, *J* = 73.2, $OCHF_2$), 7.10-7.30 (4H, m, H Ar), 9.29 (1H, s, NH). Found, %: C 54.29, H 4.05, N 8.07, S 8.92. $C_{16}H_{14}F_2N_2O_3S$. Calculated, %: C 54.54, H 4.00, N 7.95, S 9.10.

Propoxyethyl 5-cyano-4-(2-difluoromethoxyphenyl)-2-methyl-6-methylsulfanyl-1,4-dihydropyridine-3-carboxylate (2f).

Compound **2f** was prepared in the same manner as **2d** by making use propoxyethyl acetoacetate instead of cyanoethyl acetoacetate. Yield 76%, mp 118-120 °C. IR spectrum, ν, cm^{-1}: 1683 (C=O), 2206 (C≡N), 3296 (N-H). ^{1}H NMR spectrum ($CDCl_3$) δ, ppm (*J*, Hz): 0.82, 1.48 and 3.35 (7H, t, m and t, *J* = 7.0, $CH_3CH_2CH_2O$), 2.30 (3H, s, 2-CH_3), 2.39 (3H, s, SCH_3), 3.42 and 4.00 (4H, m and m, $COO(CH_2)_2$); 5.01 (1H, s, H-4), 6.03 (1H, s, NH), 6.50 (1H, q, *J* = 73.2, $OCHF_2$), 7.00-7.20 (4H, m, H Ar). Found, %: C 57.45, H 5.49, N 6.35, S 7.36. $C_{21}H_{24}F_2N_2O_4S$. Calculated, %: C 57.52, H 5.52, N 6.39, S 7.31.

Ethyl 6-carbamoylmethylsulfanyl-5-cyano-4-(2-difluoromethoxyphenyl)-2-methyl-1,4-dihydropyridine-3-carboxylate (2g).

Compound **2g** was prepared in the same manner as **2d** by making use ethyl acetoacetate instead of cyanoethyl acetoacetate and iodoacetamide (10% excess) instead of iodomethane. Yield 70%, mp 199-201 °C. IR spectrum, ν, cm^{-1}: 1672, 1695 (C=O), 2188 (C≡N), 3200, 3354 (N-H). ^{1}H NMR spectrum (DMSO-d_6) δ, ppm (*J*, Hz): 1.02 and 3.90 (5H, t and q, *J* = 7.0, $COOCH_3CH_2$), 2.34 (3H, s, 6-CH_3), 3.60 and 3.72 (2H, d and d, *J* = 15.0, SCH_2), 4.97 (1H, s, H-4), 7.18 (1H, q, *J* = 73.2, $OCHF_2$), 7.10-7.40 (4H, m, H Ar), 7.62 and 7.92 (2H, br.s and br.s, $CONH_2$), 10.43 (1H, s, NH). Found, %: C 53.94, H 4.37, N 9.95, S 7.53. $C_{19}H_{19}F_2N_3O_4S$. Calculated, %: C 53.89, H 4.52, N 9.92, S 7.57.

Ethyl 5-cyano-4-(2-difluoromethoxyphenyl)-6-ethoxycarbonylmethylsulfanyl-2-methyl-1,4-dihydropyridine-3-carboxylate (2h).

Compound **2h** was prepared in the same manner as **2d** by making use ethyl acetoacetate instead of cyanoethyl acetoacetate and ethyl bromoacetate (5% excess) instead of iodomethane. Yield 70%, mp 120-122 °C. IR spectrum, ν, cm^{-1}: 1676, 1706 (C=O), 2198 (C≡N), 3184, 3230 (N-H). ^{1}H NMR spectrum ($CDCl_3$) δ, ppm (*J*, Hz): 1.08 and 3.98 (5H, t and q, *J* = 7.0, $COOCH_2CH_3$), 1.30 and 4.28 (5H, t and q, *J* = 7.0, $SCH_2COO\underline{CH_2CH_3}$), 2.38 (3H, s, 6-$CH_3$), 3.54 (2H, s, SCH_2), 5.10 (1H, s, H-4), 6.56 (1H, q, *J* = 73.2, $OCHF_2$);7.00-7.30 (4H, m, H Ar), 8.48 (1H, s, NH). Found, %: C 55.67, H 4.83, N 6.25, S 7.17. $C_{21}H_{22}F_2N_2O_5S$. Calculated, %: C 55.74, H 4.90, N 6.19, S 7.09.

Propoxyethyl 6-carbamoylmethylsulfanyl-5-cyano-4-(2-difluoromethoxy-phenyl)-2-methyl-1,4-dihydropyridine-3-carboxylate (2i).

Compound **2i** was prepared in the same manner as **2d** by making use propoxyethyl acetoacetate instead of cyanoethyl acetoacetate and iodoacetamide (10 % excess) instead of iodomethane. Yield 82%, mp 178-180 °C. IR spectrum, ν, cm^{-1}: 1662, 1700 (C=O), 2194 (C≡N), 3184, 3356, 3466 (N-H). ^{1}H NMR spectrum (DMSO-d_6) δ, ppm (*J*, Hz): 0.79, 1.42 and 3.26 (7H, t, m and t, *J* = 7.0, $CH_3CH_2CH_2O$), 2.36 (3H, s, 6-CH_3), 3.42 and 4.00 (4H, m and m, $COOCH_2CH_2O$), 3.60 and 3.76 (2H, d and d, *J* = 15.0, SCH_2), 4.94 (1H, s, H-4), 7.12 (1H, q, *J* = 73.2, $OCHF_2$), 7.10-7.30 (4H, m, H Ar), 7.62 and 7.90 (2H, br.s and br.s, $CONH_2$), 10.47 (1H, s, NH). Found, %: C 54.80, H 5.18, N 8.68, S 6.82. $C_{22}H_{25}F_2N_3O_5S$. Calculated, %: C 54.88, H 5.23, N 8.73, S 6.66.

2-Propoxyethyl 5-cyano-4-(2-difluoromethoxyphenyl)-6-(2-methoxycarbonyl-ethylsulfanyl)-2-methyl-1,4-dihydropyridine-3-carboxylate (2j).

A sample of 2-propoxyethyl 5-cyano-4-(2-difluoromethoxyphenyl)-2-methyl-6-thioxo-1,4,5,6-tetrahydropyridine-3-carboxylate **1j** (0.85 g, 2 mmol), piperidine (0.23 ml, 2.3 mmol) and methyl 3-bromo propionate (0.25 ml, 2.3 mmol) in 20 ml methanol was heated for 1 h. The precipitated crystals were removed by filtration, washed with 2 ml of cold MeOH, 5 ml of H_2O and 1 ml of MeOH to give 0.71 g (70%) of ester **2j** as colourless crystals, mp 74-75 ^oC. IR spectrum, ν, cm^{-1}: 1705, 1719 (C=O), 2198 (C≡N), 3259 (NH). ^1H NMR spectrum ($CDCl_3$) δ, ppm (*J*, Hz): 0.86, 1.49 and 3.29 (7H, t, m and t, *J* = 7.0, $CH_3CH_2CH_2O$), 2.43 (3H, s, 2-CH_3), 2.61-2.74 (2H, m, 6-$SCH_2\underline{CH_2}$), 2.99-3.21 (2H, m, 6-$S\underline{CH_2}CH_2$), 3.46 (2H, t, *J* = 7.0, $COOCH_2\underline{CH_2}O$), 3.78 (3H, s, 6-$COOCH_3$), 4.08 (2H, t, *J* = 7.0, $COO\underline{CH_2}CH_2O$), 5.08 (1H, s, H-4), 6.18-6.93 (1H, q, *J* = 73.2, 2-$OCHF_2$), 7.06-7.29 (4H, m, H Ar), 8.00 (1H, s, NH). Found, %: C 56.27, H 5.39, N 5.38, S 6.10. $C_{24}H_{28}F_2N_2O_6S$. Calculated, %: C 56.46, H 5.53, N 5.49, S 6.28.

Methyl 5-Cyano-4-(2-difluoromethoxyphenyl)-6-(2-methoxycarbonylethyl-sulfanyl)-2-methyl-1,4-dihydropyridine-3-carboxylate (2k).

Methyl 5-cyano-4-(2-difluoromethoxy-phenyl)-2-methyl-6-thioxo-1,4,5,6-tetrahydro-pyridine-3-carboxylate **1k** (0.71 g, 2 mmol) [20], piperidine (0.23 ml, 2.3 mmol) and methyl 3-bromopropionate (0.23 ml, 2.3 mmol) in 20 ml of methanol were heated for 2 h. The precipitated crystals were removed by filtration, washed with 2 ml of cold MeOH, 5 ml of H_2O and 1 ml of MeOH to give 0.76 g (87%) of **2k** as colourless crystals, mp. 134-135 ^oC. IR spectrum, ν, cm^{-1}: 1710 (C=O), 2201 (C≡N), 3183 (N-H). ^1H NMR spectrum ($CDCl_3$) δ, ppm (*J*, Hz): 2.42 (3H, s, 2-CH_3), 2.67-2.74 (2H, m, $SCH_2\underline{CH_2}$), 2.96-3.24 (2H, m, $S\underline{CH_2}CH_2$), 3.55 (3H, s, 3-$COOCH_3$), 3.78 (3H, s, 6-$SC_2H_4COO\underline{CH_3}$), 5.06 (1H, s, H-4); 6.54 (1H, q, *J* = 73.2, $OCHF_2$); 6.96-7.27 (4H, m, H Ar), 8.03 (1H, s, NH). Found, %: C 54.66, H 4.55, N 6.36, S 7.13. $C_{20}H_{20}F_2N_2O_5S$. Calculated, %: C 54.79, H 4.60, N 6.39, S 7.31.

Ethyl 3-amino-2-carbamoyl-4-(2-difluoromethoxyphenyl)-6-methyl-4,7-dihydrothieno[2,3-b]pyridine-5-carboxylate (3g).

Sample of DHP **2g** (0.42 g, 1 mmol) in 10 ml of ethanol was treated with 0.3 ml of 4 M KOH water solution, refluxed for 15 min, stirred for 1h at ambient temperature. Then 0.5 ml 3 M HCl ethanol solution was added, stirred for 15 min and cooled to 0 ^oC. The precipitate was separated by filtration, washed with 5 ml of cold ethanol, 20 ml of water and 2 ml of ethanol to give 0.25 g (59%) of **3g** as yellow powder, mp 208-210 ^oC. IR spectrum, ν, cm^{-1}: 1624, 1636 sh, 1656 sh, 1670 sh (C=O), 3166, 3260, 3342, 3430 (NH, NH_2). ^1H NMR (DMSO-d_6) δ, ppm (*J*, Hz): 0.88 and 3.88 (5H, t and q, *J* = 7.0, $COOCH_2CH_3$), 2.37 (3H, s, 6-CH_3), 5.24 (1H, s, H-4), 7.18 (1H, q, *J* = 73.2, $OCHF_2$), 7.00-7.50 (8H, m, H Ar, NH_2, $CONH_2$); 9.88 (1H, s, NH). Found, %: C 52.94, H 4.43, N 9.62, S 7.29. $C_{19}H_{19}F_2N_3O_4S$. Calculated, %: C 53.89, H 4.52, N 9.92, S 7.57.

Propoxyethyl 3-amino-2-carbamoyl-4-(2-difluoromethoxyphenyl)-6-methyl-4,7-dihydrothieno[2,3-b]pyridine-5-carboxylate (3i).

Compound **3i** was prepared in the same manner as **3g**. Yield 73%, mp 103-105 ^oC. IR spectrum, ν, cm^{-1}: 1624, 1687 (C=O), 3218, 3316 sh., 3364, 3484 (NH, NH_2). ^1H NMR (DMSO-d_6) δ, ppm (*J*, Hz): 0.83, 1.50 and 3.28 (7H, t, m and t, *J* = 7.0, $CH_3CH_2CH_2O$), 2.45 (3H, s, 6-CH_3), 3.50 and 4.14 (4H, m and m, $COOCH_2CH_2O$), 5.00 (2H, br.s, NH_2), 5.32 (1H, s, H-4), 6.80 (1H, q, *J* = 73.2, $OCHF_2$), 7.10-7.40 (6H, m, H Ar and $CONH_2$). Found, %: C 54.57, H 5.37, N 8.59, S 6.31. $C_{22}H_{25}F_2N_3O_5S$. Calculated, %: C 54.88, H 5.23, N 8.73, S 6.66.

Ethyl 5-cyano-4-(2-difluoromethoxyphenyl)-2-methyl-6-methylsulfanyl-pyridine-3-carboxylate (4b).

Sample of 1,4-DHP **2b** (0.76 g, 2 mmol) in 7 ml of acetic acid was treated with sodium nitrite (0.35 g, 5 mmol), heated for 15 min and stirred at ambient temperature for 1h. Then 10 ml of 50% ethanol was added and cooled to 5^oC. The precipitate was separated by filtration, washed with 5 ml of 50% ethanol to give 0.22 g (29%) of pyridine **4b** as slightly yellow powder, mp 66-68 ^oC. IR spectrum, ν, cm^{-1}: 1732 (C=O), 2220 (C≡N). ^1H NMR spectrum ($CDCl_3$) δ, ppm (*J*, Hz): 0.88 and 4.00 (5H, t and q, *J* = 7.0, $COOCH_2CH_3$), 2.66 (3H, s, 6-CH_3), 2.70 (3H, s, SCH_3), 6.50 (1H, q, *J* = 73.2, $OCHF_2$); 7.00-7.60 (4H, m, H Ar). Found, %: C 56.88, H 4.14, N 7.32, S 8.50. $C_{18}H_{16}F_2N_2O_3S$. Calculated, %: C 57.14, H 4.26, N 7.40, S 8.47.

Biological evaluation of compounds 2 and 3. Passive avoidance response (PAR) test was performed according to the procedures given in [17,21]. Cerebrocrast was used as references drug. The results obtained in the experiments were expressed as the mean values ± SEM and analysed by Student's t-test. The criterion of statistical significances was $P < 0.05$.
Influence of compounds **2** on Ca^{2+} concentration in cell line SH-SY5Y in presence of agonist carbachol was determined according to the procedures given in [10].

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SYNTHESIS AND ENANTIOSELECTIVE LIPASE-CATALYZED KINETIC RESOLUTION OF METHYL 6-(METHOXYCARBONYLMETHYLSULFANYL)-1,4-DIHYDROPYRIDINE-3-CARBOXYLATES

A series of methyl 6-(methoxycarbonylmethyl)sulfanyl-1,4-dihydropyridine-3-carboxylates as more lipophilic derivatives of biologically active 6-methylthio-1,4-dihydropyridine-3-carboxylic acid esters have been prepared by alkylation of 6-thioxo-1,4-dihydropyridines **1** with methyl bromoacetate. *Candida antarctica* lipase B (Novozym 435®)-catalyzed kinetic resolution has been investigated. 70% enantiomeric excess was reached for esters **2** in water-saturated diisopropylether at 45°C. The experiments revealed that 6-(methoxycarbonylmethyl)sulfanyl group is an essentially new activating group, which being 5 bonds remote from chiral center undergoes easy enzymatic hydrolysis and could be used for kinetic resolution of racemic 1,4-dihydropyridines.

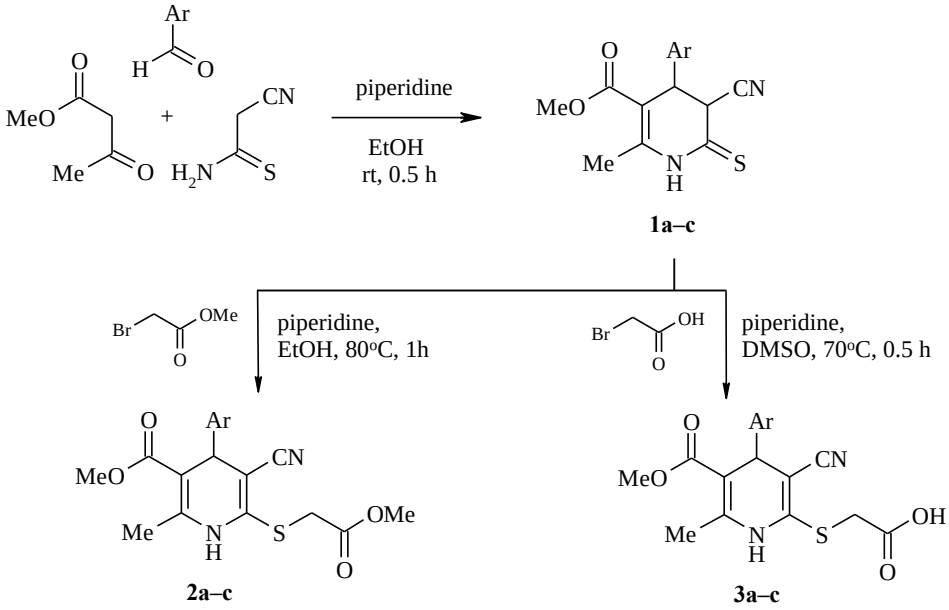
Keywords: Dihydropyridines; Candida antarctica lipase B; Enzymatic kinetic resolution.

1,4-Dihydropyridines (DHPs) are known as calcium channel effectors and antagonists. Many cardiovascular drugs on their basis are currently in use in the clinics or still are in different stages of development [1–4]. Pharmacology of DHPs is at the eve of a novel boom: the academic interest is growing towards pharmacological activities which are not related (or partially related) with their L-type calcium channel antagonist properties: neurotropic (antiamnestic, anticonvulsant, neuroregulatory) [5], membrane protecting [6–8], radioprotecting [9], analgesic [10], antidiabetic [11], antitumor [12], antitubercular [13], anti-inflammatory [14], gene-transfection [15], and as uroselective agents for treatment of benign prostatic hyperplasia [16]. Some DHPs have also showed the ability to modulate N-type calcium channel, and studied as anticonvulsants [17], stress protective agents [18] and cardio depressants [19].

6-Alkylthio-substitued 1,4-DHPs display cardiovascular [20–24], hepato-protective [25], antioxidant [26], and antiradical [27] activities, however, these compounds are still insufficiently studied.

The enantiomers of chiral 1,4-DHPs usually differ in their biological activities, and can even have an opposite action profile [28–30]. Chemoenzymatic methods for preparation of optically pure drugs have a number of advantages: it is simple, direct, efficient, mild, and cheap in case of repeated use of the enzyme. Incorporation of an enzymatically labile functional group, which allow kinetic resolution of monocyclic 1,4-DHPs, has been used as a conventional technique for the last decade [30–32].

The objective of this work was the preparation of enantiopure 1,4-DHPs containing lipophilic methoxycarbonylmethylsulfanyl group at position 6. We expected that the enzymatic hydrolysis of this group could promote kinetic resolution of the target DHPs. The starting 4-aryl-5-cyano-3-methoxycarbonyl-2-methyl-1,4-dihydropyridine-6-thiones **1** were prepared by a one-pot three-component condensation of the methyl acetoacetate, aromatic aldehyde and 2-cyanothioacetamide according to the previously mentioned synthetic protocol [33, 34].

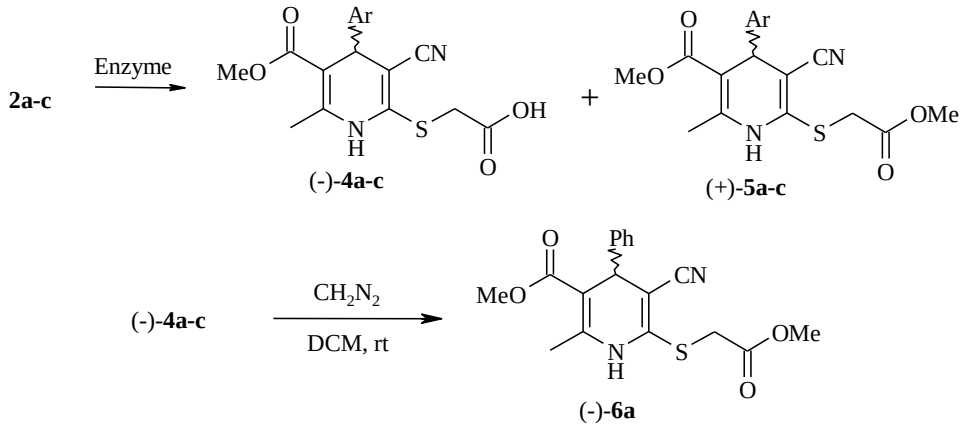


1-3 a Ar = Ph; **b** Ar = 2-Cl-C_6H_4; **c** Ar = 2-F_2HCO-C_6H_4

Alkylation of thiones **1** bearing many nucleophilic reaction centres (C-5, S, N) under mild reaction conditions with methyl bromoacetate proceeds preferably at the sulfur atom giving methyl 4-aryl-5-cyano-6-methoxycarbonylmethylsulfanyl-2-methyl-1,4-dihydropyridine-3-carboxylates **2**. Carboxylic acids **3** as references compounds for the investigation of enzyme catalyzed hydrolysis were prepared by alkylation of thiones **1** with bromoacetic acid in the presence of piperidine.

Enzymatic screening of the substrates **2** was performed in water-saturated diisopropylether (IPE), water-saturated *tert*-butyl methyl ether and phosphate buffer pH 7.5, modified (or not) with 15% MeCN at 20 to 50°C by using protease P6 (*Aspergillus melleus*), acylase 30,000 (*Aspergillus sp*), *Candida rugosa* lipase (CRL) and *Candida antarctica* lipase B (CAL-B, Novozym 435®). In the case of methyl 6-methoxycarbonylmethylsulfanyl-1,4-dihydropyridine-3-carboxylates **2**, protease P6 and acylase 30,000 were inactive. CRL-catalyzed hydrolysis of the methoxycarbonylmethylsulfanyl group in phosphate buffer was rather slow, but Novozym 435® showed significant hydrolytic activity both in water-saturated IPE and in phosphate buffer.

Kinetic resolution of methyl 6-methoxycarbonylmethylsulfanyl-1,4-dihydro-pyridine-3-carboxylates **2** in the presence of Novozym 435® were investigated in more detail. As we have found, the concentration of substrate in water-saturated IPE had to be less than 0.006% otherwise precipitation took place. To increase the solubility of methyl 6-methoxycarbonylmethylsulfanyl-1,4-dihydropyridine-3-carboxylates **2**, 1–3% of dichloromethane (DCM) was added to the reaction mixture. It increased the reaction rate, but decreased of the enantioselectivity.



2, 4, 5 a Ar = Ph; **b** Ar = 2-Cl-C_6H_4; **c** Ar = 2-$OCHF_2$-C_6H_4

The formed acid **4** and the remaining ester **5** were separated by column chromatography. Yields and the enantiomeric excess (*ee*) of esters **5** were determined by HPLC equipped with Whelk O1 and Lux Cellulose-2 (Table). The *ee* values ranged between 10 to 70%, depending on the substituent at position 4. The reaction time is longer and the enantioselectivity is better at room temperature than at 45°C.

Reaction conditions and yields of enzymatic hydrolysis

Sub-strate	DCM–IPE ratio, v/v	Temp, °C	Time, h	of **4**, %	Yield of **5**, %	*ee* of **5**, %
2a	1:57	rt	1.5	46*	49*	53*
2b	1:33	rt	1.5	47	46	70
2c	1:100	rt	1.5	45	46	10
2a	1:16	rt	1.5	46	46	43
2b	1:16	rt	1.5	48	47	51
2c	1:16	rt	1.5	44	45	10
2a	1:57	45°C	1.0	45	48	49
2b	1:33	45°C	1.0	45	45	67
2c	1:100	45°C	1.0	47	46	8

* Isolated yields and *ee* values were measured after column chromatography

Since the enantiomeric excess of carboxylic acids **4** could not be determined by given HPLC methods, acid (–)-**4a** was methylated with diazomethane and enantiomeric excess was measured for the corresponding methyl (–)-6-methoxy-carbonylmethylsulfanyl-1,4-dihydropyridine-3-carboxylate (–)-**6a**, which appeared to be 25%. Absolute configurations of the obtained enantioenriched esters **5** and **6** are still unknown because we have not succeeded in growing crystals for the X-ray analysis.

In conclusion, new methyl 6-methoxycarbonylmethylsulfanyl-1,4-DHP-3-carboxylates as more lipophilic derivatives of biologically active methyl 6-methyl-sulfanyl-1,4-DHP-3-carboxylates [20] have been prepared by alkylation of 1,4-DHP-6-thiolates with methyl bromoacetate, and their kinetic resolution catalyzed by *Candida antarctica* lipase B has been investigated. Our experiments allowed to the characterize 6-methoxycarbonylmethylsulfanyl group as an essentially new activating group, which being 5 bonds remote from the chiral center could be used for kinetic resolution of racemic 1,4-DHPs. The stereoselectivity of the imobilized *Candida antarctica* lipase B under the above mentioned conditions (water-saturated IPE, 25–45 °C, excess of substrate with regard to lipase) toward the methyl 6-methoxycarbonylmethylsulfanyl-1,4-DHP-3-carboxylates 2 was moderate.

EXPERIMENTAL

IR spectra were recorded on a Perkin-Elmer 580 B spectrometer in Nujol, or in thin layer. NMR spectra were recorded with a Varian Mercury 200BB (200 MHz) or a Varian 400-MR (400 and 100 MHz). Chemical shifts are reported in ppm relative to hexamethyldisiloxane (δ 0.055). Mass spectral data were determined on an Acquity UPLC system (Waters) connected to a Q-TOF mass spectrometer (Micromass) operating in the ESI positive or negative ion mode on an Acquity UPLC BEH C18 column (1.7 μm, 2.1×50 mm) using a gradient elution with MeCN–HCOOH (0.1%) in water. The enantiomeric excesses were analyzed by HPLC on a Lux Cellulose-2 phase column (4 μm, 4.6×150 mm), eluent 0.1% acetic acid in 2-PrOH-hexane, 50:50, flow rate 1 ml/min, detection at 254 nm. Melting points were determined on an OptiMelt (SRS Stanford Research Systems). Elemental analyses were determined on an EA 1106 (Carlo Erba Instruments). Optical rotation values were measured with a Rudolph Research Analytical autopol VI automatic polarimeter. TLC was performed on 20×20 cm Silica gel TLC-PET F254 foils (Fluka). All reagents were purchased from Aldrich, Acros, Fluka or Merck and used without further purification.

Preparation of compound **1a** was described in [33], **1b** in [34], **2a** and **3a** in [35].

Methyl 5-cyano-4-(2-difluoromethoxyphenyl)-2-methyl-6-thioxo-1,4,5,6-tetrahydropyridine-3-carboxylate (1c). A mixture of 2-difluoromethoxybenzaldehyde (0.69 g, 4.0 mmol), methyl acetoacetate (0.46 g, 4.0 mmol) and piperidine (0.04 ml, 0.4 mmol) in EtOH (20 ml) was stirred for 5 min at room temperature. Then 2-cyanothioacetamide (0.4 g, 4.0 mmol) and piperidine (0.4 ml, 4.0 mmol) were added and the reaction mixture was stirred for 30 min. The resulting reaction mixture was acidified with 2.4 ml of 3N hydrochloric acid in EtOH. The precipitate was separated by filtration, washed with cold (-10°C) MeOH (5 ml) and water (20 ml) to give 0.94 g (67%) of thione **1c** as yellow powder, mp 121–122°C. IR spectrum (Nujol), ν, cm^{-1}: 3309 (N–H), 2198 (C≡N), 1714 (C=O). ^{1}H NMR spectrum (200 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 2.45 and 2.53 (3H, two s, *cis*- and *trans*-, 2-CH_3); 3.59 and 3.61 (3H, two s, *cis*- and *trans*-, 3-$COOCH_3$); 4.11 and 4.80 (1H, two d, *J* =7.4 and *J* = 1.8, *cis*- and *trans*-, H-5); 4.17 and 5.00 (1H, two d, *J* = 7.4, *cis*- and *trans*-, H-4); 6.58 (1H, t, *J* = 73.2, $OCHF_2$); 6.84–7.30 (4H, m, H Ar); 8.81 (1H, br. s, NH); Found, %: C 54.40; H 4.11; N 7.87. $C_{16}H_{14}F_2N_2O_3S$. Calculated, %: C 54.54; H 4.00; N 7.95.

Methyl 4-(2-chlorophenyl)-5-cyano-6-methoxycarbonylmethylsulfanyl-2-methyl-1,4-dihydropyridine-3-carboxylate (2b). A mixture of thione **1b** (0.32 g, 1.0 mmol) and piperidine (0.1 ml, 1.0 mmol) in EtOH (20 ml) was stirred 10 min at room temperature. Then methyl bromoacetate (0.12 ml, 1.3 mmol) was added and the reaction mixture was stirred at 80°C for 1 h. The precipitate was separated by filtration, washed with cold (-10°C) MeOH (5 ml) and water (20 ml) to give 0.35 g (90%) of ester **2b** as white powder, mp 159–161°C. IR spectrum (Nujol), ν, cm^{-1}: 3309 (N–H), 2198 (C≡N), 1714 (C=O). ^{1}H NMR spectrum (400 MHz, $CDCl_3$) δ, ppm (*J*, Hz): 2.35 (3H, s, 2-CH_3); 3.54 (2H, ABq, *J* = 16.0, SCH_2); 3.54 (3H, s, 3-$COOCH_3$); 3.78 (3H, s, $CH_2COOC\underline{H}_3$); 5.24 (1H, s, H-4); 7.00–7.30 (4H, m, H Ar); 8.51 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, $CDCl_3$), δ, ppm: 19.3 (2-CH_3); 34.7 (SCH_2); 39.2 (C-4); 51.2 ($CH_2COO\underline{C}H_3$); 53.7 (3-$COO\underline{C}H_3$); 90.9 (C-3); 101.3 (C-5); 117.9 (C≡N); 127.4 (C Ar); 128.4 (C Ar); 129.7 (C Ar); 130.2 (C Ar); 132.3 (C Ar); 141.9 (C Ar); 145.4 (C-6); 145.5 (C-2); 167.0 (3-$\underline{C}OOCH_3$); 173.1 ($CH_2\underline{C}OOCH_3$). Mass-spectrum, *m/z* (I_{rel}, %): 393 $[M+H]^+$ (100), 281 $[M–Ar]^+$ (47). Found, %: C 55.23; H 4.21; N 7.02. $C_{18}H_{18}ClN_2O_4S$. Calculated, %: C 55.03; H 4.36; N 7.13.

Methyl 5-cyano-4-(2-difluoromethoxyphenyl)-6-methoxycarbonylmethylsulfanyl-2-methyl-1,4-dihydropyridine-3-carboxylate (2c). Compound **2c** was prepared in the same manner as **2b** using thione **1c** instead of **1b**. Yield 0.32 g (76%), mp 180–181°C. IR spectrum (Nujol), ν, cm^{-1}: 3309 (N–H), 2198 (C≡N), 1714 (C=O). ^{1}H NMR spectrum (400 MHz, $CDCl_3$) δ, ppm (*J*, Hz): 2.35 (3H, s, 2-CH_3); 3.51 (2H, ABq, *J* = 16.0, SCH_2); 3.50 (3H, s, 3-$COOCH_3$); 3.78 (3H, s, $CH_2COOC\underline{H}_3$); 5.00 (1H, s, H-4); 6.48 (1H, dd, *J* = 73.2, *J* = 2.7, $OCHF_2$); 7.00–7.30 (4H, m, H Ar); 8.36 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, $CDCl_3$), δ, ppm: 19.4 (2-CH_3); 34.7 (SCH_2); 37.2 (C-4); 51.1 ($CH_2COO\underline{C}H_3$); 53.7 (3-$COO\underline{C}H_3$); 90.5 (C-3); 100.6 (C-5); 118.1 (C≡N); 116.6 (t, *J* = 259.2, $OCHF_2$); 125.7 (C Ar); 128.4 (C Ar); 130.3 (C Ar); 132.3 (C Ar); 135.4 (C Ar); 142.0 (C Ar); 145.6 (C-6) 148.8 (C-2); 167.1 (3-$\underline{C}OOCH_3$); 173.0 ($CH_2\underline{C}OOCH_3$). Mass-spectrum, *m/z* (I_{rel}, %): 447 $[M+Na]^+$ (100), 425 $[M+H]^+$ (35), 296 $[M–Ar]^+$ (53). Found C 53.64; H 4.42; N 6.55. $C_{19}H_{18}F_2N_2O_5S$. Calculated, %: C 53.77, H 4.27, N 6.60.

Methyl 6-carboxymethylsulfanyl-4-(2-chlorophenyl)-5-cyano-2-methyl-1,4-dihydropyridine-3-carboxylate (3b). A mixture of thione **1b** (0.32 g, 1.0 mmol) and piperidine (0.1 ml, 1.0 mmol) in DMSO (20 ml) was stirred 10 min at room temperature. Then bromoacetic acid (0.14 g, 1.0 mmol) was added and the reaction mixture was stirred at 70°C for 30 min. Resulting mixture was diluted with water (30 ml) and extracted with ethyl acetate (3×30 ml). The combined organic extracts were evaporated and crystallized from dichloromethane. The precipitate was separated by filtration, washed with cold (-10°C) MeOH (5 ml) and water (20 ml) to give 0.33 g (87%) of acid **3b** as white powder, mp 165–167°C. IR spectrum (Nujol, ν, cm^{-1}: 3225 (N–H), 3175 (O–H), 2216 (C≡N), 1685 (C=O). ^{1}H NMR spectrum (400 MHz, DMSO-d_6) δ, ppm (*J*, Hz): 2.30 (3H, s, 2-CH_3); 3.44 (3H, s, 3-$COOCH_3$); 3.81 (2H, ABq, *J* = 15.6, SCH_2); 5.07 (1H, s, H-4); 7.17–7.39 (4H, m, H Ar); 9.59 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 18.5 (2-CH_3); 34.7 (SCH_2); 39.2 (C-4); 53.7 (3-$COO\underline{C}H_3$); 90.0 (C-3); 101.3 (C-5); 118.9 (C≡N); 127.2 (C Ar); 128.3 (C Ar); 129.5 (C Ar); 130.2 (C Ar); 132.1 (C Ar); 141.7 (C Ar); 145.4 (C-6) 145.5 (C-2); 166.9 (3-$\underline{C}OOCH_3$); 172.8 ($CH_2\underline{C}OOH$). Mass-spectrum, *m/z* (I_{rel}, %): 401 $[M+Na]^+$ (21), 379 $[M+H]^+$ (100), 267 $[M–Ar]^+$ (68). Found, %: C 53.80, H 3.81, N 7.22. $C_{17}H_{15}ClN_2O_4S$. Calculated, %: C 53.90, H 3.99, N 7.39.

Methyl 6-carboxymethylsulfanyl-5-cyano-4-(2-difluoromethoxyphenyl)-2-methyl-1,4-dihydropyridine-3-carboxylate (3c). Compound **3c** was prepared in the same manner as **3b** using thione **1c** instead of **1b**. Yield 0.33 g (81%), mp 122–124°C. IR spectrum (Nujol), ν, cm^{-1}: 3225 (N–H), 3175 (O–H), 2216 (C≡N), 1685 (C=O). ^{1}H NMR spectrum (400 MHz, DMSO-d_6) δ, ppm (*J*, Hz): 2.29 (3H, s, 2-CH_3); 3.45 (3H, s, 3-$COOCH_3$); 3.80 (2H, ABq, *J* = 15.6, SCH_2); 4.90 (1H, s, H-4); 6.58 (1H, t, *J* = 73.9, $OCHF_2$); 7.08–7.31 (4H, m, H Ar); 9.50 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 19.3 (2-CH_3); 34.7 (SCH_2); 37.1 (C-4); 53.7 (3-$COO\underline{C}H_3$); 90.5 (C-3); 100.4 (C-5); 118.1 (C≡N); 116.3 (t, *J* = 259.2, $OCHF_2$); 125.5 (C Ar); 128.3 (C Ar); 130.1 (C Ar); 132.2 (C Ar); 135.4 (C Ar); 141.9 (C Ar); 145.4 (C-6) 148.7 (C-2); 167.0 (3-$\underline{C}OOCH_3$); 173.0 ($CH_2\underline{C}OOH$). Mass-spectrum, *m/z* (I_{rel}, %): 433 $[M+Na]^+$ (85), 411 $[M+H]^+$ (100), 267 $[M–Ar]^+$ (24). Found, %: C 52.40, H 3.82, N 6.73. $C_{18}H_{16}F_2N_2O_5S$. Calculated, %: C 52.68, H 3.93, N 6.83.

General procedure of preparative *Candida antarctica* lipase B (Novozym 435®, CAL-B) catalysed hydrolysis of esters 2. To a solution of 1 mmol of ester **2** in 2 ml of dichloromethane and appropriate amount of diisopropylether was added to reach the desired solvent ratio, as mentioned in table 1. Then 0.4 g of Novozym 435® (≥10,000 U/g) was added and the reaction mixture was stirred (300 rpm) at the appropriate temperature. Probe samples (10–20 μl) were taken with syringe every 10–20 min, diluted with 1 ml of 75% aq. MeCN solution and analyzed by HPLC. The reaction was stopped when ~50% of acid **4** was formed (HPLC). Blank reactions without enzyme showed no conversion of substrate. Then enzyme was filtered off, the filtrate was evaporated under reduced pressure at 50°C to dry and the residue was separated by flash chromatography to give acids **4** and esters **5**, which were analyzed on HPLC.

Methyl 6-carboxymethylsulfanyl-5-cyano-2-methyl-4-phenyl-1,4-dihydropyridine-3-carboxylate ((–)-4a). Isolated yield 0.16 g (46%), mp 122–123°C, $[\alpha]^{20}_D$ -2.9° (*c* 1, MeOH).

Methyl 5-cyano-6-methoxycarbonylmethylsulfanyl-2-methyl-4-phenyl-1,4-dihydro-pyridine-3-carboxylate ((+) 5a). Isolated yield 0.18 g (49%), mp 160-161°C, $[\alpha]^{20}_D$ +55.1° (*c* 1, MeOH).

Methyl 5-cyano-6-methoxycarbonylmethylsulfanyl-2-methyl-4-phenyl-1,4-dihydro-pyridine-3-carboxylate ((–) 6a). A mixture of acid (–)-**4a** (0.1 g, 0.3 mmol) and 0.5M diazomethane ether solution (1.0 ml) in dichloromethane (7 ml) was stirred for 10 min at room temperature. The precipitate was separated by filtration, washed with cold (-10°C) MeOH (5 ml) and water (20 ml) to give 0.10 g (97%) of ester (-)-**6a** as white powder, mp 159-161°C, $[\alpha]^{20}_D$ -19.2° (c 1, MeOH). IR spectrum (Nujol), ν, cm^{-1}: 3309 (N–H), 2198 (C≡N), 1714 (C=O). ^{1}H NMR spectrum (400 MHz, $CDCl_3$) δ, ppm (*J*, Hz): 2.35 (3H, s, 2-CH_3); 3.51 (2H, ABq, *J* = 16.0, SCH_2); 3.50 (3H, s, 3-$COOCH_3$); 3.78 (3H, s, $CH_2COOC\underline{H}_3$); 5.24 (1H, s, H-4); 7.06–7.28 (5H, m, H Ph); 8.37 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, $CDCl_3$), δ, ppm: 19.4 (2-CH_3); 34.8 (SCH_2); 42.4 (C-4); 51.2 ($CH_2COO\underline{C}H_3$); 53.7 (3-$COO\underline{C}H_3$); 91.5 (C-3); 101.5 (C-5); 118.5 (C≡N); 127.2 (C Ph); 127.3 (C Ph); 128.7 (C Ph); 141.5 (C Ph); 144.3 (C-6); 145.0 (C-2); 167.2 (3-$\underline{C}OOCH_3$); 173.0 ($CH_2\underline{C}OOCH_3$). Mass-spectrum, *m/z* (I_{rel}, %): 359 $[M+H]^+$ (100), 281 $[M–Ph]^+$ (61). Found, %: C 60.22; H 5.11; N 7.86. $C_{18}H_{18}N_2O_4S$. Calculated, %: C 60.32; H 5.06; N 7.82.

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SYNTHESIS AND HYDROLYSIS OF ETHOXYCARBONYLMETHYL AND CYANOETHYL 5-CYANO-6-METHYLSULFANYL-1,4-DIHYDROPYRIDINE-3-CARBOXYLATES

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Abstract: Ethoxycarbonylmethyl and 2-cyanoethyl 6-methylsulfanyl-1,4-dihydropyridine-3-carboxylates were synthesized by making use of one-pot four- and five-component method, and by alkylation of 1,4-dihydropyridine-3-carboxylic acid with ethyl bromoacetate. By basic hydrolysis of esters, both 1,4-dihydropyridine-3-carboxylic acids and (1,4-dihydropyridine-3-carbonyloxy)acetic acids were prepared. *Candida antarctica lipase* B catalysed hydrolysis of ethoxycarbonylmethyl 1,4-dihydropyridine-3-carboxylates proceeded with low enantioselectivity yielding both type of acids as slightly enantioenriched compounds.

Keywords: Ethoxycarbonylmethyl 6-methylsulfanyl-1,4-dihydropyridine-3-carboxylates, 2-cyanoethyl 6-methylsulfanyl-1,4-dihydropyridine-3-carboxylates, one-pot four-component synthesis, hydrolysis, alkylation, *Candida antarctica* lipase B.

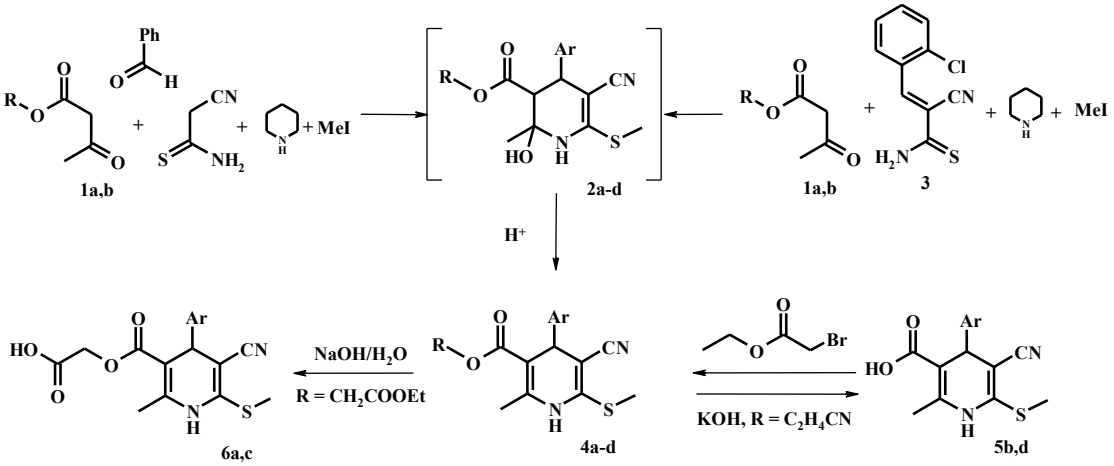
Introduction

1,4-Dihydropyridines (1,4-DHPs) are known as effective calcium channel modulators, especially antagonists[1-5]. 1,4-DHPs, bearing different carbofunctionalities at the positions 3 and 5, possess a stereogenic carbon at position 4 in the 1,4-DHP ring, and enantiomers often show different biological activities[3]. Amongst the methods for preparation of enantiopure compounds, the biotechnological approach based on enzyme-catalysed enantiomeric differentiation has become a promising way for the enantioseparation of 1,4-DHPs[6]. In most cases, the alkoxycarbonyl groups, which are directly attached to the 1,4-DHP ring, do not undergo enzymatic hydrolysis as their reactivity is diminished due to β-aminovinylcarbonyl type conjugation. To get enantiopure compounds, the modification of 1,4-DHP-3-carboxylates with enzymatically labile groups is necessary. The ethoxycarbonylmethyl esters at the position 3 of the 1,4-DHP are readily cleavable by lipases with high enantioselectivity[7,8]. Racemic 6-methylsulfanyl-1,4-DHP-3-carboxylates display antihypertensive or vasodilating activities and low toxicity[9]. Their modification to carboxymethyl 1,4-DHP-carboxylates, enzymatic hydrolysis and alkylation could lead to the desired enantiopure or enantioenriched 6-methylsulfanyl-1,4-DHP-3-carboxylates.

Results and discussion

The choice of methods for synthesis of ethoxycarbonylmethyl 6-methylsulfanyl-1,4-DHP-3-carboxylates **4** was based on our previous work[7,9] and literature data[10]. Bis(ethoxycarbonylmethyl) 1,4-DHP-3,5-carboxylates were prepared by Hantzsch synthesis of ethoxycarbonylmethyl acetoacetate, aromatic aldehyde and ammonia in ethanol in 42-67% yields. By making use of *Candida antarctica* lipase B (CAL-B), they were enzymatically hydrolysed reaching 93% enantiomeric excess[7]. On the other hand, six methods for preparation of 6-methylsulfanyl-1,4-DHP-3-carboxylates are known[9].

To prepare ethoxycarbonylmethyl esters **4**, first of all ethoxycarbonylmethyl 3-oxobutyrate **1a** as key building block was synthesized by esterification of ethyl hydroxyacetate with 4-methyleneoxetan-2-one[11]. 6-Methylsulfanyl-4-phenyl-1,4-dihydropyridine-3-carboxylates **4a,b** (see Scheme 1) were prepared in 59% and 41% yields by condensation of ethoxycarbonylmethyl or 2-cyanoethyl 3-oxobutyrates **1**, benzaldehyde, 2-cyanothioacetamide, piperidine and iodomethane (one-pot five-component, method A). In case of 4-(2-chlorophenyl) substituent, isolation of compound **4c** from reaction mixture by making use of column chromatography is rather complicated. By carrying out the condensation of ethoxycarbonylmethyl or 2-cyanoethyl 3-oxobutyrates **1**, 3-(2-chlorophenyl)-2-cyanothioacrylamide **3**, piperidine and iodomethane (one-pot four-component synthesis, method B), 1,4-DHP **4c** or **4d** crystallizes from reaction mixture in 65% and 64% yield, respectively.



a) Ar = C_6H_5, R = CH_2COOEt; b) Ar = C_6H_5, R = C_2H_4CN;
c) Ar = 2-Cl-C_6H_4, R = CH_2COOEt; d) Ar = 2-Cl-C_6H_4, R = C_2H_4CN

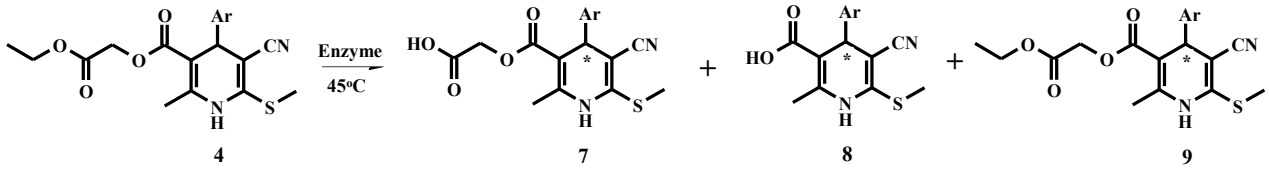
Scheme 1. Synthesis and hydrolysis of ethoxycarbonylmethyl and 2-cyanoethyl 6-methylsulfanyl-
1,4-dihydropyridine-3-carboxylates **4a-d**.

It should be noted that in case of the methods **A** and **B** 6-hydroxy-1,4,5,6-tetrahydropyridines **2** are formed as intermediates. By acidification of the reaction mixture containing compounds **2** and **4**, the dehydration of 6-hydroxy derivatives **2** takes place giving pure 1,4-dihydropyridines **4.**

Hydrolysis of cyanoethyl esters **4b,d** with KOH gave 1,4-DHP-3-carboxylic acids **5b,d** (96% and 92% yields, respectively), but hydrolysis of esters **4a,c** with $NaOH/H_2O$ – (1,4-DHP-3-carbonyloxy)acetic acids **6a,c** (82% and 63% yields, respectively). Acids **5** and **6** were authentic samples because enzymatic hydrolysis could proceed touching "inner" or "outer" ester groups (Scheme 1). Ethoxycarbonylmethyl ester **4a** in 71% yield was obtained also by alkylation of 1,4-dihydropyridine-3-carboxylic acid **5b** in DMF with ethyl bromoacetate (method **C**).

The structures of the compounds were proved by spectroscopic methods. In the IR spectra of 1,4-DHPs **4, 5** and **6**, absorption bands for $\nu_{C\equiv N}$ at 2199-2204 cm^{-1} (5-cyano, β-amino vinyl conjugation) and at 2250-2260 cm^{-1} (3-$CO_2C_2H_4$CN, no conjugation in case of **4b,d**) and bands corresponding to the type of conjugation for $\nu_{C=O}$ are observed. In the ^1H NMR spectra of **4, 5** and **6** the most characteristic are singlets from 4-H protons at 4.73-5.40 ppm, which confirm 1,4-dihydropyridine structure.

As we expected, enzymatic hydrolysis of ethoxycarbonylmethyl 6-methylsulfanyl-1,4-DHP-3-carboxylates **4a,c** yielded both type of acids **7** and **8** (Scheme 2). Hydrolysis of the substrates **4a,c** was performed in the most appropriate reaction conditions taking into account literature data[7] and our experience (phosphate buffer pH 7.5 modified with 15% acetonitrile or dimethyl sulfoxide, water-saturated diisopropyl ether - DIPE), using lipases *Candida antarctica (Novozym 435*® or CAL-B) and *Candida rugosa* (CRL). As the obtained acids and remaining esters are kinetically controlled products, enzyme catalysed hydrolysis was stopped when 45-50% conversion was occurred. When ester **4a** was hydrolysed in presence of CRL, less than 10% of enantiomeric excess was observed both for reaction product - "outer" acid **7a** and for the remaining ester **9a**. Using CRL, no enantioselective hydrolysis was observed for **4c**.



a) Ar = C_6H_5; c) Ar = 2-Cl-C_6H_4

Scheme 2. Enzymatic hydrolysis of ethoxycarbonylmethyl 6-methylsulfanyl-1,4-dihydropyridine-3-carboxylates **4a,c**.

CAL-B is an efficient catalyst for the enantioselective transformations of various kinds of substrates and is widely used in practice[12-15]. When CAL-B was used for enantioseparation of **4a,c** in phosphate buffer pH 7.5 (modified with acetonitrile at 45 °C, Table 1), hydrolysis of both ester groups ("outer" and "inner") of compounds **4a,c** was observed, but contrary to above mentioned literature data for symmetric 1,4-DHPs (CAL-B hydrolyses only the "outer" ester giving 93% enantiomeric excess)[7], the enantiomeric excess for both acids **7** and **8**, and remaining ester **9** was again very low.

Table 1. Enzyme catalysed hydrolysis of **4a,c** in different solvents at 45 °C

Substrate **4**	Enzyme	Solvent	Time, h.	Acid **7**		Acid **8**		Remaining ester **9**	
				Chem. yield, %*	ee, %	Chem. yield, %*	ee, %	Chem. yield, %*	ee, %
4a	Novozym 435®	MeCN/ Buffer	24	33	10	15	2	46	11
4a	*Candida rugosa*	MeCN/ Buffer	110	45	6	-	-	48	5
4a	Novozym 435®	DIPE/ CH_2Cl_2	20	31	-	18	-	47	-
4a	Novozym 435®	DMSO/ Buffer	24	35	-	15	-	46	-

4c	Novozym 435®	MeCN/ Buffer	34	31	9	13	2	45	10
4c	*Candida rugosa*	MeCN/ Buffer	140	49	-	-	-	49	-

* Chemical yields were determined by HPLC.

When enzymatic hydrolysis of the substrates **4a,c** with CAL-B was carried in water-saturated diisopropyl ether or phosphate buffer modified with dimethyl sulfoxide, no enantioseparation was observed. So, new enzymatically labile groups have to be introduced to get enantioseparation for unsymmetrical sulfur containing 1,4-DHPs.

Conclusion

In conclusion, ethoxycarbonylmethyl and 2-cyanoethyl 6-methylsulfanyl-1,4-dihydropyridine-3-carboxylates **4** were synthesized by making use of a one-pot four- and five-component method. Ethoxycarbonylmethyl ester **4a** was obtained also by alkylation of 1,4-dihydropyridine-3-carboxylic acid **5b** with ethyl bromoacetate. By basic hydrolysis of 2-cyanoethyl and ethoxycarbonylmethyl esters **4**, the 1,4-dihydropyridine-3-carboxylic acids **5** and (1,4-dihydropyridine-3-carbonyloxy)acetic acids **6** were prepared, subsequently.

Candida antarctica lipase B catalysed hydrolysis of the unsymmetrical sulfur containing ethoxycarbonylmethyl 1,4-dihydropyridine-3-carboxylates **4a,c** proceeded touching both "inner" and "outer" ester groups yielding slightly enantioenriched acids **7** and **8**.

Experimental

All reagents were purchased from Aldrich or Acros and used without further purification. Lipase B acrylic resin from *Candida antarctica (Novozym 435®)* ≥10,000 U/g, recombinant, expressed in *Aspergillus niger* was used. Melting points were determined on Optimelt MPA100 apparatus and are uncorrected. IR spectra were recorded on a "Shimadzu" IRPrstige-21 spectrometer (in nujol) and peak positions ν_{max} were expressed in cm^{-1}. ^1H NMR spectra were recorded on a Varian Mercury-200 (200 MHz) spectrometer using $CDCl_3$ and DMSO-d_6 as solvents. Chemical shifts are expressed in δ (p.p.m. downfield from TMS) and coupling constants (*J*) in Hz. The course of the reactions and the individuality of substances were monitored by TLC on Kieselgel 60 F Merck plates with dichloromethane/hexane/methanol (5:5:1) as eluent. Determination of enantiomeric excesses of the products **7**, **8** and **9** was performed by direct analysis on a chiral column (R,R)-Whelk-O 1, 4.6x250 mm, 5 μ (Regis) using Shimadzu LC-20AD pump, SPD-M20A diode array detector and Sil-20AC autosampler. The eluent was hexane/dichloromethane/isopropanol with 0.01% acetic acid (80:10:10) and flow rate 1.0 ml/min. Enzymatic reactions were carried out in a New Brunswick Scientific G24 environmental incubatory orbital shaker. Compounds were recrystallized from ethanol.

General procedure for synthesis of ethoxycarbonylmethyl (2-cyanoethyl) 5-cyano-2-methyl-6-methylsulfanyl-4-phenyl-1,4-dihydropyridine-3-carboxylates (4a,b) (Method A).

A mixture of 4 mmol of ethoxycarbonylmethyl 3-oxobutyrate **1a** or 2-cyanoethyl 3-oxo-butyrate **1b**, 4 mmol of aldehyde and 0.3 mmol of piperidine in 15 ml of ethanol was stirred for 5 min. Then 4 mmol of 2-cyanothioacetamide and 4.3 mmol of piperidine were added and the mixture was stirred for 25 min. After the 4.3 mmol of methyl iodide were added, the resulting reaction mixture was shortly heated till reflux, cooled to 30-40 ^oC and acidified with 1 ml of 3N hydrochloric acid in ethanol and stirred for 1 h at room temperature. The precipitate was filtered, washed with 5 ml of cold (ca. 5 °C) ethanol and 20 ml of water to give products **4a,b**.

Ethoxycarbonylmethyl 5-cyano-2-methyl-6-methylsulfanyl-4-phenyl-1,4-dihydropyridine-3-carboxylate (4a). Yellow crystals, yield 59%, mp 129-130 °C. IR: 1681, 1756 (C=O); 2200 (C≡N); 3066, 3270 (NH). ^{1}H NMR ($CDCl_3$): 1.23 and 4.16 (t and q, J = 7 Hz, 5H, Et); 2.41 (s, 3H, 2-Me); 2.48 (s, 3H, SMe); 4.54 (AB quartet, J = 16 Hz, 2H, 3-$COOCH_2$); 4.75 (s, 1H, 4-H); 6.09 (s, 1H, NH); 7.20-7.38 (m, 5H, 4-Ph). Anal. Calcd. for $C_{19}H_{20}N_2O_4S$: C 61.27, H 5.41, N 7.52, S; 8.61 Found: C 60.75; H 5.40; N 7.46; S 8.69.

2-Cyanoethyl 5-cyano-2-methyl-6-methylsulfanyl-4-phenyl-1,4-dihydropyridine-3-carboxylate (**4b).** Yellow crystals, yield 41%, mp 160-162 °C. IR: 1675, (C=O); 2197, 2260 (C≡N); 3316 (NH). ^{1}H NMR ($CDCl_3$): 2.33 (s, 3H, 2-Me); 2.44 (s, 3H, SMe); 2.47 and 4.14 (t and t, J = 6.5 Hz, 4H, 3-$COOCH_2CH_2CN$); 4.59 (s, 1H, 4-H); 6.23 (s, 1H, NH); 7.16-7.27 (m, 5H, 4-Ph). Anal. Calcd. for $C_{18}H_{17}N_3O_2S$: C 63.70, H 5.05, N 12.38, S 9.45; Found: C 63.65, H 5.17, N 12.30, S 9.34.

General procedure for synthesis of ethoxycarbonylmethyl (2-cyanoethyl) 4-(2-chlorophenyl)-5-cyano-2-methyl-6-methylsulfanyl-1,4-dihydropyridine-3-carboxylates (4c,d) (Method B).

A mixture of 4 mmol of ethoxycarbonylmethyl 3-oxo-butyrate **1a** or 2-cyanoethyl 3-oxo-butyrate **1b**, 4 mmol of 3-(2-chlorophenyl)-2-cyanothioacrylamide and 4.3 mmol of piperidine in 15 ml of ethanol was stirred for 25 min. Then 4.3 mmol of methyl iodide was added, the resulting reaction mixture was shortly heated until reflux, cooled to 30-40 °C, acidified with 1 ml of 3N hydrochloric acid in ethanol and stirred for 1 h at room temperature. The precipitate was filtered, washed with 5 ml of cold (ca. 5 °C) ethanol and 20 ml of water to give products **4c,d**.

Ethoxycarbonylmethyl 4-(2-chlorophenyl)-5-cyano-2-methyl-6-methylsulfanyl-1,4-dihydro-pyridine-3-carboxylate (4c). White crystals, yield 65%, mp 144-145 °C. IR: 1684, 1728 (C=O); 2199 (C≡N); 3336, (NH). ^{1}H NMR ($CDCl_3$): 1.14 and 4.07 (t and q, J = 7 Hz, 5H, Et); 2.34 (s, 3H, 2-Me); 2.40 (s, 3H, SMe); 4.44 (AB quartet, J = 16 Hz, 2H, 3-$COOCH_2$); 5.29 (s, 1H, 4-H); 6.20 (s, 1H, NH); 7.05-7.30 (m, 4H, 4-C_6H_4). Anal. Calcd. for $C_{19}H_{19}ClN_2O_4S$: C 56.09, H 4.71, N 6.88, S 7.88; Found: C 56.01, H 4.79, N 6.81, S 7.92.

2-Cyanoethyl ester 4-(2-chlorophenyl)-5-cyano-2-methyl-6-methylsulfanyl-1,4-dihydro-pyridine-3-carboxylate (4d). White crystals, yield 64%, mp 151-153 °C. IR: 1654, (C=O); 2199, 2250 (C≡N); 3299 (NH). ^{1}H NMR ($CDCl_3$): 2.35 (s, 3H, 2-Me); 2.41 (s, 3H, SMe); 2.48 and 4.11 (t and t, J = 6.5 Hz, 2H, 3-$COOCH_2CH_2CN$); 5.23 (s, 1H, 4-H); 6.08 (s, 1H, NH); 7.10-7.34 (m, 4H, 4-C_6H_4). Anal. Calcd. for $C_{18}H_{16}ClN_3O_2S$: C 57.83, H 4.31, N 11.24, S 8.58; Found: C 57.80, H 4.36, N 11.16, S 8.51.

General procedure for synthesis of 4-aryl-5-cyano-2-methyl-6-methylsulfanyl-1,4-dihydropyridine-3-carboxylic acids (5b,d).

A mixture of 1 mmol of cyanoethyl 6-methylsulfanyl-1,4-dihydropyridine-3-carboxylates **4b,d** and 1.3 mmol of KOH in 10 ml of absolute ethanol was stirred at ambient temperature. After 4 h the mixture was acidified until pH ~ 2 with 2M HCl/H_2O. The

precipitate was filtered, washed with 5 ml of cold (ca. 5 °C) ethanol and 15 ml of water to give products **5b,d**.

5-Cyano-2-methyl-6-methylsulfanyl-4-phenyl-1,4-dihydropyridine-3-carboxylic acid (5b). White crystals, yield 96%, mp 182-184 °C. IR: 1675 (C=O); 2211 (C≡N); 3198, 3267, (NH, OH). ^{1}H NMR (DMSO-d_6): 2.26 (s, 3H, 2-Me); 2.43 (s, 3H, SMe); 4.41 (s, 1H, 4-H); 7.07-7.28 (m, 5H, 4-Ph); 9.27 (s, 1H, NH); 11.95 (s, 1H, OH). Anal. Calcd. for $C_{15}H_{14}N_2O_2S$: C 62.92, H 4.93, N 9.78, S 11.20; Found: C 62.96, H 4.94, N 9.58, S 11.10.

4-(2-Chlorophenyl)-5-cyano-2-methyl-6-methylsulfanyl-1,4-dihydropyridine-3-carboxylic acid (5d). White crystals, yield 92%, mp 191-192 °C. IR: 1697, (C=O); 2194 (C≡N); 3326 (NH). ^{1}H NMR (DMSO-d_6): 2.28 (s, 3H, 2-Me); 2.41 (s, 3H, SMe); 5.00 (s, 1H, 4-H); 7.16-7.33 (m, 4H, 4-C_6H_4); 9.27 (s, 1H, NH); 11.87 (s, 1H, OH). Anal. Calcd. for $C_{15}H_{13}ClN_2O_2S$: C 56.16, H 4.08, N 8.73, S 10.00; Found: C 56.33, H 4.00, N 8.63, S 9.91.

Ethoxycarbonylmethyl 5-cyano-2-methyl-6-methylsulfanyl-4-phenyl-1,4-dihydropyridine-3-carboxylate 4a (method C). A mixture of 1 mmol of 6-methylsulfanyl-1,4-dihydropyridine-3-carboxylic acid **(5b)** and 1 mmol of Et_3N in 10 ml of ethanol and 3 ml of DMF was shortly refluxed and stirred for 12 h at room temperature. The precipitate was flash chromatographed with chloroform/hexane/acetone (2:1:1) to give product **4a** as colourless crystals, yield 71%, mp 129-130 °C.

General procedure for synthesis of 4-aryl-5-cyano-2-methyl-6-methylsulfanyl-(1,4-dihydropyridine-3-carbonyloxy)acetic acids (6a,b).

A mixture of 1 mmol of ethoxycarbonylmethyl 6-methylsulfanyl-1,4-dihydropyridine-3-carboxylates **4a,c** and 1 mmol of 3N NaOH/H_2O in 10 ml of ethanol was stirred at the ambient temperature. After 8 h the mixture was acidified until pH ~ 2 with 2M HCl/H_2O. The precipitate was filtered, washed with 5 ml of cold (ca. 5 °C) ethanol and 20 ml of water to give acid **6**.

5-Cyano-2-methyl-6-methylsulfanyl-4-phenyl-(1,4-dihydropyridine-3-carbonyloxy)acetic acid (**6a).** White crystals**,** yield 82%, mp 163-164 °C. IR: 1687, 1775 (C=O); 2200 (C≡N); 3188, 3273, (NH, OH). ^{1}H NMR ($CDCl_3$): 2.41 (s, 3H, 2-Me); 2.48 (s, 3H, SMe); 4.58 (AB quartet, J = 16 Hz, 2H, 3-$COOCH_2$); 4.73 (s, 1H, 4-H); 6.18 (s, 1H, NH); 7.10-7.38 (m, 5H, 4-Ph). Anal. Calcd. for $C_{17}H_{16}N_2O_4S$: C 59.29, H 4.68, N 8.13, S; 9.31 Found: C 59.75; H 4.57; N 8.03; S 9.27.

4-(2-Chlorophenyl)-5-cyano-2-methyl-6-methylsulfanyl-(1,4-dihydropyridine-3-carbonyloxy)-acetic acid (6b). White crystals**,** yield 63%, mp 119-121 °C. IR: 1644, 1717 (C=O); 2193 (C≡N); 3325, 3625 (NH, OH). ^{1}H NMR (DMSO-d_6): 2.27 (s, 3H, 2-Me); 2.40 (s, 3H, SMe); 4.33 (AB quartet, J = 16 Hz, 2H, 3-$COOCH_2$); 5.05 (s, 1H, 4-H); 7.06-7.36 (m, 4H, 4-C_6H_4); 9.50 (s, 1H, NH). Anal. Calcd. for $C_{17}H_{15}ClN_2O_4S$: C 53.90, H 3.99, N 7.39, S 8.46; Found: C 53.78, H 4.12, N 7.33, S 8.40.

General procedure for enzymatic hydrolysis of ethoxycarbonylmethyl 6-methylsulfanyl-1,4-dihydropyridine-3-carboxylates 4a,c.

0.2 mmol of 1,4-dihydropyridine **4a,c** were dissolved in 20 ml of solvent system and heated to 45 ^{o}C, after which 80 mg of enzyme was added. The resulting mixture was shaken at 350 rpm and heated at 45 ^{o}C. Reactions were monitored by HPLC and were stopped when 45-50% of acids were formed. Solvent systems: 3 ml of acetonitrile or dimethyl sulfoxide and 17 ml of 20 mM K_2HPO_4/KH_2PO_4 buffer, pH 7.5, 3 ml of dichloromethane and 17 ml of water-saturated diisopropyl ether. The results are combined in Table 1.

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SYNTHESIS AND PROPERTIES OF PARTIALLY HYDROGENATED ETHYL ([3,4']BIPYRIDIN-6'-YLSULFANYL) ACETATES

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Ethyl 5'-cyano-6'-ethoxycarbonylmethylsulfanyl-2'-hydroxy-2'-phenyl-1',2',3',4'-tetrahydro[3,4']bipyridine-3'-carboxylate (**3**) and the corresponding ethyl 1',4'-dihydro[3,4']bipyridine-3'-carboxylate (**4**) as potential cardiovascular agents have been prepared by alkylation of 2'-hydroxy-1',2',3',4'-tetrahydro[3,4']bipyridine-6'-thiolate **1** or betaine **2** with ethyl bromoacetate. The treatment of [3,4']bipyridine **4** with KOH/H_2O gave diethyl 3-amino-6-phenyl-4-(pyridin-3-yl)-4,7-dihydro-thieno[2,3-*b*]pyridine-2,5-dicarboxylate (**5)**, but subsequent treatment of the remaining reaction mixture with acetic acid excess gave ethyl 8-cyano-3-oxo-5-phenyl-7-(pyridin-3-yl)-2,3-dihydro-7H-thiazolo[3,2-*a*]pyridine-6-carboxylate (**7)** – the product of intramolecular acylation**.** Compounds **4**, **5** and **7** have been prepared by making use of one-pot synthesis method. Alkylation of [3,4']bipyridine **4** with iodomethane gave 1',4'-dihydro[3,4']bipyridin-1-ium iodide **8**.

Key words: [3,4']bipyridine, piperidinium pyridine-6-thiolate, 7H-thiazolo[3,2-a]pyridine, thieno[2,3-b]pyridine, [3,4']bipyridin-1-ium iodide.

INTRODUCTION

6-Alkylsulfanyl-1,4-dihydropyridines (DHPs) display antihypertensive and vasodilating [1–3], hepatoprotective [4], antioxidant [5], and antiradical [6] activities, however, the biological activities of these compounds in comparison with DHPs not containing sulfur atom are still insufficiently studied.

On the other hand, [3,4']bipyridines are of interest as cardiotonic agents [7] for more than 25 years. Among them milrinone, phosphodiesterase III inhibitor, is non-catecholamine, non-glycoside drug that is not associated with β-adrenergic receptors or plasma catecholamine concentrations [8-10]. Recently compounds and pharmaceutical compositions for treatment of myosin heavy chain mediated diseases, and in particular, heart failure, have been found in series of 3-acetyl-6-benzoylsulfanyl-1,4-DHPs [11]. The potential cardioto-nics – 1',4'-dihydro[3,4']bipyridines bearing carbamoylmethylsulfanyl substituent in position 6' appeared to be not enough soluble and lipophilic [12].

To improve cardiovascular activity of the low-toxic 4-aryl-5-cyano-2-methyl-6-methylsulfanyl-1,4-DHP-3-carboxylates [3], lipophilicity was optimized by introducing another ester group in the 1,4-DHP molecule [13], which is in agreement with general observations for DHPs series [14].

Partially hydrogenated ethyl (5'-ethoxycarbonyl[3,4']bipyridin-6'-ylsulfanyl) acetates bearing two ester groups could be interesting from this point of view as potential cardiotonics.

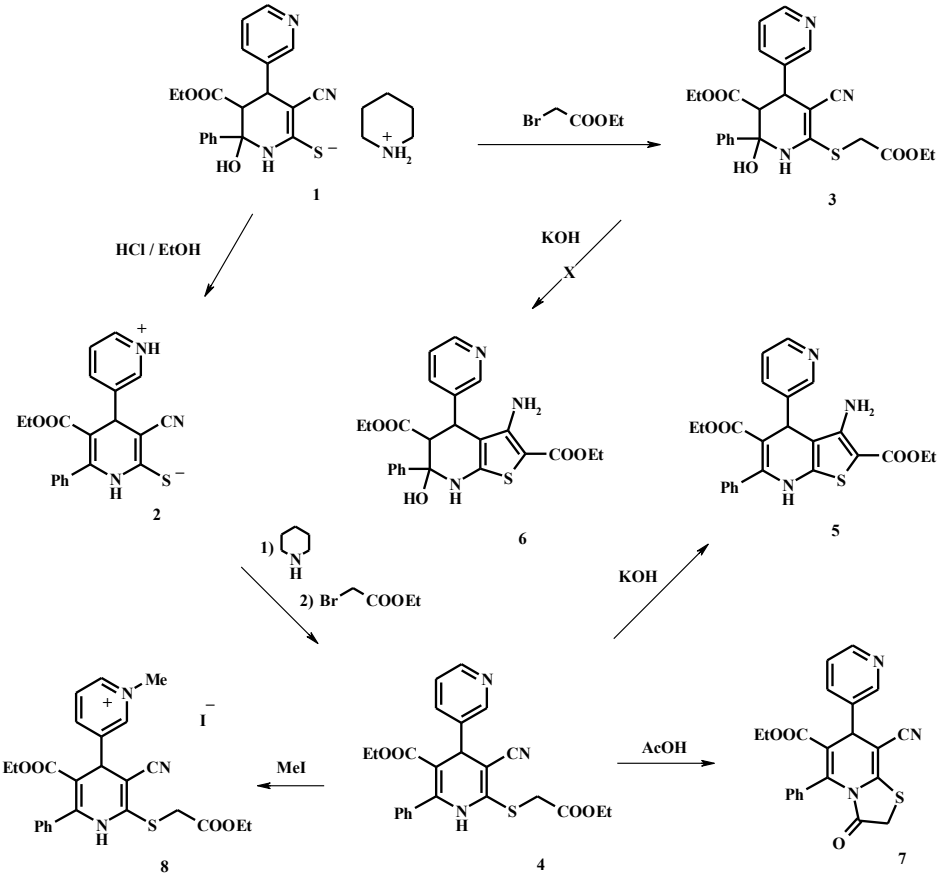
EXPERIMENTAL

Melting points were determined on a *Boetius* apparatus and were used uncorrected. IR spectra were recorded on a *Perkin-Elmer 580 B* spectrometer (in nujol) and peak positions ν_{max} were measured in cm^{-1}. NMR spectra were recorded on a *Varian Mercury 200* spectrometer using hexamethyldisiloxane (HMDSO) as internal standard. The course of the reactions and the individuality of substances were monitored by TLC on Kieselgel 60 F Merck plates with dichloromethane – hexane – acetone (2:1:1) as eluent. Synthesis of piperidinium 1',2',3',4'-tetrahydrobipyridine-6'-thiolate **1** (yield 72%) and betaine **2** (yield 80%) is described in [12]. Compounds were recrystallized from ethanol.

ETHYL 5'-CYANO-6'-ETHOXYCARBONYLMETHYLSULFANYL-2'-HYDROXY-2'-PHENYL-1',2',3',4'-TETRAHYDRO[3,4']BIPYRIDINE-3'-CARBOXYLATE (3).

Mixture of piperidinium 1',2',3',4'-tetrahydrobipyridine-6'-thiolate **1** [12] (4.67 g, 10 mmol) and ethyl bromoacetate (1.10 ml, 10 mmol) in 20 ml of ethanol was shortly heated until dissolution,

stirred for 30 min at ambient temperature, and water (2 ml) was added. The precipitate was filtered, washed with ethanol (5 ml) and water (20 ml) to give 3.83 g (82%) of compound **3** as colourless powder; mp 172–174 °C. IR spectrum, ν, cm^{-1}: 1700, 1738 (C=O); 2201 (C≡N); 3282, 3456 (NH, OH); ^{1}H NMR spectrum (DMSO-d_6; δ, ppm): 0.40 and 3.35 (5H, t and q, *J* = 7.0 Hz, 3'-COOEt); 1.17 and 4.11 (5H, t and q, *J* = 7.0 Hz, 6'-SCH_2COOEt); 2.90 and 4.12 (2H, d and d, *J* = 12.1 Hz, 3'-H and 4'-H); 3.86 and 3.97 (2H, d and d, *J* = 15.7 Hz, SCH_2); 6.49 (1H, s, OH); 7.27–8.40 (9H, m, 2'-C_6H_5 and 2-, 4-, 5- and 6-H); 8.08 (1H, s, NH). Found, %: C 61.30, H 5.44, N 8.83, S 6.72. Calculated for $C_{24}H_{25}N_3O_5S$, %: C 61.66, H 5.39, N 8.99, S 6.86.



ONE-POT REACTIONS OF BETAINE 2 WITH ETHYL BROMOACETATE, POTASSIUM HYDROXIDE AND ACETIC ACID.

To the suspension of betaine **2** [12] (3.63 g, 10 mmol) in 10 ml of ethanol, piperidine (1.0 ml, 10 mmol) and ethyl bromoacetate (1.10 ml, 10 mmol) were added, the mixture was shortly heated until dissolution, stirred for 30 min at ambient temperature, and water (20 ml) was added. The reaction mixture was treated with 10 ml of 2 *N* potassium hydroxide solution, refluxed for 5 min, cooled to ~ 0 °C and precipitate was filtered, washed with ethanol (5 ml) and water (20 ml) to give 0.55 g (12%) of **diethyl 3-amino-6-phenyl-4-(pyridin-3-yl)-4,7-dihydrothieno[2,3-*b*]pyridine-2,5-dicarboxylate** (**5)** as slightly yellow powder; mp 200–203 °C. IR spectrum, ν, cm^{-1}: 1650, 1660 (C=O); 3320, 3460 (NH, NH_2); ^{1}H NMR spectrum (DMSO-d_6; δ, ppm): 0.63 and 3.61 (5H, t and q, *J* = 7.0 Hz, 5-COOEt); 1.13 and 4.05 (5H, t and q, *J* = 7.0 Hz, 2-COOEt); 5.16 (1H, s, 4-H); 6.38 (2H, s, NH_2); 7.24–8.60 (9H, m, 6-C_6H_5 and 4-C_5H_4N); 10.05 (1H, s, NH). Found, %: C 63.70, H 5.27, N 9.20, S 7.28. Calculated for $C_{24}H_{23}N_3O_4S$, %: C 64.13, H 5.16, N 9.35, S 7.13.

The remaining reaction mixture was refluxed for 5 min with 2 ml of acetic acid, cooled to ~ 10 °C and precipitate was filtered, washed with ethanol (5 ml) and water (20 ml) to give 1.05 g (23%) of **ethyl 5'-cyano-6'-ethoxycarbonylmethylsulfanyl-2'-phenyl-1',4'-dihydro-[3,4']bipyridine-3'-carboxylate (4)** as slightly yellow powder; mp 128–129 °C. IR spectrum, ν, cm^{-1}: 1684, 1735 (C=O);

2198 (C≡N); 3148, 3240 (NH); ^{1}H NMR spectrum (DMSO-d_6; δ, ppm): 0.65 and 3.65 (5H, t and q, *J* = 7.0 Hz, 3'-COOEt); 1.14 and 4.07 (5H, t and q, *J* = 7.0 Hz, 6'-$SCH_2COO\underline{Et}$); 3.79 and 4.05 (2H, d and d, *J* = 15.3 Hz, SCH_2); 4.58 (1H, s, 4'-H); 7.27–8.46 (9H, m, 2'-C_6H_5 and 2-, 4-, 5- and 6-H); 9.98 (1H, s, NH). Found, %: C 63.90, H 5.14, N 9.27, S 7.18. Calculated for $C_{24}H_{23}N_3O_4S$, %: C 64.13, H 5.16, N 9.35, S 7.13.

The remaining second filtrate was refluxed for 5 h with 2 ml of acetic acid, cooled to ~ 10 °C and precipitate was filtered, washed with ethanol (5 ml) to give 0.20 g (5%) of **ethyl 8-cyano-3-oxo-5-phenyl-7-(pyridin-3-yl)-2,3-dihydro-7*H*-thiazolo[3,2-*a*]pyridine-6-carboxylate** (**7)** as slightly yellow powder; mp 168–170 °C [15]. IR spectrum, ν, cm^{-1}: 1672, 1757 (C=O); 2206 (C≡N); ^{1}H NMR spectrum (DMSO-d_6; δ, ppm): 0.53 and 3.52 (5H, t and q, *J* = 7.0 Hz, 6-COOEt); 3.97 and 4.04 (2H, d and d, *J* = 17.2 Hz, 2-CH_2); 4.80 (1H, s, 7-H); 7.24–8.54 (9H, m, 5-C_6H_5 and 7-C_5H_4N).

TREATMENT OF PURE ETHYL 5'-CYANO-6'-ETHOXYCARBONYLMETHYLSULFANYL-2'-PHENYL-1',4'-DIHYDRO[3,4']BIPYRIDINE-3'-CARBOXYLATE (4) WITH KOH.

Mixture of carboxylate **4** (0.31 g, 0.69 mmol) and KOH (0.04 g, 0.71 mmol) in 10 ml of absolute ethanol was refluxed for 5 min, cooled to ~ 10 °C and precipitate was filtered, washed with ethanol (2 ml) and water (10 ml) to give 0.24 g (79%) of **diethyl 4,7-dihydrothieno[2,3-*b*]pyridine-2,5-dicarboxylate (5).**

5'-CYANO-3'-ETHOXYCARBONYL-6'-ETHOXYCARBONYLMETHYLSULFANYL-1-METHYL-2'-PHENYL-1',4'-DIHYDRO[3,4']BIPYRIDIN-1-IUM IODIDE (8).

Mixture of 1',4'-dihydro-[3,4']bipyridine-3'-carboxylate **4** (0.23 g, 0.5 mmol) and iodomethane (0.62 ml, 10 mmol) in 10 ml of acetone was refluxed for 5 h, the reaction mixture was evaporated to dry and treated with 10 ml of ethanol. The precipitated crystals were filtered to give 0.22 g (74%) of **1-methyl-1',4'-dihydro[3,4']bipyridin-1-ium iodide 8** as yellow powder; mp 95-98 °C. IR spectrum, ν, cm^{-1}: 1635, 1676, 1720 (C=O); 2201 (C≡N); 3250 (NH). ^{1}H NMR spectrum (DMSO-d_6; δ, ppm): 0.64 and 3.66 (5H, t and q, *J* = 7.0 Hz, 3'-COOEt); 1.15 and 4.09 (5H, t and q, *J* = 7.0 Hz, 6'-$SCH_2COO\underline{Et}$); 3.82 and 4.08 (2H, d and d, *J* = 15.3 Hz, SCH_2); 4.35 (3H, s, 1-Me); 4.90 (1H, s, 4'-H); 7.32–8.87 (9H, m, 2'-C_6H_5 and 4-C_6H_4N); 10.16 (1H, s, NH). Found, %: C 50.31, H 4.16, N 6.87. Calculated for $C_{25}H_{26}IN_3O_4S$, %: C 50.77, H 4.43, N 7.10.

RESULTS AND DISCUSSION

In continuation of search for biologically active compounds, we have synthesized new [3,4']bipyridines – ethyl 6'-ethoxycarbonylmethylsulfanyl-1',2',3',4'-tetrahydro-[3,4']bipyridine-3'-carboxylate **3**, the corresponding 1',4'-dihydro[3,4']bipyridine **4** and investigated their transformations by treatment with KOH, acetic acid and iodomethane.

Piperidinium 5'-cyano-3'-ethoxycarbonyl-2'-hydroxy-2'-phenyl-1',2',3',4'-tetrahydro[3,4']bipyridine-6'-thiolate (1) has been obtained by three-component condensation of ethyl benzoylacetate, 3-pyridinecarboxaldehyde, 2-cyanothioacetamide and piperidine with 72% yield [12]. It is worth to mention that application of N-methylmorpholine instead of piperidine leads to the corresponding 1',4'-dihydrobipyridine-6'-thiolate [16]. Alkylation of 2'-hydroxy-1',2',3',4'-tetrahydro[3,4']bipyridine-6'-thiolate 1 bearing many nucleophilic reaction centres (O, S, N(1), N(1') and C(5')) under mild reaction conditions with ethyl bromoacetate proceeds preferably at the sulfur atom giving rise to ethyl 5'-cyano-6'-ethoxycarbonylmethylsulfanyl-2'-hydroxy-2'-phenyl-1',2',3',4'-tetrahydro[3,4']bipyridine-3'-carboxylate (3).

Usually, the corresponding 1,4-dihydropyridines are easily prepared by dehydration of 2-hydroxy-1,2,3,4-tetrahydropyridines, but treatment of 2'-hydroxy-1',2',3',4'-tetrahydro[3,4']bipyridine 3 with HCl/EtOH similarly as mentioned in [17] leads to the complicated reaction mixture, which contains products of ring cleavage and hydrolysis. By treatment of bipyridine 3 with KOH/H_2O, the desired 6-hydroxy-4,5,6,7-tetrahydrothieno[2,3-*b*]pyridine 6 was not isolated from the complicated reaction mixture.

Alkylation of betaine 2 [12] (prepared by treatment of 1',2',3',4'-tetrahydrobipyridine-6'-thiolate 1 with HCl/EtOH) with ethyl bromoacetate in the presence of stoichiometric amount of piperidine gave crude ethyl 6'-ethoxycarbonylmethylsulfanyl-1',4'-dihydro[3,4']bipyridine-3'-carboxylate 4 which did not crystallize from reaction mixture. The treatment of the latter with KOH/H_2O gave the desired diethyl 3-amino-6-phenyl-4-(pyridin-3-yl)-4,7-dihydrothieno[2,3-*b*]pyridine-2,5-dicarboxylate (5) with 12% yield (79% yield of compound 5 was reached, when pure 1',4'-dihydrobipyridine 4 was used), but subsequent treatment of the remaining reaction mixture with acetic acid gave pure 1',4'-dihydrobipyridine 4 (5 min reflux) and the product of intramolecular acylation – ethyl 8-cyano-3-oxo-5-phenyl-7-(pyridin-3-yl)-2,3-dihydro-7H-thiazolo[3,2-*a*]pyridine-6-carboxylate (7) (5 h reflux). By treatment of ethyl 1',4'-dihydro[3,4']bipyridine-3'-carboxylate 4 with iodomethane in dry acetone, dihydro[3,4']bipyridinium salt 8 was formed. The low yield of 1',4'-dihydrobipyridine 4 (complicated isolation) in the case when betaine 2 was treated with stoichiometric amount of ethyl bromoacetate is, obviously, explained by competitive alkylation both at S atom and N(1) atom of the pyridine ring.

The structures of the synthesized compounds were confirmed by spectroscopic methods. In the IR spectra, the characteristic absorption bands of C≡N group are observed for 1',2',3',4'-tetrahydrobipyridine 3, 1',4'-dihydrobipyridines 4, 8 and 7H-thiazolo[3,2-*a*]pyridine 7 at 2198–2206 cm^{-1}. Absorption bands of C=O groups of the compounds 3-5 and 7 are in agreement with the type of conjugation of C=O groups. The doublets in the ^1H NMR spectrum of compound 3 with $J_{3,4}$ = 12.1 Hz according to [18] confirm *trans*-diaxial configuration of the 3'-H and 4'-H protons. In the case of 1',4'-dihydrobipyridines 4, 8, 4,7-dihydrothieno[2,3-*b*]pyridine 5 and 7H-thiazolo[3,2-*a*]pyridine 7, the characteristic 4'-H, 4-H or 7-H proton signals at 4.58–5.18 ppm are observed.

CONCLUSIONS

Ethyl 5'-cyano-6'-ethoxycarbonylmethylsulfanyl-2'-hydroxy-2'-phenyl-1',2',3',4'-tetrahydro[3,4']bipyridine-3'-carboxylate (**3**) and the corresponding ethyl 1',4'-dihydro[3,4']bipyridine-3'-carboxylate (**4**) as potential cardiovascular agents have been prepared by alkylation of 2'-hydroxy-1',2',3',4'-tetrahydro[3,4']bipyridine-6'-thiolate **1** or betaine **2** with ethyl bromoacetate. The treatment of ethyl 5'-cyano-6'-ethoxycarbonylmethylsulfanyl-2'-phenyl-1',4'-dihydro[3,4']bipyridine-3'-carboxylate **(4)** with KOH/H_2O gave diethyl 3-amino-6-phenyl-4-(pyridin-3-yl)-4,7-dihydrothieno[2,3-*b*]pyridine-2,5-dicarboxylate (**5)**, but subsequent treatment of the remaining reaction mixture with acetic acid excess gave ethyl 8-cyano-3-oxo-5-phenyl-7-(pyridin-3-yl)-2,3-dihydro-7H-thiazolo[3,2-*a*]pyri-dine-6-carboxylate (**7)** – the product of intramolecular acylation**.** Compounds **4**, **5** and **7** have been prepared by making use of one-pot synthesis method. Alkylation of [3,4']bipyridine **4** with iodomethane gave 1',4'-dihydro[3,4']bipyridin-1-ium iodide **8**.

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SYNTHESIS AND PROPERTIES OF METHYL 6-ALKYLSULFANYL-4-(2-CHLOROPHENYL)-1,4-DIHYDROPYRIDINE-3-CARBOXYLATES

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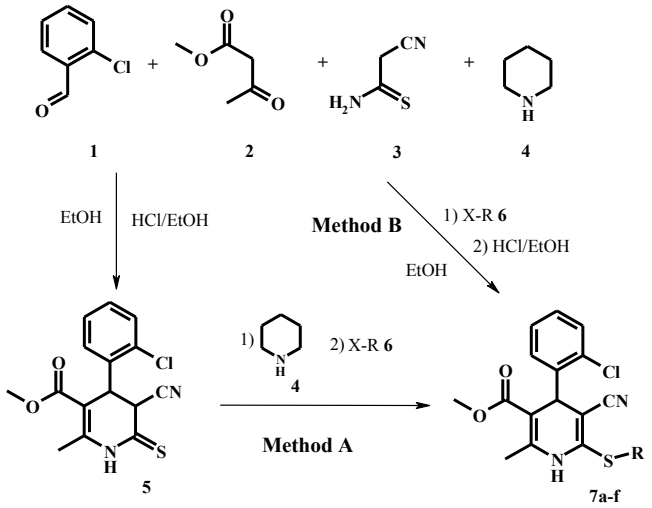
Methyl 6-alkylsulfanyl-1,4-dihydropyridine-3-carboxylates **7** with optimum lipophilicity have been obtained by treatment of methyl 4-(2-chlorophenyl)-6-thioxo-1,4,5,6-tetrahydropyridine-3-carboxylate **5** with alkyl halides **6**. Preparation of target compounds **7** was improved by making use one-pot five-component synthesis. Methyl 6-ethylsulfanyl-, 6-cyclopropylmethylsulfanyl- and 6-isobutylsulfanyl-1,4-dihydropyri-dine-3-carboxylates **7b**,**e**,**f** have shown calcium channel blocking activity in *in vitro* models – SH-SY5Y cell lines (human neuroblastoma).

Key words: 1,4-Dihydropyridine-3-carboxylate, 6-thioxo-1,4,5,6-tetrahydropyridine, one-pot five-component synthesis, calcium channel blocking activity.

INTRODUCTION

6-Alkylsulfanyl-1,4-dihydropyridines display antioxidant [1], hepatoprotective [2] and antiradical [3] activities. 6-Methylsulfanyl-1,4-dihydropyridine-3-carboxylates possess pronounced coronary circulation-stimulating and blood pressure-decreasing activities [4,5]. Moreover, the advantage of 1,4-dihydropyridines (DHP) containig sulfur atom is their low toxicity [5].

This study was performed in order to synthesize methyl 6-alkylsulfanyl-4-(2-chlorophenyl)-1,4-dihydropyridine-3-carboxylates and test their calcium channel blocking activity in *in vitro* models (SH-SY5Y cell lines – human neuroblastoma). To improve their cardiovascular activity, various alkyl groups were introduced in the position 6 in the 1,4-DHP molecule.



X = Br, I

a) R=Me, b) R=Et, c) R=Pr, d) R=*i*-Pr, e) R=$CH_2CH(CH_2)_2$, f) R=$CH_2CH(CH_3)_2$

EXPERIMENTAL

All reagents were purchased from *Aldrich, Acros, Fluka* or *Merck* and used without further purification. TLC was performed on 20×20 cm Silica gel TLC-PET F254 foils (*Fluka*). NMR spectra were recorded with a *Varian Mercury 200BB* spectrometer (200 MHz). Chemical shifts are reported in ppm relative to hexamethyldisiloxane (δ 0.055). Multiplicities are abbreviated as: s, singlet; d,

doublet; t, triplet; q, quartet; m, multiplet; br, broad. The coupling constants are expressed in Hz. Melting points were determined on an *OptiMelt* (*SRS Stanford Research Systems*). Elemental analyses were performed on an *EA 1106* (*Carlo Erba Instruments*). IR spectra have been recorded on a *Perkin-Elmer 580 B* spectrometer (in nujol) and peak positions ν_{max} were expressed in cm^{-1}. Compounds were recrystallized from methanol.

Synthesis of 5-cyano-4-(2-chlorophenyl)-2-methyl-6-thioxo-1,4,5,6-tetrahydropyridine-3-carboxylic acid methyl ester (5)

To stirred mixture of aromatic aldehyde **1** (2.0 mmol), methyl acetoacetate **2** (2.0 mmol), 2-cyanothioacetamide **3** (2.0 mmol) in ethanol (20 ml), piperidine **4a** (2.0 mmol) was added. The mixture was stirred at room temperature for 1 h, and then 5 ml of 3 *N* hydrochloric acid in ethanol were added and stirred for 1 h at room temperature. The precipitate was filtered and washed with 10 ml of ethanol and 10 ml of water to give 0.53 g (84%), of compound **5** as yellow powder; m.p. 142–143 °C. IR spectrum, ν, cm^{-1}: 1690 (C=O); 2258 (C≡N); 3242 (NH). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 2.45 and 2.56 (3H, s and s, *cis*- and *trans*-2-Me); 3.59 and 3.61 (3H, s and s, *cis*- and *trans*-OMe); 4.18 and 4.88 (1H and 1H, d and d, J = 1.96 Hz, *trans*-4-H and 5-H); 4.19 and 5.15 (1H and 1H, d and d, J = 7.43 Hz, *cis*-4-H and 5-H); 6.82-7.38 (4H, m, C_6H_4Cl); 8.67 (1H, br.s, NH).

Elemental analysis data.

Calculated for $C_{15}H_{13}ClN_2O_2S$, %: C 56.16; H 4.08; N 8.73. Found, %: C 56.14; H 3.86; N 8.70.

Synthesis of methyl 4-(2-chlorophenyl)-5-cyano-2-methyl-6-alkylsulfanyl-1,4-dihydropyridine-3-carboxylates (7). General method.

Mixture of 6-thioxo-1,4,5,6-tetrahydropyridine-3-carboxylate **5** (0.32 g, 1.0 mmol) and alkyl halide **6** (1.0 mmol) in 7 ml of ethanol was shortly heated until dissolution, stirred for 1 h at ambient temperature. The precipitate was filtered, washed with ethanol (5 ml) and water (5 ml).

Methyl 4-(2-chlorophenyl)-5-cyano-2-methyl-6-methylsulfanyl-1,4-dihydropyridine-3-carboxylate (7a)

Yield 0.31 g (93%), colourless powder; m.p. 210–212 °C. IR spectrum, ν, cm^{-1}: 1695 (C=O); 2200 (C≡N); 3200, 3260 (NH). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 2.38 (3H, s, 2-Me); 2.46 (3H, s, 6-S-Me); 3.54 (3H, s, OMe); 5.30 (1H, s, 4-H); 6.00 (1H, s, NH); 7.00-7.50 (4H, m, C_6H_4).

Found, %: C 57.34; H 4.26; N 8.59; S 9.55. Calculated for $C_{16}H_{15}ClN_2O_2S$, %: C 57.40; H 4.52; N 8.37; S 9.58.

Methyl 4-(2-chlorophenyl)-5-cyano-6-ethylsulfanyl-2-methyl-1,4-dihydropyridine-3-carboxylate (7b)

Yield 0.28 g (82%), colourless powder; m.p. 157–159 °C. IR spectrum, ν, cm^{-1}: 1697 (C=O); 2211 (C≡N); 3070, 3247 (NH). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.27 and 2.93 (5H, t and q, J = 7.0 Hz, 6-S-Et); 2.38 (3H, s, 2-Me); 3.56 (3H, s, OMe); 5.31 (1H, s, 4-H); 6.07 (1H, s, NH); 7.10–7.36 (4H, m, C_6H_4).

Found, %: C 58.34; H 4.74; N 8.00; S 9.12. Calculated for $C_{17}H_{17}ClN_2O_2S$, %: C 58.53; H 4.91; N 8.03; S 9.19.

Methyl 4-(2-chlorophenyl)-5-cyano-2-methyl-6-propylsulfanyl-1,4-dihydropyridine-3-carboxylate (7c)

Yield 0.31 g (86%), colourless powder; m.p. 125–126 °C. IR spectrum, ν, cm^{-1}: 1660 (C=O); 2200 (C≡N); 3438 (NH). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.97, 1.52–1.71 and 2.71–2.99 (7H, t, m and m, J = 7.48 Hz, 6-S-Pr); 2.37 (3H, s, 2-Me); 3.55 (3H, s, OMe); 5.29 (1H, s, 4-H); 6.15 (1H, s, NH); 7.12–7.36 (4H, m, C_6H_4).

Found, %: C 58.84; H 5.34; N 7.64; S 9.03. Calculated for $C_{18}H_{19}ClN_2O_2S$, %: C 59.58; H 5.28; N 7.72; S 8.84.

Methyl 4-(2-chlorophenyl)-5-cyano-6-isopropylsulfanyl-2-methyl-1,4-dihydropyridine-3-carboxylate (7d)

Yield 0.30 g (83%), colourless powder; m.p. 137–139 °C. IR spectrum, ν, cm^{-1}: 1660 (C=O); 2210 (C≡N); 3438 (NH). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.27 and 1.29 (6H, dd, J_{1-1} = 2.20 Hz, J_{1-2} = 4.40 Hz, 6-S-CH($\underline{CH_3}$)$_2$); 2.39 (3H, s, 2-Me); 3.45–3.58 (1H, m, 6-S-$\underline{CH}$(CH_3)$_2$); 3.56 (3H, s, OMe); 5.33 (1H, s, 4-H); 6.09 (1H, s, NH); 7.10–7.37 (4H, m, C_6H_4).

Found, %: C 59.24; H 5.28; N 7.71; S 8.72. Calculated for $C_{18}H_{19}ClN_2O_2S$, %: C 59.58; H 5.28; N 7.72; S 8.84.

Methyl 4-(2-chlorophenyl)-5-cyano-6-cyclopropylmethylsulfanyl-2-methyl-1,4-dihydropyridine-3-carboxylate (7e)

Yield 0.34 g (92%), colourless powder; m.p. 132–133 °C. IR spectrum, ν, cm^{-1}: 1660 (C=O); 2200 (C≡N); 3438 (NH). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.22 and 0.57 (4H, m and m, 6-S-$CH_2CH(\underline{CH_2})_2$); 0.90–1.07 (1H, m, 6-S-$CH_2\underline{CH}(CH_2)_2$); 2.38 (3H, s, 2-Me); 2.68–2.94 (2H, m, 6-S-$\underline{CH_2}CH(CH_2)_2$); 3.55 (3H, s, OMe); 5.31 (1H, s, 4-H); 6.19 (1H, s, NH); 7.10–7.36 (4H, m, C_6H_4).

Found, %: C 60.41; H 5.17; N 7.43; S 8.74. Calculated for $C_{19}H_{19}ClN_2O_2S$, %: C 60.87; H 5.11; N 7.47; S 8.55.

Methyl 4-(2-chlorophenyl)-5-cyano-6-isobutylsulfanyl-2-methyl-1,4-dihydropyridine-3-carboxylate (7f)

Yield 0.30 g (79%), colourless powder; m.p. 142–144 °C. IR spectrum, ν, cm^{-1}: 1660 (C=O); 2200 (C≡N); 3438 (NH). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.97 (6H, t, *J* = 6.59 Hz, 6-S-$CH_2CH(\underline{CH_3})_2$); 1.70–1.87 (1H, m, 6-S-$CH_2\underline{CH}(CH_3)_2$); 2.39 (3H, s, 2-Me); 2.65–2.88 (2H, m, 6-S-$\underline{CH_2}CH(CH_3)_2$); 3.56 (3H, s, OMe); 5.29 (1H, s, 4-H); 6.06 (1H, s, NH); 7.13–7.36 (4H, m, C_6H_4).

Found, %: C 60.30; H 5.65; N 7.69; S 8.62. Calculated for $C_{19}H_{21}ClN_2O_2S$, %: C 60.55; H 5.62; N 7.43; S 8.51.

One-pot reaction for synthesis of compounds 7

To stirred mixture of 2-chlorobenzaldehyde **1** (0.5 mmol), methyl acetoacetate **2** (0.5 mmol), 2-cyanothioacetamide **3** (0.5 mmol) in ethanol (2 ml), piperidine **4** (0.5 mmol) was added. The mixture was stirred at room temperature for 1 h. Alkyl halide **6** (0.5 mmol) was added and reaction mixture was heated for 5 min. Reaction mixture was acidified with 0.12 ml of 3*N* hydrochloric acid in ethanol and stirred for 1 h at room temperature. The precipitate was filtered and washed with 10 ml of ethanol and 10 ml of water.

***In vitro* activity assay**

Measurement of intracellular Ca^{2+}

Monolayer tumor cells SH-SY5Y (human neuroblastoma, cells were obtained from the ATTC®) (American Type Culture Collection) and were grown in standard medium DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% of fetal bovine serum. 3×10^4 cells/well were placed in 96-well plates for 24 h. Changes in intracellular concentration $[Ca^{2+}]_i$ were assayed using Fluo-4 NW Calcium assay kit (*Invitrogen*) according to modificaton of the known method [6, 7]. The cells were preincubated with compounds (100 μ*M*) for 15 min followed by stimulation with 20 n*M* carbachol. Fluorescence intensity was monitored using fluorescence spectrophotometer (*Thermo Ascient*, Finland) with excitation at 494 nm and emmision at 516 nm. The IC_{50} values were calculated using the program *Graph Pad Prism® 3.0*.

RESULTS AND DISCUSSION

The aim of this work is to elaborate the method for the synthesis of methyl 6-alkylsulfanyl-1,4-dihydropyridine-3-carboxylates **7** by modifying substituents at position 6 in DHP molecule. The introduction of prolonged and lipophilic 6-alkylsulfanyl group in DHP carboxylate could increase the bioavailability of the title compound in the human body.

Methyl 4-(2-chlorophenyl)-6-thioxo-1,4,5,6-tetrahydropyridine-3-car-boxylate 5 was prepared in 84% yield according to the standard procedure [5]. Methyl 6-alkylsulfanyl-1,4-dihydropyridine-3-carboxylates 7a–f have been obtained by alkylation of intermediate 5 with alkyl halides 6 (method A) and by one-pot five-component condensation (method B) as well (see Scheme). Using the conventional method A compounds 7 were obtained in 79–93% yield. Summary yields of compounds 7 in two-step synthesis were 69–78% (Table 1.). According to the our previous studies [5, 8, 9] target compounds 7 were obtained in five-component one-pot synthesis (method B). It is worth to mention, that in the method B, the

sequence of starting compounds 1–4 and 6 is important for reaching high yields of target products 7 [10]. Condensation of aromatic aldehyde 1, methyl acetoacetate 2, 2-cyanothioacetamide 3, piperidine 4 and alkyl halides 6, followed by acidification with HCl/EtOH solution gave methyl 6-alkylsulfanyl-1,4-dihydropyridine-3-carboxylates 7 in 77–93% yield (Table 1.). This approach enables the preparation of methyl 6-alkylsulfanyl-1,4-dihydropyridine-3-carboxylates 7 in a shorter time (1–2 hours), under mild conditions and in higher yields than in the two-step synthesis.

Table 1.

The yields and lipophilicity values of methyl 6-alkylsulfanyl-1,4-dihydropyridine-3-carboxylates (7)

Compound	R	Method A, Yield, %, (Summary yield[1], %)	Method B, Yield[2], %	log *P*[4]
a	Me	93 (78)	93 (85[3])	3.47
b	Et	82 (69)	872	3.85
c	Pr	86 (72)	77	4.35
d	*i*-Pr	83 (70)	79	4.21
e	$CH_2CH(CH_2)_2$	92 (77)	82	4.34
f	$CH_2CH(CH_3)_2$	79 (66)	88	4.59

N o t e s : [1] calculated on 2-chlorobenzaldehyde;
[2] determined by HPLC;
[3] isolated yield;
[4] theoretical calculations [14].

Theoretical calculations of lipophilicity of compounds under study show that introduction of longer alkyl chain in the position 6 of 1,4-DHP ring causes the increase of value of log *P* (partition coefficient in the system *octanol–water*). According to the Lipinski's Rule of Five [11, 12] the new synthesized compounds 7 possess the optimum lipophilicity – log *P* = 3.47–4.59 (Table 1.).

The structures of intermediate 5 and reaction products 7 were established by their spectral data (^{1}H NMR, IR) and elemental analysis data. In the IR spectra, characteristic absorption bands of 5-C≡N group for 6-thioxo-1,4,5,6-tetrahydropyridine 5 at 2258 cm^{-1} and for 1,4-dihydropyridines 7 at 2200–2210 cm^{-1} were observed. Absorption bands of $\nu_{C=O}$ of compounds are in agreement with the type of conjugation of C=O groups. In the ^{1}H NMR spectra (taken in $CDCl_3$ solution) signals characteristic of *cis-trans* isomers of 6-thioxo-1,4,5,6-tetrahydropyridines 5 were observed, the *cis* isomer being in superiority over *trans* isomer [13]. In the ^{1}H NMR spectrum the characteristic 4-H proton signals of 1,4-DHPs 7 appeared as singlets at δ 5.29–5.33 ppm.

Methyl 6-alkylsulfanyl-1,4-dihydropyridine-3-carboxylates 7 were tested on calcium channel blocking activity in *in vitro* models (SH-SY5Y cell lines) as effectors of agonist carbachol. Compounds 7a, 7c and 7d show no activity, while compounds 7b,e,f have antagonistic activity in concentration IC_{50} > 100 μ*M*.

Conclusions

Methyl 6-alkylsulfanyl-1,4-dihydropyridine-3-carboxylates **7** with optimum lipophilicity have been obtained in alkylation reactions of methyl 4-(2-chlorophenyl)-6-thioxo-1,4,5,6-tetrahydropyridine-3-carboxylate **5** with alkyl halides **6**. Preparation of target compounds **7** was improved using one-pot five-component synthesis. Methyl 6-ethylsulfanyl-, 6-cyclopropylmethylsulfanyl- and 6-isobutylsulfanyl-1,4-dihydropyridine-3-carboxylates **7b**,**e**,**f** show calcium channel blocking activity in *in vitro* models – SH-SY5Y cell lines (human neuroblastoma).

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SYNTHESIS OF 6- ALKOXYCARBONYLMETHYLSULFANYL-5-CYANO-2-METHYL-4-PHENYL-1,4-DIHYDROPYRIDINE-3-CARBOXYLATES

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Abstract: 6-Alkoxycarbonylmethylsulfanyl-5-cyano-2-methyl-4-phenyl-1,4-dihydropyridine-3-carboxylates **3** as a more lipophilic derivatives of the biologically active 6-methylsulfanyl-1,4-DHP-3-carboxylates **2** have been prepared by the alkylation of 1,4-dihydropyridine-6-thiolates **1** with alkyl bromoacetates. Reactivity of **2** with KOH/H_2O was investigated.

Introduction

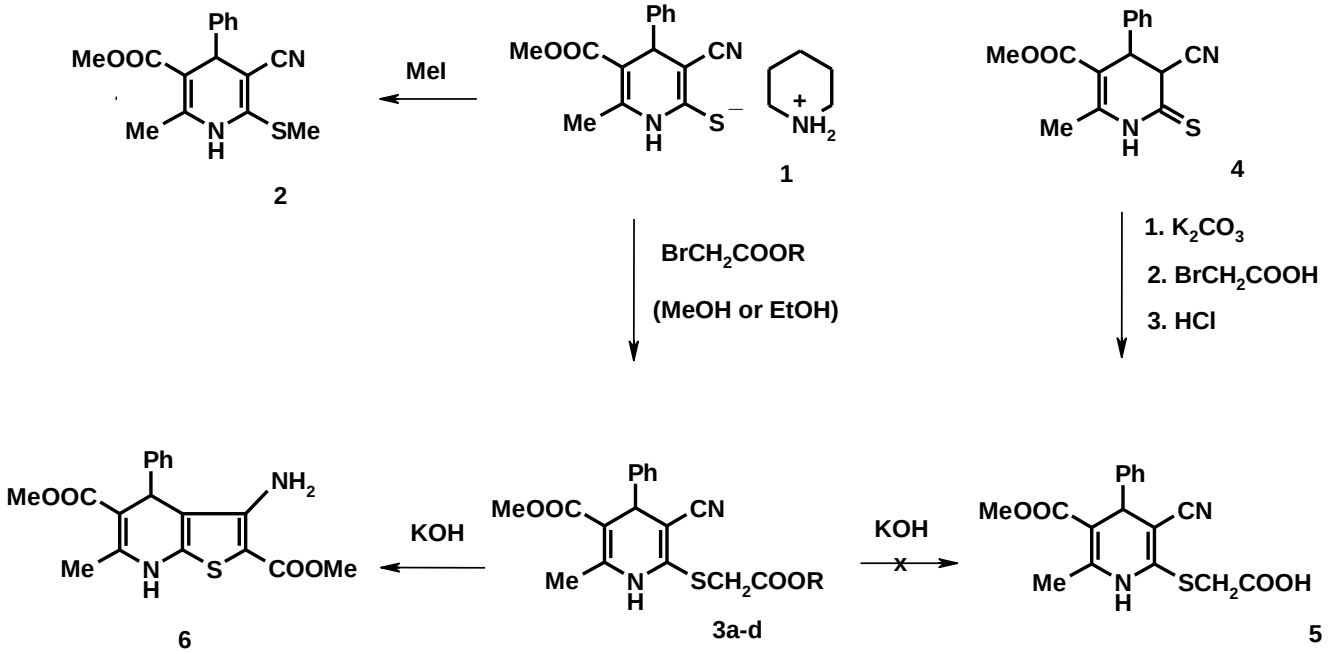
1,4–Dihydropyridines (DHPs) are known as effective calcium channel effectors, especially antagonists. Many cardiovascular drugs which are currently in the clinics or are in different stages of development are based on DHPs[1-4]. Pharmacology of DHPs is at the eve of a novel boom: after synthesis, studies, development of a set of antihypertensive and antianginal drugs, the interest is growing towards pharmacological activities not connected (or partially connected) with their calcium antagonist properties: neurotropic (antiamnesic, anticonvulsant, neuroregulatory)[5], membrane protecting[6-8], analgesic[9], antidiabetic[10], antiinflammatory[11], gene-transfection agents[12] and also as uroselective agents for benign prostatic hyperplasia treatment[13].

6-Alkylsulfanyl-1,4-DHPs display cardiovascular[14-16], hepatoprotective[17], antioxidant[18], and antiradical[19] activities, however, these compounds are still insufficiently studied. As we have shown recently, ethyl 4-aryl-5-cyano-2-methyl-6-methylsulfanyl-1,4-DHP-3-carboxylates besides being of low toxicity display antihypertensive or vasodilating activities[20] depending on the character of the substituents in the position 4, but to optimize lipophilicity, another ester group has to be introduced in the 1,4-DHP molecule[1]. 6-Alkoxycarbonylmethylsulfanyl-5-cyano-2-methyl-4-phenyl-1,4-dihydropyridine-3-carboxylates bearing two ester groups could be interesting from this point of view.

Results and discussion

The task of this publication is the synthesis of new 1,4-DHPs containing lipophilic alkoxycarbonylmethylsulfanyl group at the position 6 as the group which might undergo further chemical transformations.

3-Ethoxycarbonyl-5-cyano-2-methyl-4-phenyl-1,4-dihydropyridine-6-thiolate **1** was prepared by one-pot four-component condensation of ethyl acetoacetate, benzaldehyde, 2-cyanothioacetamide and piperidine[21]. Alkylation of thiolate **1** with iodomethane (50 % excess) and alkyl bromoacetate (5-10 % excess) gave rise to methyl 5-cyano-2-methyl-6-methylsulfanyl-4-phenyl-1,4-dihydropyridine-3-carboxylate **2** and methyl 6-alkoxycarbonylmethylsulfanyl-5-cyano-2-methyl-4-phenyl-1,4-dihydropyridine-3-



carboxylates **3**.

a) R = Me; b) R = Et; c) R = i-Pr; d) R = *t*-Bu

Our efforts to prepare methyl 6-benzyloxycarbonylmethylsulfanyl-5-cyano-2-methyl-4-phenyl-1,4-dihydropyridine-3-carboxylate **3e** under the above mentioned reaction conditions were not successful. Alkylation of thiolate **1** with benzyl 2-bromoacetate in anhydrous benzyl alcohol gave rise to an unstable product which by washing with ethanol easily underwent transesterification to give ethyl carboxylate **3b**.

Hydrolysis of ester **3a** with KOH/H_2O to the corresponding acid **5** was not successful, as the Thorpe′s cyclisation occurs instead of hydrolysis to form 3-amino-4,7-dihydrothieno[2,3-b]pyridine **6**. Carboxylic acid **5** was prepared by alkylation of thione **4**[21] with bromoacetic

acid in the presence of excess of K_2CO_3 with subsequent careful neutralization of the reaction mixture.

The structures of the compounds are proved by spectroscopic methods. In the IR spectra of 1,4-DHPs **2**, **3** and **5** absorption bands for $\nu_{C\equiv N}$ at 2197-2201 cm^{-1} (disappear in the case of thienopyridines **6**) and bands corresponding to the type of conjugation for $\nu_{C=O}$ are seen. In the ^1H NMR spectra of **2**, **3** and **5** the most characteristic are singlets of 4-H protons at 4.51 - 5.00 ppm. In the case of **3** and **5** AB-doublets of the SCH_2 group with J = 15.6 - 16.0 Hz are seen.

In conclusion, a series of 6-alkoxycarbonylmethylthio-5-cyano-2-methyl-4-phenyl-1,4-DHP-3-carboxylates **3** which are more lipophilic derivatives of the biologically active 6-methylthio-1,4-DHP-3-carboxylate **2** have been prepared by alkylation of 1,4-DHP-6-thiolates with alkyl bromoacetates. By treatment of **3a** with KOH/H_2O the corresponding 3-amino-4,7-dihydrothieno[2,3-b]pyridine **6** was prepared.

Experimental

Melting points were determined on a Boetius apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 580 B spectrometer (in nujol) and peak positions ν_{max} were expressed in cm^{-1}. ^1H NMR spectra were recorded on a Varian Mercury-200 (200 MHz) spectrometer. Chemical shifts are expressed in δ (p.p.m. downfield from TMS) and coupling constants (*J*) in Hz. The course of the reactions and the individuality of substances were monitored by TLC on Kieselgel 60 F Merck plates with dichloromethane – hexane – methanol (5 : 5 : 1) as eluent. Compounds were recrystallized from ethanol.

Methyl 5-cyano-2-methyl-6-methylthio-4-phenyl-1,4-dihydropyridine-3-carboxylate 2: A mixture of thiolate **1** (0.37 g, 10 mmol) and methyl iodide (0.12 ml, 20 mmol) in 20 ml of methanol was shortly heated until dissolution of thiolate **1** and stirred at ambient temperature for 1h. The precipitate was filtered, washed with 5 ml of cold (ca. 5°C) methanol and 20 ml of water to give ester **2** as colourless crystals**,** yield 87 %, mp 163 - 164°C. IR: 1638, 1698 (C=O); 2200 (C≡N); 3392 (NH). ^1H NMR ($CDCl_3$): 2.38 (s, 3H, 2-CH_3); 2.47 (s, 3H, SCH_3); 3.68 (s, 3H, OCH_3); 4.70 (s, 1H, 4-H); 6.20 (s, 1H, NH); 7.1 - 7.4 (m, 5H, C_6H_5). ^{13}C NMR ($CDCl_3$ - DMSO-d_6): 16.03 (q); 18.08 (q); 41.73 (d); 50.55 (q); 88.99 (s); 100.24 (s); 118.92 (s); 126.57, 127.53, 128.31, 144.38 (aromatic C); 144.90 (s), 145.85 (s); 166.74 (s). Anal. Calcd. for $C_{16}H_{16}N_2O_2S$: C 63.97, H 5.37, N 9.33; Found: C 64.40, H 5.46, N 9.53.

General procedure for synthesis of 6-alkoxycarbonylmethylthio-5-cyano-2-methyl-4-phenyl-1,4-dihydropyridine-3-carboxylates 3.

A mixture of 10 mmol of corresponding thiolate **1** and 11 mmol of alkyl bromoacetate in 20 - 25 ml of methanol (or ethanol) was shortly heated until dissolution of thiolate **1** and stirred at ambient temperature for 1 - 2 hr. The precipitated crystals (in case of **3c,d** 1 ml of water was added) were removed by filtration, washed with 10 ml of cold (ca. 5°C) ethanol and 20 ml of water to give 77 - 97% of 6-alkoxycarbonylmethylthio-1,4-dihydropyridine-3-carboxylates **3**.

Methyl 5-cyano-6-methoxycarbonylmethylthio-2-methyl-4-phenyl-1,4-dihydropyridine-3-carboxylate 3a: colourless crystals, yield 96%, mp 122 - 123°C. IR: 1681, 1714 (C=O); 2197(C≡N); 3194, 3233, 3264 (NH). ^{1}H NMR ($CDCl_3$): 2.40 (s, 3H, 2-CH_3); 3.53 and 3.57 (d and d, J = 16.0 Hz, 2H, SCH_2); 3.59 (s, 3H, 3-$COOCH_3$); 3.81 (s, 3H, $SCH_2COO\underline{CH_3}$); 4.66 (s, 1H, 4-H); 7.2 - 7.3 (m, 5H, C_6H_5); 8.40 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): 19.48 (q); 34.86 (t); 42.37 (d); 51.29 (q); 53.76 (q); 91.54 (s); 101.55 (s); 118.60 (s); 127.20, 127.33, 128.73, 141.73 (aromatic C); 144.33 (s); 145.04 (s); 167.27 (s); 173.11 (s). Anal. Calcd. for $C_{18}H_{18}N_2O_4S$: C 60.32, H 5.06, N 7.82; Found: C 60.57, H 5.10, N 8.00.

Methyl 3-Cyano-6-ethoxycarbonylmethylthio-2-methyl-4-phenyl-1,4-dihydropyridine-3-carboxylate 3b: colourless crystals**,** yield 97%, mp 136 - 137°C. IR: 1682, 1706 (C=O); 2197 (C≡N); 3194, 3234, 3263 (NH). ^{1}H NMR ($CDCl_3$): 1.32 (t, J = 7 Hz, 3H**,** $CH_2\underline{CH_3}$); 2.40 (s, 3H, 2-CH_3); 3.52 and 3.55 (d and d, J = 16 Hz, 2H, SCH_2); 3.59 (s, 3H, OCH_3); 4.27 (q, J = 7 Hz, 2H, $\underline{CH_2}CH_3$); 4.67 (s, 1H, 4-H); 7.2 - 7.4 (m, 5H, 4-C_6H_5); 8.56 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): 14.01 (q); 19.47 (q); 35.07 (t); 42.35 (d); 51.28 (q); 63.25 (t); 91.25 (s); 101.52 (s); 118.62 (s); 127.29, 127.34, 128.65, 141.47 (aromatic C); 144.53 (s); 144.72 (s); 166.78 (s); 173.09 (s). Anal. Calcd. for $C_{19}H_{20}N_2O_4S$: C 61.27, H 5.41, N 7.52; Found: C 61.65, H 5.49, N 7.68.

Methyl 5-cyano-6-isopropoxycarbonylmethylthio-2-methyl-4-phenyl-1,4-dihydropyridine-3-carboxylate 3c: colourless crystals, yield 77 %, mp 112 - 113°C. IR: 1645, 1685, 1696 (C=O); 2198 (C≡N); 3188, 3234, 3261 (NH). ^{1}H NMR ($CDCl_3$): 1.30 and 1.33 [s and s, 6H, $CH(\underline{CH_3})_2$]; 2.41 (s, 3H, 2-CH_3); 3.49 and 3.53 (d and d, J = 16 Hz, 2H, SCH_2); 3.60 (s, 3H, OCH_3); 4.68 (s, 1H, 4-H); 5.10 [m, 1H, $\underline{CH}(CH_3)_2$]; 7.1 - 7.4 (m, 5H, 4-C_6H_5); 8.63 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): 19.47 (q); 21.61 (q); 35.32 (t); 42.34 (d); 51.27 (q); 71.64 (d); 90.99 (s); 101.48 (s); 118.68 (s); 127.20, 127.29, 128.71,

141.84 (aromatic C); 144.40 (s); 145.13 (s); 167.32 (s); 172.25 (s). Anal. Calcd. for $C_{20}H_{22}N_2O_4S$: C 62.15, H 5.74, N 7.25; Found: C 62.53, H 5.74, N 7.21.

Methyl 6-*tert*-Butoxycarbonylmethylthio-5-cyano-2-methyl-4-phenyl-1,4-dihydropyridine-3-carboxylate 3d: colourless crystals, yield 86 %, mp 94 - 95°C. IR: 1645, 1684, 1700 sh (C=O); 2201 (C≡N); 3186, 3232, 3264 (NH). ^{1}H NMR ($CDCl_3$): 1.52 [s, 9H, $C(CH_3)_3$]; 2.40 (s, 3H, 2-CH_3); 3.44 and 3.48 (d and d, J = 16 Hz, 2H, SCH_2); 3.60 (s, 3H, OCH_3); 4.67 (s, 1H, 4-H); 7.1 - 7.4 (m, 5H, 4-C_6H_5); 8.68 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): 19.47 (q); 27.87 (q); 36.23 (t); 42.31 (d); 51.26 (q); 84.61 (s); 90.71 (s); 101.42 (s); 118.75 (s); 127.19, 127.26, 128.70, 142.08 (aromatic C); 144.43 (s); 145.19 (s); 167.35 (s); 171.96 (s). Anal. Calcd. for $C_{21}H_{24}N_2O_4S$: C 62.98, H 6.04, N 7.00; Found: C 63.18, H 6.03, N 6.91.

Methyl 6-carboxymethylthio-5-cyano-2-methyl-4-phenyl-1,4-dihydropyridine-3-carboxylate 5. A mixture of 10 mmol of thione **4,** 20 mmol of dried and well crushed potassium carbonate and 15 mmol of bromoacetic acid in 100 ml of methanol was heated for 3 - 5 min at 50 - 60°C with stirring, and left for 20 hr at the ambient temperature. Then the insoluble part of potassium carbonate was separated by filtration, the reaction mixture neutralised with 1.5 N HCl solution in ethanol and precipitated sodium chloride was separated by filtration. Water (200 ml) was added and reaction mixture was acidified until pH 2 - 2.5. After 3-4 hr the precipitate was separated by filtration to give 63 % of acid **5a:** colourless crystals, yield 63%, mp 145 - 146 °C. IR: 1645, 1720 (C=O); 2200 (C≡N); 3191, 3256, 3450 sh (NH, OH). ^{1}H NMR (DMSO-d_6): 2.31 (s, 3H, 2-CH_3); 3.53 (s, 3H, OCH_3); 3.83 and 3.93 (d and d, J = 15.6 Hz, 2H, SCH_2); 4.51 (s, 1H, 4-H); 7.1 - 7.4 (m, 5H, 4-C_6H_5); 9.57 (s, 1H, NH); 13.01 (br.s, 1H, OH). ^{13}C NMR (DMSO-d_6): 18.12 (q); 34.35 (q); 41.89 (4-H); 50.97 (q); 89.70 (s); 100.27 (s); 118.92 (s); 126.82, 127.06, 128.66, 142,54 (aromatic C); 144.93 (s); 146.05 (s); 166.71 (s); 169.79 (s). Anal. Calcd. for $C_{17}H_{16}N_2O_4S$: C 59.29, H 4.68, N 8.14; Found: C 59.15, H 4.60, N 8.20.

Dimethyl 3-Amino-6-methyl-4-phenyl-4,7-dihydrothieno[2,3-b]pyridine-2,5-dicarboxylate 6. A sample of 5-cyano-6-methoxycarbonylmethylthio-1,4-dihydropyridine **3** (0.72 g, 2 mmol) in 5 ml of methanol was treated with 1 ml of 2 M KOH water solution by short heating till reflux and stirring at room temperature for 1 hr. The precipitate was separated by filtration, washed with 2 ml of cold (ca. 5°C) methanol and 10 ml of water to yield 0.67 g (93 %) of **6** as light yellow powder, mp 261 - 262°C. IR: 1653, 1670 (C=O); 3310, 3364, 3480 (NH, NH_2). ^{1}H NMR ($CDCl_3$): 2.38 (s, 3H, 6-CH_3); 3.61 (s, 3H, 5-$COOCH_3$); 3.74 (s, 3H, 2-$COOCH_3$); 5.00 (s, 1H, 4-H); 5.27 (br.s, 2H, 3-NH_2); 6.24 (s, 1H, 7-NH); 7.1 - 7.3 (m, 5H, 4-C_6H_5). ^{13}C NMR (DMSO-d_6): 19.24 (q); 40.74 (d); 50.47 (q); 50.57 (q);

100.04 (s); 110.95 (s); 126.13, 127.36, 128.02, 142.27 (aromatic C); 146.65 (s); 146.78 (s); 152.50 (s); 164.11 (s); 167.42 (s). Anal. Calcd. for $C_{18}H_{18}N_2O_4S$: C 60.32, H 5.06, N 7.82; Found: C 60.48, H 5.00, N 7.74.

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