Speeding up and improving the Identification of a potent β2 agonist as a growth promoter for cattle

Initially I plan to take you through a program carried out at Zoetis

Then go back and look to see the impact of the new tools we are introducing

"What is the potential of these to stream line our research process?"

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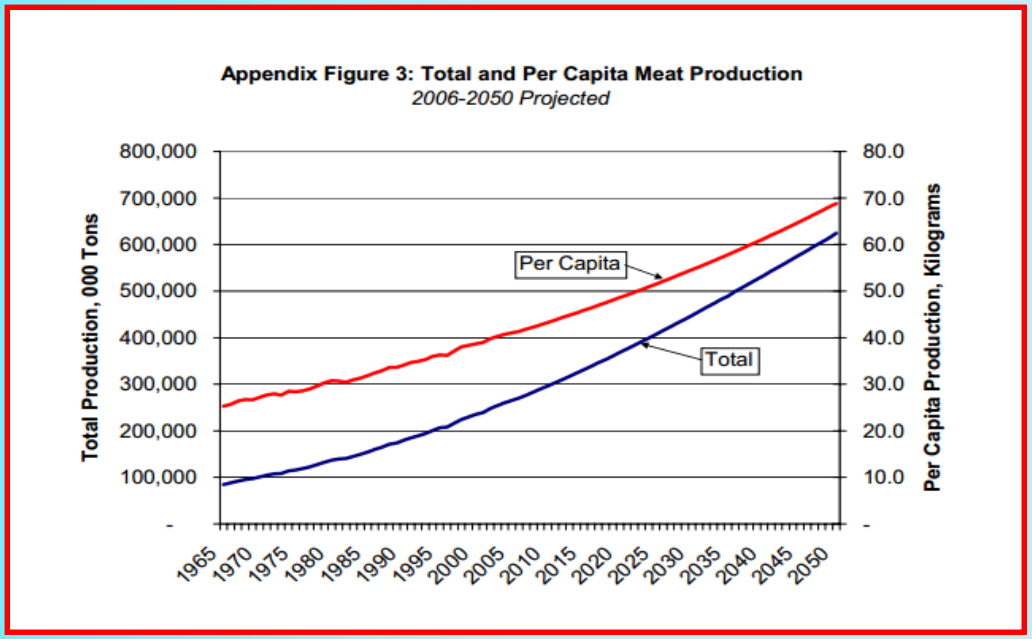
Food Security

The world population is predicted to reach 9 Billion
At the same time, GDP per capita is also increasing
Both of these factors are producing an increased demand for meat
We already produce 6 times as much meat as in the 1950's.
Set to increase by an additional 135% by 2050



Food Animal	Feed Conversion Ratio
Fish	1:1.2
Chickens	1:2
Pigs	1:3
Cattle	1:10

This will put pressure on the production of animal feed which will strain the environment
Increasing the efficiency with which animal convert feed into muscle will help alleviate this

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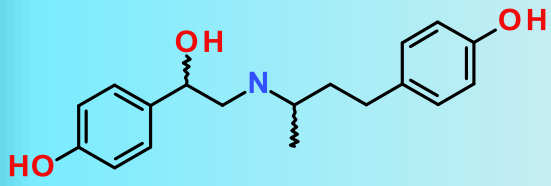
Current Market

Swine: **Paylean (Ractopamine)** On the market with a zero day withdrawal

Delivers ~10 % increase in feed conversion and growth rates



Cattle: **Optiflex (Ractopamine)** On the market with a zero day withdrawal

Efficacy below our product profile

Zilmax (Zilpaterol) On the market with a 2-3 day withdrawal

Delivers efficacy consistent with product profile

Why does Ractopamine get a zero withdrawal?

	Ractopamine	Zilpaterol
IV PK	mix 4 diasteromers	Mix 2 enantiomers
$T_{1/2}$ (hr)	3.0 (0.3)	4.8 (2.6)
V_{ss} (L/Kg)	5.8 (2.2)	2.6 (1.3)
Cl (ml/min/kg)	25.1 (9.1)	6.6 (2.1)
PO PK		
$T_{1/2}$ (hr)	BLOQ	23.1 (7.0)
T_{max} (hr)	BLOQ	11.3 (11.0)
C_{max} (ng/ml)	BLOQ	13.9 (1.4)
F_{oral} (%)	<15*	65.5 (14.6)

Even though the higher volume translates to higher tissue levels
The higher clearance means these are lower at the time of slaughter

We wish to identify a more potent, more efficacious compound with a zero day withdrawal

Ki ~1-20 nM; V_{diss} ~2-4 L; Cl ~15 ml/min/kg

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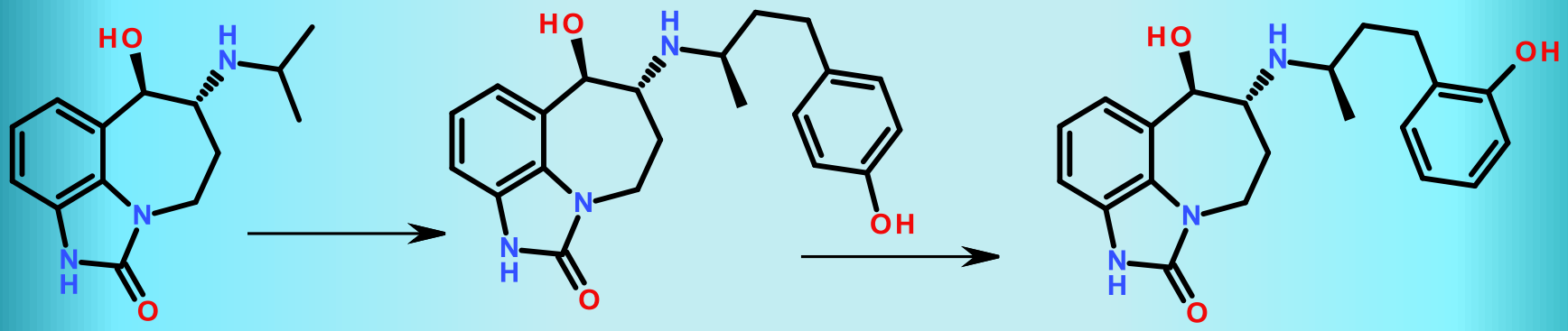
SAR Overview



Ki nM	-
EC_{50} nM	447
IV $T_{1/2}$ (hr)	4.8
V_{ss} (L/Kg)	2.6
Cl (ml/min/kg)	6.6
PO $T_{1/2}$ (hr)	23
C_{max} (ng/ml)	14
Foral (%)	66

Ki nM	16
EC_{50} nM	5.3
IV $T_{1/2}$ (hr)	2.6
V_{ss} (L/Kg)	8.6
Cl (ml/min/kg)	38
PO $T_{1/2}$ (hr)	-
C_{max} (ng/ml)	-
Foral (%)	<10

Ki nM	5.7
EC_{50} nM	2
IV $T_{1/2}$ (hr)	7.3
V_{ss} (L/Kg)	4.1
Cl (ml/min/kg)	13.8
PO $T_{1/2}$ (hr)	19
C_{max} (ng/ml)	6.8
Foral (%)	36

Simple! But it took ~400 analogues to reach this

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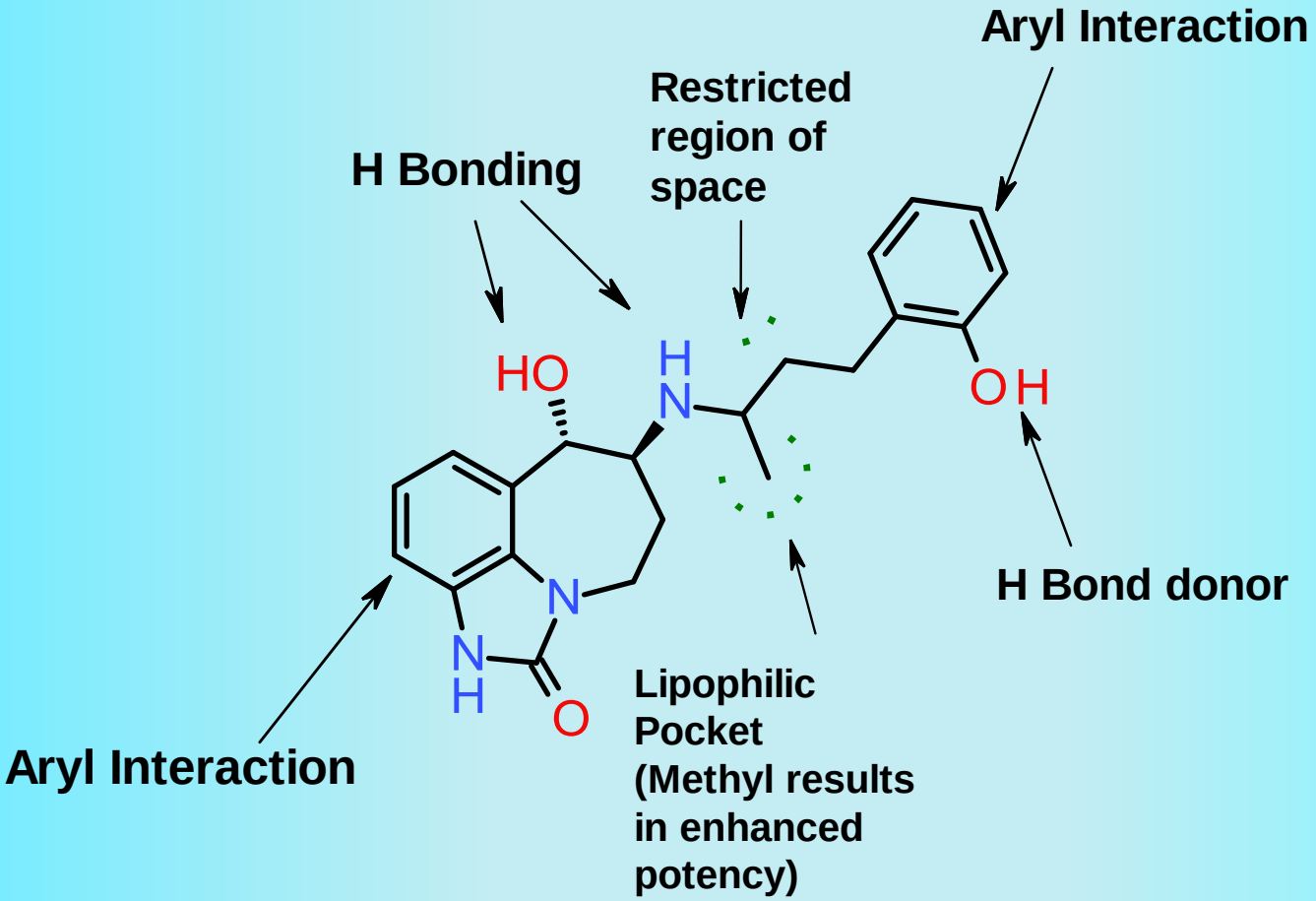
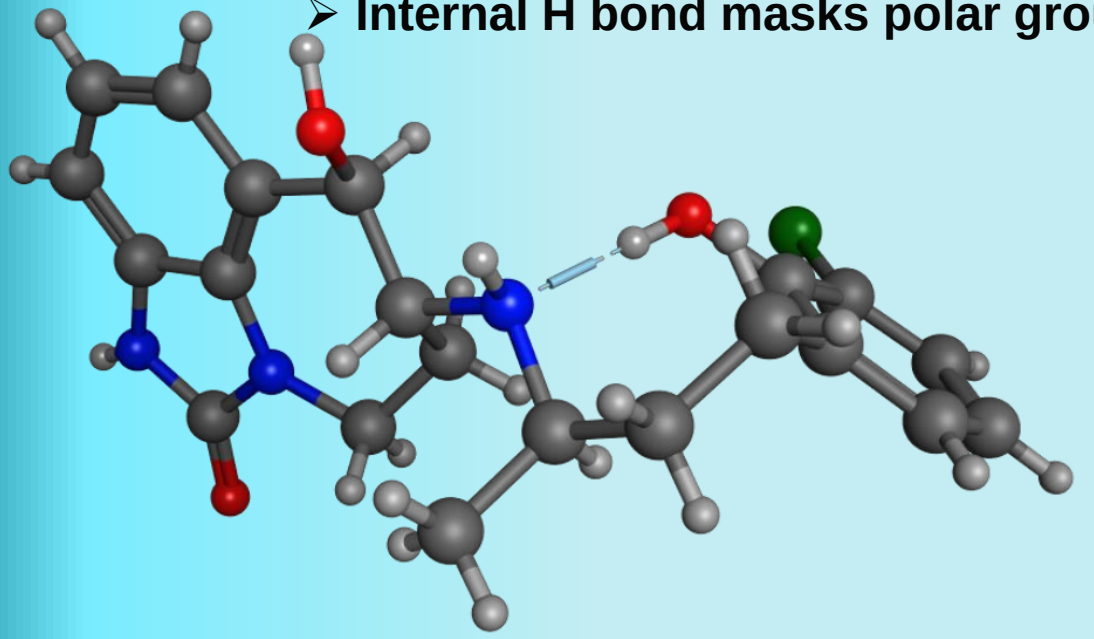
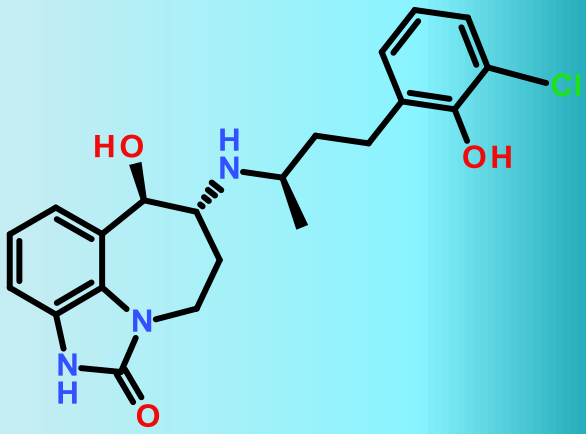
Beta agonists: SAR summary



Beta agonists: Cattle PK

- 23 Analogues evaluated for cattle PK
  - iv / po cross over design: 0.25 mg/kg iv, 0.5mg/kg po
  - 3 calves used: 2 iv 1 po then 1 iv 2 po
- No analogue has shown comparable oral availability to Zilpaterol
- Most have high clearance and low oral bioavailability
  - Generally below LLQ; 1 ng/ml
- Best orally available analogue ortho phenol; 36%
  - para phenol poor
  - Internal H bond masks polar group





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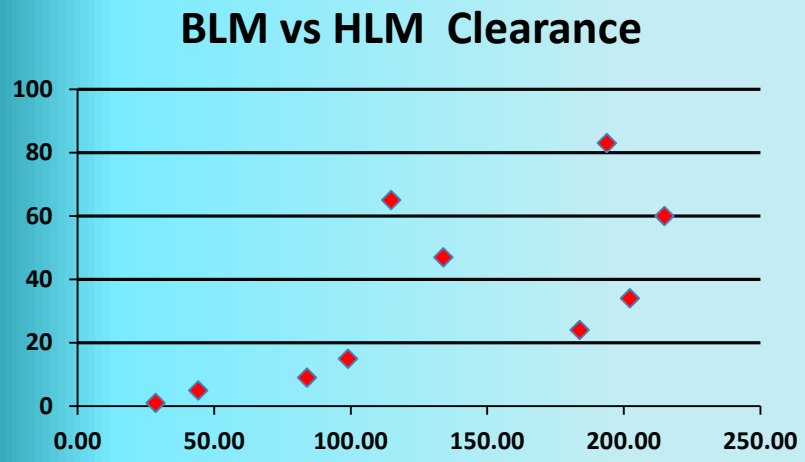
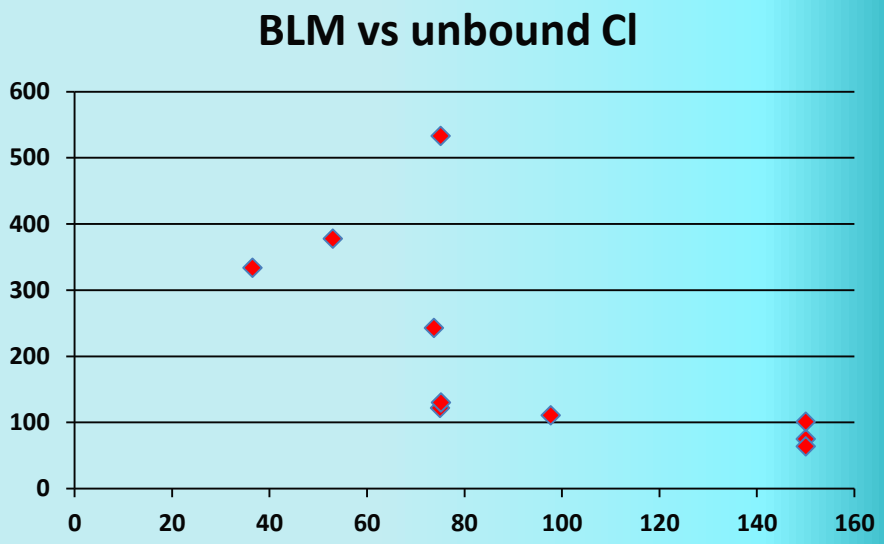
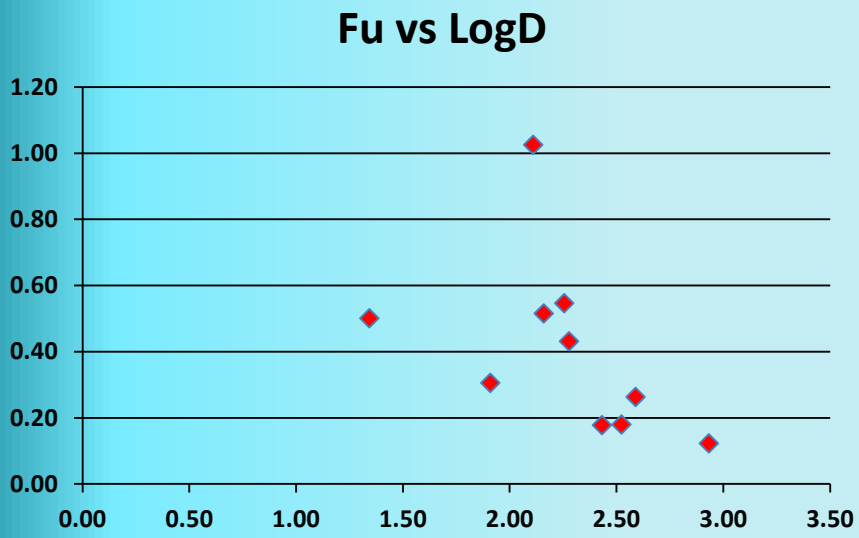
Can we predict the PK







For in vivo Clearance Yes!
this is driven by oxidative metabolism

The Volume, half life & oral availability do not correlate with properties

Oral availability does not correlate with Clearance; not a first pass effect!

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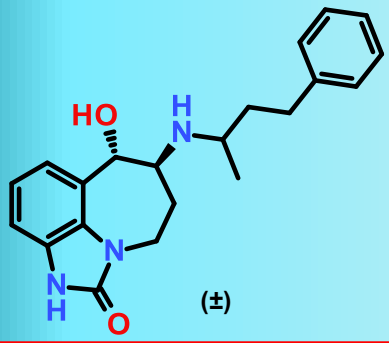
Lead Profiles



(±)

Positive Attributes:

High Cmax 33 ng/ml

Cf Zilpaterol 13 ng/ml

High clearance: good $t_{1/2}$ (10hr po)

Good potency (rbB2 Ki 14nM, EC50 6nM)

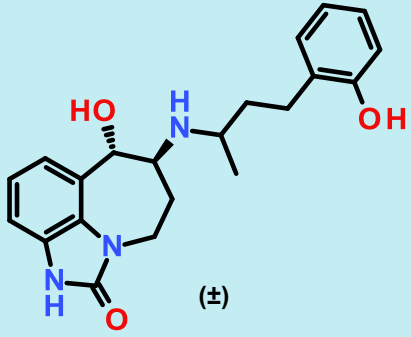
Cf Zilpaterol ~300nM

Issue:

Oral bioavailability low (15%)

High Vss (8.7L/kg)

cf Zilpaterol (2.6L/kg)



(±)

Positive attributes:

Oral bioavailability (36%)

Cmax 6 ng/ml

Cf Zilpaterol 13 ng/ml

Excellent potency (rbB2 Ki 5.7nM, EC50 2nM)

Cf Zilpaterol ~300nM

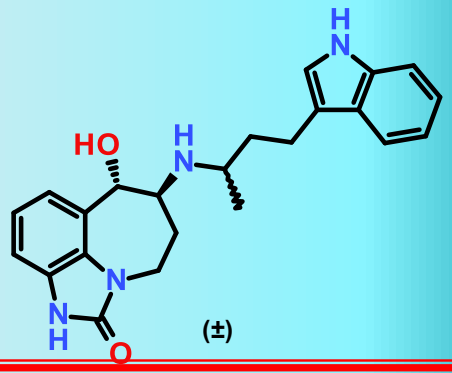
Issue:

Long oral t1/2 (19h)

Cf Zilpaterol oral $t_{1/2}$ 24h

Vss (4.1L/kg)

cf Zilpaterol (2.6L/kg)



(±)

Positive Attributes:

Efficacy (19% FCR @ 1ppm in cattle mixed isomers)

Acceptable $t_{1/2}$ (6hr po, 1h iv)

Good potency (rbB2 Ki 10nM, EC50 4nM)

Cf Zilpaterol ~300nM

Issue:

Oral bioavailability poor/variable

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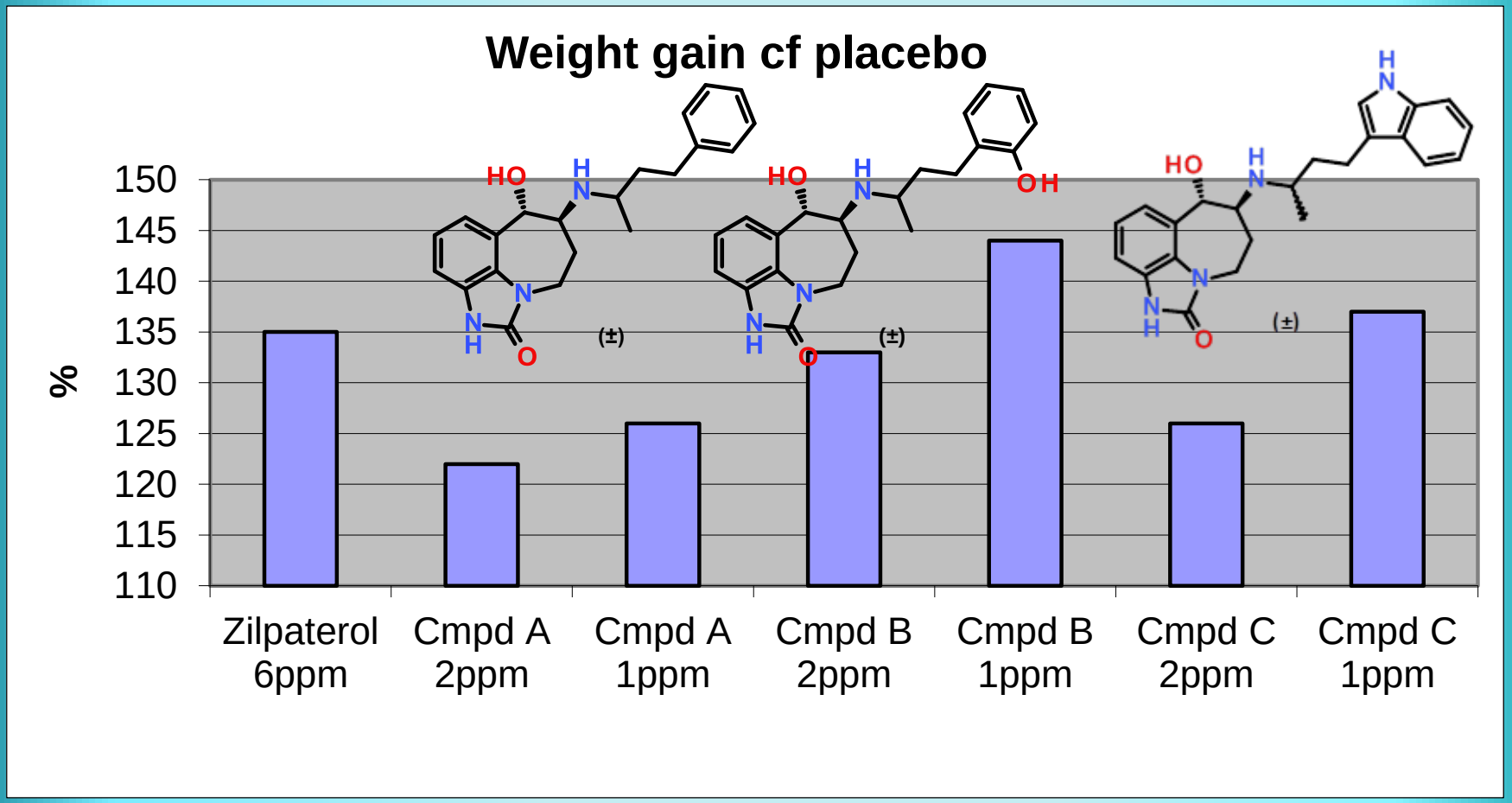
Cattle Efficacy



All treatment groups were significantly different to placebo for weight gain at the 5% level

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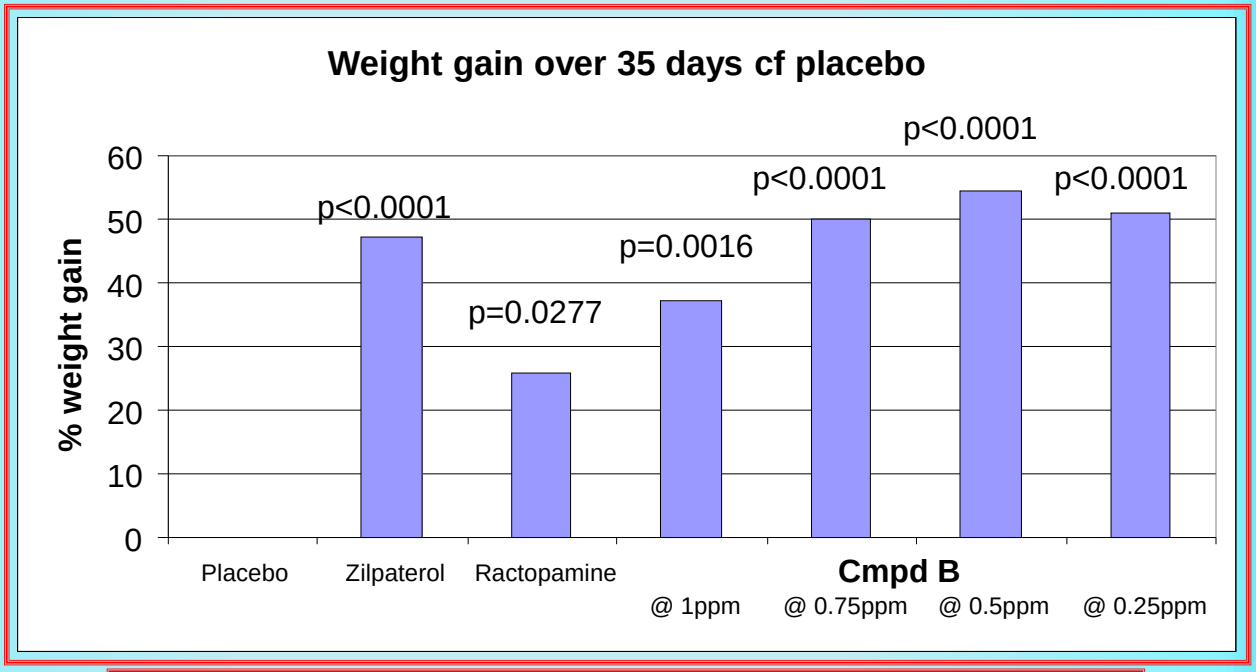
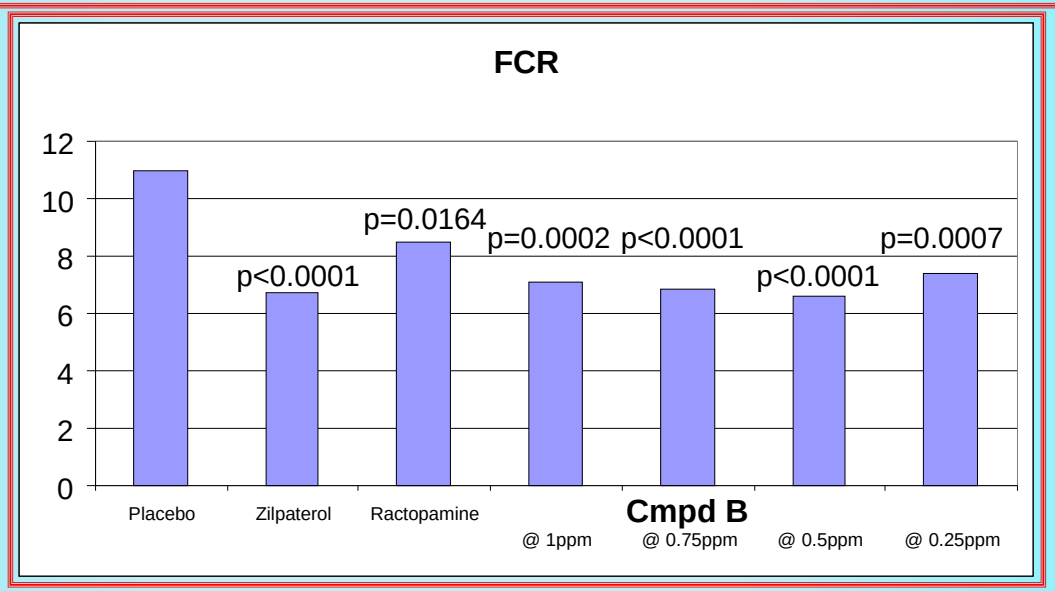
Cattle Efficacy: Lead compound



Distinct Bell Shaped Response seen

Optimal dose 0.25 ppm

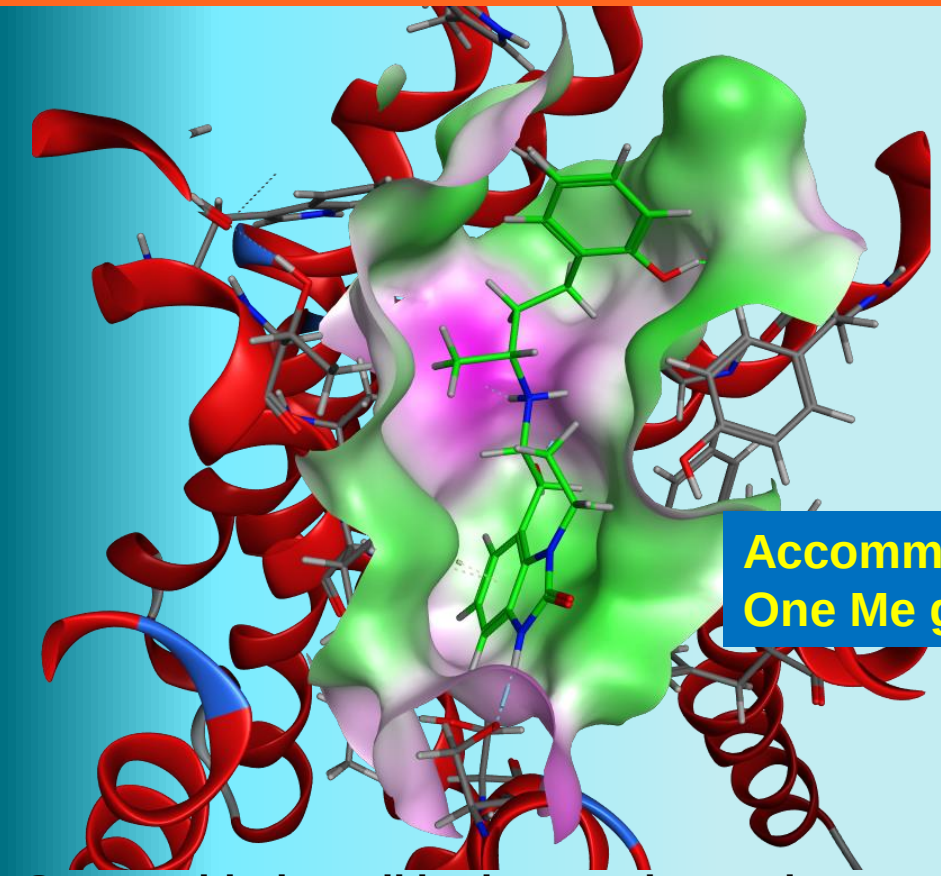


Moved into Development

**After completion of the optimization phase,
the X-ray structure of beta 2 was released**

We have looked back to see how our Ligand based approach compared to Structure Based Drug Design

Model of our antagonist bound into X-ray structure



Potential for Aryl interaction & H bond to Phenol

Accommodate Only One Me group

H bond to both OH & NH

Aryl interaction

In addition H bond

Can see binds well in the agonist mode of the receptor
Overlaying with the bound agonist and minimizing, allowing the residues within 4.5A to move

Explains many of the features we had identified in our Ligand Based SAR

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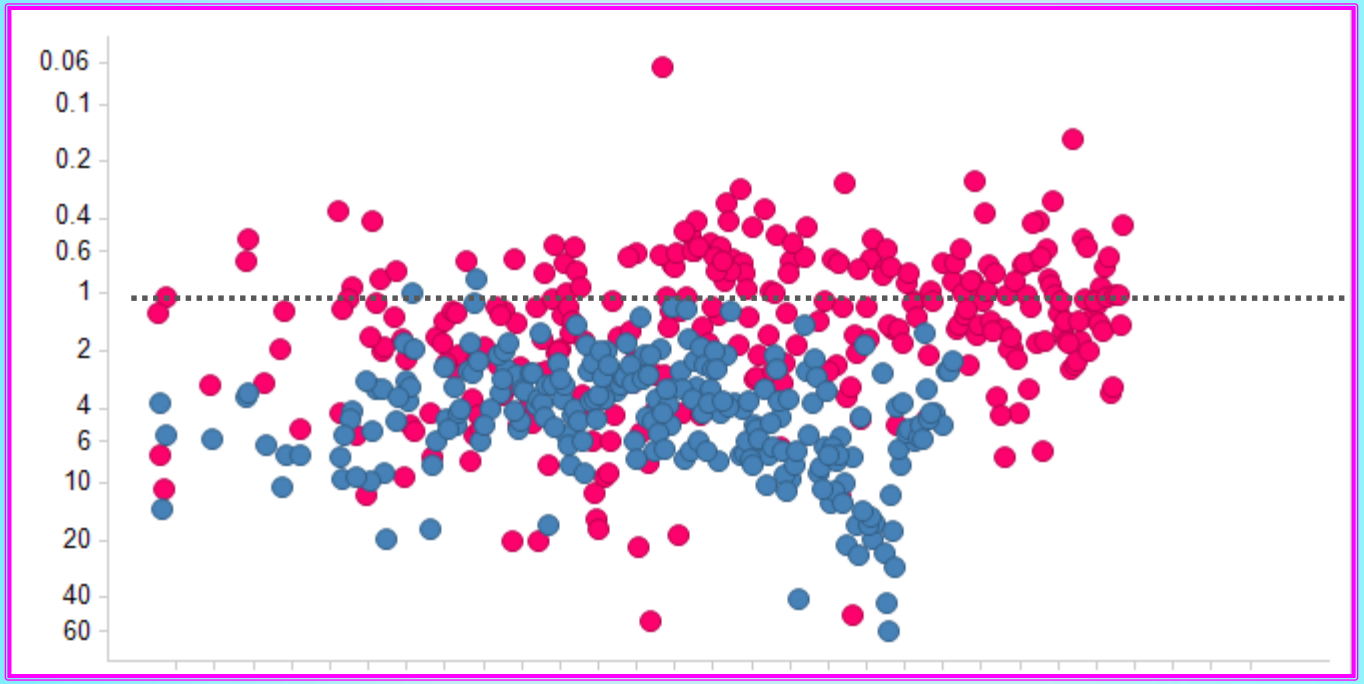
Selectivity between species



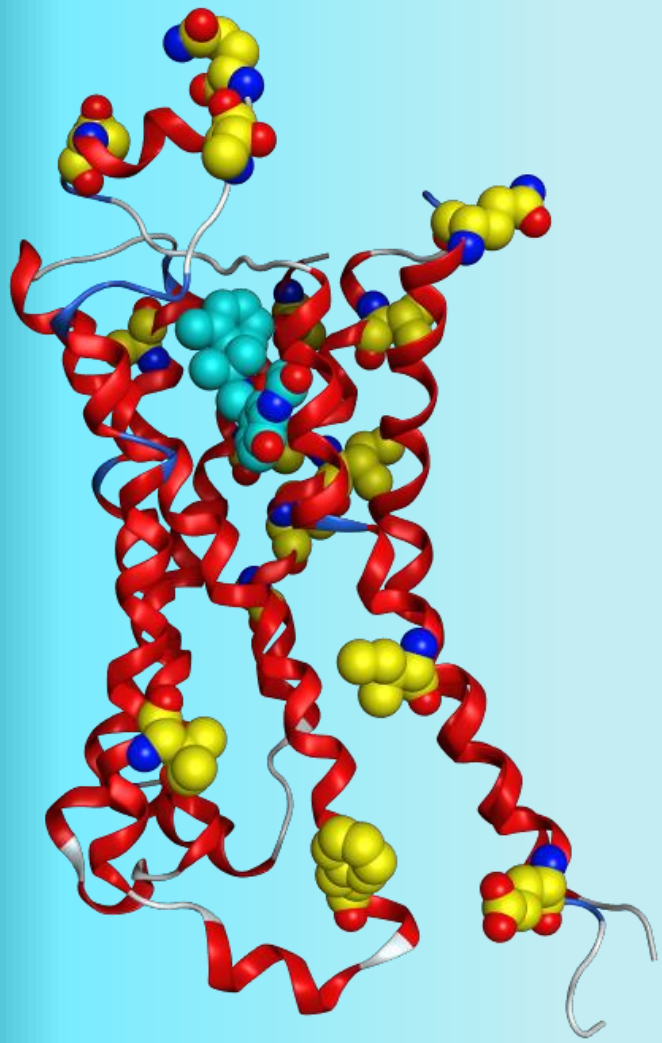
Can get selectivity Human over Bovine but not other way round

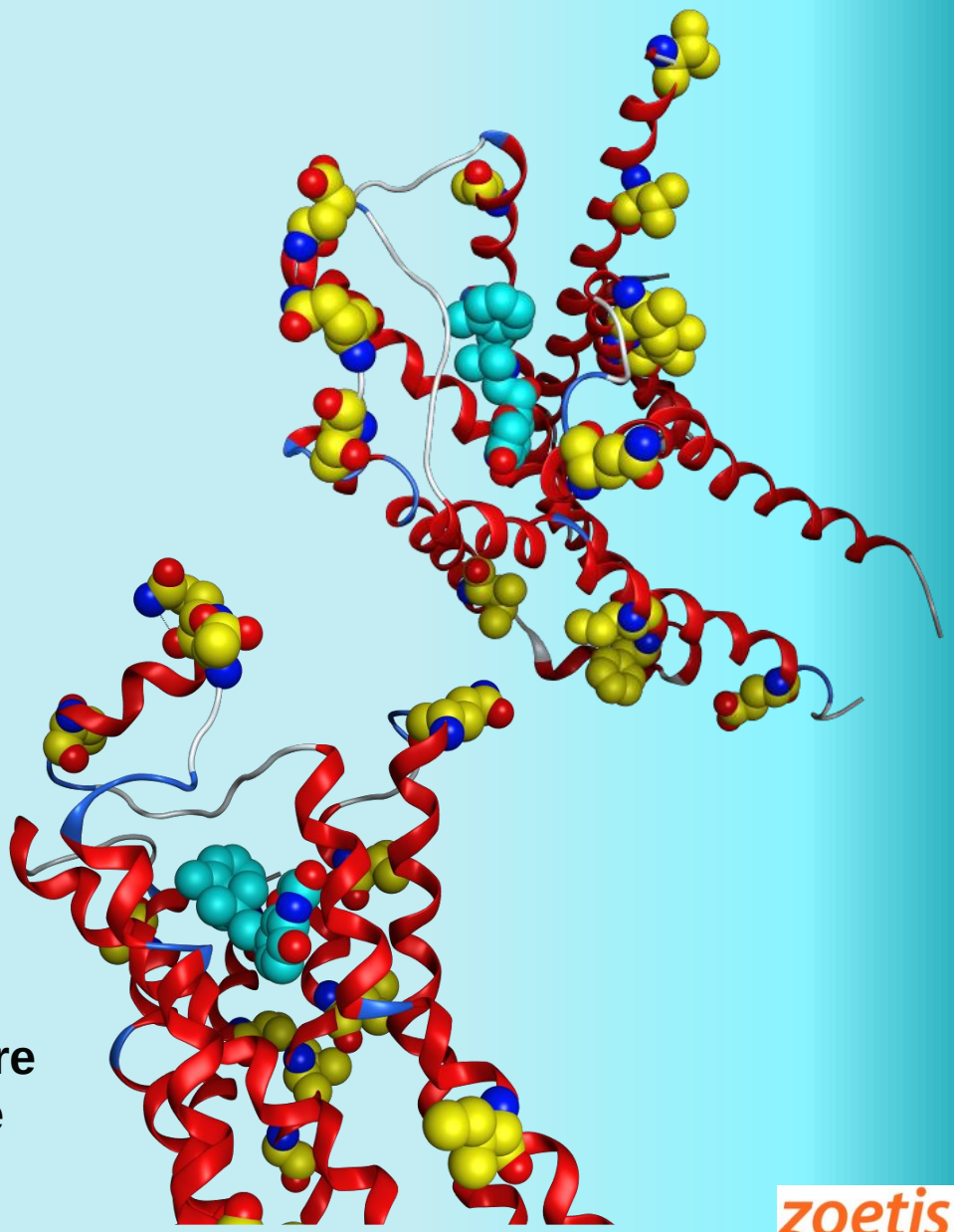
Range for ratio 0.84 – 59

Can get selectivity either way between Bovine and Porcine

Range 0.06 - 54

Cattle – Human residues differences





None of the different residues are close to the agonist binding site

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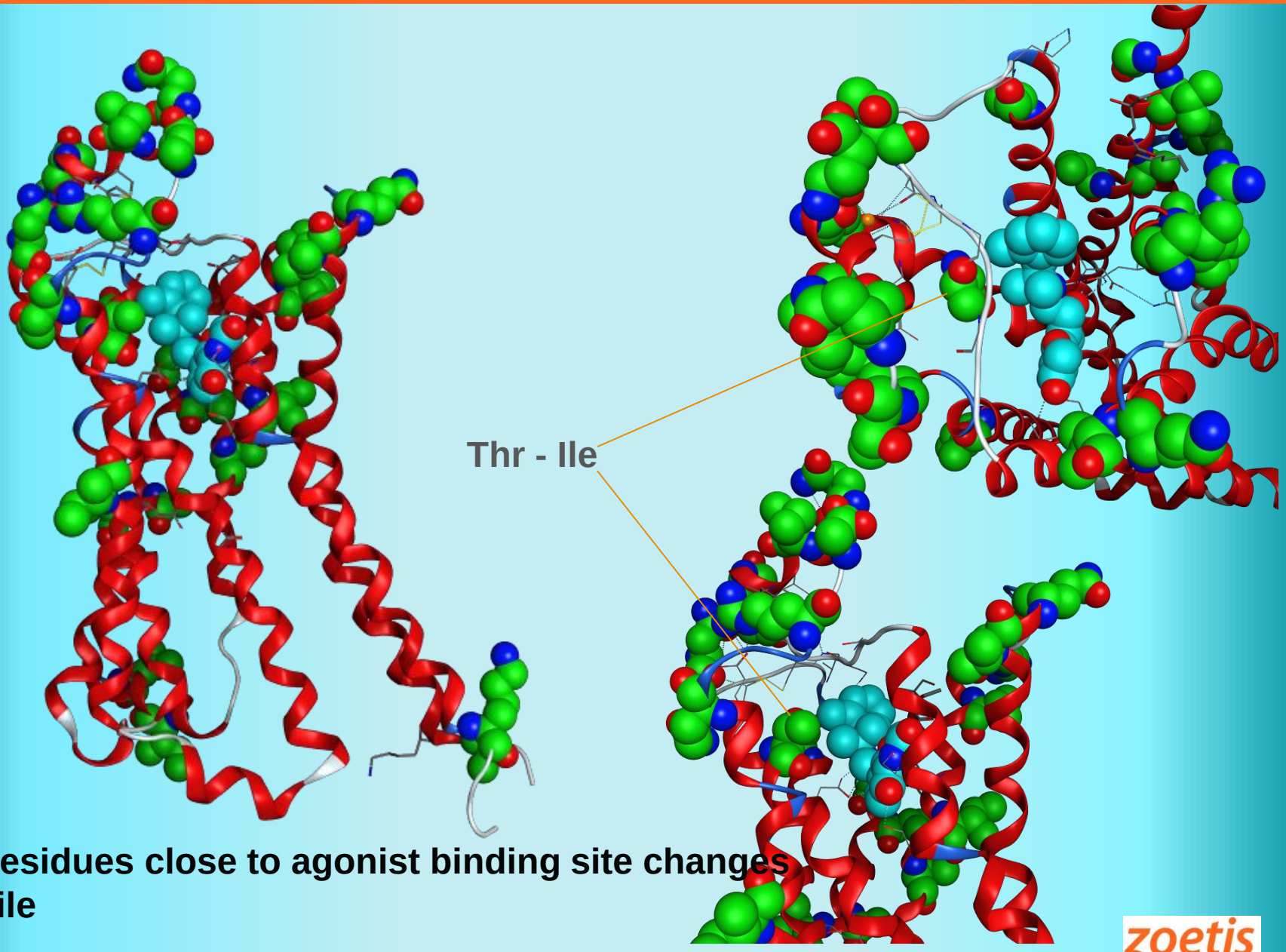
Cattle – Porcine residues differences



One residues close to agonist binding site changes
Thr - ile

Acknowledgments:

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Large Scale : Jerome Dauvergne, David Walker, Neil Cheesman

Separation Group: Dave Koss

Farm: Sandra Johnson

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Zoetis Spun out of Pfizer 2013: What we lost

SAR Visualization Tools

Design Tools
Library Design
Clustering
Desk Top Modelling

Comp Chem Tools (HPC)

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LIMS

Analytical Data Handling

Assay Management

Chemist Electronic Note Book

Corporate Database

Inventory Control

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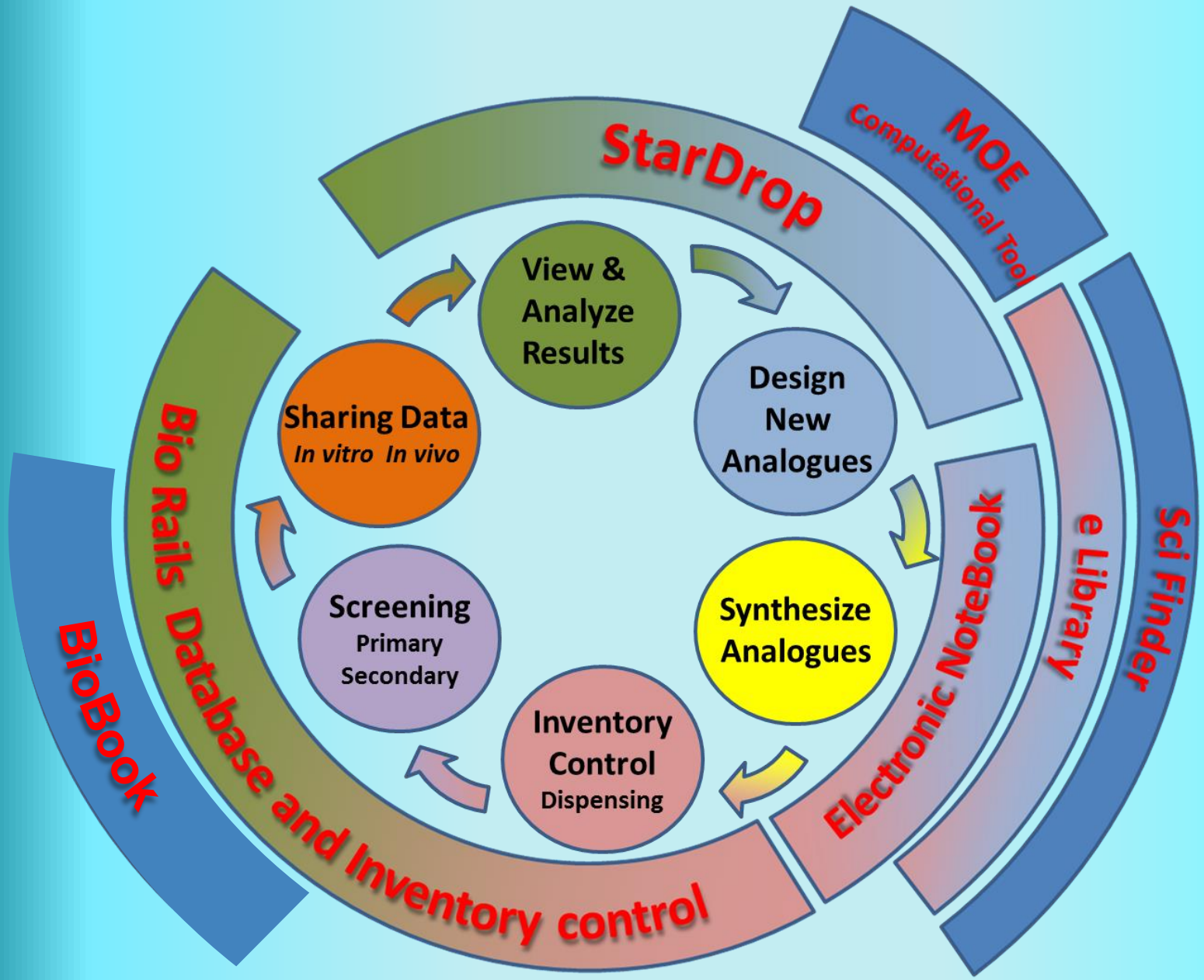
Opportunity to look again at what we do

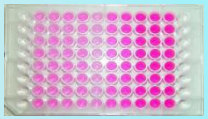
"In the middle of difficulty lies opportunity."

Albert Einstein

Zoetis New IT structure



Bio Rails: Central role in Screening & Logistics



Assay Request

Manage Requests
Manage Plate
Interrogate Stores
biorails
Store Results
Manage Assays
Release to screens

Results

Initiate Cascades

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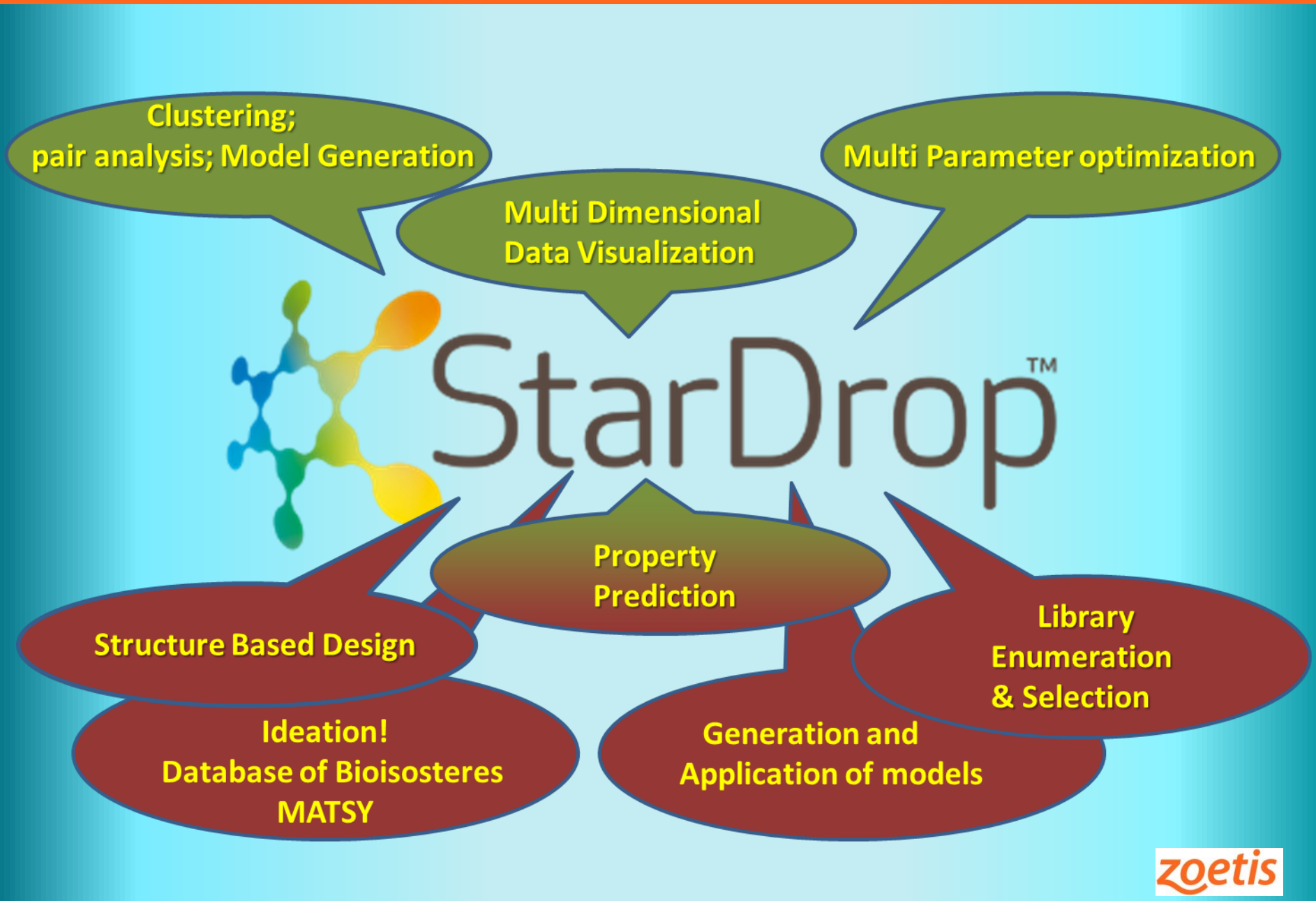
StarDrop: Central to Design and Analysis



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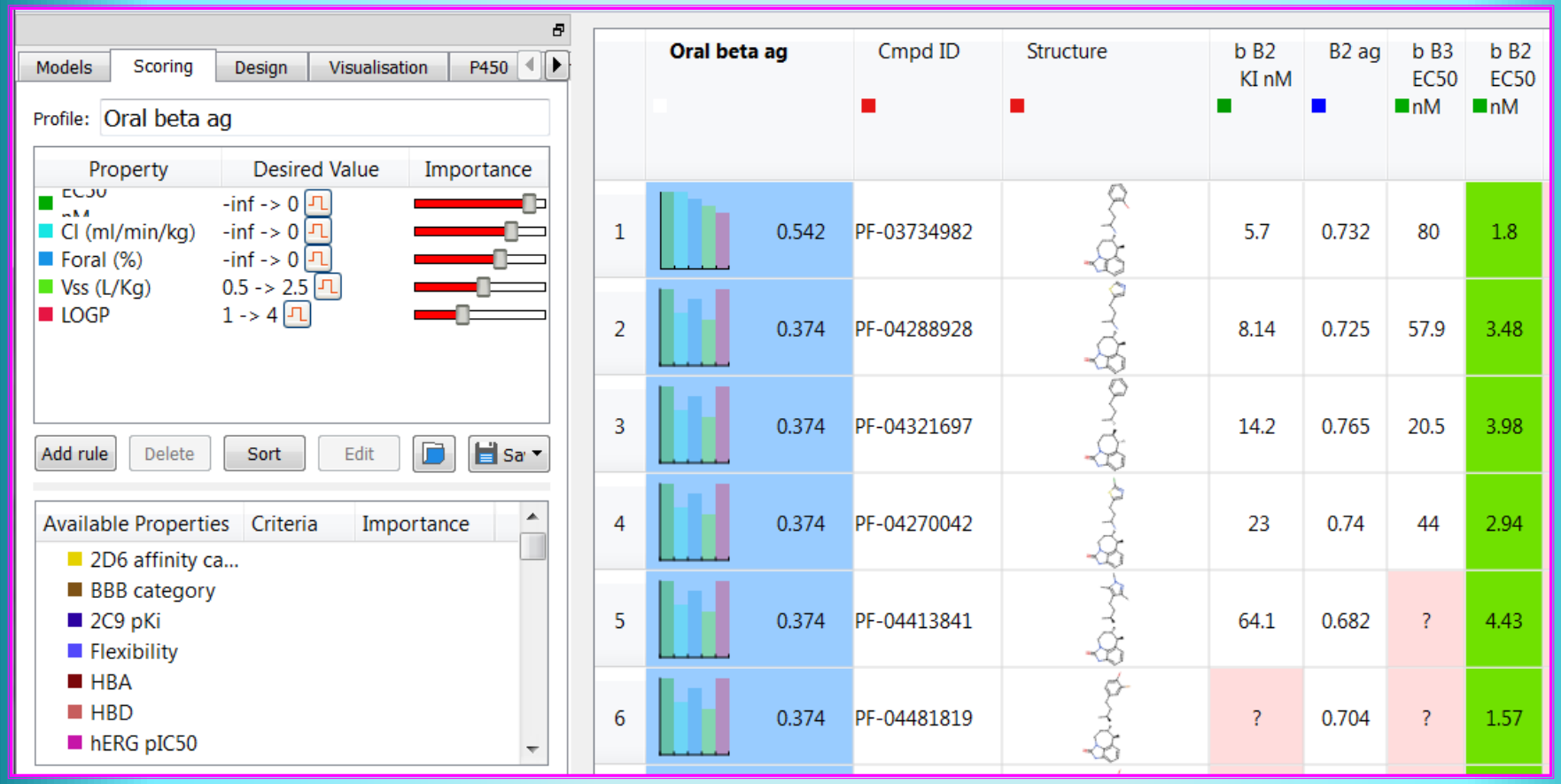
Star Drop: Profiling tool



Desired Profile set as:

EC_{50} Bovine B2: Desirable <10 nM; Acceptable <50 nM

Clearance Desirable 10-20 ml/min/kg

Oral availability Desirable >40%; Acceptable 25-40%

Vdiss: Desirable 0.5-2.5 L/kg; acceptable 2.5-5 L/kg

LogP: Desirable 1-4; Acceptable 0-1 & 4-5

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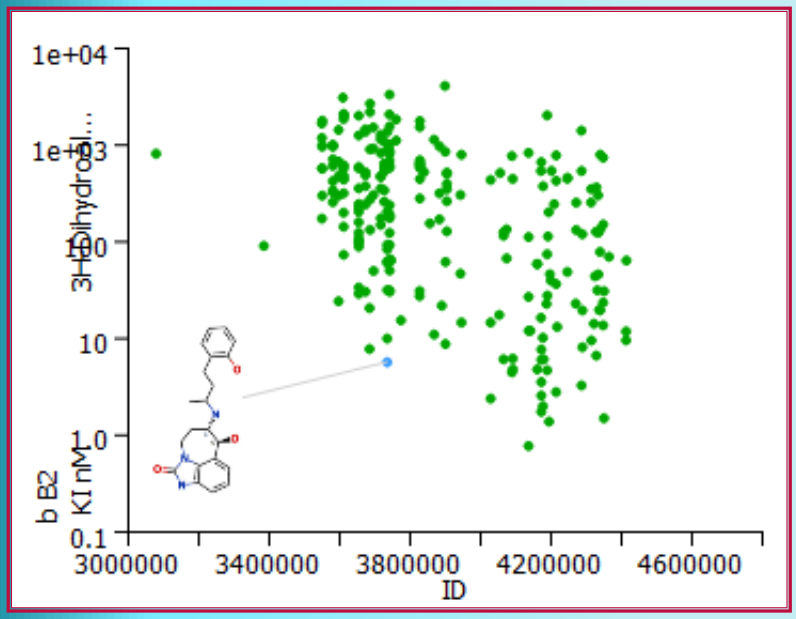
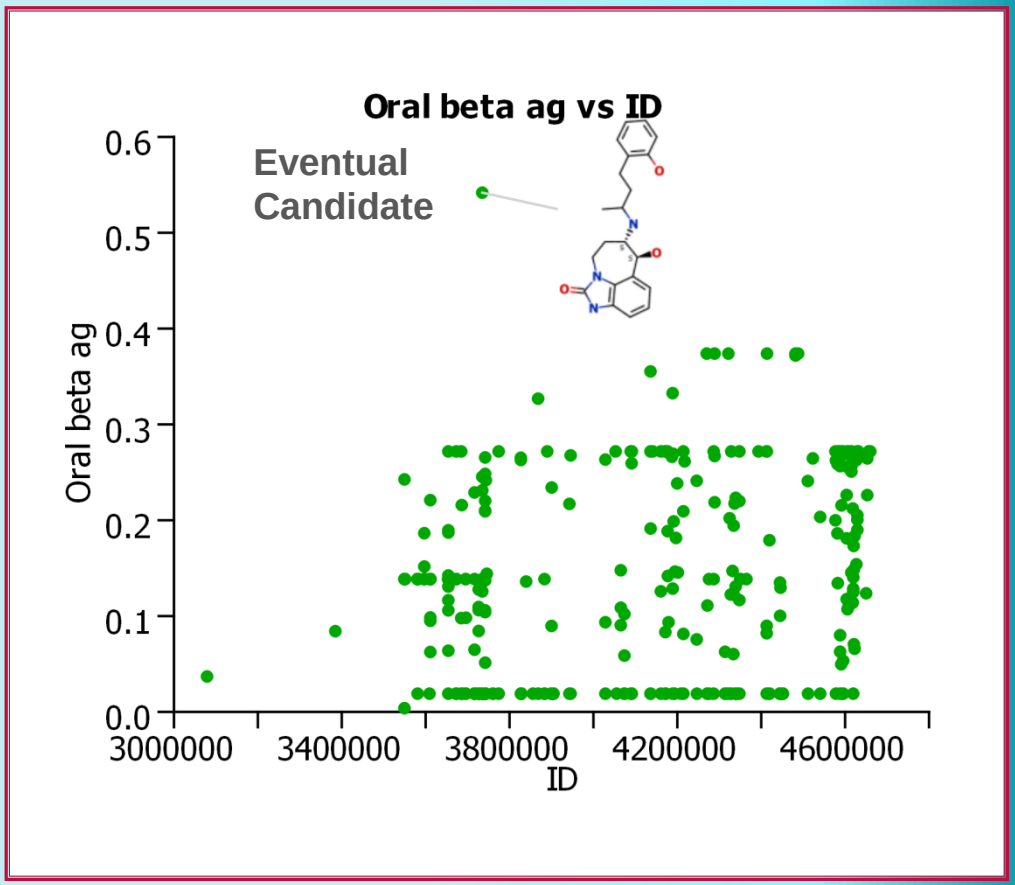
How could this have helped us





Just looking at Potency did not make the final development compound standout

The eventual candidate achieved 0.54 and was the 99th analogue made. It was not identified as such until over 250 analogues had been made and tested

If we achieved 90% of all 5 parameters in a single compound or 2 at 100%, 1 at 90% and 2 at 80% we would achieve a score of 0.59

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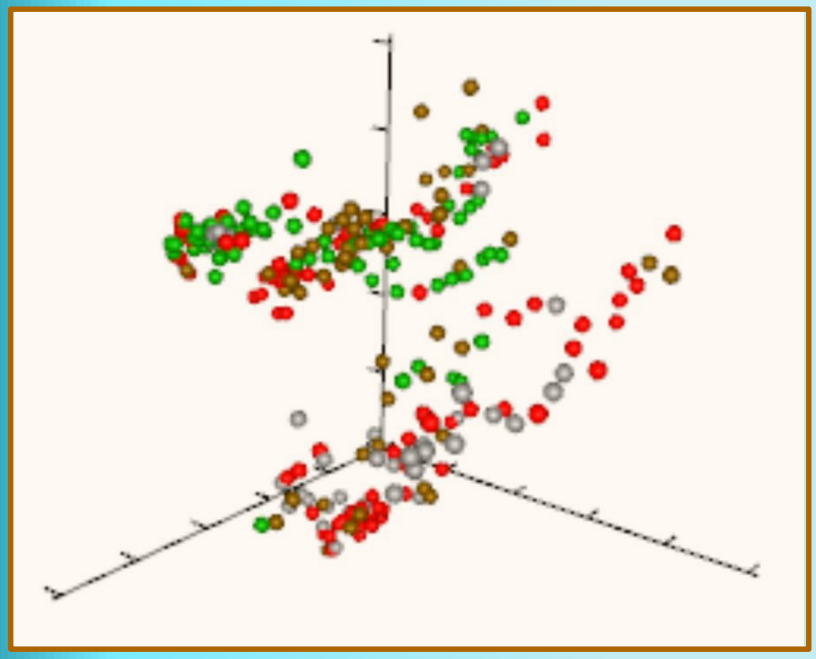
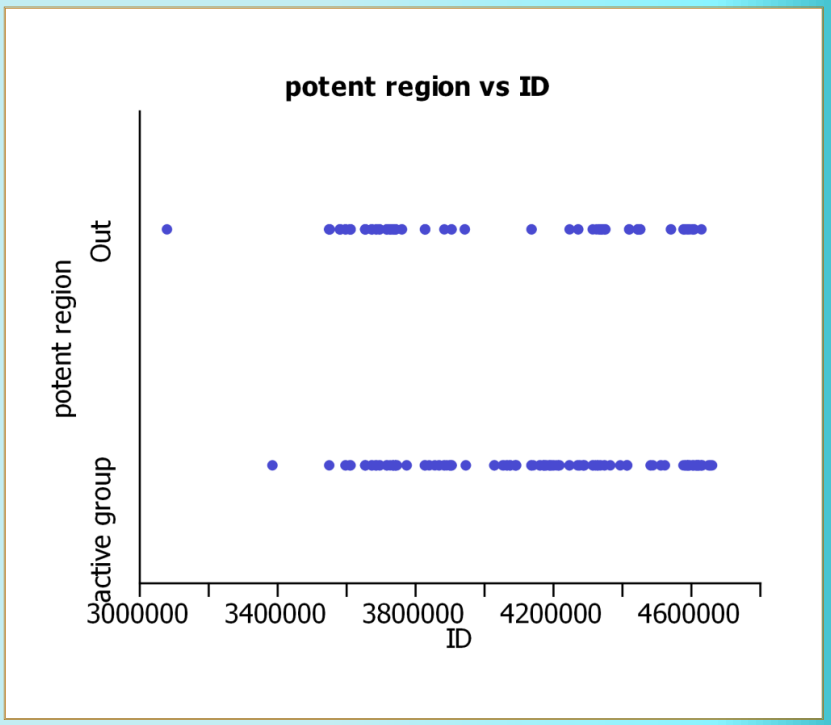
3D PCA of chemical space based on Structure





Looking at chemical space using a PCA based on Structure splits the analogues into two main groups
Most of the actives (green, <25nM) are located in just one of these

Both of these groups of compounds were made over the life time of the Project
Had this been recognized earlier, we could have made less compounds

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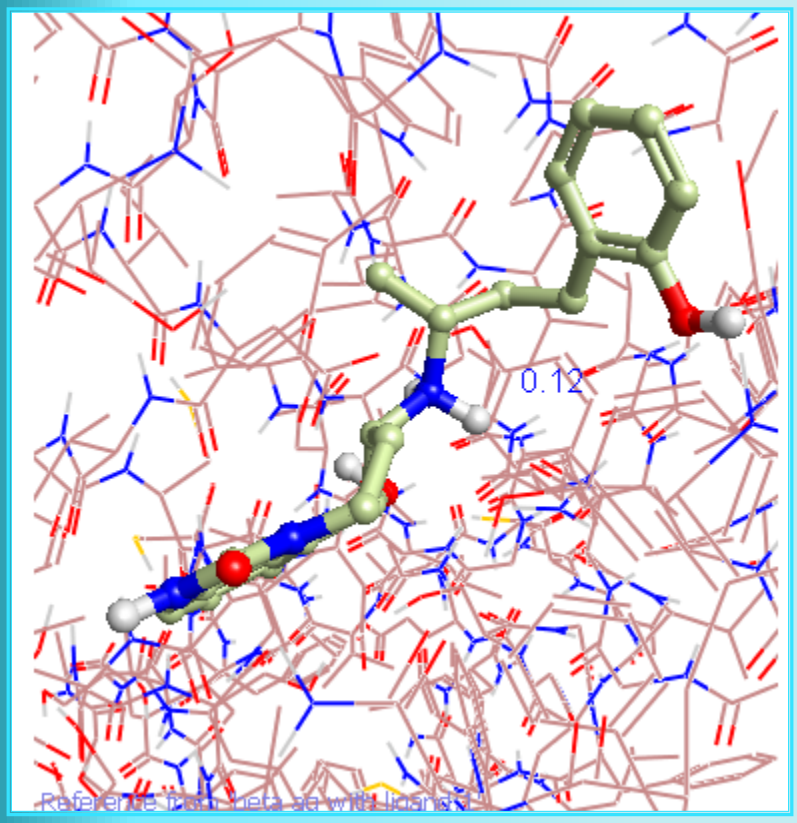
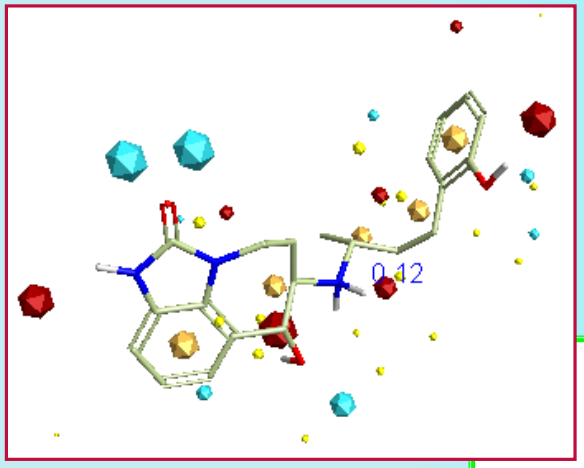
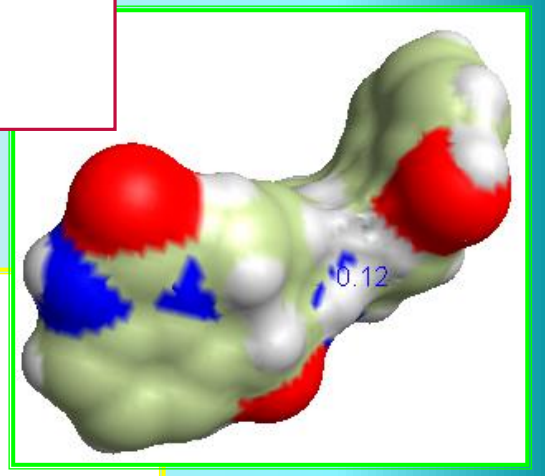
Star Drop: Torch

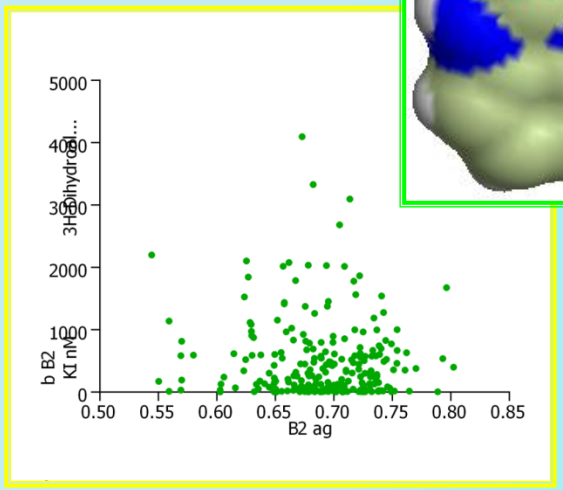




Program projects
Required electronic
& Lipophilic points
In space, “ZEDS”.

Can also put surfaces
onto analogues





Can load X-ray of β2 with Ligand docked
Define Ligand as a reference
Align analogues with reference and score fit

Score is not highly predictive of Ki
However, performs no worse than Docking scores generated with MOE
Good Desk top tool to explore ideas and focus design

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Would these new tools help?

- Integrating a lot of previous functions into a single platform will simplify and speed up logistics and cycle times
  - Inventory searching; assay requests;
- Automated requesting of additional assays will improve cycle times

- Having both analysis and design tools in a single program will improve design and reduce the number of analogues synthesised
  - Simple to view new analogues with the real data
  - Access to literature based databases to spur ideas
  - Simple way to use SBDD in the same tool
- A simple way to view the overall profile and compare this between analogues should enable earlier identification of potential candidates

We could do most, but not all of these things with our legacy systems, but they were spread over several programs.
Now they are available in two easy to use programs

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Thank you for your attention

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