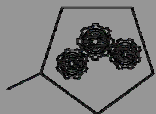




Hit-finding against GPCR Targets

Discovery of Potent, Selective MCH-1 Receptor
Antagonists by Virtual Screening



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Computer-Aided Drug Design

Argenta Discovery Ltd., Harlow



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Outline



- Background
- Virtual screening – process
- Virtual screening – results
- Hit-to-lead optimisation
- Conclusions



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Background



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Argenta Discovery



- ❑ Independent drug discovery company
- ❑ Formed August 2000 by former Aventis employees and Imperial College spin-out
- ❑ Located in purpose-built labs in Harlow
- ❑ Grown from 26 to >90 employees
- ❑ Two business streams
 - High-quality outsourced medicinal chemistry
 - Proprietary drug discovery programmes
- ❑ Clients include GSK, AstraZeneca, Lundbeck, Corcept
- ❑ Pipeline of therapeutics projects



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RRT Hit-finding Paradigm

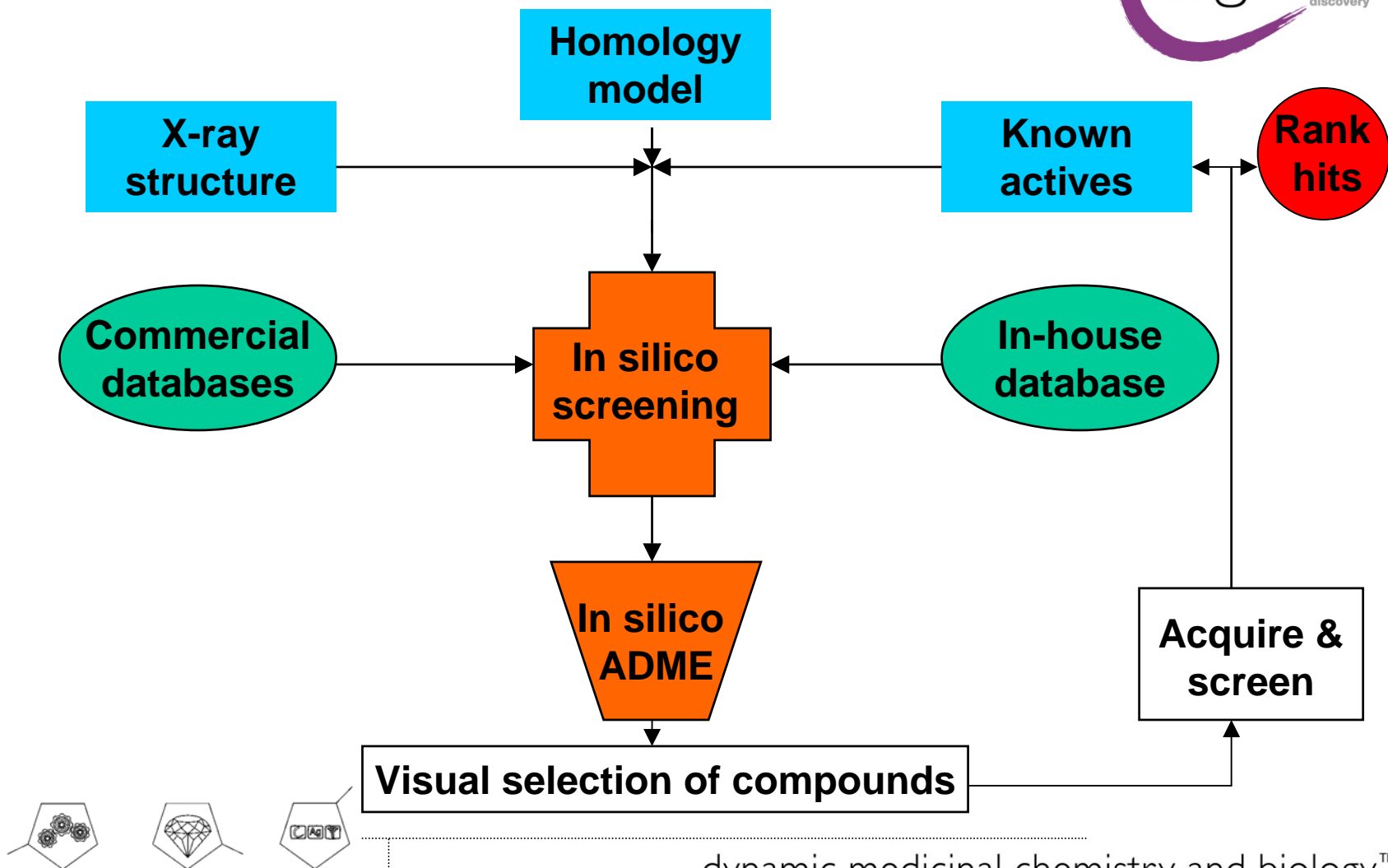


- ❑ Hit-finding for client and therapeutics projects
- ❑ RRT = Rapid Reaction Team
- ❑ Driven by *in silico* screening coupled with biochemical screening - limited chemistry resource required
- ❑ Aim: 6-month hit-finding phase
 - Encompasses two rounds of (virtual) screening
 - Aim to screen up to 1000 compounds in primary assay
 - Enables rapid follow-up of early hits, generation of SAR
- ❑ But, can move to hit-to-lead earlier if first round identifies suitable compounds
- ❑ Target selection criteria for RRT projects
 - Compelling biological rationale
 - Structural information on biochemical target/homology model and/or known ligands



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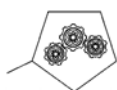
RRT Hit-finding Paradigm



MCH-1R and Obesity



- ❑ Urgent need for obesity therapeutics
 - Obesity is linked to potential fatal conditions
 - Costs >12% of US national healthcare budget (\$118bn)
 - Lost US productivity due to obesity: \$3.93bn per annum
- ❑ Melanin-concentrating hormone (MCH) is an orexigenic neuropeptide found in the lateral hypothalamus
 - Fasting increases expression of MCH mRNA in normal and obese animals
 - Injection of MCH into the lateral ventricles of rats has been shown to increase food intake acutely
 - MCH knockout mice have reduced body weight and are lean, due to hypophagia and increased metabolic rate
 - Transgenic mice over-expressing MCH are obese and insulin resistant



MCH-1R and Obesity



- ❑ Two G-protein coupled receptors have been identified as MCH receptors: MCH-1R (SLC-1) and MCH-2R
 - MCH-1R is widely expressed in multiple regions of the brain including areas of the hypothalamus implicated in the control of energy homeostasis
 - MCH-1R knockout mice have normal body weight but are lean with reduced fat mass. These mice are resistant to diet-induced obesity
 - *In vivo* experiments with MCH-1R antagonists (SNAP-7941, T-226296) suggest potential utility in the treatment of obesity
 - A “hot” target – much interest from pharmaceutical industry

Browning, A. Recent developments in the discovery of melanin-concentrating hormone antagonists: novel antiobesity agents. *Exp. Opin. Ther. Patents* 2004, 14, 313-325.



Virtual Screening - Process

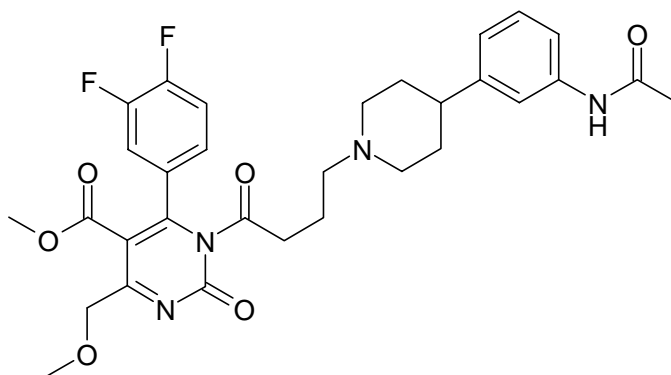


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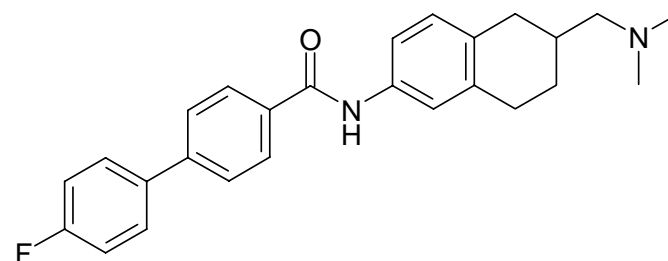
Virtual Screening – Starting Points



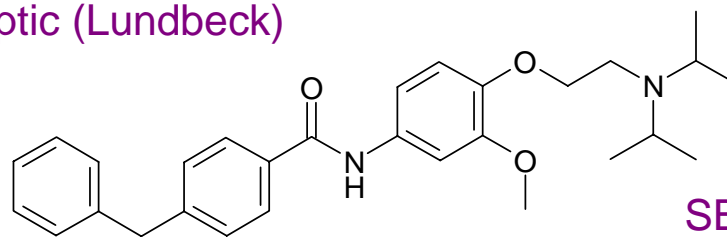
- Although it is possible to construct homology models of GPCRs, virtual screening was primarily ligand-based
- 11 competitor compounds used as queries including:



SNEC-3, Synaptic (Lundbeck)



T-226296, Takeda



SB568849, GSK



Virtual Screening - Databases



- ❑ 24 vendor collections collated: ~2 million structures
- ❑ Filtered for drug-likeness: ~615,000 structures
- ❑ Databases created from this set:
 - Daylight fingerprinted TDT file
 - Unity 3-D database
 - 3-D structures generated with Concord
 - Smaller tertiary amine databases for use with FlexS
 - 2-4 aromatic rings, $300 < MW < 450$, Nrotbonds < 7 (~13,600)
 - Further refined: $360 < MW < 450$, tPSA $< 100\text{\AA}^2$ (~8,600)
 - Concord structures minimised using MMFF94



Virtual Screening – Search Techniques



- ❑ Multiple search techniques applied to each query
 - 2-D substructure: Daylight SMARTS
 - 2-D similarity: Daylight and Unity fingerprints, data fusion (Daylight fingerprints, Tanimoto + Russell-Rao)
 - 3-D similarity: FlexS
 - Flexible superposition mode
 - Conformation of query molecule fixed (generally obtained from Batchmin LowMode conformational search, MMFFs + GB/SA)
 - 3-D substructure: Unity
 - 3- or 4-point queries based on individual compounds
- ❑ Structure-based pharmacophore searches based on alignment of query compounds in MCH-1R homology model
- ❑ Clustering of subset of small FlexS database



Virtual Screening - Results



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Compound Selection



- ❑ From all the searches, 3015 hits were selected
- ❑ Analysed in conjunction with an experienced medicinal chemist
 - Drug-likeness
 - Synthetic tractability, potential for optimisation chemistry
 - Key computed properties: MW, ClogP, PSA
- ❑ 1490 compounds selected
- ❑ Clustered using Daylight fingerprints (Tanimoto similarity threshold = 0.85): 874 clusters
- ❑ 877 compounds selected for purchase
- ❑ After investigating availability and price, 806 compounds ordered, of which 795 were received



Biochemical Screening



- ❑ A scintillation proximity assay using ^{125}I -[Phe¹³, Tyr¹⁹]-MCH binding to MCH-1R membranes was developed
- ❑ 795 compounds were tested at a single concentration (10 μM)
- ❑ 62 actives (active: >40% inhibition) were re-tested in duplicate at 10 μM
- ❑ IC₅₀ values were determined for 19 compounds (6-point curves)
- ❑ Compounds were classed into 7 structurally distinct series with IC₅₀ values ranging from 55nM to 27 μM

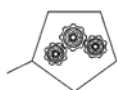


Which Searches Produced Which Hits?



Compound	IC ₅₀ (μ M)	Search 1	Search 2	Search 3	Search 4
A	0.055	10_3d_sub	10_flexs		
B	0.70	3_flexs			
C	0.73	10_flexs	8_flexs		
D	0.82	3_3d_sub	9_3d_sub		
E	1.8	4_flexs			
F	2.9	4_2d_sim			
G	4.0	1_3d_sub	11_3d_sub		
H	8.2	clustering	10_flexs		
I	11.0	1_3d_sub	3_flexs	8_flexs	
J	11.4	1_3d_sub	10_3d_sub		
K	12.9	2_3d_sub	9_2d_sim		
L	12.9	7_flexs			
M	14.5	10_flexs			
N	14.7	clustering			
O	18.1	2_flexs			
P	19.9	1_3d_sub	3_3d_sub	10_3d_sub	11_3d_sub
Q	24.0	clustering			
R	25.0	10_3d_sub			
S	26.7	1_3d_sub			

- Only 2 query compounds did not yield hits
- 2-D substructure and structure-based pharmacophore searches did not yield hits
- Lesson: use as many queries and search types as possible!



Hit Rates for Various Search Types



Search Type	Number of Compounds Screened	Number of Hits	Hit Rate (%)
flexs	526	11	2.1
3d_sub	350	15	4.3
2d_sub	11	0	0.0
2d_sim	36	2	5.6
clustering	124	3	2.4
structure-directed	49	0	0.0
pharmacophore			

- All submicromolar compounds found by 3-D searches
- But simple 2-D searches can still be effective

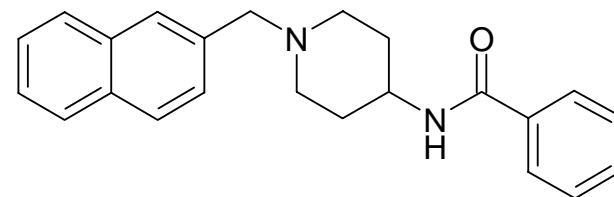


Two Examples of Hit Compounds



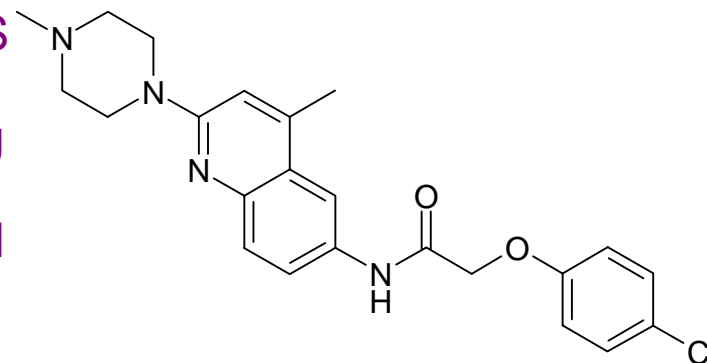
□ Compound F ($IC_{50} = 2.93mM$)

- Found by 2-D similarity search based on a Synaptic compound
- 3-methoxy derivative reported as MCH-1R antagonist by Amgen at 224th ACS meeting ($IC_{50} = 150nM$)

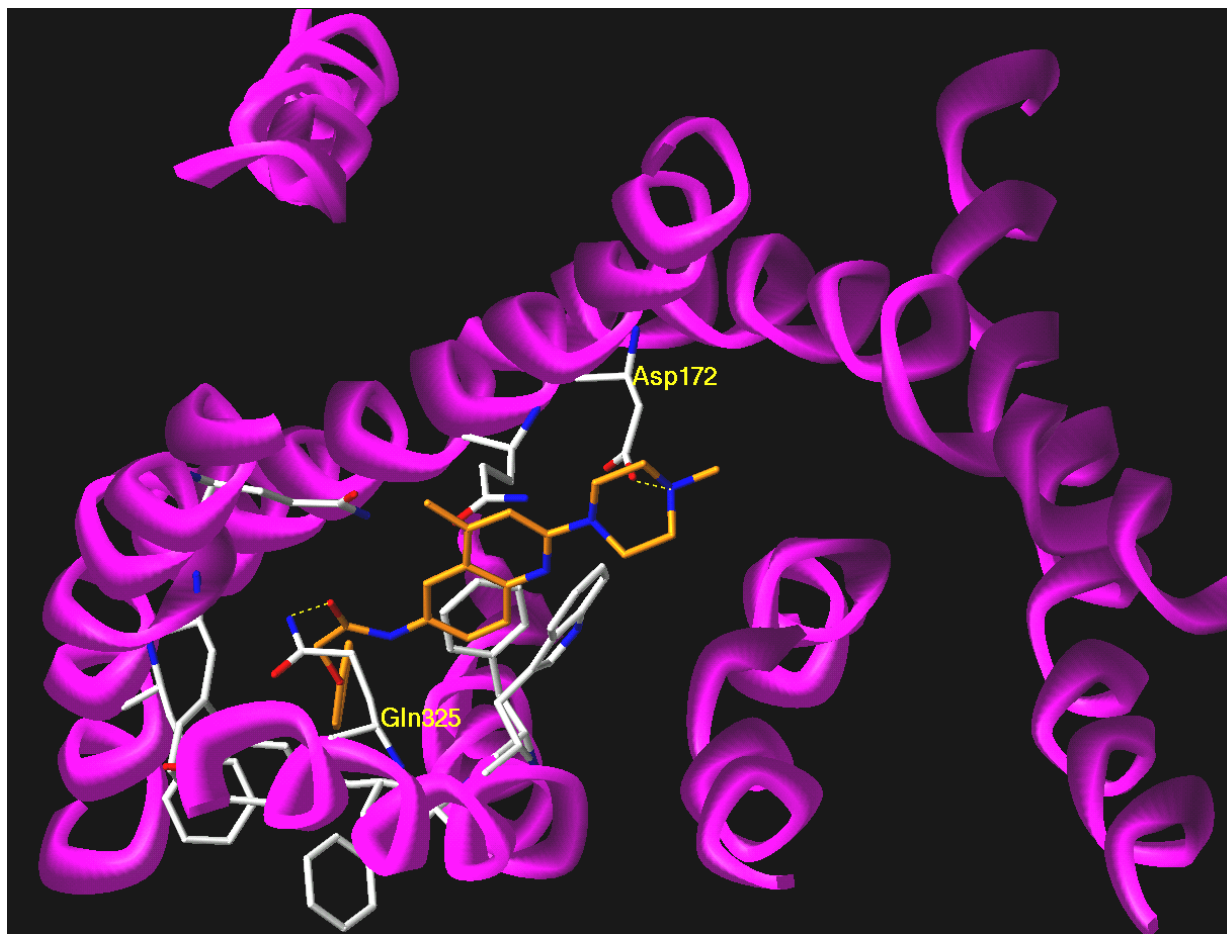


□ Compound A ($IC_{50} = 55nM$)

- Found by 3-D substructure and FlexS searches based on SB568849
- Tested in functional assay measuring Ca^{2+} release
- No agonist activity was observed and compound antagonised the Ca^{2+} response to $0.1\mu M$ MCH (EC_{80})
- Chosen for initial hit-to-lead work



Proposed Binding Mode of Compound A



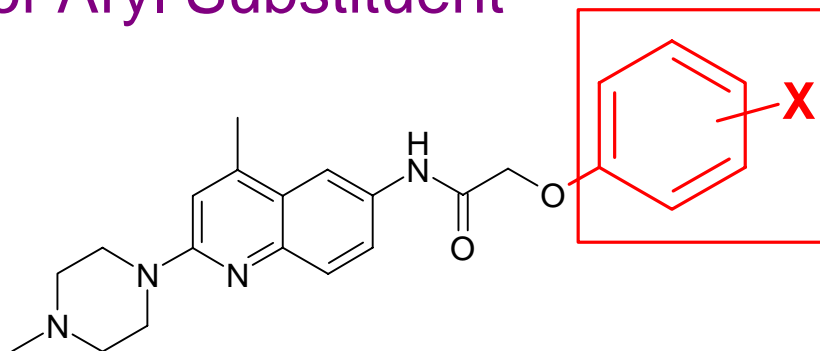
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Hit-to-Lead Optimisation



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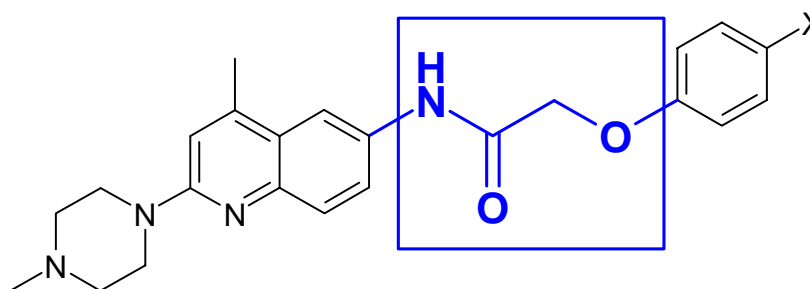
Optimisation of Aryl Substituent



Compound	X	IC ₅₀ , nM	Compound	X	IC ₅₀ , nM or % @ 1μM
A	4-Cl	55	A6	2-Cl	470
A2	4-CH ₃	80	A7	3-Cl	12%
A3	4-CF ₃	14	A8	3-CH ₃	19%
A4	4-OCH ₃	152	A9	2,4-di Cl	24
A5	4-CN	370	A10	H	2%



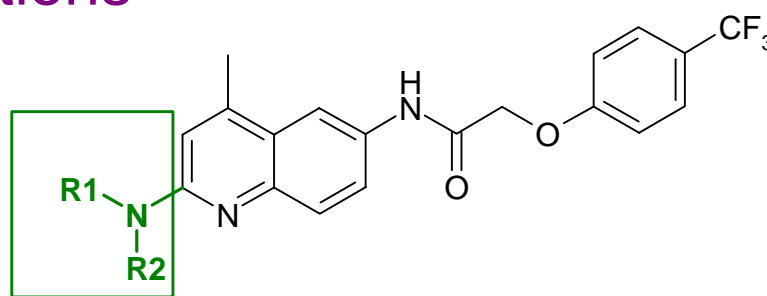
Linker Variations



- Phenoxyacetamide proved to be the best linker
- Phenylpropionamides also gave good activity
- A shorter linker resulted in loss of activity
- All longer linkers resulted in loss of activity
- Ureas and sulphonamides resulted in weak activity
- Methylation of the amide NH abolished activity



Amine Modifications



Compound	NR ¹ R ²	IC ₅₀ , nM	Compound	NR ¹ R ²	IC ₅₀ , nM or % @ 1μM
A3		14	A14		38
A11		91	A15		11
A12		226	A16		174
A13		15	A17		40%



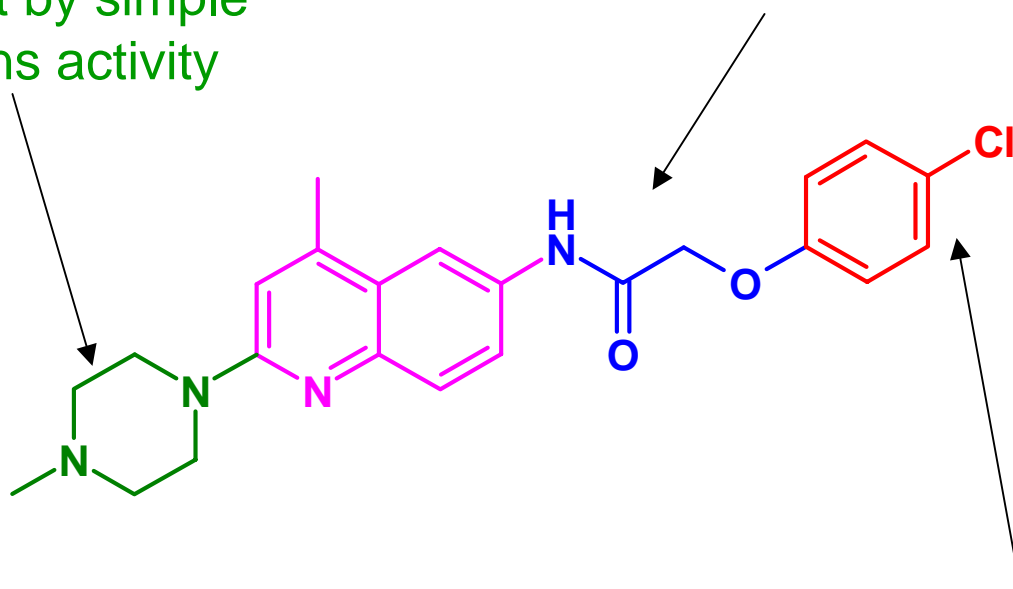
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Summary of SAR



Limited variations possible,
2-atom spacer optimal

Replacement by simple
amines retains activity



4-substitution required,
electron withdrawing
groups preferred

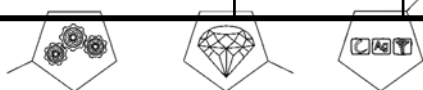


Specificity of Selected Compounds



Compound	MCH-1 IC ₅₀ nM	5-HT _{2A} IC ₅₀ nM or % @ 1μM	5-HT _{2B} IC ₅₀ nM or % @ 1μM	5-HT _{2C} IC ₅₀ nM or % @ 1μM	D2 %@10μM	α _{1A} %@10μM
A	55	2180	349	970	35%	84%
A3	14	27%	442	39%	41%	85%
A4	24	10%	201	33%	35%	55%
A11	91	27%	19%	27%	*43%	*5%
A13	15	11%	>3000	7%	*7%	*10%
(±)T226296	64	90	35%	60	44%	63%

*% inhibition
@ 1μM



Summary

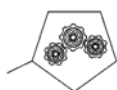


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Summary



- ❑ 795 compounds identified by virtual screening have been tested in an MCH-1R binding assay
- ❑ Multiple hit series identified with most potent compound having $IC_{50} = 55nM$
- ❑ Hit-finding completed in 6 months (~3 FTEs)
- ❑ Structure-activity relationships have been delineated and key features required for binding have been identified
- ❑ Representative compounds showed some affinity at $5HT_{2A}$, $5HT_{2B}$, $5HT_{2C}$, D2 and α_{1A} receptors, but some were selective for MCH-1R
- ❑ Optimisation has provided an improvement in potency and selectivity
- ❑ All compounds tested were functional antagonists in the calcium release assay. No agonism was observed



Conclusions



- ❑ Virtual screening can be a rapid and cost-effective approach to hit-finding
- ❑ For GPCRs, ligand-based virtual screening can provide hits of sufficient potency, novelty and diversity for optimisation
- ❑ Similar successes have been achieved at Argenta against other GPCRs and enzyme targets (e.g. HDAC) using ligand-based and/or structure-based approaches

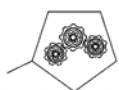


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□ Argenta Discovery

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