Potent and selective, orally active GPBAR1 agonists as chemical biology probes

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Type 2 diabetes

- Type 2 diabetes
  - a disease resulting from a combination of defective insulin secretion and defective responsiveness to insulin.
  - 285 M people (6.4% of world adult population) affected in 2010.
    (source: International Diabetes Federation, http://www.diabetesatlas.org)
  - a manageable chronic condition but risk of long-term complications.

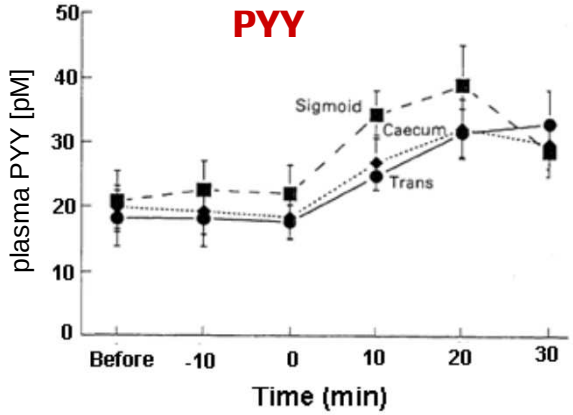
- Initial therapy of type 2 diabetes starts with metformin (in addition, lifestyle changes are strongly recommended: diet and exercise)

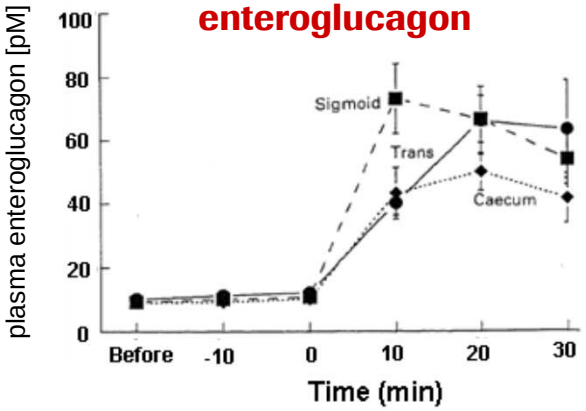
- If sufficient glucose control is not achieved within 3 months, add one of the following to metformin (ADA/EASD consensus report: *Diabetes Care* **2012**, *35*, 1364):
  - sulfonylurea (e. g., glibenclamide)
  - PPARγ agonist (pioglitazone)
  - DPP4 inhibitor (e. g., sitagliptin)
  - GLP–1 receptor agonist (e. g., liraglutide)
  - insulin

GLP–1 is a potent inducer of glucose-dependent stimulation of glucose secretion

Bile acids as incretin secretagogues

- Deoxycholic acid administered through intracolonic infusion in humans stimulates **PYY and GLP-1 release** through activation of L-cells, to extent similar to that of a normal meal (*Gut* **1993**, *34*, 1219).



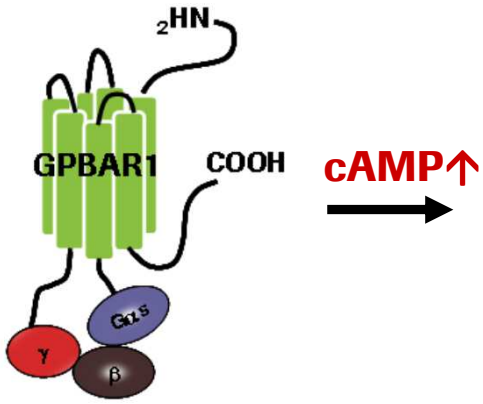


- Discovery of the target, **GPBAR1** (TGR5, M-BAR) independently at Takeda and Banyu (2002), leading to intracellular increase of cAMP. Bile acids are endogenous ligands:
  - deoxycholic acid EC_{50} = 1 µM
  - lithocholic acid EC_{50} = 0.53 µM

- Bile acids promote **GLP-1 secretion** through GPBAR1 (*BBRC* **2005**, *329*, 386) in STC1 enteroendocrine cell line

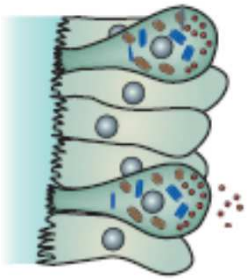
GPBAR1 action on L-cells and macrophages

Both anti-diabetic and anti-inflammatory effects



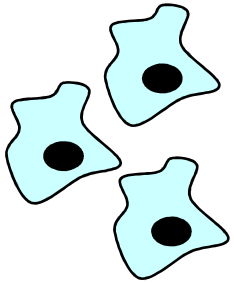
cAMP↑

L-cells



Secretagogue:
PYY ↑
GLP-1 ↑

- Glucose-dependent glycemic control
  - low risk of hypoglycemia
  - synergistic action of peptides
  - parallels to bariatric surgery
- Islet preservation
- Body weight loss



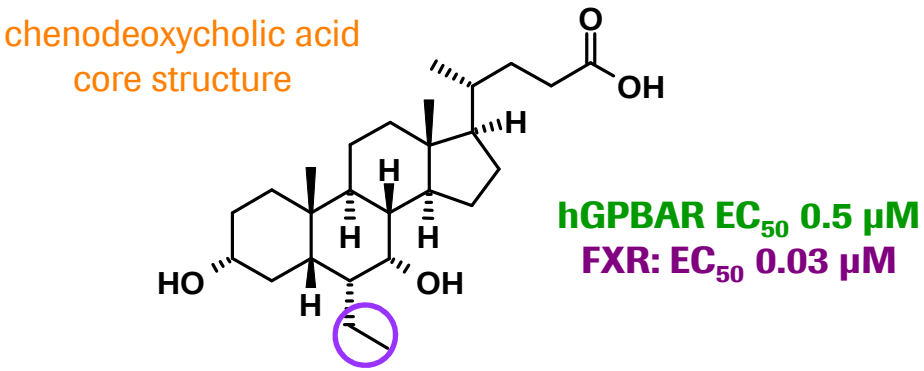
Kupffer cells
Monocytes

cytokine release:
TNFα ↓
IL6 ↓

- Anti-inflammatory actions
  - Improved insulin sensitivity
  - Reduced liver inflammation
  - Reduced liver fibrosis

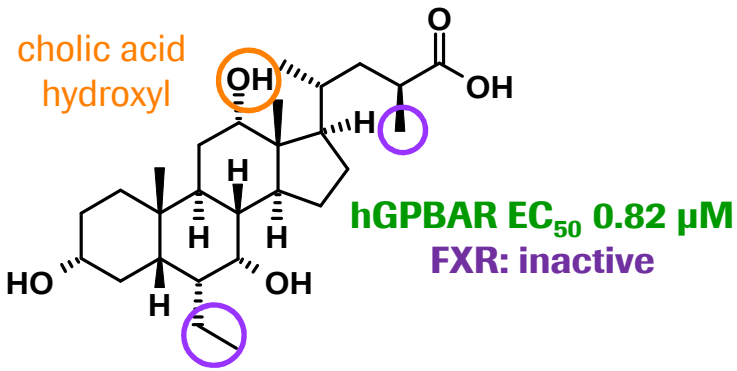
Reference compounds (1)
Synthetic bile acids

Intercept/Università di Perugia: Obeticholic acid (INT-747)



- Mixed **FXR**/**GPBAR1 agonist**.
- Phase 3 (primary biliary cirrhosis); Phase 2/3 (non-alcoholic steatohepatitis)

Intercept/Università di Perugia: INT-777 [6-EMCA]



- Selective **GPBAR1 agonist**.
- Anti-diabetic effects: *Cell Metabolism* **2009**, *10*, 167.
- Reduction of atherosclerosis by reducing macrophage inflammation and lipid loading: *Cell Metabolism* **2011**, *14*, 747.
- "lead candidate to advance into clinical studies" (*J. Med. Chem.* **2009**, *52*, 7958).

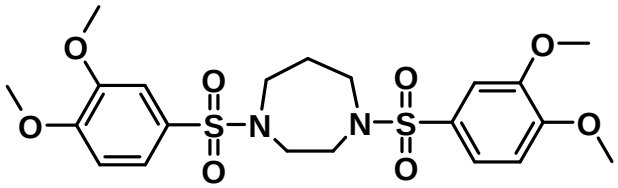
Reference compounds (2)
Non-bile acids

Exelixis/Bristol-Myers Squibb: XL-475

structure not disclosed

- Orally *administered* GPBAR1 agonist. **Designed to selectively target the GPBAR1 receptors in the intestine without significant systemic exposure** to enhance the therapeutic index for potential chronic administration.
- Effective in lowering blood glucose, improving glucose tolerance, improving plasma and hepatic lipid levels, and reducing hepatic steatosis.

GlaxoSmithKline: SB-050



hGPBAR1 EC_{50} 1.2 µM

- **Compound SB-050 progressed to the clinic** but was stopped due to inconsistent PD effects/large inter-individual variations (e.g. GLP-1 release) across doses.
- Not clear whether systemic exposure is required for PD effect.
- *241st ACS National Meeting & Exposition, Anaheim* **2011**, MEDI-335.

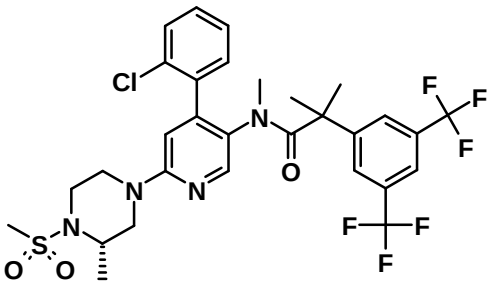
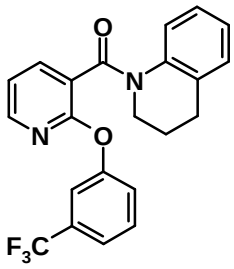
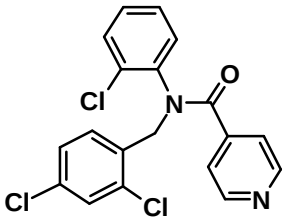
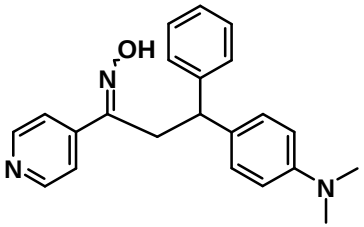
Today's topics

- A new class of non-bile acid derived, orally bioavailable GPBAR1 agonists
  - ⇨ Start from scratch, by way of high-throughput screening

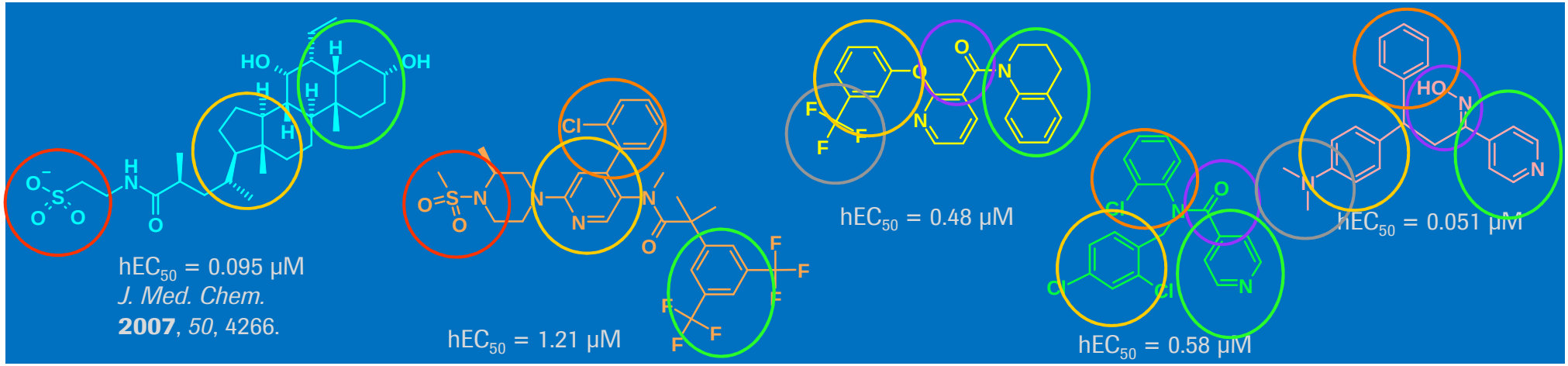
- Characterisation of the new GPBAR1 agonists as antidiabetic agents
  - ⇨ Glucose, PYY, GLP–1

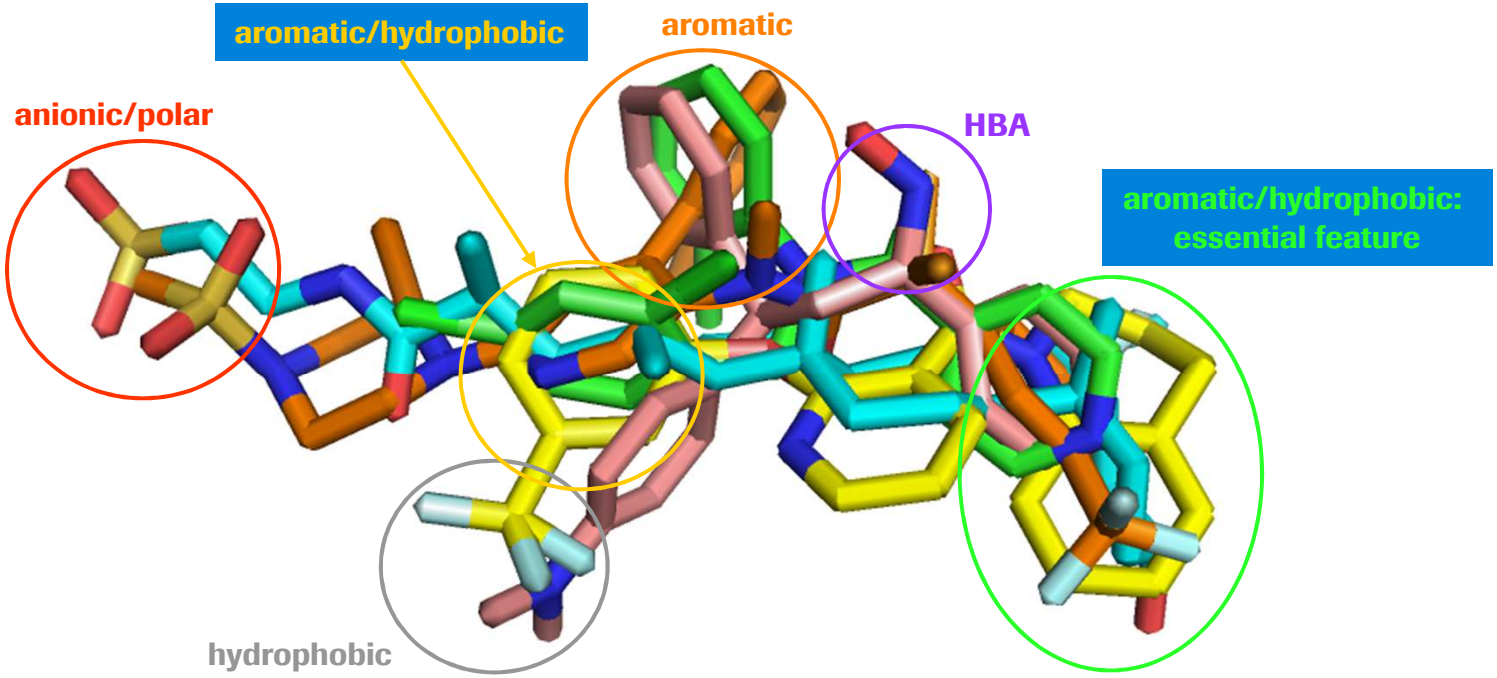
GPBAR1 – HTS

- High-throughput screening of the whole Roche library (n = 940000)
- 5147 validated hits, of these 847 at hEC_{50} < 3 µM
- Examples (other than bile acids, steroids):

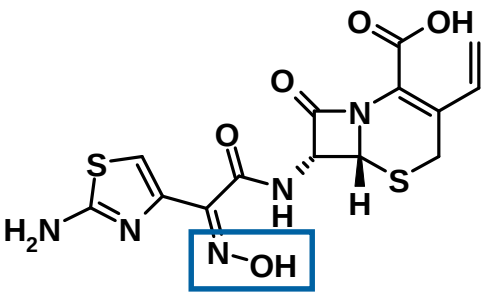
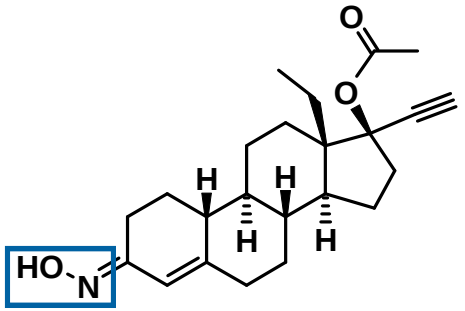
	NK1 antagonists (n = 43)	**nicotinamides** (n = 1)	**"Novartis-type"** (n = 4)	**oximes** (n = 8)
				
hEC_{50} [µM]	1.21	0.49	0.58	0.051
mEC_{50} [µM]	>20	2.7	>20	0.28

Ligand alignment: HTS hits/synthetic bile acid

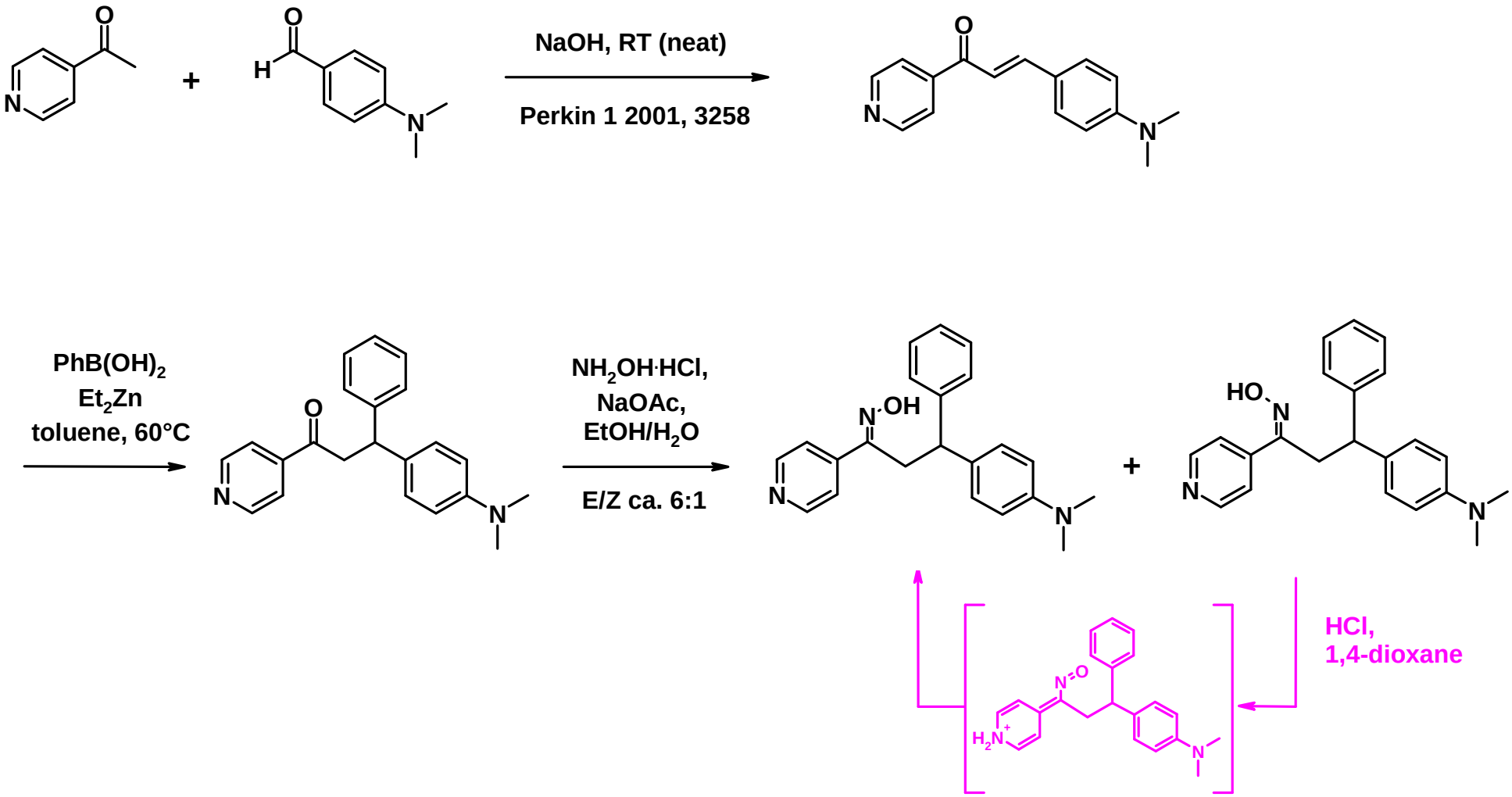




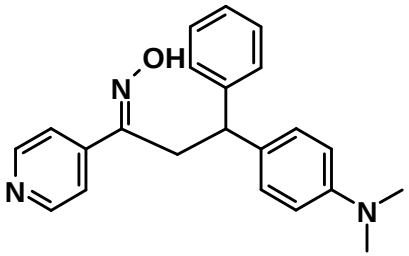
Should we consider oximes at all?
Marketed drugs

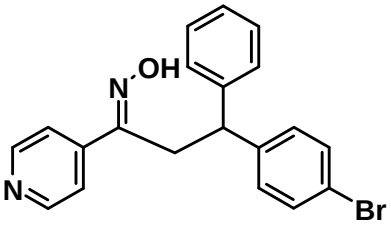
	Antibiotics	Hormonal contraceptives
		
INN	cefdinir	norgestimate
marketed by	Astellas/Abbott + generics	Johnson & Johnson + generics
peak sales (year)	ca. USD 900 M (2006)	n/a
dose, administration route	oral, 300 -600 mg/day	Oral, 0.18 – 0.25 mg per tablet, predominantly in combination with ethynylestradiol
half life	1.7 h	n/a
clogP	-0.5	5.1
metabolism	none (renal excretion of parent)	Complete first-pass metabolism (intestine, liver) to active metabolites norelgestromine (deacetylation), and norgestrel (deacetylation + ketone formation)

Resynthesis of the HTS hit



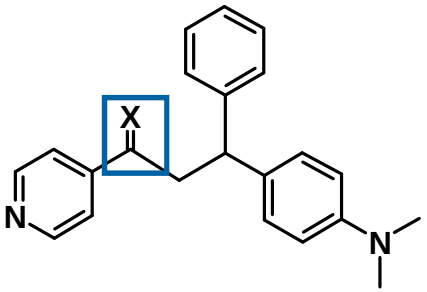
Profile of the oxime hits

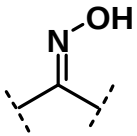


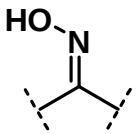


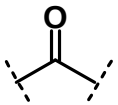
human/mouse EC_{50} [nM]	**45**	**19**
mouse EC_{50} [nM]	**2000**	**760**
solubility [mg/L] (LYSA, pH 6.5)	**<1**	**<1**
logD (pH 7.4)	**3.4**	**>3**
aqueous stability (pH 1, 4, 6, 8, 10)	n. d.	**stable**
permeability (prediction)	medium/high	medium/high
Cl_{mic} [mL/min/mg protein] (h/m)	**169 / 460**	**242 / 388**
CYPs [µM] (3A4, 2D6, 2C9)	**<0.2 / 3.1 / <0.2**	**<0.2 / 2.2 / <0.2**
hERG IC_{20} [µM]	n.d.	**5.5**
Mouse PK Cl [mL/min/kg]		**77**
Mouse PK V_{ss} [L/kg]		1.6
Mouse PK F [%]		**32**
Mouse PK $t_{1/2}$ [h]		**0.2 – 0.5**
protein binding f_u [%] (h/m)		0.2 / 0.5

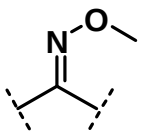
Initial SAR: Oxime, pyridine



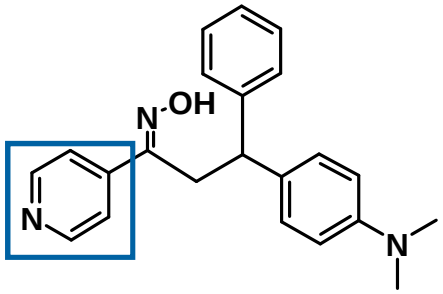


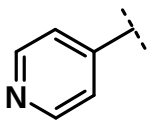


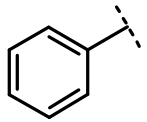


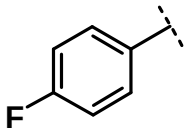


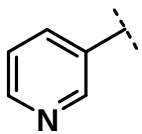
hEC_{50}	0.045 µM	0.26 µM	>10 µM	>10 µM

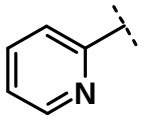










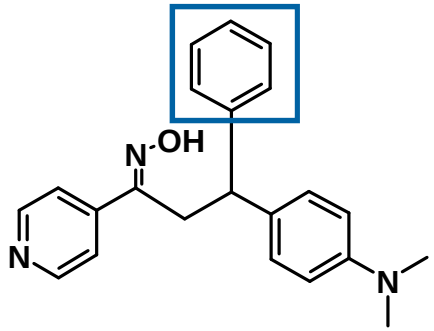


hEC_{50}	0.045 µM	>10 µM	>10 µM	>10 µM	>10 µM

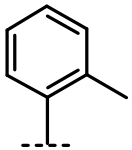
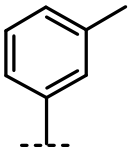
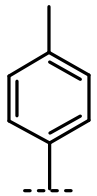
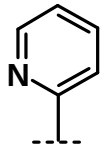
(–)-Enantiomer: 0.028 µM

(+)-Enantiomer: 0.105 µM

Optimisation of "northern" vector

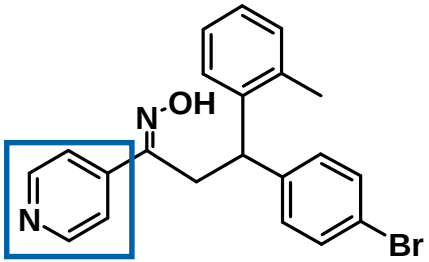


hEC_{50} = 0.045 µM

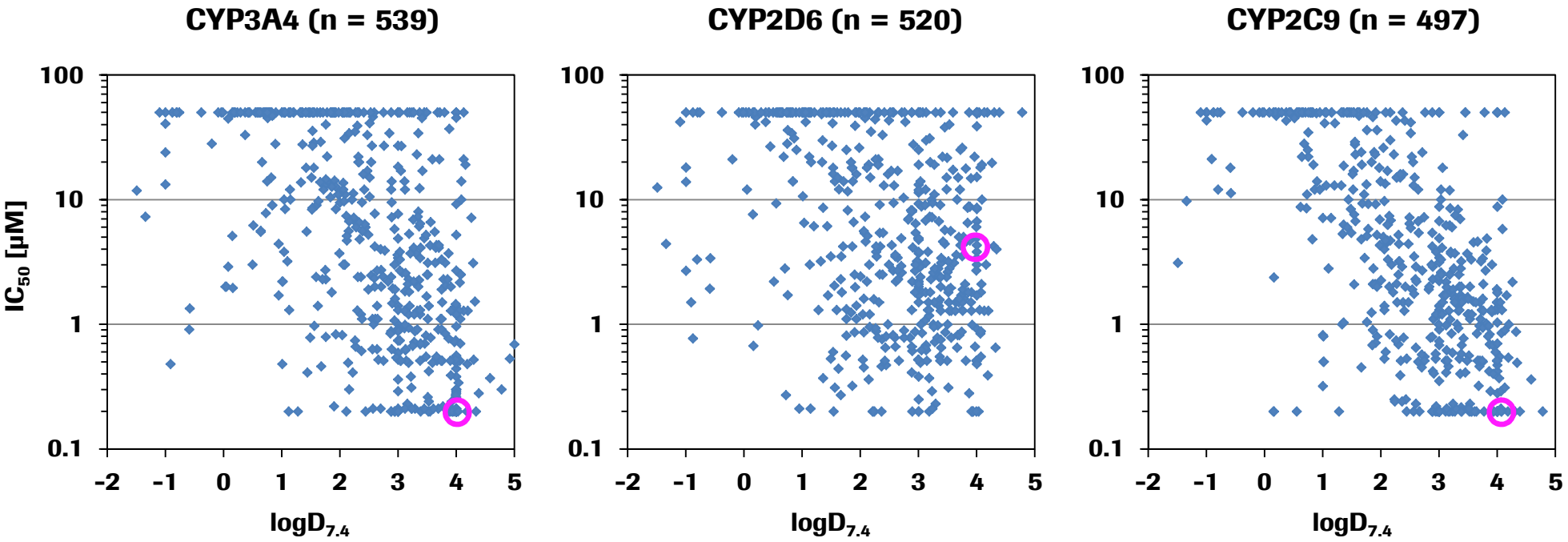
						
hEC_{50}	0.025 µM	0.077 µM	0.1 µM	0.38 µM	>10 µM	>10 µM

Lipophilic unsubstituted 4-pyridyl derivatives

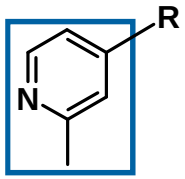
3A4/2C9 pharmacophore

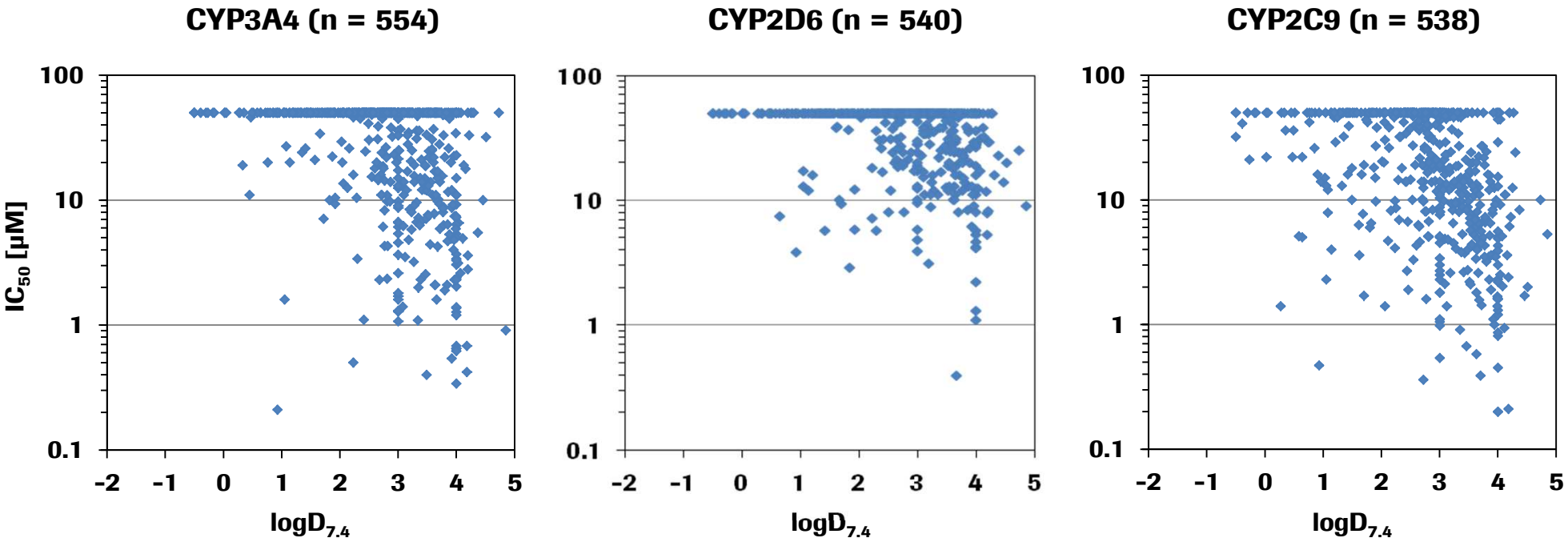


hEC_{50}	0.012 µM
CYP3A4, IC_{50}	<0.2 µM
CYP2D6, IC_{50}	4.3 µM
CYP2C9, IC_{50}	<0.2 µM
logD	>4

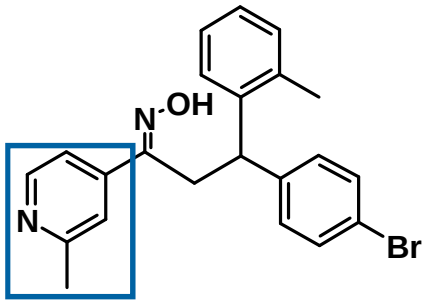


3-Methyl-4-pyridyl is not a CYP450 pharmacophore

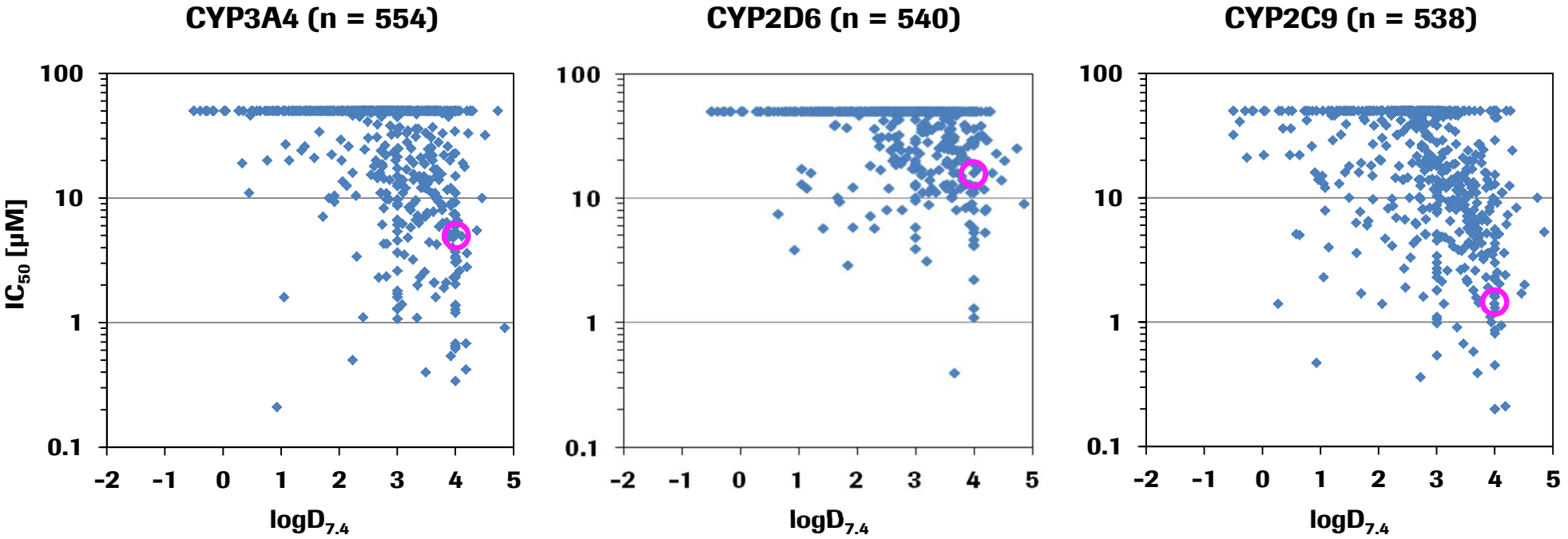




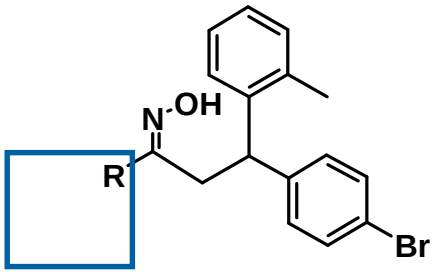
3-Methyl-4-pyridyl is not a CYP450 pharmacophore

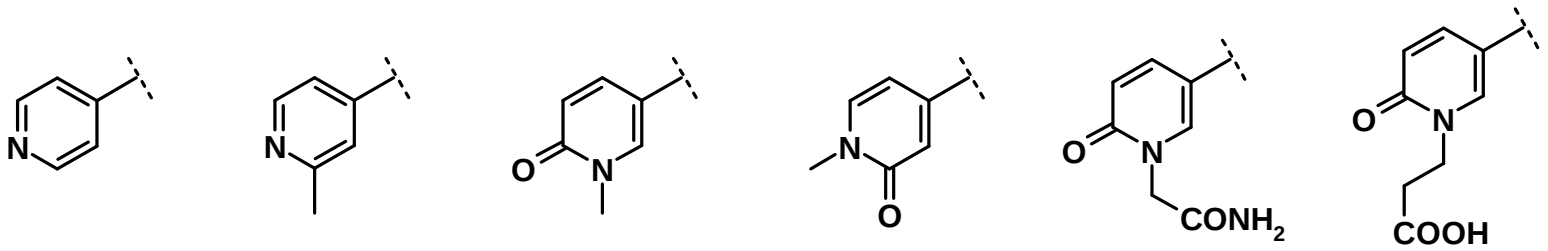


hEC_{50}	0.028 µM
CYP3A4, IC_{50}	5 µM
CYP2D6, IC_{50}	19 µM
CYP2C9, IC_{50}	1.6 µM
logD	>4



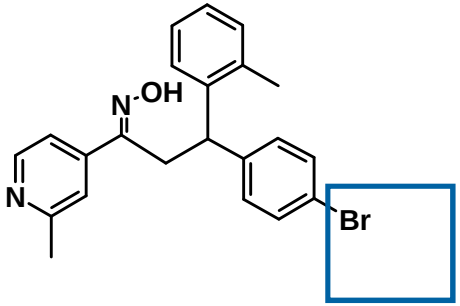
Other head groups with reduced CYP interaction

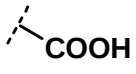


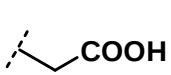


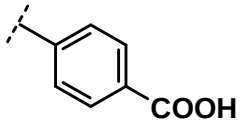
hEC_{50}	**0.012 µM**	**0.028 µM**	**0.008 µM**	**>10 µM**	**0.179 µM**	**0.87 µM**
CYP3A4, IC_{50}	**<0.2 µM**	**5 µM**	**>50 µM**	**15 µM**	n. a.	**>50 µM**
CYP2D6, IC_{50}	**4.3 µM**	**19 µM**	**24 µM**	**25 µM**	**50 µM**	**>50 µM**
CYP2C9, IC_{50}	**<0.2 µM**	**1.6 µM**	**5.1 µM**	**4.4 µM**	**32 µM**	**6.6 µM**
logD	**>4**	**>4**	**3.8**	**3.8**	**2.9**	**1.0**

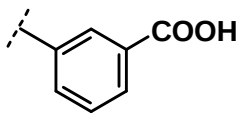
Introduction of polarity at "south eastern" exit vector

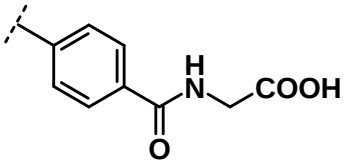




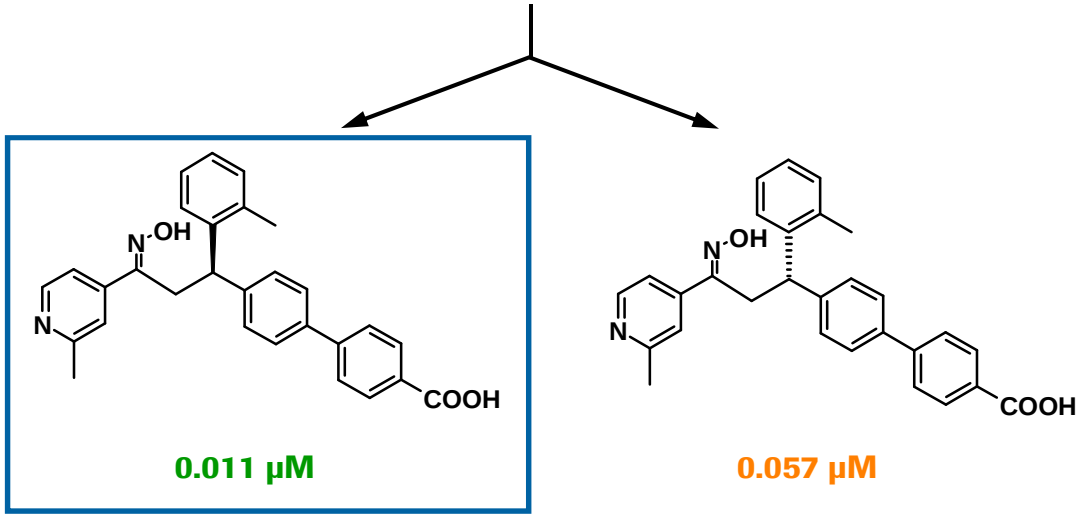






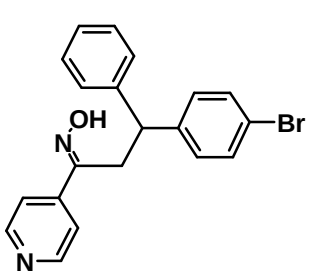
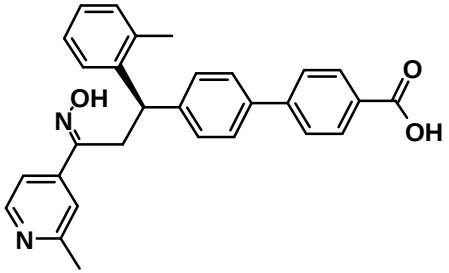


hEC_{50} = 0.028 µM	0.26 µM	0.7 µM	0.02 µM	0.29 µM	0.01 µM
mEC_{50} = 0.74 µM	3.7 µM	6.1 µM	0.093 µM	0.86 µM	0.07 µM
logD = >4	1.1	0.9	2.5	2.4	1.0
permeatility = medium/high	medium/high	medium/high	medium/high	medium/high	LOW



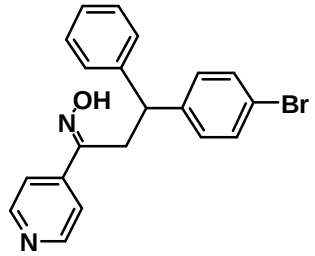
hEC_{50} 0.011 µM 0.057 µM

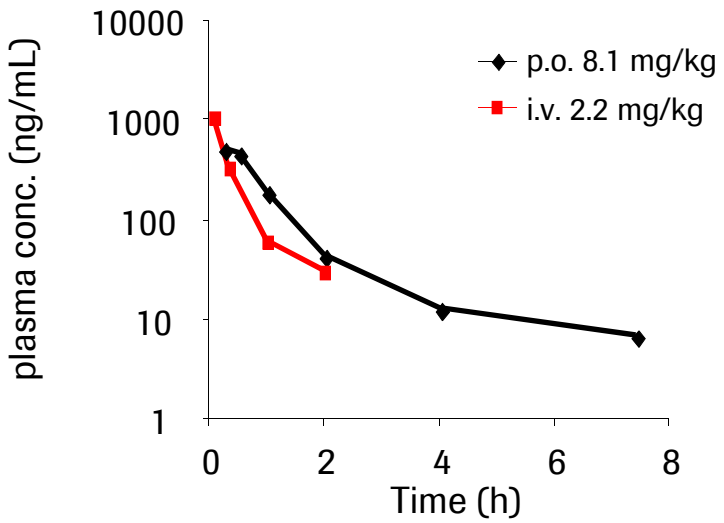
Comparison of in vitro profiles

	HTS hit	benzoic acid lead compound
		
human EC_{50} [nM]	19	11
mouse EC_{50} [nM]	760	130
FXR transactivation	inactive	inactive
off-target activity: PPARs, LXR, PXR, RXR	n.d.	inactive
solubility [mg/L] (LYSA, pH 6.5)	<1	9
logD (pH 7.4)	>3	2.7
aqueous stability (pH 1, 4, 6, 8, 10)	stable	stable
permeability (prediction)	medium/high	medium/high
Cl_{hep} [mL/min/mg protein] (h/m)	n.d.	16 / 53
CYPs [µM] (3A4, 2D6, 2C9)	<0.2 / 2.2 / <0.2	45 / >50 / 14
hERG IC_{20} [µM]	5.5	>10
protein binding f_u [%] (h/m)	0.2 / 0.5	0.04 / 0.2

Improved properties translate into an improved PK

HTS hit

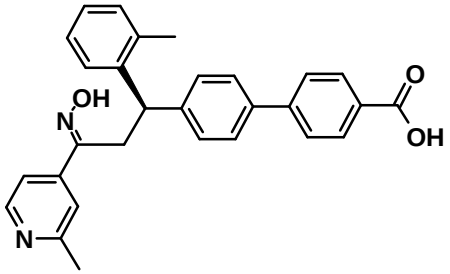


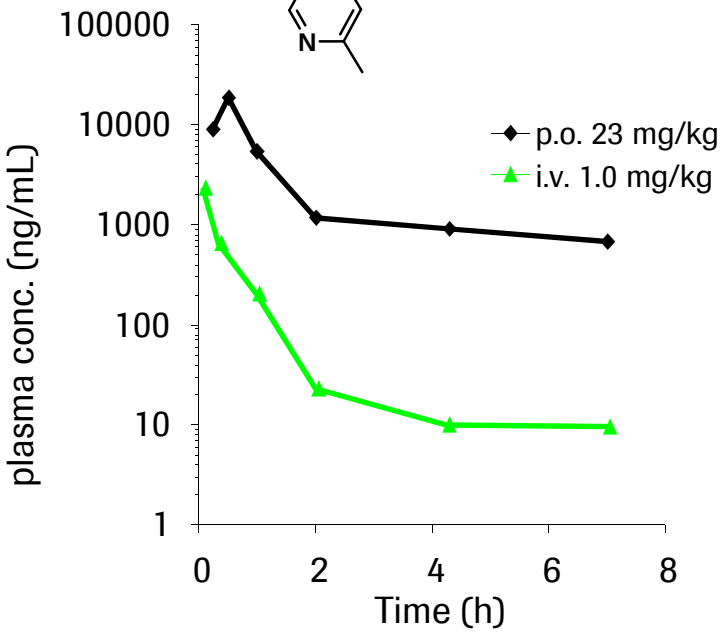


Cl [mL/min/kg]	V_{ss} [L/kg]	$t_{1/2}$ [h]	F [%]	c_{max} norm. [ng/mL]/[mg/kg]
77	1.6	0.2-0.5	32	65

mouse

benzoic acid lead compound

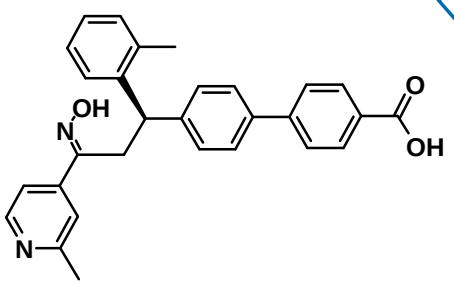




Cl [mL/min/kg]	V_{ss} [L/kg]	$t_{1/2}$ [h]	F [%]	c_{max} norm. [ng/mL]/[mg/kg]
15	0.5	2.5	80	800

PK/PD for benzoic acid lead compound

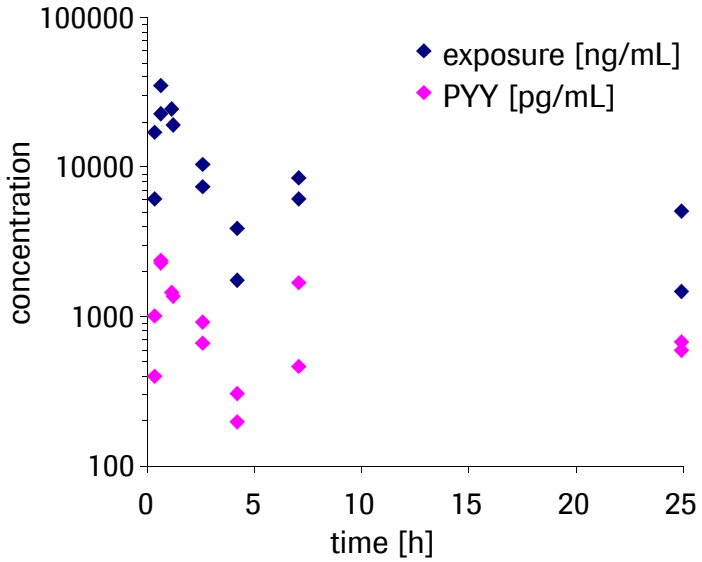
PYY as mechanistic readout



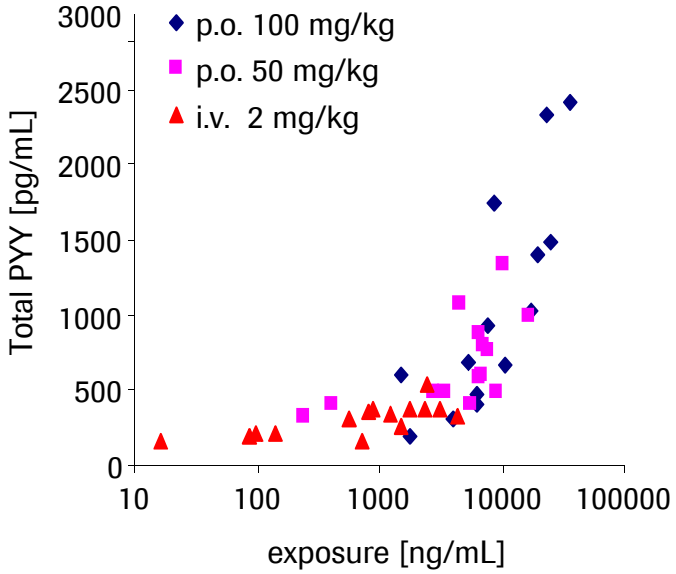
C57BL6 mice

concentration vs. time

dose: 100 mg/kg p.o.



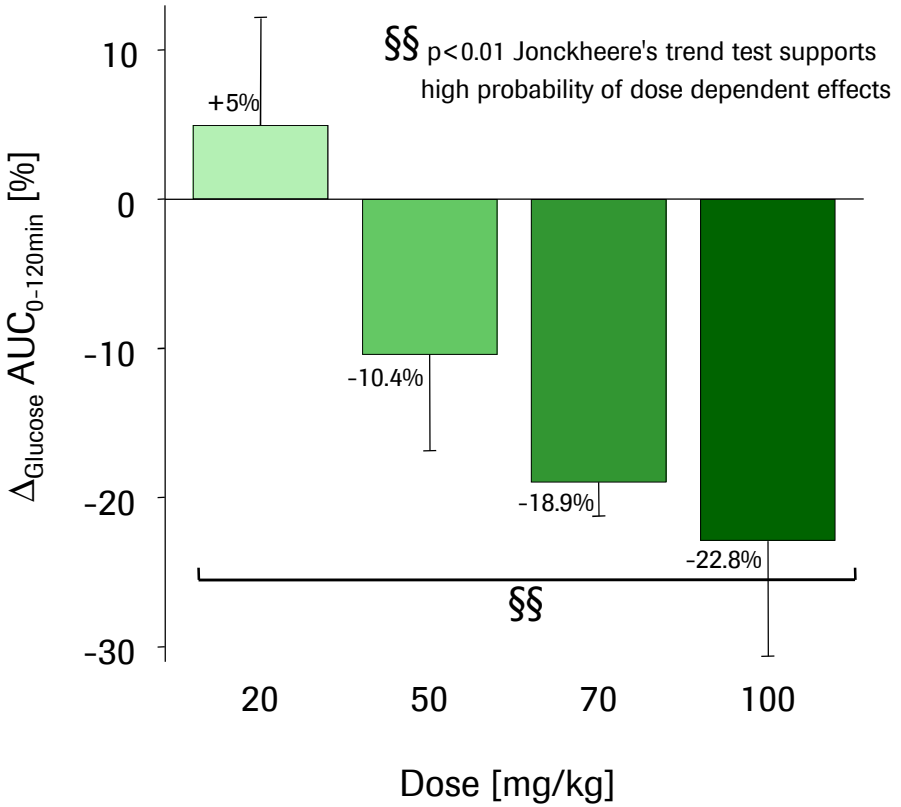
PK/PD relationship



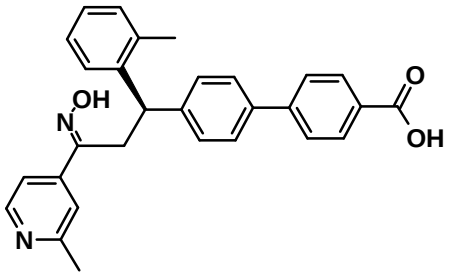
- No indirect or delayed effect
- Plasma exposure appears to be a good surrogate for GPBAR1 action.

Oral glucose tolerance test of lead compound

Dose dependent improvement of glucose tolerance

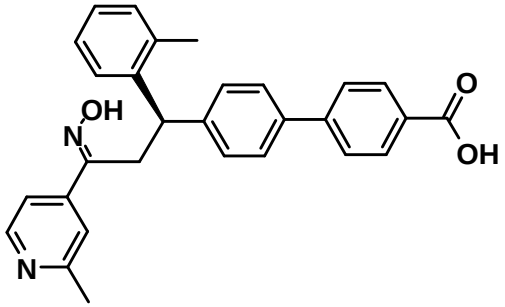


C57BL6 mice



compound administration 1 h before glucose challenge

Second generation: Reduce logD/protein binding

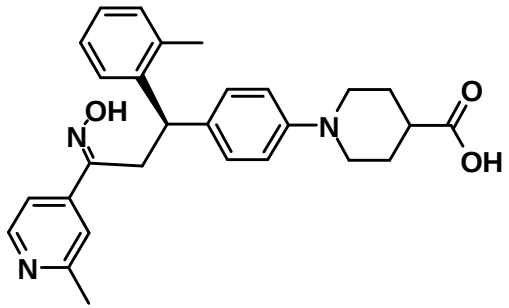


in vitro:

hEC_{50} [nM]	mEC_{50} [nM]	solubility [mg/L]	logD	f_u (h/m) [%]
11	130	9	2.7	0.04/0.2

mouse PK:

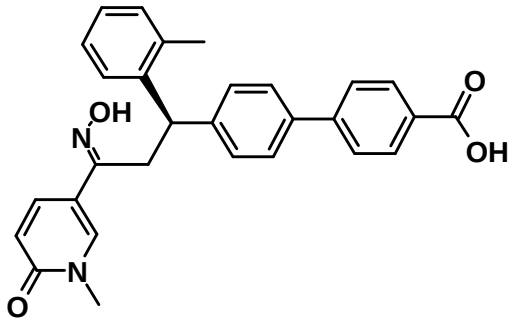
Cl [mL/min/kg]	Vss [L/kg]	$t_{1/2}$ [h]	F [%]	c_{max} norm. [ng/mL]/[mg/kg]
15	0.5	2.5	80	800



RO5527239

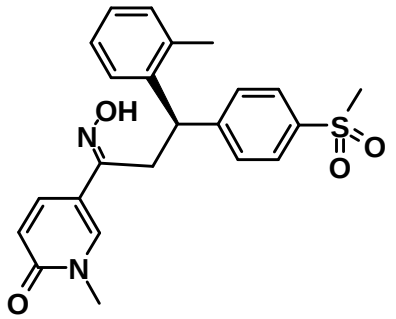
hEC_{50} [nM]	mEC_{50} [nM]	solubility [mg/L]	logD	f_u (h/m) [%]
4	28	273	1.5	0.4/1.9

Cl [mL/min/kg]	Vss [L/kg]	$t_{1/2}$ [h]	F [%]	c_{max} norm. [ng/mL]/[mg/kg]
5	0.4	1.7	100	650



hEC_{50} [nM]	mEC_{50} [nM]	solubility [mg/L]	logD	f_u (h/m) [%]
60	450	250	1.3	0.4/0.4

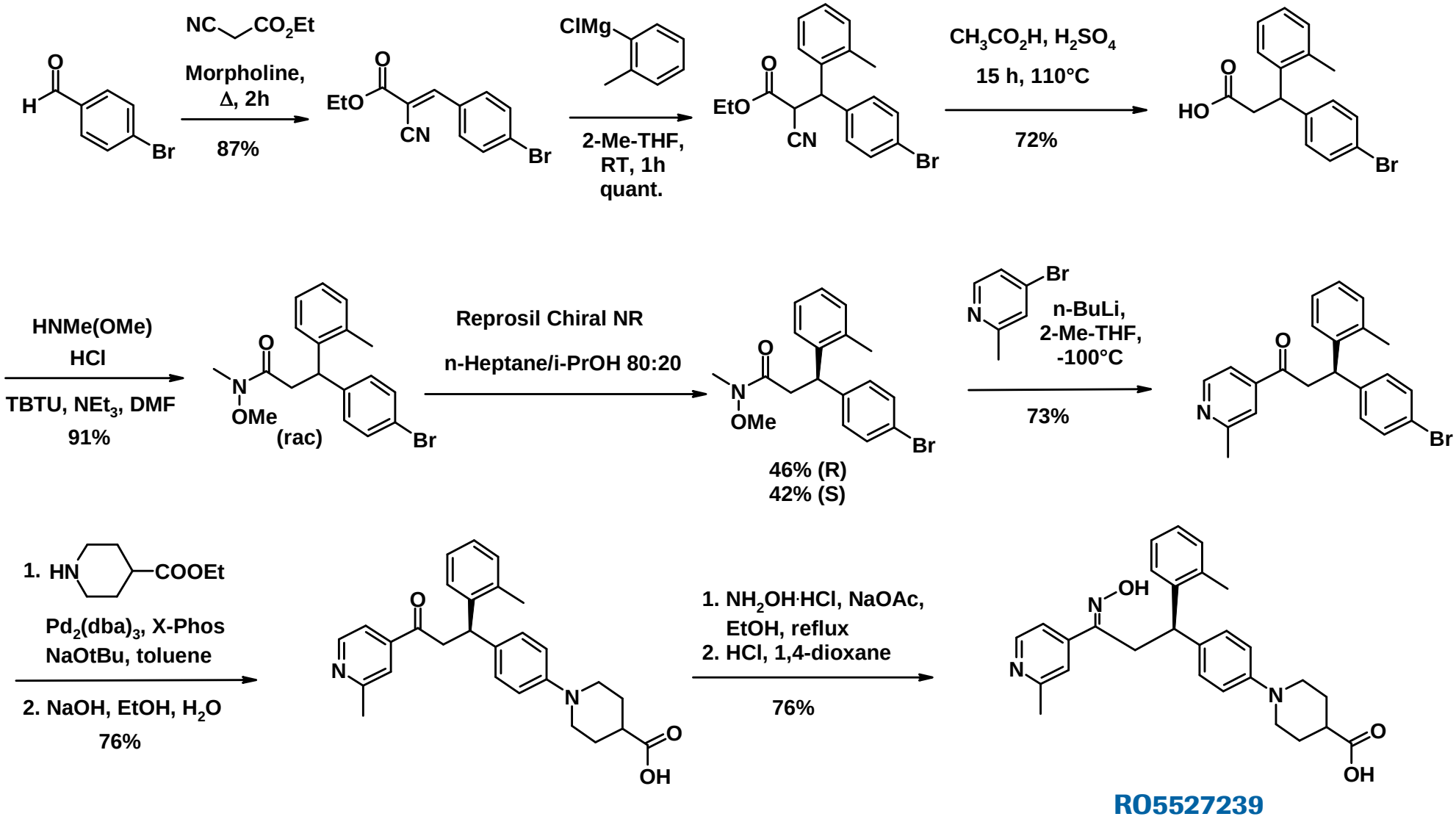
Cl [mL/min/kg]	Vss [L/kg]	$t_{1/2}$ [h]	F [%]	c_{max} norm. [ng/mL]/[mg/kg]
5	0.5	0.7	87	600



hEC_{50} [nM]	mEC_{50} [nM]	solubility [mg/L]	logD	f_u (h/m) [%]
26	1300	300	1.7	2.1/11

Cl [mL/min/kg]	Vss [L/kg]	$t_{1/2}$ [h]	F [%]	c_{max} norm. [ng/mL]/[mg/kg]
67	3.3	2.2	29	40

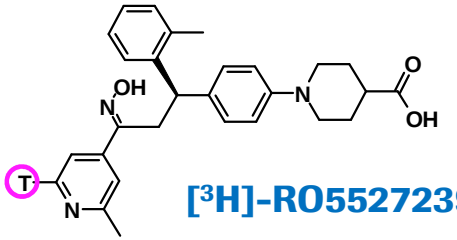
Synthesis of RO5527239



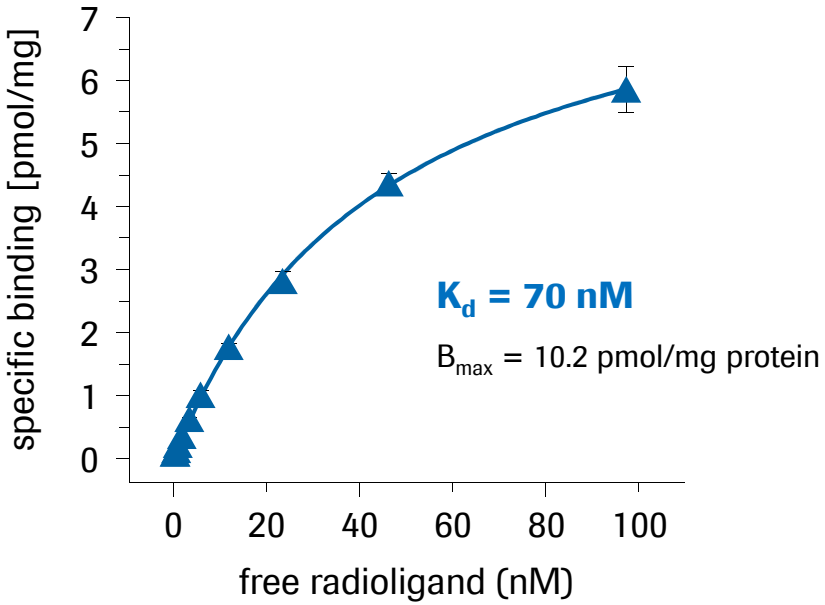
RO5527239

RO5527239 binds specifically to GPBAR1

Same binding site as lithocholic acid



[^{3}H]-RO5527239



No meaningful off-target activity identified

- radioligand binding panel (Cerep, n = 97)
- transactivation assays:
  - FXR
  - LXRα, LXRβ
  - PPARα, PPARγ, PPARδ
  - RXRα

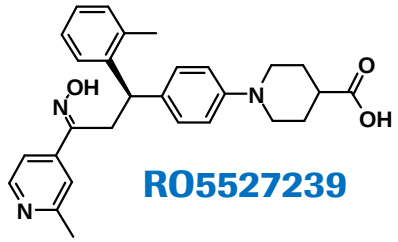
Cellular activity of RO5527239

GPBAR1 expressing enteroendocrine STC-1 cells

cAMP

EC_{50} = 1.59 µM

EC_{50} > 10 µM



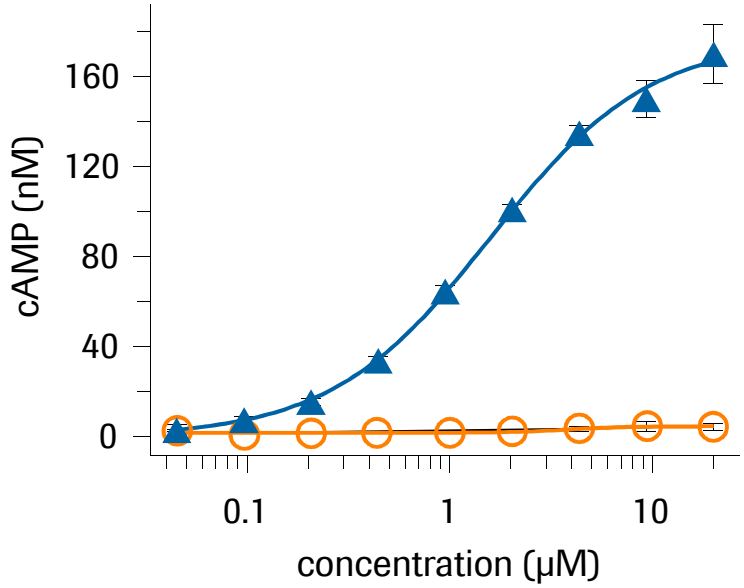
RO5527239

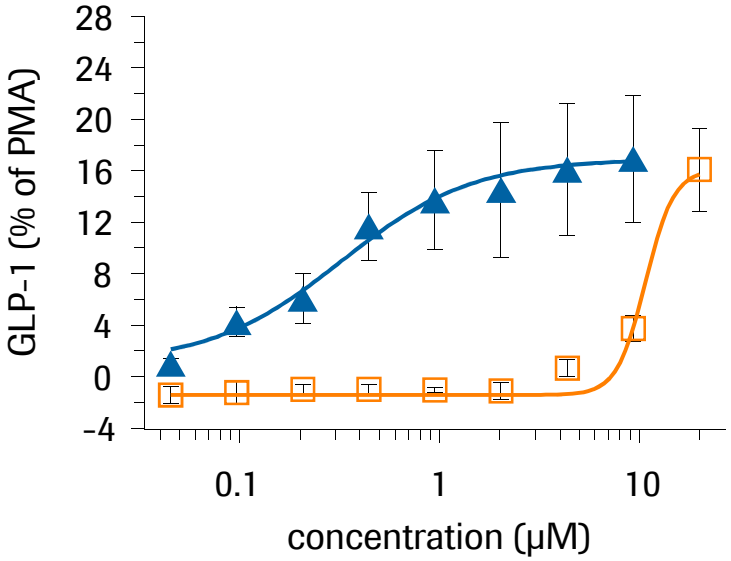
deoxycholic acid

GLP–1

EC_{50} = 0.32 µM

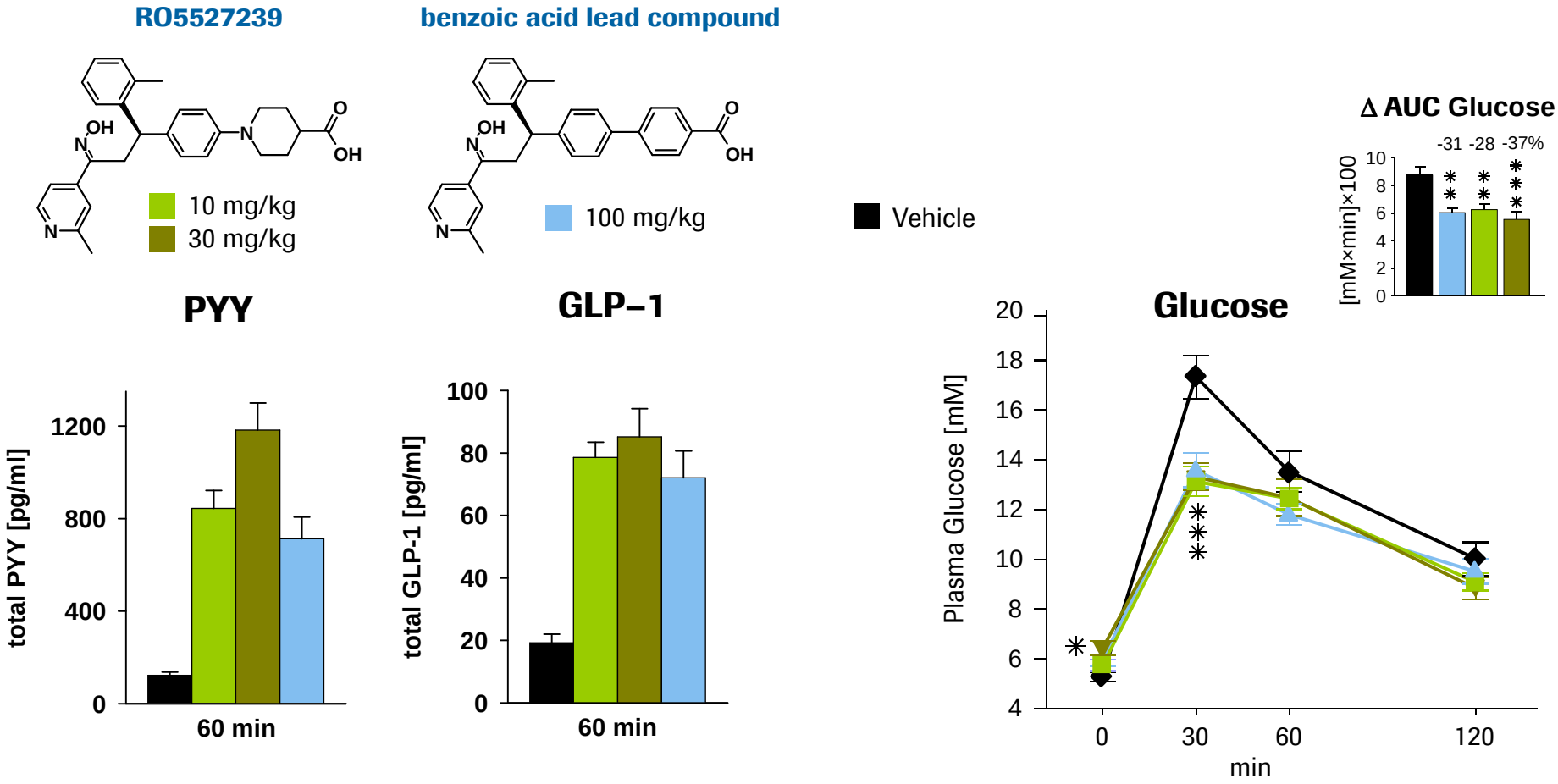
EC_{50} = 11 µM





RO5527239 in humanised DIO (C57/Bl6) mice

GLP-1/PYY secretion and glucose tolerance



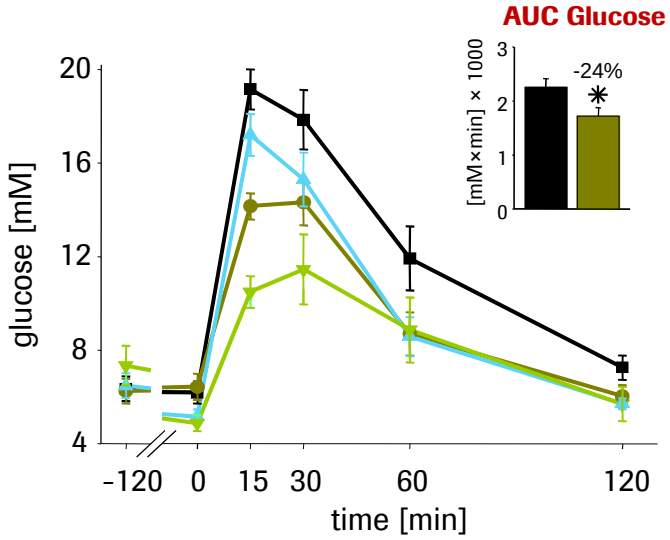
- Higher in vivo efficacy of RO5527239 in GLP-1/PYY release translates into superior glucose tolerance.

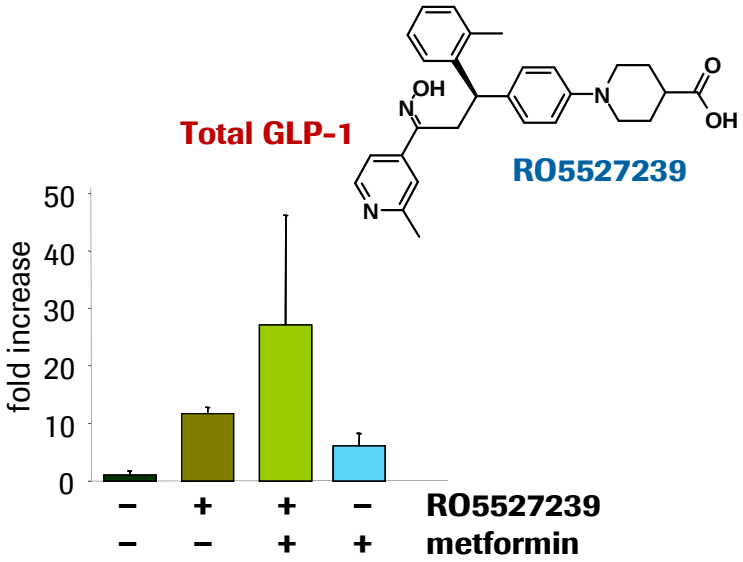
Acute improvement in glucose tolerance in db/db mice

Synergistic effects with metformin and sitagliptin

a) with metformin

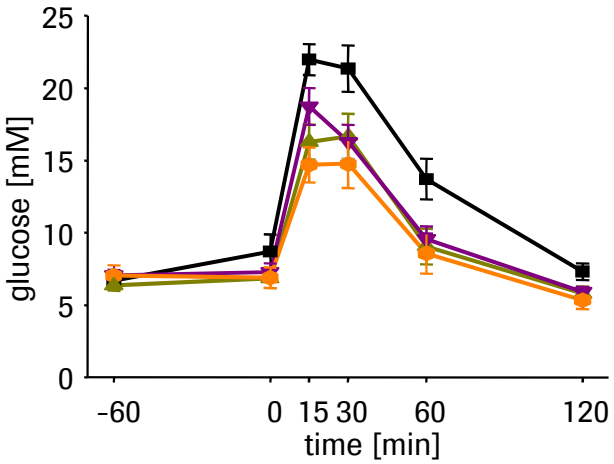
- vehicle
- RO5527239 (30 mg/kg)
- metformin (100 mg/kg)
- metformin (100 mg/kg) + RO5527239 (30 mg/kg)

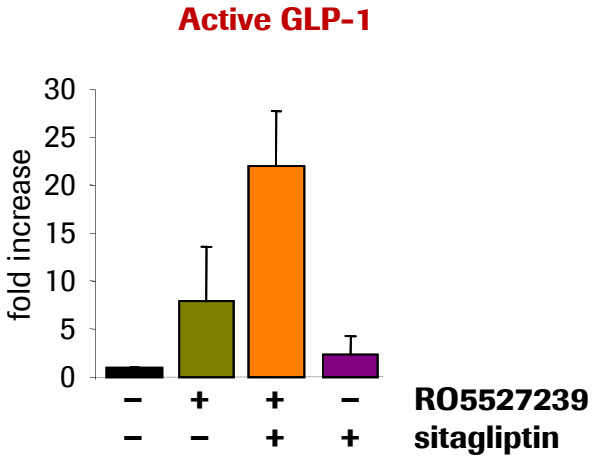




b) with sitagliptin (DPP4 inhibitor)

- vehicle
- RO5527239 (30 mg/kg)
- sitagliptin (10 mg/kg)
- sitagliptin (10 mg/kg) + RO5527239 (30 mg/kg)





Summary

- We have identified a new class of orally available GPBAR1 agonists starting from high-throughput screening hits.
- An unsubstituted oxime group is required for this class of compounds.
- Optimised GPBAR1 agonists bind to the target in the same manner as bile acids, resulting in secretion of PYY and GLP-1.
- RO5527239, the most potent compound of this class, improves glucose tolerance in rodents and shows synergistic effects with sitagliptin and metformin.

We Innovate Healthcare