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Bioorganic & Medicinal Chemistry Letters 13 (2003) 179–183

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

A New Series of Potent Benzodiazepine γ-Secretase Inhibitors

Ian Churcher,[a,*] Kate Ashton,[a] John W. Butcher,[b] Earl E. Clarke,[c] Timothy Harrison,[a] Huw D. Lewis,[c] Andrew P. Owens,[a] Martin R. Teall,[a] Susie Williams[a] and Jonathan D. J. Wrigley[c]

[a]*Department of Medicinal Chemistry, The Neuroscience Research Centre, Merck, Sharp and Dohme Research Laboratories, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR, UK*

[b]*Department of Medicinal Chemistry, Merck Research Laboratories, PO Box 4, Sumneytown Pike, West Point, PA 19486, USA*

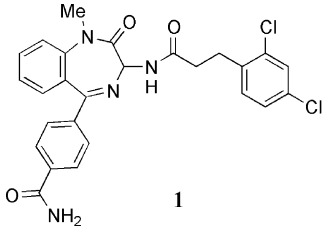
[c]*Department of Biochemistry and Molecular Biology, The Neuroscience Research Centre, Merck, Sharp and Dohme Research Laboratories, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR, UK*

Received 28 August 2002; revised 4 October 2002; accepted 22 October 2002

Abstract—A new series of benzodiazepine-containing γ-secretase inhibitors with potential use in the treatment of Alzheimer's disease is disclosed. Structure–activity relationships of the pendant hydrocinnamate side-chain which led to the preparation of highly potent inhibitors are described.

The 40–42 amino acid peptide amyloid-β (Aβ) is the major component of the extracellular proteinaceous plaques seen in Alzheimer's disease (AD) and much evidence suggests a pivotal role for Aβ in the disease process.[1] In particular, individuals possessing autosomal dominant mutations in the genes encoding for amyloid-β precursor protein (βAPP) or the membrane-bound protein homologues presenilin 1 and 2 have elevated Aβ levels and suffer from aggressive forms of early onset AD.[2] These observations have led to the hypothesis that Aβ, either in its soluble form or when aggregated into fibrils and subsequently plaques, is responsible for neuronal toxicity and cell death.[3,4] Inhibition of Aβ synthesis is thus an attractive target for therapeutic intervention in AD.

Aβ is derived by processing of the 695–770 residue, type I transmembrane protein βAPP.[5] The major metabolic pathway of βAPP involves cleavage by the proteases α-secretase and γ-secretase leading to non-amyloidogenic fragments.[6] Alternative processing by stepwise cleavage mediated by β-secretase and γ-secretase leads to the production of Aβ and it is inhibitors of the latter enzyme that were targeted in the current work.



1

A whole-cell γ-secretase inhibition assay using SHSY5Y cells in which human γ-secretase catalyzes the breakdown of the overexpressed exogenous substrate hspbA4CTF has been developed.[7] Using this assay, screening of the Merck sample collection identified **1** which inhibited the secretion of Aβ with an $IC_{50} = 33$ nM.

Initial studies directed towards the optimization of **1** involved changes to the aromatic substitution pattern in the hydrocinnamoyl C-3 side-chain. A synthesis of the benzodiazepine which allows introduction of the side-chain at a late stage is outlined in Scheme 1.[8]

Dimethyl anthranilate was transformed into benzodiazepinedione **2**[9] which in turn allowed the introduction of the C-5 benzamide substituent in protected form by way of addition of the appropriate Grignard reagent.[10] Subsequent deprotection and cyclization gave benzodiazepine **3**. The C-3 amino substituent was introduced

*Corresponding author. Tel.: +44-1279-440000; fax: +44-1279-440390; e-mail: ian_churcher@merck.com

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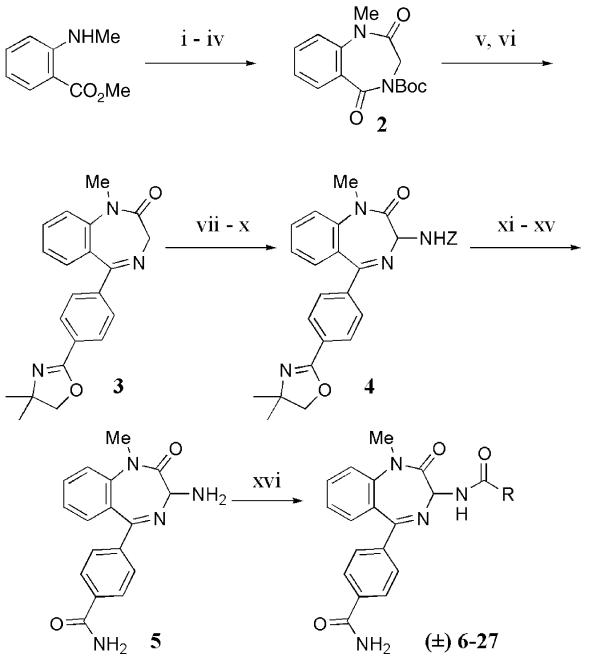
PII: S0960-894X(02)00909-5



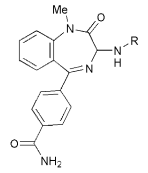
Scheme 1. (i) $BrCH_2COBr$, DCM; (ii) aq NaOH, 0 °C; (iii) NH_3, MeOH; (iv) Boc_2O, NaH, THF; (v) 2-(4-bromophenyl)-4,4-dimethyl-4,5-dihydro-oxazole, Mg, I_2, THF; (vi) HCl, EtOAc then aq $NaHCO_3$; (vii) KO^{t}Bu, ^{i}AmONO, PhMe; (viii) EtNCO, Et_3N, THF; (ix) H_2/Pd/C, MeOH; (x) benzylchloroformate, Na_2CO_3, aq dioxan; (xi) 1 N HCl/dioxan; (xii) AcCl, Et_3N, DCM; (xiii) NaOH, aq THF; (xiv) NH_4Cl, EDC, HOBt, Et_3N, DMF; (xv) HBr/AcOH; (xvi) RCO_2H, EDC, HOBt, Et_3N, DCM.

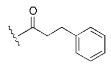
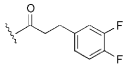
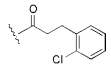
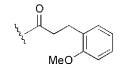
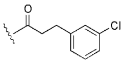
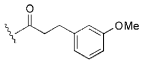
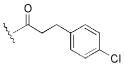
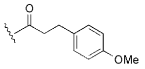
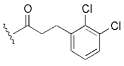
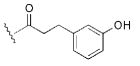
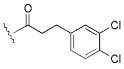
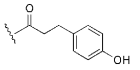
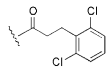
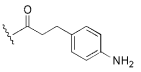
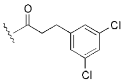
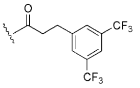
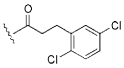
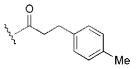
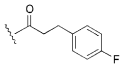
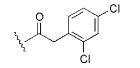
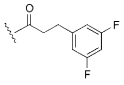
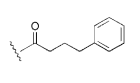
by nitrosylation and hydrogenation of the derived carbamate[11] which, after protection gave racemic aminobenzodiazepine **4**. Unmasking of the carboxamide followed by benzyl carbamate cleavage gave the versatile, racemic amine **5** which was coupled with a range of carboxylic acids to give the amides **6**–**27**. The required hydrocinnamic acids were available from rhodium-catalyzed hydrogenation of the corresponding cinnamic acids or via alkylation of the anion of ethyl acetate followed by hydrolysis.

The structure–activity relationships of a variety of C-3 amide substituents are summarized in Table 1. In general, more polar functionality was poorly tolerated (compounds **18**–**23**) relative to unsubstituted hydrocinnamate **6**. Of the more lipophilic substituents, halogens in particular gave the most potent compounds. Investigation of representative halogen substituent patterns showed 2,4- and 3,4-dihalo (e.g., **11**, **17**) to be amongst the best and these were selected for further elaboration. Variation of chain length of the linking group was briefly investigated but both truncation (**1**→**26**) and homologation (**6**→**27**) were attended with large decreases in potency.

Concurrently, investigations into reducing the flexibility of the side-chain were carried out (Table 2).[12] Whilst use of the cinnamate side-chain in **28** resulted in a large reduction in potency, introduction of a methyl group at the α-position of the hydrocinnamate, as in **29**, gave an increase. Introduction of a further α-substituent as in

Table 1. Structure–activity relationships of hydrocinnamate-based C-3 substituents

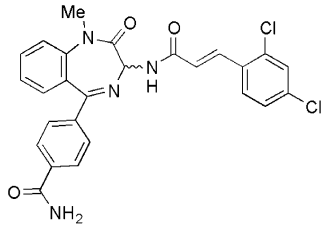
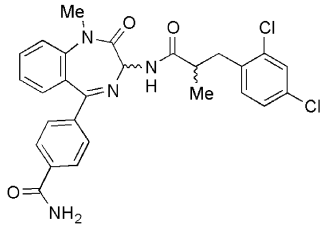
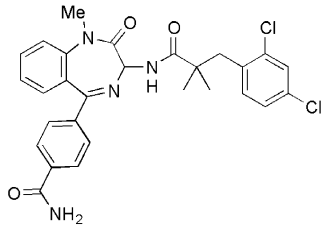
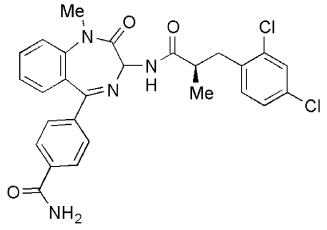
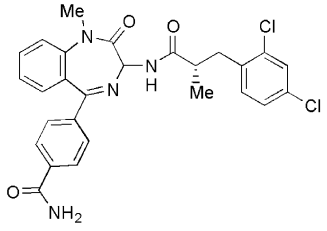


Entry	R	IC_{50}, nM (n)	Entry	R	IC_{50}, nM (n)
6		340 (3)	**17**		29 (6)
7		305 (3)	**18**		5100 (3)
8		93 (4)	**19**		1600 (3)
9		15 (3)	**20**		1340 (3)
10		22 (3)	**21**		6800 (3)
11		12 (6)	**22**		> 10,000 (3)
12		3200 (3)	**23**		> 10,000 (3)
13		40 (3)	**24**		400 (3)
14		60 (3)	**25**		180 (3)
15		110 (3)	**26**		> 10,000 (3)
16		110 (3)	**27**		5000 (3)

the geminally substituted analogue **30** was poorly tolerated. The four stereoisomers of **29** were prepared individually by reaction of a single enantiomer of the requisite α-methyl hydrocinnamate[13] with racemic benzodiazepine **5** followed by separation of the resultant diastereomers. This demonstrated that it was the (*S*)-configured methyl hydrocinnamate that was preferred and also that the activity resided mainly in one stereoisomer at the benzodiazepine C-3 centre. Subsequent work (vide infra, compounds **34** and **35**) suggested that it was the (*S*)-configured benzodiazepine that was preferred but at this stage this was not proven.

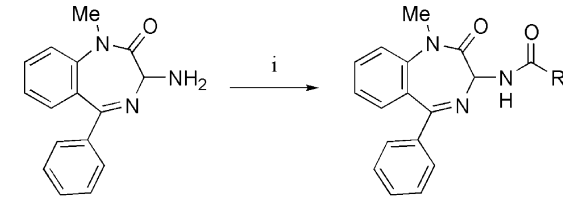
The use of an (*S*)-2-methyl-(2,4-dichloro) hydrocinnamate side-chain had thus shown that substituted

Table 2. Introduction of conformational restrictions into hydrocinnamate side-chain

		IC_{50}, nM (n)	
28		4700 (3)	
29		8 (3)	
30		400 (8)	
31		diastereomer A diastereomer B	42 (3) 920 (4)
32		diastereomer A diastereomer B	500 (3) 1.9 (4)

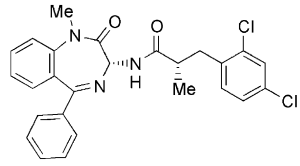
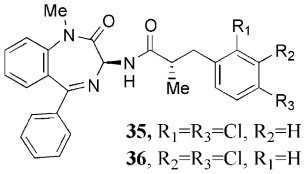
benzodiazepines could function as highly potent γ-secretase inhibitors.

In addition to the above work, investigation into the effect of carboxamide deletion was undertaken utilizing the readily available C-5 phenyl benzodiazepine **33**[14] allowing the synthesis of amides **34–52** as shown in Scheme 2. Immediately evident was that removal of the carboxamide moiety resulted in a substantial reduction in inhibitory activity (Table 3). Further, the availability of both enantiomers[15] of **33** showed the C-3 (*S*) stereoisomer to be preferred. The structure–activity



Scheme 2. (i) RCO_2H, EDC, HOBt, DCM.

Table 3. Determination of preferred stereochemistry using C-5 phenyl benzodiazepine

		IC_{50}, nM (n)
34		> 10,000 (2)
		
	35, R_1=R_3=Cl, R_2=H	820 (3)
	36, R_2=R_3=Cl, R_1=H	320 (6)
	37, R_2=R_3=F, R_1=H	220 (9)

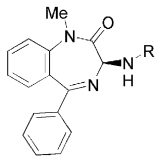
relationships of the aromatic substituents on the hydrocinnamate moiety mirrored that seen previously, with halogen substituents again giving the best results (**35–37**; cf. Table 1).

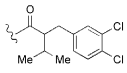
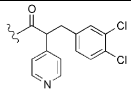
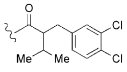
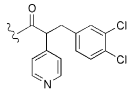
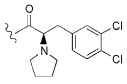
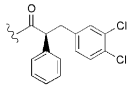
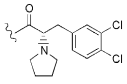
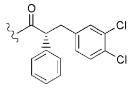
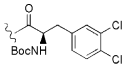
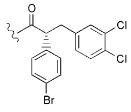
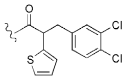
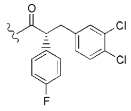
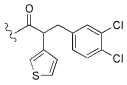
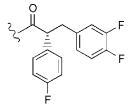
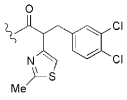
In order to recover potency back to the levels seen with the C-5 benzamide substituent, a variety of α-substituted hydrocinnamates were screened at the C-3 position and the activity of representative compounds is summarized in Table 4.

The necessary carboxylic acids were prepared in racemic form via alkylation of the relevant substituted acetate ethyl ester by 3,4-dichlorobenzyl bromide followed by saponification. Amide bond formation with (***S***)-**33** gave a pair of diastereomers which usually could be separated chromatographically. In cases where the substituted cinnamate was prepared as a defined, single enantiomer, alkylation was carried out utilizing the Evans benzyl oxazolidinone[13] as auxilliary or else commercially available substituted phenylalanines were employed and subsequently modified.

A range of compounds were found to possess potency similar to or better than methyl analogue **36**. Basic centres were well tolerated (**40**, **41**, **47**) as were other, lipophilic substituents. In particular, aromatic and heteroaromatic groups (e.g., **43–44**) gave improved potency. In general, both stereoisomers of the side-chain showed similar levels of inhibition and the marked stereochemical preference noted with the isomers of **31** and **32** (Table 2) was not usually observed.

Table 4. Structure–activity relationships of α-substituted hydrocinnamates



Entry	R	IC_{50}, nM (n)	Entry	R	IC_{50}, nM (n)
38		440 (3) (diast. A)	46		1360 (3) (diast. A)
39		240 (3) (diast. B)	47		190 (3) (diast. B)
40		405 (4)	48		72 (3)
41		350 (4)	49		180 (3)
42		750 (3)	50		100 (3)
43		37 (3) (1:1 diast mix)	51		15 (4)
44		100 (3) (1:1 diast mix)	52		3.8 (4)
45		640 (3) (1:1 diast mix)			

The optimal α-substituent was found to be an (*R*)-configured 4-fluorophenyl (**51**, 15 nM) and substitution of the chlorine atoms for fluorine (**52**) gave a further increase in potency to a level which now matched that seen with the carboxamide-substituted benzodiazepines.

In summary, we have developed a new series of benzodiazepine γ-secretase inhibitors containing a substituted hydrocinnamate C-3 substituent. High potency could be attained by use of a benzodiazepine bearing a C-5 carboxamide group. A further series of compounds utilizing a C-5 phenyl-substituted benzodiazepine was developed in which the potency lost by omission of the carboxamide moiety was successfully recovered by elaboration of the hydrocinnamate side-chain. In particular, the (*R*)-3-(3,4-difluoro)-2-(4-fluorophenyl)-propionyl substituent allowed the preparation of a further series of highly potent, benzodiazepine-derived γ-secretase inhibitors.

Acknowledgements

The authors wish to thank Drs. M. S. Shearman and D. Beher for their valuable contributions and Beth Oxley for assistance in screening.

References and Notes

1. For recent reviews on the role of β-amyloid in Alzheimer's disease see: Coughlan, C. M.; Breen, K. C. *Pharmacol. Ther.* **2000**, *86*, 111. Jhee, S.; Shiovitz, T.; Crawford, A. W.; Cutler, N. R. *Exp. Opin. Invest. Drugs* **2001**, *10*, 593. Sambamurti, K.; Grieg, N. H.; Lahiri, D. K. *NeuroMolec. Med.* **2002**, *1*, 1.
2. Reviews on the presenilins see: Sisodia, S. S.; St George-Hyslop, P. H. *Nat. Rev. Neurosci.* **2002**, *3*, 281. Steiner, H.; Haass, C. *Nat. Rev. Molec. Cell Biol.* **2000**, *1*, 217. Wolfe, M. S. *J. Neurochem.* **2001**, *76*, 1615.
3. Selkoe, D. J. *Physiol. Rev.* **2001**, *81*, 741.

4. Selkoe, D. J. *Nature* **1999**, *99*, A23.
5. Selkoe, D. J.; Podlisny, M. B.; Joachim, C. L.; Vickers, E. A.; Lee, G.; Fritz, L. C.; Oltersdorf, T. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 7341.
6. For a review of the secretases see: Wolfe, M. S. *J. Med. Chem.* **2001**, *44*, 2039. For recent speculation as to the identity of γ-secretase see: Tsai, J.-Y.; Wolfe, M. S.; Xia, W. *Curr. Med. Chem.* **2002**, *9*, 1087.
7. Clarke, E. E.; Shearman, M. S. *J. Neurosci. Meth.* **2000**, *102*, 61.
8. Churcher, I.; Nadin, A. J.; Owens, A. P., PCT Int. Appl. **2002**, WO 0230912.
9. Siegl, P. K. S.; Goldberg, A. I.; Goldberg, M. R.; Chang, P. I. PCT Int. Appl. **1997**, WO 9749690.
10. Meyers, A. I.; Temple, D. L.; Haidukewych, D.; Mihelich, E. D. *J. Org. Chem.* **1974**, *39*, 2787.
11. Bourrain, S.; Showell, G. A. *Synthesis* **1994**, *505*.
12. Castro Pineiro, J. L.; Churcher, I.; Guiblin, A. R.; Harrison, T.; Kerrad, S.; Madin, A.; Nadin, A. J.; Owens, A. P.; Sparey, T. J.; Teall, M. R.; Williams, S. PCT Int. Appl. **2001**, WO 0190084.
13. The required (*R*) and (*S*)-3-(2,4-dichlorophenyl)-2-methylpropionic acids were prepared by the alkylation of (*S*)-(+)-4-benzyl-3-propionyl-2-oxazolidinone and (*R*)-(−)-4-benzyl-3-propionyl-2-oxazolidinone respectively followed by LiOH/H_2O_2 hydrolysis cf. Evans, D. A.; Ennis, M. D.; Mathre, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 1737.
14. Bock, M. G.; DiPardo, R. M.; Evans, B. E.; Rittle, K. E.; Veber, D. F.; Freidinger, R. M.; Hirshfield, J.; Springer, J. P. *J. Org. Chem.* **1987**, *52*, 3232.
15. Reider, P. J.; Davis, P.; Hughes, D. L.; Grabowski, E. J. J. *J. Org. Chem.* **1987**, *55*, 955.