

Identification of a novel BACE1 inhibitor scaffold via an *in silico* screening approach

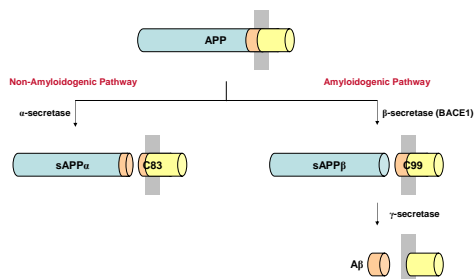
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Introduction

Aging is a major risk factor for Alzheimer's disease (AD) and the number of AD patients will increase as our population grows older. For this reason, therapeutic approaches are urgently sought to treat this debilitating disease. The accumulation of amyloid β -peptide ($A\beta$) into insoluble plaques in the brain is a key event in the pathogenesis of AD. The first step in $A\beta$ formation is the cleavage of the amyloid precursor protein (APP) by the β -site APP cleaving enzyme (BACE1).

Amyloid precursor protein (APP) processing leading to $A\beta$ formation



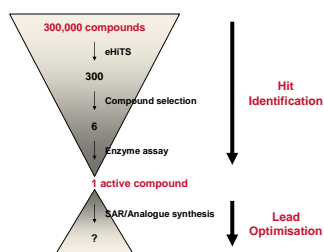
As $A\beta$ appears to initiate the neurodegeneration seen in AD, BACE1 is considered an attractive target for the development of small molecule inhibitors to treat the disease.

Virtual high throughput screening

Virtual high throughput screening (VHTS) provides a quick and efficient method for lead identification, and is becoming an integral part of the drug design process. With the recent availability of X-ray crystallographic structures of BACE1 inhibitor complexes, *in silico* screening provides an attractive approach to identifying potential BACE1 inhibitors.

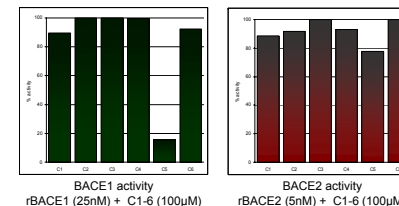
We have used the computer programme eHiTS (Electronic High Throughput Screening) to screen 300,000 compounds from three commercially available compound libraries against BACE1. The essential elements of *in silico* screening are a fragment-based docking procedure coupled with an efficient evaluation of the binding interaction.

Six compounds were selected for purchase based upon their predicted binding affinity and physicochemical characteristics (i.e. are they likely to cross the blood-brain barrier).

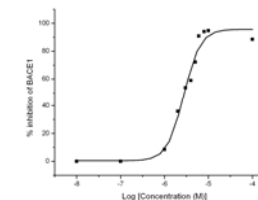


Compound analysis

Compounds were tested in an *in vitro* enzyme activity assay using a quenched fluorescent peptide substrate based upon the Swedish mutant APP sequence (SEVNLDAEFK).



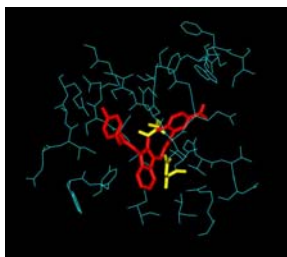
Compound 5 showed selective inhibition of BACE1 over BACE2



Concentration-response curve showing inhibition of BACE1 by Compound 5
IC₅₀(rBACE1) = 2.4 μ M

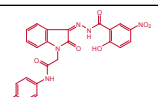
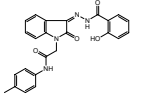
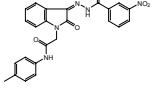
Analogue synthesis

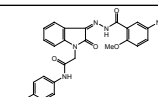
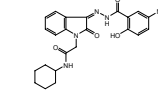
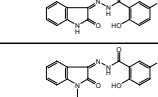
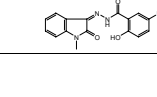
Based upon the predicted binding mode of the hit compound and the observed selectivity for BACE1 over BACE2, several analogues were prepared to explore the structure-activity relationship for activity.



Predicted binding pose of hit compound identified by VHTS with eHiTS

The inhibitor is shown in red, the enzyme in blue with the two catalytic aspartate residues depicted in yellow.

	Structure	BACE1 inhibition at 100 μ M (%)
1		84.5
2		40.2
3		10.1

	Structure	BACE1 inhibition at 100 μ M (%)
4		20.2
5		92.0
6		34.0
7		10.9

Conclusions

- The discovery of a BACE1 inhibitor with low micromolar activity was achieved by virtual high throughput screening.
- Several analogues were prepared to explore structure-activity relationships.
- It was observed that subtle changes in the inhibitor structure can lead to changes in the activity of these compounds against BACE1.
- The presence of free hydroxy and nitro groups appears to play an important role in determining activity for this class of compounds (entries 2, 3 and 4 in the table).
- As demonstrated by the drop in activity when removing the amide portion, this group also appears to be essential for inhibition (entries 6 and 7).
- It may also be possible to vary the nature of this amide portion to prepare more potent compounds (entry 5).

The combination of an *in silico* approach with validation by *in vitro* enzyme assays has led to the identification of a new BACE1 inhibitor scaffold.

Acknowledgements

We would like to thank the Alzheimer's Research Trust (JC and KABD) and the Hong Kong Croucher Foundation (NYM) for funding.