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

Graeme Semple
Arena Pharmaceuticals
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).
• 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 Prostacyclin analogues for PAH

- **Epoprostenol** (Flolan)
  - Continuous i.v. or s.c. infusion (Epoprostenol, Treprostinil); Inhaled aerosol 6-12x/day (Iloprost)
- **Treprostinil** (Remodulin)
- **Iloprost** (Ventavis)
  - Treatment 6-9 x/day
  - Intermittent coverage
- **Beraprost** (Dorner)
  - 4x/day
  - Intermittent coverage

- **Prostacyclin analogues have sub-optimal delivery routes**
  - 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
$\text{t}_{1/2}$ 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 (PGI₂)

Beraprost

Nonprostanoids

NS304

FK788 (Fujisawa)

ONO-1301
Compound Design

Nonprostanoids

NS304  FK788 (Fujisawa)  ONO-1301

Biphenyl Group  Scaffold  Acidic Moiety

O—CO₂H
Initial Design for IP receptor Agonists

- Biphenyl Group
- Scaffold
- Acidic Moiety

- pyrimidine
- carbamate
- urea
- pyridazinone
- pyridazinone
Initial Design for IP receptor Agonists

Biphenyl Group  Scaffold  Acidic Moiety

pyrimidine  carbamate  urea  pyridazinone  pyridazinone
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

1. PhCH₂NHNH₂ → O
2. POCl₃ → Cl
3. R₂·PhB(OH)₂ → NH
4. AlCl₃ → i) tBuOK, A
   ii) HCl/dioxan
5. Pd(PPh₃)₄ → R₁-PhB(OH)₂

1. NH₂NH₂ → O
2. R₁·PhB(OH)₂ → NH
3. Pd(PPh₃)₄ → R₁-PhB(OH)₂
4. HCl/dioxan → CO₂H
### 3,4-Diphenylpyridazinones SAR

<table>
<thead>
<tr>
<th></th>
<th>R₁</th>
<th>R₂</th>
<th>EC₅₀ hIP* (nM)</th>
<th>IA (%)</th>
<th>EC₅₀ rIP* (nM)</th>
<th>IA (%)</th>
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</table>

* = EC₅₀ in the HTRF cAMP human or rat IP receptor assay
¶ = Intrinsic activity (efficacy) relative to 1 μM iloprost as the positive control
# = EC₅₀ 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

<table>
<thead>
<tr>
<th>Enantiomer</th>
<th>hIP, cAMP</th>
<th>rIP, cAMP</th>
<th>h Platelet</th>
<th>r Platelet</th>
<th>DP1 Mel</th>
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<td>33nM (n=3)</td>
<td>170nM (n=2)</td>
<td>35nM (n=2)</td>
<td>4000 nM (n=1)</td>
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<tr>
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<th>rIP, cAMP</th>
<th>h Platelet</th>
<th>r Platelet</th>
<th>DP1 Mel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isomer 1</td>
<td>21nM (n=3)</td>
<td>88nM (n=3)</td>
<td>22nM (n=2)</td>
<td>1200 nM (n=1)</td>
<td>23 nM (n=3)</td>
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<tr>
<td>Isomer 2</td>
<td>230nM (n=4)</td>
<td>6000nM (n=4)</td>
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<table>
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<tr>
<th>Enantiomer</th>
<th>hIP, cAMP</th>
<th>rIP, cAMP</th>
<th>h Platelet</th>
<th>r Platelet</th>
<th>DP1 Mel</th>
</tr>
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<td>68nM (n=3)</td>
<td>15nM (n=2)</td>
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<tr>
<td>Isomer 2</td>
<td>320nM (n=4)</td>
<td>1600nM (n=4)</td>
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Synthesis of Substituted 4,5-diphenyl-pyridazinones

\[
\begin{align*}
\text{Cl} & \quad \text{NH} & \quad \text{Cl} & \quad \text{NH} \\
\text{O} & \quad \text{O} & \quad \text{O} & \quad \text{O} \\
\end{align*}
\]

\[
\begin{align*}
\text{PhMgBr} & \quad \text{K}_2\text{CO}_3, \text{A} \\
\text{NaOMe, MeOH} & \quad \text{R}_2\text{PhB(OH)}_2, \text{Pd(Ph}_3\text{P)}_4 \\
\text{PhB(OH)}_2, \text{Pd(Ph}_3\text{P)}_4 & \quad \text{HCl/dioxan} \\
\text{POCl}_3 & \quad \text{K}_2\text{CO}_3, \text{A} \\
\end{align*}
\]

2

\[
\begin{align*}
\text{R}_1 & \quad \text{R}_2 \\
\end{align*}
\]
## 4,5-Diphenylpyridazinones SAR

<table>
<thead>
<tr>
<th></th>
<th>R₁</th>
<th>R₂</th>
<th>EC₅₀ hIP* (nM)</th>
<th>IA (%)*</th>
<th>EC₅₀ rIP* (nM)</th>
<th>IA (%)*</th>
<th>EC₅₀ hDP1# (nM)</th>
<th>Human platelet IC₅₀ (nM)#</th>
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<td>H</td>
<td>H</td>
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<td>75</td>
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<td>2b</td>
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<td>3-OMe</td>
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<td>101</td>
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<td>106</td>
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<td>85</td>
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* = EC₅₀ in the HTRF cAMP human or rat IP receptor assay
† = Intrinsic activity (efficacy) relative to 1µM iloprost as the positive control
# = EC₅₀ in a melanophore assay
@ = Inhibition of ADP-induced human platelet aggregation
Compound **2g** Extended Profile

- **hiP, cAMP** = 38 nM (n = 18)
- **rIP, cAMP** = 110 nM (n = 17)
- **h Platelet** = 90 nM (n = 6)
- **DP1, cAMP** = 850 nM (n = 8)
- **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; PO $t_{1/2}$ = 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

Active in vivo s.c. but ‘optimized’ compounds from this series not active p.o.
Second Generation Design for IP receptor Agonists

- Bliphenyl Group
- Scaffold
- Acidic Moiety

Chemical structures:
- Pyrimidine
- Carbamate
- Urea
- Pyridazinone
- Pyridazinone
Second Generation Design for IP receptor Agonists

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Chemical structures:
- Pyrimidine
- Carbamate
- Urea
- Pyridazinone
- Pyridazinone
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

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

\[ \text{* = EC}_{50} \text{ in the HTRF cAMP human IP receptor assay} \]

**trans-cyclohexyl**

- R=H; hIP EC\textsubscript{50} = 9.6 nM\textsuperscript{*}
- R=4-Cl; hIP EC\textsubscript{50} = 8.5 nM
- R=4-OMe; hIP EC\textsubscript{50} = 3.3 nM

\[ \text{cis-cyclohexyl} \]

- R=H; hIP EC\textsubscript{50} = 68 nM
- R=4-Cl; hIP EC\textsubscript{50} = 46 nM
- R=4-OMe; hIP EC\textsubscript{50} > 1000nM
## Carbamate Series SAR

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<tr>
<th>R₁</th>
<th>R₂</th>
<th>EC₅₀ hIP* (nM)</th>
<th>IA (%) ¶</th>
<th>EC₅₀ rIP* (nM)</th>
<th>IA (%) ¶</th>
<th>EC₅₀ hDP1# (nM)</th>
<th>Human platelet IC₅₀ (nM)@</th>
<th>PK Properties (rat)</th>
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# = EC₅₀ in a HTRF cAMP assay @ = Inhibition of ADP-induced human platelet aggregation
Further Profiling of Potential Lead Compounds

**3b**

- hIP $K_i$ = 8 nM
- hIP, cAMP = 8 nM
- rIP, cAMP = 530 nM
- hPlatelet = 38 nM
- DP1 cAMP = 750 nM
- EP2 cAMP = NR
- EP4 cAMP = NR

hERG (astemizole binding) = NR
HepG2 cell (intracellular calcium, cell proliferation, membrane integrity) = NR
Water solubility = 1.0 mg/ml (pH 7)
Microsomal stability $t/12 > 60'$, $r > 60'$
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/kg L/kg.
Cmax (po): 3.703 µg/mL at 1.5 hr.
Oral bioavailability (%F) = 57.4%.

**3f**

- hIP $K_i$ = 14 nM
- hIP, cAMP = 8 nM
- rIP, cAMP = 280 nM
- hPlatelet = 18 nM
- DP1 cAMP = 3000 nM
- EP2 cAMP = NR
- EP4 cAMP = NR

hERG (astemizole binding) = NR
HepG2 cell (intracellular calcium, cell proliferation, membrane integrity) = NR
Water solubility = 2.6 mg/ml (pH 7)
Microsomal stability $t/12 > 60'$, $r > 60'$
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

**Right Ventricular Weight**

- **Comparison:** RV/LV+S

- **Graph:**
  - Y-axis: RV/LV+S
  - X-axis: Sham, MCT + Vehicle, MCT + 3b 10mg/kg, MCT + 3b 30mg/kg
  - Data points with error bars
  - Statistical significance:
    - * p<0.001 vs. MCT + Vehicle

**Mortality**

- **Graph:**
  - Y-axis: Survival Rate (%)
  - X-axis: Day
  - Data points:
    - Sham + Vehicle
    - MCT + Vehicle
    - MCT + AR392830 10mg/kg
    - MCT + AR392830 30mg/kg
  - Survival rates for different treatments
  - Statistical significance:
    - * P<0.001 vs. MCT
    - * P<0.05 vs. MVT
3b MCT data

Pulmonary artery pressure

![Graph showing % wall thickness and mPAP for different groups: Sham, MCT, and MCT + 3b.](image)

- **% wall thickness (%)**
  - Sham: (5)
  - MCT: (5)
  - MCT + 3b: (5)

- **mPAP (mmHg)**
  - Sham: (9)
  - MCT + Vehicle: (8)
  - MCT + 3b 30mg/kg: (5)

*Significance:* p<0.01
### Pharmacokinetics of 3b in Male Sprague-Dawley Rats

#### IV Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg)</td>
<td>2</td>
</tr>
<tr>
<td>T1/2 (hr)</td>
<td>6.7</td>
</tr>
<tr>
<td>Cmax (µg/mL)</td>
<td>6.540</td>
</tr>
<tr>
<td>AUC(0-INF)(hr*µg/mL)</td>
<td>5.093</td>
</tr>
<tr>
<td>Cl_obs (L/hr/kg)</td>
<td>0.427</td>
</tr>
<tr>
<td>MRTlast (hr)</td>
<td>4.2</td>
</tr>
<tr>
<td>Vss (L/kg)</td>
<td>2.742</td>
</tr>
</tbody>
</table>

#### PO Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg)</td>
<td>10</td>
</tr>
<tr>
<td>T1/2 (hr)</td>
<td>5.4</td>
</tr>
<tr>
<td>Tmax (hr)</td>
<td>1.5</td>
</tr>
<tr>
<td>Cmax (µg/mL)</td>
<td>3.703</td>
</tr>
<tr>
<td>AUC(0-INF)(hr*µg/mL)</td>
<td>14.629</td>
</tr>
<tr>
<td>MRTlast (hr)</td>
<td>3.8</td>
</tr>
<tr>
<td>%F</td>
<td>57.4</td>
</tr>
</tbody>
</table>
**Pharmacokinetics of 3f in Male Sprague-Dawley Rats**

### IV Parameters

- **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

- **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)
Scale-Up Synthesis Route

- Efficient 2 pot synthesis from available building blocks
- Avoids Rh catalysed diazoacetate chemistry
- Extra PPE required for handling API
• APD811 had an extended terminal phase across species

Enterohepatic recycling?
APD811 : Bile Duct Cannulated Rats

- Male SD rat bile duct cannulated
- IV administration at 2 mg/kg
- Collected plasma, bile and urine from 0 to 48 hr

**Graph:**
- Y-axis: Plasma Conc. (ng/mL)
- X-axis: Time (hr)
- Data points for IV and Historical Control

**Table:**

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

- APD811 undergoes enterohepatic recirculation
- APD811 biliary and renal elimination account for <1% of the total dose
- Metabolism is the primary elimination pathway for APD811
Taurine conjugate formed in liver is excreted in bile (low levels in plasma) and can be reabsorbed leading recirculation of parent compounds. Metabolites were all significantly less active at IP receptor.
APD811 Pharmacokinetics in Cynomolgus Monkeys

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Tmax (hr)</th>
<th>T1/2 (hr)</th>
<th>Cmax (nM)</th>
<th>Ctrough (nM)</th>
<th>Cmax/Ctrough</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>2.67</td>
<td>17.5</td>
<td>28.0</td>
<td>5.67</td>
<td>5</td>
</tr>
<tr>
<td>0.5</td>
<td>4.17</td>
<td>33.5</td>
<td>215</td>
<td>67.8</td>
<td>3</td>
</tr>
</tbody>
</table>

- Low peak to trough ratio
- Suitable for once-a-day dosing
• 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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Anna Shifrana
Anthony Blackburn

**Process Chemistry**
Sagar Shakya

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Mike Morgan
Woo Hyun Yoon

**Biology**
John Adams
Zhuangjie Li
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