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Triazoles as γ-secretase modulators

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ABSTRACT

Synthesis, SAR, and evaluation of aryl triazoles as novel gamma secretase modulators (GSMs) are presented in this communication. Starting from the literature and in-house leads, we evaluated a range of five-membered heterocycles as replacements for olefins commonly found in non-acid GSMs. 1,2,3-*C*-aryl-triazoles were identified as suitable replacements which exhibited good modulation of γ-secretase activity, excellent pharmacokinetics and good central lowering of Aβ42 in Sprague–Dawley rats.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder which in 2010 affected over 5 million patients in the US alone.[1] To date, only symptomatic treatments are available, which provide short-term stabilization but have no effect on subsequent disease progression. A widely accepted hypothesis for AD describes oligomers of amyloid β Aβ, and specifically the longer fragment Aβ42, as neurotoxic species. Such oligomers have led to cell death and neurodegeneration in vitro and in vivo, causing symptoms such as cognitive impairment.[2]

The ultimate step in the formation of Aβ fragments[3] is the proteolytic cleavage of C-99[4] by the γ-secretase complex, wherein presenilin acts as the protease which forms Aβ fragments as well as an intracellular domain (AICD).[5] The molecular biology of γ-secretase is complex and the intramembrane proteolysis of this multi-component enzyme is poorly understood.[6]

γ-Secretase inhibitors (GSIs) have been studied for over a decade and several patents and publications have described the efforts in this area.[7] Inhibition of γ-secretase has been associated with mechanism-based side-effects linked to the processing of Notch, one of several other substrates of γ-secretase. Such side-effects potentially limit the long-term use of GSIs.

An alternative approach to interrogate the amyloid hypothesis came from the observation that the longer Aβ42 fragment elicits significantly greater neurotoxicity than their shorter counterparts Aβ36–Aβ40.[2] It has therefore been postulated that modulation of the γ-cleavage to favor production of shorter fragments, while not affecting total Aβ levels, might be a safe approach to a disease-modifying therapy.[8] γ-Secretase modulators (GSMs) modulate the cleavage of C-99 to decrease Aβ42 and increase the shorter Aβ fragments (e.g., Aβ37/38[9]) whilst not affecting cleavage of other substrates such as Notch.[10]

(*R*)-Flurbiprofen (Tarenflurbil, Flurizan™) (**1**, Fig. 1)[11] is an example of the first generation of carboxylic acid GSMs,[12] and we have previously reported the discovery and SAR of several exquisitely selective (Aβ IC_{50} 40/42 > 100) classes of GSMs such as piperidine carboxylic acids **2**.[13] In the evolving arena of non-acid GSMs, following the early work from Neurogenetics, Eisai disclosed aryl imidazoles as represented by **3**.[14] In our own program aimed at identifying novel non-carboxylic acids as modulators of γ-secretase, we previously published our lead optimization strategy for pyrimidine and purine derivatives (e.g., **4**) as GSMs.[15,16] Additionally, we recently detailed the discovery of olefinic quinazolinones (**5**) as GSMs, where we noted that the physicochemical properties of such compounds were poor, hence limiting their oral bioavailability.[17]

Following the discovery of quinazolinone GSMs (**5**), we sought to identify related GSMs with improved solubility and oral exposure in order to assess their in vivo PK/PD profile. Additionally, compounds of the general structures **3** or **5** were also of concern

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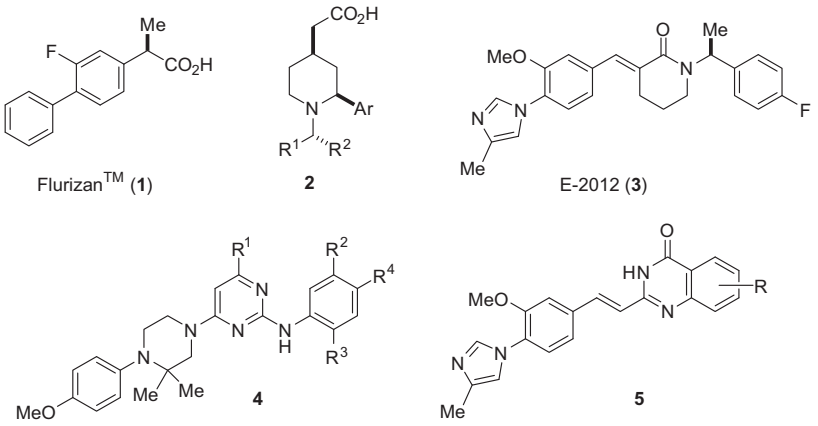


Figure 1. Select gamma secretase modulators.

to us by virtue of their styrene moiety and we aimed to address these concerns by replacing the olefin of compounds **5** with heterocycles (Fig. 2).[18]

Initial studies were aimed at replacing the olefin with simple five-membered heterocycles, which were readily accessible from commercially available starting materials. *C*-aryl triazoles were prepared according to Schemes 1, and 2 describes a range of additional heterocycles that were synthesized.

In Scheme 1, alkyl azides were generally prepared from the corresponding halide or pseudohalide and sodium azide in polar aprotic solvents at room temperature. Aldehyde **8** was readily prepared from commercially available 4-fluoro-3-methoxybenzaldehyde (**7**) and 4-methyl-1*H*-imidazole with potassium carbonate as the inorganic base and heating overnight in DMF. Separation of the undesired regioisomer was achieved through a wash procedure and subsequent purification on silica gel.[19]

Alkynylation of aldehyde **8** with the Bestmann–Ohira reagent gave the desired alkyne **9** in quantitative yield. The click reaction of alkyne **9** with azides in ethanol/water with copper(II)sulfate and sodium ascorbate as the reductant furnished triazoles **10** in good to excellent yields.[20]

Other heterocylic GSMs were prepared as described in Scheme 2 by standard cross-coupling and condensation chemistry from commercially available materials.

S_NAr of compounds **11** and **18** with 2-methyl-imidazole furnished the corresponding building blocks **12** and **19**. Nitro compound **12** was further reduced and then diazotized and treated with sodium bromide or sodium azide to form bromide **13a** and azide **13b**, respectively. Azide **13b** was then converted to the *N*-aryl triazoles **17** under standard click conditions (vide supra).

Oxadiazoles **15** were prepared from bromide **13a** in a two-step procedure that involved carbonylation and trapping with acylhydrazides followed by cyclodehydration. Bromide **13a** was also used as a starting material in Suzuki reactions with heteroaromatic boronates to form pyrazoles **16**, for example. Nitrile **19** was converted to oxadiazoles **20**, tetrazoles **21**, and 1,2,4-triazoles **22** by standard condensation chemistry.

During the course of this initial exploration, a wide range of heterocyclic GSMs were prepared according to the general chemistry described in Schemes 1 and 2. Cell biochemical data for selected examples are reported in Table 1.[21]

Our initial set of five-membered ring heterocyclic replacements revealed a strong preference for triazoles and particularly *C*-aryl-linked 1,2,3- and 1,2,4-triazoles. Further evaluation and screening of both 1,2,3- and 1,2,4-triazoles revealed very similar potencies and modulation potential for both scaffolds, however 1,2,3-triazoles consistently preformed better in vivo (data not shown). After establishing 1,2,3-triazoles as a viable replacement for the olefin in compounds **3** or **5**, we proceeded to explore the SAR of this new triazole series. Rapid exploration of the chemical space was aided by a convergent click chemistry approach from alkyne **9** and azides under Cu-catalysis (see Scheme 1).[22]

We initially proceeded to examine the SAR around benzyl triazole **23**. Substitution in the *meta*- and *para*-positions gave a boost in potency, with 4-substituted and 3,5-disubstituted benzylic triazoles being the most promising in terms of potency (Table 2).

Compound **39** displayed particularly potent GSM activity with an excellent window over Notch (IC_{50} > 50 μM; >100-fold selective).[23] Consequently, we decided to further profile compound **39** in vivo. Initial PK studies in Sprague–Dawley (SD) rats established an excellent profile and triazole **39** was taken into an acute PK/PD experiment to assess brain penetration and Aβ42 lowering (Fig. 3). Six hours after an oral dose of 100 mg/kg in SD rats, an excellent plasma and brain exposure (brain to plasma ratio 1.2:1) was achieved and we were delighted to observe a significant reduction of whole brain Aβ42 levels.

While triazoles **31**–**39** (Table 2) showed a substantial improvement in potency relative to our initial screening hit **23**, we also

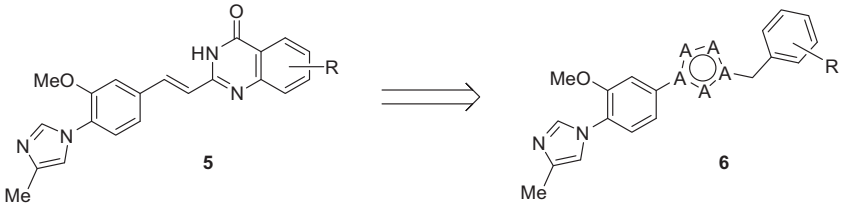
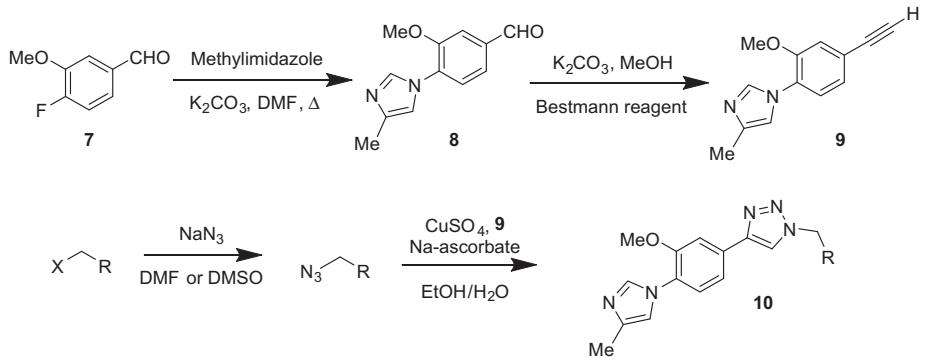
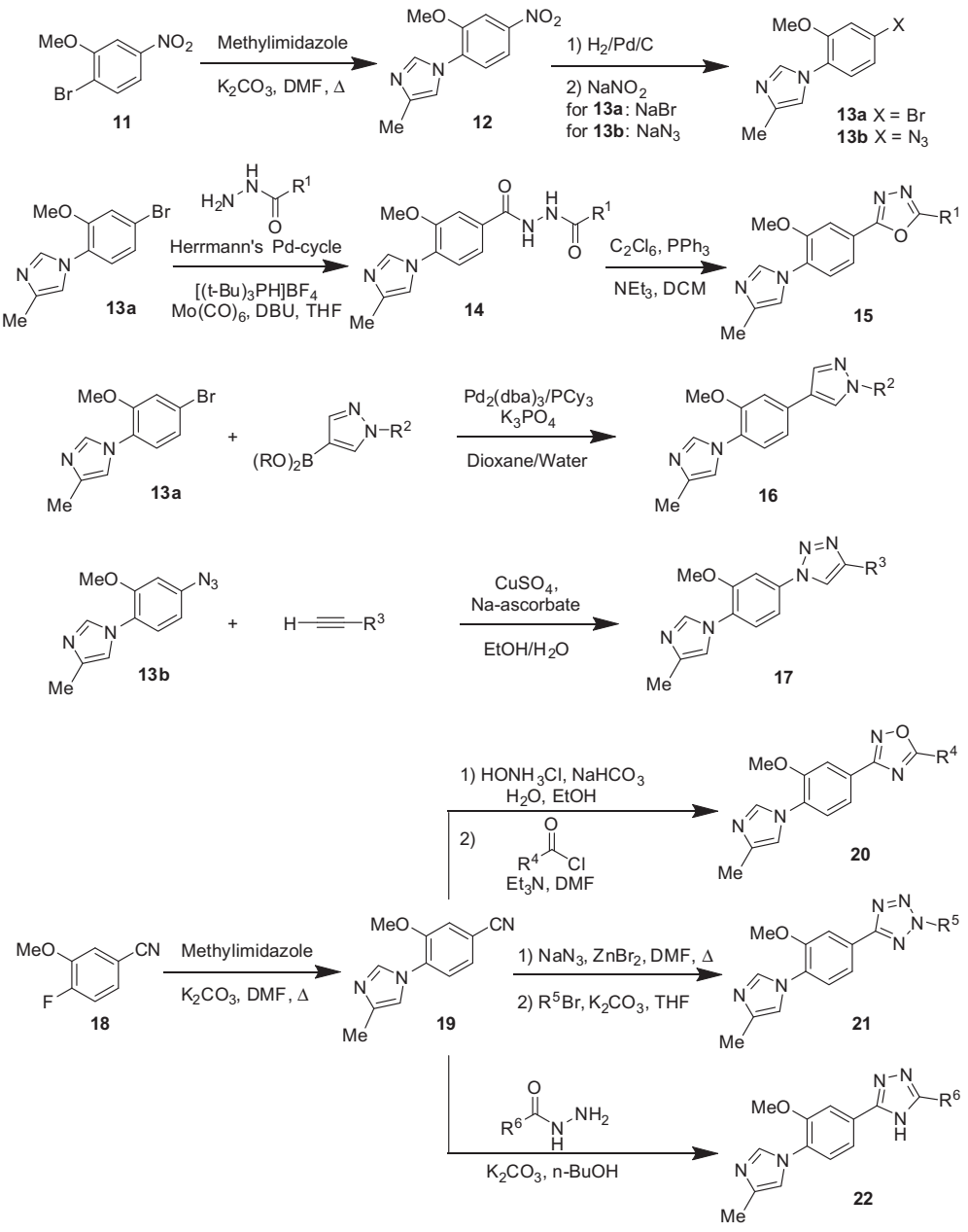


Figure 2. Olefin replacement strategy.



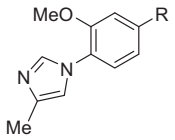
Scheme 1. Synthesis of *C*-aryl-1,2,3-triazole GSMs.

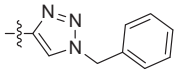
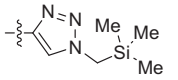
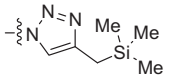
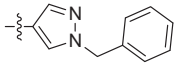
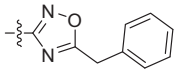
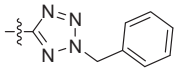
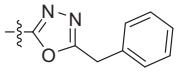
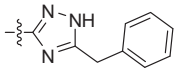
observed an increased binding to the hERG channel. Encouraged by the in vivo activity of compound **39**, we proceeded to further optimize this series with a particular focus on reducing the hERG liability. We speculated that the hydrophobic benzyl substituents



Scheme 2. Synthesis of representative heterocyclic GSMs.

Table 1
Screen of heterocyclic replacements

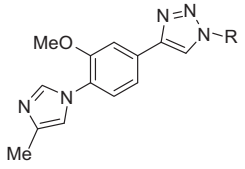


Compound	R	Aβ42 IC_{50} (μM)	Aβ40 IC_{50} (μM)
23		1.44	8.10
24		1.94	7.61
25		7.06	>10
26		>10	>10
27		7.01	>10
28		5.71	>10
29		>10	>10
30		0.78	3.01

All compounds were tested at least twice in independent experiments. For a description of the assay conditions. See Ref. 21.

on the triazole were responsible for the increase in hERG binding[24] and proceeded to look for suitable replacements of the phenyl group as well as introduction of polar functionality (Table 3).

Table 2
Modulation of Aβ40/42 processing by triazoles **31**–**39**



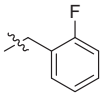
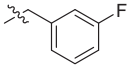
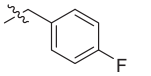
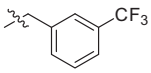
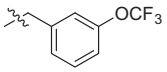
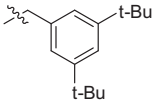
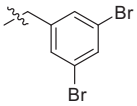
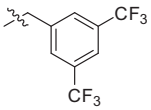
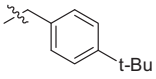
Compound	R	Aβ42 IC_{50} (μM)	Aβ40 IC_{50} (μM)	hERG IC_{50} (μM)
31		2.64	>10	2.49
32		1.44	6.24	2.70
33		1.22	>10	2.19
34		0.49	6.02	1.25
35		0.44	2.53	0.72

Table 2 (*continued*)

Compound	R	Aβ42 IC_{50} (μM)	Aβ40 IC_{50} (μM)	hERG IC_{50} (μM)
36		0.45	1.71	0.32
37		0.32	2.77	0.10
38		0.22	1.19	0.39
39		0.30	4.80	0.39

All compounds were tested at least twice in independent experiments (at least once for hERG). For a description of the assay conditions. See Refs. 21,24.

Dose (iv; po) (mg/kg)	1; 2
Clp (mL/min/kg)	24
Vd_{ss} (L/Kg)	7.1
t½ (iv) (h)	7.7
%F	41
AUC_N 0–24 po (μM h kg/mg)	1.4

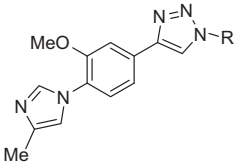
PK/PD assessment 6 hours after an oral dose of 100 mg/kg (n=5)

[Plasma] = 47 μM; [Brain] = 56 μM

Aβ42 (brain): **-48 %**

Figure 3. SD rat PK and PK/PD assessment of triazole **39**.

Table 3
Modulation of Aβ40/42 processing by triazoles **40**–**47**



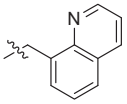
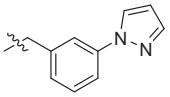
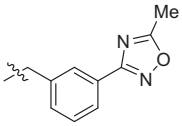
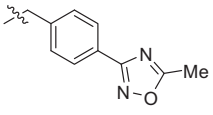
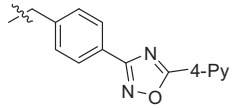
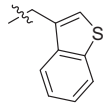
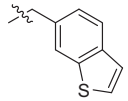
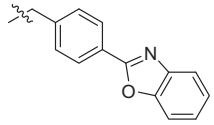
Compound	R	Aβ42 IC_{50} (μM)	Aβ40 IC_{50} (μM)	hERG IC_{50} (μM)
40		0.82	4.04	5.43
41		1.41	>10	>10
42		1.64	6.61	>10
43		1.00	5.46	8.1

Table 3 (*continued*)

Compound	R	Aβ42 IC_{50} (μM)	Aβ40 IC_{50} (μM)	hERG IC_{50} (μM)
44		0.82	7.60	>10
45		0.27	0.77	2.15
46		0.41	1.13	>10
47		0.20	2.06	>10

All compounds were tested at least twice in independent experiments (at least once for hERG). For a description of the assay conditions. See Refs.[21,24]

Dose (iv; po) (mg/kg)	1; 2
Clp (mL/min/kg)	13.7
Vd_{ss} (L/Kg)	2.8
t½ (iv) (h)	5.4
%F	49
AUC_N 0–24 po (μM h kg/mg)	2.6

PK/PD assessment 6 hours after an oral dose of 60 mg/kg (n=5)

[Plasma] = 40 μM; [Brain] = 9.4 μM

Aβ42 (brain): **-42%**

Figure 4. SD rat PK and PK/PD assessment of triazole **47**.

Gratifyingly, both strategies led to a significant attenuation of hERG binding while maintaining good on-target potency and GS modulation. In particular, compound **47** stood out as one of the most potent compounds in this series, accompanied with reduced binding to the hERG channel. Further evaluation of triazole **47** revealed an acceptable overall profile without affecting the processing of Notch (IC_{50} > 50 μM; >100-fold selective). The in vivo rat PK profile of triazole **47** was sufficient for further profiling in rat PK/PD studies and we were pleased to observe central Aβ42 lowering 6 h after a 60 mg/kg dose in SD rats (Fig. 4). However, brain exposure and the brain to plasma ratio[25] were significantly lower compared to compound **39**.

In summary, we have reported the discovery and SAR of triazoles as modulators of γ-secretase. Starting from the initial lead benzyl triazole **23**, we proceeded to optimize potency, brain penetration and hERG binding. We found that the selectivity of Aβ42 over Aβ40 was generally moderate (5- to 20-fold), which is consistent with other non-acid GSMs of this type.[14] However, compounds in this series behaved as GSMs[26] and the selectivity over Notch processing was generally very high (>100-fold), irrespective of the Aβ 42/40 ratio. During the course of this optimization program we identified several potent and selective GSMs with in vivo PD activity upon oral dosing. Future directions in this series and novel strategies for non-acid GSM will be reported in due course.

References and notes

1. Alzheimer's Association: 2010 Alzheimer's disease facts and figures http://www.alz.org/national/documents/report_alzfactsfigures 2010.pdf.
2. See for example: (a) Hung, L. W.; Ciccotosto, G. D.; Giannakis, E.; Tew, D. J.; Perez, K.; Masters, C. L.; Cappai, R.; Wade, J. D.; Barnham, K. J. *J. Neurosci.* **2008**, *28*, 11950; (b) Moreno, H.; Yu, E.; Pigino, G.; Hernandez, A. I.; Kim, N.; Moreira, J. E.; Sugimori, M.; Llinas, R. R. *PNAS* **2009**, *106*, 5901.
3. Thinakara, G.; Koo, E. H. *J. Biol. Chem.* **2008**, *283*, 29615.
4. C-99 is the C-terminal fragment of APP, which is formed from APP by BACE1 mediated proteolysis.
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Consistent with the profile of γ-secretase modulators, total Aβ peptide levels were constant; selected GSMs were confirmed in a SELDI experiment confirming the appearance of shorter fragments while Aβ42 formation was suppressed. (a) Best, J. D.; Jay, M. T.; Otu, F.; Ma, J.; Nadin, A.; Ellis, S.; Lewis, H. D.; Pattison, C.; Reilly, M.; Harrison, T.; Shearman, M. S.; Williamson, T. L.; Atack, J. R. *J. Pharmacol. Exp. Ther.* **2005**, *313*, 902; (b) Clarke, E. E.; Shearman, M. S. *J. Neurosci. Methods* **2000**, *102*, 61; (c) Dyrks, T.; Dyrks, E.; Monning, U.; Urmoneit, B.; Turner, J.; Beyreuther, K. *FEBS Lett.* **1993**, *335*, 89.
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