

Identification of fragment inhibitors of phosphodiesterase 10a (PDE10a)

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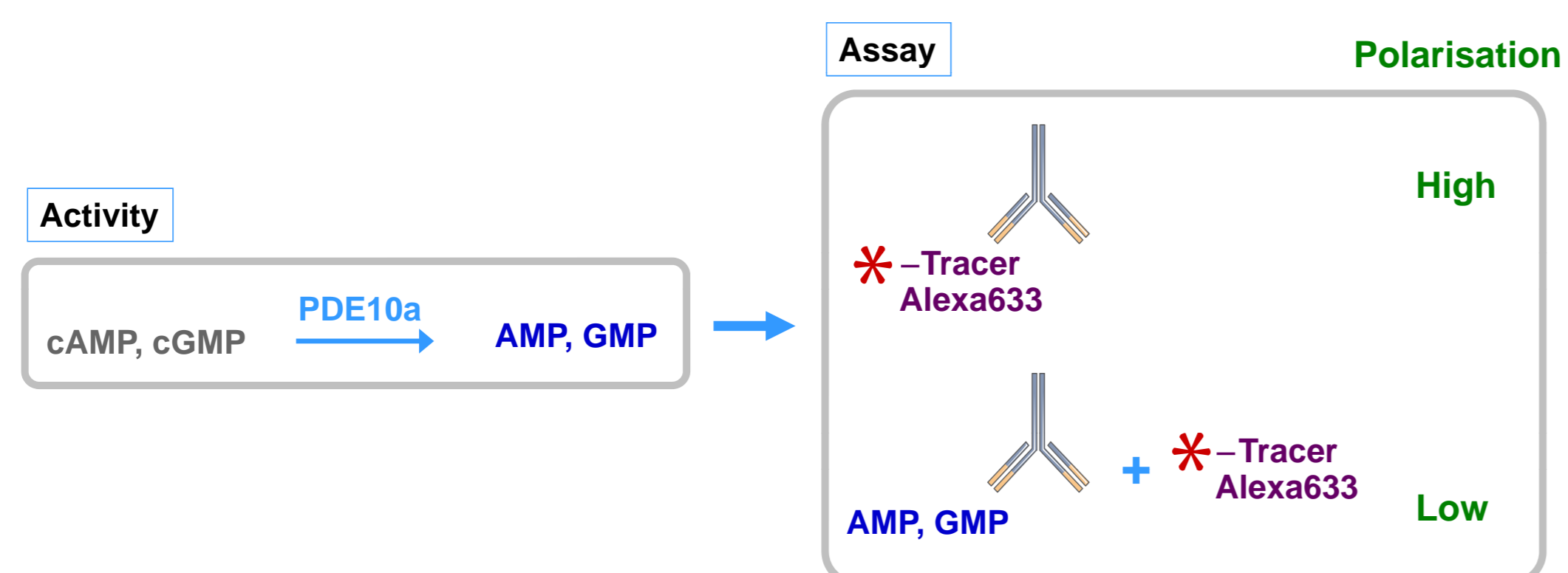
Abstract

Phosphodiesterase 10a (PDE10a) modulates cellular cyclic nucleotide signalling and is highly expressed in the striatal medium spiny neurons (MSNs) of the basal ganglia,¹ dysfunction of which has been implicated in various CNS disorders including Schizophrenia. Thus, inhibition of PDE10a may modulate behaviours regulated by this circuit. Recent studies on PDE10a knockout-mice support the hypothesis that PDE10a inhibition represents a novel approach to the treatment of psychosis.²

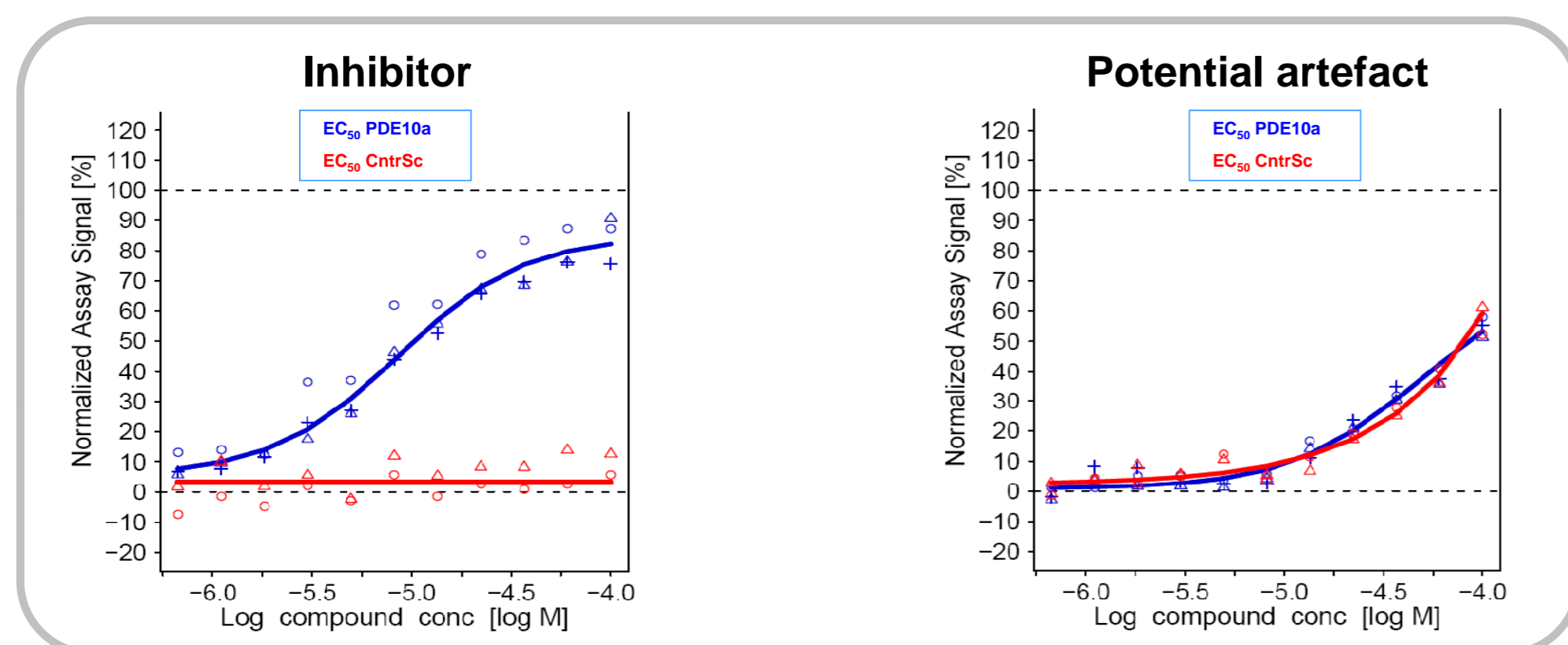
By employing a fragment-based screening approach, Evotec has identified novel inhibitors of PDE10a. Promising chemical scaffolds have been confirmed with secondary assays and co-crystal structures for several fragment inhibitors have been obtained, forming the basis for a structure-based medicinal chemistry program. For the most efficient starting compounds we have achieved nanomolar on-target potency and excellent selectivity with few, design-driven chemical steps and we are currently selecting an *in vivo* candidate from this work.

High throughput fragment screening of PDE10a

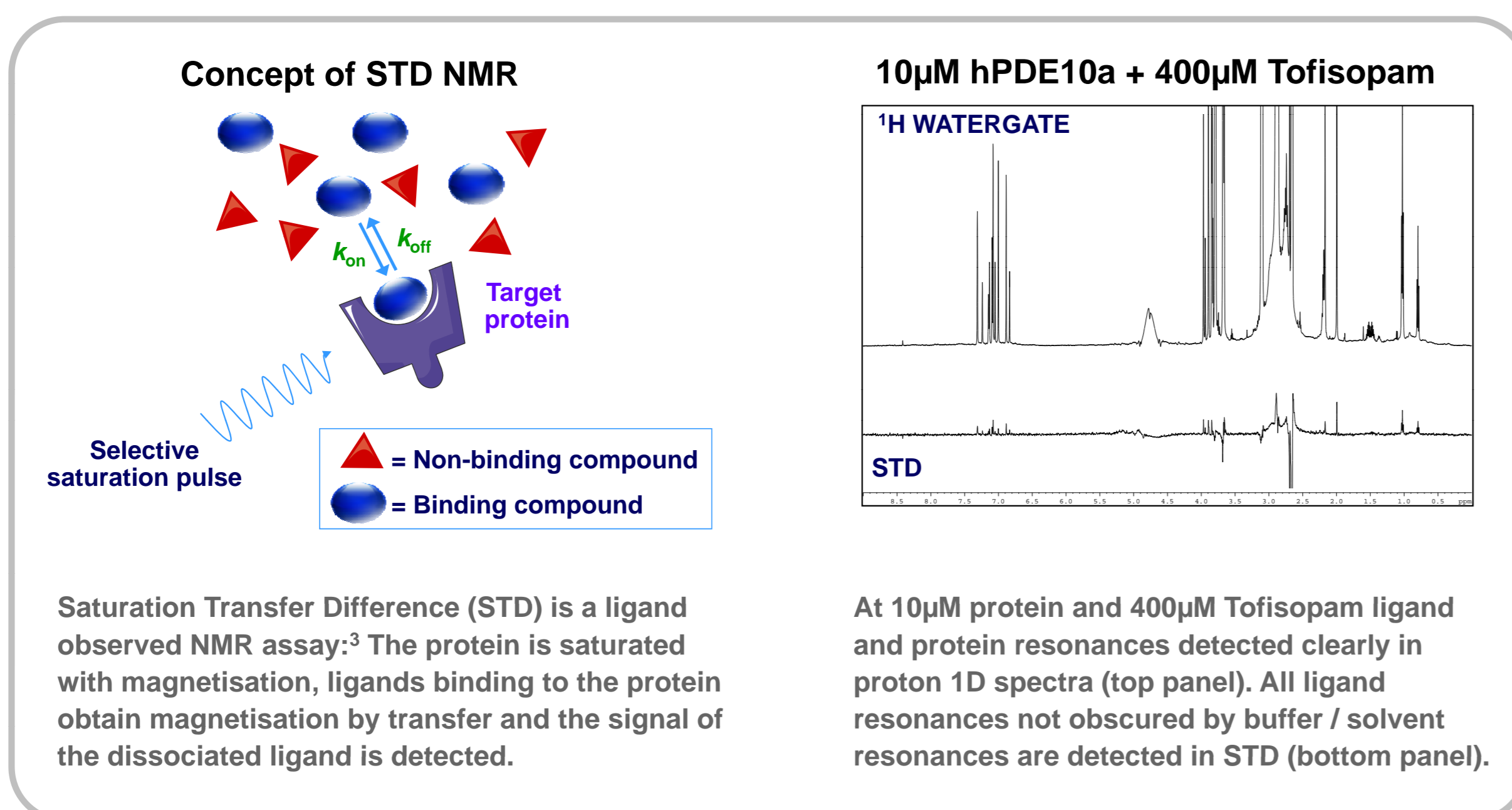
Evotec's fragment library (22,000 compounds) was screened against human PDE10a using the DMSO-free NanoStore™ concept and BellBrook Labs Transcreener™ PDE assay principle adapted to Evotec's sensitive FCS⁺plus fluorescent technology. Overall statistics were excellent with a mean Z' >0.8. A total of 905 fragment hits were identified with IC₅₀ below 1 mM. Subsequent screening against rat PDE10a demonstrated no species differences.



Assay Principle (Transcreener™ PDE Assay, BellBrook Labs): The observed fluorescence polarisation signal is reduced upon displacement of AMP/GMP Alexa633 tracer bound to the antibody by AMP/GMP substrate generated by PDE10a. The specificity of the signal was successfully controlled in a counter assay by adding the compound after stopping the PDE10a-mediated reaction with EDTA.



Confirmation of fragment binding by saturation transfer difference NMR



Representative fragment hits from preferred series

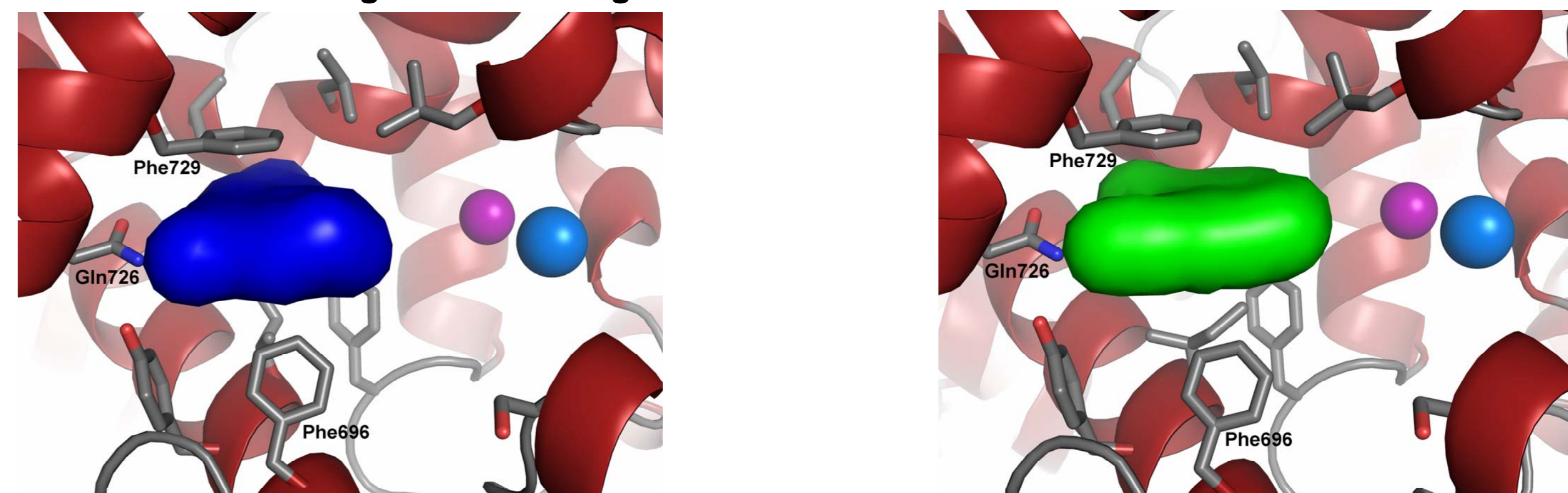
Series	hPDE10a IC ₅₀ [µM]	Molecular Weight [D]	Ligand Efficiency ⁴
1	2.4	258	0.43
2	4.0	234	0.44
3	3.1	286	0.38
4	3.5	292	0.34
5	49	268	0.30
6	17	234	0.39
7	284	176	0.38

Screening identified 905 fragment hits, from which 93 hits from 31 classes were prioritised by chemical tractability, novelty, ligand efficiency and aqueous solubility. Following confirmation in the secondary NMR assay described earlier, 10 classes were selected for SAR expansion and crystal trials – 16 crystal structures have been obtained thus far from 8 series.

Virtual high throughput screen

A database of ~3 million commercial compounds was filtered on MW, cLogP, TPSA and Lipinski fails to give ~140,000 fragment-like examples which were then scored by high throughput docking into the active site of PDE10a using GOLD. The top 500 scored compounds were further refined by removing molecules with 'undesirable' moieties; the remaining 357 compounds were then wet-screened with a hit rate >50% (IC₅₀ <1 mM). A novel chemical series was subsequently prioritised containing numerous examples with IC₅₀'s in the range 0.2-1µM (LE ≤ 0.4). A co-crystal structure of the lead compound has recently been obtained.

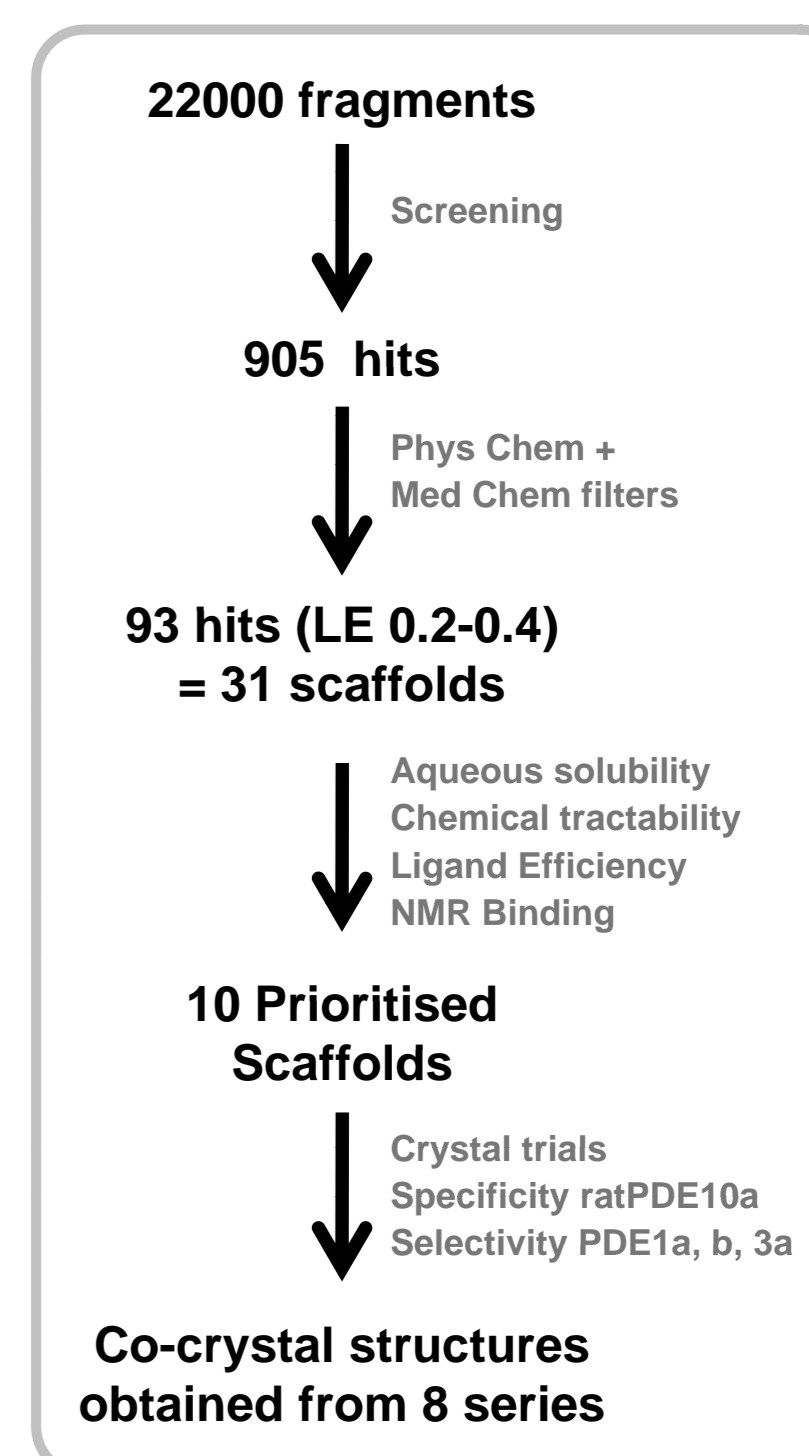
Structure-based design of PDE10a ligands



Example co-crystal structures of 2 preferred inhibitors in the active site of hPDE10a @ 2.0 Å and 1.9 Å respectively. Phe729 and Phe696 form the 'Phe clamp' which interacts with the nucleobase of the natural substrate (cAMP / cGMP). A further proposed key binding interaction is between cAMP / cGMP and Gln726. Tractable vectors have been identified for accessing key interactions and the proposed "PDE10a selectivity pocket" for a number of the fragment series. Guided by crystallographic information, a single round of analogues was prepared based on a fragment from series 2. A 34-fold increase in potency was achieved whilst maintaining ligand efficiency; further crystal structures of the more potent compounds were obtained to direct synthesis.

Evotec's fragment-based approach to identifying novel PDE10a inhibitors includes:

- A high throughput fragment screen (HTFS) of Evotec's 22,000 fragment library against the hPDE10a, utilising Evotec's sensitive FCS⁺plus fluorescent functional PDE10a assay.
- Prioritisation of hits according to aqueous solubility, chemical tractability and ligand efficiency (LE) and confirmation by protein NMR experiments.
- A complementary virtual screening campaign had a hit rate >50% and identified a further series.
- 16 Co-crystal structures of PDE10a with proprietary fragments and optimised inhibitors have been obtained.
- Initial structure-based optimisation has increased the selectivity and potency of the fragment hits.



References

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- 2 Siuciak *et al.*, *Neuropharmacology*, 2006, **51**(2), 374-85
- 3 Mayer and Meyer, *J. Am. Chem.Soc.*, 2001, **123**, 6108
- 4 Carr *et al.*, *Drug Discovery Today*, 2005, **10**(14), 987-992