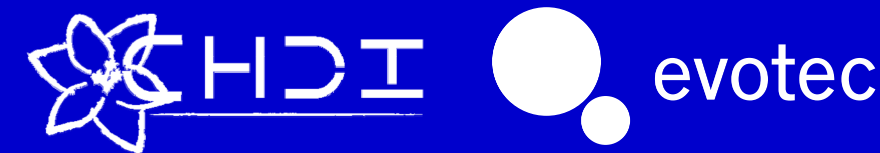
The Development of Potent and Selective Transglutaminase-2 Inhibitors for the Treatment of Huntington's Disease



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Introduction

Tissue transglutaminase-2 (TG2; TGM2; human Gene ID# 7052) is a multi-functional protein primarily known for its calcium-dependent enzymatic activity of crosslinking proteins via an isopeptide bond formation between glutamine and lysine residues, but has also recently been shown to act as a GTPase and ATPase. TG2 over-expression and activity has been found to be associated with Huntington's disease (HD) by several investigators; specifically, TG2 is up-regulated in the brains of HD patients as well as in *in vivo* models of the disease. Interestingly, knocking out TG2 in two different HD mouse models, R6/1 and R6/2, results in improved phenotypes including a reduction in neuronal death and prolonged survival. Thus we are interested in the discovery of potent and selective TG2 inhibitors for proof of concept experiments and eventual clinical development as an HD modifying agent.

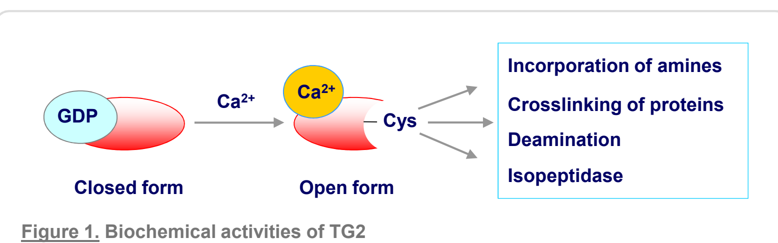
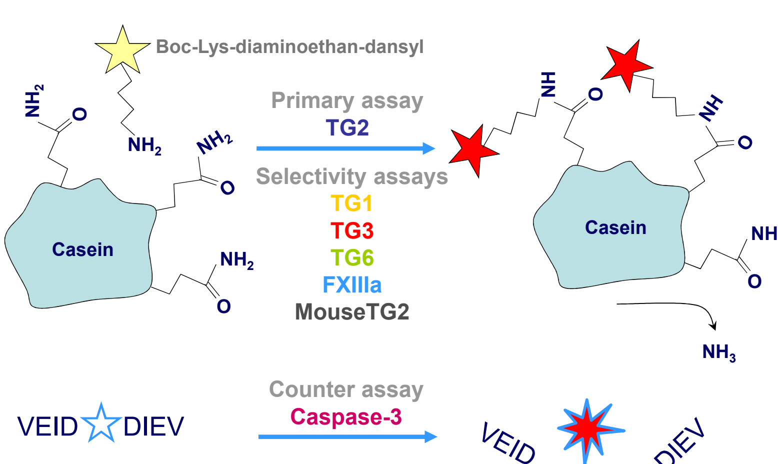


Figure 1. Biochemical activities of TG2

TG2 Transamidation Inhibition Assay



Cellular TG2 Inhibition Assay/Toxicity Assay

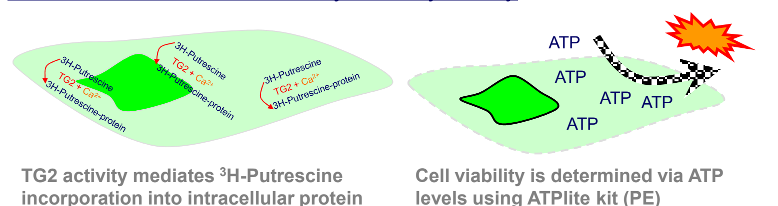


Figure 2. Assay portfolio established at Evotec to validate TG2 inhibitors

Mechanism of Action Assays

- Analyse reaction kinetics // pre-incubate at high concentration and dilute → reversible or irreversible binding?
- Reversible inhibitor: vary $CaCl_2$ and substrates → competitive or non-competitive interaction towards Ca^{2+} / substrate sites likely?
- Monitor GTP binding site using fluorescent GTPγS → does inhibitor binding influence GTP binding (direct or indirect?)

Target Profile and Hit Identification

Target inhibitor profile

Compounds to possess specific mode of inhibition (active site, GTP site)
TG2 IC_{50} (human and mouse) <0.1 μM
TG2 selective (>100 fold over TG1, TG3 and FXIIIa),
Cellular IC_{50} <0.5 μM,
Microsome stability >45 min human & mouse, reasonable PK profile with trough brain exposure three times the cellular IC_{50}.

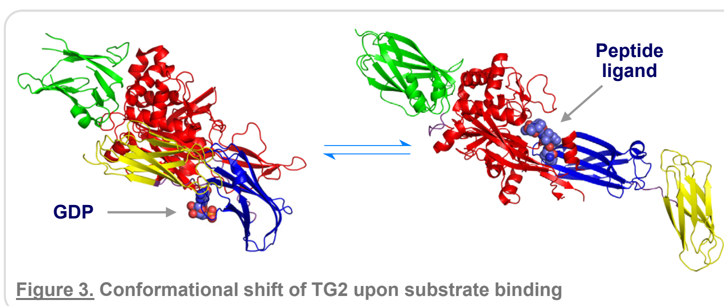
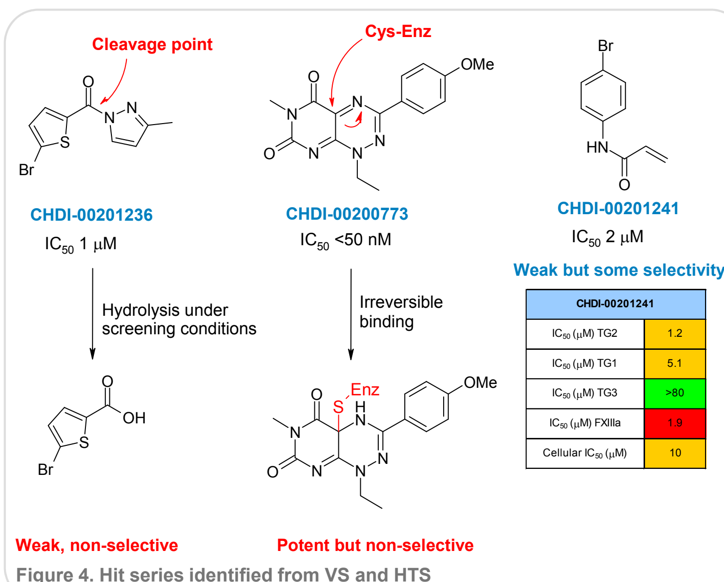


Figure 3. Conformational shift of TG2 upon substrate binding

Four approaches were employed to discover inhibitors of TG2:

- Virtual screening (carried out open and closed forms of TG2)
- High throughput screening (HTS)
- Fragment-based screening (FBS)
- Optimisation of literature TG2 inhibitors

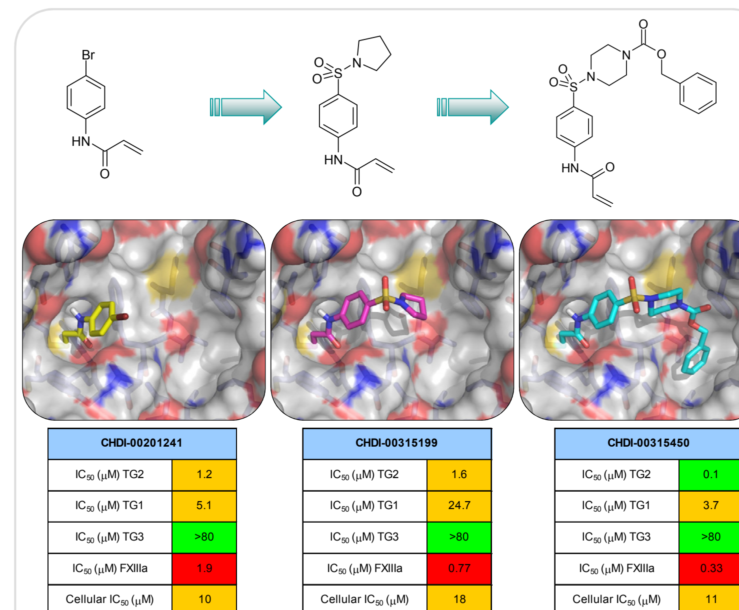


CHDI-00201241	
IC_{50} (μM) TG2	1.2
IC_{50} (μM) TG1	5.1
IC_{50} (μM) TG3	>80
IC_{50} (μM) FXIIIa	1.9
Cellular IC_{50} (μM)	10

Figure 4. Hit series identified from VS and HTS

Medicinal Chemistry

Initial SAR exploration of screening hit CHDI-00201241 and computational generation of a docking pose based around binding to active site cysteine 277 led to the generation of further SAR ideas. Significant improvements in biochemical potencies were achieved by designing compounds to reach the hydrophobic pocket observed in the crystal structure of TG2



CHDI-00201241	
IC_{50} (μM) TG2	1.2
IC_{50} (μM) TG1	5.1
IC_{50} (μM) TG3	>80
IC_{50} (μM) FXIIIa	1.9
Cellular IC_{50} (μM)	10

CHDI-00315199	
IC_{50} (μM) TG2	1.6
IC_{50} (μM) TG1	24.7
IC_{50} (μM) TG3	>80
IC_{50} (μM) FXIIIa	0.77
Cellular IC_{50} (μM)	18

CHDI-00315450	
IC_{50} (μM) TG2	0.1
IC_{50} (μM) TG1	3.7
IC_{50} (μM) TG3	>80
IC_{50} (μM) FXIIIa	0.33
Cellular IC_{50} (μM)	11

Figure 5. Docking poses for initial hit development

Homology modelling indicated that the hydrophobic pocket present within the active site of TG2 in a fully activated open conformation varies significantly between the TGase isoforms.

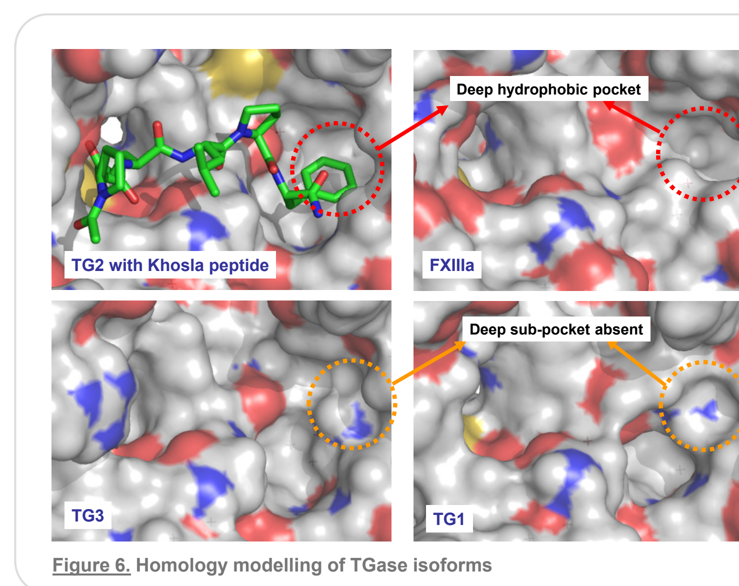
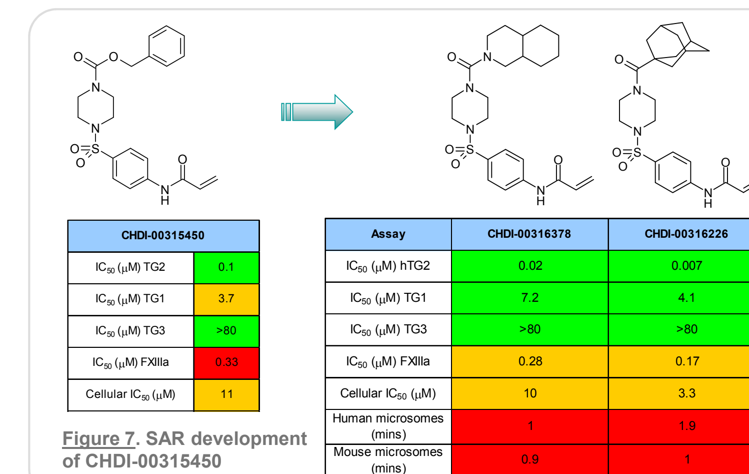


Figure 6. Homology modelling of TGase isoforms

Only FXIIIa appears to contain a hydrophobic pocket of similar size and depth to that observed in the crystal structure of open form TG2. We postulated that increasing the space occupancy of this hydrophobic pocket would lead to an increase in selectivity over TG1 and TG3.

Initially, increasing the size of the hydrophobic head group only afforded minimal improvements to the selectivity profile. Replacement of the carbamate unit with a cyclic urea or a cyclic amide led to a dramatic increase in both primary potency and selectivity. To achieve target potency against TG2 with selectivity over TG1 and TG3, a fairly rigid, bulky head group was required.

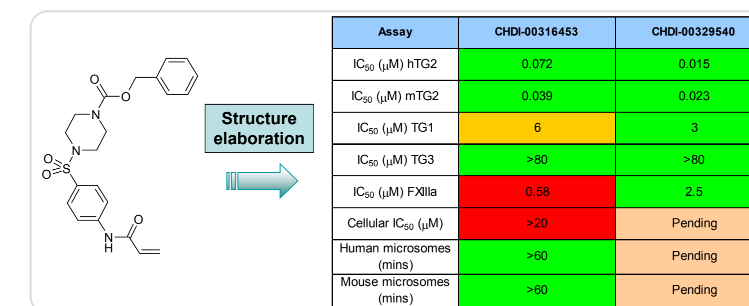


CHDI-00315450	
IC_{50} (μM) TG2	0.1
IC_{50} (μM) TG1	3.7
IC_{50} (μM) TG3	>80
IC_{50} (μM) FXIIIa	0.33
Cellular IC_{50} (μM)	11

Assay	CHDI-00316378	CHDI-00316226
IC_{50} (μM) hTG2	0.02	0.007
IC_{50} (μM) TG1	7.2	4.1
IC_{50} (μM) TG3	>80	>80
IC_{50} (μM) FXIIIa	0.28	0.17
Cellular IC_{50} (μM)	10	3.3
Human microsomes (mins)	1	1.9
Mouse microsomes (mins)	0.9	1

Figure 7. SAR development of CHDI-00315450

Whilst nM potencies against TG2 were obtained and >100 fold selectivity over TG1 and TG3 were achieved, FXIIIa activity and cellular activity remained an issue. Further to this, microsomal stability studies suggested that the compounds will be rapidly cleared in both human and mouse. Subsequent manipulations led to the development of a second generation of inhibitors showing improved selectivity and improved ADMET profiles.



Assay	CHDI-00316453	CHDI-00329540
IC_{50} (μM) hTG2	0.072	0.015
IC_{50} (μM) mTG2	0.039	0.023
IC_{50} (μM) TG1	6	3
IC_{50} (μM) TG3	>80	>80
IC_{50} (μM) FXIIIa	0.58	2.5
Cellular IC_{50} (μM)	>20	Pending
Human microsomes (mins)	>60	Pending
Mouse microsomes (mins)	>60	Pending

Summary

Traditional high throughput screening as well as fragment and virtual screening enabled the identification of hit compounds that display inhibition of the transamidation activity of TG2. These compounds have been significantly developed to show excellent biochemical potencies, excellent selectivity over other TGase isoforms and promising *in vitro* ADMET profiles. This series of compounds is currently undergoing pharmacokinetic profiling with a view towards carrying out proof of concept studies in animal models of HD.

References

Liu S, Cerione RA, Clardy J.(2002) Structural basis for the guanine nucleotide-binding activity of tissue transglutaminase and its regulation of transamidation activity. *Proc. Natl. Acad. Sci. USA*, **2002**, *99*, 2743-7.

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