Discovery of Lu AA47070

a phosphonooxymethylene prodrug of a potent and selective hA_{2A} receptor antagonist

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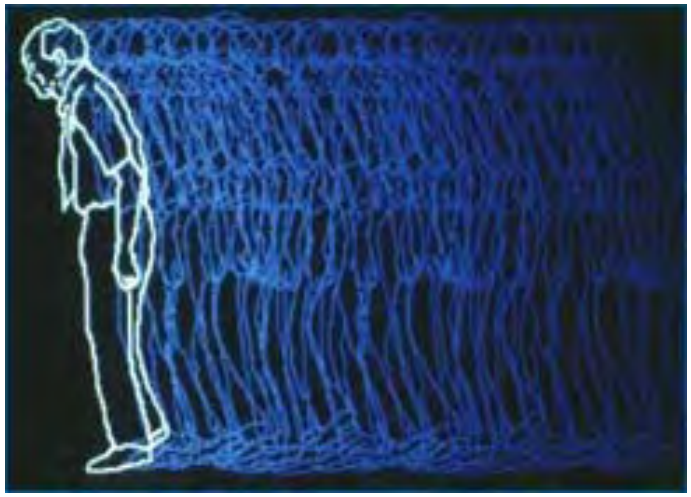
Parkinson's Disease

Parkinson’s Disease

First described in "*Essay on the Shaking Palsy*", published in 1817 by the english physician James Parkinson (1755-1824)



- Affects 1-2% of the population over 65
  - ca. 500,000 patients in Europe
  - Anual cost of treatment ca. 3 billion Euro

- A chronic progressive neurological disorder
  - Resting tremor
  - General slowness of movement (bradykinesia)
  - Stiffness of limbs
  - Gait/balance problems (postural instability)

Parkinsons Disease

- Disease etiology is unknown
- Symptoms are caused by progressive preferential loss of dopaminergic neurons in substantia nigra
- Current drug therapy focuses on dopaminergic replacement strategies for symptomatic relief

Current treatments of Parkinsons Disease

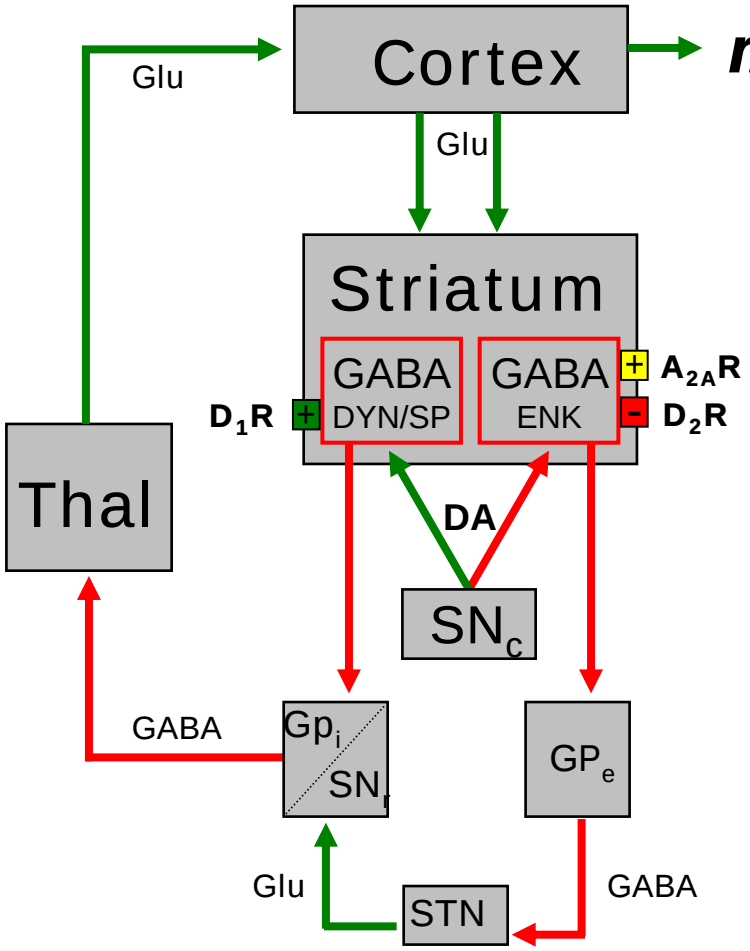
- Dopamine agonists
  - D_1/D_2 agonists (L-DOPA (dopamine precursor) and apomorphine)
  - Selective D_2 agonists

- D_1/D_2 agonists remain gold standard of treatment – but:
  - Dosing issues - side effects related to dose peaks:
  - Long term use is associated with induction of motor fluctuations ("wearing off", "on-off")
  - risk of inducing dyskinesias (inability to control muscles)

Main current treatments of Parkinsons Disease

- Monoamine Oxidase B (MAO-B) inhibitors
  - Increase dopamine levels in the brain by preventing its breakdown
  - Monotherapy in early PD, adjunct to L-DOPA in late PD
- Catechol-*O*-methyl transferase (COMT) inhibitors
  - Prevent inactivation of catecholamines, incl. dopamine and L-DOPA
  - Only adunct to L-DOPA treatment
- A_{2A} antagonists
  - Functional positive modulation of dopamine signal
  - Monotherapy in early PD? Adjunct to L-DOPA in late PD

Rationale: Locomotor control in basal ganglia



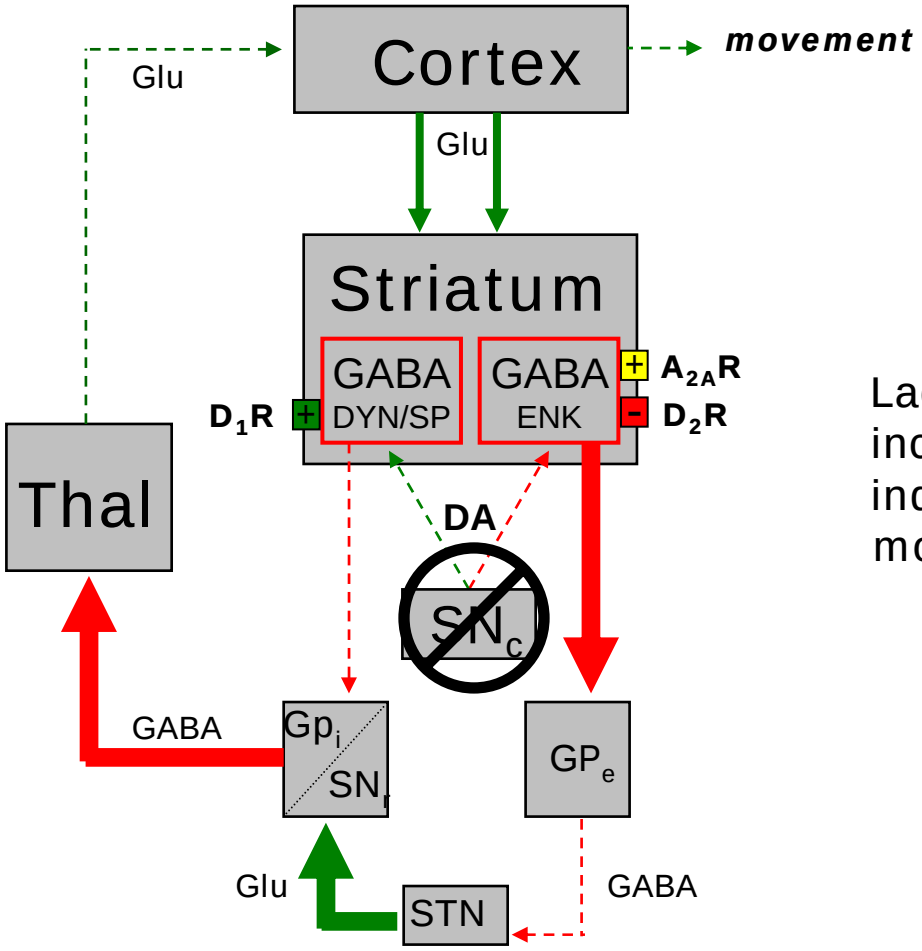
A_{2A}- and D_2 receptors inversely regulate GABA output of the indirect pathway

Excitatory signal

Inhibitory signal

modified from M. Schwartzschild/Albin, Young & Penney (1989) *TINS* 12:366-375.

PD - Pathophysiology



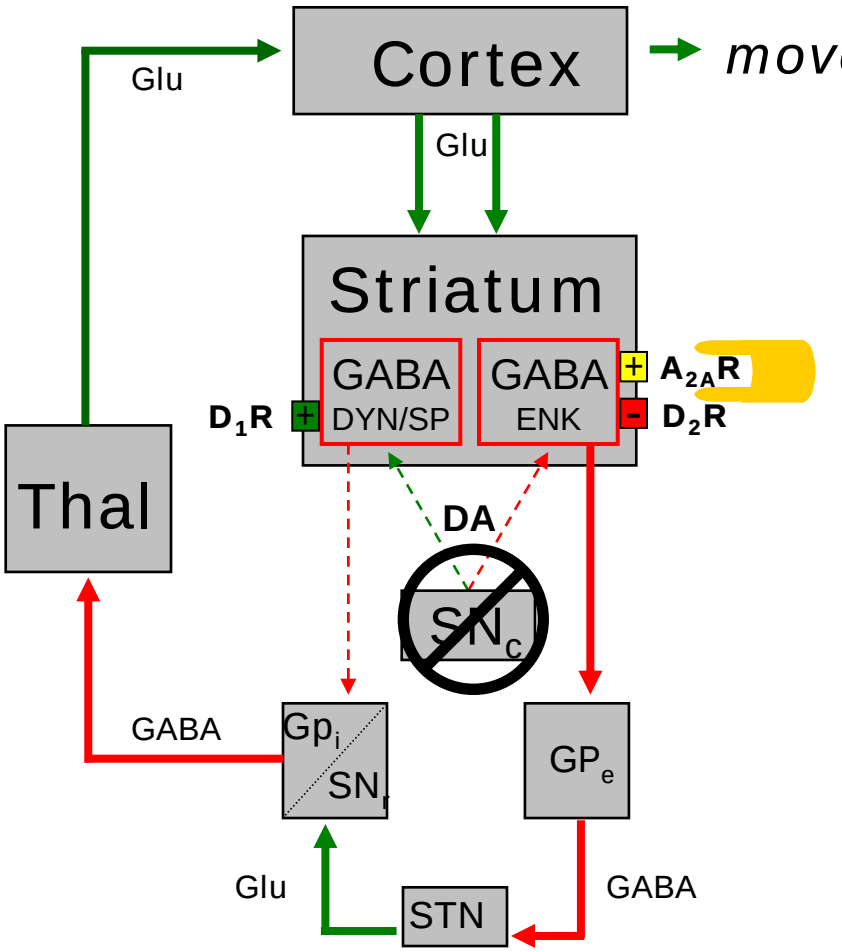
Lack of D_2 stimulation leads to increase in GABA output of the indirect pathway, and reduced motor control

Excitatory signal

Inhibitory signal

modified from M. Schwartzschild/Albin, Young & Penney (1989) *TINS* 12:366-375.

Symptomatic treatment of PD



The imbalance of the indirect pathway is compensated by A_{2A} receptor blockade

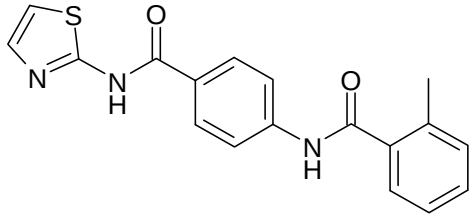
A_{2A} antagonism is functionally equivalent to D_2 agonism

Excitatory signal

Inhibitory signal

modified from M. Schwartzschild/Albin, Young & Penney (1989) *TINS* 12:366-375.

Screening hits



Hit1

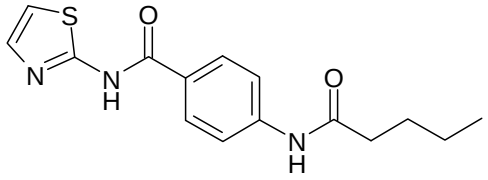
hA_{2A} K_i = 220 nM
hA_1: 47% inhib. @10uM
(K_i>2000 nM)

LE: 0.38

HLM: 0.5 L/min (LBF: 1.4)
Caco-2:
Papp = 84 cm/s
BA/AB: 0.8

CYP's (IC_{50}, uM):

CYP1A2	>40
CYP2C9	40
CYP2C19	23
CYP2D6	>40
CYP3A4	1.4



Hit2

hA_{2A} K_i = 190 nM
hA_1: 69% inhib. @10uM
(K_i>2000 nM)

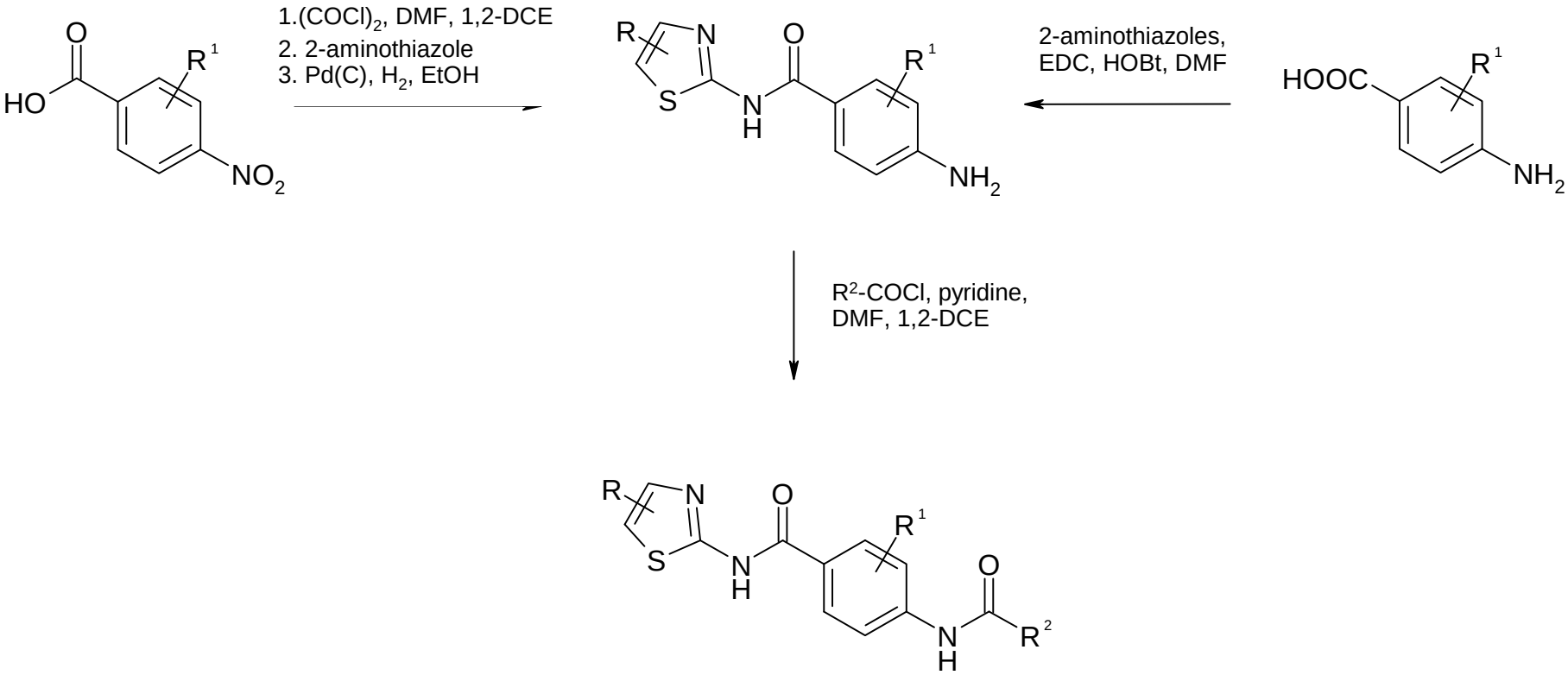
LE: 0.44

HLM: 2.3 L/min (LBF: 1.4)
Caco-2:
Papp = 77 cm/s
BA/AB: 0.7

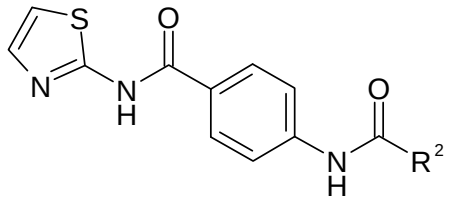
CYP's (IC_{50}, uM):

CYP1A2	>40
CYP2C9	40
CYP2C19	>40
CYP2D6	>40
CYP3A4	>40

Parallel synthesis



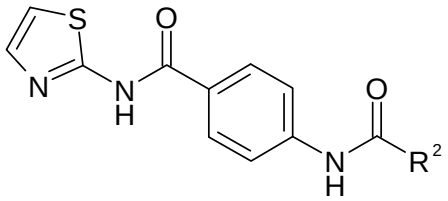
SAR of R^2



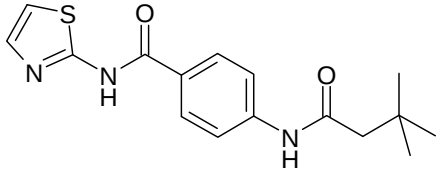
Hit1

R^2	hA_{2A} Ki (nM)	hA_1 inhib @10uM
phenyl	360	-
2-Me-phenyl	220	47%
2-OMe phenyl	530	-
2-Cl phenyl	290	-
3-Me phenyl	310	-
4-Me phenyl	370	-
benzyl	220	310

SAR of R^2

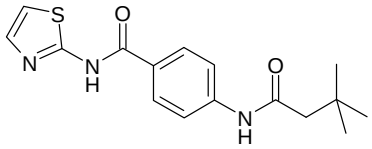
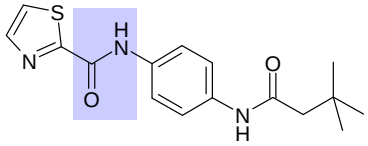
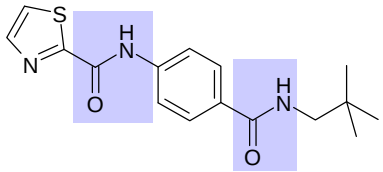
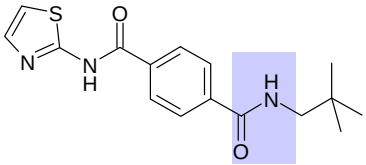


	R^2	hA_{2A} Ki (nM)	hA_1 inhib @10uM	HLM (L/min)	CYPs IC50 μM	Caco2 Papp (cm/s)	Caco2 Ratio
Hit 2	n-butyl	190	69%	2.3	>40	77	0.7
	n-propyl	350	54%	0.8	>40	120	0.7
	ethyl	890	31%	-	-	-	-
	isopropyl	300	-	-	-	-	-
	isobutyl	90	64%	1.6	>40	110	0.7
	neo-pentyl	36	39%	0.7	>40	110	0.7



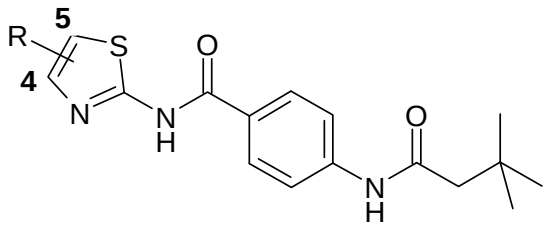
LE:0.46

SAR of amide inversion

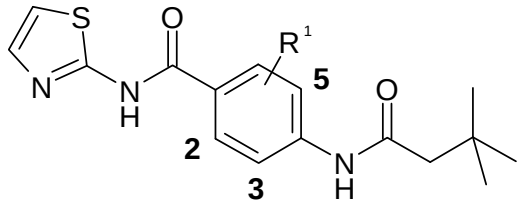
Compound	hA_{2A} Ki (nM) or % inhib @10uM
	36
	18%
	0%
	110

SAR of thiazole substitution

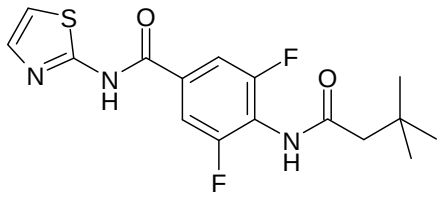
R	hA_{2A} Ki (nM)	hA_1 Ki (nM) or % inhb @10uM
H	36	39%
5-Cl	28	220
5-Me	120	1100
4,5-di Me	220	-



SAR of R^1



R^1	hA_{2A} Ki (nM)	hA_1 Ki (nM) or inhib @10uM	HLM (L/min)	CYPs IC50 μM	Caco2	
					Papp (cm/s)	ratio
H	36	39%	0.7	>40	110	0.7
2-OMe	50	1100	2.2	>40	110	0.7
2-Me	200	36%	2.2	-	86	0.9
2-Cl	180	14%	-	-	-	-
3-OMe	17	340	2.2	1A2: 5.5	120	0.7
3-Me	19	460	0.6	>40	130	0.6
3-Cl	22	210	1.1	>40	92	0.9
3-F	9.7	650	0.4	>40	-	-
3,5-di F	5.9	410	0.8	>40	77	0.7
3-F, 5-Me	32	580	1.3	-	-	-
2-OMe, 3-F	12	220	1.4	-	-	-



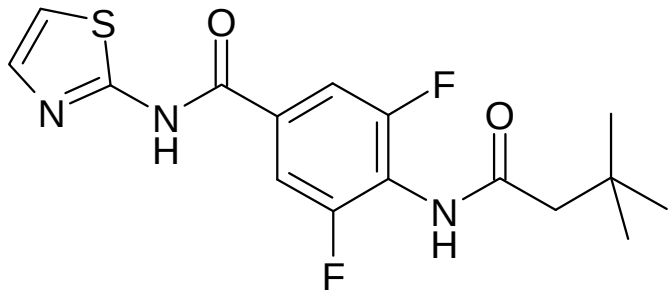
LE:0.47

Lu AA41063

Profile

Lu AA41063

hA_{2A} K_i = 5.9 nM
hA_1 K_i = 410 nM (68 fold)
hA_{2B} K_i = 260 nM (43 fold)
hA_3 K_i > 10000 nM (> 1000 fold)



LE: 0.47

Broad-screen:
< 30% inhib@10μM across 60 targets

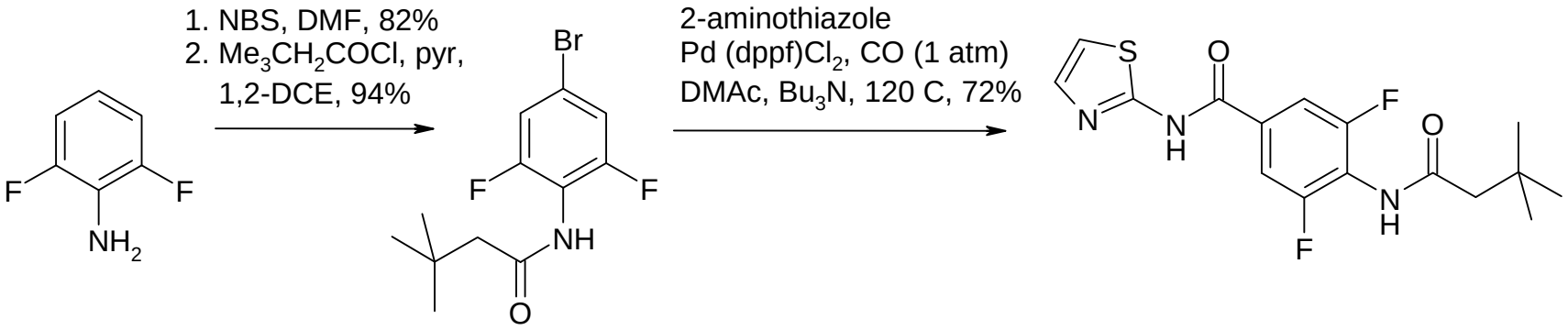
CL(rat) = 0.3 L/h/kg (LBF = 1.5)
F (rat) = 88%
Predicted human(70kg) CL = 29 L/h (LBF = 90 L/h)

CYPs: IC_{50} > 40 μM

Caco-2 Papp = 77 cm/s
Caco-2 BA/AB = 0.7
B/P (mouse) = 0.8
Plasma protein binding (human) = 85%

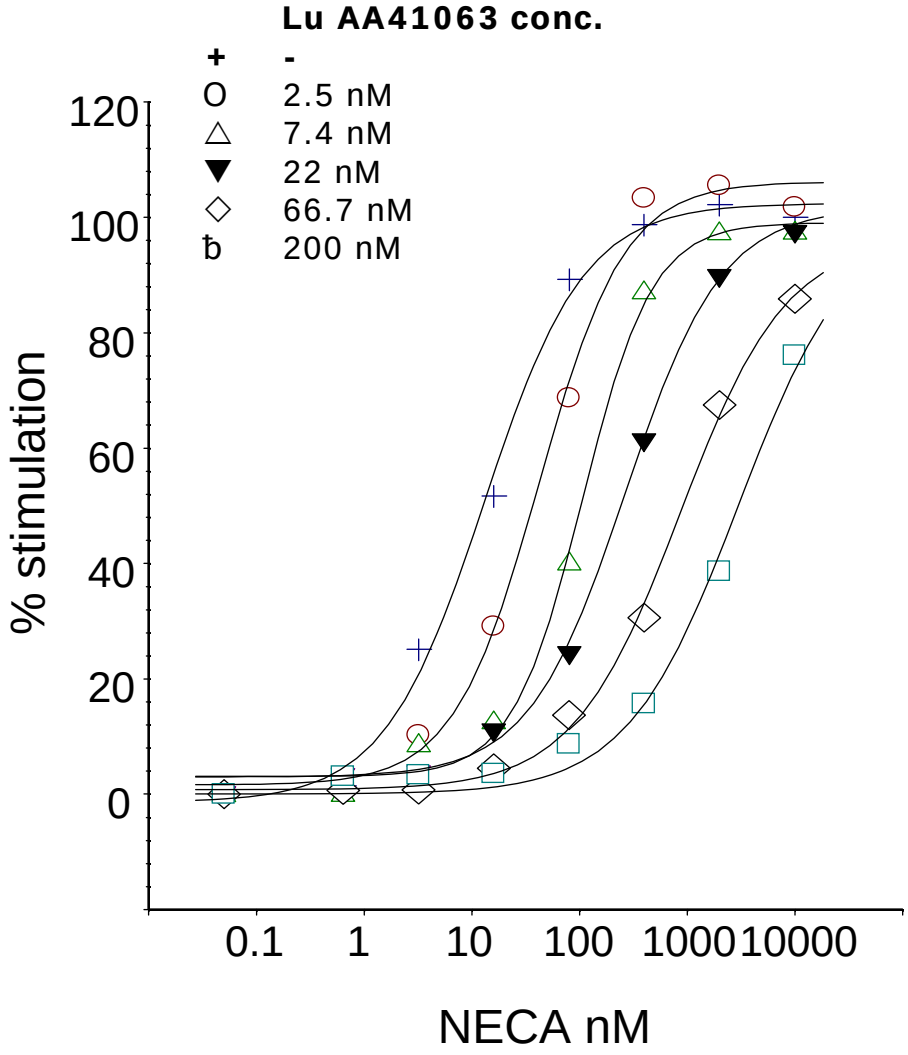
Solubility: 1 μg/mL (pH 7.4)

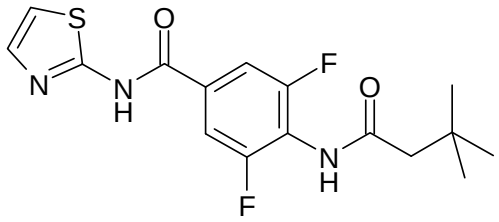
Kg scale synthesis



Lu AA41063

Antagonistic properties



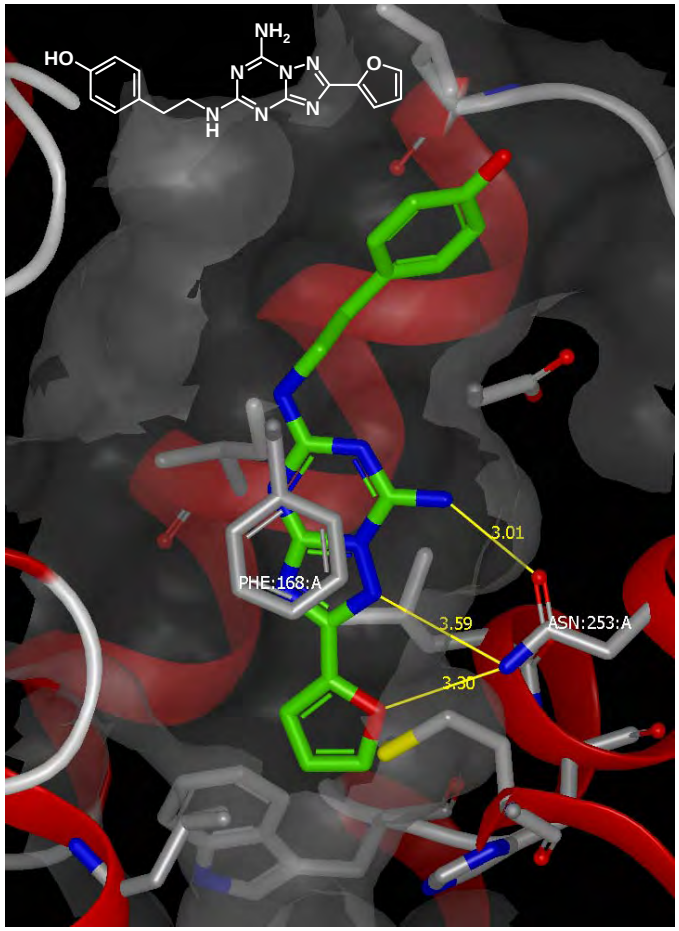


Lu AA41063 is a competitive antagonist at the hA_{2A} receptor

K_b = 1.3 nM

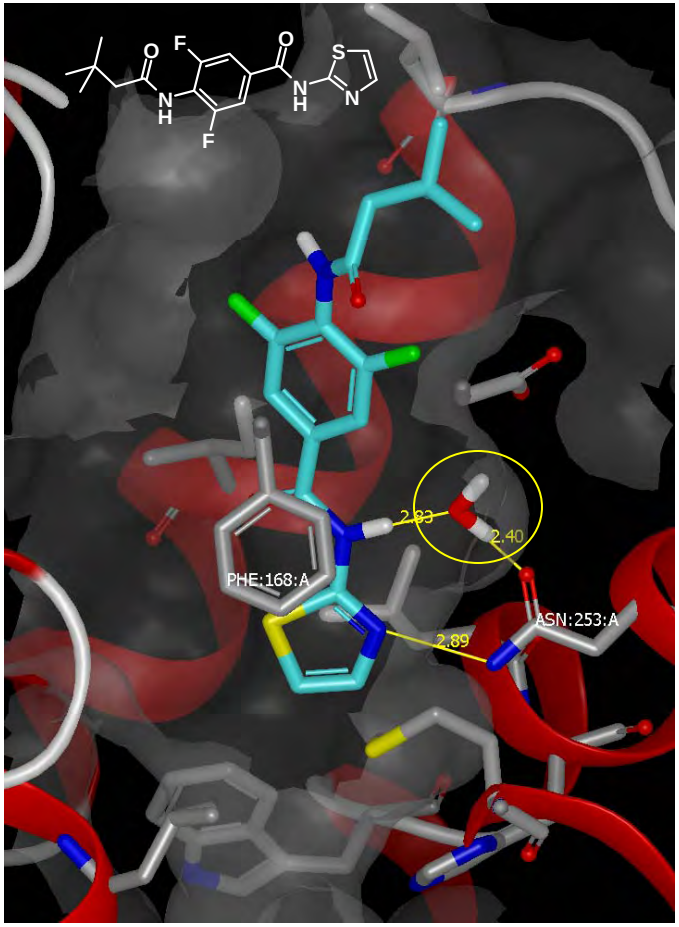
Receptor docking

A_{2A} receptor x-ray structure w. ZM241385



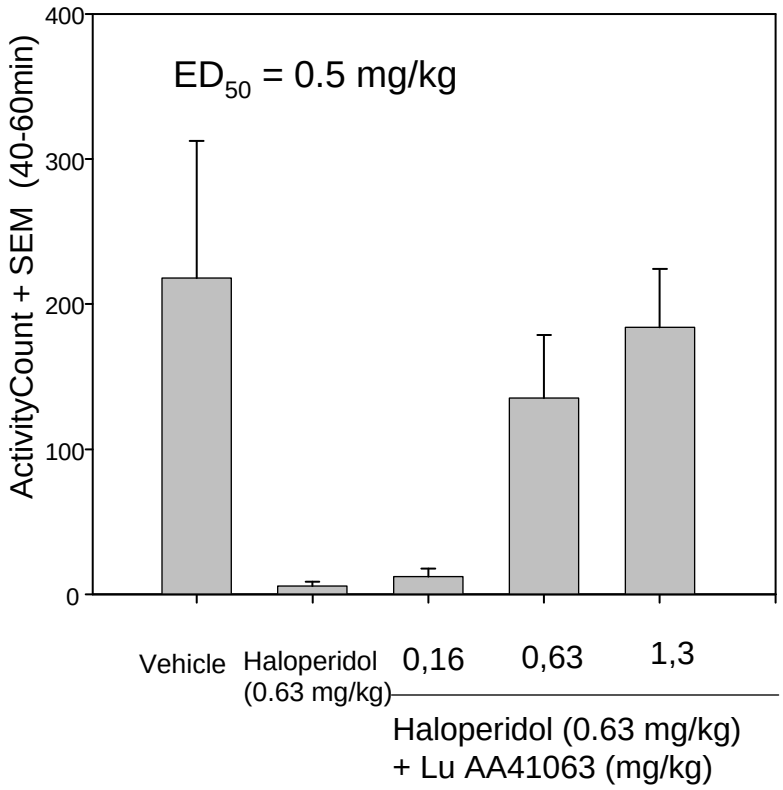
view from TM5 (removed for clarity)

Docking pose of Lu AA41063



Effect of Lu AA41063 *in vivo*

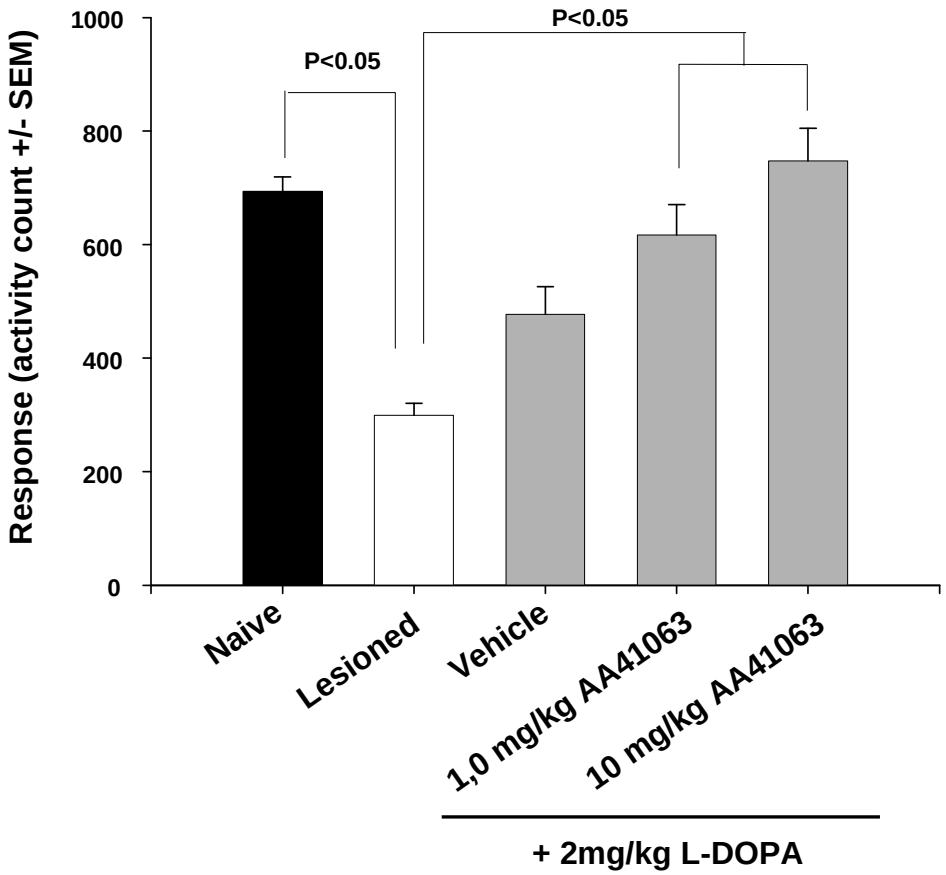
Model of early PD:
Reversal of haloperidol induced hypolocomotion in mice



Lu AA41063 dose-dependently reverses locomotor deficits induced by haloperidol

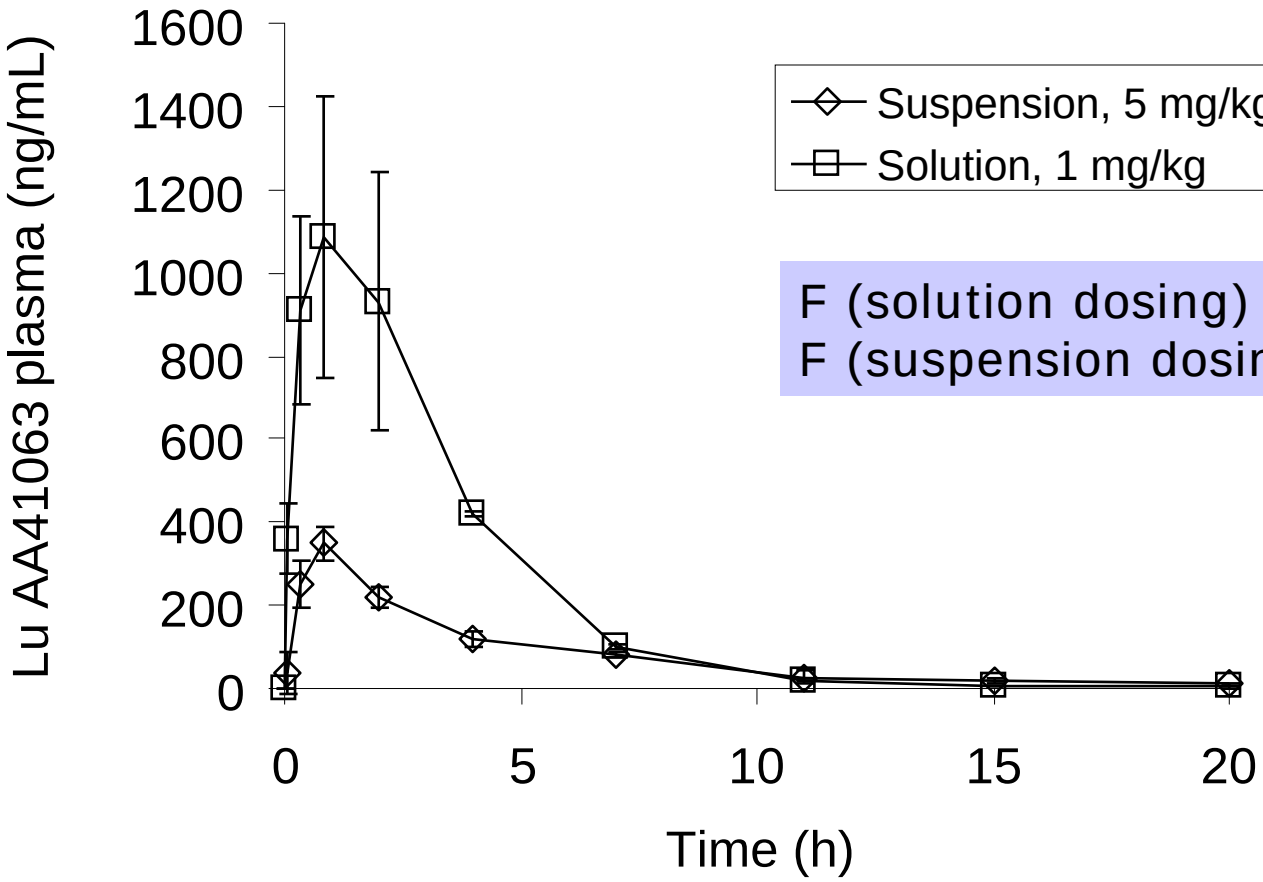
Effect of Lu AA41063 *in vivo*

Model of advanced PD:
Reversal of hypoactivity induced by unilateral 6-OHDA lesion in rats



Lu AA41063 dose-dependently reverses induced locomotor deficits as adjunct to a sub-optimal L-DOPA dose

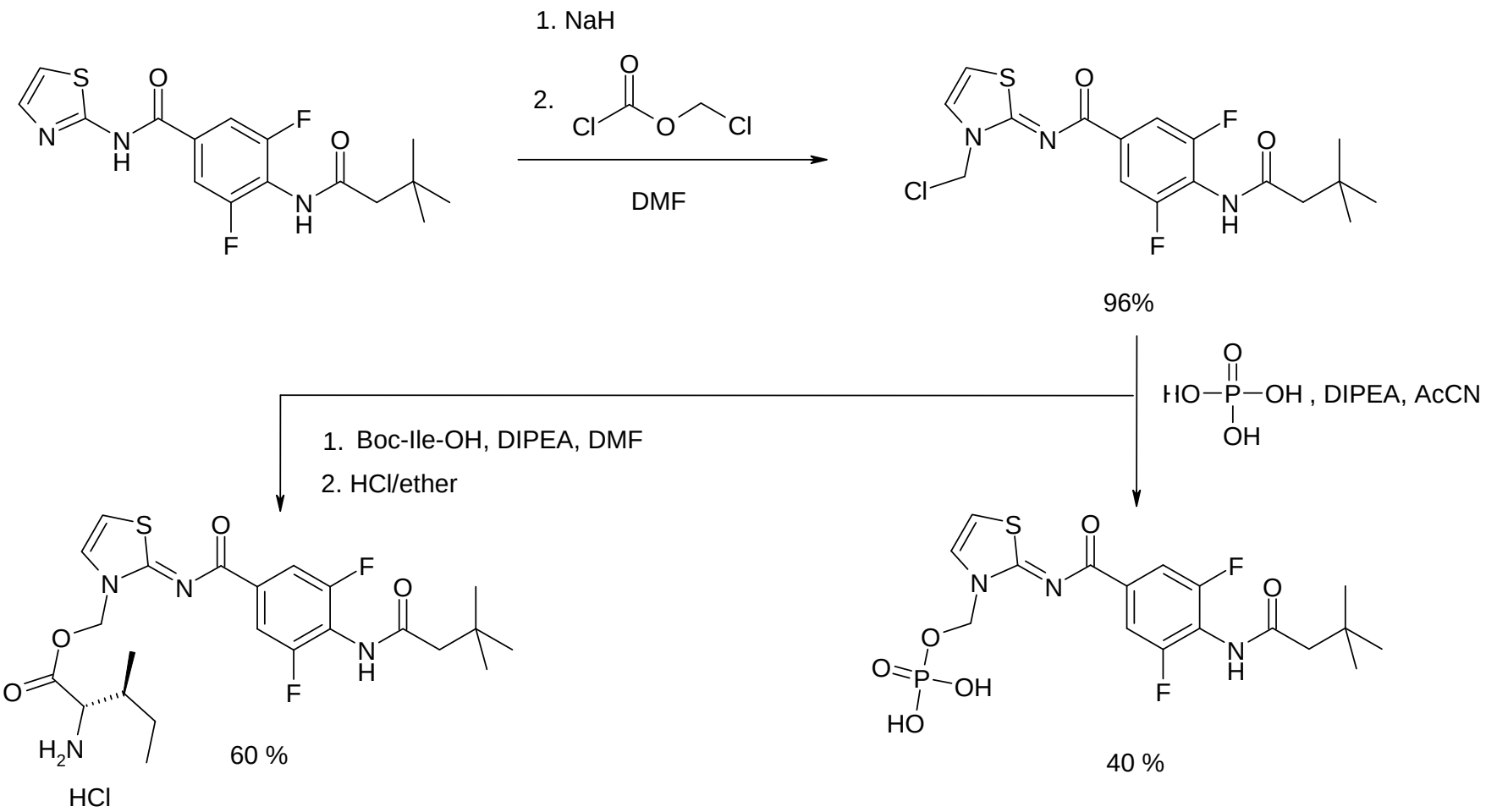
Solid dose oral bioavailability



F (solution dosing) = 88%
F (suspension dosing) = 10%

The low solubility (1 μg/mL) is potentially limiting for the developability of Lu AA41063

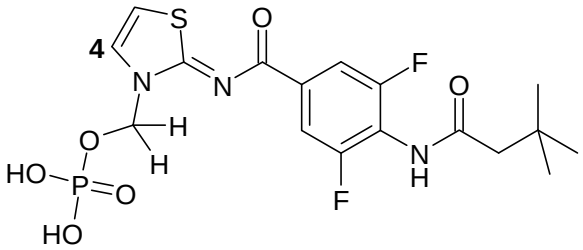
Derivatisation as prodrugs

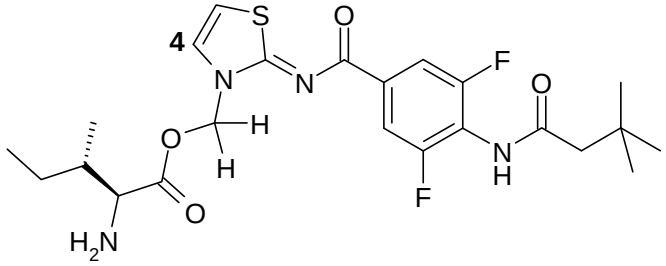


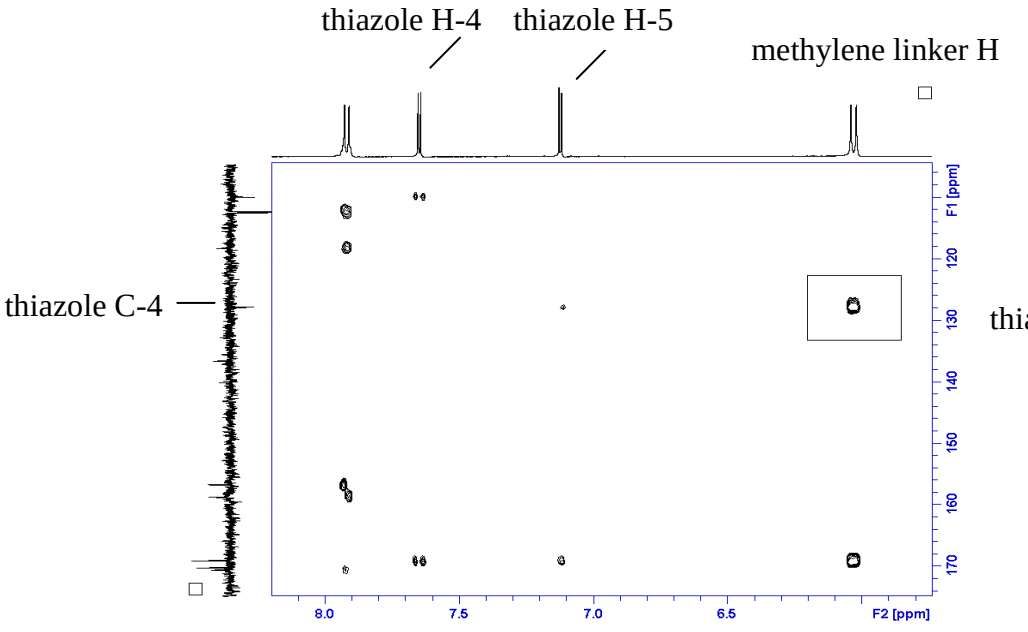
Lu AA47070

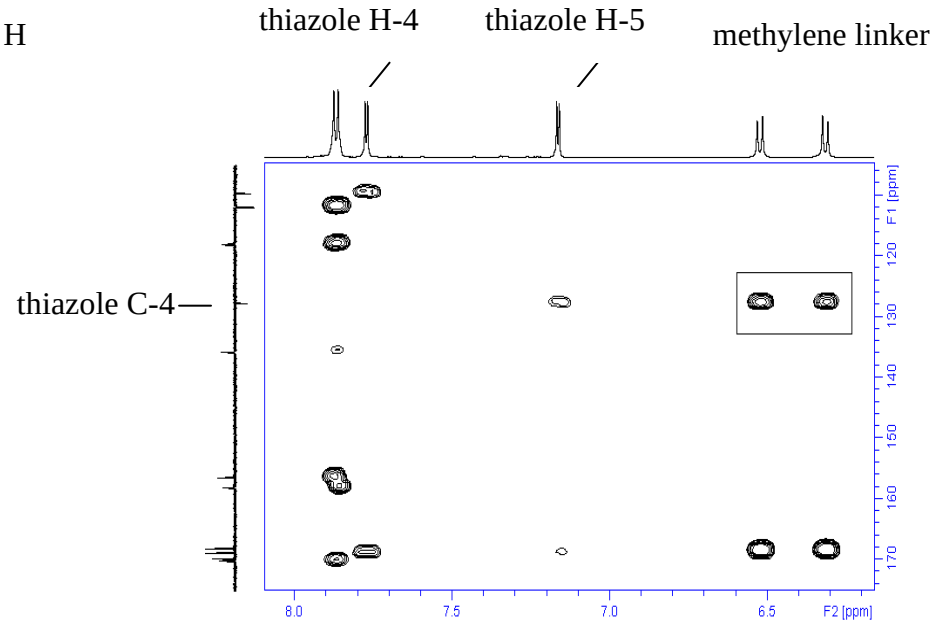
Prodrugs regiochemistry

HMBC ^{1}H-^{13}C correlation NMR spectra:

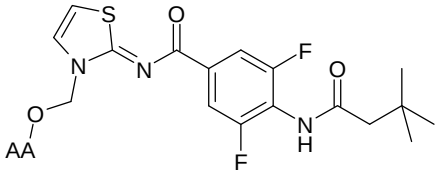


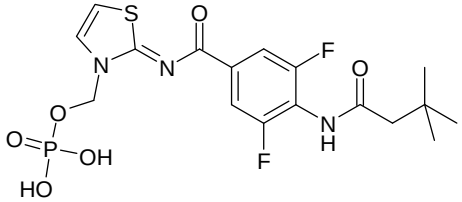






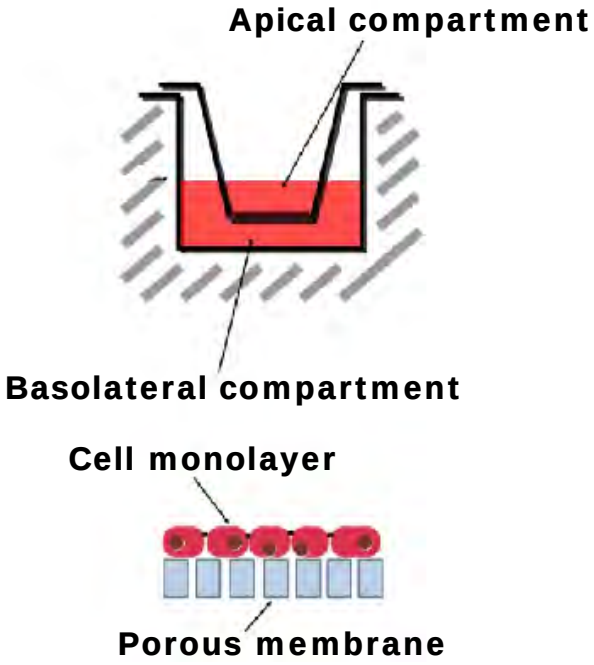
Prodrugs - hydrolytic stability and solubility

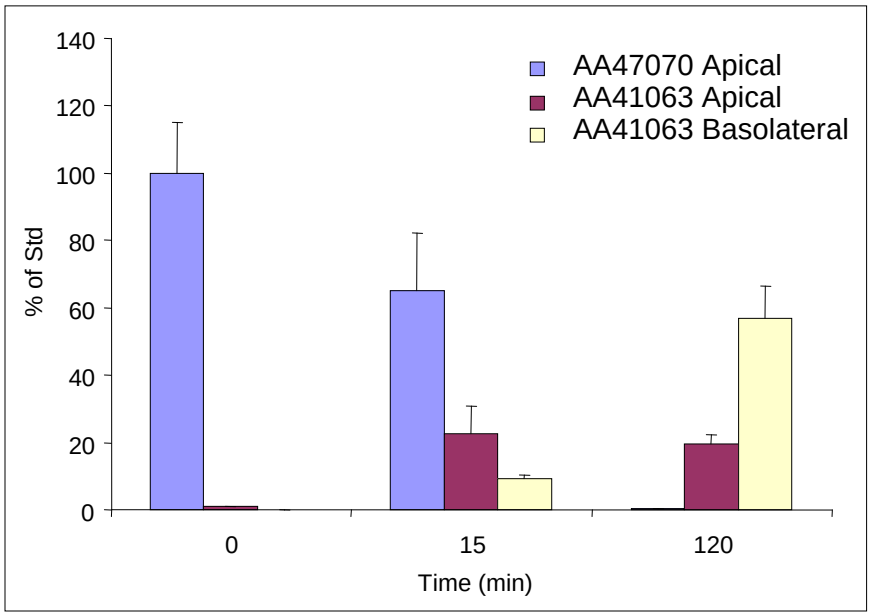




Solubilising group	t½ @ pH 7 (h)	pH for optimal stability	t½ (h) @ optimal pH	Solubility mg/mL @ optimal pH
Valine	0.3	2.1	46	>5
Isoleucine	0.4	2.1	75	19.4
β-Alanine	0.6	3.9	44	>5
4-carboxy piperidine	5.4	2.1	165	>5
phosphate (Lu AA47070)	>1600	>7	>1600h (pH7)	8.6

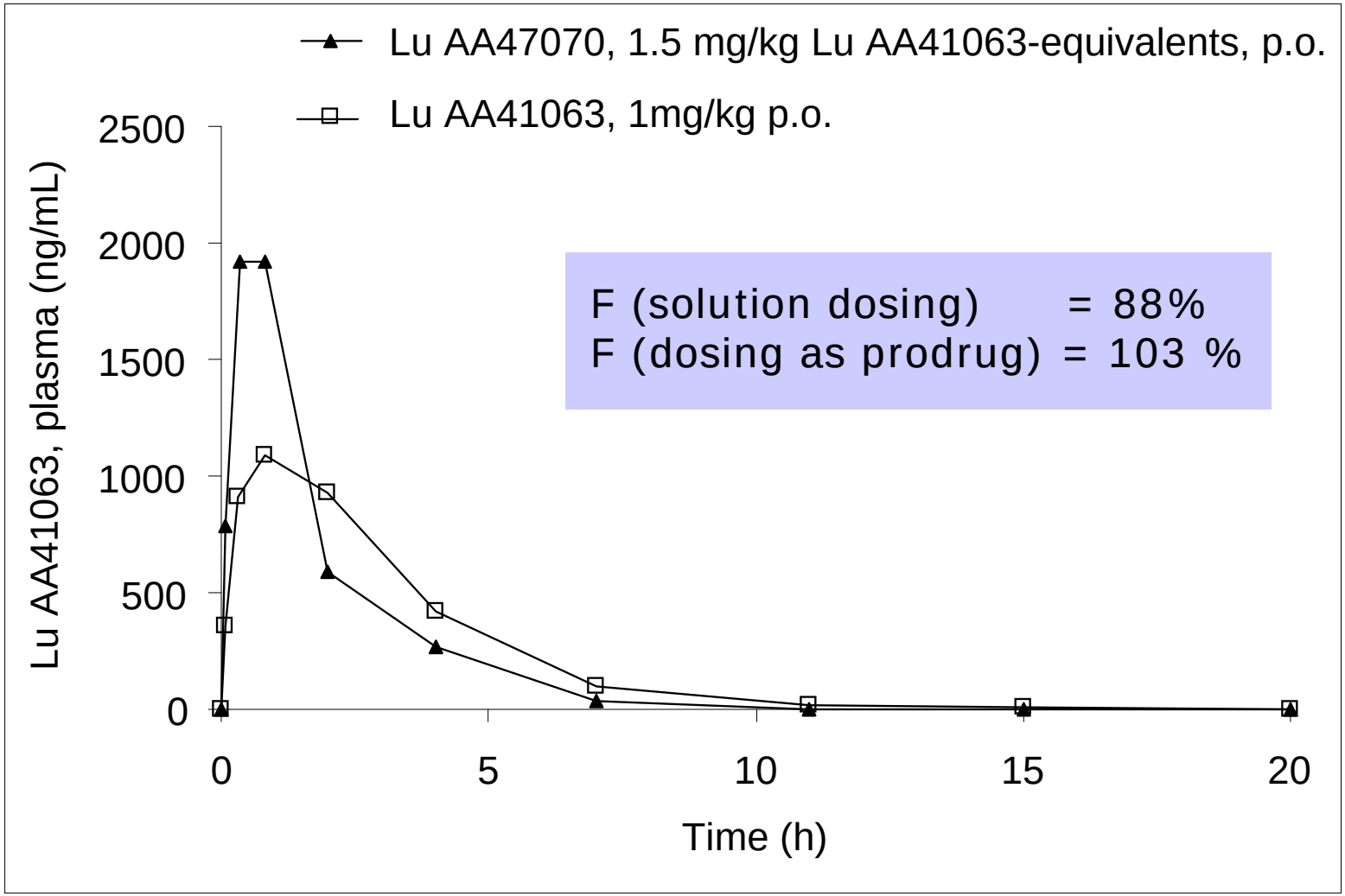
Lu AA47070 bioactivation *in vitro*



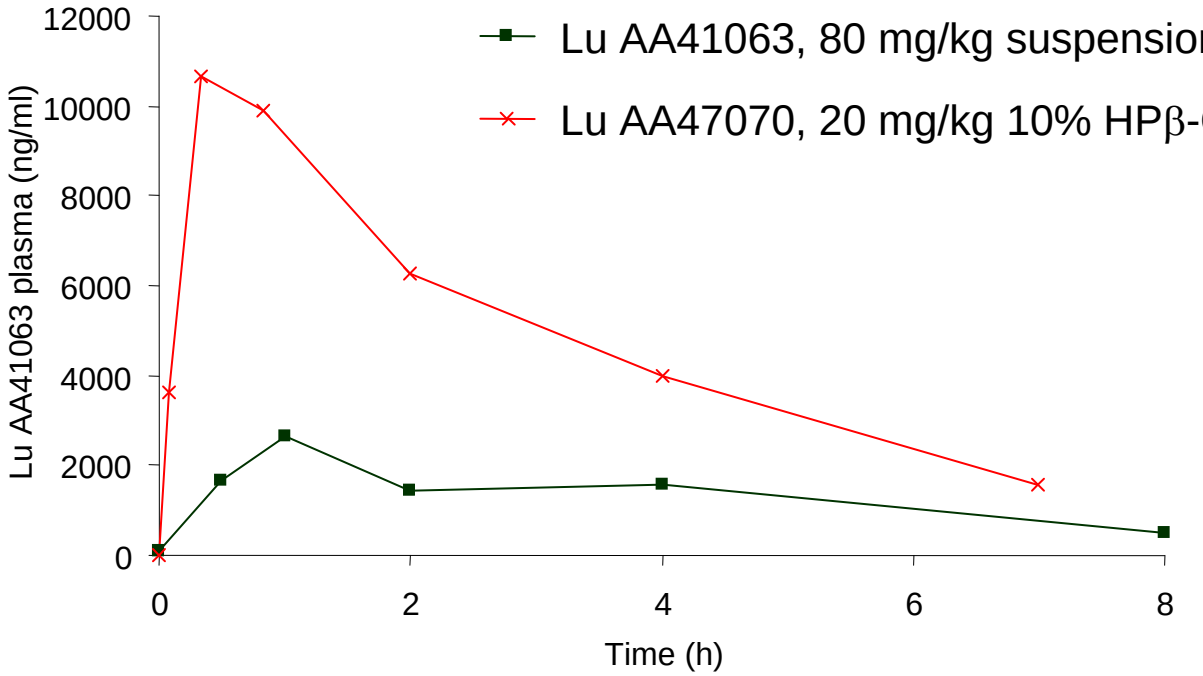


- Lu AA47070 was applied on the apical side of a Caco-2 cell monolayer
- Only Lu AA41063 was detected on the basolateral side of the monolayer
- This suggests that Lu AA47070 is converted to AA41063 in the intestinal epithelia (e.g. by alkaline phosphatases), and is not itself (significantly) absorbed

Oral bioavailability from Lu AA47070



High dose oral bioavailability



	"F"$_{0\text{-}8\ h}$
Lu AA41063, 80 mg/kg suspension	5%
Lu AA47070, 20 mg/kg 10% HPβ-CD	59%

- High solubility of prodrug overcomes limitation in bioavailability (dissolution limiting)
- Prodrug allowed dose escalation in toxicology and safety assesments!

Therapeutic index, Lu AA41063

Effective plasma exposure levels (ng/mL)					
Mouse			Rat		
Haloperidol hypoactivity	MTD NOAEL*	TI	6-OHDA hypoactivity	MTD NOAEL**	TI
160	2000	12	1810	86700	48

* Observed adverse effects: Piloerection, hyperactivity, aggression, loss of body weight (5%)

** Observed adverse effects: Hyperactivity in females and lower body weight

Conclusion

- Lu AA41063 is a potent and selective hA_{2A} antagonist
- Lu AA41063 is effective in rodent models of early and advanced Parkinson's Disease
- Lu AA47070 is a phosphonooxymethylene prodrug of Lu AA41063
- The improved solubility of Lu AA47070 overcomes dissolution limited reductions in oral bioavailability of Lu AA41063 at higher doses
- Lu AA47070 quantitatively delivers Lu AA41063 into systemic circulation upon oral dosing, with no detectable prodrug in circulation
- Lu AA47070 was progressed into Phase 1 clinical trials but was discontinued, as “the Phase 1 results did not live up to Lundbeck's requirements as Lu AA47070 did not have the intended pharmacological properties”

Thanks to:

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Toxicology:

Tomas Mow

Anders Lassen

In vivo pharmacology:

Lise Tøttrup Brennum

Lars Torup

ADME:

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