G-proteīnu receptoru ligandi kā mazmolekulārās signālmolekulas dislipidēmijas un diabēta terapijā

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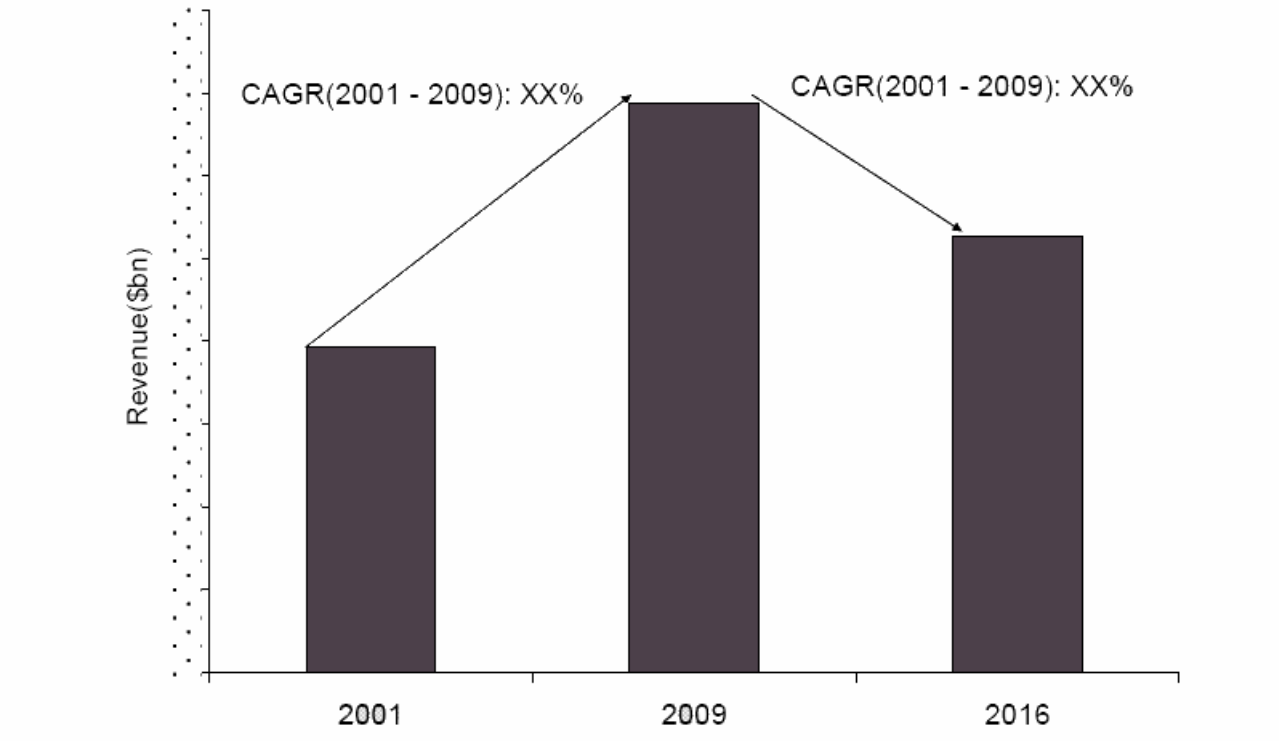
30.09.2010.

Dyslipidemia – Pipeline Assessment and Market Forecasts to 2016

The Dyslipidemia Market is Forecast to Decline until 2016

Global Dyslipidemia Therapeutics Market, Revenue ($bn), 2001–2016



Source: GlobalData

Competition in the Dyslipidemia Market is Strong

GlobalData found that the current competition in the dyslipidemia market is becoming intense due to the increased entry of combinational therapies and the use of statins, fibrates, niacin and omega-3 fatty acid derivatives as antidyslipidemic agents.

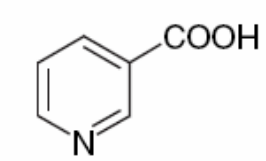
In 2010, the global prevalence of diabetes was estimated to have reached 285 million and is predicted to reach 438 million in 2030. This $29 Billion market brings many exciting developments and emerging companies developing oral agents and injectable products!

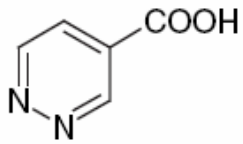
Dyslipidemia – Pipeline Assessment and Market Forecasts to 2016

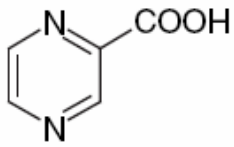
Unmet needs Exists in Terms of the Safety and Efficacy of the Currently Marketed Products

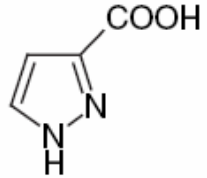
GlobalData has found that several conventional therapies for the treatment of dyslipidemia such as statins, fibrates, combinational therapies, niacin and prescription omega-3 esters are mainstay therapies for dyslipidemia. These molecules offer substantial efficacy combined with durable responses and convenient dose frequencies. Combination therapies lead the market and are the preferred first-line therapies for dyslipidemia. Dyslipidemic drugs do not completely cure the disease; rather they offer symptomatic treatments and are associated with safety concerns. Thus, there are **unmet needs** in the market in terms of efficacy and safety. Any new treatment in the pipeline targeting safety and efficacy – with pricing as another prime factor – is likely to attain blockbuster drug status.

Nicotinic acid receptor subtypes and their ligands

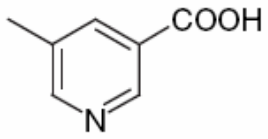


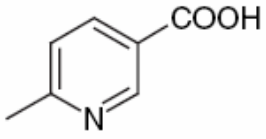


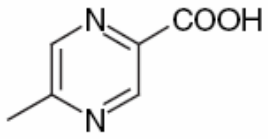


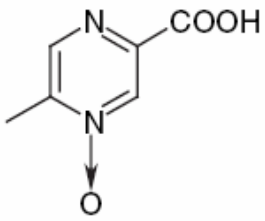


Nicotinic acid	Pyridazine-4-COOH	Pyrazine-2-COOH	Pyrazole-3-COOH
K_i = 0.033 µM	K_i = 0.121 µM	K_i = 0.762 µM	K_i = 0.594 µM
EC_{50} = 0.703 µM	EC_{50} = 3.14 µM	EC_{50} = 21.8 µM	EC_{50} = 21.5 µM
RIA = 100%	RIA = 100%	RIA = 100%	RIA = 85.3%





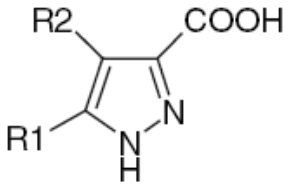


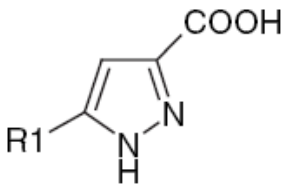


5-Me-nicotinic acid	6-Me-nicotinic acid	5-Me-pyrazine-2-COOH	Acipimox
K_i = 0.717 µM	K_i = 1.700 µM	K_i = 0.652 µM	K_i = 0.309 µM
EC_{50} = 30.0 µM	EC_{50} = 53.7 µM	EC_{50} = 14.5 µM	EC_{50} = 6.56 µM
RIA = 100%	RIA = 100%	RIA = 100%	RIA = 100%

Lorenzen A, Stannek C, Lang H, Andrianov V, Kalvinsh I, Schwabe U. Characterization of a G protein-coupled receptor for nicotinic acid. *Mol Pharmacol* 2001;59:349–357.

Potencies (K_i and EC_{50} Values) and Relative Intrinsic Activities (RIA) of Pyrazole-Derived Partial Agonists for the Rat Nicotinic Acid Receptor



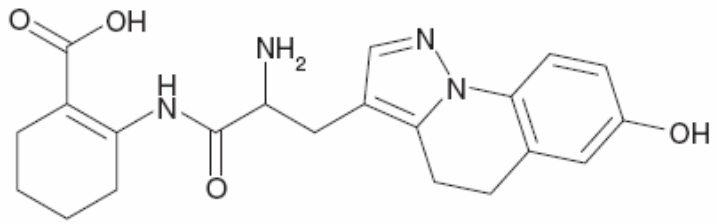


R_1	R_2	K_i (μM)	EC_{50} (μM)	RIA (%)		R_1	Ki (μM)	EC_{50} (μM)	RIA (%)
nicotinic acid		0.033	0.74	100		C_6H_5-	101	n.d.	n.d.
H	H	0.594	21.5	85.3		3Cl-C_6H_4-	63.7	n.d.	n.d.
i-C_3H_7	H	0.683	21.8	67.7		4Cl-C_6H_4-	107	n.d.	n.d.
C_3H_7	H	0.143	5.09	69.9		4CH_3-C_6H_4-	37.9	n.d.	n.d.
C_4H_9	H	0.072	2.26	80.7		C_6H_5-CH_2-	1.25	86.9	50.3
$C_{11}H_{23}$	H	21.4	n.d.	n.d.		C_6H_5-$(CH_2)_2$-	1.57	n.d.	n.d.
-C_3H_6-		0.156	6.97	55.9		C_6H_5-$(CH_2)_3$-	6.30	n.d.	n..d
-C_4H_8-		3.54	64.6	47.2		3Cl-C_6H_4-CH_2-	0.504	46.6	39.4
						4Cl-C_6H_4-CH_2-	3.61	n.d.	n.d.
						4-CH_3-C_6H_4-CH_2-	20.3	n.d.	n.d.
						4-OCH_3-C_6H_4-CH_2-	66.0	n.d.	n.d.

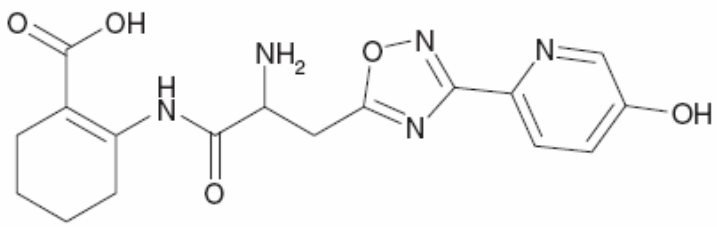
n.d., notdetermined.

Van Herk T, Brussee J, van den Nieuwendijk AMCH, van der Klein PAM, IJzerman AP, Stannek C, Burmeister A, Lorenzen A. Pyrazole derivatives as partial agonists for the nicotinic acid receptor. *J.Med.Chem.* 2003; 46:3945–3951.

Novel patent publications on high-affinity nicotinic acid receptor agonists



28



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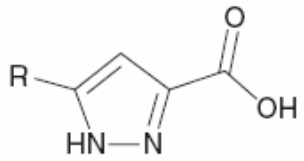

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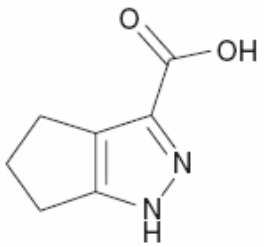

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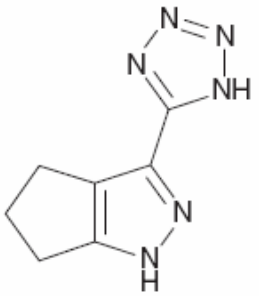
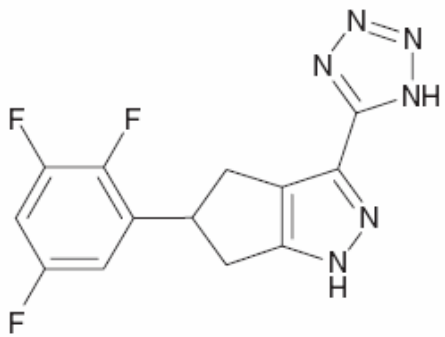


32

^{3}H-niacin binding (rat spleen memberanes)
IC_{50} = 0.16 μM
rGTPγS (rat adipocyte memberanes)
EC_{50} = 6.4 μM (52%)*
*Relative intrinsic activity with respect to niacin
h-cAMP
EC_{50} = 0.86 μM (106%)*
*Relative intrinsic activity with respect to niacin

h-cAMP
EC_{50} = 1.65 μM (59%)*
*Relative intrinsic activity with respect to niacin

Novel patent publications on high-affinity nicotinic acid receptor agonists



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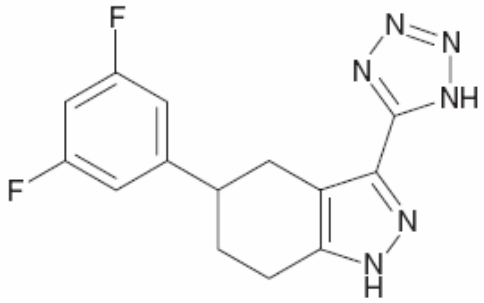

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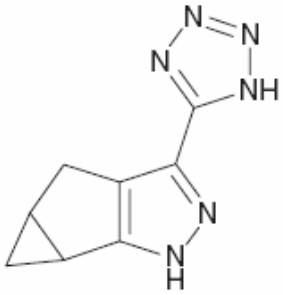
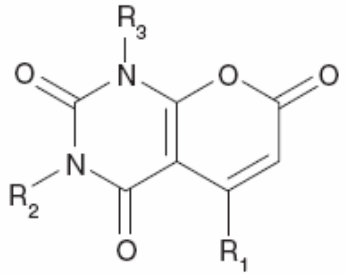
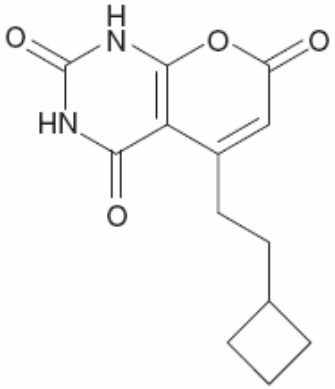


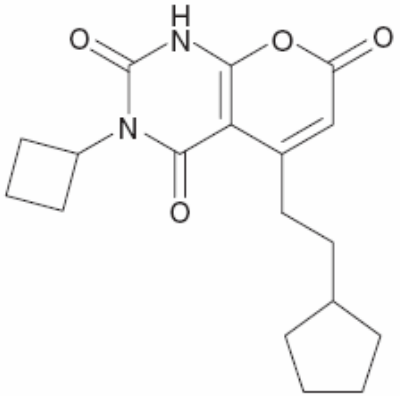
35



36
R_2 and/or R_3 = H



37
h-cAMP
EC_{50} = 1.2 nM



38
h-cAMP
EC_{50} = 13 nM

Pyrazole derivatives as partial agonists for the nicotinic acid receptor

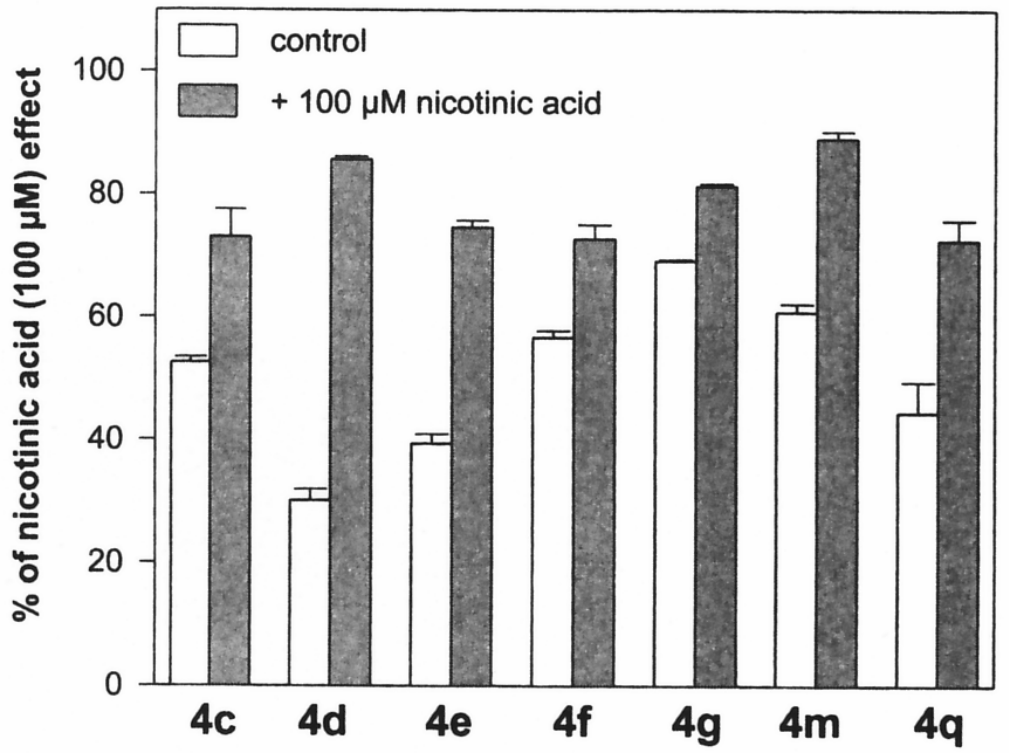


Figure 5. Stimulation of basal [^{35}S]GTPγS binding and inhibition of nicotinic acid-stimulated [^{35}S]GTPγS binding by pyrazole derivatives. [^{35}S]GTPγS binding was determined with 1 μg of adipocyte membrane protein in absence or presence of 100 μM nicotinic acid. Pyrazole derivatives (1 mM) stimulate basal [^{35}S]GTPγS binding (open columns) and inhibit the stimulatory effect of nicotinic acid (grey columns). Unstimulated binding of [^{35}S]GTPγS was 1191 ± 86 cpm/μg, binding in the presence of 100 μM nicotinic acid was 2634 ± 178 cpm. All experiments (n = 3) were performed in triplicate.

Niacīnskābes receptoru ligandi

Receptors GPR 109A

Žurku receptori

3-hidroksibutirāts
Nikotīnskābes atvasinājumi
Nikotīnskābei līdzīgie savienojumi (pieclocekļu, sešlocekļu heterociklu karbonskābes)
Tetrazola atvasinājumi
Barbitūrskābes atvasinājumi
Piridopirimidona atvasinājumi
Antranilskābes atvasinājumi
Tetrahidroantranilskābes atvasinājumi

Cilvēka receptori

3-hidroksibutirāts
Nikotīnskābes atvasinājumi
Nikotīnskābei līdzīgie savienojumi (pieclocekļu, sešlocekļu heterociklu karbonskābes)
Kondensētu heterociklisko savienojumu karbonskābes
Piesātinātu heterociklisko savienojumu karbonskābes
Tetrazola atvasinājumi

Niacīnskābes receptoru ligandi

Receptors GPR 109A

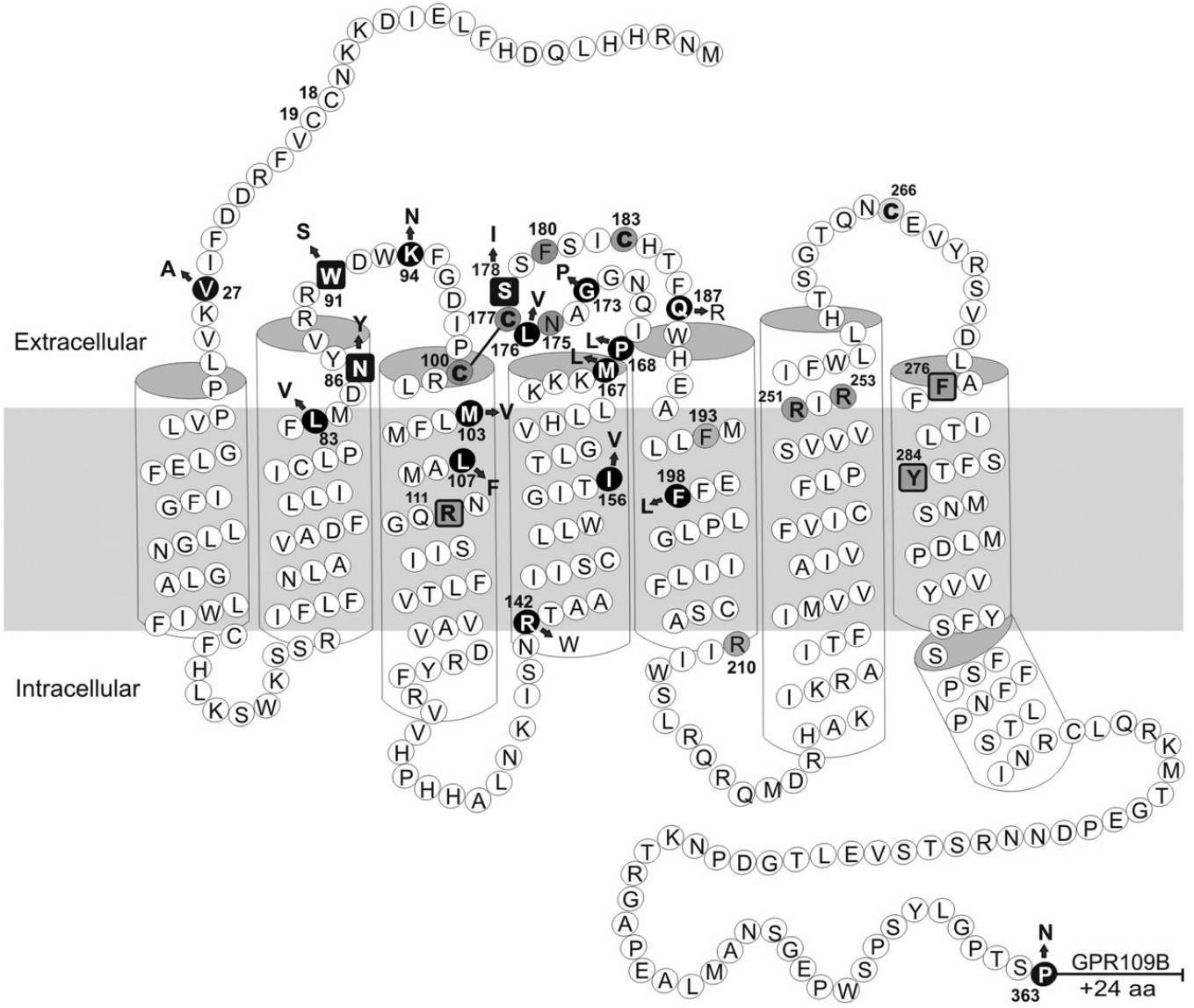
Peļu receptori
3-Hidroksibutirāts

Kāmju receptori
Pirazola karbonskābju atvasinājumi
Antranilskābju atvasinājumi
(aptuveni aktivitātes un afinitātes dati)

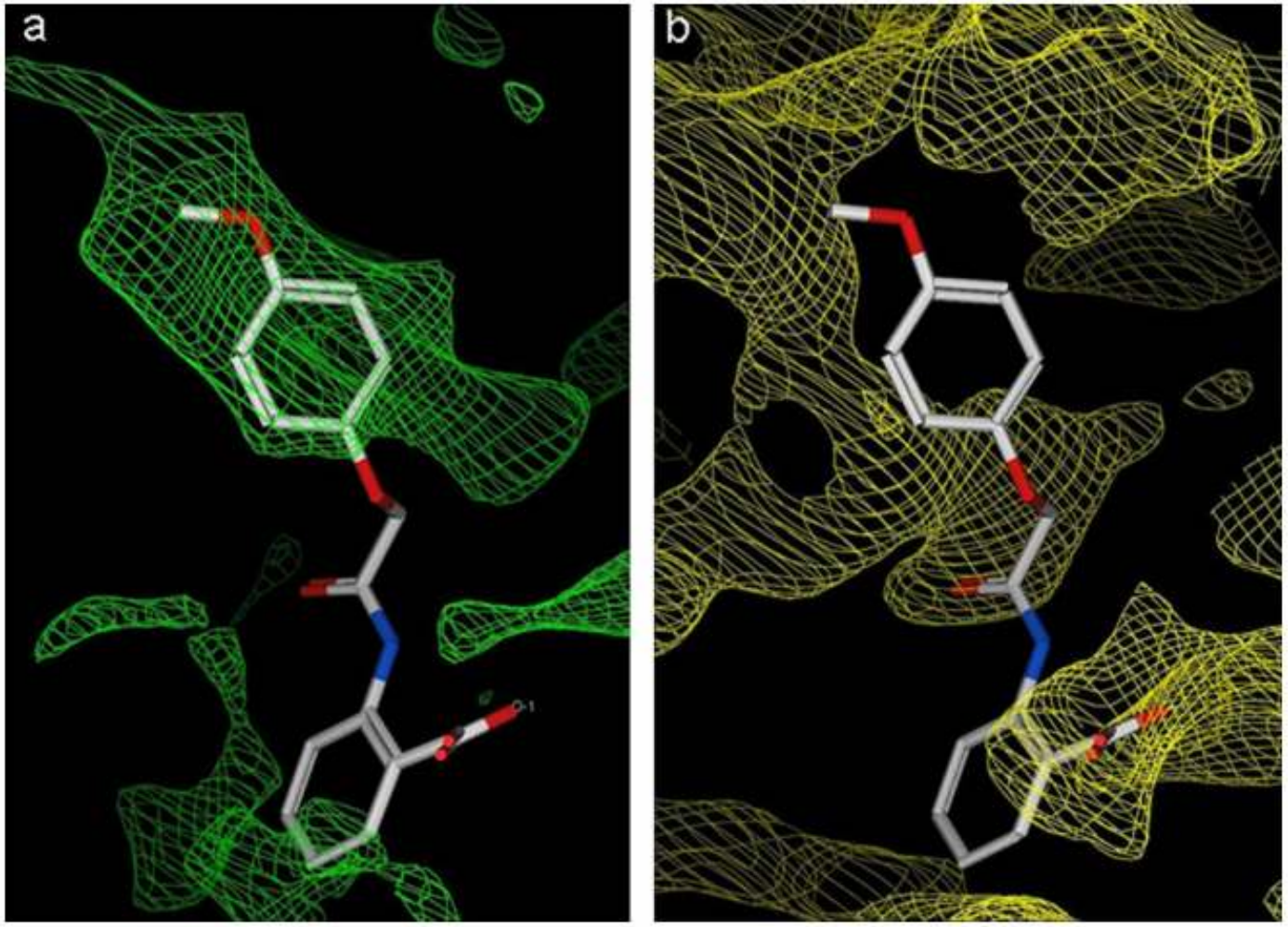
Receptors GPR 109B

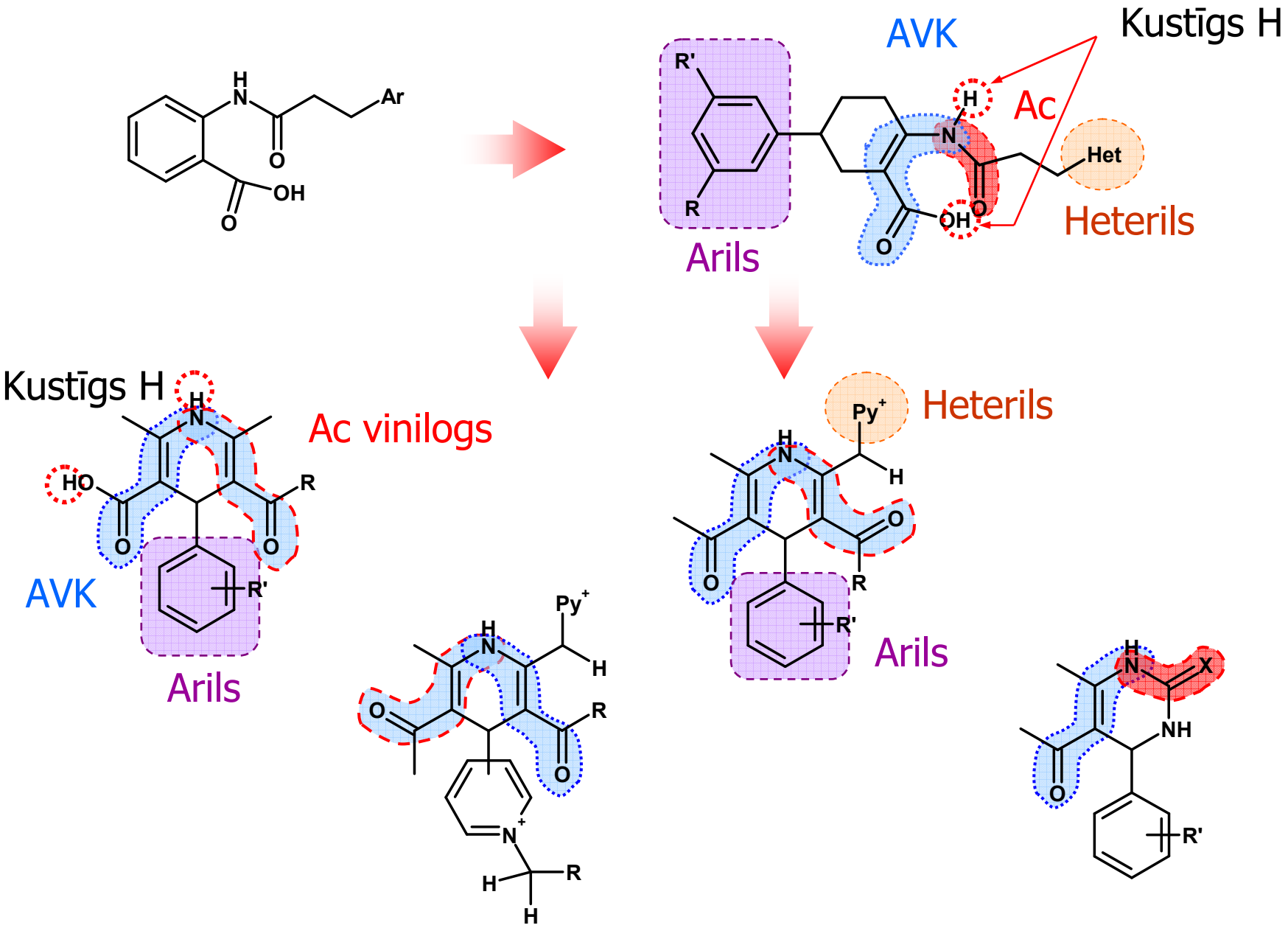
Nikotīnskābes atvasinājumi
Nikotīnskābei līdzīgie savienojumi (pieclocekļu, sešlocekļu heterociklu karbonskābes)
Kondensētu heterociklisko savienojumu karbonskābes
Tetrazola atvasinājumi

Secondary structure of the human nicotinic acid receptor GPR109A

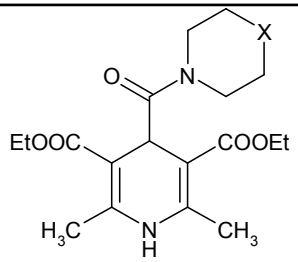
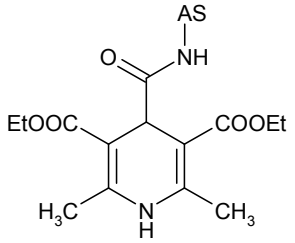
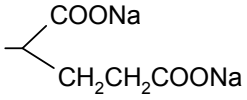
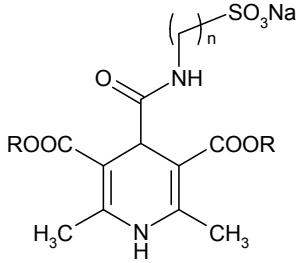


Molecular modeling aided design of nicotinic acid receptor GPR109A agonists

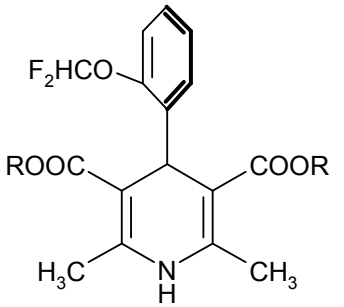
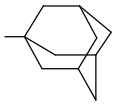
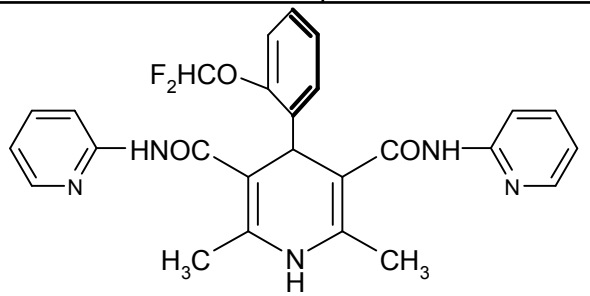
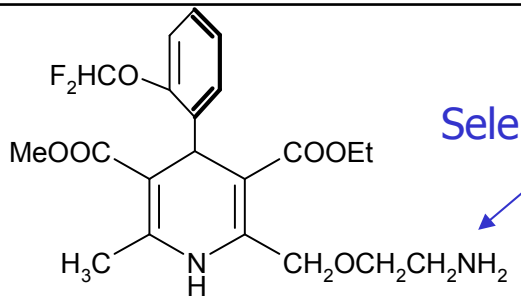



Kustīgs H
AVK
Ac
Het
Heterils
Arils
Kustīgs H
Ac vinilogs
AVK
Arils
Heterils
Arils

1,4-Dihidroizonikotīnskābes rindas peptidomimētiķu iedarbība uz niacīna receptoriem

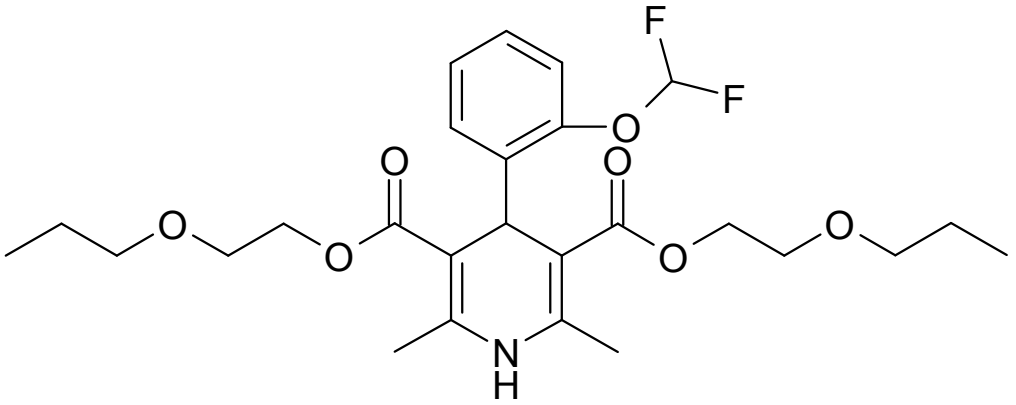
	GPR109A	**GPR109B**		
OSI-5443	Ag 84±1%	Ag 70±14%		X=O *Uz abiem*
P-5-76	0	Ag 80±1%		X=S *Tikai uz 109B*
Glutapirons	Ag 66±17%	Ag 89±12%		AS= 
Alapirons	Ag 74±14%	Ag 71±2%		AS=
OSI-3093	0	Ag 51±25%		R=Me n=2 *Tikai uz 109B*
Tauropirons	Ag 63±8%	Ag 62±9%		R=Et n=2
Homotauropirons	0	0		R=Et n=3 *Homologs- neaktīvs!*

4-Aril-1,4-dihidropiridīna atvasinājumu iedarbība uz niacīna receptoriem GPR 109A, GPR 109B

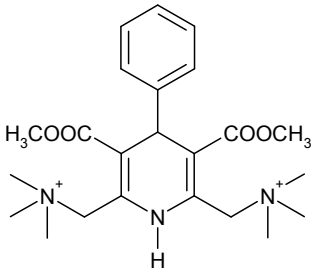
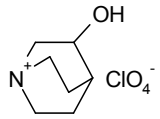
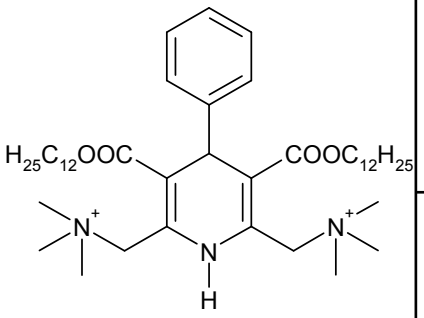
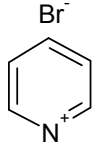
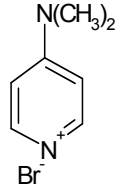
	GPR109A	**GPR109B**	Estera alkilgrupas apjoma un amīda ietekme	
Prosidīns	NA	NA		R = Pr-*i*
Foridons	NA	Ag 64±1%		R = Me
Diflurons	Ag 73±8%	Ag 71±5%		R = 
OSI-541	Ag 74±6%	Ag 52±1%		
OSI-9791	NA	Ag 69±1%	 Selektīvi uz 109B	

3. Paaudzes 1,4-dihidropiridīna Ca antagonista cerebrokrasta antidiabētiskās īpašības

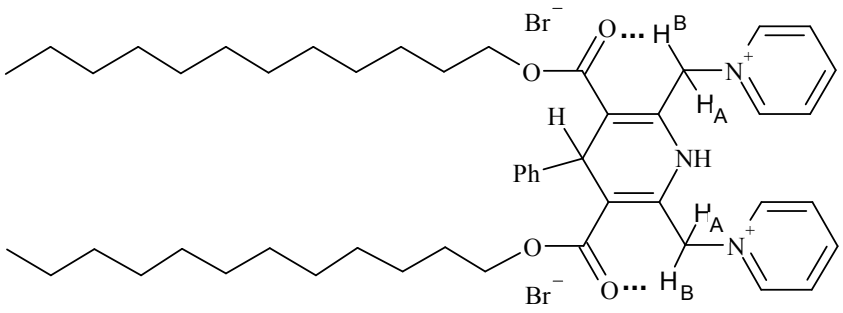
Cerebrokrasts ilgstoši samazina plazmas glikozes līmeni STZ-diabētisko žurku asinīs, sekmē glikozes transportu uz smadzenēm, izolētos eritrocītos, izolētā sirdī; palielina ATF veidošanos miokardā, normalizē plazmas lipīdu sastāvu, inhibē 3H-arahidonskābes atbrīvošanos no trombocītu plazmatiskās membrānas fosfolipīdiem, mobilizē insulīnu no beta šūnām.

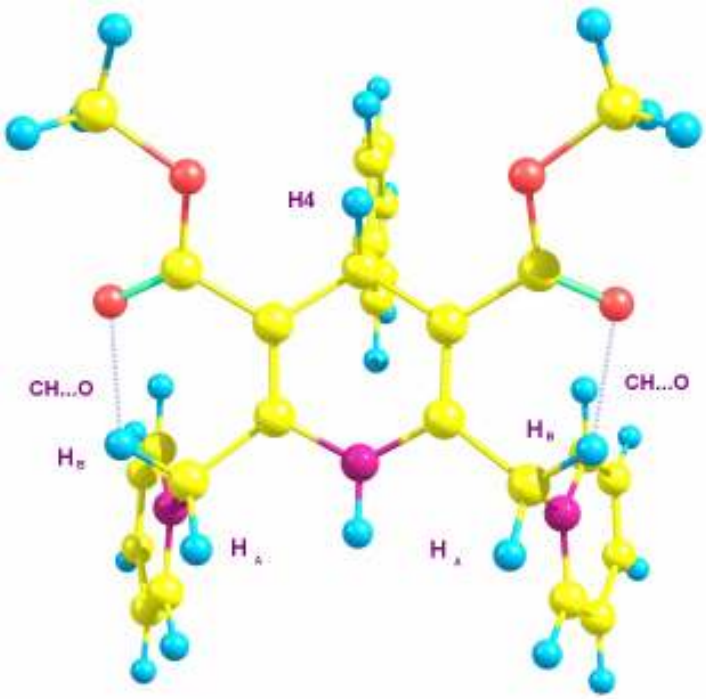


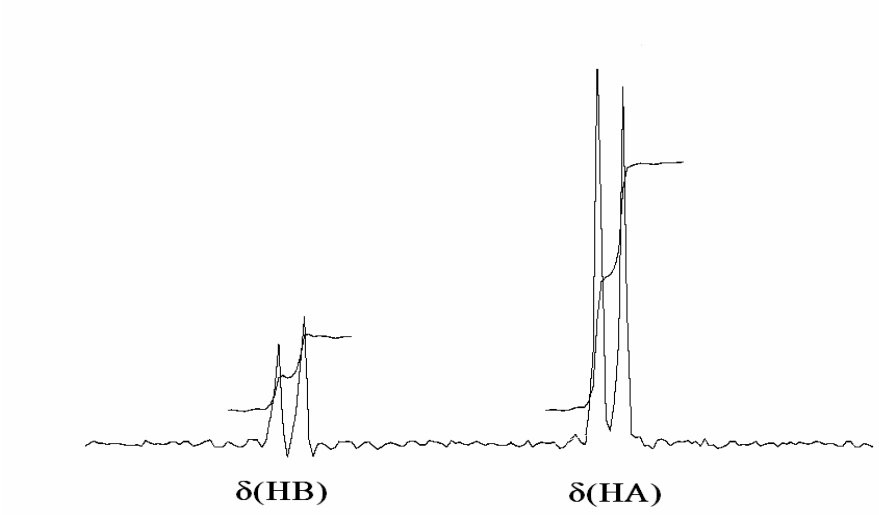
Katjonie 1,4-dihidropiridīni kā GPR 109A, GPR 109B un adenozīna A2A, A2B receptoru efektori

	GPR 109A	**GPR 109A**	**A2A**	**A2B**		
Z-41-67-A	Ag 29±5%	Ag 47±14%	Ant 36±8% (Parkinsona slimība)	Ant 46±5% (astma, **diabēts**, vēzis)		
Z-41-74	NA	NA	NA	NA		$N^+(CH_3)_3\ Br^-$
D-3-14	NA	Ag 70±10%	Ago 157±96% Allost. 259±142% (pret plaušu iek.)	Ago 15±10% Allost. 155±17%		
L-6	Ag 16±1%	Ag 29±5%	Ant IC_{50}=46±19 μM (Parkinsona sl.)	Ant 7±3%		

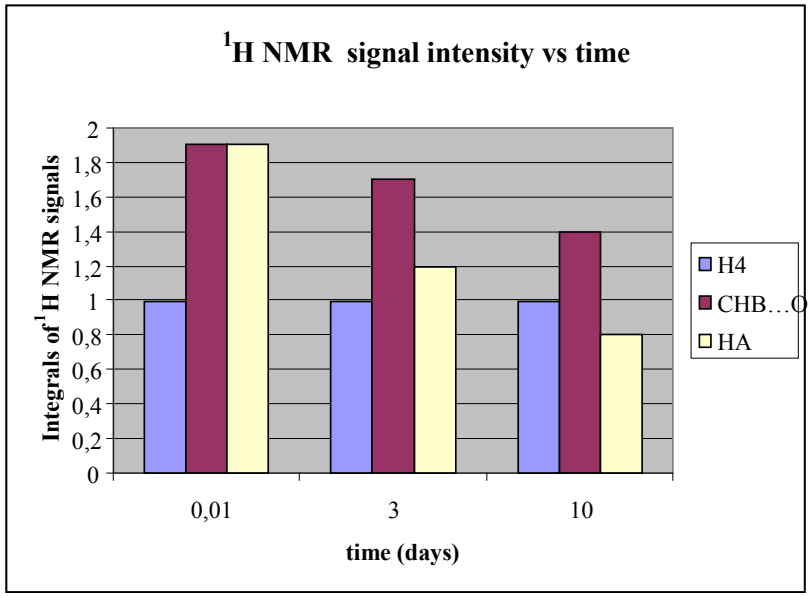
NMR and theoretical data show that HB protons of the methylene group form CH…O intramolecular H-bond with carboxyl group





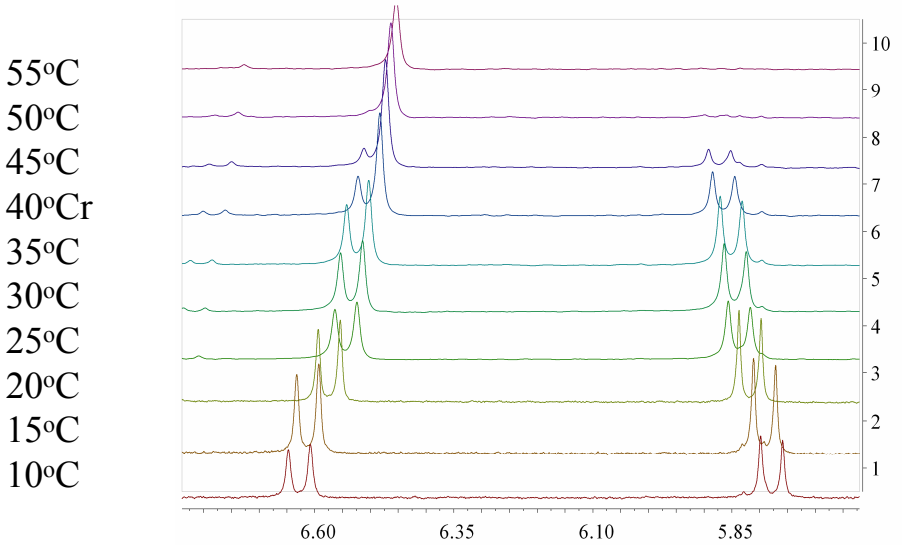


More intensive NOE registered between NH and H_A protons shows preferable orientation of H_A towards to NH



Time dependence of the H/D exchange of the methylene protons.

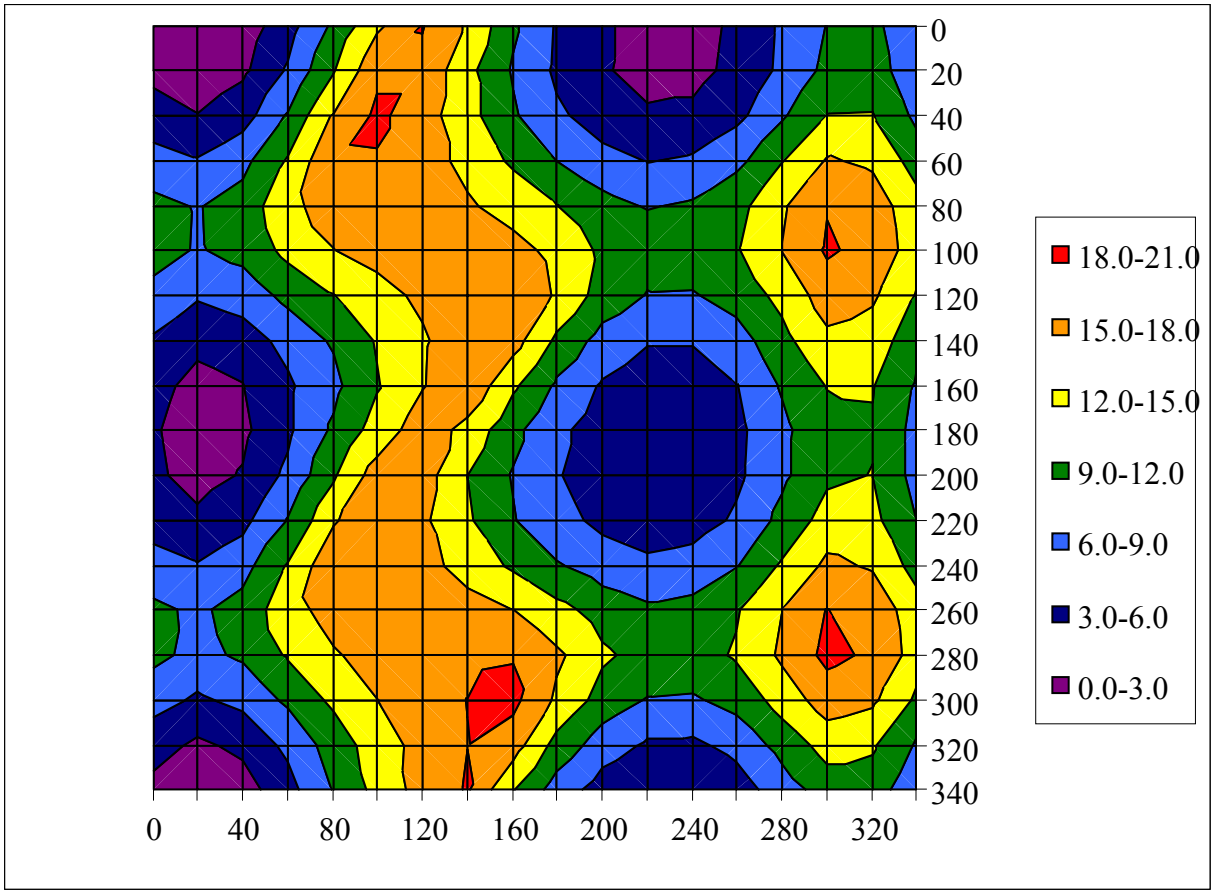
NMR spectra showing the temperature dependence of the methylene protons and H/D exchange in CD_3OD.



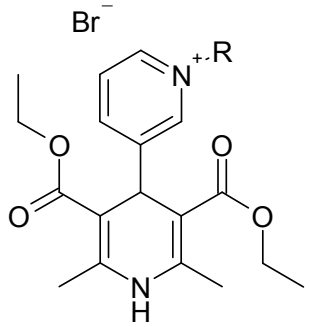
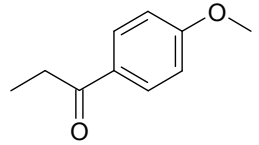
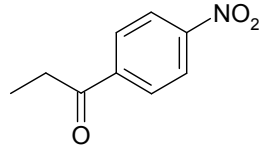
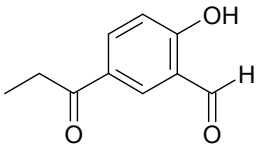
^{1}H NMR chemical shifts, (ppm).

The *ab initio* calculations show that the most stable conformation corresponds to the minimum on the potential energy surface and is stabilized with CH…O bond by ~1.5 kcal/mol.

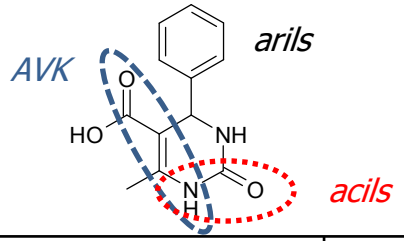
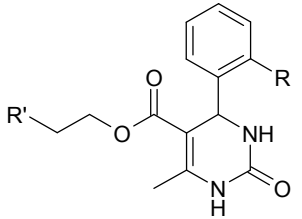
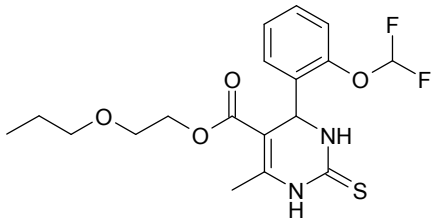
The hindered rotation around O=C----C3=C2 bond is about twice more restricted than around the C5-CH2 one.



Fenacilpiridīnija atvasinājumu ietekme uz GPR 109A, GPR 109B receptoriem

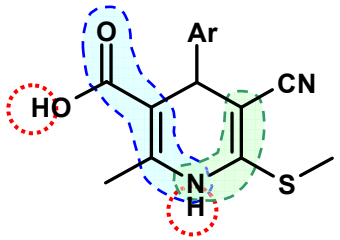
	GPR109A	**GPR109B**		R
IB-120	NA	NA		
IB-125	Ag 64±1%	NA		
IB-149	Ag 79±5%	Ag 71±20%		

Tetrahidropirimidīnonu-5-karbonskābes atvasinājumu iedarbība uz niacīna receptoriem

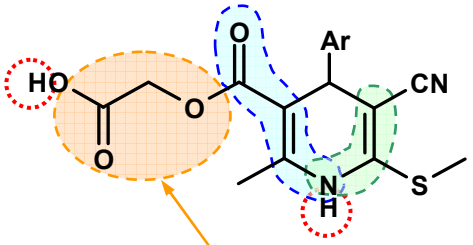
	GPR 109A	**GPR 109B**			
8-105-2	Ag 71±5%	Ag 63±23%			
8-105-1	NA	Ag 66±10%	R'=NC		R=H
J-9-21	NA	NA	R'=$H_3CCH_2CH_2O$		R=$OCHF_2$
J-9-20	Ag 68±14%	Ag 74±15%			

Tetrahidropirimidīnoni nedaudz atgādina Roche patentētos pirīdopirimidīnonus. Karbonskābes atvasinājums ir GPR 109A un GPR 109B agonists, skābes cianoetilesteris tikai GPR 109B agonists, bet propoksietilesterim aktivitātes nav. Tomēr analogais tions atkal ir aktīvs gan uz GPR 109A gan GPR 109B. Zināmā mērā aktivitātes var būt saistītas ar protonu kustīgumu.

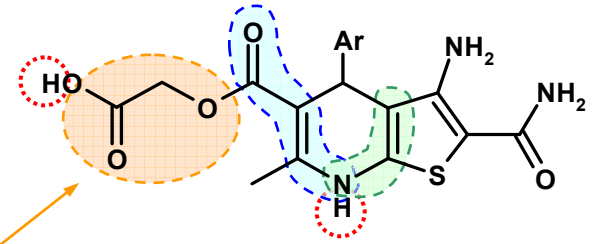
Aminovinilkarboksi un acilamino izostēru fragmentus saturošie hidrēto antranilskābju analogi



OSI-7703

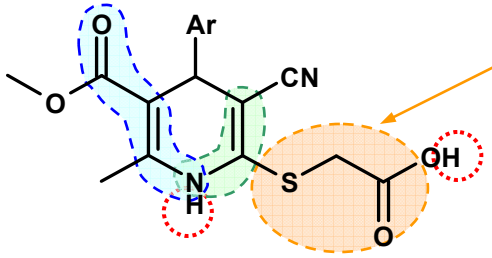


OSI-9774



OSI-9603

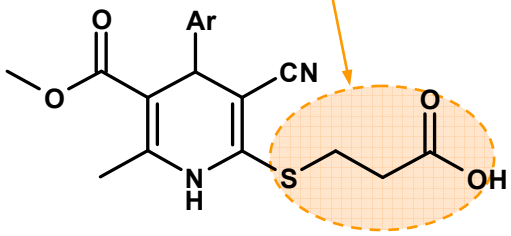
Linkers



OSI-9790

OSI-9911

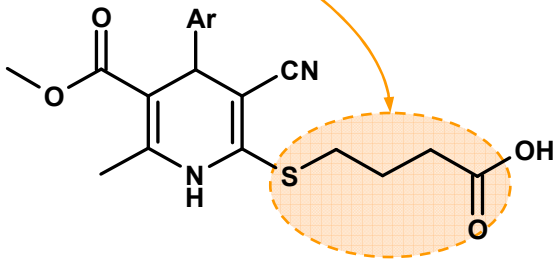
OSI-9913



OSI-9901

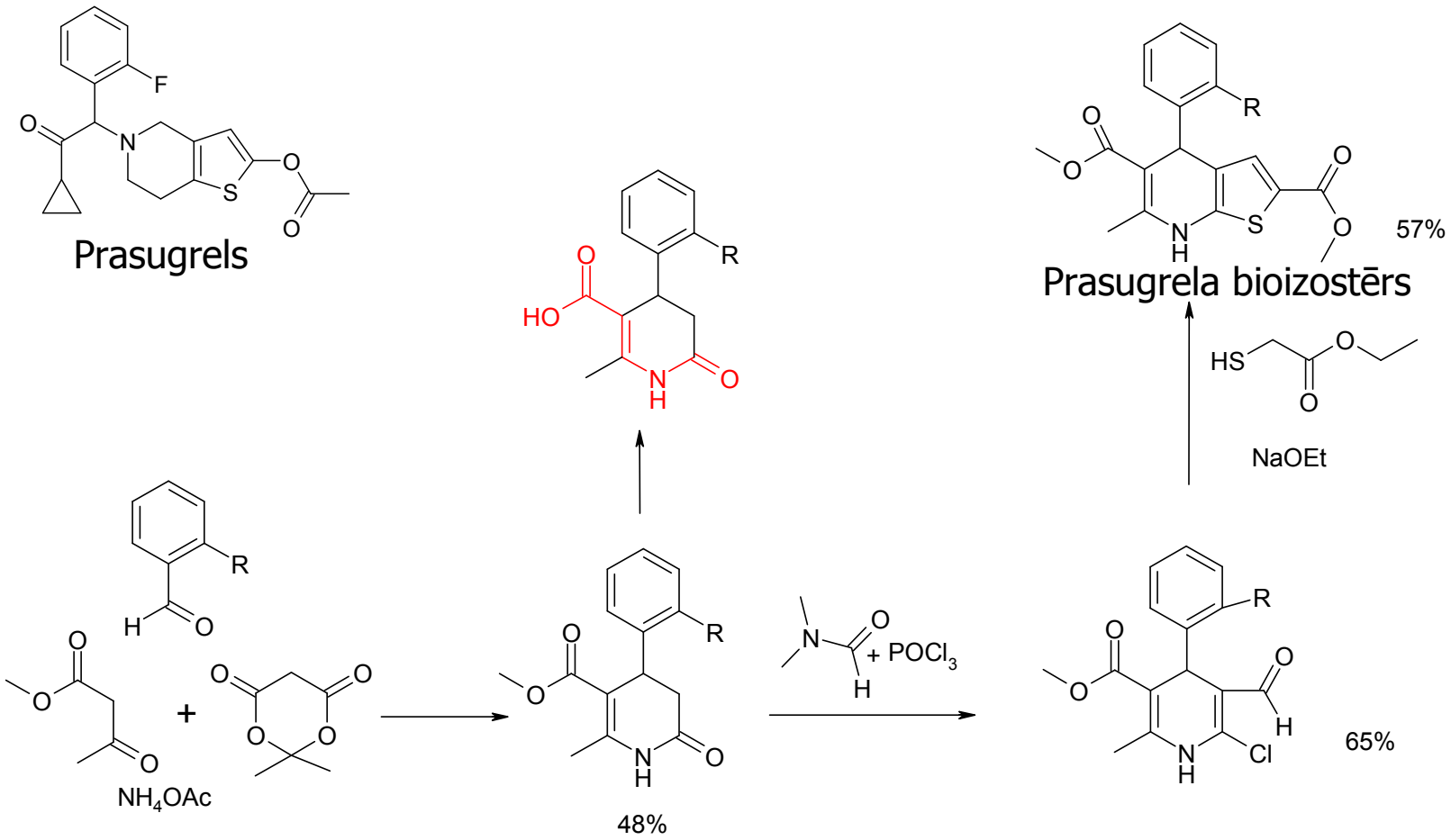
OSI-9910

OSI-9914

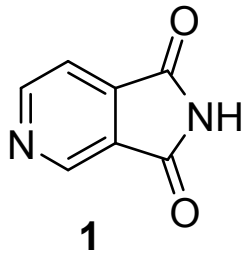


OSI-9915

GPR 109A, GPR 109B ligandu un klopidogrela un 3. klīniskajā fāzē esošā prasugrela bioizostēra sintēze



1H-Pirolo[3,4-c]piridīn-1,3(2H)-dions (**1**) un tā atvasinājumi **2** - **5**

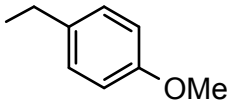


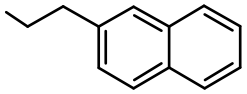
1

1*H*-Pirolo[3,4-*c*]piridīn-1,3(2*H*)-dions

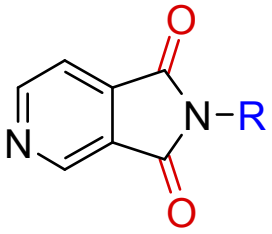
Pašlaik sintezēti:

1 un **5a**, R = H

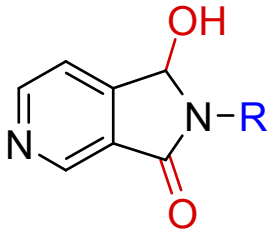
2b - **5b** , R = 

2c un **5c** , R = 

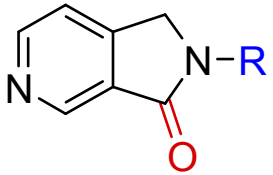
SINTEZĒJAMO SAVIENOJUMU VISPĀRĪGĀ STRUKTŪRA



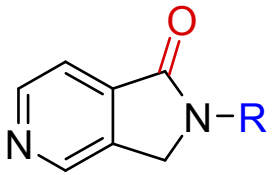
2



3



4

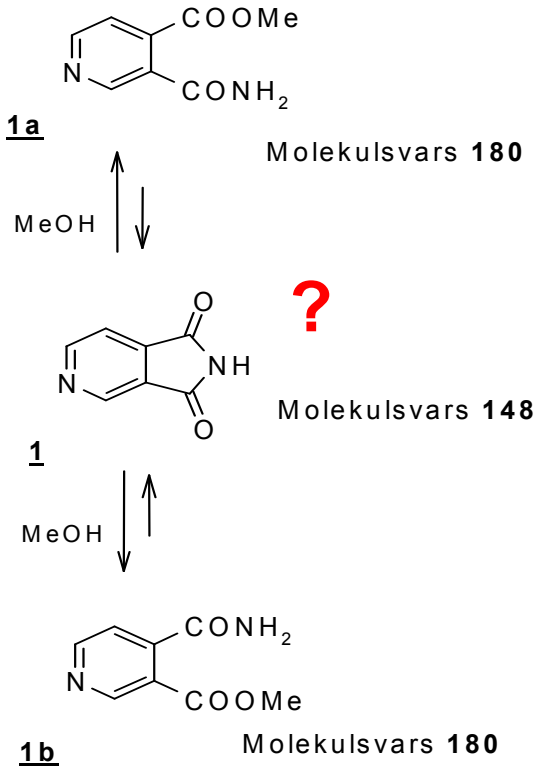


5

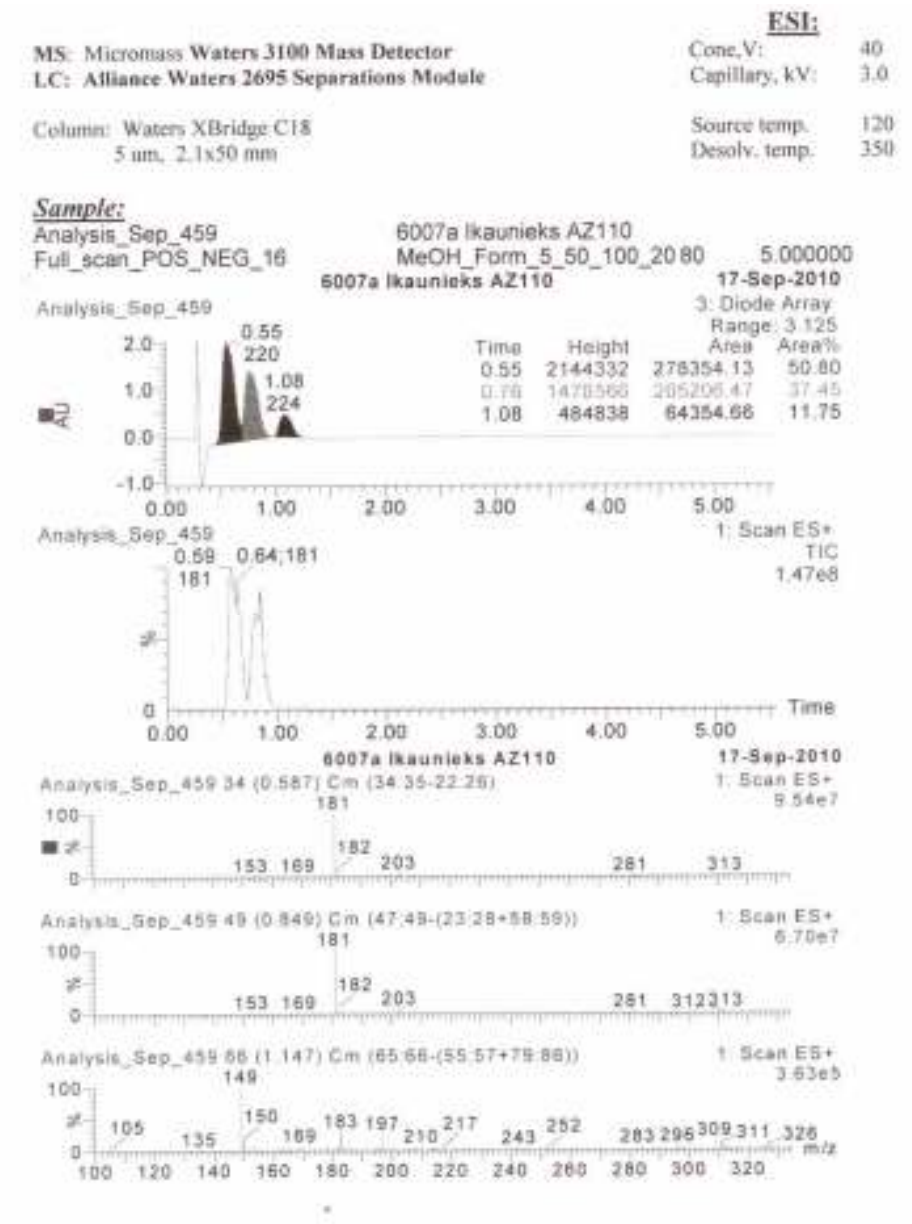
R = H vai attiecīgi modificēta sānu ķēde

1H-Pirolo[3,4-c]piridīn-1,3(2H)-diona (**1**) īpašības

Savienojums 1 LC-MS analīzē parādās kā dinamisks vielu maisījums (paraugs tiek šķīdināts MeOH un tad analizēts):



Līdzīgi savienojumam 1 vajadzētu reaģēt ar H_2O, veidojot niacīna atvasinājumus (Py kā nukleofīlais katalizators?)

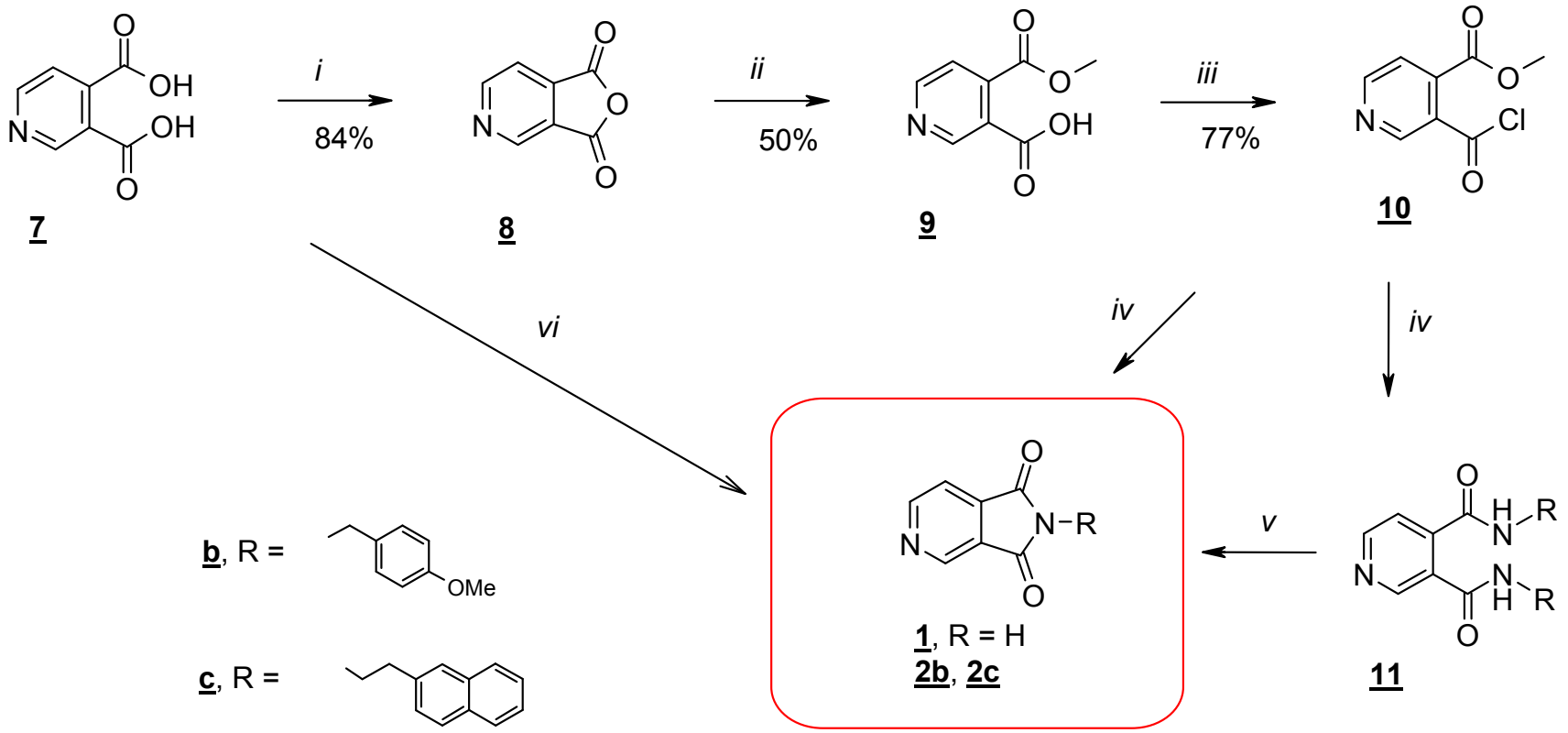


1H-Pirolo[3,4-c]piridīn-1,3(2H)-diona (**1**) un tā 2-aizvietoto atvasinājumu **2b** un **2c** sintēze

Aprobētas 1,3-dionu 2 sintēzes metodoloģijas

1) sakausējot piridīndikarbonskābi 7 ar attiecīgu amīnu NH_2R (ja tie ir termiski stabili);

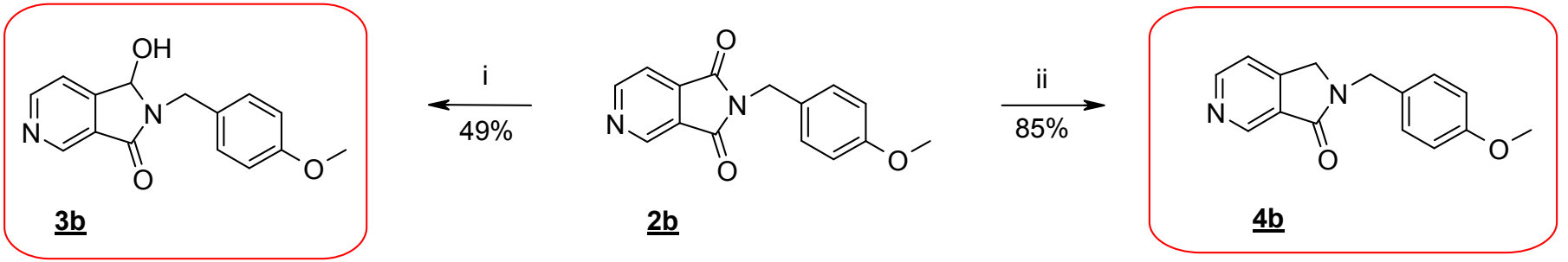
2) vispirms pārvēršot dikarbonskābi 7 par hloranhidrīdu 10 un tad to apstrādājot ar attiecīgu amīnu NH_2R (ja tie ir gaistoši vai termiski nestabili):



Reaģenti un reakciju apstākļi: *i)* Ac_2O, 112ºC, 1,5 st., 84%; *ii)* 1) NaOMe, THF, MeOH, -70º→25°C; 2) k.HCl, ist. temp., 50%; *iii)* $SOCl_2$, benzols, 80ºC, 16 st., 77%; *iv)* NH_2R,Et_3N, DMAP, CH_2Cl_2; *v)* 185ºC; *vi)* 170ºC, NH_2R.

2-(4-Metoksibenzil)-1,2-dihidro-3H-pirolo[3,4-c]piridīn-3-ona **4b** un tā 1-hidroksianaloga **3b** sintēze

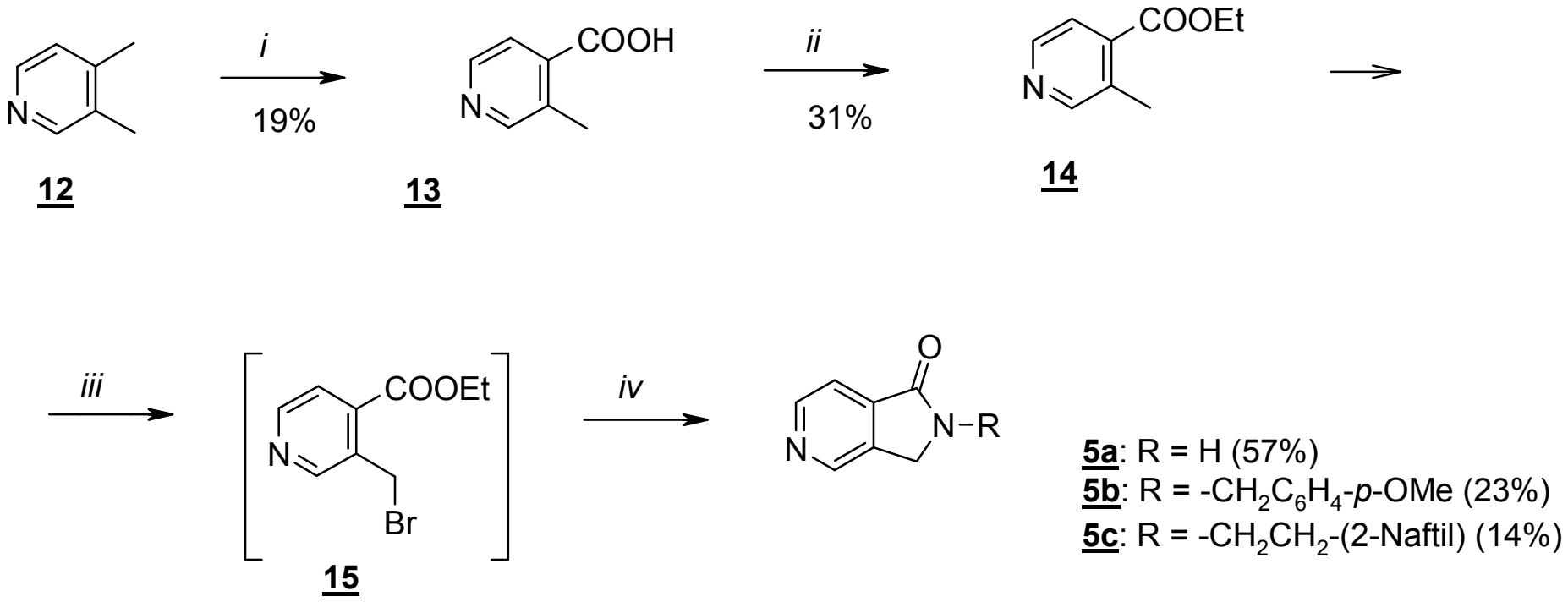
Savienojumiem 2 ir iespējama selektīva 1-okso grupas reducēšana par spirtiem 3 vai pat pilnīga tās deoksigenēšana, veidojot savienojumus 4:



Reaģenti un reakciju apstākļi: *i)* Sn (granulas), AcOH, HCl, 25°C, 24 st., 49%; *ii)* Sn (pulv.), AcOH, HCl, 25°C, 48 st., 85%.

2,3-Dihidro-1H-pirolo[3,4-c]piridīn-1-ona 5a un tā 2-aizvietoto analogu 5b,c sintēze

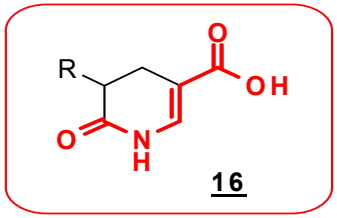
Savienojumu 5, kuri satur tikai vienu okso grupu 1.pozīcijā, sintēzei ir aprobēta to iegūšanas metode no 3,4-lutidīna (12):



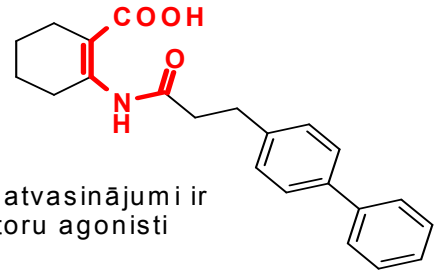
5a: R = H (57%)
5b: R = -$CH_2C_6H_4$-*p*-OMe (23%)
5c: R = -CH_2CH_2-(2-Naftil) (14%)

Reaģenti un reakciju apstākļi: *i)* Se_2O, 1,4-dioksāns, 102ºC, 24 st., 19%; *ii)* 1) NaOH, ist. temp., 1 st., 2) EtBr, 40ºC, 15 st., 31%; *iii)* NBS, Bz_2O_2, CCl_4, 76ºC, 3 st. ; *iv)* H_2NR, EtOH, ist. temp., 48 st.

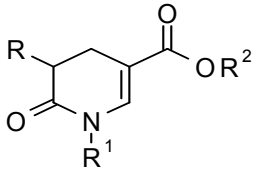
6-Okso-1,4,5,6-tetrahidro-3-piridīnkarbonskābes 16 atvasinājumu retrosintēzes shēma



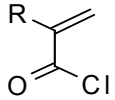
16



Cikloheksēna atvasinājumi ir niacīna receptoru agonisti

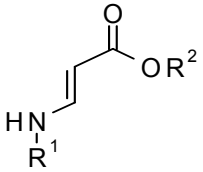


17

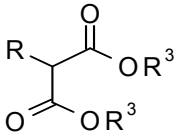


18

+

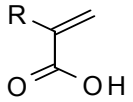


19



21

+ HCHO

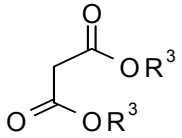


20

24

+

25



22

+ RBr

23

Kopsavilkums

1. Savākti un apkopoti literatūras dati par mazmolekulāro savienojumu ietekmi uz GPR109A, GPR109B receptoriem.

2. Konstatēti galvenie struktūrelementi, kas raksturīgi aktīvajiem GPR109A, GPR109B ligandiem, sākta kvantitatīvās struktūras-aktivitātes sakarību noskaidrošana, gatavošanās ligandu dokingam.

3. Izvērtēta oriģinālā informācija par 3 dažādu savienojumu grupu pārstāvju ietekmi uz GPR109A, GPR109B receptoriem.

4. Izvērtēti četri sintēžu virzieni, izstrādātas sintēžu shēmas, iegūti, attīrīti un raksturoti 12 savienojumi – potenciālie GPR109A, GPR109B ligandi.

Pateicība

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Protective Role of SIRT1 in Diabetic Vascular Dysfunction

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Objective—Calorie restriction (CR) prolongs the lifespan of various species, ranging from yeasts to mice. In yeast, CR extends the lifespan by increasing the activity of silencing information regulator 2 (Sir2), an NAD-dependent deacetylase. SIRT1, a mammalian homolog of Sir2, has been reported to downregulate p53 activity and thereby prolong
the lifespan of cells. Although recent evidence suggests a link between SIRT1 activity and metabolic homeostasis during CR, its pathological role in human disease is not yet fully understood.

Methods and Results—Treatment of human endothelial cells with high glucose decreases SIRT1 expression and thus activates p53 by increasing its acetylation. This in turn accelerates endothelial senescence and induces functional abnormalities. Introduction of SIRT1 or disruption of p53 inhibits high glucose–induced endothelial senescence and dysfunction. Likewise, activation of Sirt1 prevents the hyperglycemia-induced vascular cell senescence and thereby protects against vascular dysfunction in mice with diabetes.

Conclusions—These findings represent a novel mechanism of vascular cell senescence induced by hyperglycemia and suggest a protective role of SIRT1 in the pathogenesis of diabetic vasculopathy.

Small molecule activators of SIRT1 replicate signaling pathways riggered by calorie restriction *in vivo*

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***BMC Systems Biology* 2009, 3:31**

Results: Here we demonstrate that SIRT1 activators recapitulate many of the molecular events downstream of CR *in vivo*, such as enhancing mitochondrial biogenesis, improving metabolic signaling pathways, and blunting pro-inflammatory pathways in mice fed a high fat, high calorie diet.

Conclusion: CNM of gene expression data from mice treated with SRT501 or SRT1720 in combination with supporting *in vitro* and *in vivo* data demonstrates that SRT501 and SRT1720 produce a signaling profile
that mirrors CR, improves glucose and insulin homeostasis, and acts via SIRT1 activation *in vivo*. Taken together these results are encouraging regarding the use of small molecule activators of SIRT1 for therapeutic intervention into type 2 diabetes, a strategy which is currently being investigated in multiple clinical trials.

Small molecule activators of SIRT1 replicate signaling pathways riggered by calorie restriction *in vivo*