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Design and synthesis of benzoazepinone-derived cyclic malonamides and aminoamides as potent γ-secretase inhibitors

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Abstract—We report the synthesis of benzoazepine-derived cyclic malonamides (**2**) and aminoamides (**3**) as γ-secretase inhibitors for the potential treatment of Alzheimer's disease. The in vitro structure–activity relationships of **2** and **3** along with dog pharmacokinetic results are described.

Alzheimer's disease (AD) is the most common form of dementia and affects 7–10% of those over 65 years of age and up to 40% of those over age 80.[1] The progressive cognitive impairment and memory loss in AD is accompanied pathologically by an accumulation in brain of plaques consisting of 40–42-amino acid β-amyloid peptides (Aβ), and neurofibrillary tangles made up of hyperphosphorylated tau.[2] Considerable evidence points to a causative role for Aβ in AD, and numerous strategies for reducing the production and accumulation of Aβ are under investigation.[3] Aβ peptides are generated by the cleavage of membrane-bound β-amyloid precursor protein (APP) by the sequential action of β-secretase (BACE), an aspartyl protease, and γ-secretase, a heteromeric complex consisting of presenilin, Aph-1, Pen-2, and nicastrin, in which presenilin appears to be the catalytic component.[4,5] Although APP processing produces more Aβ40 than Aβ42, Aβ42 is most closely associated with AD pathogenesis.[6] Inhibition of γ-secretase reduces production of all forms of Aβ, and represents a potential strategy for therapeutic intervention in AD.[7]

Previously we reported that succinamide **1** potently inhibits γ-secretase activity in APP-transfected Chinese hamster ovary (CHO) cells (IC_{50} = 13 nM).[8] Like succinamide **1**, many benzodiazepine-derived γ-secretase inhibitors in the literature contain one or more chiral centers in the side chain.[9] In an ongoing study of the structure–activity relationships (SAR) of succinamides related to **1**, we questioned whether it would be possible to eliminate the α- and β-stereocenters by making gem-disubstituted analogs **2** and **3** while maintaining the desired γ-secretase activity (Fig. 1). In this strategy, the R′ and R of malonamides **2** and aminoamides **3** could replace the *n*-propyl (R′) and *i*-butyl (R) groups of **1** and the terminal amide bonds of **2** and **3** could function as H-bond acceptor/donors, mimicking the primary amide (see Fig. 1).[10] Herein we report the syntheses of **2** and **3** and their γ-secretase inhibitory activities.

Amino-benzoazepinones **4** and **5** (Fig. 2) have been used in the synthesis of γ-secretase inhibitors and their synthesis has been reported.[11] The synthesis of trifluoromethylphenyl-benzodiazepinone **6** is shown in Scheme 1. Deacylation of phenyl acetamide **7**[12] with 1N HCl in EtOH gave aniline **8**. Benzotriazole **9**[13] was then coupled to aniline **8** and the resulting product **10** was cyclized to benzodiazepinone **11** using NH_4OH/MeOH. Methylation of **11**, followed by resolution using preparative chiral HPLC, gave the desired single isomer **12**, which was treated with HBr gas in DMF to provide the amine **6**. The absolute configuration of **6** was confirmed by a related crystal structure (vide infra).

A general synthetic scheme for the preparation of cyclic malonamides (**2a**) is shown in Scheme 2. Dialkylation of

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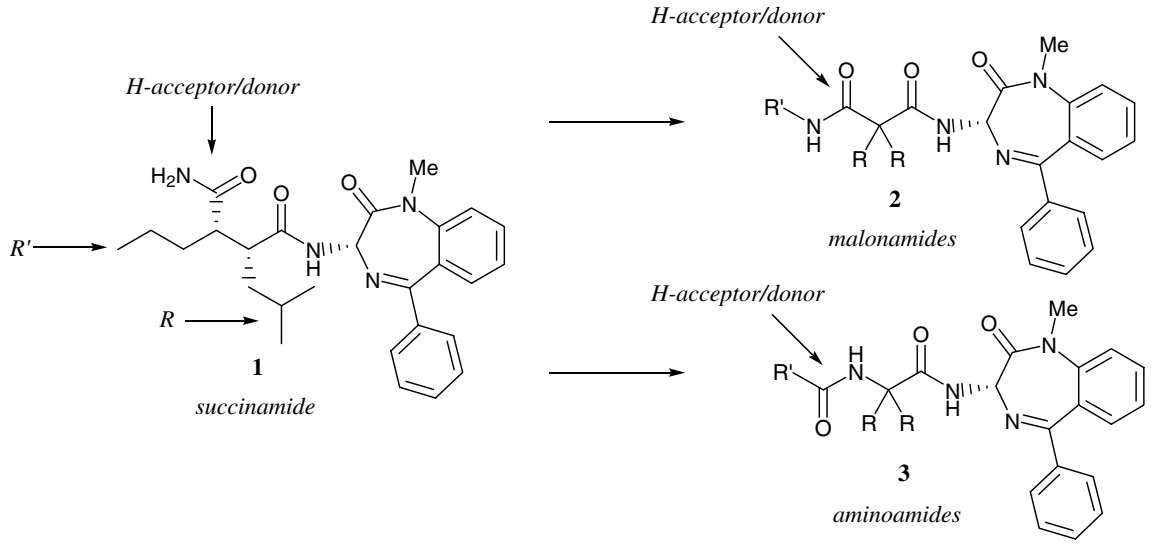


Figure 1. Proposed synthetic targets.

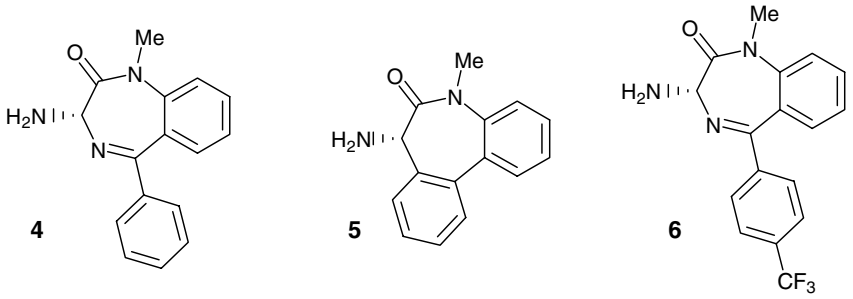
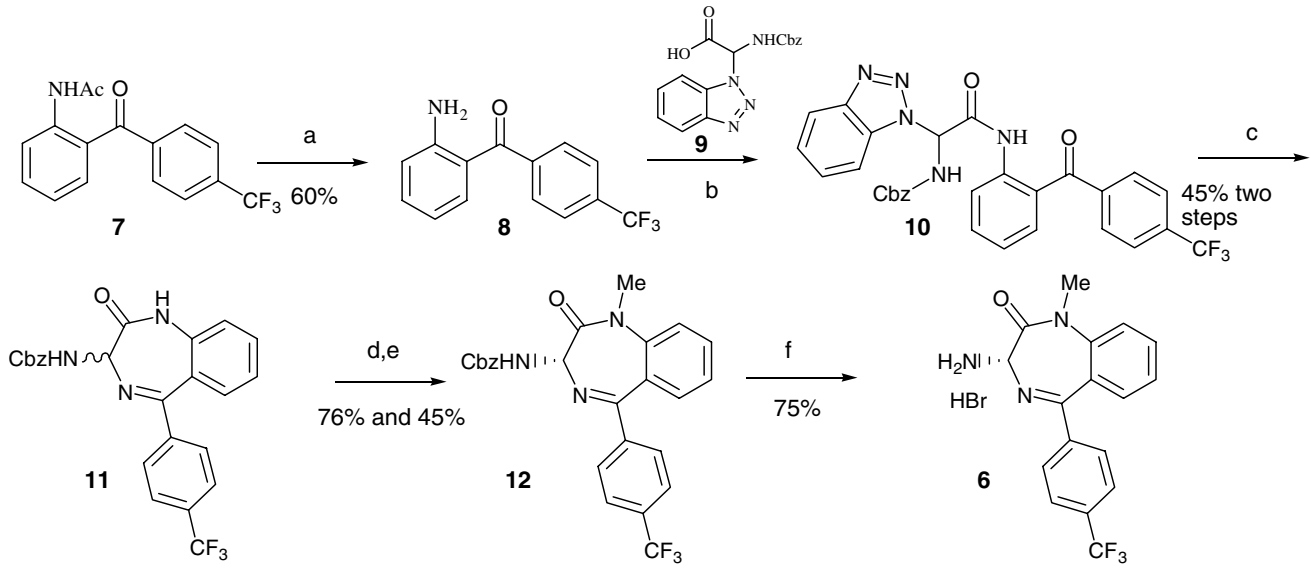
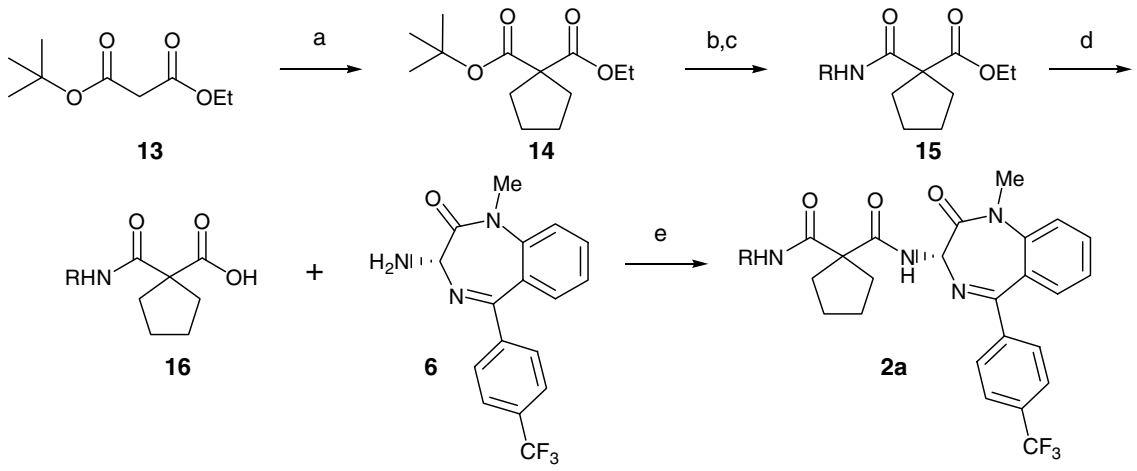


Figure 2. Amino-benzoazepinones.

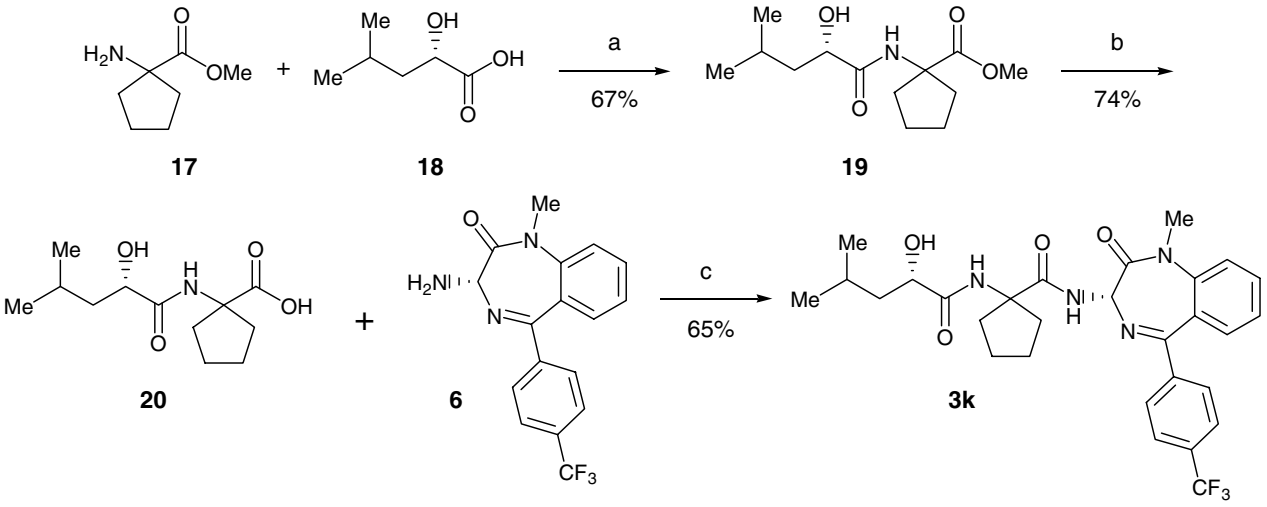


Scheme 1. Reagents: (a) HC1, EtOH; (b) $(COC1)_2$, NMM; (c) NH_4OH, MeOH; (d) NaH, Mel, DMF; (e) chiral separation using a CHIRALCEL AD column with 100% acetonitrile; (f) HBr gas, DMF.

malonate **13**[14] with 1,4-dibromobutane gave cyclic dicarboxylate **14** in 65% yield. Upon removing the *tert*-butyl group, the resulting acid was coupled to amines and saponified to provide carboxylic acids **16**, which were coupled to aminobenzodiazepinone **6** to give the desired amides (**2a**).



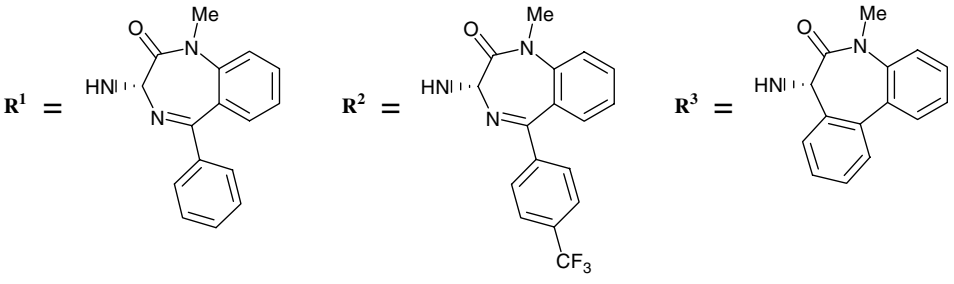
Scheme 2. Reagents: (a) NaH, l,4-dibromobutane, DMF; (b) TFA, CH_2C1_2; (c) RNH_2, DIEA, HATU, DMF; (d) LiOH, THF, H_2O; (e) DIEA, HATU, DMF.



Scheme 3. Reagents: (a) DIEA, HATU, DMF; (b) LiOH, THF, H_2O; (c) DIEA, HATU, DMF.

The synthesis of aminoamides **3** is straightforward and is exemplified with hydroxyl analog **3k** (Scheme 3). Commercially available amine **17** and acid **18** were used for the initial coupling reaction to yield intermediate **19**. Saponification of **19**, followed by the coupling reaction with benzodiazepinone **6**, gave the final product **3k** in 32% overall yield. X-ray crystallographic analysis of **3k** confirmed the stereocenter of the benzodiazepinone to be (*S*) (Fig. 3).

Inhibitors **2** and **3** were initially screened for their ability to displace a radiolabeled γ-secretase inhibitor (^{3}H-**1**) from membranes derived from THP-1 cells,[15] then evaluated in APP-transfected CHO cells for their ability to block Aβ production.[16] Binding K_i and cellular IC_{50} values for malonamide-derived inhibitors are presented in Table 1. Examination of the data reveals the following SAR trends: (I) branched lipophilic side chains (R′), such as *i*-Bu, were optimal for γ-secretase inhibition



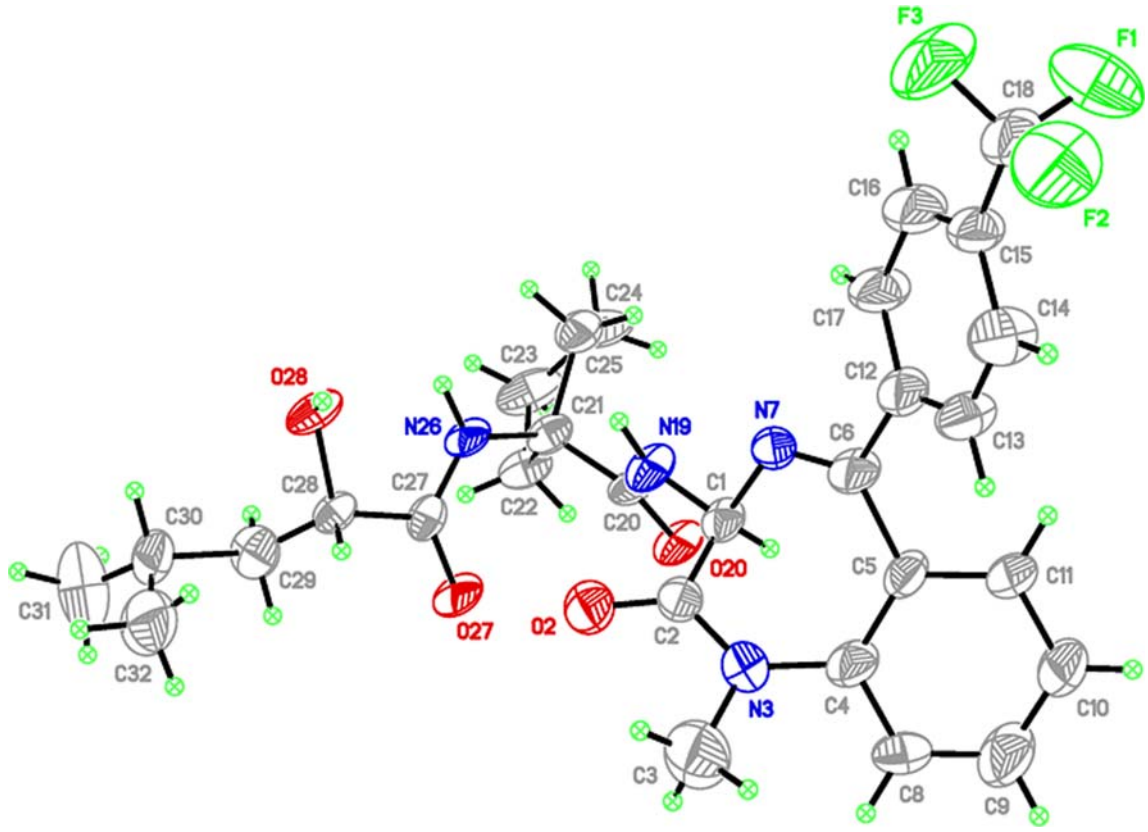
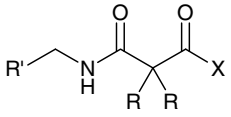


Figure 3. Crystal structure of compound **3k**.

Table 1. Binding K_i and APP IC_{50} of malonamides[a]



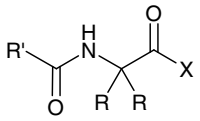
Compound	R	X	R/R	Binding[b] (APP IC_{50})[b]
2b	Et	$\mathbf{R}^1$	Et/Et	1000 nM (NT)
2c	*n*-Pr	$\mathbf{R}^1$	Et/Et	227 nM (NT)
2d	*n*-Pr	$\mathbf{R}^1$	*c*-C_4H_8	15 nM (82 nM)
2e	*n*-Bu	$\mathbf{R}^1$	*c*-C_4H_8	11 nM (20 nM)
2f	*i*-Bu	$\mathbf{R}^1$	*c*-C_4H_8	3.5 nM (21 nM)
2g	*i*-Bu	$\mathbf{R}^2$	*c*-C_4H_8	1.2 nM (28 nM)
2h	*i*-Bu	$\mathbf{R}^3$	*c*-C_4H_8	1.4 nM (5.8 nM)
2i	*i*-Bu	$\mathbf{R}^1$	*c*-C_5H_{10}	6.2 nM (19.5 nM)
2j	*i*-Bu	$\mathbf{R}^2$	*c*-C_5H_{10}	2.0 nM (12.5 nM)
2k	*i*-Bu	$\mathbf{R}^3$	*c*-C_5H_{10}	3.0 nM (26.1 nM)

[a] Binding K_i and APP IC_{50} are 2.6 and 13 nM, respectively, for compound **1**.
[b] Data are reported as means of three determinations. See Ref. 8 for assay protocol.

(e.g., **2f** vs **2e** and **2d**); (II) cyclic malonamides were more potent than their corresponding acyclic analogs (e.g., **2c** vs **2d**); among the cyclic inhibitors, there was no difference in affinity between five- and six-membered cyclic compounds; and (III) the incorporation of different benzoazepinones ($\mathbf{R}^1$–$\mathbf{R}^3$) had minimal impact on the binding activity, but in some cases cellular potency could be modestly improved with the bisbenzoazepinone ($\mathbf{R}^3$) group (e.g., **2h** vs. **2g**).

Binding affinities and cellular IC_{50} values of amino-amide-derived inhibitors are presented in Table 2. The lipophilic side chain R′ was essential for γ-secretase inhibition. As noted in the malonamide series, there was no significant difference in affinity between five- and six-membered ring analogs (e.g., **3c** vs. **3d**). In addition, we observed that the incorporation of a hydroxy moiety in R′ was well tolerated by the enzyme. The (*S*)-hydroxyl diastereoisomer **3n** exhibited superior potency

Table 2. Binding K_i and APP IC_{50} of aminoamides[a]



Compound	R′	X	R/R	Binding[b] (APP IC_{50})[b]
3a	Thiophene-CH_2	**R**[1]	*c*-C_5H_{10}	41 nM (19 nM)
3b	*c*-C_5H_9-CH_2CH_2	**R**[1]	*c*-C_5H_{10}	36 nM (NT)
3c	3,5-Difluorophenyl-CH_2	**R**[1]	*c*-C_5H_{10}	9.9 nM (96 nM)
3d	3,5-Difluorophenyl-CH_2	**R**[1]	*c*-C_4H_8	13.5 nM (115 nM)
3e	*i*-Bu-CH_2	**R**[2]	*c*-C_4H_8	4.9 nM (14 nM)
3f	*i*-Bu-CH_2	**R**[3]	*c*-C_4H_8	4.0 nM (15 nM)
3g	*c*-C_5H_9-CH_2CH_2	**R**[2]	*c*-C_4H_8	2.2 nM (13 nM)
3h	*c*-C_5H_9-CH_2CH_2	**R**[3]	*c*-C_4H_8	1.3 nM (21 nM)
3i	(*S*)-*i*-Pr-CH(OH)	**R**[2]	*c*-C_4H_8	1.1 nM (6.7 nM)
3j	(*S*)-*I*-Pr-CH(OH)	**R**[3]	*c*-C_4H_8	3.9 nM (15 nM)
3k	(*S*)-*i*-Bu-CH(OH)	**R**[2]	*c*-C_4H_8	2.1 nM (6.1 nM)
3l	(*S*)-*i*-Bu-CH(OH)	**R**[3]	*c*-C_4H_8	0.9 nM (4.2 nM)
3m	*i*-Bu-CF_2	**R**[3]	*c*-C_4H_8	22 nM (280 nM)
3n	(*S*)-*c*-C_6H_{11}CH(OH)	**R**[2]	*c*-C_4H_8	3.1 nM (11 nM)
3o	(*R*)-*c*-C_6H_{11}-CH(OH)	**R**[2]	*c*-C_4H_8	111 nM (280 nM)
3p	*c*-C_6H_n-C(O)	**R**[2]	*c*-C_4H_8	93 nM (NT)
3q	(*S*)-*c*-C_6H_{11}-*CR*(*OK*)	**R**[3]	*c*-C_4H_8	0.9 nM (3.6 nM)

[a] Binding K_i and APP IC_{50} are 2.6 and 13 nM, respectively, for compound **1**.
[b] Data are reported as means of three determinations. See Ref. 8 for assay protocol.

(binding K_i = 3.1 nM), as compared to either the (*R*)-isomer **3o** (binding K_i = 111 nM) or ketone **3p** (binding K_i = 93 nM). Similarly, the gem-difluoro analog **3m** was approximately 25-fold less active as compared to the parent hydroxyl analog **3l**.

Representative compounds from Tables 1 and 2 were tested in the beagle dog for pharmacokinetic (PK) properties and the resulting in vivo data are summarized in Table 3. Compounds were dosed in a single animal (cassette format) with up to nine other compounds. The doses were kept low (0.25 mg/kg iv and 0.5 mg/kg po) to help minimize potential compound interactions. All compounds exhibit good oral bioavailability and a moderate (or long) half-life. Compound **3k** was noteworthy in giving plasma levels of 2975 nM (bioavailability = 84%) in this study.

Notch-1 is a cell surface receptor important in cell fate decisions.[17] Previous studies have reported that γ-secretase inhibitors block Notch-1 signaling and impair Notch function.[18] Therefore, we tested aminoamide **3k** in NIH 3T3 cells using murine Notch Δ*E* to assess its ability to block γ-secretase-mediated Notch signaling. The potency of **3k** for inhibition of Notch signaling (IC_{50} = 9.8 nM)[10] was similar to that observed for inhibition of Aβ production (IC_{50} = 6.1 nM).

Table 3. Pharmacokinetics of selective compounds

Parameter	2f	2h	3e	3h	3k	3f
po Dose (m pk)	0.5	0.5	0.5	0.5	0.5	0.5
Fu (%, dog)	0.4	0.1	0.5	0.2	1.7	0.5
Cl (l/h/kg)	1.3	0.5	0.2	0.3	0.04	0.2
V_{ss} (V kg)	3.1	2.1	1.9	2.9	0.2	0.9
$t_{1/2}$ (h)	5.3	4.6	13.2	13.4	6.5	5.3
C_{max} (nM)	84	184	567	365	2975	172
F (%)	42	47	61	71	84	23

Data are reported as means of two or more determinations. See Ref. 10 for full experimental details.

In summary, we have found that benzoazepinone-derived cyclic malonamides and aminoamides are potent inhibitors of γ-secretase, as demonstrated by inhibition of cellular Aβ production and low-nanomolar binding to presenilin. SAR studies indicated that a cyclic moiety was preferred at the R position; cyclopentyl and cyclohexyl-derived inhibitors exhibited similar inhibitory activity. The introduction of a hydroxyl moiety in the aminoamide side chain improved both binding and cellular activity. Aminoamide **3k** has fewer stereocenters than succinamide **1** and exhibits similar potency against cellular Aβ production. As shown from preliminary in vivo results, compound **3k** has an attractive overall PK profile in dog. Further studies are needed to assess the effects of **3k** on Aβ levels in the central nervous system.

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