Discovery of PF-3635659
An Inhaled Once-daily M3 Antagonist for Asthma & COPD

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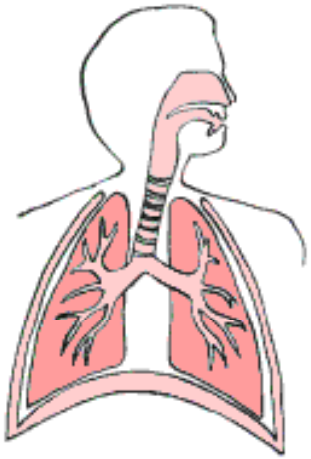
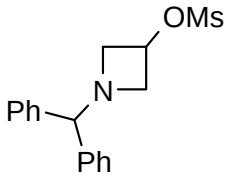


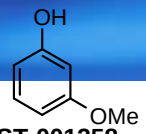
Overview

Synthesis
Asthma and COPD
Inhalation by design
Slow offset kinetics (PD)
PK
Safety
Solid form
Phase I
People

Synthesis



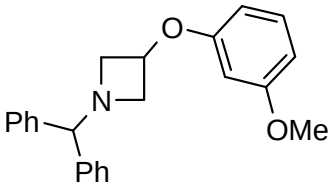
PF-347683
317.41



ST-001258
124.14

EtCN, K_2CO_3

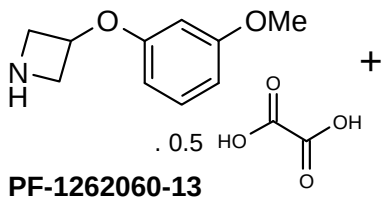
Step A



PF-1261660
346.45

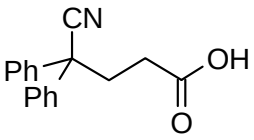
(i) EtOH, $Pd(OH)_2$, H_2
(ii) oxalic acid

Step B



PF-1262060-13
free base - 179.22
salt (0.5) - 224.24

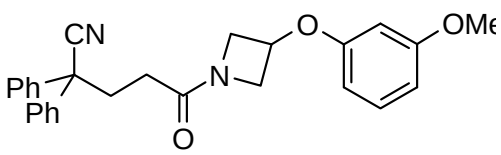
+



PF-1152632
265.32

Step C

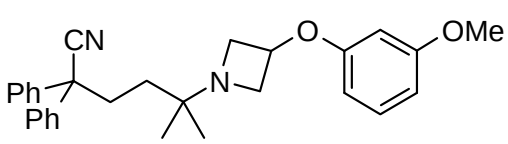
WSCDI, DMAP
EtCN



PF-4473518
426.51

$ZrCl_4$, THF
MeMgCl

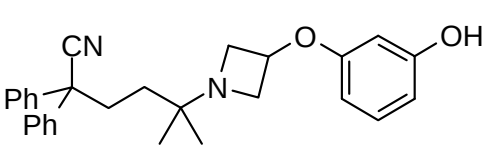
Step D



PF-3623564
440.58

Methionine
MsOH

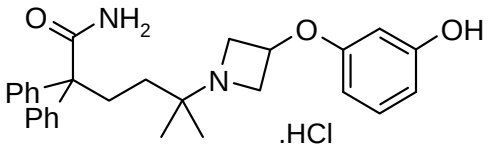
Step E



PF-3843465
426.56

Step F

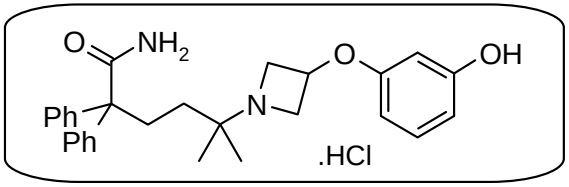
(i) KOH,
t-amyl-OH
(ii) c.HCl



PF-3635659-01
444.57 (free base)
481.07 (HCl salt)

MEK or IPA
reslurry

Step F/X

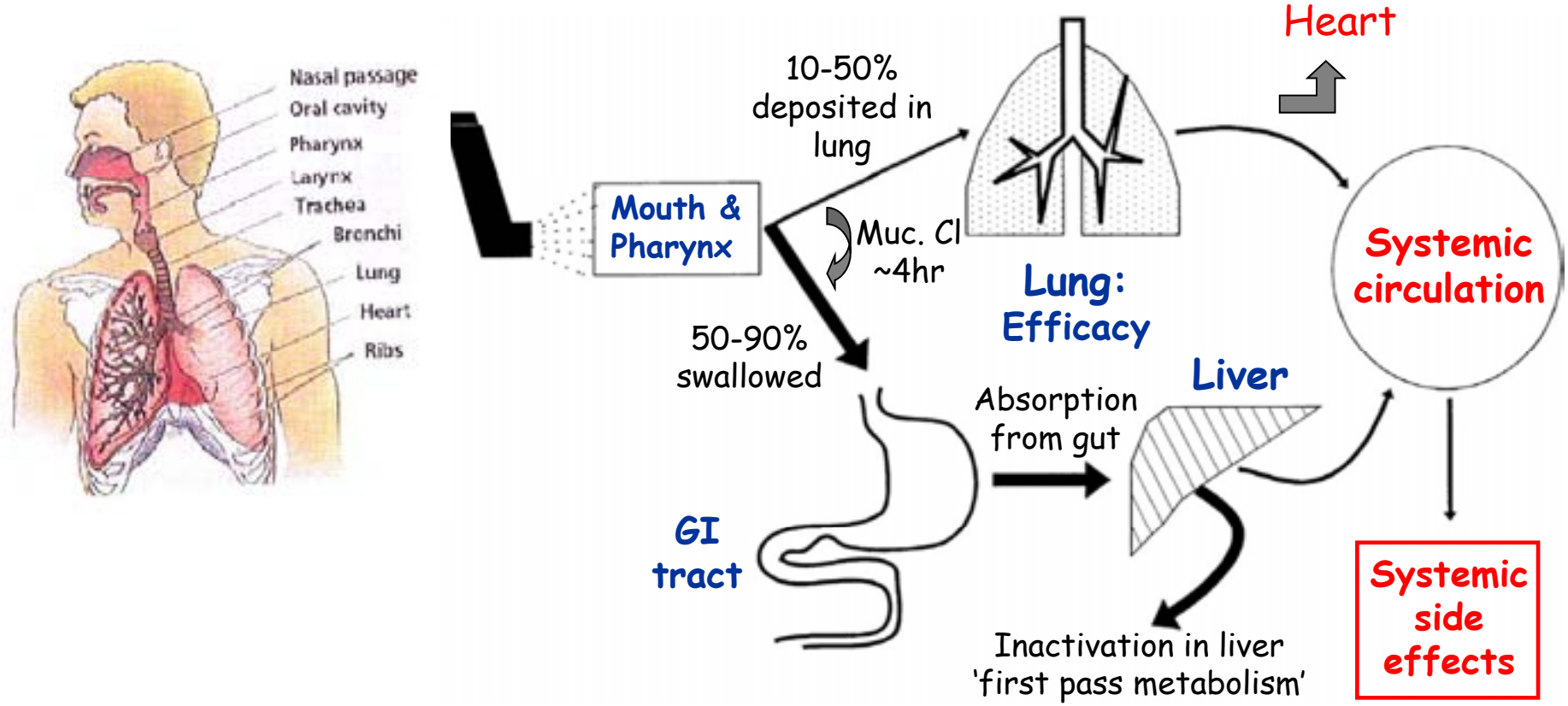


PF-3635659-01
444.57 (free base)
481.07 (HCl salt)

Asthma & COPD

- Asthma:
  - Chronic inflammatory disorder of the airways in response to allergen challenge, causing recurrent episodes of wheezing, breathlessness, chest tightness and coughing
  - Symptoms often result in decreased quality of life
- COPD:
  - COPD is the fourth leading cause of death in the US
  - Characterized by airflow limitation that is not fully reversible – cigarette smoke is the biggest cause
  - Symptoms are typically breathing-related (*e.g.* chronic cough, exertional dyspnoea & wheeze)
- Asthma & COPD affect millions of people worldwide with numbers continuing to increase (~300M for asthma alone)
- Global asthma/COPD market forecast: $30 billion by 2015

Drug Delivery *via* Inhalation



- Good inhaled agents require different design strategy to oral drugs
  - 'Inhalation by design' – importance of efficacy & safety
- Drug generally delivered *via* dry-powder inhaler (DPI) or metered-dose inhaler (MDI) - variety of devices used

Inhalation Devices

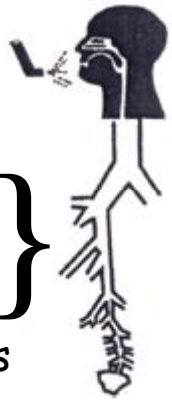
- Dry Powder Inhalers (DPI) highly preferred
- Stringent material properties required for DPI formulation
- As important as drug substance

'Inhalation by Design' - 5 Key Requirements

- Material properties
  - Suitable for DPI formulation & delivery
- High level of potency
  - Low doses required for DPI (<2mg)
- Lung solubility & absorption
  - Sufficient solubility so absorption > mucociliary clearance
- Lung pharmacodynamics
  - Design strategy to achieve long duration of action (*q.d.*)
- Low systemic exposure
  - Optimise ADME properties to maximise TI

Lung Deposition – Dry powder

Upper/central airways are main site of action for bronchodilators

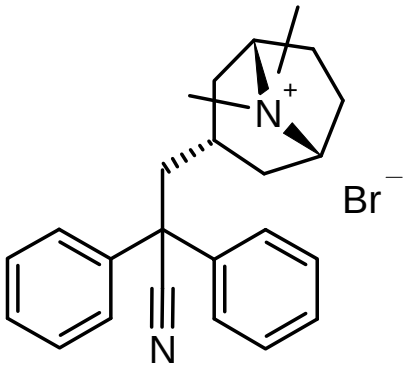


Particle Size (μM)	Regional Deposition	Bronchodilator Efficacy	Safety	
>5	Mouth/ oesophageal region	No clinical effect	Absorption from GI tract if swallowed	← swallow
1-5	Upper/ central airways	Clinical effect	Subsequent absorption from lung	← **target zone**
<1	Peripheral airways/ alveoli	Low clinical effect	High systemic absorption	← rapid absorption

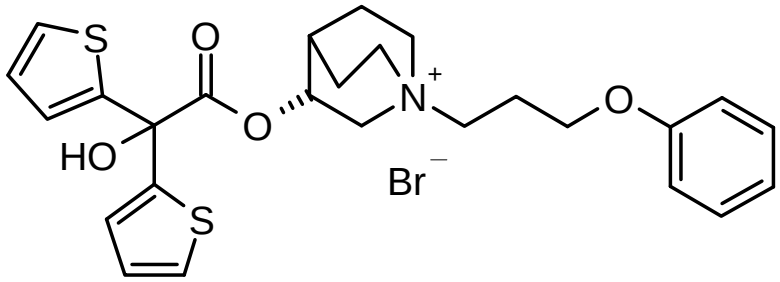
- Inhaled particle size important (2-3μM ideal)[10] for optimal efficacy, duration of action & therapeutic-index

[10] Mobley, C.; Hochhaus, G., *DDT*, **2001**, *6*, *7*, 367.

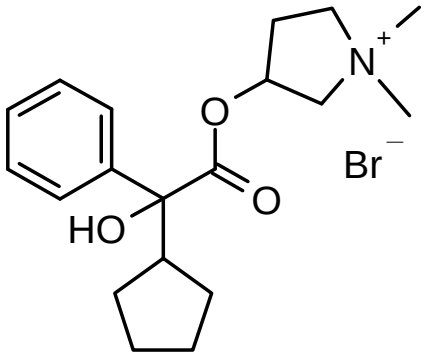
External environment



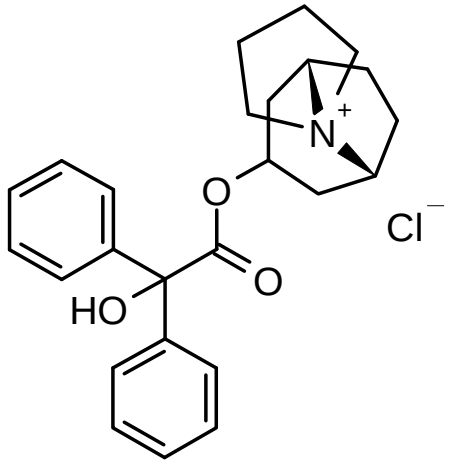
Darotropium bromide



Aclidinium bromide



Glycopyrronium bromide



Trospium chloride

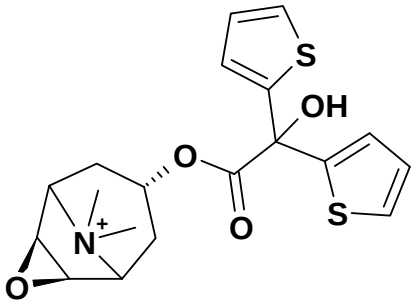
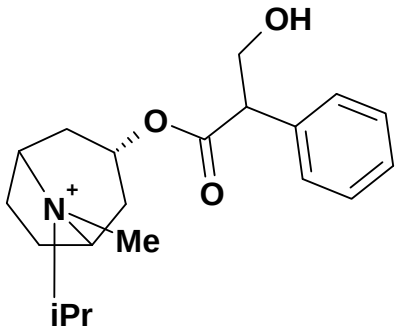


Table 1. Binding affinities and dissociation half-lives of tiotropium and ipratropium to select muscarinic receptor subtypes [5].

	Tiotropium		Ipratropium	
	K_D (nM)	$t_{½}$ (h)	K_D (nM)	$t_{½}$ (h)
M_1	0.041	14.6	0.183	0.110
M_2	0.021	3.6	0.195	0.035
M_3	0.014	34.7	0.204	0.260

K_D: Kinetically determined dissociation constant; $t_{½}$: Dissociation half-life.



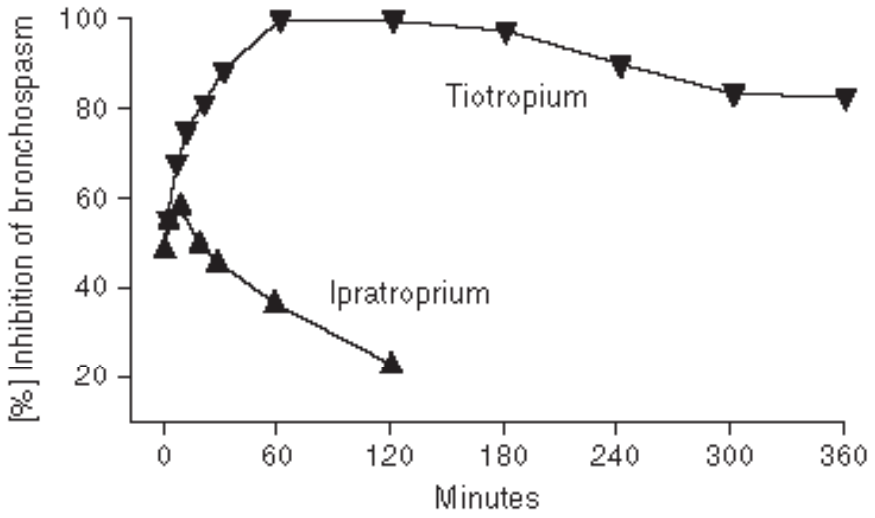


Figure 2. Time course of the protection against acetylcholine-induced bronchospasm by inhaled ipratropium bromide (▼, 1.0 mg/mL) or tiotropium (▲, 1.0 mg/mL) in dogs. N = 6 for

Balancing k_{on} & k_{off}

k_{on}						
10^8						
10^7		10nM	1 nM	0.1nM ◆#	10pM	1pM
10^6				1 nM	0.1nM	10pM
10^5				10 nM	1 nM	0.1nM
10^4					10nM	1 nM
	10	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
	(0.7s)	(7s)	(70s)	(11m)	(1.9h)	(19h)

k_{off}

* tiotropium

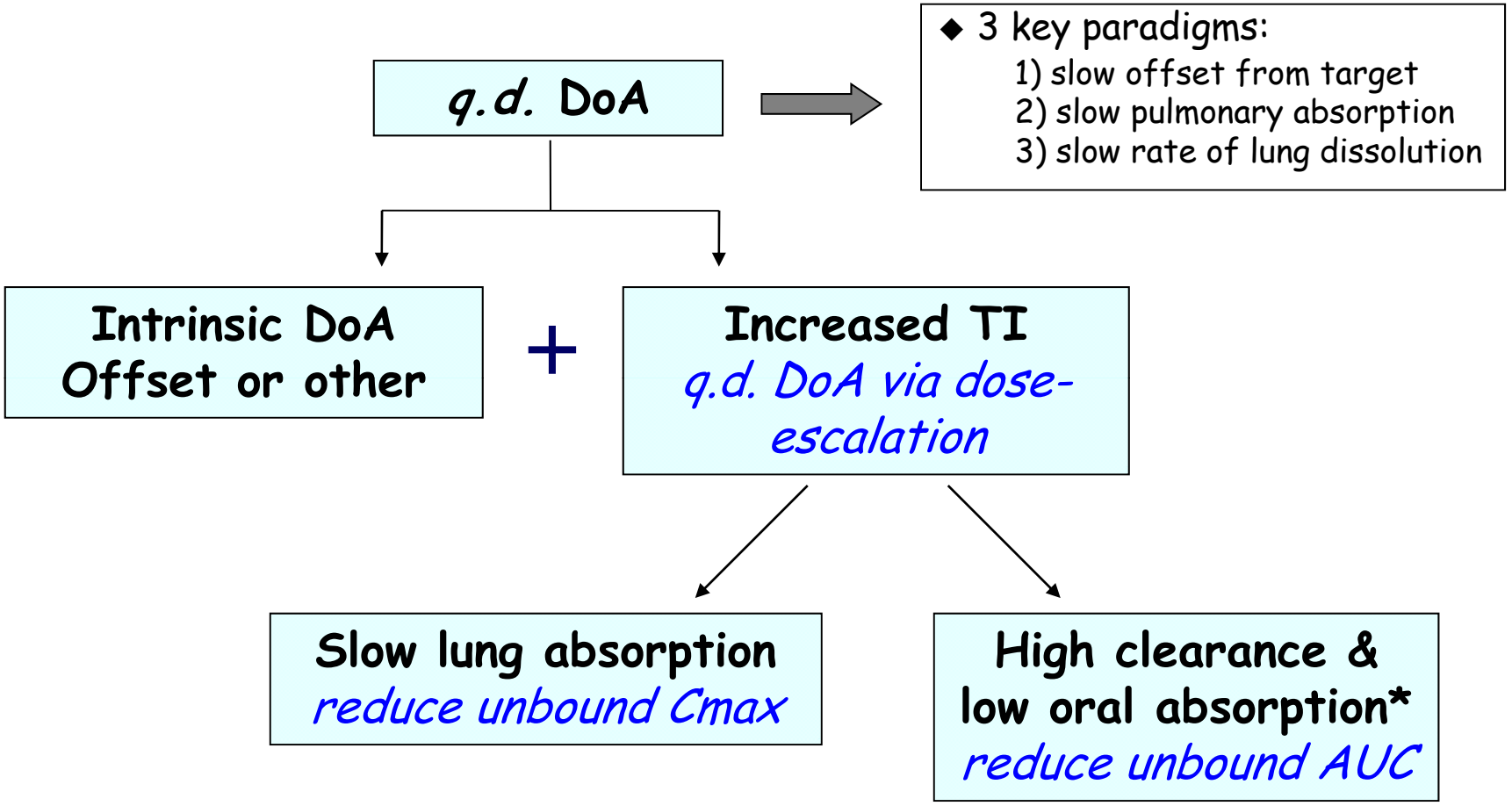
ipratropium

- $K_i = k_{off} / k_{on}$

Tiotropium

- High local conc. key to driving association in lung & loading up receptors
- Low systemic Cmax & rapid clearance to very low conc. fails to allow significant occupancy
- 20μg tiotropium gives 4μg dose to lung
- Dissolution in epithelial lung fluid (20mL) gives 2μM soln.
- 100,000 X higher than Ki (time to equilibrate < 3 mins)
  - local conc drives receptor occupancy
- Plasma Cmax 30pM (5 mins post dose) falling to trough of 4 pM
  - insufficient for occupancy in systemic compartment
  - projected 6% to 1% occupancy at steady state
- Offset profile helps drives once daily efficacy & lung focus to aid toleration

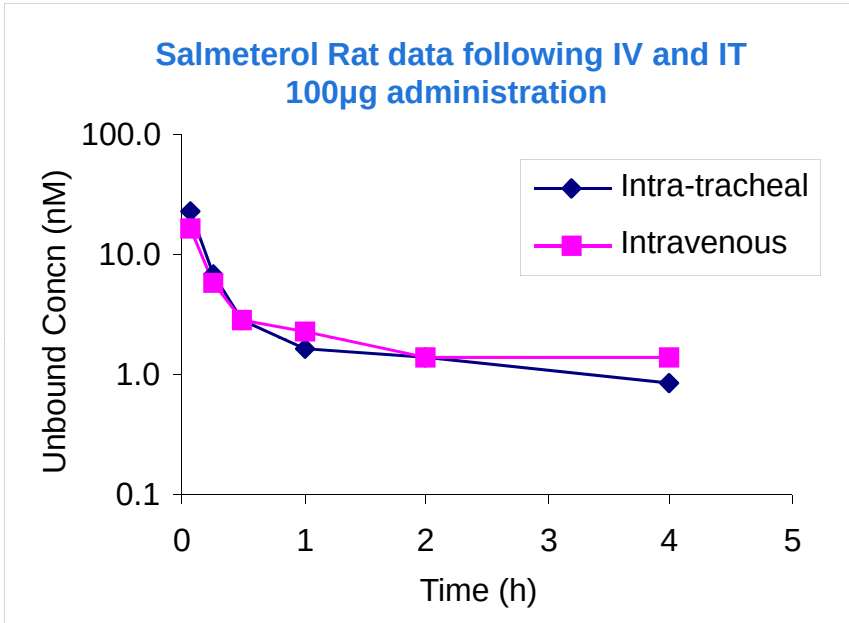
Strategy for *q.d.* DoA

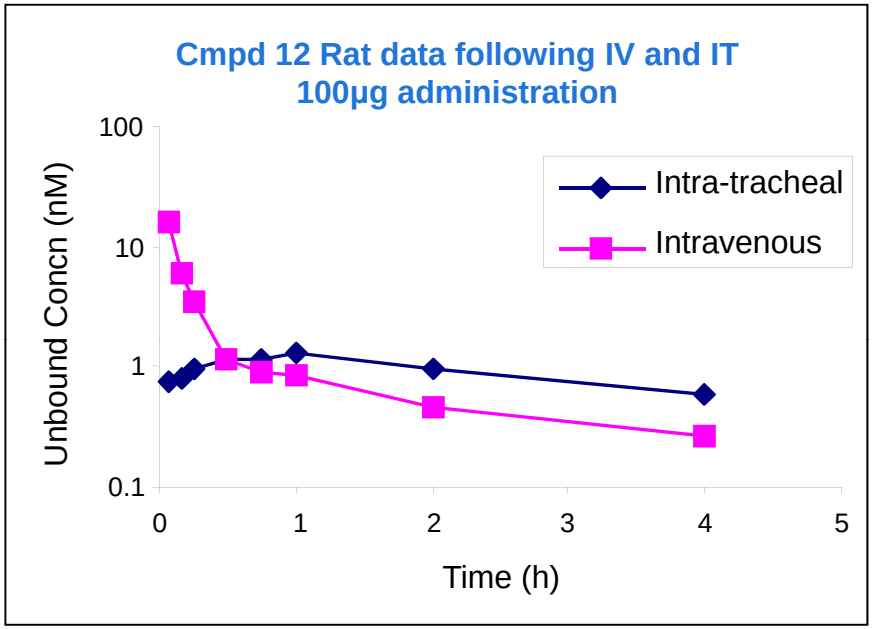


* Salmeterol: ~30% of systemically mediated AEs occur *via* the <u>swallowed</u> fraction of an 'inhaled' 400µg dose[1]

[1] Bennett, J. A. et al. *Eur. Respir. J.* **1999**, *13*, 445.

Delayed Lung Absorption: *i.t.* sol'n PK

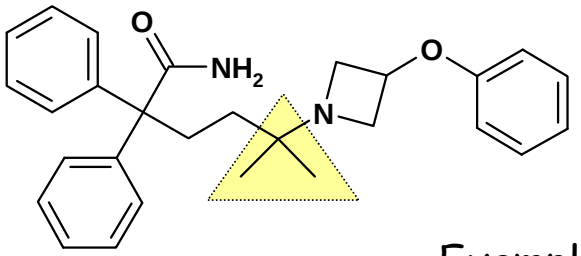




- PF-610355 exhibits improved *i.t.* pharmacokinetics *vs.* salmeterol
  - Delayed lung absorption (T_{max}= 0.5hr), ↓free Cmax (10-20x)

Potential for ↑DoA and/or ↑TI *in vivo*

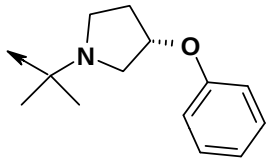
Gem-diMe drives slow offset/DoA



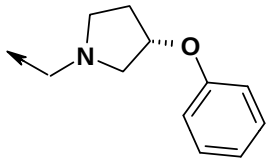
M_3 diss. $t\frac{1}{2}$ >23hr
GPT T_{25} DoA >16hr first prepped 1998

Examples:

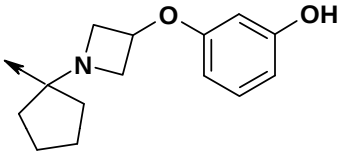
M_3 diss. $t\frac{1}{2}$ =



32.5hr



38mins



30.5hr

- Gem-dimethyl key for slow offset/DoA
  - Removal has dramatic impact on diss. $t\frac{1}{2}$ and DoA *in vitro*
  - Diss. $t\frac{1}{2}$ = 49.5hr $\Rightarrow$ K_{off} ~3.9×10^{-6} s^{-1} : large ΔE for dissociation
  - K_i = 0.128nM $\Rightarrow$ K_{on} ~3×10^{4} $M^{-1}s^{-1}$: <u>slow onset</u> due to significant re-ordering of antagonist/binding site - gem-dimethyl reduces conformational freedom and/or increases steric hindrance of protonated azetidine for key salt-bridge interaction
- Slow onset is observed experimentally and has screening implications……

Exhaustive file mining from legacy projects key to success

Slow onset compounds extended assay

Example: pK_B determination in the human bronchial ring *in vitro* for PF-3635659 and tiotropium using increasing pre-incubation times

Compound	1h pre-incubation pK_B	2h pre-incubation pK_B	4h pre-incubation pK_B
Tiotropium	10.1 (9.2,11.5) n=3	10.8 (8.8,12.2) n=4	10.2 (8.8,11.5) n=3
PF-3635659	**7.8*** **(7.2,8.4)** **n=3**	**8.5, 8.7** **n=2**	**9.6†** **(8.1,11.0)** **n=3**

- PF-3635659 potency increases with increasing pre-incubation times
  - Within 10x of tiotropium @ 4hrs, 100x @ 1hr standard pre-incubation
- *In vitro* screens were re-configured with extended pre-incubation times
  - Binding/offset assay 2⇒24hr, GPT & human/dog bronchus 1⇒4hr
  - Increased potency observed – compound data more accurate for decision making
- Slow onset also seen in the conscious dog: ~4hrs *vs.* ~1hr for tiotropium
  - Fast onset of action is not a pre-requisite for maintenance therapy of COPD

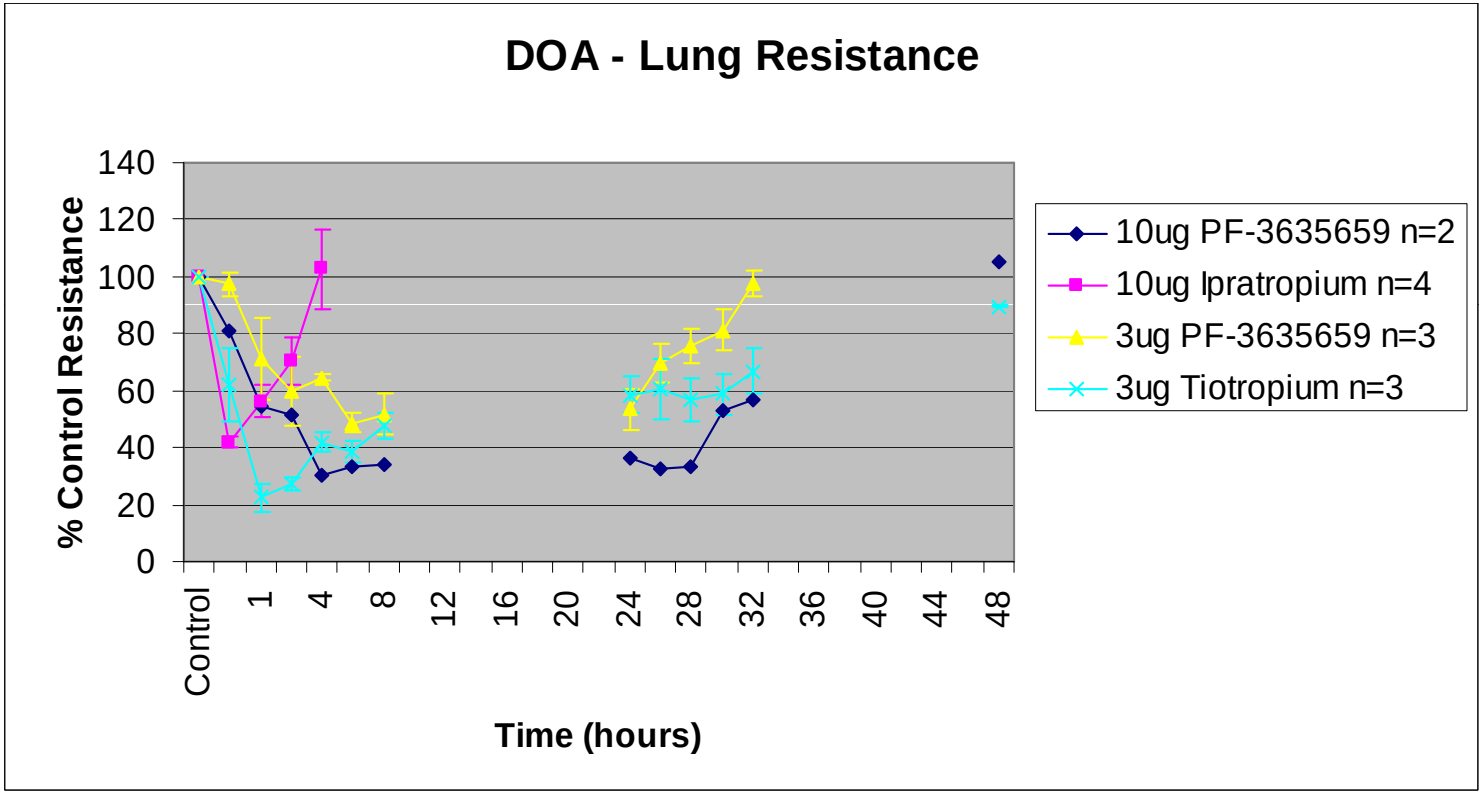
In vitro pharmacology: kinetics

- PF-3635659 shows comparable M_3 receptor offset to tiotropium
- Suggests potential for *q.d.* dosing

M2				M3		
n	95 % CI	50% Recovery (Mins)	Compound	50% Recovery (Mins)	95 % CI	n
24	<15	<15	Ipratropium	<15	<15	46
24	(634-1016)	>803	Tiotropium	>1410	(1360-1450)	41
8	<15	<15	Oxytropium Bromide	<15	<15	3
8	(223-310)	263	PF-3635659	>1440	>1440	8

PF-3635659-01: Conscious dog DoA

◆Duration of action of a single *i.t.* administration of 3µg or 10µg PF-3635659 on methacholine induced bronchoconstriction in the conscious dog



- ID_{50} dose (3µg) provides intrinsic 24hr DoA
- 10µg dose gives ↑efficacy/DoA – similar profile to 3µg dose of tiotropium
- Human Dose projection: 23-190µg *q.d.*

Oral bioavailability & absorption

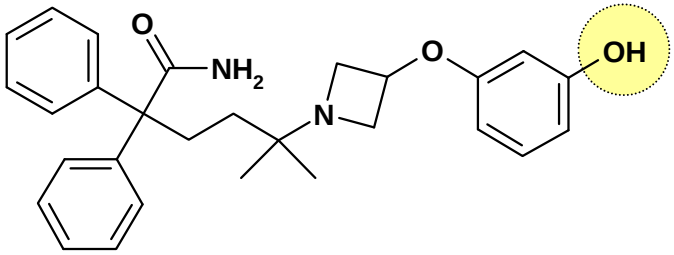
- Key opportunity to ↑TI of inhaled agents by minimising oral bioavailability (<5%)
  - Minimise gut wall permeability & ensure high first pass extraction

- Factors influencing good oral absorption are well understood
  - 'Rule of 5' – MW <500, HBD/A ≤5/10, clogP <5[2]
  - Rot. bonds ≤10, PSA ≤140Å^2 (HB count ≤12)[3]

- Key design criteria to minimise oral absorption:
  - Molecular properties consistent with non-'Ro5' compliance
  - Incorporate 2° amides for ↑PSA & the limiting role of their hydration on membrane permeation[3]
  - Screen for reduced permeability *in vitro* using Caco-2 cell monolayers

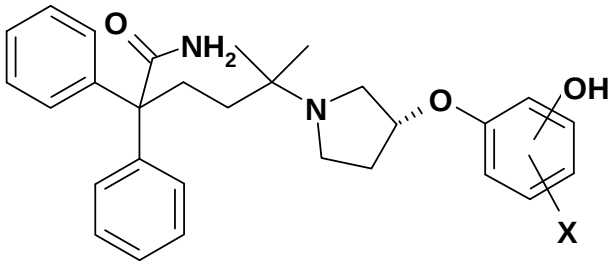
[2] Lipinski, C.A. et al., *Advanced Drug Delivery Reviews* **1997**, *23*, 3.
[3] Veber, D. F. et al., *J. Med. Chem.* **2002**, *45*, 2615.

Glucuronidation and low DDI risk



- HLM gluc'n = 221µl/min/mg with *meta*-phenol
  - ⇒ Human Cl_{gluc} pred'n ~11ml/min/kg (50%LBF)
  - ⇒ Human Cl_{P450} pred'n ~13ml/min/kg
- Gluc'n provides ↑Cl & mitigates DDI
- Phenol also gives P-gp efflux - ↓ oral absp'n
  - ◆- MDCK: AB=7, BA=64

SAR from other examples:



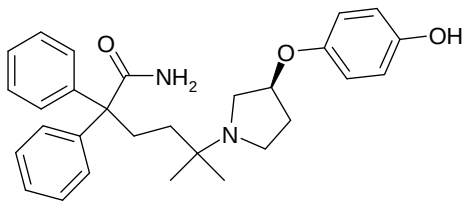
	X = H			*m*-OH		
	o-OH	*m*-OH	*p*-OH	X=2-F	X=4-Cl	X=5-CN
Gluc Cl_{int}	13	52	<4	92	146	202

(units = µl/min/mg)

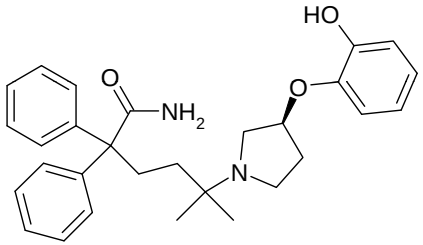
- SAR suggests gluc'n Cl_{int} influenced significantly by acidity of phenol
- Structural impact also important: Ph → Bn ↓Cl_{int}, pyrrolidine → azetidine ↑Cl_{int}
- SAR suggests that within a series gluc'n can be tuned up or down

Glucuronidation SAR: M_3 antagonists

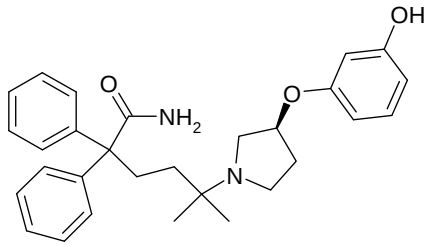
Pyrrolidines:



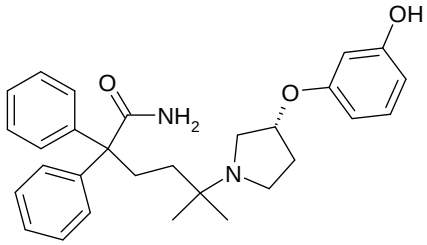
<4 µl/min/mg
est. logD 2.3



13 µl/min/mg
◆logD 2.3

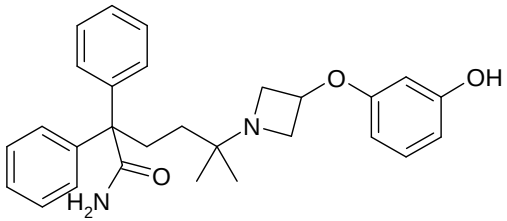


52 µl/min/mg
logD 2.4

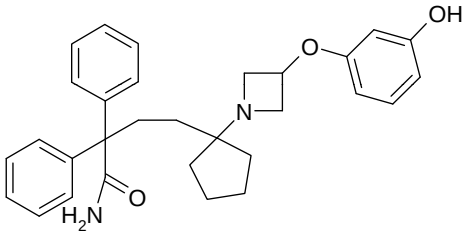


82µl/min/mg
logD 2.6

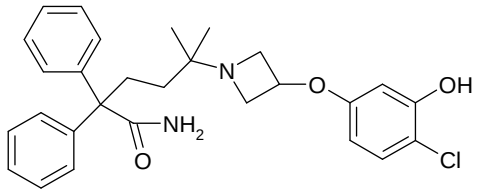
Azetidines:



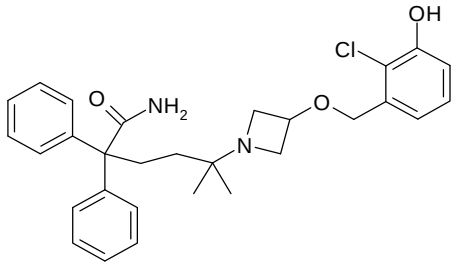
221 µl/min/mg
logD 2.9



142 µl/min/mg



430 µl/min/mg
est. logD 3.4



20 µl/min/mg
logD 3.0

Pharmacokinetics

Compound		*In vitro* PK		Rat *in vivo* PK				
				i.v.				*p.o.*
	Gluc. Cl_{int} (uL/min)/ mg	HLM Cl_{int} (uL/min)/ mg	Caco-2 P_{app} x10^{-6}cm/s		Vd L/kg	Cl_T ml/min/kg	t½ h	Rat ppb %	F %
			A-B	B-A					
	221	>282	7	64	8.4	134	0.9	94	<5

- *In vitro* PK:
  - Rapid CYP3A4 metabolism & **glucuronidation**, P-gp transporter mediated efflux
- *In vivo* PK:
  - High Cl_u, short $T_{\frac{1}{2}}$, low oral bioavailability (<5%)
- Major metabolites:
  - **Phenol glucuronide**, oxidative species
- Prediction to human:
  - Low oral bioavailability *cf* salmeterol (~30% AEs *via* swallowed fraction)[1]
  - High clearance ~ LBF (phaseI/II)

Ideal PK profile for an inhaled agent

Systemic exposure prediction safety

- Projected human dose: 23-190µg
- Projected human systemic exposure for PF-3635659
  - Oral rat PK: free C_{max} 3-23pM
  - Oral dog PK: free C_{max} 3-720pM
- 240-fold variability in predictions due to dose range & species diff

However, even with 'worst case' numbers excellent TI

Drug Safety Profile

- *In vitro* safety studies - no significant findings
  - CEREP panel >100nM
  - hERG patch clamp IC_{50} >1µM
  - Genetic Toxicity
- *In vivo* toxicology studies - no significant findings
- Rabbit lung cough model – measures afferent traffic from rapidly adapting sensory nerves (Aδ)
  - Enables early assessment of key attrition risk for inhaled agents

Pre-clinical safety profile suggests will be well tolerated in humans at all clinically relevant inhaled doses

Rabbit cough model details disclosed at ERS in 2009 – Louise Sladen

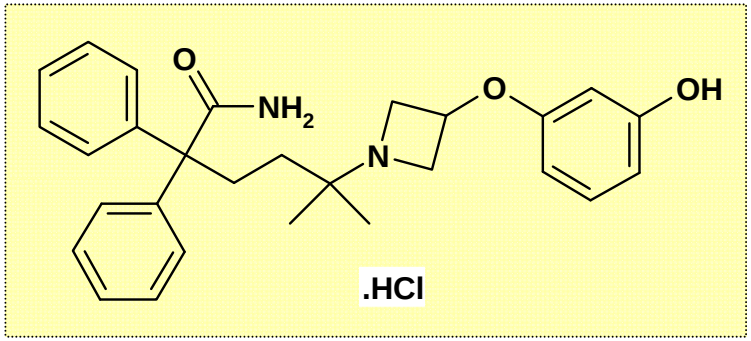
Solid Form Challenges & Strategy

- Significant solid form challenge with inhaled molecules
  - Often conformationally flexible & lipophilic
  - Limited options re: salt forms for inhalation
- Objective: identify series with higher intrinsic crystallinity to simplify solid form identification
- Key design criteria:
  - Retain key structural features responsible for activity (SAR)
  - Modify template with polar groups to increase potential H-bonding & salt bridge interactions
    - *e.g.* carboxamide present in lead

Fit-for-purpose DPI compatible form

- Crystalline HCl salt, mp=223°C, anhydrous, non-hygroscopic
- Good chemical & physical stability +/- lactose
- Solubility <5-905µg/ml @ pH 1-10
- Polymorph/hydrate screening completed with no issues

PF-3635659-01

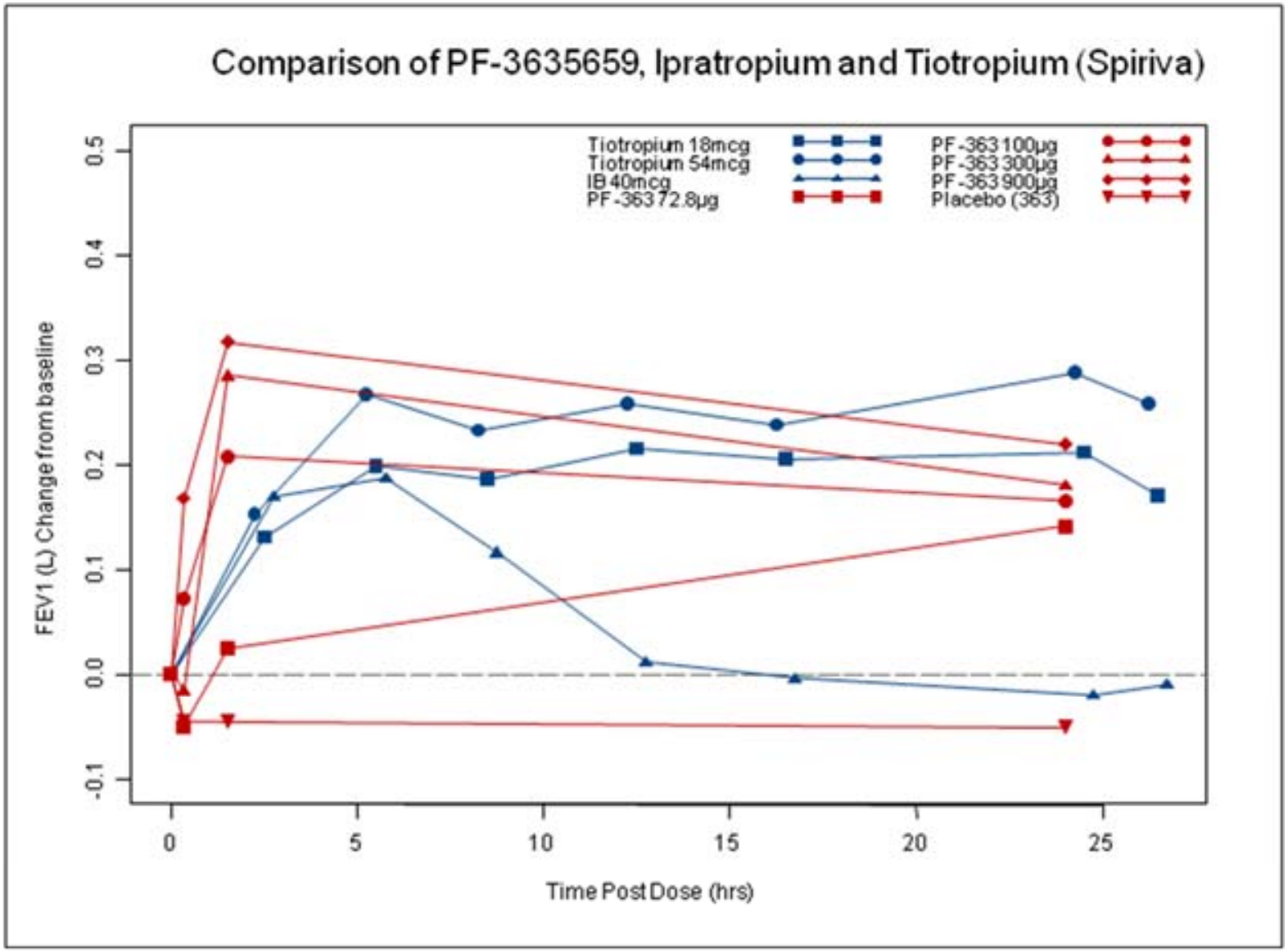


- M_3 Ki βLac = 0.201nM
- M_3 Ki bind = 0.128nM
- M_2 Ki bind = 0.138nM
- M_1 Ki bind = 0.088nM
- M_3 diss. $t_{\frac{1}{2}}$ = >23hrs
- GPT T_{25} >16hrs
- MW = 444.24
- logD = 2.9, clogP = 4.55
- HLM >337µl/min/mg
- HHeps = 96µl/min/million
- Gluc HLM = 221µl/min/mg
- MDCK: AB=7, BA=64

- Potent M_3 receptor antagonist with slow offset kinetics similar to tiotropium (tiotropium is the only marketed *q.d.* inhaled M_3 antagonist)
- Superior PK profile to tiotropium - high confidence in high clearance, multiple routes of metabolism (P450 & gluc'n) and low DDI risk
  - Human PK pred'n (rat): Vd_{ss} = 5.4L/kg, Cl_T >LBF, $t_{\frac{1}{2}}$ = 5hr, PPB >95%, ↓↓F%
- Predicted *q.d.* profile with inhaled dose of 23-190µg – conscious dog
- Pharmaceutical properties consistent with DPI formulation

Profile justifies progression to man

PF-3635659: Phase I data



What I hope I have said…

Potency is more than just a number

Product concept includes device

Preclinical dog model was essential

If you have a compound file use it well

Solid form is rarely given the respect it deserves

Thank you for your questions

ARD

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Ed Hammersley

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Tim Davies
Steve Evans
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