

Fragment-based GPCR screening: Histamine receptor sub-type specific fragments identified

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Introduction

Fragment-based drug discovery (FBDD) has emerged as a new tool for drug discovery in recent years. The rationale for doing this is to cover as much chemical diversity as possible in a small screening campaign and to ensure the chemical space for the binding pockets of the target of interest are effectively addressed. While the majority of investigations focused towards biochemical targets only few examples have been published in which this method has been used to identify ligands for GPCRs.

There are several pieces of evidence suggesting that FBDD might have some success for GPCRs:

- Ligands and compounds for biogenic amine receptors that show both good affinity and efficacy have structures not unlike fragments
- Molecular modelling prove that these ligands bind within the cavity defined by the seven transmembrane helices
- Scientist from Merck reported the design, synthesis and testing of a series of indole-based libraries that demonstrated measurable activities across a broad range of Class-A GPCRs

In this study we have screened a subset from the Evotec fragment collection to identify subtype specific antagonists for the histamine receptors (H1, H3 and H4).

Key data of the fragment screen on the histamine receptors H1, H3 and H4

	H1		H3		H4	
Concentration	2 μ M	20 μ M	2 μ M	20 μ M	2 μ M	20 μ M
# Compounds	1,708	1,708	1,708	1,708	1,708	1,708
Z' value	0.82		0.83		0.59	
Threshold	15.3%	18.4%	19.1%	16.7%	32%	31.4%
Confirmed hits	28	52	19	64	9	21
Hit overlap	18		17		5	

1,708 fragments were tested in quadruplicates at 2 μ M and 20 μ M in functional Ca²⁺ flux assays on cell lines expressing either histamine receptor H1, H3 or H4 to identify sub-type specific antagonists. In total 106 confirmed hits were identified.

Tripos 3D Topomere search – Neighbour screening

- To extend the chemical space 35 fragments out of the 106 confirmed hits were selected based on their activity of over 50% on one of the three receptors
- A Tripos 3D Topomere search (based on shape and pharmacophore interactions) was conducted with these 35 fragments in the part of the Evotec fragment library that was not screened
- 30 out of the 35 fragments had neighbours – whereby the number of hits/neighbours varied between the search structures
- The number of neighbours were limited per search structure to a maximum of 10
- As a result of this exercise 251 fragments (106 hits + 145 neighbours) were picked and tested for their potency on H1, H3 and H4

Hit profiling: Sub-type specificity

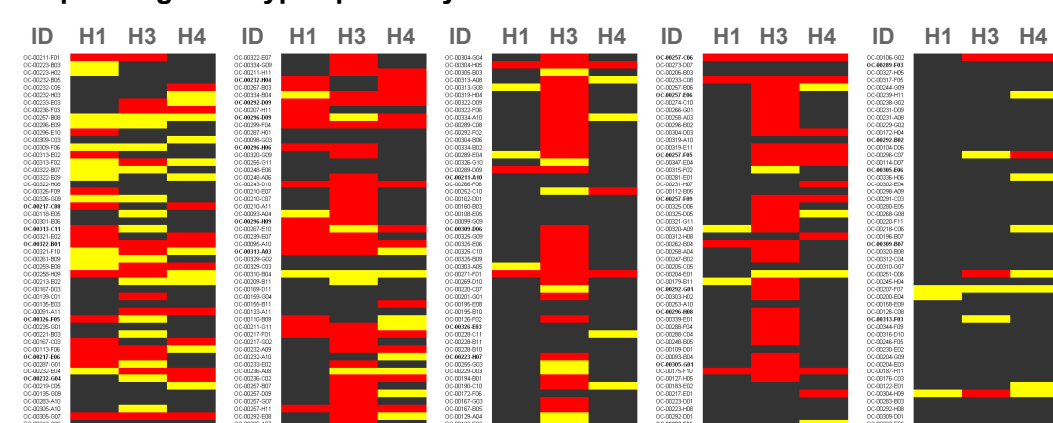
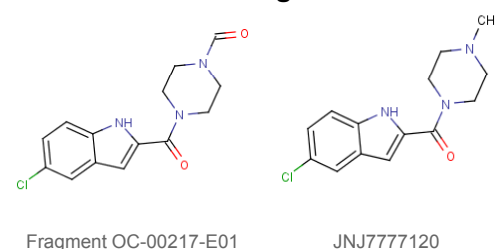


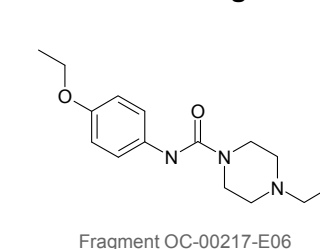
Figure 1: Selectivity of 251 fragments. H1=histamine H1 receptor subtype; H3=histamine H3 receptor subtype; H4=histamine H4 receptor subtype; The different colours reflect the potency of the fragments on the three different receptors in Ca²⁺ flux assays: **Black:** Inactive, **Yellow:** IC₅₀ >100 μ M, **Red:** IC₅₀ <100 μ M

Using a limited amount of primary screening and a single round of structure-based analoging afforded a rich list of hits with many target selective starting points.

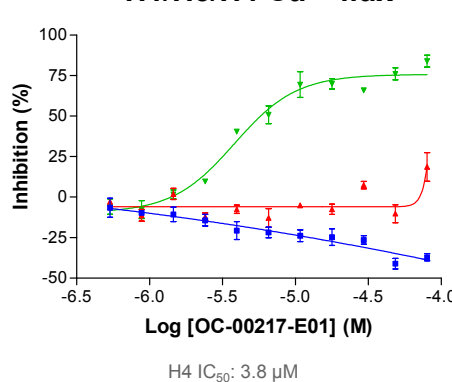
H4 selective fragment



H3 selective fragment

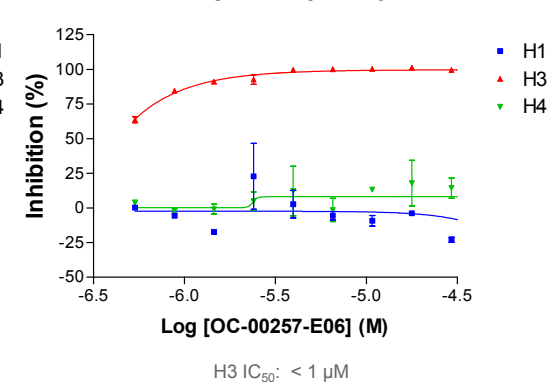


H1/H3/H4 Ca²⁺ flux



H4 IC₅₀: 3.8 μ M

H1/H3/H4 Ca²⁺ flux



H3 IC₅₀: < 1 μ M

Figure 2: Histamine H3 and H4 receptor selective fragments OC-00217-E01 and OC-00217-E06. JNJ7777120 is a drug being developed by Johnson & Johnson Pharmaceutical Research & Development which acts as a potent and selective antagonist at the histamine H4 receptor. It has been nominated as clinical candidate. *In vivo* efficacy was shown in inflammation models.

Fragment hit-based virtual screening

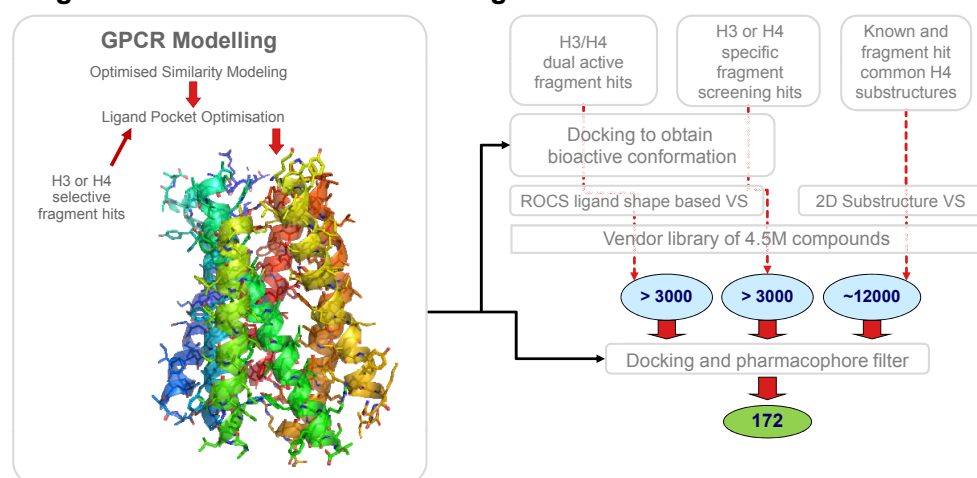


Figure 3: Virtual screening: Either histamine H3 or H4 receptor selective fragments were used to optimise the ligand pockets for either histamine H3 or H4 receptor models. These models were used in docking studies to obtain the bioactive conformation for various fragment hits which were then used for a virtual screening in vendor libraries. The identified hit structures of the virtual screen were subsequently scrutinised by docking them into the receptor models and by applying pharmacophore filters associated with the models. As a result of this procedure 172 compounds were identified.

Enrichment for H3 and H4 antagonists – Comparison of the three stages

172 compounds identified by virtual screening and structural modelling were tested in triplicates at 20 μ M in functional Ca²⁺ flux assays on cell lines expressing either histamine receptor H3 or H4 to identify sub-type specific antagonists.

	Fragment screening	Neighbour screening	Virtual Screening
# Compounds screened	1,708	145	172
H3 Confirmed hits	64	63	79
H3 Rate of confirmed Hits	3.7%	43.4%	54.5%
H4 Confirmed hits	21	23	58
H4 Rate of confirmed hits	1.2%	15.9%	40.0%

Summary

- Screening of fragments on biogenic amine receptors is a valid approach to identify attractive hit compounds for these receptors
- The combined fragment screening and *in silico* modelling approach can:
 - Identify neighbours to hit structures to establish a SAR
 - Reduce time and costs to identify potential starting points for medicinal chemistry
- Identified fragment hits can be used as tools to refine existing GPCR models
- In turn, the refined GPCR models can be used to drive the next round of compound optimisation
- The staged screening process result in a dramatic enrichment of active compounds