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Bioorganic & Medicinal Chemistry Letters 13 (2003) 4143–4145

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

High Affinity, Bioavailable 3-Amino-1,4-benzodiazepine-Based γ-Secretase Inhibitors

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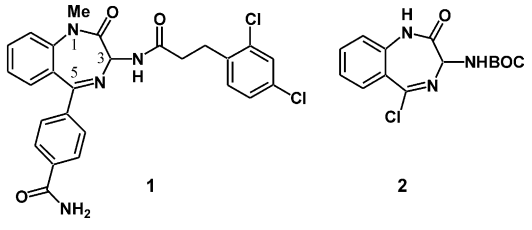
Received 13 May 2003; revised 10 June 2003; accepted 4 July 2003

Abstract—In this paper, we describe the development of a novel series of high affinity, orally bioavailable 3-amino-1,4 benzodiazepine-based γ-secretase inhibitors for the potential treatment of Alzheimer's disease. We disclose structure–activity relationships based around the 1, 3 and 5 positions of the benzodiazepine core structure.

Alzheimer's disease (AD) is a common neurodegenerative disease that is clinically characterized by a progressive cognitive and memory decline. It has been thought for some time that the deposition and accumulation of amyloid-β (Aβ) peptides into extracellular proteinaceous plaques may be responsible for this neurodegeneration.[1] These Aβ peptides are produced by sequential cleavage of membrane-bound amyloid-β precursor protein (βAPP) by two proteases known as β- and γ-secretases. One particularly attractive approach to the treatment of AD may therefore be to inhibit either β- or γ-secretase, thereby reducing the formation of Aβ peptides and consequently their aggregation into amyloid plaques. Based on this hypothesis, we embarked on a γ-secretase inhibitor discovery program to find an orally bioavailable, high potency compound.



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Recently, we reported identification of a novel series of γ-secretase inhibitors based on the screening lead **1** and described some initial structure–activity relationships (SARs) based on changes to the aromatic ring and carbon backbone of the hydrocinnamoyl C3 side chain.[2] The original synthesis of **1** involved a 16-step synthesis[2,3] which was not ideal for the preparation of analogues to investigate SARs. A recent publication from our laboratories[4] has described a novel strategy for the synthesis of 3-amino-1,4-benzodiazepines based on reactions of the chloroimidate (imidoyl chloride) **2**, thereby allowing convergent construction of structures such as **1** from a central intermediate. Herein, we describe an improved synthesis of **1** and present the in vitro potencies of a range of analogues at the C5, C3, and N1 positions.

A new route for the synthesis of **1** is shown in Scheme 1. Methylation of **2** using sodium hydride and methyl iodide in dimethylformamide gave **3** in excellent yield. Palladium-catalyzed cross-coupling of **3** with 4-carboxamidophenyl boronic acid under standard aqueous conditions gave **4**. Removal of the BOC protecting group with trifluoroacetic acid and acylation with 3-(2,4-dichlorophenyl)propionic acid gave **1** (IC_{50} 33 nM).[2,5] A range of homochiral 2-(*S*)-methyl 3-phenylpropionic acids (prepared via benzylation of Evans' homochiral 4-benzyl-3-propionyl-2-oxazolidinones)[6] were reacted with the amine derived from **4**. It was then

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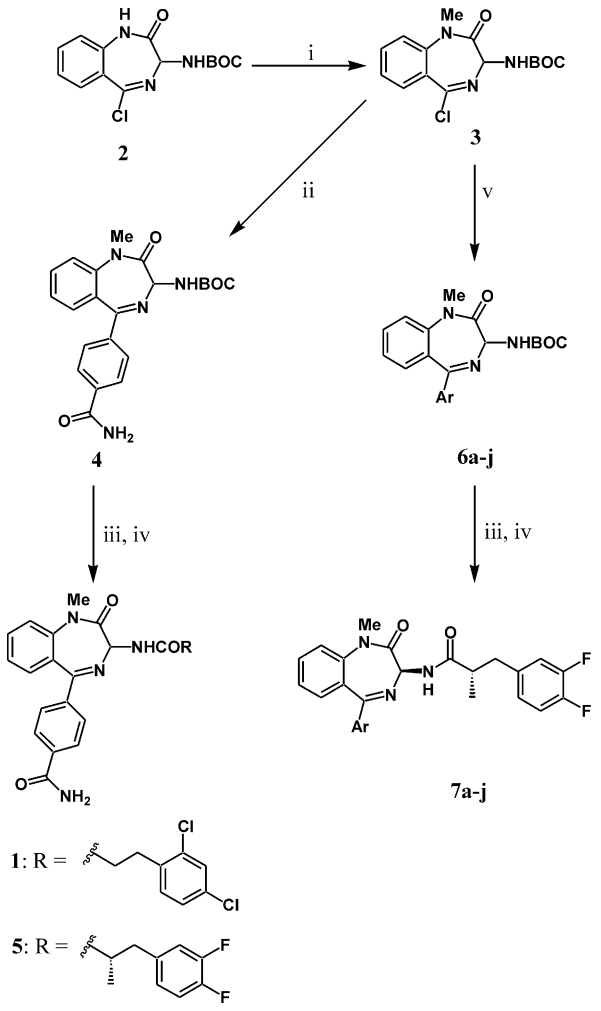
doi:10.1016/j.bmcl.2003.07.031

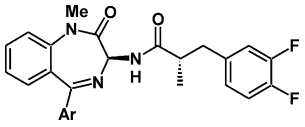


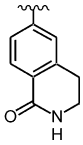
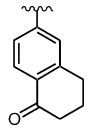
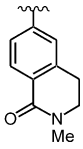
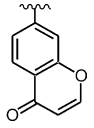
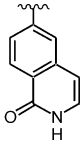
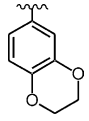
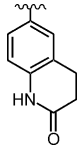
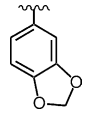
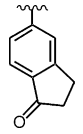
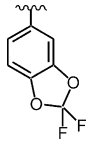
Scheme 1. (i) ICH_3, NaH, DMF, 87%; (ii) $Pd(PPh_3)_4$, Na_2CO_3, H_2O–DME, 4-$(CONH_2)$–$C_6H_4B(OH)_2$, 62%; (iii) TFA, DCM, 100%; (iv) EDC, HOBT, RCO_2H, separation of diastereomers, 70%; (v) $Pd(PPh_3)_4$, Na_2CO_3, H_2O–DME, $ArB(OH)_2$ or ArB(–$OCMe_2$-CMe_2O–), 52–93%.

possible to separate the mixtures into homochiral single diastereomers by flash silica chromatography.[7] Incorporation of the homochiral 2-(*S*)-methyl-3-(3,4-difluorophenyl)propionic acid side chain, identified in a previous publication,[2] resulted in compound **5**, which showed a hundred fold increase in potency (IC_{50} = 0.49 nM) compared to **1**. However, neither compound had significant systemic exposure in rat.

We next went on to investigate changes to the 4-carboxamidophenyl region of compound **5**. These compounds were synthesized from the intermediate **3** by palladium-catalyzed cross-coupling with commercially available aryl boronic acids, or borate esters (prepared by reaction of bis(pinacolato)diboron with the corresponding bromides)[8] under standard aqueous Suzuki conditions to give intermediates **6a–j** (Scheme 1). Subsequent BOC deprotection followed by acylation with homochiral 2-(*S*)-methyl-3-(3,4-difluorophenyl)propionic acid and separation of diastereomers gave **7a–j** (Table 1).

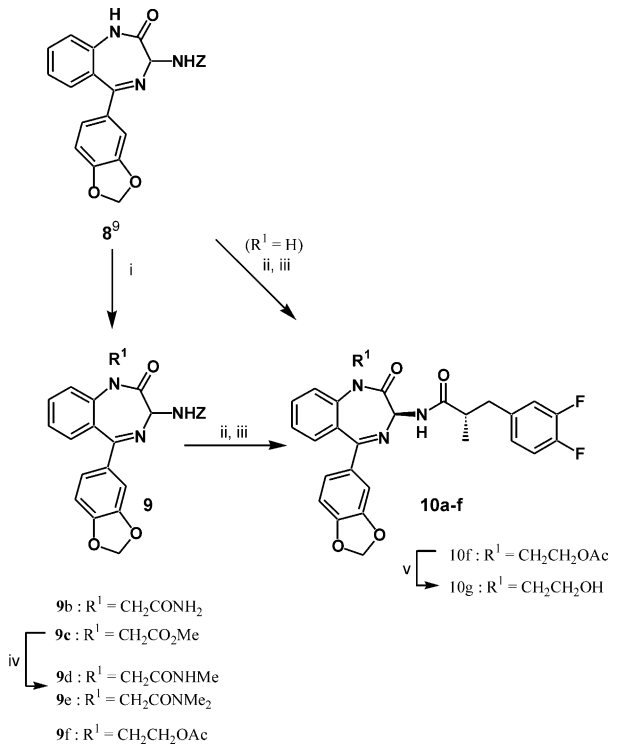
Table 1. Structure–activity relationships of C5 substituted enzodiazepines



Entry	Ar	IC_{50} (nM)	Entry	Ar	IC_{50} (nM)
7a		0.3	**7f**		2.2
7b		188.4	**7g**		1.2
7c		0.2	**7h**		4.7
7d		100.6	**7i**		13.2
7e		3.6	**7j**		28.0

This study revealed some interesting SARs. For example, tethering the carboxamide to the phenyl ring resulted in identification of **7a** and **7c**, compounds with comparable in vitro potency to **5**, whereas the isomer **7d** was much less potent. Compound **7a** was evaluated in vivo, but was found to have very low exposure in rat (systemic $AUC_{0-04\ h} < 0.004$ μM h, $C_{max} < 0.002$ μM, 1 mg/kg po). Our first approach to address this issue was to modify the polarity of the C5 substituent. Methylation of **7a** to give **7b**, resulted in a 600-fold decrease in activity which initially led us to believe in the essential requirement of a hydrogen bond donating group in the C5 region. However, further investigation of SAR by deletion of the nitrogen atom, changing ring size and polarity (compounds **7e–j**) offered evidence to negate this theory, resulting in compounds retaining high inhibitory potency.

Evaluation of these compounds in vivo showed that all had low systemic exposure in rat with the exception of compound **7i** ($AUC_{0-4\ h}$ = 0.09 μM h, C_{max} 0.04 μM, 5 mg/kg po). Although systemic exposure of **7i** remained poor, we reasoned that increasing polarity of the molecule by modification of the substituent at the N1



Scheme 2. (i) NaH or Cs_2CO_3, R^1-X (X = Br, I), DMF, 86–94%; (ii) HBr–AcOH, 90–96%; (iii) EDC, HOBT, RCO_2H, DMF, separation of diastereomers, 70%; (iv) NH_2Me or $NHMe_2$, sealed tube, MeOH, 80–90%; (v) LiOH, dioxane–H_2O, 48%; Z = CO_2CH_2Ph.

Table 2. Structure–activity relationships of N1-substituted benzodiazepines

Entry	R^1	IC_{50} (nM)
10a	H	1.8
7i	Me	13.2
10b	CH_2CONH_2	1.2
10c	CH_2CO_2Me	12.7
10d	$CH_2CONHMe$	1.8
10e	CH_2CONMe_2	5.5
10f	CH_2CH_2OAc	3.7
10g	CH_2CH_2OH	0.9

position may improve absorption. The synthesis of compounds **10a–10g**, starting from known benzodi−azepine **8**,[9] is shown in Scheme 2.

Evaluation of the in vitro potencies of **10a–10g** revealed some interesting observations. Deletion of the N1 methyl substituent gave compound **10a**, which had an 8-fold improvement in potency (Table 2). We then introduced a range of polar groups, compounds **10b–10g**, the majority of which, although tolerated, offered no significant improvement in exposure in rat. However, the carboxamidomethyl moiety of compound **10b** uniquely offered excellent potency (IC_{50} 1.2 nM) together with improved systemic exposure ($AUC_{0-4\ h}$ = 2.9 μM h, C_{max} 0.9 μM, 5 mg/kg po). Further iv studies showed that compound **10b** had rat bioavailability of 22% and $t_{1/2}$ = 1.4 h. Furthermore, compound **10b** entered the brain, although levels were relatively low (AUC_{0-4h} = 0.17 μM h, 5 mg/kg po).

In conclusion, we have demonstrated a practical application of a new approach to the synthesis of 3-amino-1,4-benzodiazepines from a single intermediate **2**, which allowed rapid evaluation of a range of benzodiazepines optionally substituted at N1, C3 and C5. This approach resulted in identification of **10b**, a potent γ-secretase inhibitor, with reasonable oral bioavailability.

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