Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 3535–3538
Bioorganic & Medicinal Chemistry Letters

# 2,3-Benzodiazepin-1,4-diones as peptidomimetic inhibitors of γ-secretase

C. V. C. Prasad,[a,*] Shikha Vig,[a] David W. Smith,[a] Qi Gao,[b] Craig T. Polson,[c] Jason A. Corsa,[c] Valerie L. Guss,[c] Alice Loo,[c] Donna M. Barten,[c] Ming Zheng,[d] Kevin M. Felsenstein[c] and Susan B. Roberts[c]

[a]*Department of Discovery Chemistry, Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492, USA*
[b]*Department of Analytical R&D, Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492, USA*
[c]*Department of Neuroscience Biology, Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492, USA*
[d]*Department of Drug Metabolism and Pharmacokinetics, Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492, USA*

Received 20 November 2003; revised 14 April 2004; accepted 19 April 2004

**Abstract**—2,3-Benzodiazepin-1,4-diones were designed as peptidomimetics at the carboxy terminus of hydroxyamides. Inhibition of brain Aβ production was improved by one of the compounds containing constrained modification.


Alzheimer's Disease (AD) is a progressive neurodegenerative disorder marked by loss of memory, cognition, and behavioral stability. Genetic analysis provides the best clues for a logical therapeutic intervention to AD.[1] Familial early onset forms of AD have been linked to missense mutations in the β-amyloid precursor protein (β-APP) and in the presenilin proteins 1 and 2.[2] All mutations found to date affect the quantitative or qualitative product of the amyloidogenic peptide known as Aβ-protein (Aβ). Transgenic animals have been developed overexpressing β-APP bearing familial mutations. The animals exhibit certain aspects of AD histopathology and cognitive deficits.[3,4] Additionally, Aβ is neurotoxic in several cell systems including cultured neurons.[5] The hypothesized link between Aβ and AD pathology emphasizes the need for development of small-molecule secretase inhibitors that modulate Aβ production in brain.

In a recent communication,[6] we described a series of potent inhibitors of γ-secretase identified using a cell-based assay.[7] These agents are triamides with two secondary amide bonds and a third tertiary amide bond. The greatest reduction of Aβ formation, and, hence, greatest inhibition of γ-secretase, was observed in the tripeptide series as represented by **1** (Fig. 1).

Oral administration of **1** in Tg2576 βAPP-Swedish transgenic mice[3] resulted in insignificant reductions (15%) of Aβ levels in the CNS. This single dose of 200 μmol/kg gave 1833 ± 196 nM concentration in the plasma, but only 65 ± 7 nM in the brain 3 h after dosing. The lack of significant activity in the brain appears to be due to the low brain to plasma ratio of this compound.[6] To circumvent the potential liabilities of these triamides, we investigated conformationally constrained analogs. Several conformationally restricted γ-secretase inhibitors have also been described in the patent literature (Fig. 2).[8]

Since both the N-terminal and internal secondary amide bonds were critical for recognition, our starting point

* Corresponding author. Tel.: 1-203-677-6699; fax: 1-203-677-7702; e-mail: prasad.chaturvedula@bms.com

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.04.056

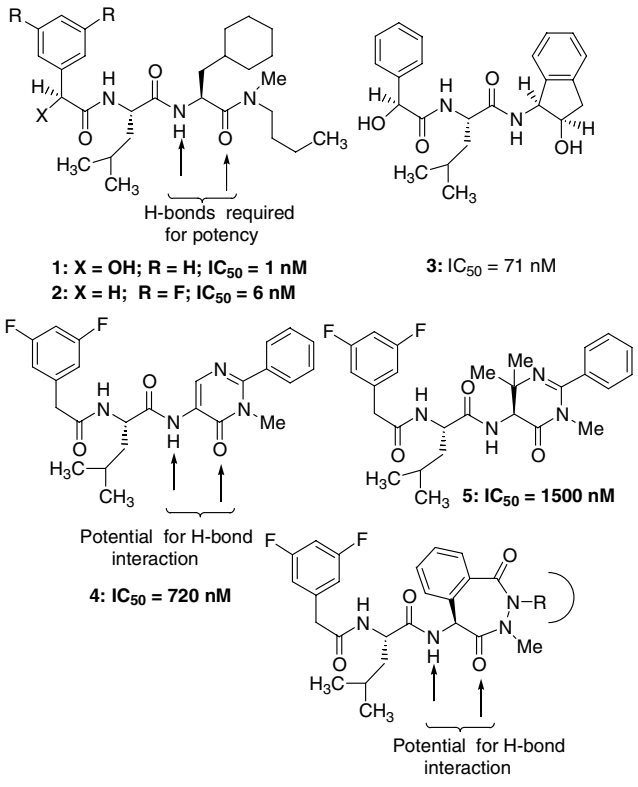



**Figure 1.** Design considerations of diazepinediones based on H-bond motiff recognition patterns (γ-secretase $IC_{50}$'s shown).

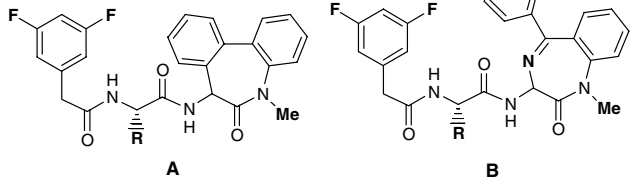



**Figure 2.** Known conformationally restricted γ-secretase inhibitors.

for peptidomimetic design was at the carboxy terminus. We had only modest success in replacing the tertiary amide bond with the β-sheet mimetic *cis*-aminoindanol in **3**.[9] Although the *cis*-aminoindanol moiety was able to satisfy the carbonyl hydrogen bond requirement,[6] it lacked the binding contribution from the N-methylbutyl functionality. To satisfy the integrity of hydrogen bond pattern as well as the contribution from the N-methylbutyl moiety, we examined the utility of a 5-amino-2-methylpyrimidinone moiety. It was hoped that the aryl group of pyrimidinone would occupy the adjacent hydrophobic pocket where *n*-butyl group of **1** was believed to reside (Fig. 1). A pyrimidinone based mimetic has been successfully used in the design of orally active human leukocyte elastase inhibitors.[10] Thus, a series of inhibitors incorporating pyrimidinone were synthesized and tested for γ-secretase inhibition. Compound **4** exhibited weak activity, as it was planar at the carboxy terminus. Two methyl groups were introduced onto the pyrimidinone **5** to afford greater facial discrimination. However, compound **5** proved to be

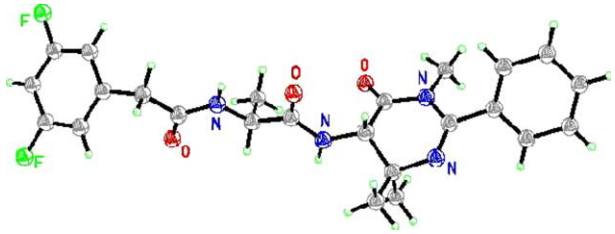
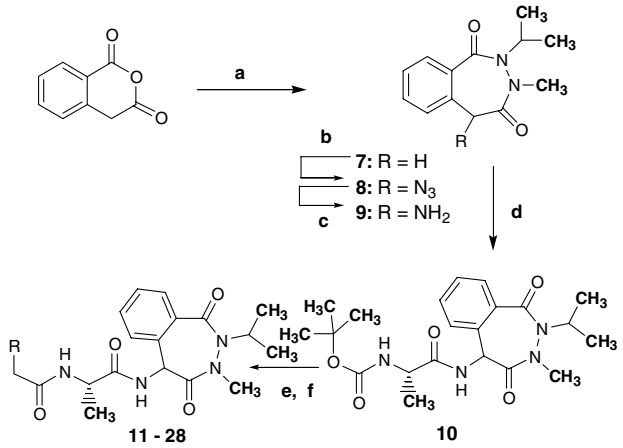


**Figure 3.** ORTEP drawing of **5**.

even less active than **4**. X-ray crystallographic analysis of **5** revealed considerable distortion of key carbonyl group at the carboxy terminus (Fig. 3). To bring the carbonyl group to the preferred orientation and incorporate facial bias, a diazepinedione ring system was examined. The design features of diazepinedione ring system are shown in Figure 1.

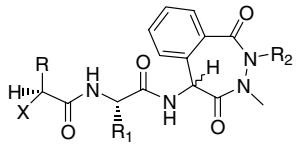
A convergent approach to the synthesis of inhibitors containing the diazepinedione moiety is shown in Scheme 1. The approach involved coupling aminodiazepinedione **9** with N-protected alanine using standard amide bond coupling reagents. Aminodiazepinediones **8** were prepared from the diazepinediones **7** using Evan's enolate chemistry and 2,4,6-triisopropylbenzenesulfonyl azide (trisyl azide).[11] The diazepinediones in turn were prepared by reacting homophthalic anhydride with substituted hydrazines in refluxing acetic acid. The regioselective formation of **7** may result from the reaction of less hindered nitrogen of 1-isopropyl-2-methylhydrazine with the more reactive carbonyl group of the anhydride. The coupled product **10** was isolated by flash chromatography as a inseparable diastereomeric mixture.

Exposure of **10** to trifluoroacetic acid resulted in removal of the N-terminal *t*-butoxycarbonyl group to afford the amine salt in 70% overall yield. This material was coupled with various carboxylic acids mediated by




**Scheme 1.** Reagents and conditions: (a) $(Me)HNNHCH(Me)_2$, HOAc, pyridine, reflux, 12 h, 60%; (b) $KN(SiMe_3)_2$, −78 °C, trisyl azide, then HOAc; (c) 10% Pd–C, EtOAc, $H_2$ (1 atm), 40 min; (d) Boc alanine, EtOC(O)Cl, $Et_3N$, 0 °C, 3 h; (e) $CF_3CO_2H$–$CH_2Cl_2$, rt, 2 h; (f) P-EDC, $CH_2Cl_2$, 18 h.

**Table 1.** Modifications at the amino and the carboxy terminus



| Compound | X | R | $R_1$ | $R_2$ | $IC_{50}$ (nM)[a] |
|---|---|---|---|---|---|
| **1** | NA | NA | NA | NA | 1 |
| **2** | NA | NA | NA | NA | 6 |
| **11** | H | Phenyl | Me | $CH(Me)_2$ | 140 |
| **12** | H | 3,5-F,F-phenyl | Me | $CH(Me)_2$ | 47 |
| **13** | H | 3-F-phenyl | Me | $CH(Me)_2$ | 57 |
| **14** | H | Cyclohexyl | Me | $CH(Me)_2$ | 150 |
| **15** | H | 2-Naphthyl | Me | $CH(Me)_2$ | >2500 |
| **16** | H | 2-Pyridyl | Me | $CH(Me)_2$ | Na |
| **17** | H | 3-Pyridyl | Me | $CH(Me)_2$ | >2500 |
| **18** | H | 4-Pyridyl | Me | $CH(Me)_2$ | >2500 |
| **19** | H | 3,5-$(OMe)_2$phenyl | Me | $CH(Me)_2$ | >2500 |
| **20** | H | 3,5-$(CF_3)_2$phenyl | Me | $CH(Me)_2$ | >2500 |
| **21** | H | 2-Furyl | Me | $CH(Me)_2$ | 86 |
| **22** | H | 3-Furyl | Me | $CH(Me)_2$ | 38 |
| **23** | H | 3-Thienyl | Me | $CH(Me)_2$ | 14 |
| **24** | OH | 3,5-F,F-phenyl | Me | $CH(Me)_2$ | 5 |
| **25** | H | 3,5-F,F-phenyl | $CH_2CH(Me)_2$ | $CH(Me)_2$ | 37 |
| **26** | H | 3-Thienyl | $CH_2CH(Me)_2$ | $CH(Me)_2$ | 4 |
| **27** | H | 3,5-F,F-phenyl | $CH(Me)_2$ | $CH(Me)_2$ | 190 |
| **28** | H | 3,5-F,F-phenyl | Me | Me | 5 |

[a] Values are means of two experiments, with 16 data points in each experiment.; intra-assay variance <10% (NA = not applicable). $IC_{50}$s were determined using a cell-based assay.[7]

polymer bound EDC to afford the desired products as inseparable diastereomeric mixtures in excellent yields.[12] Although, the diastereomeric mixture was inseparable via conventional silica gel chromatagraphic techniques, the mixture could easily be separated into pure diastereoisomers with a chiral HPLC column (Chiracel OD, $5 \times 50$ cm, particle size 20 μ) using 10% ethanol in hexane as eluent.

γ-Secretase inhibition data for compounds **11**–**28** is presented in Table 1. Direct comparison of the potency of agents **12** and **24** to their triamide congeners **1**–**2** demonstrates that the diazepinediones are reasonably effective mimetics of the carboxy terminus present in **1** and **2**. Within the diazepinedione-based inhibitors bearing the N-isopropyl substituent (**11**–**27**), modifications of the N-terminal phenylacetyl group were examined. Introduction of halogens (e.g., 3,5-difluorophenylacetyl (**12**), 3-fluorophenylacetyl (**13**) improved potency. Similar trends also were seen in the SAR of the triamide congeners.[6] In examples **14**–**15**, the phenylacetyl group was replaced with hydrophobic N-terminal groups (e.g., cyclohexyl, 2-naphthyl). As the data suggests, incorporation of larger hydrophobic groups was detrimental to potency (**12** vs **15**). In examples **16**–**18**, the phenylacetyl group was exchanged with hydrophilic N-terminal groups (e.g., 2-pyridyl, 3-pyridyl, and 4-pyridyl). These groups were introduced to enhance the solubility of the inhibitors, but they suffered a significant loss in potency compared to the parent inhibitor **11**. Attenuation in potency was observed for inhibitors **19** and **20** when the phenylacetyl group is replaced with either the 3,5-bis(trifluoromethyl)phenylacetyl (**20**) or 3,5-dimethoxyphenylacetyl (**19**) groups. Inhibitors **22**–**23** are equipotent with **12**, although a slight increase in potency was observed for the diazepinedione bearing the 3-thienyl N-capping group. Thus, in addition to halogenated aryl moieties, the 3-furylacetyl and 3-thiopheneacetyl functionalities afforded excellent potency. The most potent compound in this series was obtained by replacing the phenylacetyl group with the (*S*)-hydroxyphenylacetyl moiety.

Structure–activity relationships were also investigated at the diazepinedione moiety. Improvement in potency is seen when N(2)-isopropyl diazepinedione ring was replaced by a smaller methyl group (**12** vs **28**).

The role of the hydrophobic amino acid of the central amide bond also was investigated (**12** vs **25** and **27**). It was evident that leucine was the preferred residue for highest potency. The stereochemical role of the diazepinedione asymmetric carbon on potency was investigated by resolving compound **12** into pure diastereoisomers (absolute stereochemistry not determined) **12a** and **12b** by employing chiral HPLC column. Not unexpectedly, the isomers differed substantially in potency (**12a**: $IC_{50} = 26$ nM; **12b**: $IC_{50} = 710$ nM).[13]

In further studies, compound **28** was selected for oral administration in Tg2576 mice at 200 μmol/kg. Three hours after dosing, **28** gave a 43% reduction in brain Aβ. The plasma level of compound was $2987 \pm 415$ nM and brain was $77 \pm 11$ nM. Although the mean plasma level was 63% higher than that obtained for **1**, this was not a statistically significant difference, and, surprisingly, there

was no major improvement in total brain exposure of the compound compared to **1**. This suggests that the brain efficacy observed with **28** is not due to improved pharmacokinetic properties, at least as can be inferred from a single dose and time point. The reproducible reduction in brain Aβ measured after dosing **28** in these mice may reflect better brain exposure at the γ-secretase target.

In summary, we have discovered that a diazepinedione moiety can function as a peptidomimetic at the carboxy terminus of our previously disclosed triamide series of gamma-secretase inhibitors. We have introduced aminodiazepinediones as novel β-sheet mimetics and described synthetic procedures ideal for analog generation in a combinatorial setting. The design strategy we pursued sought to retain key hydrogen bond interactions of the triamides while enforcing conformational restriction at the carboxy terminus. An inhibitor containing the new peptidomimetic moiety displayed improved inhibition of Aβ production in the brain over their triamide congeners without significant improvements in brain penetration. Investigations are underway in our laboratories to continue to improve the brain activity of these agents.

## References and notes


1. (a) Hardy, J.; Higgins, G. A. *Science* **1992**, *256*, 184–185; (b) Selkoe, D. J. *Trends Neurosci.* **1993**, *16*, 403–409; (c) Selkoe, D. J. *Science* **1997**, *275*, 630–631.
2. (a) Hardy, J. *Ann. Med.* **1996**, *28*, 255–258; Citron, M.; Oltersdorf, T.; Haass, C.; Teplow, D. B.; Selkoe, D. J. *Nature* **1992**, *360*, 672–674; (b) Suzuki, N.; Cheung, T. T.; Cai, X. D.; Odaka, A.; Otvos, L., Jr.; Eckman, C.; Golde, T. E.; Younkin, S. G. *Science* **1994**, *264*, 1336–1340; (c) Borchelt, D. R.; Thinakaran, G.; Eckman, C. B.; Lee, M. K.; Davenport, F.; Ratovisky, T.; Prada, C. M.; Kim, G.; Seekins, S.; Yager, D.; Slunt, H. H.; Wang, R.; Seeger, M.; Levey, A. I.; Gandy, S. E.; Copeland, N. G.; Jenkins, N. A.; Price, D. L.; Younkin, S. G.; Sisodia, S. S. *Neuron* **1996**, *17*, 1005–1013.
3. Hsiao, K.; Chapman, R.; Nilsen, S.; Eckman, C.; Harigaya, Y.; Younkin, S.; Yang, R.; Cole, G. *Science* **1996**, *274*, 99–102.
4. (a) Johnson-Wood, K.; Lee, M.; Motter, R.; Hu, K.; Gordon, M.; Tan, H.; Games, D.; Lieberburg, I.; Schenk, D.; Seubert, P.; McConlogue *Proc. Natl Acad. Sci. U.S.A.* **1997**, *94*, 1550–1555; (b) Games, D.; Adams, D.; Alessandrini, R.; Barbour, R.; Berthelette, P.; Blackwell, C.; Carr, T.; Clemens, J.; Donaldson, T.; Gillespie, F.; Guido, T.; Hagopian, S.; Johnson-Wood, K.; Khan, K.; Lee, M.; Leibowitz, P.; Lieberburg, I.; Little, S.; Masliah, E.; McConlogue, L.; Montoya-Zavala, M.; Mucke, L.; Paganini, L.; Penniman, E.; Power, M.; Schenk, D.; Seubert, P.; Snyder, P.; Snyder, B.; Soriano, F.; Tan, H.; Vitale, J.; Wadsworth, S.; Wolozin, B.; Zhao, J. *Nature* **1995**, *373*, 523–527.
5. (a) Mattson, M. P.; Cheng, B.; Davis, D.; Bryant, K.; Lieberburg, I.; Rydel, R. E. *J. Neurosci.* **1992**, *12*, 379–389; (b) Yankner, B. A.; Duffy, L. K.; Kirschner, D. A. *Science* **1990**, *250*, 279–282.
6. Prasad, C. V. C.; Wallace, O. B.; Noonan, J.; Sloan, C. P.; Lau, W.; Vig, S.; Parker, M. F.; Smith, D. W.; Hansel, S. B.; Polson, C.; Barten, D. M.; Felsenstein, K. M.; Roberts, S. *Bioorg. Med. Chem.* **2004**, *14*, 1917.
7. H4 human neuroglioma cells expressing HPLAP•βAPP$^{164SFAD}$ were grown in high glucose (4.5 g/L) DMEM (Invitrogen) media supplemented with 10% FBS, 100 μg/mL pen-strep, 2 mM glutamine, and 100 μg/mL geneticin. Cells were aliquoted into a 96 well plate, and after attachment the medium was replaced with Ultraculture (Whittaker Bioproducts) containing individual compounds of interest (final DMSO concentration of 1%. After an overnight incubation the conditioned medium was removed and evaluated for the presence of Aβ in a sandwich ELISA using a monoclonal C-terminal Aβ40 specific capture antibody and an HRP labeled monoclonal antibody to the N-teminus of Aβ for detection. The endpoint measurement of Aβ1-40 level was developed using TMB reagent followed by the addition of 1 M phosphoric acid. The plates were read at 450 nm.
8. Audia, J. E.; Thompson, R. C.; Wilkie, S. C.; Britton, T. C.; Porter, W. J.; Huffman, G. W.; Latimer L. H. U.S. Patent 6509331, 2003, and references cited therein.
9. Lyle, T. A.; Wiscount, C. M.; Guare, J. P.; Thompson, W. J.; Anderson, P. S.; Darke, P. L.; Zugay, J. A.; Emini, E. A.; Schleif, W. A.; Quintero, J. C.; Dixion, R. A. F.; Sigal, I. S.; Huff, J. R. *J. Med. Chem.* **1991**, *34*, 1228–1230.
10. Brown, F. J.; Andisik, D. W.; Bernstein, P. R.; Bryant, C. B.; Ceccarelli, C.; Damewood, J. R., Jr.; Edwards, P. D.; Earley, R. A.; Feeney, S.; Green, R. C.; Gomes, B.; Kosmider, B. J.; Krell, R. D.; Shaw, A.; Steelman, G. B.; Thomas, R. M.; Vacek, E. P.; Veale, C. A.; Tuthill, P. A.; Warner, P.; Williams, J. C.; Wolanin, D. J.; Woolson, S. A. *J. Med. Chem.* **1994**, *37*, 1259–1261.
11. Evans, D. A.; Britton, T. C.; Ellman, J. A.; Dorow, R. L. *J. Am. Chem. Soc.* **1990**, *3*, 1919–1924.
12. Desai, M. C.; Stephens Stramiello, L. M. *Tetrahedron Lett.* **1993**, *34*, 7685–7688.
13. Experimental conditions: For compound **12**: To a solution of 2,3,4,5-tetrahydro-3-methyl-5(*R*,*S*)-[[(2*S*)-2-amino-3-methyl-1-oxobutyl]amino]-2-(1-methylethyl)-1*H*-2,3-benzodiazepin-1,4-dione (1.44 mL, 0.28 mmol, 0.2 M solution in dichloromethane) in dichloromethane (2 mL) was added the solution of 3,5-difluorophenyl acetic acid (2.16 mL, 0.43 mmol, 0.2 M solution in dichloromethane) and *N*-(3-dimethylaminopropyl)-*N*′-ethyl-carbodiimide polymer (0.72 g, 1.00 mmol). The resulting mixture was shaken on the shaker for 18 h and the resin was filtered-off and washed with dichloromethane (2×1 mL). The combined solvent was collected and evaporated and product was analyzed by HPLC using the column YMC S7 C18 (3.0×50 mm) with a flow rate of 5.0 mL/min and gradient time of 2.0 min, starting with solvent A (10% MeOH–90% $H_2O$–0.1% TFA), and ending with solvent B (90% MeOH–10% $H_2O$–0.1% TFA). The crude product was purified by HPLC using the column YMC S5 ODS (30×100 mm) with a flow rate of 40.0 mL/min and gradient time of 8.0 min, starting with solvent A (10% MeOH–90% $H_2O$–0.1% TFA), and ending with solvent B (90% MeOH–10% $H_2O$–0.1% TFA) to afford compound **12** in 70% yield: $^1$H NMR ($CDCl_3$): 0.80–1.25 (m, 12H), 1.88–2.05 (m, 1H), 3.25–3.49 (s, 5H), 4.32–4.52 (m, 2H), 5.18 (m, 1H), 6.08–6.18 (m, 1H) 5.09–5.10 (d, 1H), 6.66–7.80 (m, 7H); MS (ESI) 501.23 (M+H).