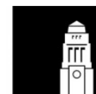

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Expansion of the structure-activity relationships of BACE1 inhibitors by harnessing diverse building blocks prepared using a unified synthetic approach

Joan Mayol-Llinàs,[a,b] Shiao Chow[a,b] and Adam Nelson*[a,b]

The structural diversity of β-site amyloid precursor protein cleaving enzyme 1 (BACE1) inhibitors was expanded by harnessing diverse building blocks that had been prepared via a unified lead-oriented synthetic approach. It was shown that the lipophilic cyclohexylmethyl group within a known series of BACE1 inhibitors could be productively replaced with a range of alternative ring systems.

Introduction

The exploration of chemical space, whilst controlling molecular properties at each stage of discovery, is an intrinsic challenge in medicinal chemistry.[1] Historically, chemical space has been explored unevenly and unsystematically,[2] which has limited the scaffold diversity of exemplified medicinal chemistry space.[3] Furthermore, discovery practices have driven medicinal chemists away from optimal property space, and have increased the focus on flatter and more lipophilic compounds.[4] Molecular weight (MW), lipophilicity and molecular complexity tend to increase during lead optimisation.[5] The challenge of controlling molecular properties in CNS discovery programmes is further heightened by the need to cross the blood-brain barrier.[6] To assist discovery, a multi-parameter optimisation (MPO) system for scoring CNS drug-likeness[7] has been developed that integrates scores for six key molecular properties.[§]

Previously, we adapted[8] the CNS Lead MPO scoring system[7] to guide the development of a unified synthetic approach to diverse scaffolds that, on decoration, would provide high-quality starting points for CNS drug discovery.[9] Here, we demonstrate that these building blocks may be harnessed to expand the structural diversity of inhibitors of the CNS target BACE1. BACE1 is an aspartic protease that is responsible for the formation of amyloid β by sequential cleavage of amyloid precursor protein (APP).[10] The aminoquinoline fragment **1**, which interacts with the two catalytic Asp residues, previously served as a starting point for fragment-based inhibitor discovery of BACE1 inhibitors.[11] Optimisation had initially involved the addition of an amide chain (to give **2**) bearing a lipophilic cyclohexylmethyl group. Successive addition of an *o*-tolyl group (to give **3**) and a methyl group (to give **4**) resulted, respectively, in ~900- and 9-fold increases in activity (Figure 1).

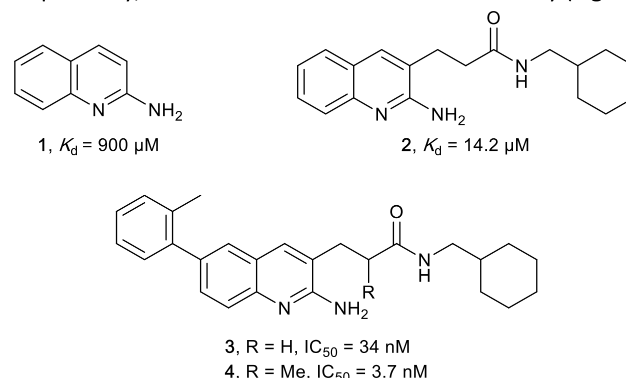

Figure 1. Fragment-based discovery of a new class of BACE1 inhibitors (ref. 11).

Results and Discussion

Initially, we investigated the replacement of the lipophilic cyclohexylmethyl group in the inhibitor **2** with more highly functionalised substituents. Here, we exploited diverse building blocks that had been prepared using a unified synthetic approach developed to yield lead-like scaffolds for CNS drug discovery.[9] We initially prepared analogues of the aminoquinoline **2** in two steps from the ester[11] **5** (Table 1). In each case, the ester **5** was aminolysed[12] with the deprotected analogue of the appropriate racemic amine-containing building block **6** (see Figure 2); TFA-induced deprotection then gave the corresponding free aminoquinoline.[11] For comparison, the known inhibitor **2** and its analogues **15**-**18** were also prepared from commercially-available amines.

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†Electronic Supplementary Information (ESI) available: synthetic procedures, compound characterisation, biochemical methods and computational approaches. See DOI: 10.1039/x0xx00000x

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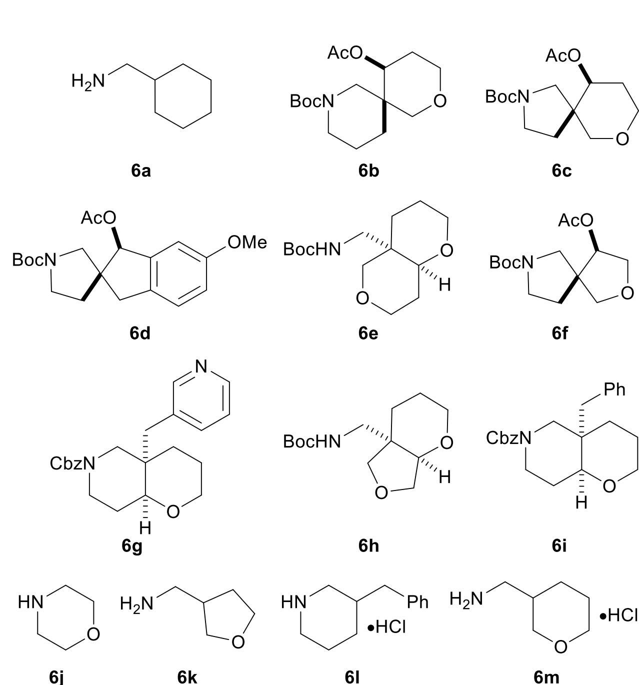

Figure 2. Building blocks used in this study. The chiral building blocks were racemic.

Inhibition of BACE1 was initially assessed at 100 μM using a fluorescence-quenching assay[13] based on the cleavage of a peptide substrate bearing a donor and a quencher at its termini. The dose-dependent activity of compounds that displayed significant inhibition at 100 μM was subsequently determined. Three compounds, in which the cyclohexylmethyl group had been replaced, had broadly comparable activity to the parent aminoquinoline **2** (IC_{50}: 31 μM): the analogue **10** (IC_{50}: 29 μM) in which the secondary amide linkage had been retained, and the tertiary amides **9** (IC_{50}: 55 μM) and **14** (IC_{50}: 84 μM). The discovery of tertiary amide inhibitors was interesting because the secondary amide NH of **2** has been shown to form a key hydrogen bond interaction with a glycine residue in the BACE1 active site.[11] Notably, simplified analogues of the active compounds were inactive: for example, the secondary amide **18** (with a tetrahydropyran replacing the bicyclic ring system of **10**) and the tertiary amide **17** (with a 3-benzyl piperidine replacing the benzyl-substituted bicyclic ring system of **14**).

To provide some structural insights into these structure-activity relationships, the aminoquinoline **2** and both enantiomers of **10** and **14** were docked onto BACE1 using GOLD (Figure 3 and ESI).[14] The structure of BACE1 in complex with the aminoquinoline fragment **1** (PDB 2OHL) was used as a starting point for these *in silico* studies. Since alternative conformations of Tyr71 have been observed in BACE complexes with different aminoquinoline ligands,[11] this residue was allowed to be flexible.

Table 1. Synthesis and evaluation of potential BACE1 inhibitors.

CO_2Et ... NH^{t}Bu **5** → Building block **6** (see below for methods) → NR^1R^2 amide, NH_2

Building block	Product (Methods[a])	NR^1R^2	Inhibition at 100 μM	IC_{50} (μM) [pIC_{50}]
6a	**2** (A, 98%; B: 83%)		>95%	31 [4.51 ± 0.06]
6b	**7**[b] (C,D,A, 21%; B, >99%)		<10%	–
6c	**8**[b] (C,D,A, 60%; B, 93%)		<10%	–
6d	**9**[b] (C,D,A, 75%; B, 32%)		>95%	55 [4.26 ± 0.01]
6e	**10**[b] (D,A, 28%; B, 98%)		>95%	29 [4.54 ± 0.02]
6f	**11**[b] (C,D,A, 72%; B, >99%)		<10%	–
6g	**12**[b] (E,A, 20%; B, 99%)		<10%	–
6h	**13**[b] (D,A, 31%; B, >99%)		<10%	–
6i	**14**[b] (E,A, 23%; B, >99%)		67%	84 [4.07 ± 0.01]
6j	**15** (A, 56%; B, 93%)		<10%	–
6k	**16** (A, 90%; B, 47%)		<10%	–
6l	**17**[b] (A, 37%; B, >99%)		<10%	–
6m	**18**[b] (A, 43%; B, >99%)		<10%	–

[a]Methods: A: TBD, toluene, 75 °C; B: TFA, 75 °C; C: NaOMe, MeOH; D: TFA, CH_2Cl_2; E: H_2, Pd/C, MeOH. TBD, triazabicyclodecene; TFA, trifluoroacetic acid. [b]Prepared as a racemate.

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Crystallographic studies had previously shown that the aminoquinoline **2** has two binding modes[11] in which its cyclohexyl substituent occupies either the P1 (magenta) or the P1′ (purple) pocket (Figure 3, Panel A). The docked pose of **2** (green) was similar to the binding mode in which the P1 pocket was targeted, with a similar orientation of Tyr71 (cyan) as the reported crystal structure (blue). Both enantiomers of **10** and **14** were also docked (see ESI). For **10**, the binding mode is predicted to be broadly similar to **2**, with a hydrogen bond maintained between Gly230 and the secondary amide NH in the ligand (Panel B). The tertiary amides **9** and **14** lack the capacity to hydrogen bond with Gly230; however, the benzyl substituent of both enantiomers of **14** is predicted to bind in the same pocket as the cyclohexyl group of **2** (Panel C).

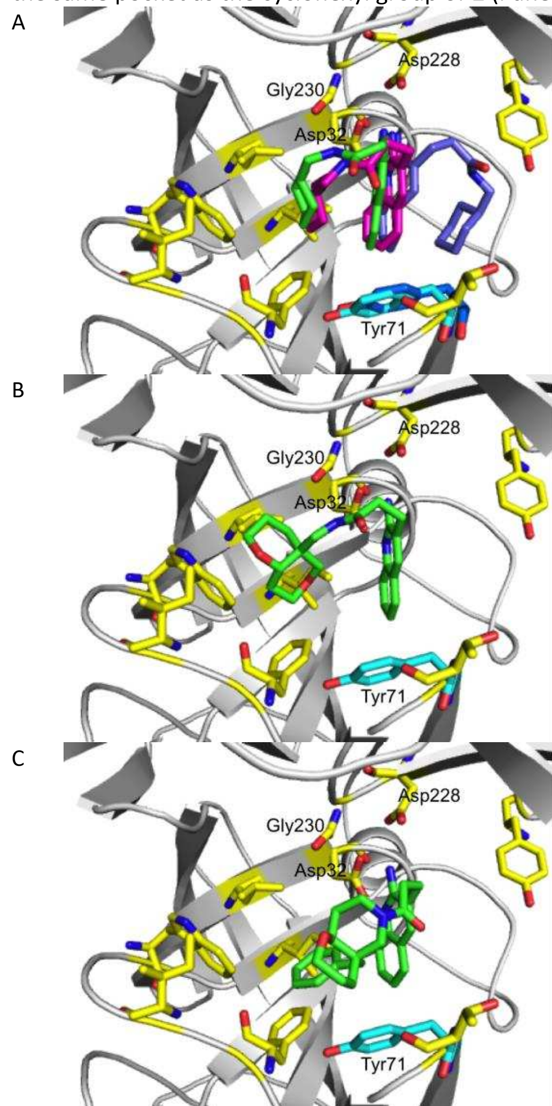

Figure 3. Interaction of inhibitors with BACE1. Panel A: Docked pose of the aminoquinoline **2** superimposed on the alternative binding modes determined by X-ray crystallography (magenta and purple; PDB 3RU1). Panel B: Docked pose of (4a*S*,8a*S*)-**10** (green). Panel C: Docked pose of (4a*S*,8a*S*)-**14** (green).

By analogy with the parent inhibitor **2** (Figure 1), we investigated the addition of an *o*-tolyl group and/or a methyl group to the compounds **9**, **10** and **14** (Table 2). In each case, aminolysis of the appropriate ester (**19**, **20** or **21**) was followed TFA-induced deprotection; compounds prepared from the chiral esters **19** and **21** were obtained as 50:50 mixtures of racemic diastereomers. The dose-dependent activity of the resulting nine products was determined.

As with the secondary amide **2**, the addition of an *o*-tolyl group to **10** (IC_{50}: 29 μM) to give **23** (IC_{50}: 120 nM) resulted in a dramatic (~240-fold) improvement in activity. Furthermore, the subsequent addition of a methyl group to **23** (to give **24**; IC_{50}: 31 nM) resulted in a further ~4-fold improvement in potency. The CNS drug-likeness scores[7] of these two compounds (3.47 for **23** and 3.00 for **24**) were broadly comparable with their cyclohexylmethyl analogues (3.20 for **3** and 3.10 for **4**). It is therefore possible to replace the cyclohexylmethyl substituent with a less lipophilic group (ΔclogP for each pair: ~2.1) whilst maintaining both potency and CNS drug-likeness.

The inhibitor **24**, in which both an *o*-tolyl group and a methyl group have been incorporated, was docked onto BACE1 (see ESI for all stereoisomers). The structure of BACE1 in complex with the aminoquinoline fragment **1** (PDB 2OHL) was used as a starting point, with alternative conformations of Tyr71 (cyan) allowed. The docked poses were substantially different to those of its unsubstituted analogue **10** (compare Figure 4, Panel A and ESI with Figure 3, Panel B): the P1′ pocket was now predicted to be targeted and the Tyr71 had a different orientation and formed a hydrogen bond with the secondary amide of the ligand. In the crystal structure[11] of 3-(2-amino-6-*o*-tolylquinolin-3-yl)-*N*-cyclohexylpropanamide in complex with BACE1 (PDB 3RTN), the cyclohexyl substituent also targeted the P1′ pocket and Tyr71 had a similar orientation (Figure 4, Panel B).

With the tertiary amides **9** (IC_{50}: 55 μM) and **14** (IC_{50}: 84 μM), the addition of an *o*-tolyl group had a much less dramatic effect: ~8-fold (to give **26**; IC_{50}: 6.1 μM) and ~14-fold (to give **29**; IC_{50}: 3.2 μM) improvements in activity respectively were observed. In addition, the subsequent addition of a methyl groups to **26** and **29** (to give **27** and **30**) resulted in only modest improvements in activity.

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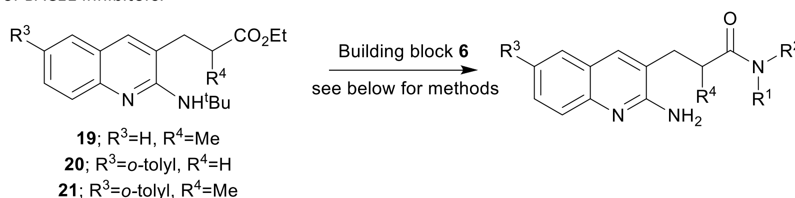
Table 2. Synthesis and evaluation of BACE1 inhibitors.



Substrate	Building block	Product	NR^1R^2	R^3	R^4	Method (Yield)	pIC_{50}	IC_{50} (μM)
19	6e	22		H	Me	D, A, B (43%[b])	4.48 ± 0.08	34
20	6e	23		*o*-tolyl	H	D, A, B (60%)	7.11 ± 0.07	0.12
21	6e	24		*o*-tolyl	Me	D, A, B (37%[b])	7.51 ± 0.06	0.031
19	6d	25		H	Me	C, D, A, B (29%[b])	5.43 ± 0.02	3.7
20	6d	26		*o*-tolyl	H	C, D, A, B (7%)	5.21 ± 0.06	6.1
21	6d	27		*o*-tolyl	Me	C, D, A, B (16%[b])	5.29 ± 0.06	5.1
19	6i	28		H	Me	E, A, B (18%[b])	4.47 ± 0.03	34
20	6i	29		*o*-tolyl	H	E, A, B (39%)	5.50 ± 0.03	3.2
21	6i	30		*o*-tolyl	Me	E, A, B (15%[b])	5.57 ± 0.03	2.7

[a]Methods: A: TBD, toluene, 75 °C; B: TFA, 75 °C; C: NaOMe, MeOH; D: TFA, CH_2Cl_2; E: H_2, Pd/C, MeOH. [b]The use of two racemic reactants resulted in a 50:50 mixture of diastereomeric products. TBD, triazabicyclodecene; TFA, trifluoroacetic acid.

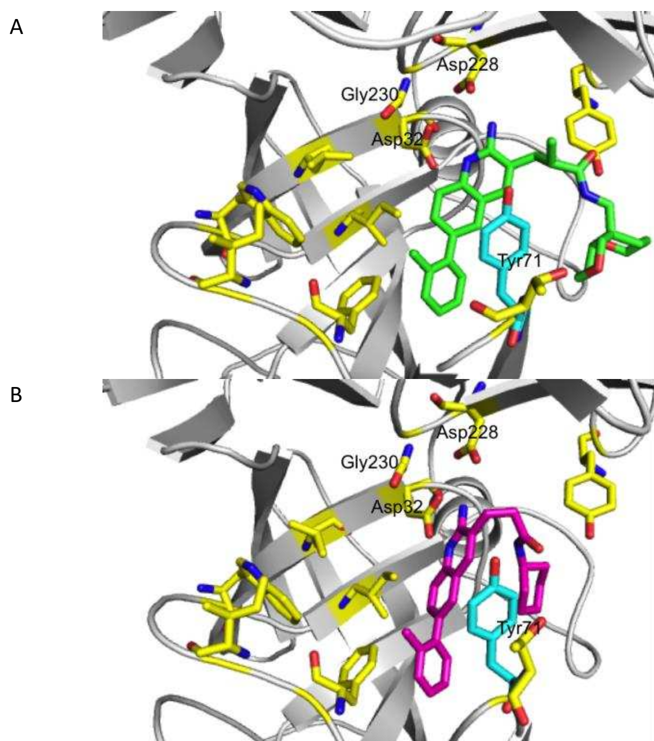


Figure 4. Complexes of 2-amino-6-*o*-tolylquinoline inhibitors with BACE1 with Tyr71 highlighted (cyan). Panel A: Docked pose of a representative stereoisomer of the ligand **24** [with 2*R* configuration in linker and (4a*S*,8a*S*)-configured building block] Panel B: Co-crystal structure of 3-(2-amino-6-*o*-tolylquinolin-3-yl)-*N*-cyclohexylpropanamide with BACE1 (PDB 3RTN).

Conclusion

We have demonstrated that structurally-diverse, sp^3-rich building blocks that were prepared using a unified lead-oriented approach can significant enrich structure-activity relationships. In particular, we showed that the cyclohexylmethyl group in a series of known BACE1 inhibitors could be replaced productively with alternative structurally-diverse ring systems. Notably, the incorporation of simplified analogues of these alternative ring systems was not productive, and did not yield active BACE1 inhibitors. The availability of diverse building blocks via a lead-oriented synthetic approach therefore enabled the replacement of a lipophilic substituent with maintenance of both potency and CNS drug-likeness. The exploitation of diverse building blocks with controlled molecular properties within drug discovery programmes thus may significantly expand opportunities in lead discovery and optimisation.

Acknowledgements

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Conflicts of interest

There are no conflicts to declare.

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§ Scores for six molecular properties (MW; clogP; clogD$_{7.4}$; pK_a; polar surface area; number of hydrogen bond donors) contribute to the CNS drug-likeness score (maximum MPO score: 6) (ref. 7).

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