Discovery of APD811: an orally available prostacyclin receptor agonist for the treatment of Pulmonary Arterial Hypertension (PAH)

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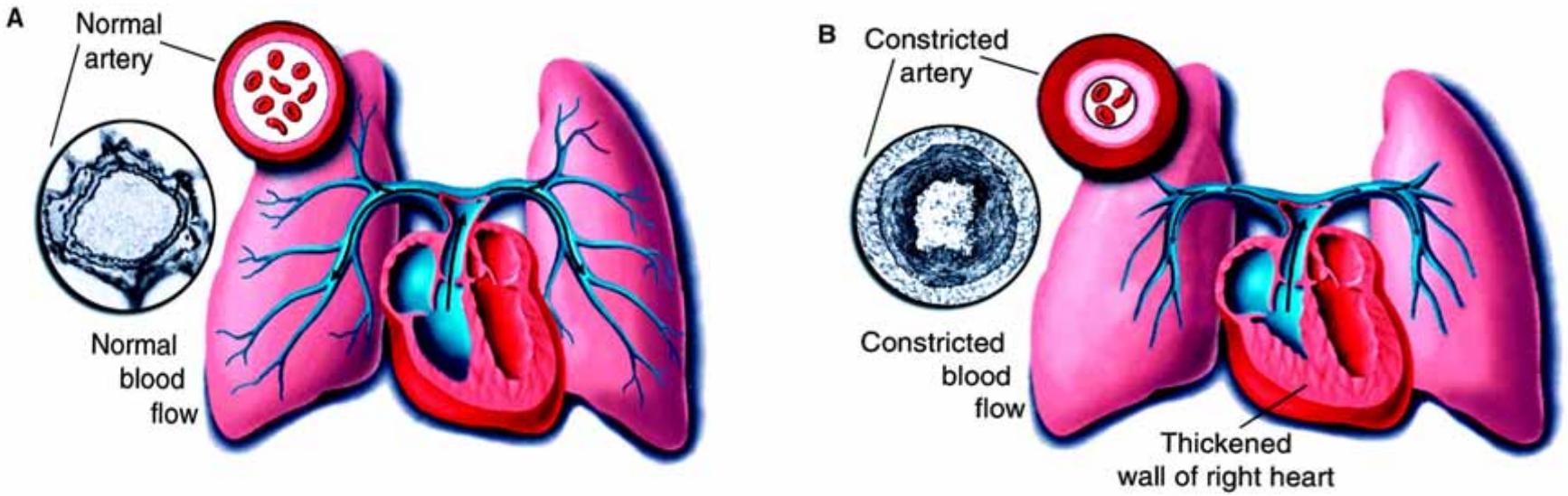
Pulmonary Arterial Hypertension

- PAH is a disease of the small pulmonary arteries characterized by vascular proliferation and remodeling
- Impaired production of vasoactive mediators, such as prostacyclin and NO, accompanied by prolonged overexpression of vasoconstrictors like ET-1 are thought to be responsible for the pathogenesis of PAH
- PAH results in a progressive increase in pulmonary vascular resistance and, ultimately, right ventricular failure and death (50% survival 5 years post diagnosis)



PAH Classification and Current Therapies

- Class I: Patients who have no symptoms of any kind, and for whom ordinary physical activity does not cause fatigue, palpitation, dyspnea or anginal pain.
- Class II: Patients who are comfortable at rest but have symptoms with ordinary physical activity
- Class III: Patients who are comfortable at rest but have symptoms with less-than-ordinary effort
  - Class II and III patients are treated with ET antagonists and PDE5 Inhibitors
- Class IV: Patients who have symptoms at rest.
  - Currently treated with Prostacyclin analogues

Epoprostenol for Class IV PAH – Clinical Data

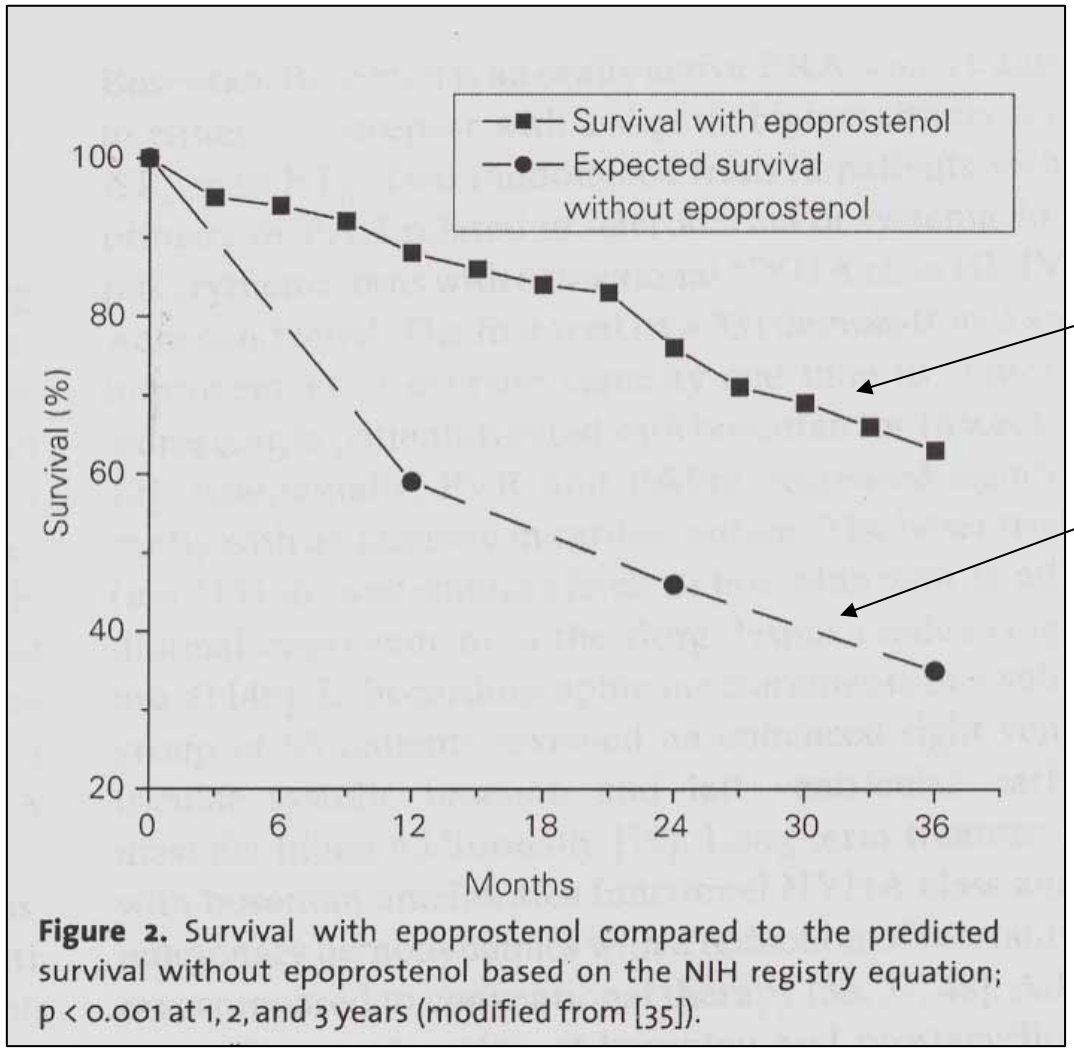
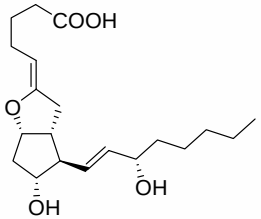
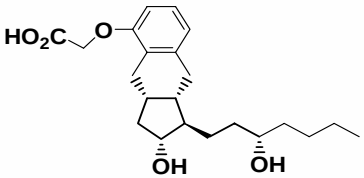
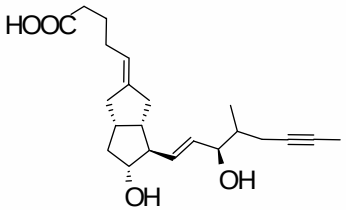
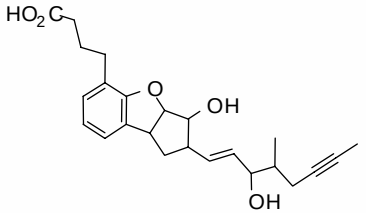
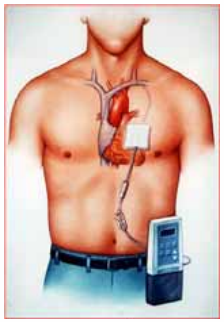
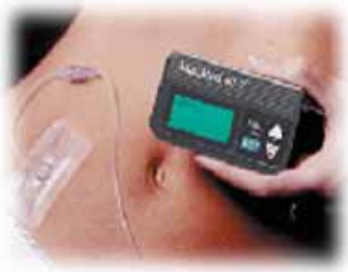


Figure 2. Survival with epoprostenol compared to the predicted survival without epoprostenol based on the NIH registry equation; $p < 0.001$ at 1, 2, and 3 years (modified from [35]).

Current therapy
With Epoprostenol

Current therapy
Without Epoprostenol

Current Prostacyclin analogues for PAH

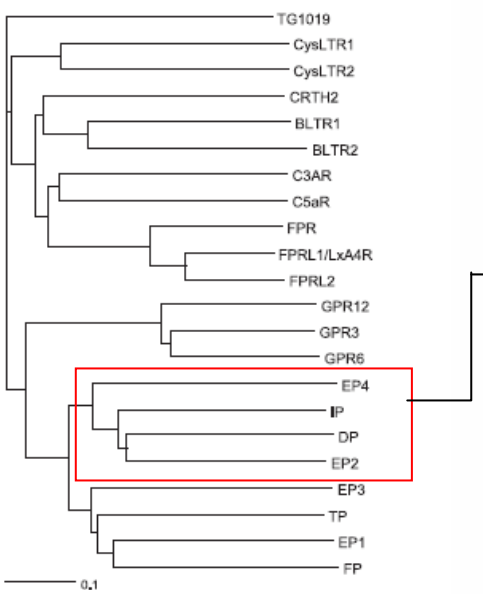
Epoprostenol (Flolan)	Treprostinil (Remodulin)	Iloprost (Ventavis)	Beraprost (Dorner)
			
			
Infection Sepsis Severe rebound PAH	Infection Injection site pain sepsis	Treatment 6-9 x/day Intermittent coverage	4x/day Intermittent coverage

- **Prostacyclin analogues have sub-optimal delivery routes**
  - Continuous i.v. or s.c. infusion (Epoprostenol, Treprostinil); Inhaled aerosol 6-12x/day (Iloprost)
  - Oral 4x/day with efficacy limited to < 6 mo (Beraprost)
- **An orally active qd IP agonist could be a useful addition for the treatment of PAH**

Prostacyclin Receptor Agonist: Product Profile

- Indication: pulmonary arterial hypertension
  - Efficacy equal or greater than I.V. or inhaled prostacyclin analogs in Class IV patients
  - Ideally, able to be used in less severe cases
- High potency and selectivity for IP receptor
- Oral delivery
- PK profile suitable for once daily dosing
  - Long half-life with low peak-trough changes in drug level
  - Key to tolerability in clinic
- Compatible with co-administration of other PAH drugs
- Clean off-target safety profile

IP agonists – testing scheme for PAH



Human/rat IP receptor cAMP assay
Human IP RBA

↓

Melanophores (EP1, EP3, TP, FP)
cAMP (DP1/EP2/EP4)
RBA DP1/EP2/EP4
GPCR panel (spot check)

↓

Human platelet aggregation

↓

PK, CyP inhibition, hERG, rat PRP

↓

PAH model (rat)

Potency EC_{50} < 50 nM

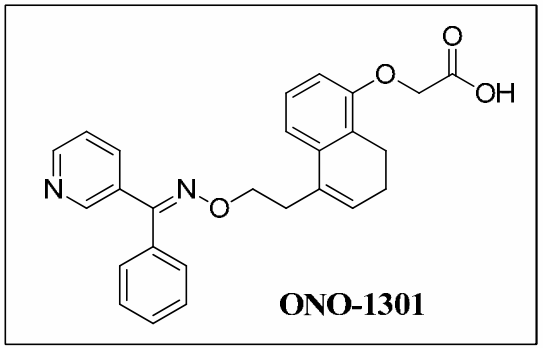
Selectivity > 300x
DP1 > 100x

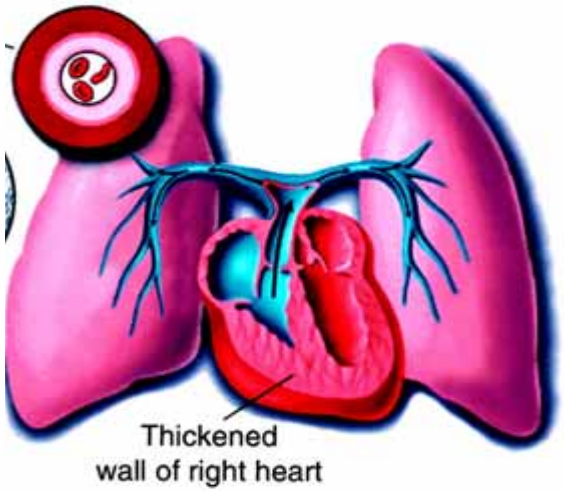
IC_{50} <100 nM

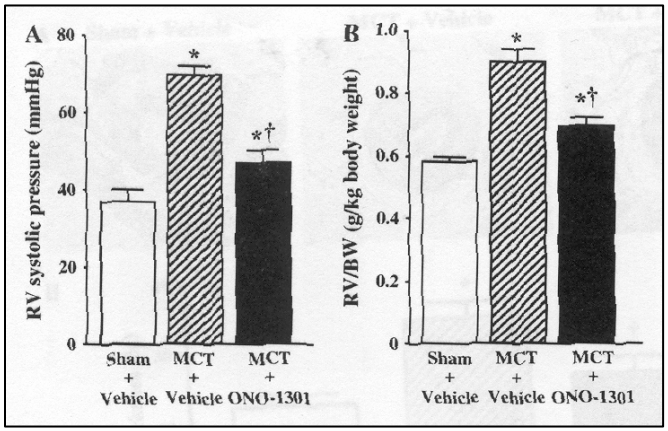
$t_{½}$ suitable for
1-2x/day dosing
%F > 30
CyP > 10uM
hERG > 5uM

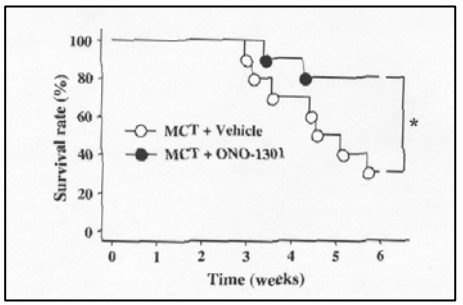
Animal Model of PAH

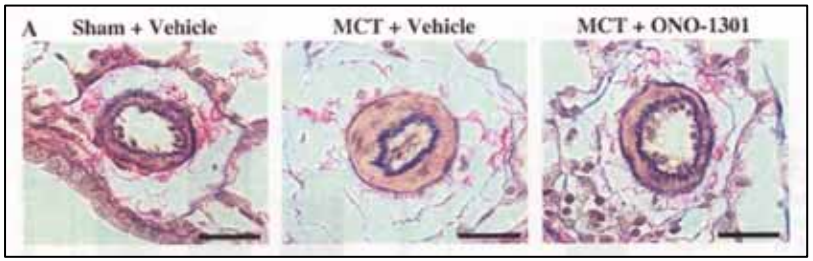
- **Monocrotaline (MCT), a pyrrolizidine alkaloid from *Crotalaria spectabilis (showy rattlebox)*, is activated metabolically in the liver to monocrotaline pyrrole which is then transported to the lungs and becomes pneumotoxic.**
- **Since subcutaneous injection of MCT can cause PH, medial hypertrophy of the pulmonary arteries, and severe pressure overload-induced right ventricular hypertrophy, MCT has been widely used as an animal model of PAH**





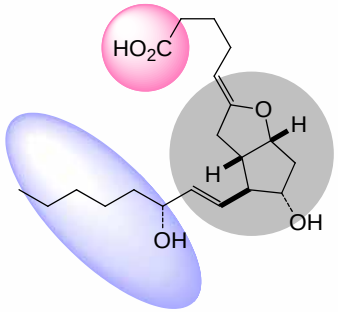




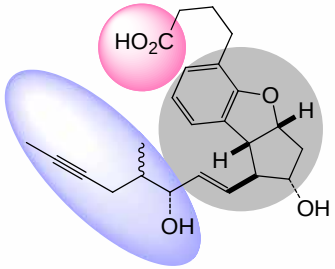


Comparison of Structures of Prostanoid and Known Nonprostanoid Ligands

Prostanoids

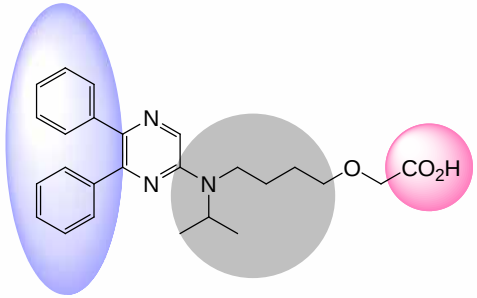


Prostacyclin (PGI_2)

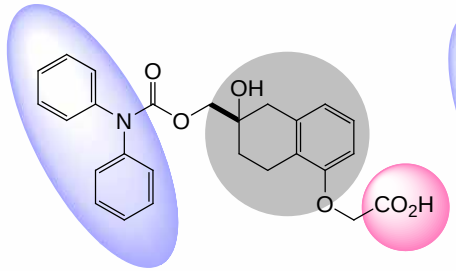


Beraprost

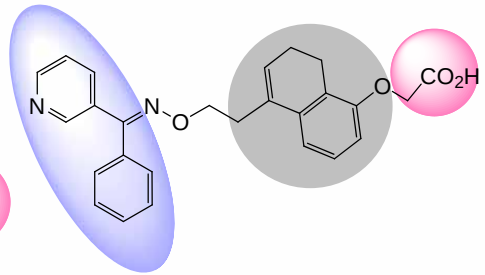
Nonprostanoids



NS304



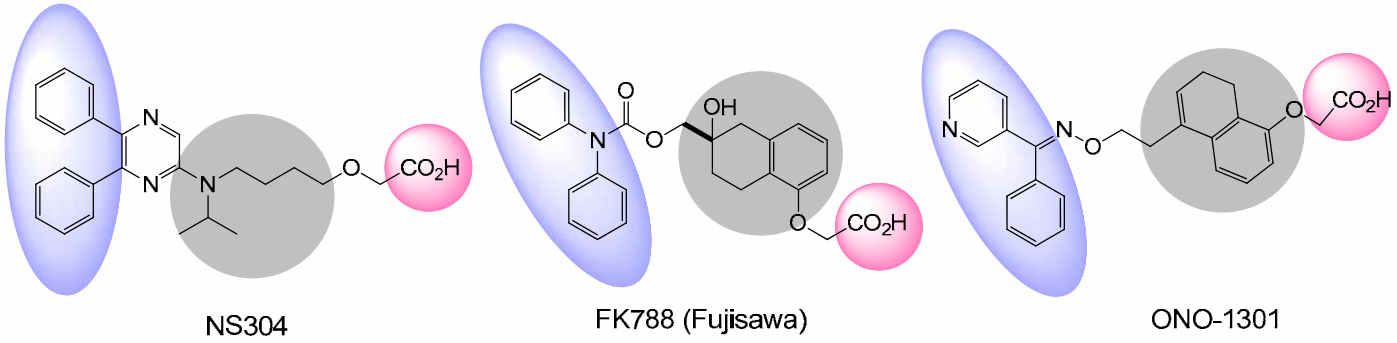
FK788 (Fujisawa)



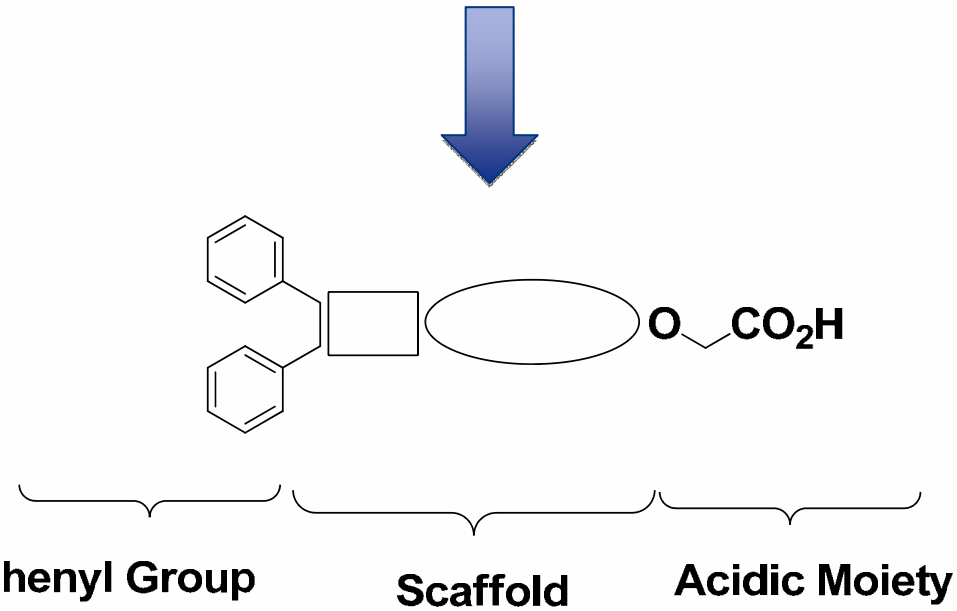
ONO-1301

Compound Design

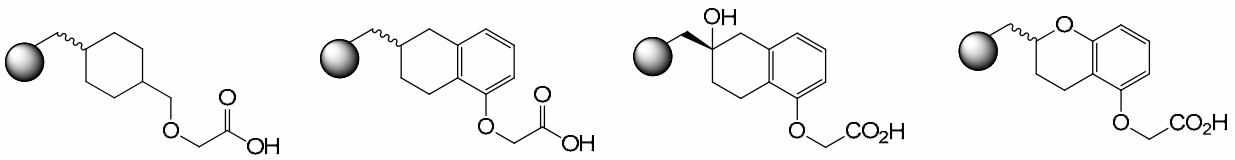
Nonprostanoids

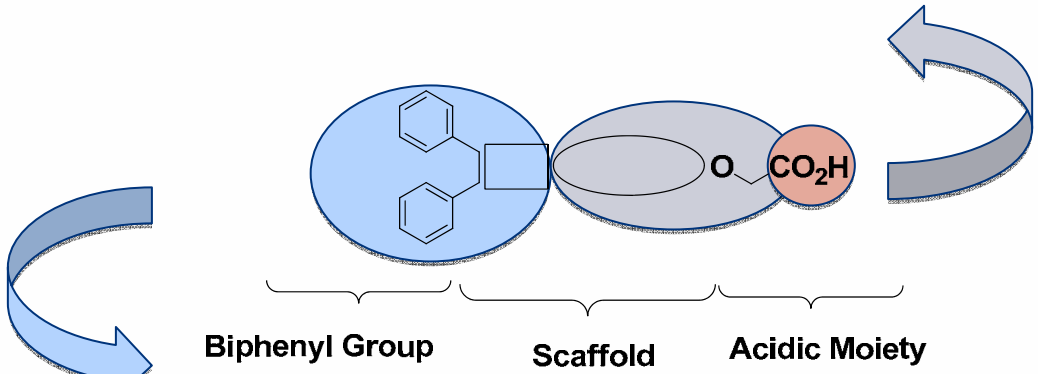


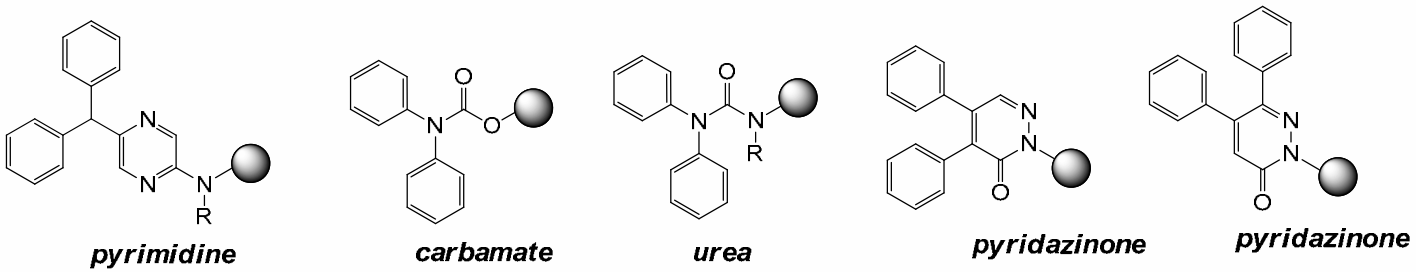
NS304 FK788 (Fujisawa) ONO-1301



Initial Design for IP receptor Agonists







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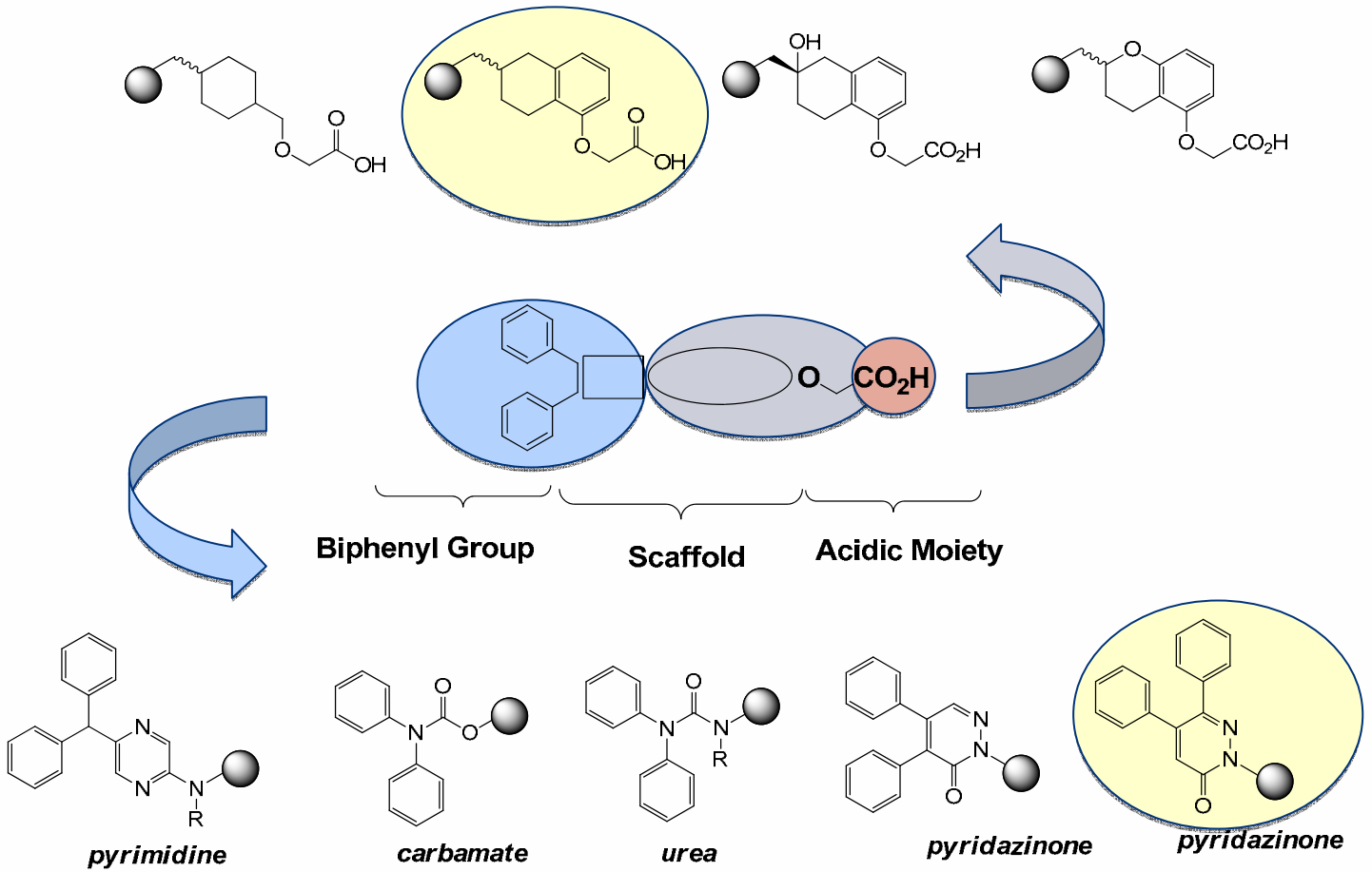
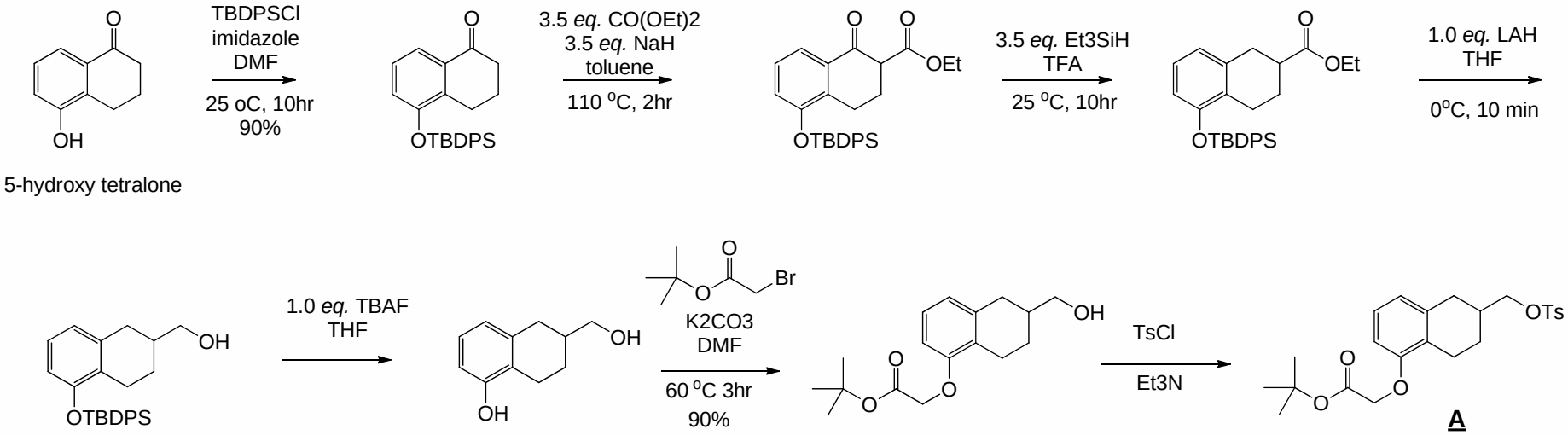


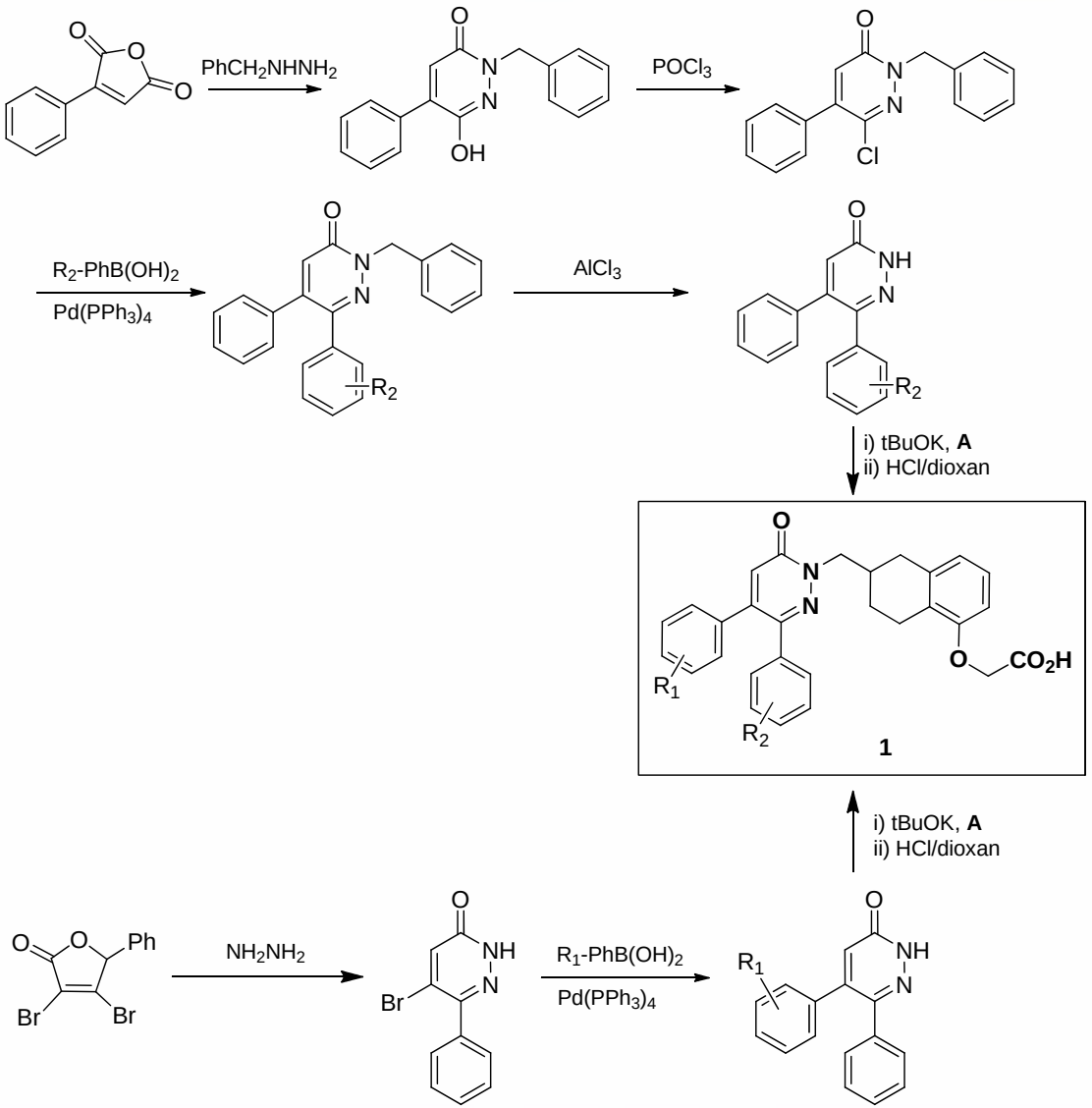
Synthesis of tetrahydronaphthalene core



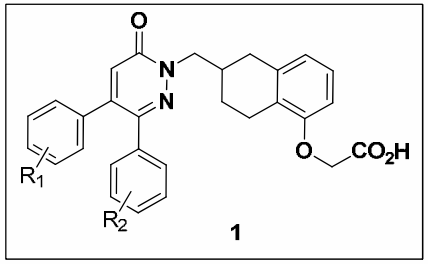
Yield 90% X 80% X 80% X 90% X 70% X 90% X70% = 23% (7 steps) **|** Two purifications on SiO2

- 7 Steps to protected scaffold/acid portion with appropriate leaving group for alkylation reactions

Synthesis of Substituted 3,4-diphenyl-pyridazinones



3,4-Diphenylpyridazinones SAR



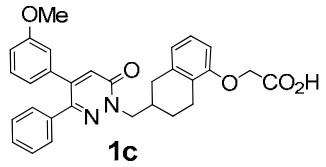
1

	R_1	R_2	EC_{50} hIP* (nM)	IA (%)¶	EC_{50} rIP* (nM)	IA (%)¶	EC_{50} hDP1# (nM)
1a	H	H	969	60	2190	70	21
1b	4-OMe	H	2670	47	>10000	-	n.d.
1c	3-OMe	H	60	88	310	96	17
1d	3-Me	H	180	97	370	72	25
1e	3-F	H	380	63	900	97	44
1f	3-Cl	H	60	77	360	62	61
1g	3-OMe, 2-F	H	37	97	350	85	6
1h	H	3-OMe	1720	87	>10000	-	n.d.
1i	H	3-F	1060	88	2370	99	n.d.
1j	H	4-OMe	8.5	80	12.5	93	1.7
1k	H	4-Me	24	67	120	99	n.d.
1l	H	4-F	114	89	1500	93	n.d.
1m	H	4-OMe, 2-F	20	78	112	76	31
1n	H	4-OMe, 3-F	10	80	51	75	3
1o	3-OMe	4-Me	9.5	81	28	107	11
1p	3-OMe	4-F	62	91	620	102	24

* = EC_{50} in the HTRF cAMP human or rat IP receptor assay ¶ = Intrinsic activity (efficacy) relative to 1μM iloprost as the positive control
# = EC_{50} in a melanophore assay

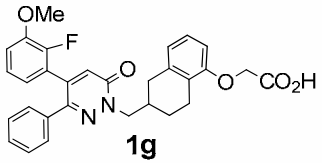
Separation of Enantiomers

- Chiral resolution of the tetrahydronaphthyl building block allowed synthesis of each enantiomer separately
- The bulk of the activity was observed in one isomer
- However, no improvement in selectivity was seen



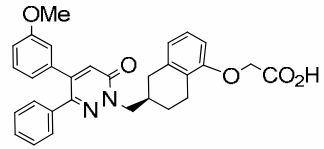
1c

hIP, cAMP = 62 nM (n = 5)
rIP, cAMP = 250 nM (n = 5)
h Platelet = 40 nM (n = 2)
r Platelet = 1300 nM (n = 1)
DP1 Mel = 24 nM (n = 3)



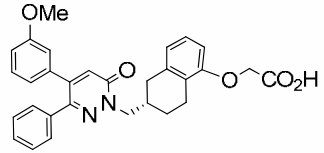
1g

hIP, cAMP = 33nM (n = 3)
rIP, cAMP = 170nM (n = 2)
h Platelet = 35nM (n = 2)
r Platelet = 4000 nM (n =1)
DP1 Mel = 7 nM (n = 4)



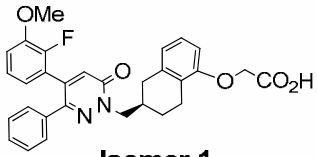
Isomer 1

hIP, cAMP = 21nM (n =3)
rIP, cAMP = 88nM (n = 3)
h Platelet = 22nM (n = 2)
r Platelet = 1200 nM (n = 1)
DP1 Mel = 23 nM (n = 3)



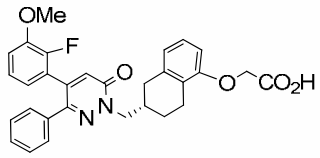
Isomer 2

hIP, cAMP = 230nM (n = 4)
rIP, cAMP = 6000nM (n = 4)



Isomer 1

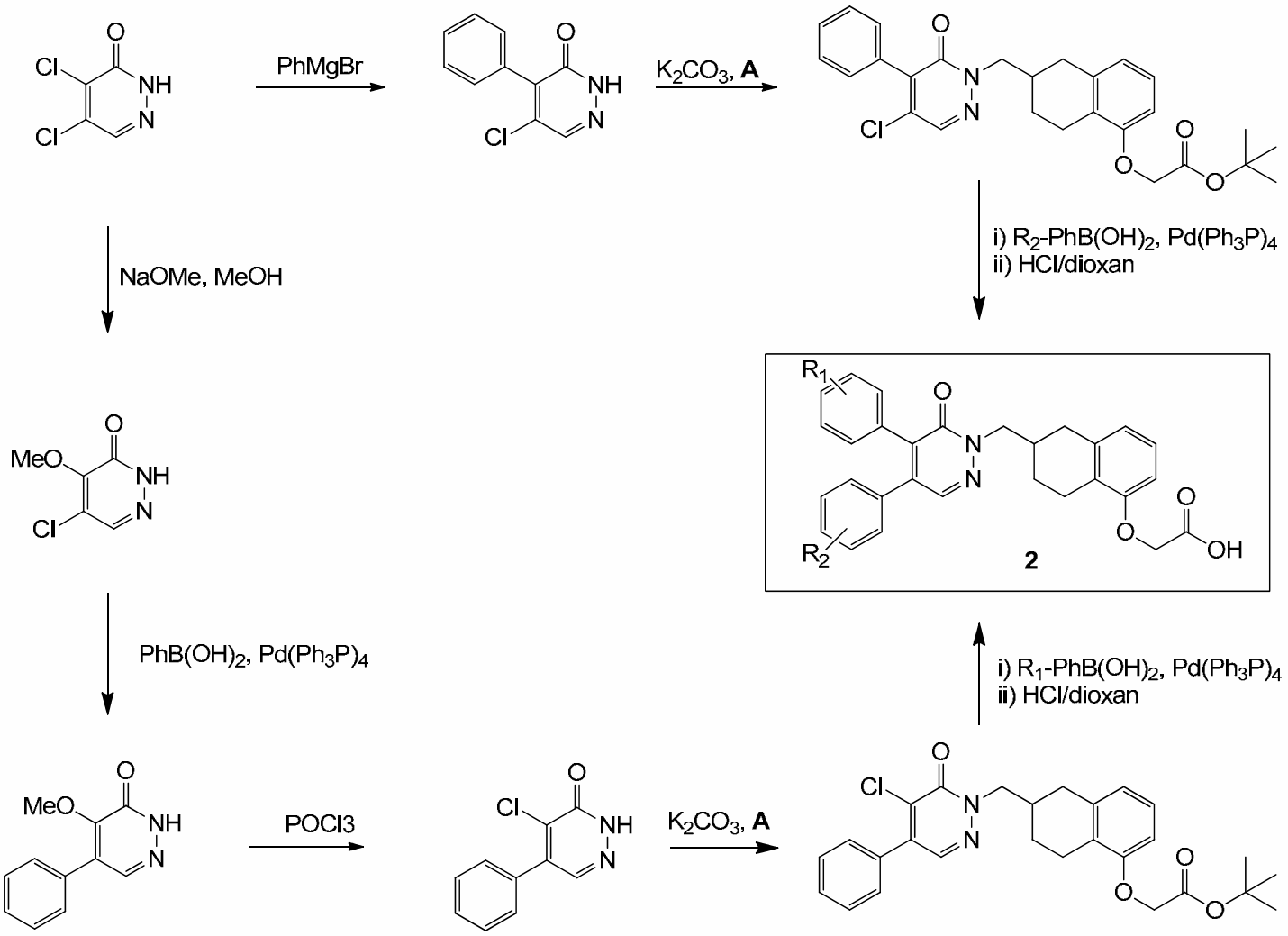
hIP, cAMP = 16nM (n = 3)
rIP, cAMP = 68nM (n = 3)
h Platelet = 15nM (n = 2)
r Platelet = 1200 nM (n = 1)
DP1 Mel = 6 nM (n = 3)



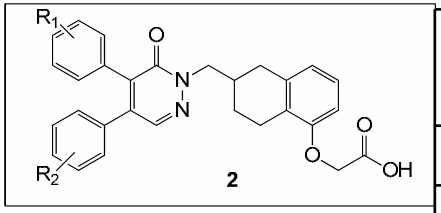
Isomer 2

hIP, cAMP = 320nM (n = 4)
rIP, cAMP = 1600nM (n = 4)

Synthesis of Substituted 4,5-diphenyl-pyridazinones



4,5-Diphenylpyridazinones SAR



2

	R_1	R_2	EC_{50} hIP* (nM)	IA (%)¶	EC_{50} rIP* (nM)	IA (%)¶	EC_{50} hDP1# (nM)	Human platelet IC_{50} (nM)@
2a	H	H	100	75	260	88	180	325
2b	H	3-OMe	140	101	690	81	48	n.d.
2c	H	3-F	60	82	410	103	88	191
2d	H	3-Cl	86	93	260	98	n.d.	n.d.
2e	H	4-OMe	1060	73	> 3000	-	n.d.	n.d.
2f	H	2-F	120	106	420	99	31	220
2g	H	2,3-F_2	38	87	110	101	36	87
2h	H	2-F, 3-OMe	10	78	40	98	40	64
2i	2-F	H	140	91	370	116	n.d.	n.d.
2j	3-F	H	260	100	790	121	n.d.	n.d.
2k	4-F	H	20	80	170	104	n.d.	230
2l	3-OMe	H	770	74	3500	99	n.d.	n.d.
2m	4-OMe	H	4	72	11	105	n.d.	330
2n	4-F	2,3-F_2	6	85	122	96	44	62

* = EC_{50} in the HTRF cAMP human or rat IP receptor assay ¶ = Intrinsic activity (efficacy) relative to 1μM iloprost as the positive control
# = EC_{50} in a melanophore assay @ = Inhibition of ADP-induced human platelet aggregation

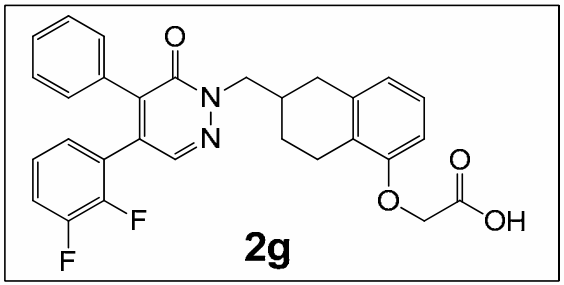
Compound **2g** Extended Profile

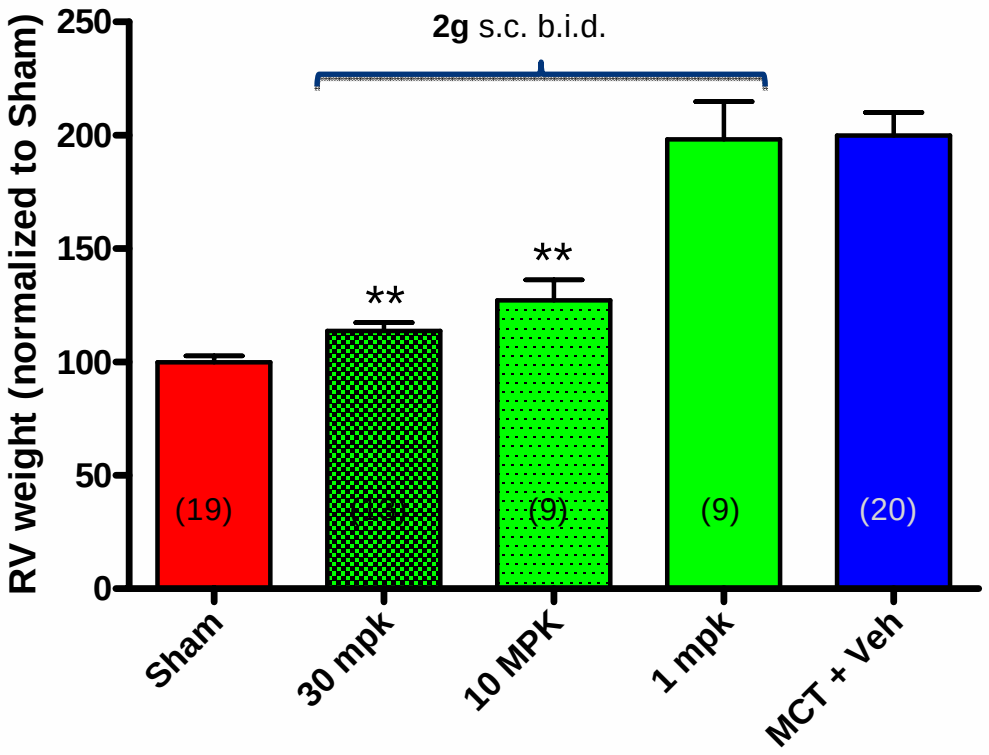
2g

- **hIP, cAMP = 38 nM (n = 18)**
- **rIP, cAMP = 110 nM (n = 17)**
- **h Platelet = 90 nM (n = 6)**
- **DP1, cAMP = 850 nM (n = 8)**
- **DP2, EP1, EP2, EP3, EP4, FP, TP , cAMP = NR**
- **CYP Inhibitions : Clean**
- **hERG Patch Clamp: 16% inhibition @ 3 μM**
- **Water Solubility (sodium salt): 2.2 mg/mL (pH 7)**
- **PK profile: Dose(mg/kg) IV = 2, PO = 3**
  IV $t_{1/2}$ = 2.3 h; P.O $t_{1/2}$ = 3.2 hr
  F = 44.2%

In Vivo POC compound

- Monocrotoline administered on Day 1
- Rats dosed twice daily with test compound or vehicle for 21 days
- Right ventricular weight measured on day 21

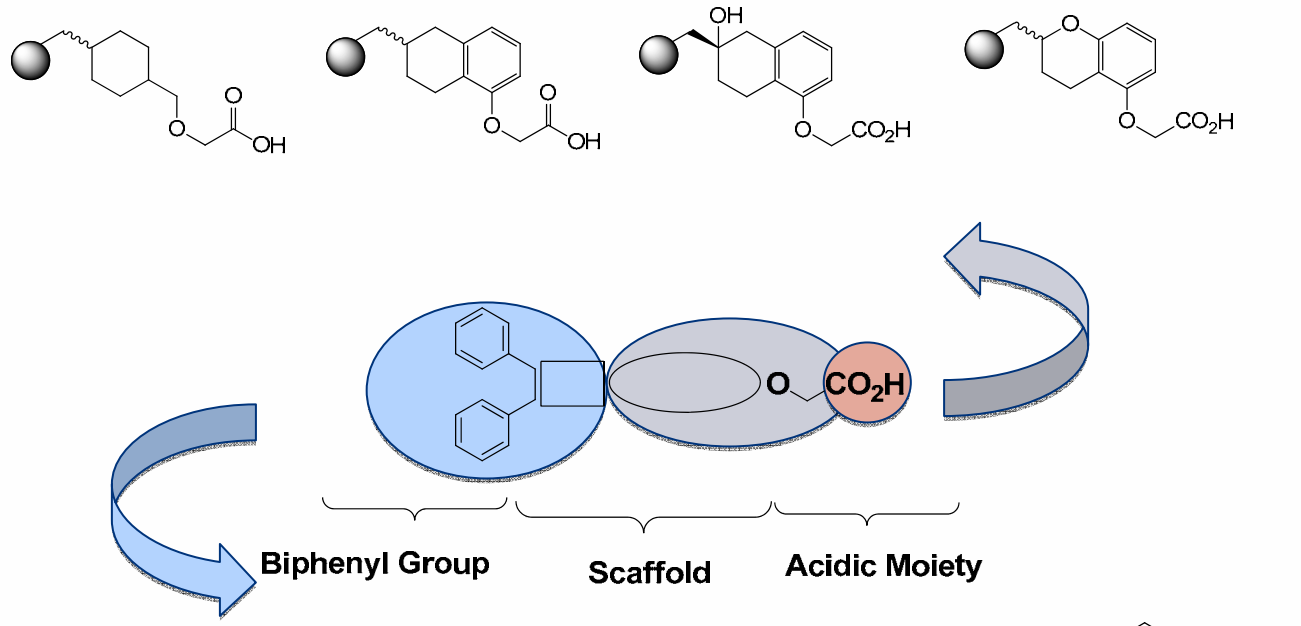




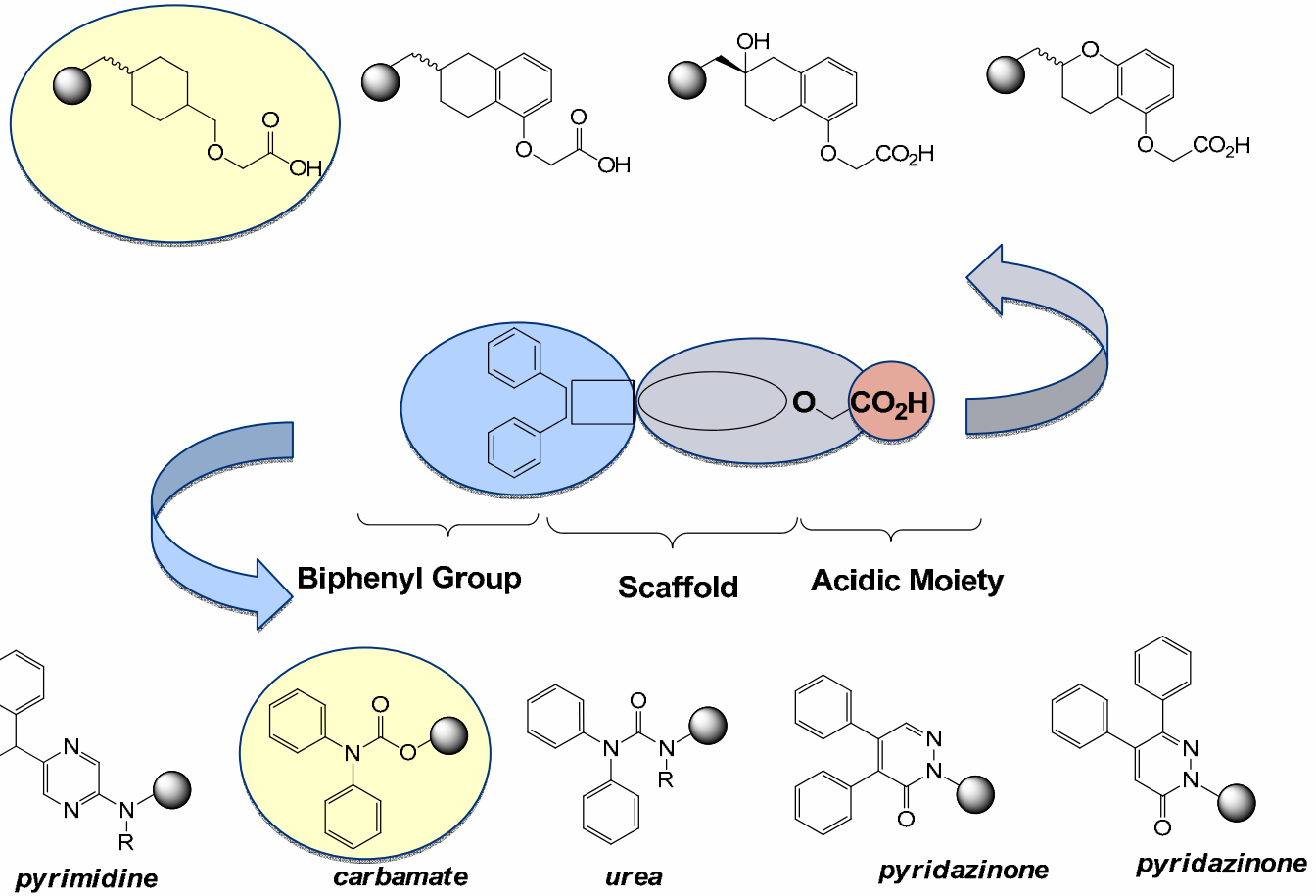
**P<0.001 vs. MCT + Vehicle

Active in vivo s.c. but 'optimized' compounds from this series not active p.o.

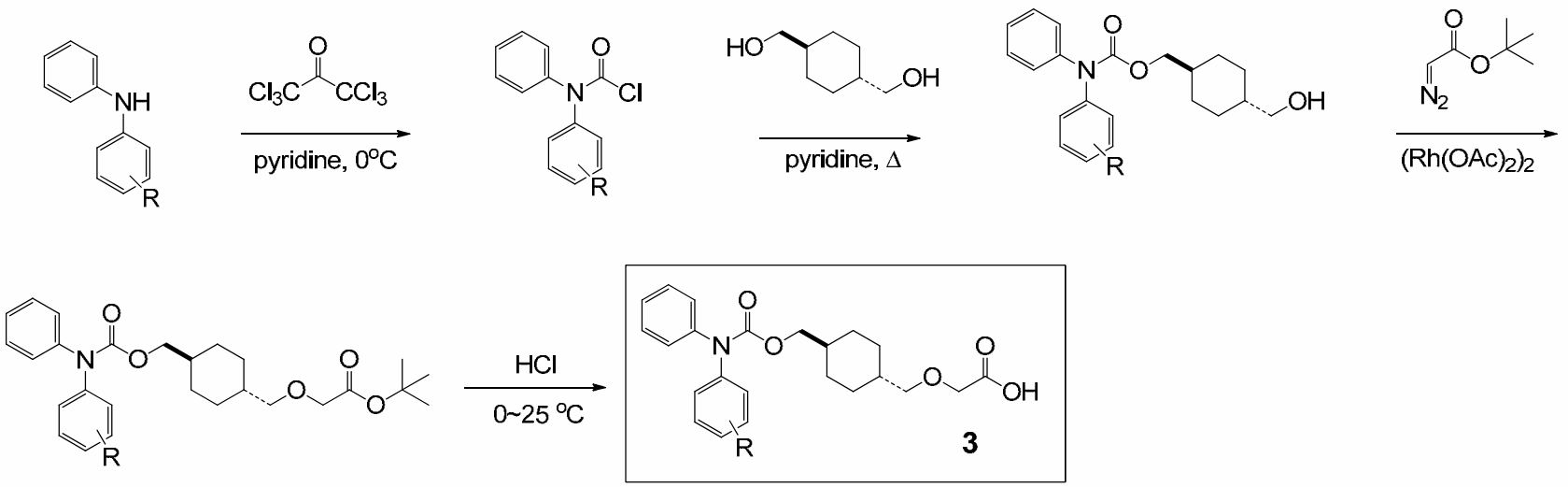
Second Generation Design for IP receptor Agonists



Second Generation Design for IP receptor Agonists

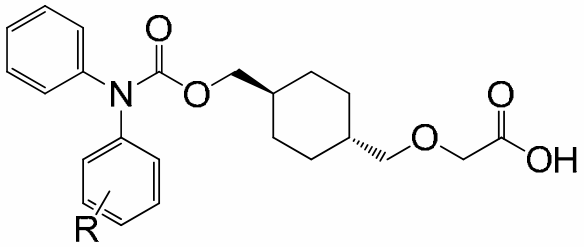


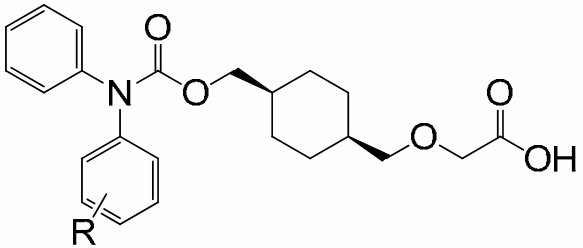
Cyclohexyl-Carbamate Synthetic Route



- Significantly shorter, high-yielding synthesis
  - Overall yields typically >40%
- Reduced lipophilicity, no chiral centre
- Improved selectivity vs DP1

Early SAR : Relative Stereochemistry Requirements





trans-cyclohexyl

- R=H; hIP EC_{50} = 9.6 nM*
- R=4-Cl; hIP EC_{50} = 8.5 nM
- R=4-OMe; hIP EC_{50} = 3.3 nM

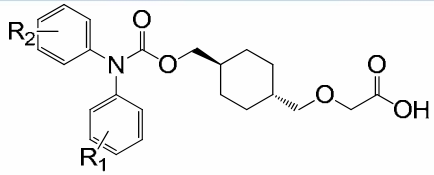
(* = EC_{50} in the HTRF cAMP human IP receptor assay)

cis-cyclohexyl

- R=H; hIP EC_{50} = 68 nM
- R=4-Cl; hIP EC_{50} = 46 nM
- R=4-OMe; hIP EC_{50} > 1000nM

- A clear preference for the *trans*-stereochemistry was noted
- Further analogues were prepared using only the *trans*-cyclohexyl core

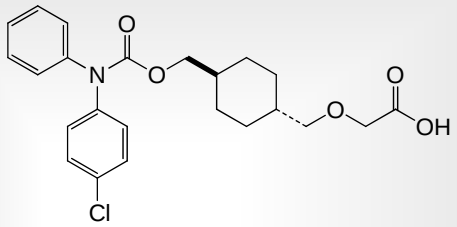
Carbamate Series SAR



	R_1	R_2	EC_{50} hIP* (nM)	IA (%)¶	EC_{50} rIP* (nM)	IA (%)¶	EC_{50} hDP1# (nM)	Human platelet IC_{50} (nM)@	PK Properties (rat)
3a	H	H	9.6	91	365	89	355	25	-
3b	H	4-Cl	8.5	85	530	88	850	38	$T_{1/2}$: 6.7h; F: 57%
3c	H	4-OMe	3.3	83	165	91	240	22	$T_{1/2}$: 0.3h; F: 100%
3d	H	4-Me	3.9	81	174	99	320	39	-
3e	H	4-F	4.8	89	535	91	560	12	$T_{1/2}$: 3.9h; F: 100%
3f	H	3-F	8.7	85	284	83	2760	18	$T_{1/2}$: 3.4h; F: 69%
3g	H	4-Cl, 3-F	3.4	85	400	92	2050	23	$T_{1/2}$: 5h; F: 88%
3h	4-OMe	3-F	5.2	70	1570	60	1030	43	-
3i	4-Cl	3-F	18.5	70	3190	44	1140	n.d.	-
3j	4-F	4-F	41	65	n.d.		862	n.d.	-

* = EC_{50} in the HTRF cAMP human or rat IP receptor assay ¶ = Intrinsic activity (efficacy) relative to 1μM iloprost as the positive control
# = EC_{50} in a HTRF cAMP assay @ = Inhibition of ADP-induced human platelet aggregation

Further Profiling of Potential Lead Compounds

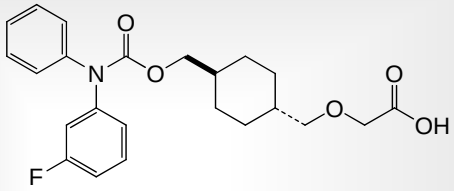


3b

hIP Ki = 8 nM
hIP, cAMP = 8 nM
rIP, cAMP = 530nM
hPlatelet = 38 nM
DP1 cAMP = 750nM
EP2 cAMP = NR
EP4 cAMP = NR

hERG (astemizole binding) = NR
HepG2 cell (intracellular calcium,
cell proliferation, membrane intergrity) : NR
Water solubility = 1.0mg/ml (pH7)
Microsomal stability t/12 h > 60' , r > 60'
CYP Inhibitions HLM : clean
Ames, hERG Patch, DP1 RBD, GPCR- screen, clean

Rat PK Dose(mg/kg) IV = 2, PO = 10
T1/2: IV = 6.7 hr; PO = 5.4 hr.
CL = 0.427 L/hr/kg
(Vss) = 2.742 L/kgL/kg).
Cmax (po): 3.703 µg/mL at 1.5 hr.
Oral bioavailability (%F) = 57.4%.



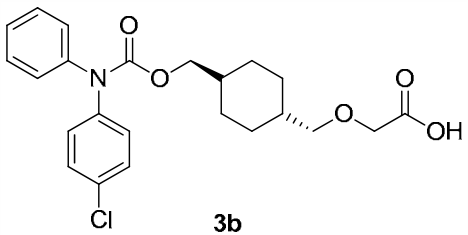
3f

hIP Ki = 14 nM
hIP, cAMP = 8 nM
rIP, cAMP = 280nM
h Platelet = 18 nM
DP1 cAMP = 3000nM
EP2 cAMP = NR
EP4 cAMP = NR

hERG (astemizole binding) = NR
HepG2 cell (intracellular calcium,
cell proliferation, membrane intergrity) : NR
Water solubility = 2.6 mg/ml (pH7)
Microsomal stability t/12 h > 60' , r > 60'
CYP Inhibitions HLM : clean
Ames, hERG Patch, DP1 RBD, GPCR- screen, clean

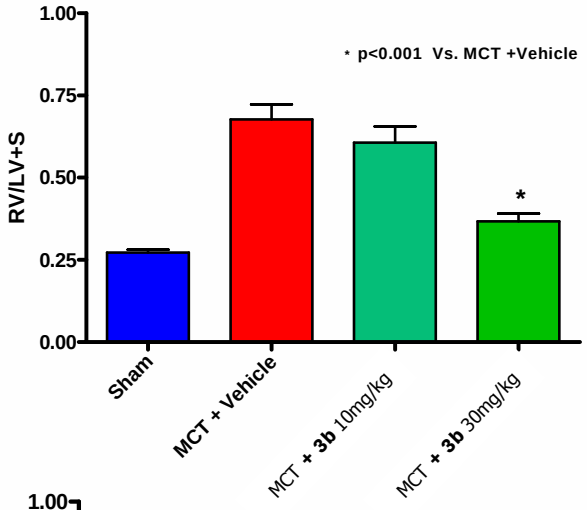
Rat PK Dose(mg/kg) IV = 2, PO = 10
T1/2: IV = 3.4 hr; PO = 2.9 hr.
CL = 1.205 L/hr/kg
(Vss) = 2.749 L/kg
Cmax (po): 3.423 µg/mL at 0.3 hr.
Oral bioavailability (%F) = 69.0%.

Rat PAH model: Carbamates

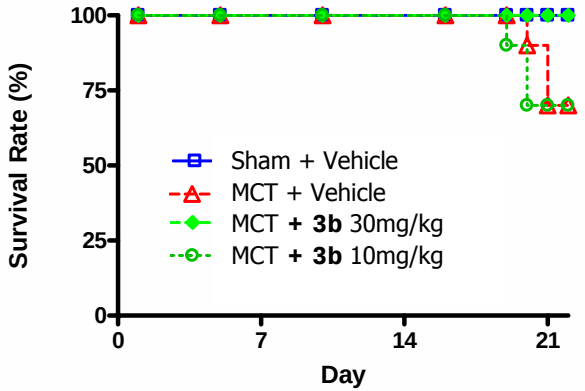


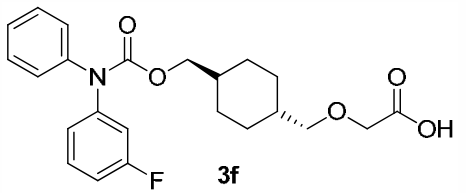
3b

Right Ventricular Weight

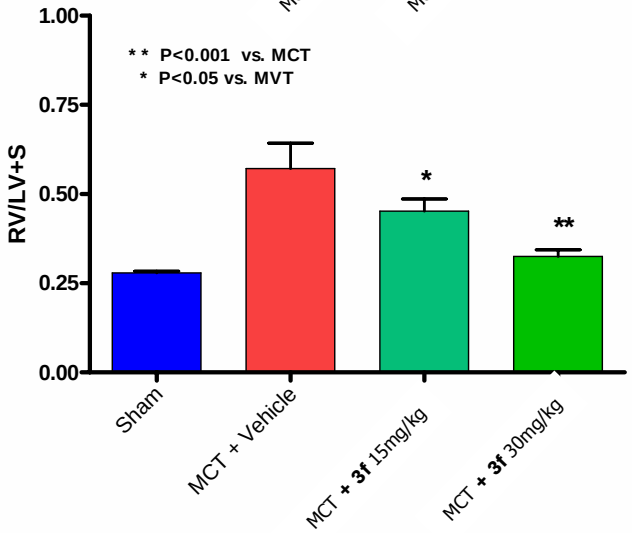


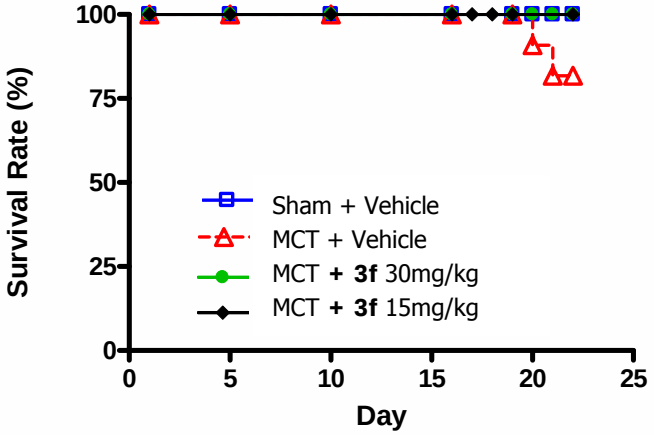
Mortality



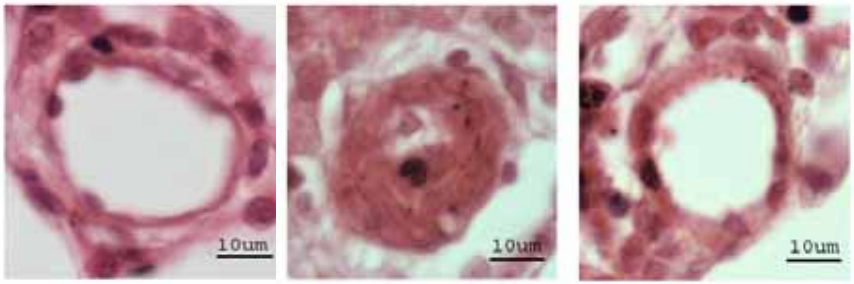


3f

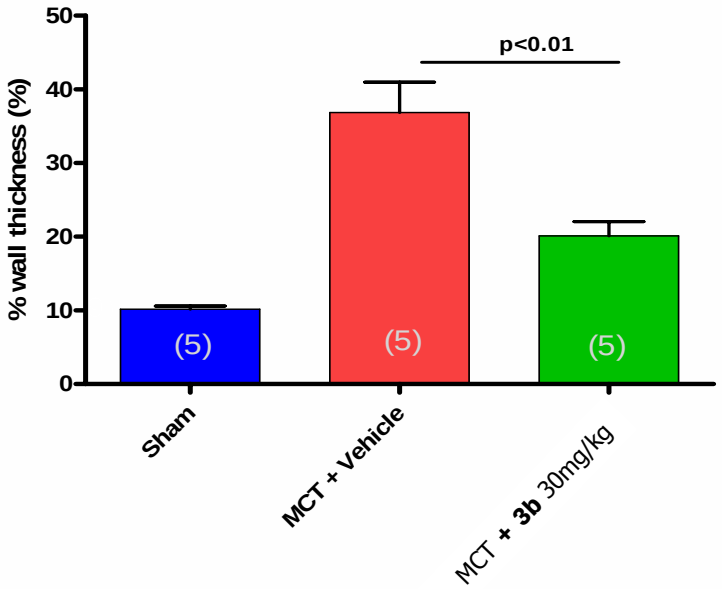




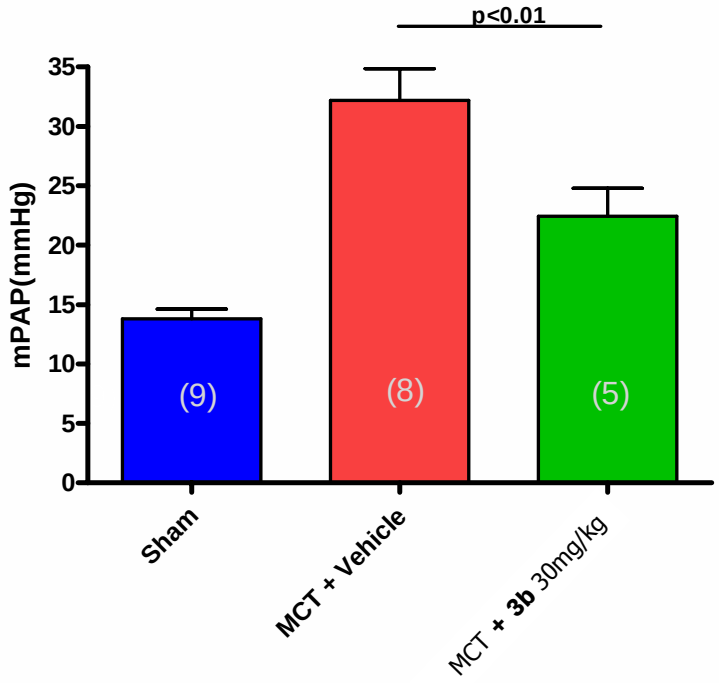
3b MCT data



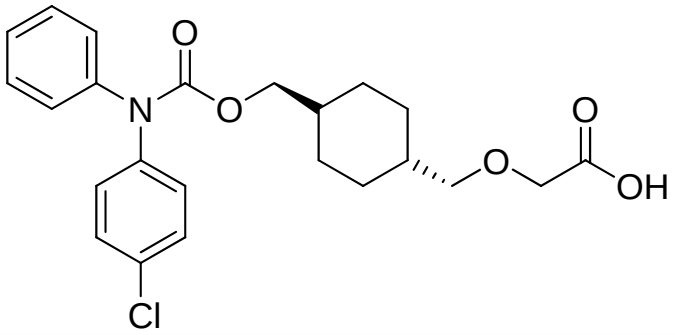
Sham **MCT** **MCT + 3b**

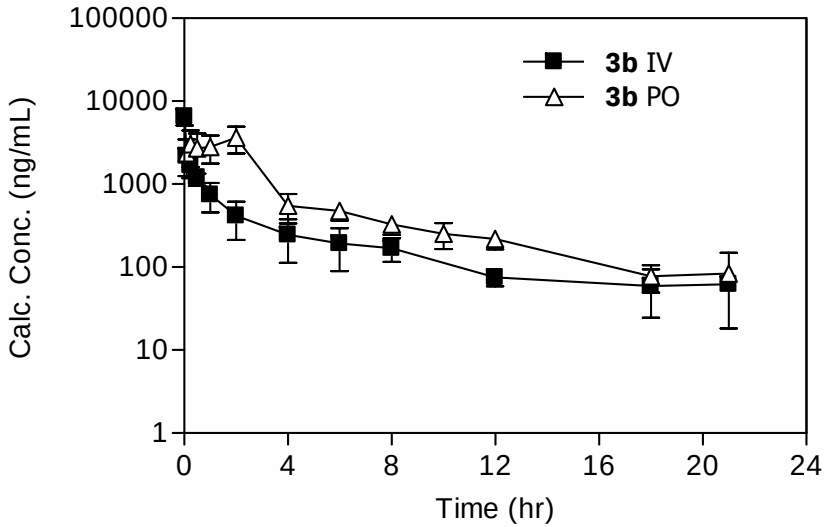


Pulmonary artery pressure



Pharmacokinetics of **3b** in Male Sprague-Dawley Rats

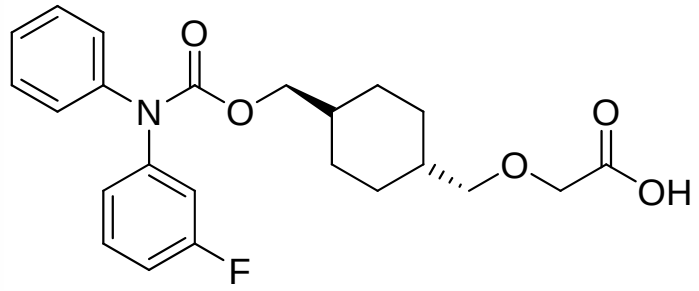


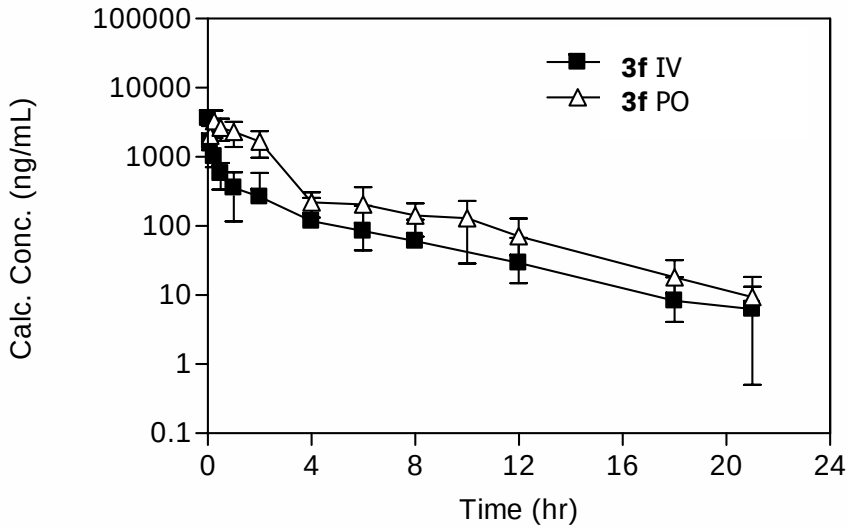


IV Parameters	3b
Dose (mg/kg)	2
T1/2 (hr)	**6.7**
Cmax (µg/mL)	6.540
AUC(0-INF)(hr*µg/mL)	5.093
Cl_obs (L/hr/kg)	**0.427**
MRTlast (hr)	4.2
Vss (L/kg)	**2.742**

PO Parameters	3b
Dose (mg/kg)	10
T1/2 (hr)	**5.4**
Tmax (hr)	**1.5**
Cmax (µg/mL)	**3.703**
AUC(0-INF)(hr*µg/mL)	14.629
MRTlast (hr)	3.8
%F	**57.4**

Pharmacokinetics of **3f** in Male Sprague-Dawley Rats





IV Parameters	3f
Dose (mg/kg)	2
T1/2 (hr)	**3.4**
Cmax (µg/mL)	3.630
AUC(0-INF)(hr*µg/mL)	2.300
Cl_obs (L/hr/kg)	**1.205**
MRTlast (hr)	2.5
Vss (L/kg)	**2.749**

PO Parameters	3f
Dose (mg/kg)	10
T1/2 (hr)	**2.9**
Tmax (hr)	**0.3**
Cmax (µg/mL)	**3.423**
AUC(0-INF)(hr*µg/mL)	7.938
MRTlast (hr)	2.7
%F	**69.0**

Physical Characterization

- **3b** sodium salt

  Early Candidate Solid-state Testing :

  – Crystalline, anhydrous form with high melting onset

  – Non hygroscopic (pure sample uptake <2% of water at 90%RH)

  – High critical water activity (>0.75)

  – Hydrate form solubility ≈ 2.6 mg/mL

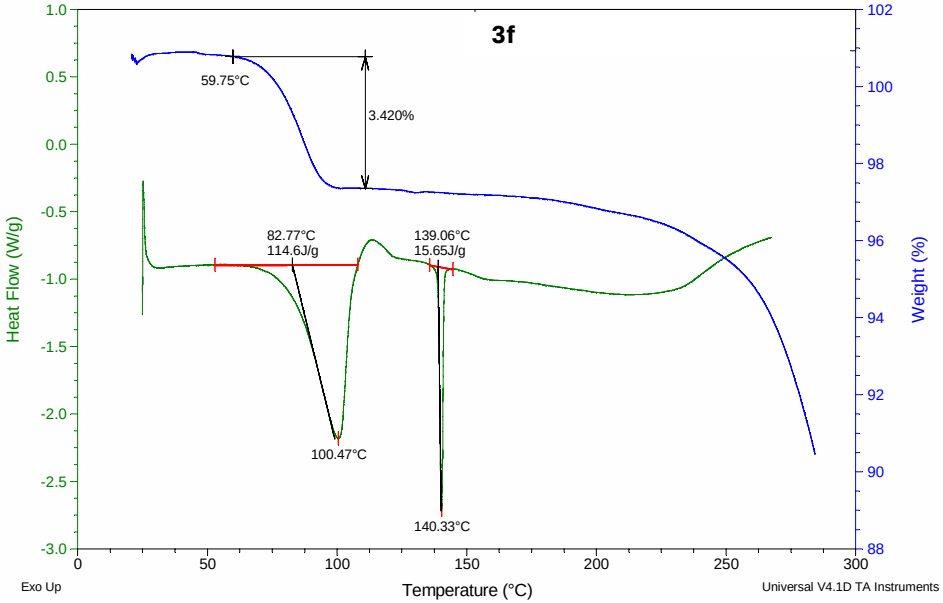
- **3f** sodium salt

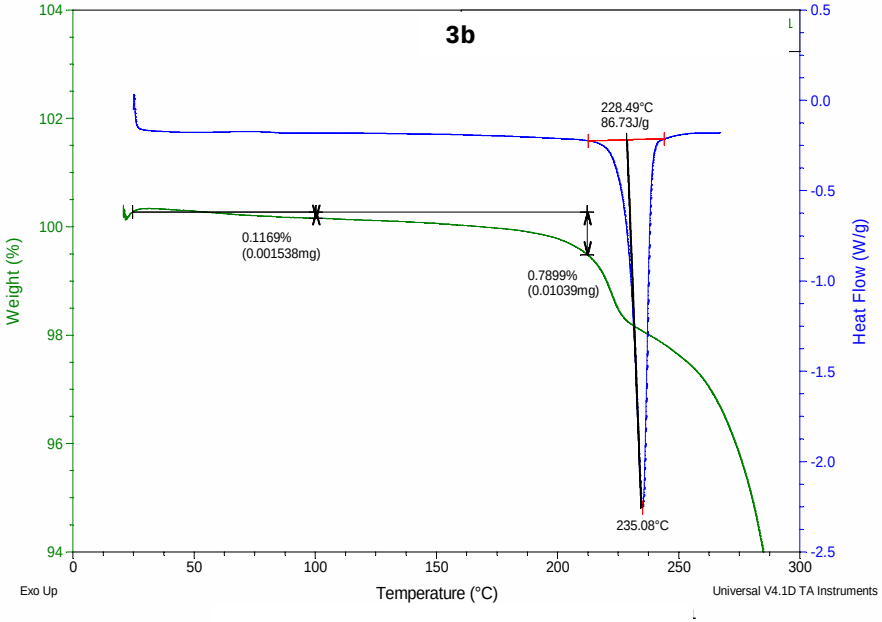
  Early Candidate Solid-state Testing :

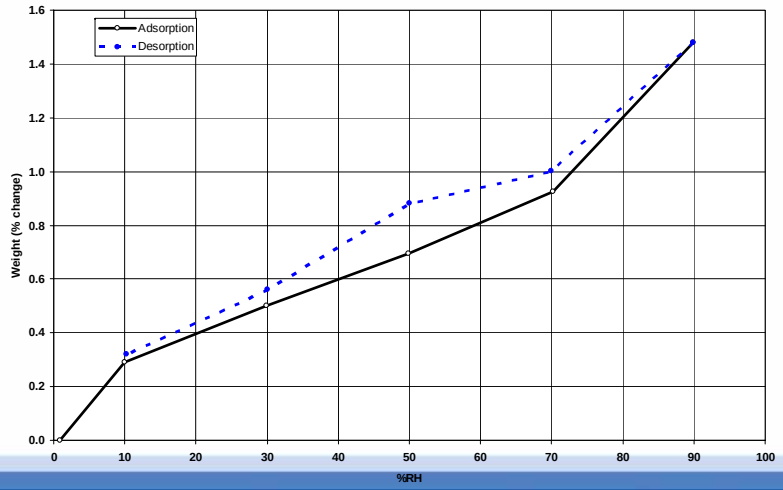
  – Crystalline, but a hydrate

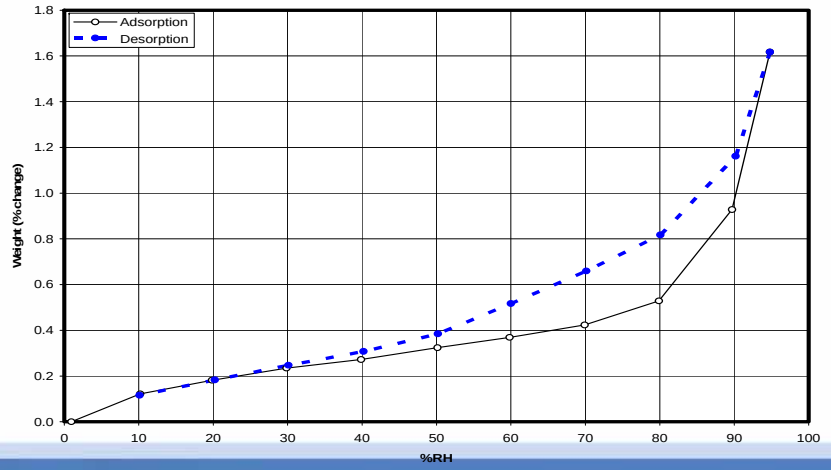
  – Non hygroscopic (pure sample uptake <2% of water at 90%RH)

3b & **3f** Na-salts

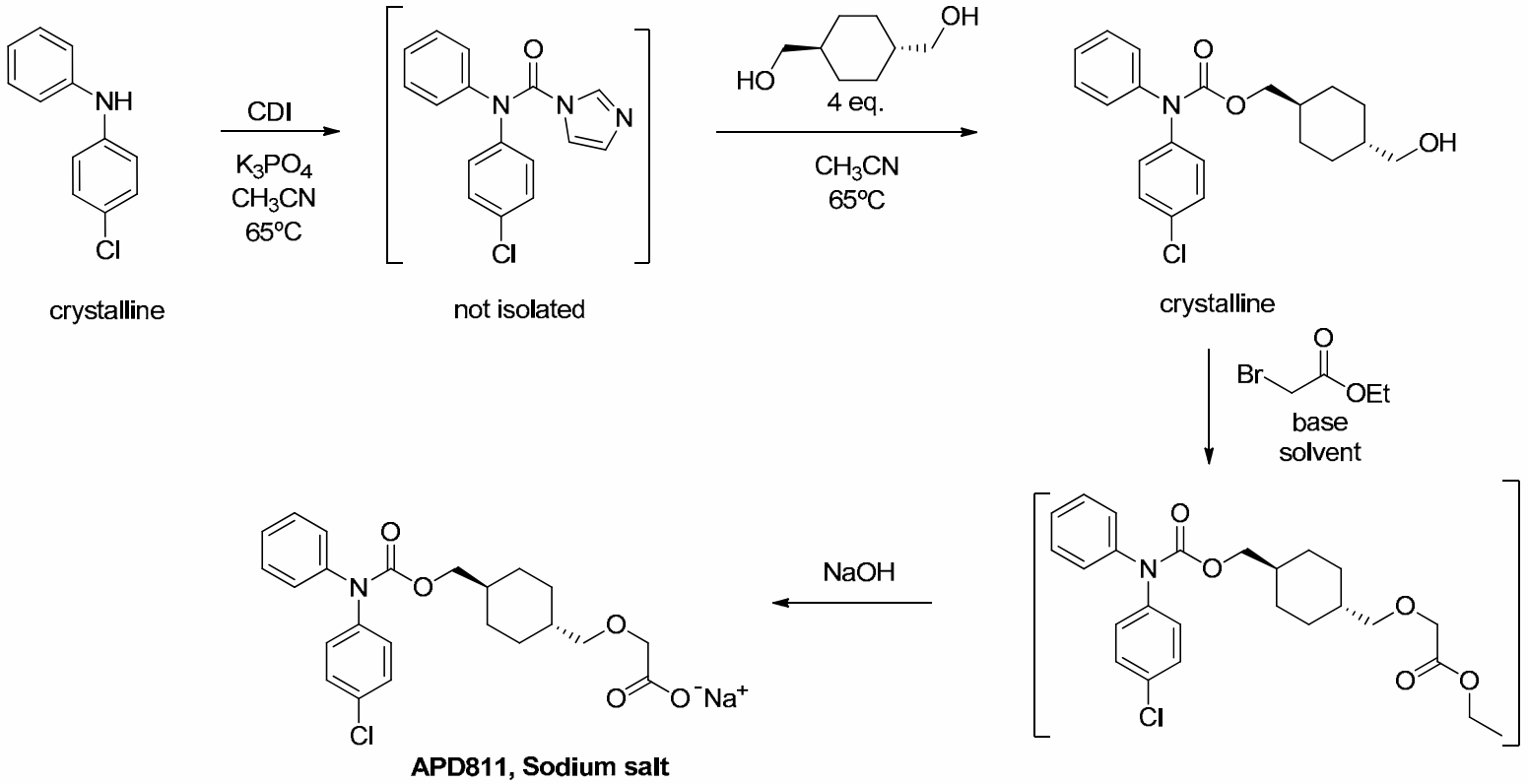






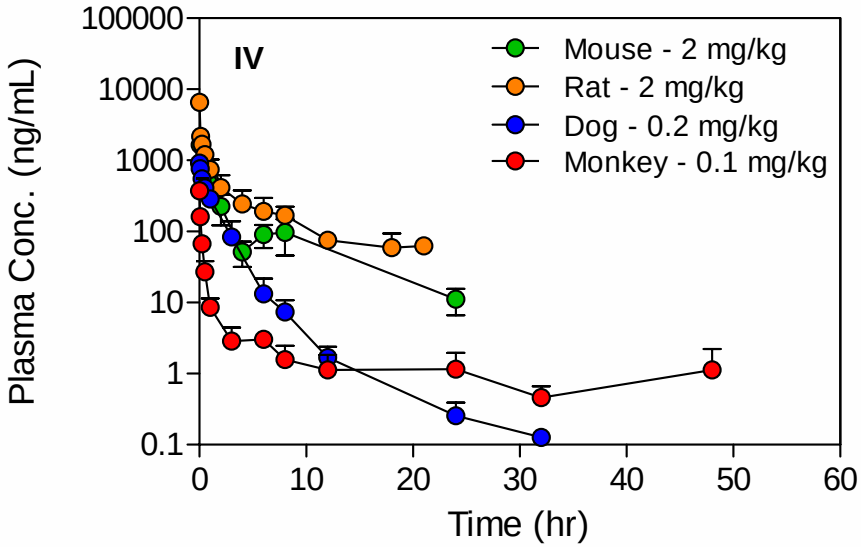


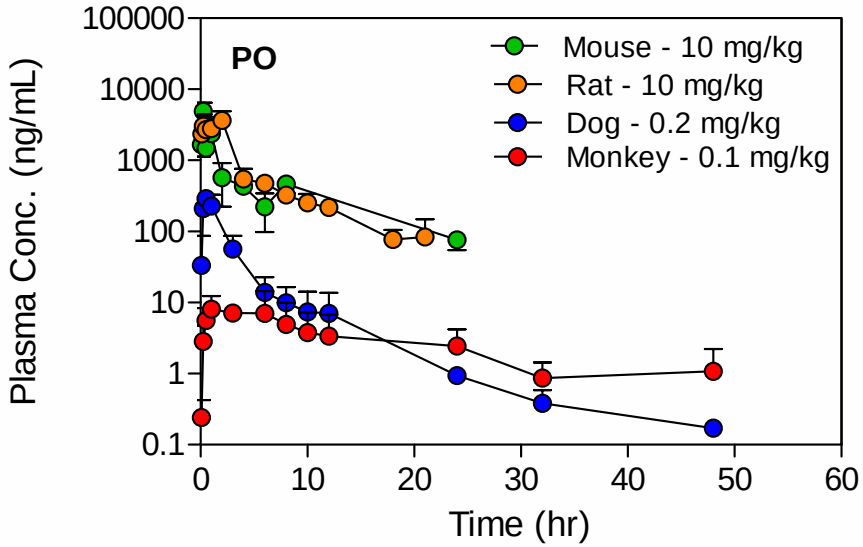
Scale-Up Synthesis Route



- Efficient 2 pot synthesis from available building blocks
- Avoids Rh catalysed diazoacetate chemistry
- Extra PPE required for handling API

Cross-Species Pharmacokinetic Profile Comparison

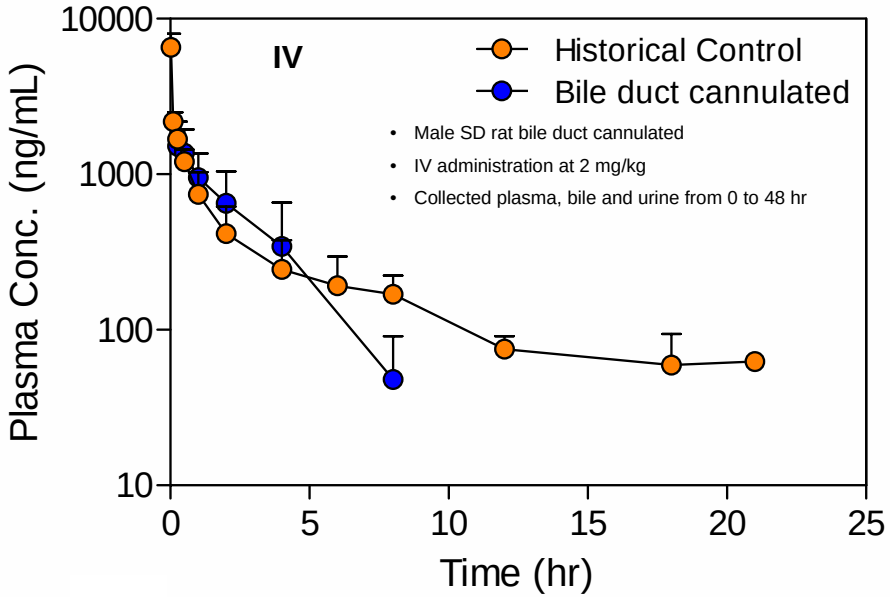


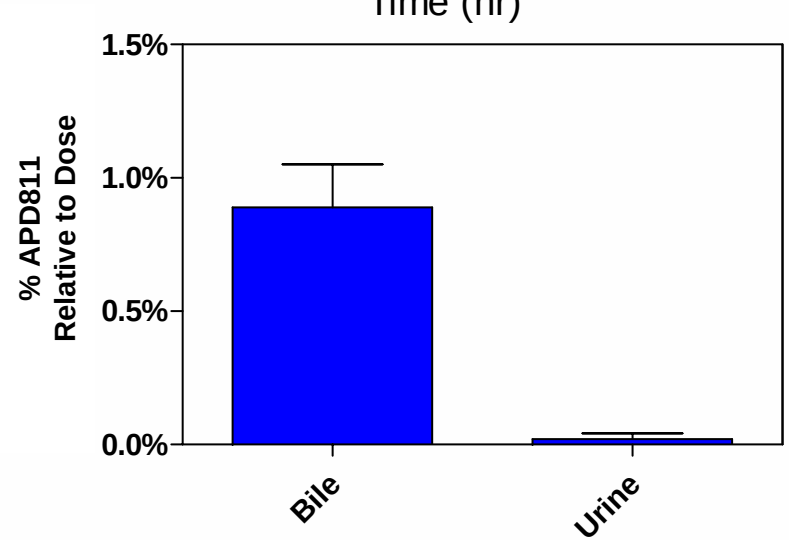


- **APD811 had an extended terminal phase across species**

Enterohepatic recycling?

APD811 : Bile Duct Cannulated Rats

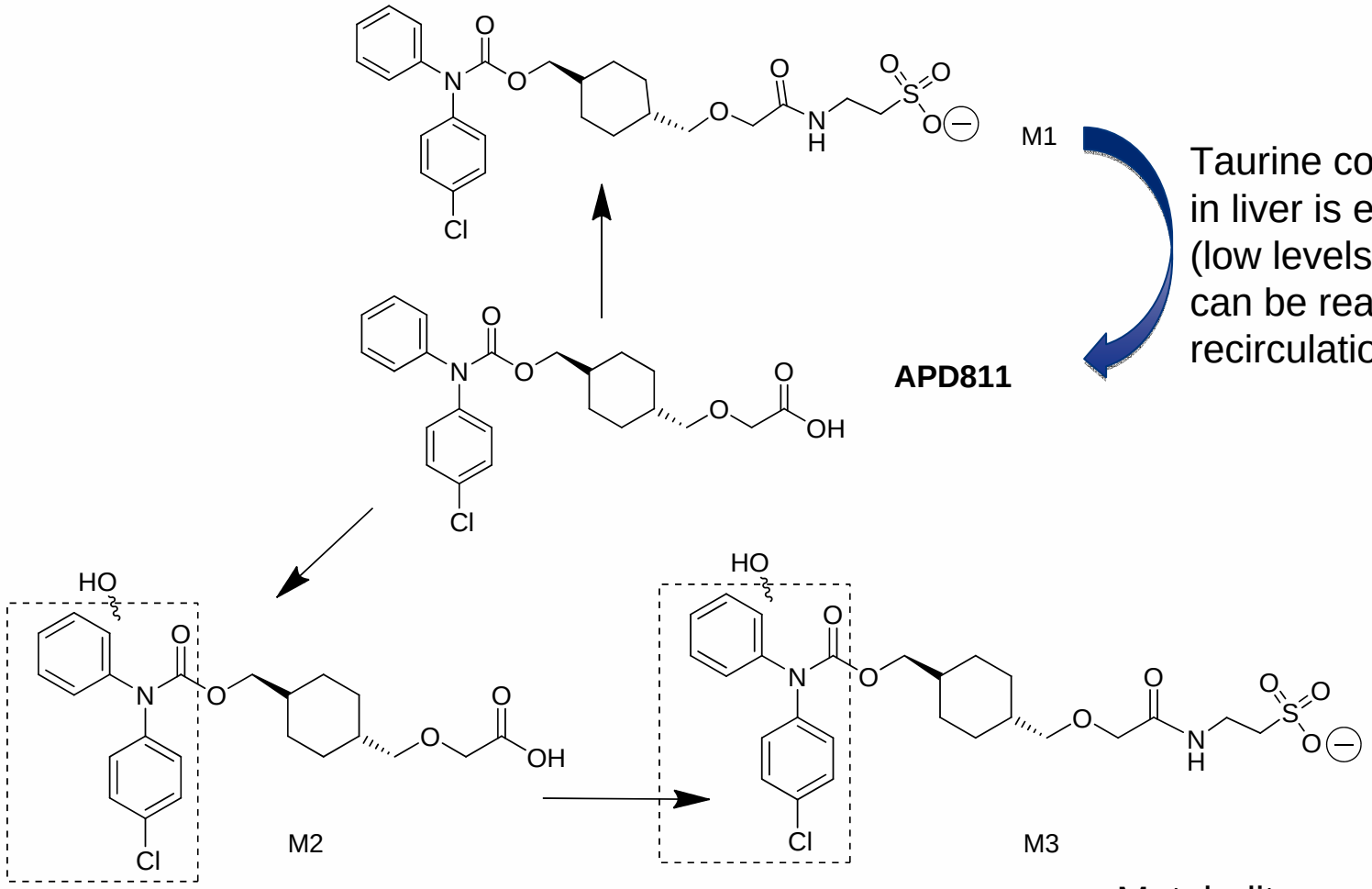




Parameter	Bile Duct Cannulation	Historical Control
Dose (mg/kg) IV	2.00	2.00
$T_{1/2}$ (hr)	**2.21**	**6.72**
AUC(0-INF) (hr*µg/mL)	4.53	5.09
Vss (L/kg)	1.31	2.74
$Cl_{systemic}$ (L/hr/kg)	**0.551**	0.427
Cl_{bile} (L/hr/kg)	**0.00518**	-
Cl_{renal} (L/hr/kg)	**0.0000815**	-
% of Dose in Bile	**0.889**	-
% of Dose in Urine	**0.0208**	-

- **APD811 undergoes enterohepatic recirculation**
- **APD811 biliary and renal elimination account for <1% of the total dose**
- **Metabolism is the primary elimination pathway for APD811**

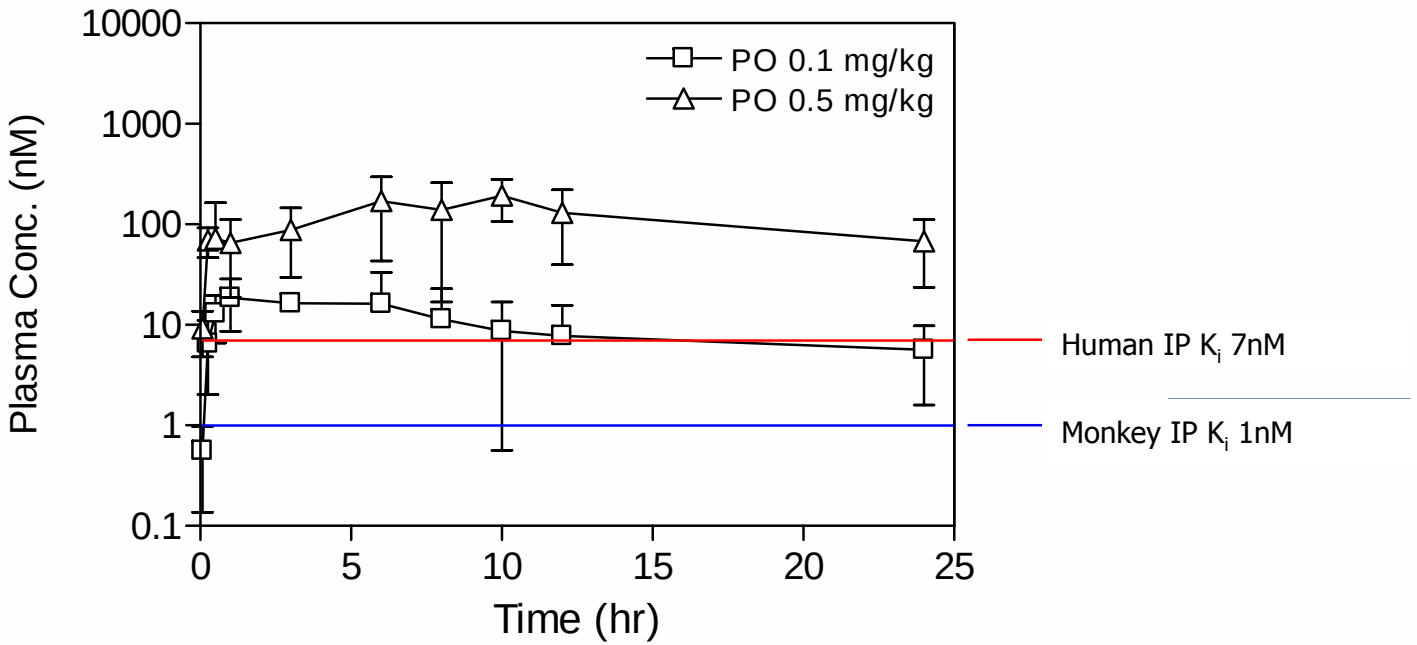
APD811 In Vivo Metabolic Pathways in the Rat



Taurine conjugate formed in liver is excreted in bile (low levels in plasma) and can be reabsorbed leading recirculation of parent

Metabolites were all significantly less active at IP receptor

APD811 Pharmacokinetics in Cynomolgus Monkeys



Dose (mg/kg)	Tmax (hr)	$T_{1/2}$ (hr)	C_{max} (nM)	C_{trough} (nM)	C_{max}/C_{trough}
0.1	2.67	17.5	28.0	5.67	**5**
0.5	4.17	33.5	215	67.8	**3**

- **Low peak to trough ratio**
- **Suitable for once-a-day dosing**

Prostacyclin Receptor Agonist Program at Arena: Overview

- Several series of novel, orally available, highly potent and selective IP receptor agonists identified
- Our lead compound APD811 had good bioavailability across species and was efficacious in a rat model of PAH
- DMPK, safety and pharmaceutical profiles suggest once daily dosing, with minimal peak-to-trough ratio
- Clinical Development is underway
  - Similar PK profile observed in human subjects in SAD

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Tong Zhang

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Anthony Blackburn

Process Chemistry
Sagar Shakya

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