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Quinazolinones as γ-secretase modulators

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This communication is dedicated to George N. Nikov

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ABSTRACT

Synthesis, SAR and evaluation of styrenyl quinazolinones as novel gamma secretase modulators are presented in this communication. Starting from literature and in-house leads we evaluated a range of quinazolinones which showed good modulation of γ-secretase activity.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, which in 2009 affected over 5 million patients in the US alone.[1] Current standard of care relies on symptomatic treatment, which only provides short-term stabilization and to date no disease modifying therapy has been clinically validated. Late-onset, sporadic AD is a poorly understood disease and while many hypotheses exist, no causative relationship has been established yet. The most widely accepted hypothesis for AD describes oligomers of the β-amyloid (Aβ) peptides, specifically the longer fragment Aβ42, as a neurotoxic species which in vitro and in vivo result in neurodegeneration, thus causing the symptoms of AD such as cognitive impairment.[2]

The last step in the formation of Aβ is the proteolytic cleavage of C-99[3] by the γ-secretase complex, wherein presenilin acts as the protease producing Aβ fragments as well as an intracellular domain (AICD) of unknown function.[4] The biology of γ-secretase is complex and the mechanism of intra-membrane proteolysis by this multi-component enzyme is to date poorly understood.[5]

We and others have investigated inhibitors of γ-secretase for the past decade and a number of patents and publications have described the efforts in this area.[6] Several γ-secretase inhibitors (GSIs) have progressed into clinical trials, but only limited pharmacodynamic and no efficacy data have been released yet. While the efficacy of GSIs is still awaiting clinical validation, it is widely accepted that inhibition of γ-secretase is associated with mechanism-based side-effects potentially limiting the long-term use of GSIs. Most adverse events have been associated with inhibiting the processing of Notch, one of several other substrates of γ-secretase in addition to C-99. Efforts to develop GSIs that dissociate inhibition of C-99 and Notch cleavage are a field of intense research and several promising leads have been documented.[7]

An alternative approach to interrogate the amyloid hypothesis came from the observation that the longer Aβ42 fragments elicit significantly greater neurotoxicity than their shorter counterparts Aβ36–40.[2] It has therefore been postulated that modulation of the γ-cleavage to increase production of shorter fragments at the expense of longer fragments such as Aβ42 might be a safer approach to a disease modifying therapy. Gamma secretase modulators (GSMs) modulate the cleavage of C-99 to decrease Aβ42, increase Aβ37/38[8] and leave total Aβ levels unchanged whilst not affecting cleavage of other substrates, such as Notch.[9]

The discovery that (*R*)-flurbiprofen (Tarenflurbil, Flurizan™) (**1**, Fig. 1)[10], amongst other nonsteroidal anti-inflammatory drugs, showed a GSM-like profile fueled the research in this area. Recently Myriad reported disappointing Phase III data for Flurizan™ leading to the discontinuation of clinical development.[11] While this has been a major setback for the field of γ-secretase modulation, the cellular potency of Flurizan™ and brain exposure is very low. Additionally, studies have recently suggested that Flurizan™ might bind to amyloid precursor protein (APP) and not to γ-secretase it-

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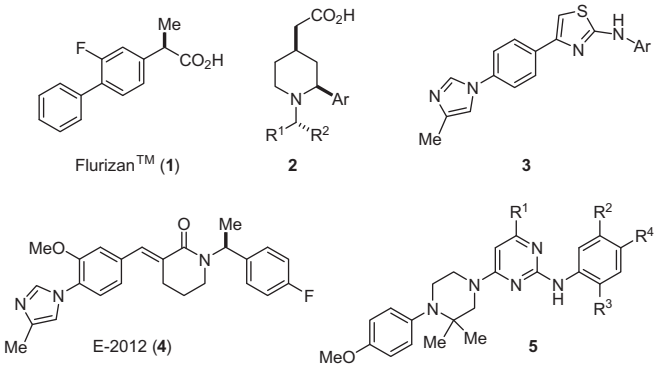


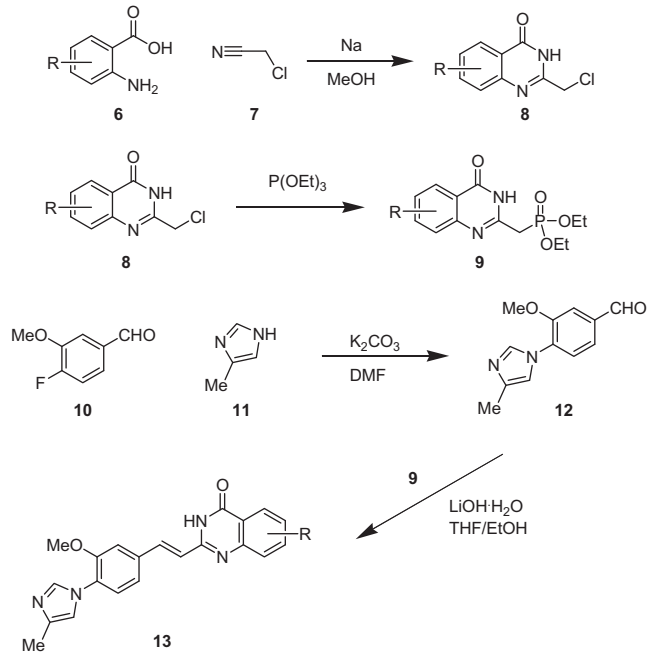
Figure 1. Selected gamma secretase modulators.

self.[12] Therefore, despite negative Phase III data, the discovery of potent modulators of γ- secretase which directly target the enzyme rather than APP is still worth pursuing.

We have reported the discovery and SAR of exquisitely selective (Aβ40 IC_{50}/Aβ42 IC_{50} >100) carboxylic acid GSMs such as piperidine carboxylic acids **2**[13] and Torrey-Pines[14] as well as Eisai[15] disclosed aryl imidazoles as represented by **3** and **4** (Fig. 1).

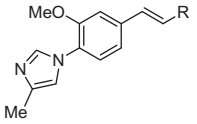
In an effort to diversify our GSM portfolio we embarked on a program to identify novel non-carboxylic acids as modulators of γ-secretase. Recent publications have detailed our lead optimization strategy in pyrimidine derivatives (e.g., **5**) as GSMs.[16,17] Herein we present our work on olefinic quinazolinones.[18]

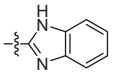
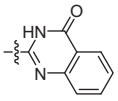
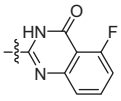
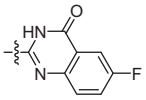
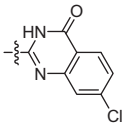
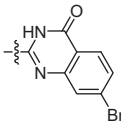
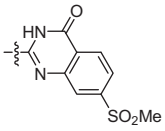
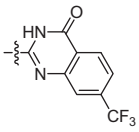
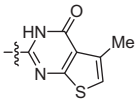
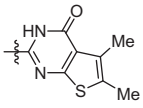
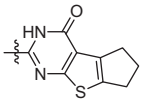
Our efforts in this area started with lessons learned from literature examples **3** and **4** as well as our in-house compounds such as **5**. Initially we focused our efforts on maintaining the olefinic spacer of **4** while investigating amide replacements such as benzimidazoles (**14**, Table 1).[19] While we were able to identify several imidazole derivatives which behaved as potent γ-secretase modulators (data not shown), they all suffered from significant hERG binding.[20] We hypothesized that a ring-expansion from benzimidazoles to quinazolinones would lower their basicity and attenuate hERG binding while maintaining their salient properties.



Scheme 1. Representative synthesis of compounds **14**–**32**.

Table 1
Modulation of Aβ40/42 processing by compounds **14**–**24**



Compound	R	Aβ42 IC_{50} (μM)	Aβ40 IC_{50} (μM)	hERG IC_{50} (μM)
14		0.84	>10	0.68
15		3.89	4.95	>10
16		4.87	>10	n.d.
17		4.28	>10	>10
18		0.66	>10	>10
19		0.34	4.82	>10
20		2.18	6.74	>10
21		0.37	3.74	0.87
22		1.29	>10	>10
23		1.65	>10	>10
24		0.37	3.89	4.73

Synthesis of the cinnamyl quinazolinones (**13**) commenced with the preparation of phosphonates (**9**) from chloromethyl quinazolinones (**8**), which were either commercially available or alternatively prepared from the corresponding anthranilic acids (**6**) with chloroacetonitrile (**7**) and freshly prepared sodium methoxide (Scheme 1). The solvent-free reaction of chloromethyl quinazolinones (**8**) with triethylphosphite gave the corresponding phosphonates (**9**) in generally good yields and most analogues could be readily precipitated from diethylether requiring no purification.

The left-hand piece of the molecule was readily prepared from commercially available 4-fluoro-3-methoxybenzaldehyde (**10**)

Dose (iv; po) (mg/kg)	1; 2
Clp (mL/min/kg)	6.8
Vd_{ss} (L/Kg)	1.8
t½ (iv) (h)	5.8
%F	59
AUC_N 0–24 po (μM h kg/mg)	3.7

Figure 2. Pharmacokinetic profile of **18** in Sprague–Dawley rats.

and 4-methyl-imidazole (**11**) with potassium carbonate as the inorganic base and heating over night in DMF. Separation of the undesired regio-isomer was achieved through a wash procedure and subsequent purification on silica gel.[21]

Horner–Wadsworth–Emmons reaction of 3-methoxy-4-(4-methyl-1*H*-imidazol-1-yl)benzaldehyde (**12**) with quinazolinone phosphonates (**9**) yielded the final products in good yields after purification by normal or reversed-phase chromatopgraphy.[22]

Quinazolinones **14**–**24** were profiled in our hAPP-overexpressing SH-SY5Y cell line and showed potent modulation of Aβ formation (Table 1).[23]

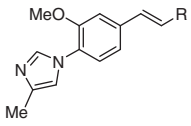
Substitution on the 7-position of the quinazoline ring proved optimal for potency (e.g., **18**, **19**, **21**) and we were delighted to see significant attenuation of hERG binding as measured by displacement of MK-499 from hERG (e.g., **18**–**20**).[24] Compounds in this series were variably selective for inhibition of Aβ42 versus Aβ40 production, with some compounds having no selectivity (e.g., **15**) and others having at least 15-fold selectivity (e.g., **18**).

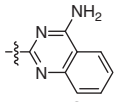
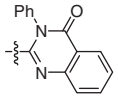
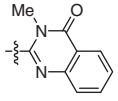
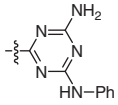
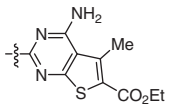
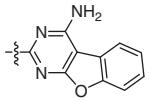
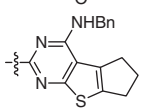
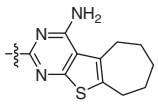
Quinazolinone **18** was early on identified as a compound with reasonable cell potency, Aβ42 selectivity and attenuated hERG binding.[25] Compound **18** was further evaluated in vivo and displayed excellent rat pharmacokinetics with acceptable oral bioavailability (Fig. 2).

The quinazolinones in Table 1 proved to be poorly soluble even after substantial formulation efforts, which led to variable exposures at higher doses. We hypothesized that the pyrimidone group was responsible for the poor physicochemical properties and began to investigate *N*-alkylated and arylated quinazolinones as well as aminopyrimidines, which were prepared either from commercially available building blocks or in a one-pot procedure from the corresponding pyrimidones.[26] Gratifyingly, both approaches led to compounds with significantly improved solubility.[27] However, a slight loss in potency and/or increased hERG binding offset the gain in solubility (Table 2).

In summary, we have reported the discovery and SAR of styrenyl quinazolinones as modulators of γ-secretase. Starting from benzimidazole **14**, which suffered from significant hERG binding, we discovered that ring-expansion to quinazolinones attenuated binding to the hERG channel. Compound **18**, with a functional hERG IC_{50} >10 μM, was a potent GSM with an excellent pharmacokinetic profile. Future directions in this series and novel strategies for non-acid GSMs will be reported in due course.

Table 2
Aβ40/42 data for compounds **25**–**32**



Compound	R	Aβ42 IC_{50} (μM)	Aβ40 IC_{50} (μM)	hERG IC_{50} (μM)
25		1.69	6.10	0.14
26		2.08	>10	0.99
27		>10	>10	0.31
28		1.07	3.33	0.30
29		0.54	3.72	0.98
30		0.32	2.96	0.51
31		1.86	>10	1.12
32		0.20	2.03	0.39

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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. *Proc. Natl. Acad. Sci.* **2009**, *106*, 5901.
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7. For a recient reivew on APP processing, see: Thinakaran, G.; Koo, E. H. *J. Biol. Chem.* **2008**, *283*, 29615; For a recent review on secretase biology and modes of γ-secretase inhibition/modulation, see: De Strooper, B.; Vassar, R.; Golde, T. *Nat. Rev. Neurol.* **2010**, *6*, 99.
8. All GSMs lower Aβ42 selectively over Aβ40, however, some raise the levels of Aβ38 and/or Aβ37. The consequence of this difference is unknown.
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17. (a) Rivkin, A.; Ahearn, S. P.; Chichetti, S. M.; Kim, Y. R.; Li, C.; Rosenau, A.; Kattar, S. D.; Jung, J.; Shah, S.; Hughes, B. L.; Crispino, J. L.; Middleton, R. E.; Szewczak, A. A.; Munoz, B.; Shearman, M. S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1269; (b) Rivkin, A.; Ahearn, S. P.; Chichetti, S. M.; Hamblett, C. L.; Garcia, Y.; Martinez, M.; Hubbs, J. L.; Reutershan, M. H.; Daniels, M. H.; Siliphaivanh, P.; Otte, K. M.; Li, C.; Rosenau, A.; Surdi, L. M.; Jung, J.; Hughes, B. L.; Crispino, J. L.; Nikov, G. N.; Middleton, R. E.; Moxham, C. M.; Szewczak, A. A.; Shah, S.; Moy, L. Y.; Kenific, C. M.; Tanga, F.; Cruz, J. C.; Andrade, P.; Angagaw, M. H.; Shomer, N. H.; Miller, T.; Munoz, B.; Shearman, M. S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2279.
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19. The olefin spacer is crucial; the more flexible ethylene spacer is significantly less potent. The saturated analogues of **14** and **15** have Aβ42 IC_{50}s >10 μM.
20. We have also investigated several other heterocyclic amide replacements, such as oxazoles, oxadiazoles, thiazoles and thiadiazoles. While all of them behaved as GSMs, their binding to the hERG channel was generally equal or greater than their cellular potency as GSMs.
21. Careful work-up and purification was necessary to remove the unwanted regio-isomer. See Ref. 18 for detailed synthetic procedures.
22. Other analogues, including amino quinazolines used in Table 2, were prepared in a similar manner. For detailed synthetic procedures, see Ref. 18
23. IC_{50} measurements for inhibition of Aβ40 and Aβ42 production were determined using electrochemiluminescent detection of these peptides secreted by SH-SY5Y cells stably overexpressing the APP C-terminal fragment SPA4CT. Consistent with the profiles of γ-secretase modulators, total Aβ peptide levels were constant. The GSM profile of selected compounds was confirmed by mass spectrometry (SELDI), which confirmed the appearance of shorter (Aβ37) 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.
24. For a description of the hERG radioligand displacement assay, see: Butcher, J. W.; Claremon, D. A.; Connolly, T. M.; Dean, D. C.; Karczewski, J.; Koblan, K. S.; Kostura, M. J.; Liverton, N. J.; Melillo, D. G. WO 200205860.
25. In a functional evaluation of hERG (I_{Kr}) currents with PatchXpress, compound **18** could not be fully evaluated due to solubility limitations; at the maximum soluble concentration (10 μM) a 39% ($N = 4$) mean blockade was observed.
26. Compound **31** was prepared in a one-pot procedure from the corresponding fused pyrimidone **24** according to: Wan, Z.-K.; Binnun, E.; Wilson, D. P.; Lee, J. *Org. Lett.* **2005**, *7*, 5877.
27. Solubility of compounds **15**–**24** was not quantifiable at pH 7 in phosphate buffered saline (PBS) buffer. Compounds **25** and **27** had 6 and 33 μM solubility, respectively, in PBS.