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# Novel orally active, dibenzazepinone-based γ-secretase inhibitors

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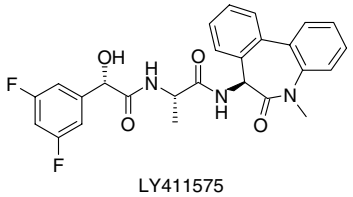
**Abstract**—Structural modifications of the γ-secretase inhibitor, LY411575, led to a malonamide analogue (*S*),(*S*)-**1** with potent inhibitory activity in vitro, but disappointing activity in a mouse model of Alzheimer's disease. Identification and replacement of a metabolically labile position provided an improved compound (*R*/*S*),(*S*)-**13** with high in vitro activity ($IC_{50}$ = 1.7 nM), and in vivo activity after oral administration (MED = 3 mg/kg). Further modifications gave an equipotent carbamate analogue **14** with improved molecular properties.


Alzheimer's disease (AD) is an invariably fatal neurodegenerative disease associated with progressive dementia, and the deterioration of the patient's memory and personality.[1] Current AD therapies can achieve a limited improvement of the patient's declining cognitive functions, but a causal therapy for this devastating disease is not yet available.

Although AD was first described 100 years ago,[2] the exact mechanism of its pathogenesis is still not completely known. A hallmark of AD is the formation of cortical plaques consisting mainly of Aβ peptides, and it is widely accepted that the Aβ peptides and their oligomers play a causal role in the pathology of the disease.[3] Aβ peptides are produced from β-Amyloid Precursor Protein (APP) by sequential cleavage by two proteases, β-secretase and γ-secretase. In a first proteolytic step, β-secretase produces a C-terminal fragment of APP (CTFβ). In a subsequent step, γ-secretase cleaves the CTFβ and produces Aβ peptides of varying lengths. Inhibition of β-secretase or γ-secretase should thus provide a causal therapy for AD. Whereas inhibitors of β-secretase with the potential to cross the blood-brain-barrier have remained elusive for a long time,[4] inhibition of γ-secretase has proven to be a better tractable target for drug discovery.[5] One of the first disclosed γ-secretase inhibitors with high activity and drug-like properties was LY411575,[6] a compound which efficaciously inhibited Aβ production in animal models of AD after oral administration.[7]

To evaluate the potential of LY411575 as a lead structure, we prepared a number of close analogues, which we tested (a) in a HEK293 reporter-gene assay ('cellular') based on a construct of the γ-secretase substrate Notch 1 fused with a Gal4/VP16-transcription factor which activates a firefly luciferase reporter-gene,[8] and (b) in a second assay based on an ELISA technique[9] measuring Aβ40 peptide produced from a recombinant APP C-terminal fragment by HEK293 derived membrane fractions containing γ-secretase activity ('cell-free').[10] Among the tested compounds, we identified malonamide **1** (Fig. 1) as a γ-secretase inhibitor with good potency in both assays (Table 1). **2** was identified



LY411575

**Figure 1.** Elan/Lilly's γ-secretase inhibitor, LY411575.

*Keywords*: Alzheimer's Disease; Gamma secretase; Inhibitor; LY411575; Dibenzazepinone.
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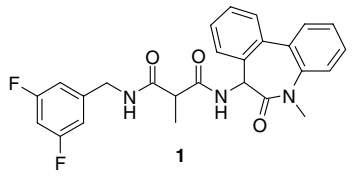
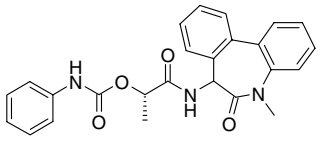
**Table 1.** Malonamide **1** was identified as a potent γ-secretase inhibitor with low microsomal stability



**1**



**2**

| | $IC_{50}$ (cellular) (nM) | $IC_{50}$ (cell free) [nM] | MAB[a] (rat) [%] | MAB[a] (human) [%] |
|---|---|---|---|---|
| **1** | 10 | 22 | 9 | 10 |
| **2** | 1500 | 2700 | Not determined | |

Carbamate **2** was indentified as another analogue of LY411575 with however reduced inhibitory activity. Both **1** and **2** were tested as mixtures of diastereomers.

[a] Maximal achievable bioavailability estimated from incubations with liver microsomes.

as another compound with relatively poor activity, which should however become important during the further course of our studies (vide infra).

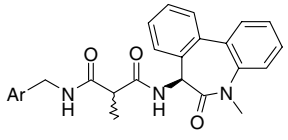
To evaluate the importance of the stereogenic centres of **1**, and also to assess the stability of the diastereomers towards epimerisation, we prepared (*R*/*S*),(*S*)-**1** and (*R*/*S*),(*R*)-**1** (Table 2) from chiral dibenzazepinone building blocks (vide infra). The biologic activity was found to reside mainly in (*R*/*S*),(*S*)-**1**, in which the stereogenic centre of the dibenzazepinone ring was of the same configuration as in LY411575. Using preparative HPLC, (*R*/*S*),(*S*)-**1** was then separated into epimers, which were obtained in a ~1:1 ratio. Both epimers had single-digit nanomolar activities in the two assays; the malonamide stereocentres were arbitrarily defined. The dimethyl substituted or unsubstituted malonamide analogues were found to be less active in our in vitro assays (data not shown).

We anticipated that the malonic acid stereogenic centre of (*S*),(*S*)-**1** and (*R*),(*S*)-**1** would be susceptible to epimerisation, but we found practically no epimerisation (<0.5%) or racemisation in DMSO solutions of (*S*),(*S*)-1 and (*R*),(*S*)-**1** during 10 days. This surprising stability was corroborated by deuteration experiments with **1** in $d^6$-DMSO, to which we had added a few drops of $D_2O$ or DCl. No deuterium was incorporated into the malonamide α-position of **1**, which suggested that the kinetics of a keto–enol equilibrium as a prerequisite for epimerisation are very slow in DMSO, even under acidic conditions.

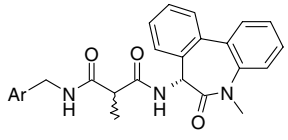
LY411575 and (*S*),(*S*)-**1** were evaluated in a transgenic mouse model of AD which expresses a cDNA of the human APPsw and a human FAD presenilin 2.[11] After subchronic administration of the test compounds, the CTFβ/APP ratio in the brain homogenate was determined by Western blot analysis and was used as a readout for inhibition of γ-secretase activity. Inhibition of γ-secretase leads to an accumulation of CTF, and thus to an increased CTF–APP ratio (see introductory paragraphs). Whereas LY411575 increased the CTF–APP ratio in this experiment after an administration of 5 doses of 20 mg/kg po over 2 days, (*S*),(*S*)-**1** showed an only weak activity after 3 doses of 50 mg/kg ip.

The permeability through an artificial membrane was high for (*S*),(*S*)-**1** and similar compounds (PAMPA,[12] $>10^6$ cm $s^{-1}$). The weak activity of (*S*),(*S*)-**1** in vivo was thus attributed to a low metabolic stability, as predicted by a low stability of **1** in rat and human liver microsome

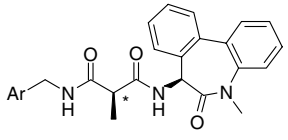
**Table 2.** A separation of diastereomers revealed that the biologic activity resides in the (*S*),(*S*) and (*R*),(*S*) diastereomers of 1



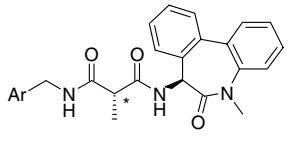
(*R*/*S*),(*S*)-**1**



(*R*/*S*),(*R*)-**1**



(*S*),(*S*)-**1**



(*R*),(*S*)-**1**

| | $IC_{50}$ (cellular) [nM] | $IC_{50}$ (cell-free) [nM] |
|---|---|---|
| (*R*/*S*),(*S*)-**1** | 8.8 | 3.1 |
| (*R*/*S*),(*R*)-**1** | 580 | 620 |
| (*S*),(*S*)-**1** | 1.6 | 2.0 |
| (*R*),(*S*)-**1** | 7.1 | 8.7 |

Significant epimerisation or racemisation of the diastereomers was not observed (see text).

*Stereochemistry arbitrarily assigned.

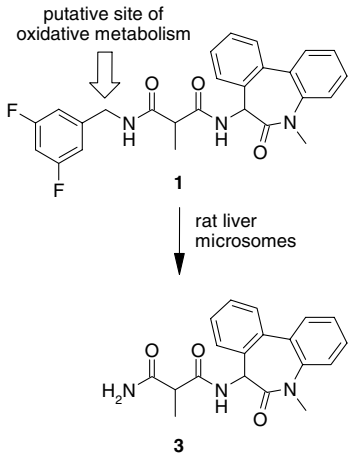
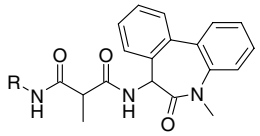
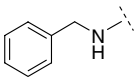
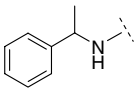
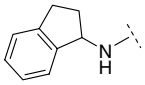
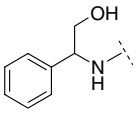
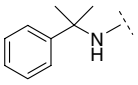
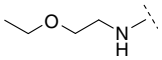
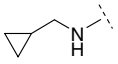
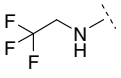
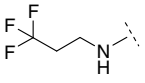
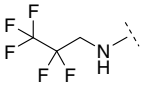



**Figure 2.** Compound **3** was identified as the main metabolite from an incubation of **1** with rat liver microsomes. This metabolite suggests oxidative metabolism in the benzylic position of **1**.

**Table 3.** Inhibitory activities and microsomal stabilities of malonamides **3**–**13** (diastereomeric mixtures)



| | R–NH= | $IC_{50}$ (cellular) [nM] | $IC_{50}$ (cell-free) [nM] | MAB[a] (rat) [%] | MAB[a] (human) [%] |
|---|---|---|---|---|---|
| **3** |  | >25 | 5.3 | | |
| **4** |  | 0.075 | 0.015 | | |
| **5** |  | 6.3 | 5.7 | | |
| **6** |  | 14 | 12 | | |
| **7** |  | >25 | 22 | | |
| **8** |  | 20 | >20 | 9 | 14 |
| **9** |  | 3.3 | 3.0 | | |
| **10** |  | 0.21 | 0.09 | 12 | 18 |
| **11** |  | 0.042 | 0.02 | 37 | 55 |
| **12** |  | 0.024 | 0.037 | | |
| **13** |  | 0.002 | | | |

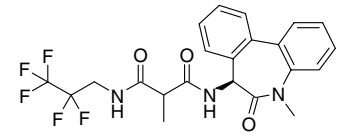
Attempts to stabilise the metabolically labile benzylic position were not successful (**5**–**8**). Fluoroalkyl R groups were finally found as stable replacements (**11**–**13**).

[a] Maximal achievable bioavailability estimated from incubations with liver microsomes.

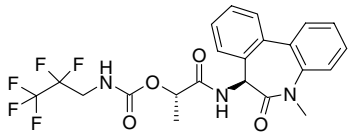
preparations (Table 1). An HPLC-MS/MS analysis of an incubation of **1** with rat liver microsomes indicated **3** as the main metabolite (Fig. 2). This finding points to an oxidation of the benzylic position of **1** to form an unstable aminal (not shown) as a precursor of **3**. Compound **3** has an only marginal inhibitory activity (Table 3).

In an attempt to improve the low metabolic stability of **1**, we prepared analogues **5**–**8** (Table 3), with sterically blocked benzylic positions. However, these compounds had a much reduced inhibitory activity (compare with **4**, Table 3). Furthermore, when we assayed **8** for microsome stability, we found that this compound was similarly unstable as **1**, although an oxidation of the quaternary benzylic position is not possible. We therefore abandoned the concept of stabilising the benzylic position and rather looked for replacements of this motif. However, the difluorobenzyl substituent proved quite critical for activity. We prepared ~50 compounds by parallel chemistry, most of which were inactive and often had low microsomal stabilities. Compounds **9** and **10** are examples which showed marginal in-vitro activity. Interestingly, similar benzoazepinone-derived, cyclic malonamides were very recently disclosed to be potent γ-secretase inhibitors with good pharmacokinetic properties.[13] Finally we prepared fluoro-alkyl derivative **11**, which had an almost restored inhibitory activity as compared to **1**, and moreover a sufficient metabolic stability. Follow-up compound **12** was similarly active. Increasing the degree of fluorination led to **13**, which now even surpassed the inhibitory activity of **1**. For the ease of synthesis, we prepared and tested **3**–**13** as diastereomeric mixtures.

**Table 4.** Malonamide (*R*/*S*),(*S*)-**13** and carbamate **14** emerged as the best γ-secretase inhibitors from the described studies



(*R*/*S*),(*S*)-**13**



**14**

| | (*R*/*S*),(*S*)-**13** | **14** |
|---|---|---|
| $IC_{50}$ (cellular) [nM] | 1.7 | 1.7 |
| $IC_{50}$ (cell-free) [nM] | 3.0 | 8.6 |
| solubility [g/L] | 28 | 290 |
| MAB[a] (human) [%] | 38 | 70 |
| MAB[a] (mice) [%] | 55 | 72 |
| CL (mice) [ml/min/kg] | 53 | 32 |
| $F$[b] [%] | 21 | 23 |
| Brain-to-plasma ratio | 0.5–1.2 | 0.5–0.9 |
| MED[c], po [mg/kg] | 3 | 3 |

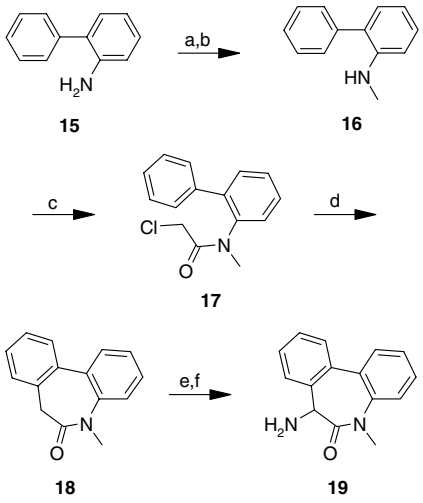
[a] Maximal achievable bioavailability estimated from incubations with liver microsomes.
[b] Mice, 7.8 mg/kg po.
[c] Transgenic mouse model of AD, see text.

From a chiral dibenzazepinone building block (vide infra), we prepared (*R*/*S*),(*S*)-**13** (Table 4). In contrast to (*R*/*S*),(*S*)-**1** (Table 2), a further separation of the epimeric mixture was unsuccessful. The unsuccessful or tedious separation of epimers by HPLC began to emerge as an obstacle that might seriously hamper our future efforts in this series. We recalled that with **2** (Table 1), we had found that a carbamate in place of an amide was tolerated. Although **2** was an only weak γ-secretase inhibitor, it offered the possibility to derive the stereogenic centre from the chiral pool, for example, lactic acid, thus avoiding any separation of diastereomers during the assembly of inhibitors from building blocks. Simply changing malonamide **13** into its carbamate analogue, **14**, gave a γ-secretase inhibitor that was equipotent in both our in-vitro assays.

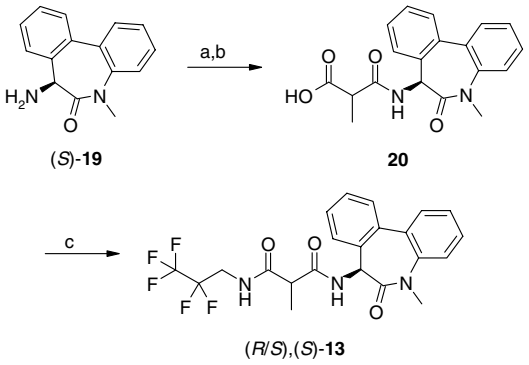
In comparison with malonamide **13**, carbamate **14** had a much improved solubility, and a further improved microsomal stability, which translated into a reduced clearance in vivo. The carbamate functionality is occasionally encountered as a biodegradable motif, however, **14** was found to be stable with plasma and in buffer at a pH of 1.7, 4.7 and 7.8. Only at a pH of 12.8, slow degradation was observed ($t_{1/2}$ = 57 h).

Both (*R*/*S*),(*S*)-**13** and **14** were active in our in-vivo AD model, and dose-dependently increased the CTF–APP ratio after an oral administration of 3 doses of 3 mg/kg over 2 days as a minimal effective dose.

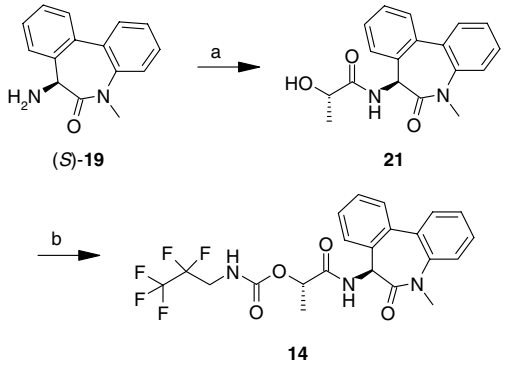
Schemes 1–3 outline the syntheses of our key compounds as representative procedures. Dibenzoxazepine building block **19** was prepared in close analogy to the method of Wu et al.[6] We obtained substantially improved yields when we cyclised the tertiary amide **17** (Scheme 1) instead of a secondary amide as described. This finding can be rationalised by the assumption that




**Scheme 1.** Reagents and conditions: (a) ClCOOEt, $K_2CO_3$, THF/$H_2O$ = 1.5/1, overnight rt, 93%; (b) RedAl, THF, 6 h 50 °C, then overnight rt, 89%; (c) $ClCH_2COCl$, DIPEA, $CH_2Cl_2$, 3 h rt, 71%; (d) $AlCl_3$, 90–180 °C, 2 h, 81%; (e) KHMDS, isoamyl nitrite, toluene, 1 h 0 °C, 65%; (f) $H_2$ (2.4 bar), Pd/C, EtOH, 24 h rt, 83%.




**Scheme 2.** Reagents and conditions: (a) methylmalonic acid mono-*tert*-butyl ester, HOBt, EDCI, DIPEA, THF, overnight rt, 48%; (b) TFA/$CH_2Cl_2$ 1:1, overnight rt 73%; (c) $CF_3CF_2CH_2NH_2$, TPTU, DMF, overnight rt.




**Scheme 3.** Reagents and conditions: (a) L-(+)-lactic acid, HOBt, EDCI, DIPEA, THF, 0 °C, then overnight rt ~quant.; (b) 4-nitrophenyl chloroformate, $Et_3N$, toluene, overnight rt, then $CF_3CF_2CH_2NH_2$, overnight rt.

a cis orientation of the amide bond is a prerequisite for an intramolecular reaction: the preferred trans-orientation of secondary amide bonds might disfavour a cyclisation, whereas tertiary amides adopt no predominant amide orientation and thus undergo cyclisation more readily. The tertiary amide **17** was obtained from biphenylamine **15** via **16** in 3 steps and was then further converted by the reported procedure[6] via **18** to **19**. Enantiomeric separation of racemic **19** by preparative, chiral HPLC (chiralpak AD, ethanol/heptane) provided enantiopure dibenzazepinone building blocks, (*S*)-**19** and (*R*)-**19**.

(*R*/*S*),(*S*)-**13** was prepared according to Scheme 2: (*S*)-**19** was coupled with methylmalonic acid mono-*tert*-butyl ester, and the *tert*-butyl protecting group was cleaved under standard conditions to give **20**. In a second coupling step, **20** was condensed with pentafluoropropyl amine to give the desired product.

Compound **14** was prepared from (*S*)-**19** as outlined in Scheme 3: Coupling with lactic acid gave **21**, which was converted to **14** via a nitrophenyl carbonate as an activated in-situ intermediate. Full experimental details can be found in Ref. 14.

In summary, scaffold modifications of the γ-secretase inhibitor, LY411575, led to malonamide and carbamate analogues. The initially low metabolic stability of these compounds was greatly improved by the replacement of the difluorobenzyl for a pentafluoropropyl substituent, as in malonamide (*R*/*S*),(*S*)-**13** and carbamate **14** (Table 4). Both γ-secretase inhibitors, (*R*/*S*),(*S*)-**13** and **14**, had single-digit nanomolar inhibitory activities in vitro, and were efficacious in a mouse model of AD after oral administration.

## Acknowledgements

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