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Fragment-based discovery and optimization of BACE1 inhibitors

James Madden [a,*], Jenny R. Dod [a], Robert Godemann [b], Joachim Kraemer [b], Myron Smith [a], Marion Biniszkiewicz [b], David J. Hallett [a], John Barker [a], Jane D. Dyekjaer [a], Thomas Hesterkamp [b]

[a] Evotec UK Ltd, 114 Milton Park, Abingdon OX14 4SA, UK
[b] Evotec AG, Schnackenburgallee 114, 22525 Hamburg, Germany

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ABSTRACT

A novel series of 2-aminobenzimidazole inhibitors of BACE1 has been discovered using fragment-based drug discovery (FBDD) techniques. The rapid optimization of these inhibitors using structure-guided medicinal chemistry is discussed.

Alzheimer's disease (AD) presents one of the greatest unmet medical needs facing aging populations worldwide. Beta secretase (BACE1) catalyzes the formation of β-amyloid (Aβ), which can form amyloid plaques, one of the pathological hallmarks of AD. The discovery of orally bioavailable, brain-penetrant, small molecule inhibitors of BACE1 has been the goal of a number of drug discovery companies. The widely acknowledged failure of high-throughput screening (HTS) approaches to identify brain-penetrant BACE1 inhibitors[1] has given extra impetus to alternative hit discovery strategies such as fragment-based drug discovery (FBDD) screening techniques such as NMR, crystallography, and surface plasmon resonance (SPR).[2]

Herein, we describe the use of a high-throughput BACE1 assay in combination with orthogonal hit confirmation using SPR and structural elucidation by X-ray crystallography to identify a number of fragment inhibitors of BACE1. We go on to describe the initial optimization and profiling of one such example.

Evotec's fragment library of 20,000 compounds,[3] with an average molecular weight of 250, was screened at 1 mM against BACE1.[4] The resulting hits were clustered by chemotype and ranked according to their novelty with reference to known BACE1 inhibitors and their ligand efficiency. Exemplars from each cluster were submitted for confirmation of binding to BACE1 using SPR.[2] Figure 1 shows example structures of fragment hits and Table 1 their assay IC_{50} compared to their BACE1 inhibition in SPR.

Although SPR did not recapitulate the affinity seen in the functional assay, all of the compounds did show some degree of binding to BACE1 in the SPR experiment. Compounds were further ranked for submission to crystallography according to their aqueous solubility (data not shown). Top ranked compounds were submitted either for co-crystallization or soaking with BACE1.[2] Fragment **3** was successfully co-crystallized with BACE1 and the structure solved to 1.8 Å resolution.[5] Figure 2 shows **3** bound in the active site of BACE1.

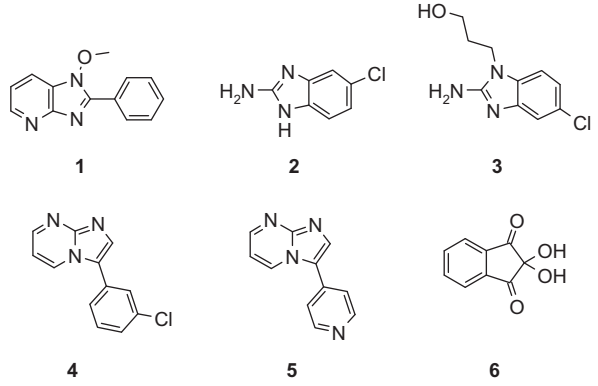

Figure 1. Example fragment inhibitors identified from high-throughput functional assay.

* Corresponding author. Tel.: +44 (0)1235441368.
E-mail address: james.madden@evotec.com (J. Madden).

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Table 1
Comparison of functional assay with SPR

Compd id	Functional assay IC_{50}[a] (μM)	Ligand efficiency[b]	SPR[c] % inhibition @ 2 mM
1	928	0.25	36
2	731	0.40	34
3	770	0.29	63
4	862	0.27	29
5	1008	0.28	20
6	776	0.34	27

[a] $n \geqslant 2$.
[b] LE = $-RT \ln(IC_{50})$/non-H atom count.
[c] $p < 0.05$.

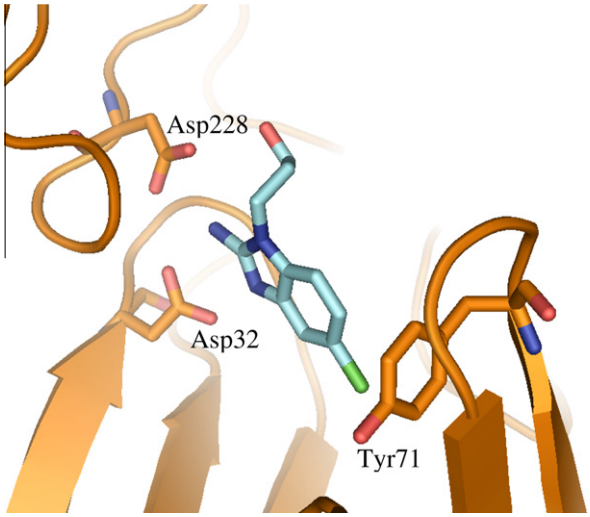


Figure 2. Fragment **3** bound in the active site of BACE1.

The endocyclic N in the 3-position forms a hydrogen bond with Asp32. The exocyclic N forms a hydrogen bond with both catalytic aspartates and the hydroxyl hydrogen bonds to Asp228. The aryl unit forms a face-to-edge π-interaction with Tyr71 and the 5-chloro substituent sits in a pocket formed between Tyr71 and Trp76. The BACE1 protein adopts the flap open conformation typical of many of the published small molecule-BACE1 structures.[6] A comparison of the structure of fragment **3** with known BACE1 inhibitors highlighted the similarity to Johnson & Johnson's BACE1 inhibitors.[7] The published crystal structure of one of their compounds, (4*S*)-4-(2-amino-7-phenoxyquinazolin-3(4*H*)-yl)-*N*,4-dicyclohexyl-*N*-methylbutanamide (**7**, Fig. 3), was overlaid with that of **3** and is shown in Figure 4.

The J&J compound contains an aminoquinazoline core that binds to both catalytic aspartates in a similar manner to **3**. In lieu of the 5-chloro substituent of **3** the J&J inhibitor features a 5-phenoxy substituent that forces Tyr71 up out of the active site and allows the phenoxy to pick up a face-to-edge interaction with Phe108. A branched chain at the 3-position of the quinazoline accesses further interactions with the prime and non-prime

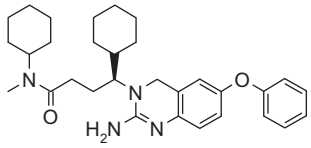


Figure 3. Johnson & Johnson's BACE1 inhibitor.

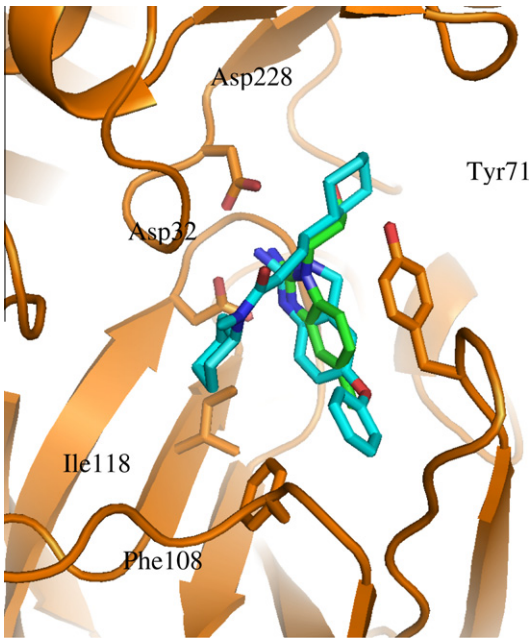


Figure 4. Fragment **3** (green) overlaid with J&J inhibitor **7** (blue) in the active site of BACE1.

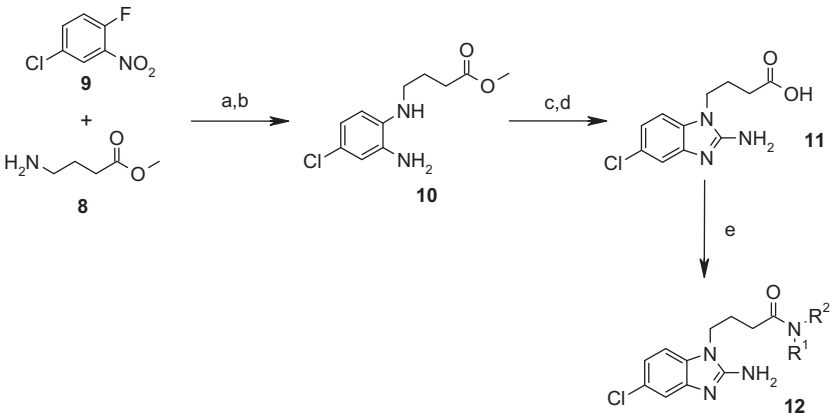
sites of the BACE1 protein. The cyclohexyl occupies a largely hydrophobic pocket in the prime side of the site while the propanamide branches towards the S3 pocket. In silico docking of analogs of **3** suggested that the hydroxypropyl moiety could be modified or replaced to target the non-prime side of the active site and improve affinity. Indeed, numerous groups have successfully employed a strategy to target the S3 pocket and its conserved water molecules as a means of improving potency.[8] However, before progressing fragment **3**, the available published data from J&J showed that compound **7** suffered from a hERG liability (hBACE1 K_i 8 nM, hERG K_i 84 nM).[9] Given the similarity of fragment **3** with the J&J lead, our rationale was to improve the affinity of compound **3** for BACE1 to a point where selectivity over hERG could be measured and used as a decision point for progressing the series. Our calculated pK_a for fragment **3** of 7.7 was thought to be sufficiently different from that reported by J&J (pK_a 10.6)[7] to permit a possible change in recognition of our molecules in the hERG binding site.

Analogs of **3** were rapidly accessed using commercially available building blocks (BB) as shown in Scheme 1. SnAr of methyl 4-aminobutanoate with 3-chloro-6-fluoronitrobenzene, reduction of the nitro group and aminoimidazole formation using cyanogen bromide all proceeded cleanly. Saponification and concentration afforded the final step precursor as the 2-aminobenzimidazole lithium carboxylate salt. Efforts to couple this salt with amines resulted in the intramolecular cyclization to the lactam by-product. It was found that acidification of the crude saponification concentrate with excess HCl in dioxane and subsequent amidation using the HCl salt of the 2-aminobenzimidazole acid resulted in minimal intramolecular cyclization.

Using this chemistry a range of 12 analogs was prepared. Their activities are given in Table 2.

Demonstrating a 29-fold increase in potency over fragment **3** and a small decrease in LE, compound **12b** was selected for crystallography and its structure was solved to 2.6 Å.[3] Figure 5 shows **12b** overlaid with J&J compound **7**.

The central 2-aminobenzimidazole core of **12b** retains the same position as **3** and the butanamide branches towards the non-prime



Scheme 1. Synthesis of analogs of **3**. Reagents and conditions: (a) Et_3N, dioxane, 80 °C, 85%; (b) Fe powder, NH_4Cl (aq), 60 °C; (c) BrCN, EtOH, 80 °C; (d) (i) $LiOH{\cdot}H_2O$, H_2O/THF, (ii) 4 M HCl in dioxane, 23% (three steps); (e) R^1R^2NH, EDCI·HCl, $HOBt{\cdot}H_2O$, DMF.

Table 2
BACE1 IC_{50} for analogs of **3**

Compd id	R^1	R^2	Functional assay IC_{50}[a] (μM) (LE)[b]
12a	Benzyl	H	1403 (0.17)
12b	Cyclohexyl	Me	26 (0.27)
12c	Isopropyl	H	193 (0.26)
12d	2-Morpholin-4-ylethyl	H	827 (0.17)
12e	2-Pyrrolidin-4-ylethyl	H	444 (0.20)
12f	Pyridine-2-ylmethyl	H	426 (0.20)
12g	Cyclohexylmethyl	H	83 (0.24)
12h	1-Methylpiperidin-4-yl	H	325 (0.21)
12i	3-Fluorobenzyl	H	249 (0.20)
12j	Cyclopropylmethyl	H	2854 (0.17)
12k	3-Fluorobenzyl	Me	194 (0.20)
12l	Cyclohexylmethyl	Me	59 (0.24)

[a] $n \geqslant 2$.
[b] LE = $-RT \ln(IC_{50})$/non-H atom count.

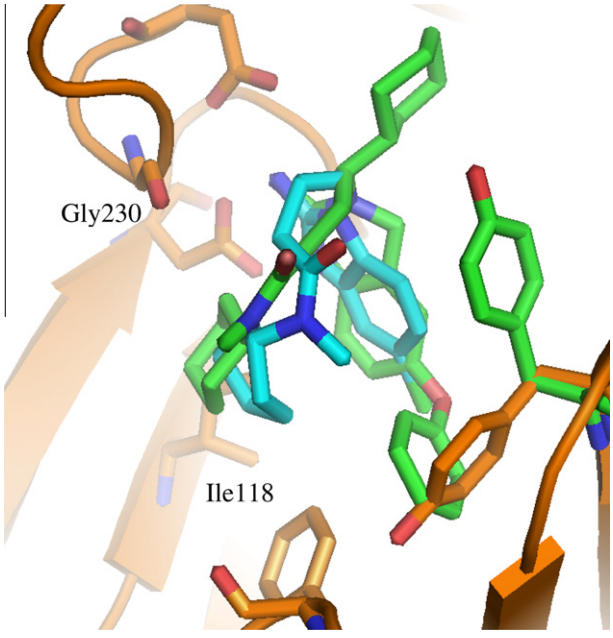


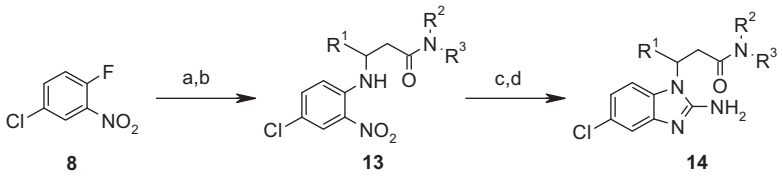
Figure 5. Compound **12b** (blue) overlaid with J&J inhibitor **7** (green) in the active site of BACE1.

side of the protein. Instead of picking up any interactions with the protein, **12b** is seen to fold in on itself; the cyclohexyl sits in a hydrophobic pocket close to Ile118 and Phe108. The amide does not appear to form any H-bonding interactions with the protein. The SAR summarized in Table 2 highlights a preference of the protein for medium sized hydrophobic amides. It was rationalized that an additional α-branching group targeting the prime side of the protein in combination with a range of polar or non-polar amides might yield additional gains in potency by interacting with residues or water molecules in or near the S3 pocket. In order to quickly access compounds of this type, it was envisaged that commercially available β-amino acids could be employed in chemistry analogous to that described in Scheme 1. Although these building blocks would afford compounds with **1** carbon fewer in the branched chain, it was rationalized that the desired binding interactions could still be achieved through the use of extended amine inputs. Scheme 2 describes the route that was used to synthesize these analogs. With this route the use of the HCl salt of the 2-aminobenzimidazole acid, as with Scheme 1, was not effective in reducing the formation of cyclic lactam by-product. Therefore the route was redesigned such that β-amino acids were used in the initial SnAr and the amide diversity introduced at the 2nd step of the synthesis.

Analysis of the crystal structure of **12b** suggested that the preferred stereochemistry around the α-branched carbon would be *S*[10] and indeed this proved to be the case. However, initially two pairs of analogs were synthesized using both enantiomers of 3-aminopentanoic acid and 3-amino-5-methylhexanoic acid. Once the preferred stereochemistry had been established, the *S* enantiomers of both acids were utilized for further analog synthesis. The results are shown in Table 3.

As with the non-branched analogs, the addition of polarity in the non-prime site was not tolerated by BACE1 and small or medium sized hydrophobic substituents appeared to be preferred. The cyclohexylmethyl and adamantyl groups (**14i** and **14m**) afforded modest improvements in potency and LE over **12b**. Attempts to introduce a H-bond acceptor into **14i** as in **14k** resulted in a drastic loss in potency. Compounds **14i** and **14m** were selected for crystallography and their structures solved to 2.4 Å and 2.6 Å, respectively.[3]

As shown in Figure 6 the binding mode of both compounds is highly conserved. As expected, the α-branched ethyl occupies the prime side of the protein with both amide substituents positioned in the hydrophobic pocket close to Ile118 and Phe108. Although the amide N–H's are both within hydrogen bonding distance of Gly230 at the edge of the S3 pocket, the torsional angle forced on the amide by the location of the cyclohexylmethyl



Scheme 2. Synthesis of analogs of **12b**. Reagents and conditions: (a) β-amino acid, Et_3N, dioxane, 80 °C; (b) R^2R^3NH, EDCI·HCl, HOBt·H_2O, DMF; (c) $SnCl_2·2H_2O$, DMF, EtOH; (d) BrCN, EtOH, 80 °C.

Table 3
BACE1 IC_{50} for analogs of **12b**

Compd id	R^1 (stereochem.)	R^2	R^3	Functional assay IC_{50}[a] (μM) (LE)[b]
14a	Et (*R*)	H	Benzyl	516 (0.19)
14b	Et (*S*)	H	Benzyl	72 (0.24)
14c	^{i}Bu (*R*)	H	Isopropyl	>1000
14d	^{i}Bu (*S*)	H	Isopropyl	246 (0.22)
14e	Et (*S*)	H	Isopropyl	695 (0.21)
14f	Et (*S*)	H	2-Morpholin-4-ylethyl	440 (0.18)
14g	Et (*S*)	H	Cyclopropylmethyl	243 (0.23)
14h	Et (*S*)	H	2-Pyrrolidin-4-ylethyl	270 (0.20)
14i	Et (*S*)	H	Cyclohexylmethyl	7.0 (0.29)
14j	Et (*S*)	Me	Cyclohexylmethyl	375 (0.19)
14k	Et (*S*)	H	Tetrahydropyran-4-ylmethyl	>1000
14l	Et (*S*)	H	Pyridine-2-ylmethyl	>1000
14m	Et (*S*)	H	2-Adamantyl	8.9 (0.26)
14n	Et (*S*)	H	3,3-Dimethylbutyl	95 (0.24)
14o	Et (*S*)	H	3-Fluorobenzyl	340 (0.19)
14p	Et (*S*)	H	4-Fluorobenzyl	264 (0.20)
14q	Et (*S*)	H	*O*-^{t}butyl-L-serineOMe	168 (0.18)
14r	Et (*S*)	H	*O*-^{t}butyl-D-serineOMe	75 (0.20)
14s	Et (*S*)	H	*O*-^{t}butyl-L-serineOH	87 (0.21)
14t	^{i}Bu (*S*)	H	Pyridine-2-ylmethyl	235 (0.19)
14u	^{i}Bu (*S*)	H	3-Fluorobenzyl	54 (0.22)

[a] $n \geqslant 2$.
[b] LE = $-RT \ln(IC_{50})$/non-H atom count.

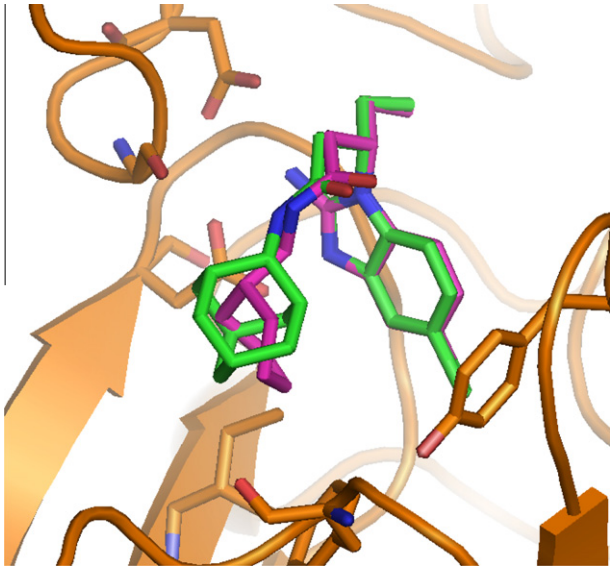


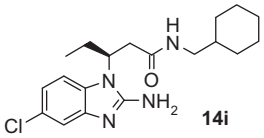
Figure 6. Compound **14i** (magenta) and **14m** (green) in the active site of BACE1.

or adamantyl in the hydrophobic pocket appears to preclude this interaction.

Compound **14i** was profiled and showed comparable activity in the BACE1 cellular Aβ secretion assay, was clean in Ames test with and without S9 metabolic activation and was not cytotoxic. The compound is equipotent against hBACE2 and hERG and has slight selectivity over Cathepsin D (see Table 4).

Despite a 100-fold improvement in the affinity of the series for BACE1 over two iterations of synthesis, the activity of **14i** and other analogs (data not shown) at hERG led us to deprioritize this series in favor of better fragment starting points and these will be the subject of a future publication.

Table 4
Profile of **14i**



Profile	Value
TPSA	73 Å^2
MW	363
pK_a	7.7
hBACE1 IC_{50}	7.0 μM
Aβ secretion IC_{50}	12 μM
% Cytotox @ 50 μM	0
Ames ± S9	Negative
Cathepsin D	42 μM
hBACE2 IC_{50}	8.0 μM
hERG IC_{50}	7.5 μM
Human/rat liver microsomes $t_{1/2}$	5.5/6.7 min
$PAMPA_{maxflux}$	12%

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5. Structure deposited with PDB. PDB ID Codes: 3MSJ (Fragment **3**), 3MSK (Ex. **12b**), 3MSL (Ex. **14i**), 3MSM (Ex. **14m**). The co-crystal for fragment **3** was obtained by Proteros Biostructures GmbH, Martinsried, Germany. The co-crystals, for examples, **12b**, **14i**, and **14m** were obtained at Evotec UK. For details of the human BACE construct employed and crystallization conditions see Ref. 4.
6. PDB Examples: 2OHQ (Astex), 3BRA (Roche), 2Q15 (Johnson & Johnson), and 2QMD (Schering-Plough).
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10. Note change of priority when assessing stereochemistry of Evotec compounds versus J&J compound.